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Antioxidant and Antibacterial Activities of Coriander (*Coriander sativum* L.) Seeds Essential Oil

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Natural products offer a wide range of possibilities for the development of new remedies. Coriander (*Coriandrum sativum* L.) seeds have been reported to contain numerous biological activities. This study aims to determine the antioxidant and antibacterial activities of coriander seeds essential oil. The essential oil was obtained using the hydrodistillation technique from non-soaked and soaked coriander seeds. The antioxidant activity was determined using the DPPH assay, while the antibacterial activity of the essential oil was determined using the Kirby-Bauer disc diffusion method against three Gram-positive bacteria: *Staphylococcus aureus* (ATCC 12600), *Staphylococcus epidermidis* (ATCC 12228), and *Bacillus cereus* (ATCC 14579). The yield of essential oil for soaked coriander seeds (0.79%) was higher than that of non-soaked coriander seeds (0.54%). The DPPH assay disclosed that the essential oil obtained from non-soaked coriander seeds had inactive antioxidant activity. Furthermore, the antibacterial activity of the essential oil from soaked seeds showed a potent antibacterial effect on *B. cereus* and *S. aureus* bacteria at 14.99 mm and 14.90 mm, respectively, while the essential oil from non-soaked seeds showed a potent antibacterial effect on *S. epidermidis* at 8.70 mm. In conclusion, coriander seeds essential oil can be developed as a new and effective antibacterial agent by showing the inhibition zone on bacteria through these studies, which can treat infections that cause human diseases.

Keywords: *Coriandrum sativum* L; soaking; non-soaking; antioxidant; antibacterial

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Free radicals, or oxidative stress, are one of the factors that cause the aging process [1]. Free radicals accumulate in cells due to excessive reactive oxygen species (ROS) and inadequate antioxidant defence mechanisms, leading to oxidative stress. Oxidative stress in cells causes damage to DNA, cell membranes, and mitochondrial DNA [2]. One class of chemicals known as ROS are oxygen-based or oxygen-containing active compounds. Superoxide anion ($\bullet O_2^-$), hydrogen peroxide (H_2O_2), the extremely active hydroxyl radical ($\bullet OH$), singlet oxygen, lipid peroxides, and nitrogen oxides are examples of ROS in the body. The human body normally has enzymatic and non-enzymatic anti-oxidant defence mechanisms to maintain the balance between free radicals and antioxidants by eliminating excess ROS [3]. According to Kassahun et al. (2020), the function of antioxidants is to stop or slow down these chain reactions by eliminating or oxidizing free radicals to prevent subsequent oxidation reactions [4]. Antioxidants, including vitamins C and E and glutathione, can be found in plants and mammals. They also possess some enzyme systems that catalyze antioxidant reactions, including catalase, superoxide dismutase (SOD), and peroxidase [5].

Meanwhile, antibacterial resistance has become a serious threat to public health as bacterial resistance is rapidly developing and spreading. The increase in bacterial resistance has resulted in several antibacterial agents being less effective [6]. This limits the therapeutic options for treating bacterial infections and leaves patients with few alternatives. According to the World Health Organization (WHO), infectious diseases are the second leading cause of death worldwide [7]. Therefore, it is crucial to search for new alternative antibacterial agents to combat the problem of bacterial resistance. The antibacterial agents in coriander seeds are potent enough to fight microorganisms [8]. For example, the double bonds in the structure of the monoterpenoid alcohol compounds, such as linalool, confer strong antibacterial properties [8].

Coriandrum sativum L. (*C. sativum*) is a plant from the Apiaceae or Umbelliferae family [5]. The medicinal plant is commonly used as a spice to enhance flavor in food preparation. *C. sativum*, which can grow up to 1.4 meters tall, is known by different names in different countries, including 'yuan sui' in China, 'ketumbar' in Malaysia, 'cilantro' in Spain, 'geshniz' in Iran, and 'coriander' in America [9, 10]. Valuable parts of the plant's dry schizocarp include two ovoid round mericarps and several longitudinal grooves on the surface, which are found in the seeds or fruits [11]. The taste is sweet, spicy, citrusy, and

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slightly reminiscent of sage [10]. Due to the differences in the leaves, two species of coriander are recognized in Islamic Traditional Medicine (ITM) textbooks [12]. One has tiny and round leaves and seeds, while the other has larger leaves and seedlings. The most utilized part of the coriander plant is the fruit, which consists of the seeds and pericarp [13]. Essential oil (EO) and fatty acids are the two major components that can be discovered in the seeds of *C. sativum* plant. The fatty acids have been reported to contain triglyceride oil, petroselinic acid, and monounsaturated fatty acids [10, 11]. The EO content in coriander seeds ranges between 0.2% and 1.5%, although it can be as high as 2.6%, while the fatty acid content ranges between 13% and 20% [10, 14].

Past studies reported that the yield of coriander seed essential oil (CSEO) ranged from 0.03% to 2.6% [8] and 0.18 to 1.40% [15]. The aromatic EO of coriander seeds is composed of several monoterpenoids. The main component is linalool, which is an acyclic monoterpene alcohol that usually accounts for more than 60% of EO [1, 16]. It has the potential to become an effective natural preservative or therapy due to its extensive biological activities. CSEO has been reported to possess antibacterial, antioxidant, antidiabetics, antiaging, and antihyperlipidemic activity [17]. Additionally, CSEO exhibits antioxidant properties by scavenging free radicals (DPPH and galvinoxyl) and preventing oxidative damage containing lipids [8]. Kassahun (2020) reported that the EO in coriander seeds can inhibit microorganisms in their growth zone [4, 18]. It can also penetrate the cytoplasmic membrane and cell wall due to its hydrophobic nature, which disrupts the structure of the cell wall and makes it more permeable. Cell death is the result of macromolecules and other cellular components leaking through the permeable membrane. In bacteria, membrane permeabilization is associated with ion loss, a decrease in membrane potential, failure of the proton pump, and depletion of the ATP pool. In addition, EO-induced coagulation of the cytoplasm can damage proteins and lipids [19].

The majority of past studies have focused on the antibacterial activity against some pathogenic bacteria and the antioxidant activity of specific medicinal plants [20]. The ability of microorganisms to undergo genetic variability (mutation), the use of antibiotics in food preservation, the overuse and underuse of prescription drugs, and general drug misuse have all been associated with an increase in the resistance of microorganisms to drugs. Moreover, oxidative stress is a harmful process that can have negative effects on various cellular structures such as membranes, lipids, proteins, lipoproteins, and DNA. This occurs when there is an imbalance between the formation of free radicals and the body's ability to remove them, ultimately contributing to the development of diseases such as cardiovascular disease, neurological disorders, and respiratory illnesses [36]. In order to use medicinal plants for treatment, it is necessary to identify them correctly and to know their antibacterial and antioxidant properties. These effects can be assessed by studying the EO of plants containing various bioactive compounds, which have been propounded as an effective alternative to counter various diseases and infections. Hence, this study aims to explore the antioxidant and antibacterial activities of EO of coriander (*C. sativum*) seed.

EXPERIMENTAL

Material and Methods

Plant Materials

Coriander seeds (2 kg) were purchased from a local supermarket (i.e., Mydin) located in Ipoh, Perak, Malaysia on March 2023.

Extraction of Coriander Seed Essential Oil

Approximately 200 g of sample was soaked in 500 mL of distilled water in a round bottom flask for one hour at room temperature. It was followed by the extraction process using a Clevenger apparatus to produce EO. Next, for the preparation of non-soaked coriander seed, 200 g of sample was used and filled with 500 mL of distilled water in a round bottom flask. Then, the extraction process was proceeded with using a Clevenger apparatus without a soaked step. Then, the extraction process proceeded to produce EO using the Clevenger apparatus [21].

The coriander seed essential oil (CSEO) was extracted by hydrodistillation at room temperature for 4 hours. The oily layer in the collecting flask was collected in a glass bottle and dried with anhydrous sodium sulphate to remove water. The CSEO was stored at 4 °C and protected from light until further use [22]. The following formula was used to determine the percent yield of CSEO [23].

Determination of Antioxidant Activity

DPPH Radical Scavenging Activity Assay

Radical scavenging activity is a mechanism for the reduction of 1,1-diphenyl-2-picrylhydrazil (DPPH) free radicals by antioxidants, resulting in a transformation of the DPPH methanol solution from purple to yellow.

$$\text{Percentage of essential oil yield (\%)} = \frac{\text{Mass of essential oil obtained (g)}}{\text{Mass of dry matter (g)}} \times (100)$$

100 mL of methanol was used to dilute 2.4 mg of DPPH powder. Since DPPH is sensitive to light, the beaker was wrapped in aluminium foil to protect the solution from light. Ascorbic acid was used as a positive control (standard), whereby 2 mg of ascorbic acid was dissolved in 2 mL of distilled water at 1 mg/1 mL [20].

The ascorbic acid solution was then serially diluted, starting with the concentrations of 1000, 500, 250, 125, and 62.5 µg/mL in a test tube containing 4 mL of the solution. The procedure was performed in triplicate. After serial dilutions, 300 µL ascorbic acid and 2.7 µL DPPH solution were added to the test tube, and the mixture was stored in a dark cabinet for 15 minutes. A similar procedure was applied to the non-soaked CSEO. For the blank solution, 300 µL of methanol was mixed with 2.7 µL of DPPH solution and the mixture was stored in a dark cabinet for 15 minutes. An aluminium foil was placed over each test tube to protect it from light. The absorbance was measured at 517 nm using methanol as a blank on a UV-Vis spectrophotometer [19]. The activity of the DPPH radical scavenger was calculated using the following equation:

$$\text{Scavenging activity (\%)} = [(A_0 - A_1) / A_0)] \times (100\%)$$

Where A_1 is the sample's absorbance while A_0 is the absorbance of the blank.

Determination of Antibacterial Properties

The bacterial organisms used in this study consisted of three bacterial strains from the American Type Culture Collection (ATCC). The bacterial strains were composed of Gram-positive bacteria: *Staphylococcus aureus (S. aureus*) (ATCC 12600), *Staphylococcus epidermidis* (*S. epidermidis*) (ATCC 12228), and *Bacillus cereus (B. cereus)* (ATCC 14579). These bacteria were obtained from the laboratory of UniKL–RCMP and cultivated in a solid medium of Nutrient Agar (NA) plates by streaking and stored at 4 °C.

Preparation of Agar Medium

Mueller-Hinton (MH) agar was prepared using the dehydrated medium according to the supplier's recommended recipe (HIMEDIA of Technical Data-Muller Hinton Agar ref 173). Approximately 38 g of MH agar powder was dissolved in 1000 mL of distilled water and the powder was swirled in the water solution until dissolved. All items were sterilized by autoclave for 15 minutes at 121 °C prior to use.

Once the sterilization process was completed, the pH level of each preparation was measured to ensure that it ranged between 7.2 and 7.4 at room temperature. This could be done in two ways: to allow a small amount of medium to get around a pH meter electrode or to macerate a small amount of medium in a little distilled water. The medium was cooled to 40 to 50 °C before the agar solution was poured into a sterile glass petri dish, with about 4 mm of agar evenly spread from one side [18]. The agar plates were dried in an incubator preheated between 30 to 37 °C for a maximum of 30 minutes or until the remaining surface moisture had evaporated. Two agar plates remained in the incubator for 24 hours to check for possible microbial contamination [24].

Preparation of Essential Oil using Blank Paper Disc

A blank paper disc (6 mm in diameter) was drizzled with 10 µL of pure CSEO [3]. It was held for 10 minutes before being placed on the MH agar. The paper discs created a concentration gradient of CSEO that diffused outward. Regarding the studies on CSEO, two types of paper disc samples were used to evaluate the zone of inhibition for different types of bacteria: one sample came from soaked CSEO and the other came from non-soaked CSEO.

On different bacteria of agar medium, the antibiotic disc acted as a positive control in conducting the disc diffusion method. Several commercially available antibiotic discs, including ampicillin, ciprofloxacin, gentamicin, and vancomycin, were utilized in the evaluation against Gram-positive bacteria.

Isolation of Bacterial Culture into a Colony

The isolation of the bacteria was conducted using the streak plate method to bring out one single colony from the bacterial culture. The single colony in an agar plate was then used to ensure that the bacteria were easier to take for dilution on bacterial suspension. Three types of bacteria colonies were used in this study, namely *S. epidermidis*, *B. cereus*, and *S. aureus*—they were taken from a pure bacterial culture (no longer than 48 hours), transferred, and diluted to 3 mL of 0.9% saline. The broth was later evaluated by a UV spectrophotometer at 625 nm, whereby the absorbance must be in the range of 0.8000 to 1.000 for each suspension [24].

Kirby-Bauer Disc Diffusion Method

An antibacterial susceptibility test was performed using the Kirby-Bauer diffusion method for *S. epidermidis*, *B. cereus*, and *S. aureus*. A bacterial lawn was prepared by dropping 100 uL of the bacterial suspension onto an MH agar plate, followed by spreading and streaking on the agar surface with cotton swab [25]. The agar plate was divided into two quadrants and carefully placed with antibiotic and CSEO paper discs using sterile forceps. The plates were incubated at 37 °C for 24 hours [18]. All studies were performed in three independent replicates for soaked and non-soaked CSEO with different types of bacteria. The appearance of the zone of inhibition around the antibiotic and CSEO paper discs indicates a positive result of antibacterial activity [9].

Table 1. Yield of coriander seed essential oil.

Sample	Non–soaked	Soaked
Weight of sample (g)	5.50	5.61
Yield of essential oil (%)	0.54	0.79

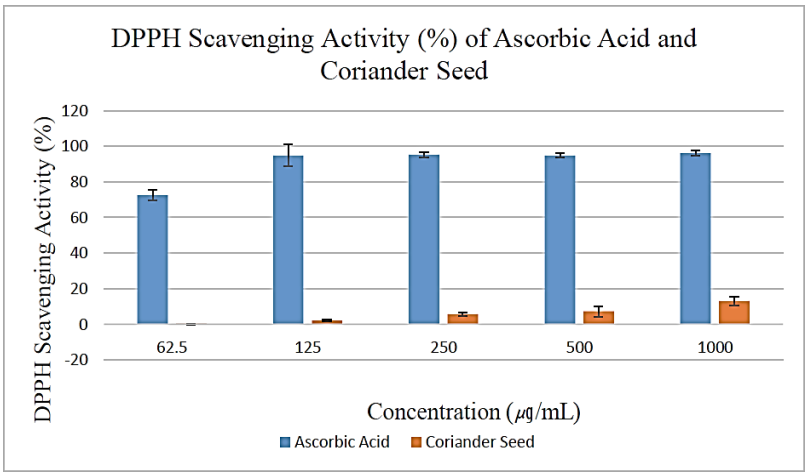

Figure 1. DPPH scavenging activity (%) between ascorbic acid and non-soaked CSEO.

Measurement of Zone Inhibition

The inhibition zones visible after 24 hours of incubation were measured in millimeters (mm) for each quadrant. The measurement of inhibited bacterial growth was determined based on the test data collected from the information plates [18]. The control quadrant of the corresponding plate was compared with the sample test quadrants. All studies were performed in three independent replicates and the diameter of the inhibition zone was determined.

RESULTS AND DISCUSSION

Percentage Yield of the Coriander Seeds Essential Oil

The EO content of coriander seeds depends on whether they are soaked. In this study, the hydrodistillation of non-soaked and soaked coriander seeds yielded 0.54% and 0.79% of essential oils, respectively (**Table 1)**. The result was based on the weight of 1 kg per two samples of coriander seeds used. A colorless oil with a characteristic odor and a mild, sweet, warm, and aromatic taste was obtained from the fully ripened and dried coriander seeds. During hydrodistillation, the color changes of CSEO were evaluated based on the physical appearance of the oil. At the beginning of the production, the CSEO appeared in clear form and it started to become cloudy after one hour of the process. The temperature was set at 100 °C during the process to ensure that the sample remained in a boiling state. Additionally, continuous condensation took place throughout the 4-hour process.

Some researchers have conducted various studies on coriander seeds. For instance, the study by Huzar et al. (2018) found that CSEO yield ranged between 0.2% to 1.5%, 0.03% to 1.5%, and 0.655 to 2.2%, respectively [2,16,17]. Based on the results in **Table 1**, the CSEO yields of soaked and non-soaked coriander seeds agree with those reported by previous studies. This can be attributed to the different stages of immature or mature coriander seeds, geographical cultivation of coriander seeds, and agronomics. Furthermore, this study found that the soaking method provided a high yield of CSEO compared to the non-soaking method. According to [26], pre-treatment by soaking showed a high amount of EO obtained. Additionally, the soaking method has been propounded to help open and expand the pores of the seed's cell structure and increase the production of oil [21].

Antioxidant Properties

The DPPH radical scavenging assay is commonly used to evaluate the antioxidant activity of chemical compounds or extracts. DPPH stands for 2,2-diphenyl-1-picrylhydrazyl, a stable free radical with a deep purple color. When the purple color of the DPPH radical fades, it indicates the scavenging or antioxidant activity of the tested compound. The extent of color reduction is proportional to the antioxidant activity of the compound tested. The DPPH radical is photosensitive

and can be photochemically degraded upon exposure to light, especially sunlight and UV radiation [27]. **Figure 1** shows a comparison of antioxidant activity between ascorbic acid and non-soaked CSEO for various concentrations. The percentage inhibition ranged between 2.01% to 13.02% for CSEO and 62.5% to 95.98% for ascorbic acid. Moreover, non-soaked coriander EO was found to have 66.48% of DPPH activity [28] and a 51.05% inhibition of radical scavenging activity [19].

The disparities in results could be due to the differences in plant collection. The coriander seeds used for this experiment were mainly from the local Malaysian market, but in the previous study, it was purchased from a grocery store in Iran [29]. Different sources of plant collection can affect the results in numerous ways. Plants from different sources may have genetic differences due to factors such as geographic location, environmental conditions, breeding history, and maturity [13]. These genetic differences can lead to variations in plant characteristics, including chemical composition. Furthermore, plants are highly responsive to their environment, variations in climate, soil conditions, and growing methods, which can affect their growth and development [30]. These environmental differences can affect the plants' metabolic processes, resulting in variations in the composition of bioactive compounds and other chemical constituents. Factors such as soil contamination, pesticide use, and nearby industrial activities can also introduce contaminants into the plants, thus affecting test results [13].

Antibacterial Activity of CSEO

Mueller-Hinton (MH) agar, which has good reproducibility, contains low concentrations of sulphonamide, trimethoprim, and tetracycline inhibitors, and also allows satisfactory growth of most pathogenic bacteria, was used to perform the disc diffusion method [31]. For this study, both non-soaked and soaked CSEO were tested against *S. epidermidis, B. cereus,* and *S. aureus.* Gram-positive bacteria were chosen in this study as they may facilitate the penetration of hydrophobic EO compounds because their cell membranes contain the lipophilic ends of lipoteichoic acid [11]. The antibiotics disc used in this study contained ampicillin, ciprofloxacin, gentamicin, and vancomycin to determine the appropriate zone of inhibition as a positive control. According to **Table 2**, all antibiotics have the potential to inhibit bacterial growth. To ensure optimal use, it is crucial to be aware of the appropriate zone of inhibition for each antibiotic. For *B. cereus*, ampicillin achieved an appropriate zone of inhibition of 44 mm. Ciprofloxacin achieved a zone of inhibition of 41 mm for *S. epidermidis*, while gentamicin had an appropriate zone of inhibition of 32 mm. For *S. aureus*, vancomycin achieved an appropriate zone of inhibition of 18 mm.

Table 3 shows the values of zones of inhibition induced by non-soaked and soaked CSEO, while **Figures 2 to 7** show images of their zones of inhibition. The soaked sample demonstrated higher values than the non-soaked sample against *B. cereus* and *S. aureus,* with 14.99 mm and 14.90 mm, respectively. As for *S. epidermidis*, the non-soaked CSEO showed greater levels (8.7 mm) than the soaked CSEO (8 mm). This proves that soaked CSEO can have potent antibacterial effects, particularly against *B. cereus* and *S. aureus,* while non-soaked CSEO has a low antibacterial effect against *S. epidermidis.* The zone inhibition also compared the strength of antibacterial activity on the inoculum. These three types of bacteria are usually associated with an environment by humans. Furthermore, Gram-positive bacteria can facilitate the penetration of hydrophobic EO compounds because their cell membranes contain lipophilic ends of lipoteichoic acid [19].

Among the clinical strains of multidrug-resistant bacteria, *B. cereus* was the most susceptible. Studies by Onder (2018) showed that the antibacterial activity of CSEO was significantly potent against *B. cereus* [32]. In another study by Bag and Chattopadhyay (2015), CSEO was found to have effective antibacterial activity as it had greater antibacterial potency and range compared to non-soaked CSEO, with a zone of inhibition of about 25.00 ± 1.97 mm [33]. According to Rezaei et al. (2016), studies on *S. aureus* using CSEO found that this CSEO has high potency in antibacterial activity with a zone of 12.50 ± 1.32 mm [34]. Meanwhile, Khalil et al. (2018) evaluated CSEO by conducting a qualitative and quantitative antibacterial study for *S. aureus*, leading to a zone of inhibition of 17.00 mm. These previous studies supported the results of the current study, which advocates the antibacterial potency of CSEO against *B. cereus* and *S. aureus* [35].

Table 2. Zones of inhibition induced by positive controls (mm).

Antibiotics	*S. epidermidis*	*B. cereus*	*S. aureus*
Ampicillin	17.00 ± 0.00	44.00 ± 0.00	8.00 ± 0.00
Ciprofloxacin	41.00 ± 0.00	34.00 ± 0.00	27.00 ± 0.00
Gentamicin	32.00 ± 0.00	22.00 ± 0.00	22.00 ± 0.00
Vancomycin	17.00 ± 0.00	17.00 ± 0.00	18.00 ± 0.00

Table 3. Zones of inhibition induced by non-soaked and soaked coriander seeds essential oils (mm).

Bacteria	Coriander seeds essential oil	
	Non–soaked	Soaked
S. epidermidis	8.70 ± 0.07	8.00 ± 0.00
B. cereus	14.06 ± 0.05	14.99 ± 0.08
S. aureus	12.90 ± 0.08	14.90 ± 0.07

(a) (b) (c)

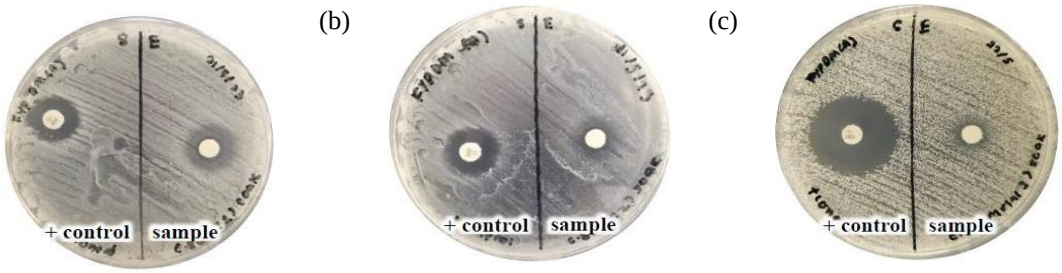

Figure 2. *S. epidermidis* for non-soaked essential oil, (a) Trial 1; (b) Trial 2; (c) Trial 3.

(a) (b) (c)

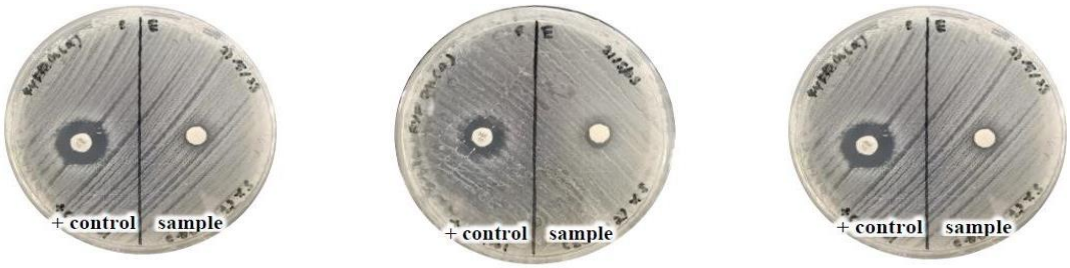

Figure 3. *S. epidermidis* for soaked essential oil, (a) Trial 1; (b) Trial 2; (c) Trial 3.

(a) (b) (c)

Figure 4. *B. cereus* for non-soaked essential oil, (a) Trial 1; (b) Trial 2; (c) Trial 3.

(a) (b) (c)

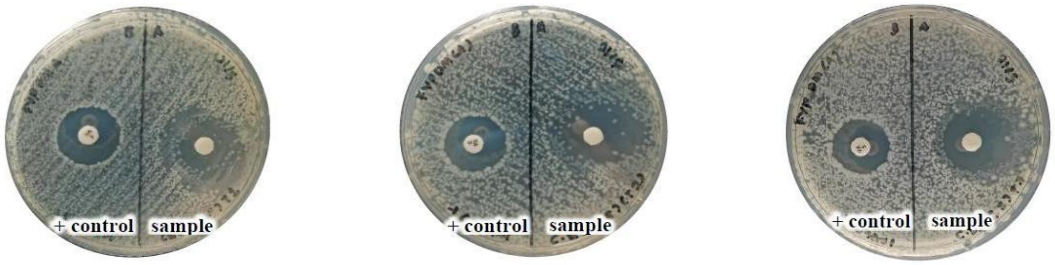

Figure 5. *B. cereus* for soaked essential oil, (a) Trial 1; (b) Trial 2; (c) Trial 3 1.8 ± 0.00.

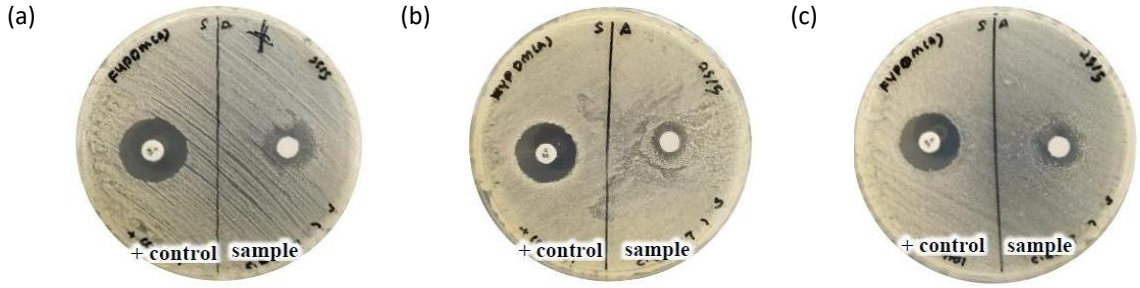

Figure 6. *S. aureus* for non–soaked essential oil, (a) Trial 1; (b) Trial 2; (c) Trial 3.

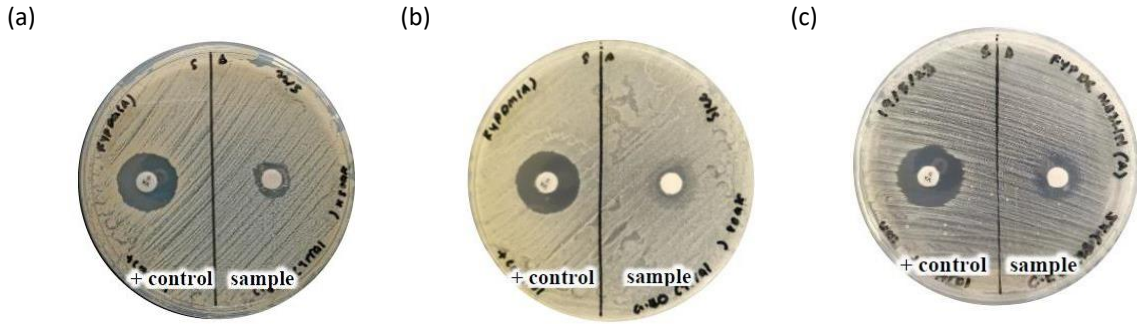

Figure 7. *S. aureus* for soaked essential oil, (a) Trial 1; (b) Trial 2; (c) Trial 3.

CONCLUSION

The findings of this study denote that CSEO in the soaked state can produce a higher yield of essential oil than in the non–soaked state. This can be demonstrated by the increased production of the yield of CSEO. The DPPH assay also showed that CSEO possessed inactive antioxidant activity. Moreover, CSEO demonstrated good potential for antibacterial activity against *S. epidermidis, B. cereus,* and *S. aureus,* with potent activities reported in soaked samples for *B. cereus* (14.99 mm) and *S. aureus* (14.90 mm). Due to CSEO's promising antibacterial activity, it can be further developed as a new agent for antibacterials to battle human infections. This study proposes the need to further analyze the phytochemical profile of essential oils to confirm the compound's suitability for a specific biological activity. More specifically, GC-FID and GC-MS can be used to determine the phytochemical profile of the essential oil. This can be complemented by conducting a docking analysis of the compounds. To further improve the efficacy of essential oils, nanotechnology can be employed to introduce nanoencapsulation of these oils. The use of nanosuspensions can provide a viable alternative to synthetic products because of the broad spectrum of antimicrobial activity and antioxidant activity possessed by essential oil.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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