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Research Article

Chemical Compositions of Essential Oil from Aerial Parts of *Cyclospermum leptophyllum* and Its Application as Antibacterial Activity against Some Food Spoilage Bacteria

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Cyclospermum leptophyllum is plant species known for its medicinal value and pleasant aroma. The aerial part and plant seeds are traditionally used as food additives as a spice. This study aims to isolate the chemical constituents of essential oil of the aerial part of the plant and study their potential antibacterial activities against some food contaminating bacteria. The essential oil of *C. leptophyllum* (CSEO) was isolated from aerial parts of the plant species and studied using GC-MS and FTIR techniques. The first four major chemical constituents determined from GC-MS analysis of CSEO (for peak area % ≥ 1.15%) were 2,5-dimethoxy-*p*-cymene (87.09%), 2-methoxy-1-methyl-4-(1-methylethyl) benzene (3.09%), 2-methoxy-4-methyl-1-(1-methylethyl) benzene (1.71%), and humulene (1.15%). 60%, 30%, 15%, 7.5%, and 3.75% of CSEO solutions were prepared and evaluated for their potential antibacterial activities against six food spoilage pathogenic bacterial strains. Three Gram-positive strains: *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (ATCC 12228), *Streptococcus agalactiae* (ATCC 12386) and three Gram-negative strains: *Escherichia coli* (ATCC 25922), *Proteus mirabilis* (ATCC 35659), and *Pseudomonas aeruginosa* (ATCC 27853) were used as test microorganisms. Compared to ciprofloxacin, a positive control, the promising antibacterial activity was observed for CSEO against *S. aureus* at minimum and maximum test solutions as the values of the zone of inhibition diameter (ZID, mm) were recorded as 14.33 ± 0.58 for 3.75% CSEO solution and 30.67 ± 0.58 for 60% CSEO solution. Tests of CSEO solutions generally showed stronger antibacterial activities against Gram-positive than Gram-negative strains. Therefore, CSEO contains potent chemical constituents that might be applicable in treating pathogenic bacterial species.

1. Introduction

Medicinal plants have been widely used for various applications supported by *in vivo* and *in vitro* studies due to their easy affordability and fewer side effects [1–5]. The World Health Organization (WHO) has reported that about 80% population of the world uses plants and natural products to treat different pathogenic diseases [6, 7]. Medicinal plants have also been reported as the source for the invention of novel drugs, and 25% of modern drugs contain one or more active components of plant origin [8, 9]. Similarly, the world's top 25 best-selling medicines were obtained from the natural products of plants [10]. Some reports showed that about 17,500 aromatic plants are known for producing essential oils (EOs) [11, 12]. EOs are the mixtures of secondary metabolites with characteristic flavour and odour.

These phytochemicals protect plants from various bacterial, fungal, and viral diseases [13–17].

Plant ecology and growth conditions can affect the quantity and quality of isolated EOs [18]. These natural products are volatile oils and naturally occurring organic compounds in plants with various physical and chemical properties with multiple functions and health benefits [19]. Some studies showed that the EOs of different parts of medicinal plants have been widely applied for diverse biological and pharmacological applications because of their wide-spectrum bioactive compounds [20–22]. Various literature reviews also reported that EOs or their significant components are used as plausible alternatives for treating pathogenic bacteria due to their complex composition of secondary metabolites [23–26]. Currently, EOs of plants are known to be employed in food as preservatives/additives, medicine, and agricultural commodities for their potential antibacterial activities [27–33].

Cyclospermum leptophyllum (Pers.) belongs to the family *Apiaceae*. This family contains approximately 450 genera and 3,700 species [34]. *C. leptophyllum* is a small, spreading, erect, and much-branched annual herb (Figure 1) [35]. Its fruit is traditionally used to treat flatulence, dyspepsia, diarrheal, laryngitis, rheumatoid arthritis, bronchitis, asthma, and folk medicine [36]. The leaves and seeds of *C. leptophyllum* were reported to be applied to treat loss of appetite and disease, which are caused by sweet inflammation locally known as "mitch" in Ethiopia and food additives, respectively [37]. The EO of *C. leptophyllum* displayed significant activities against Gram-positive and Gram-negative pathogenic bacterial strains [38]. To the best of our knowledge, there have been a limited number of reports regarding the investigation of antibacterial activities of EO of the aerial part of *C. leptophyllum* against food-related pathogenic bacterial species. Hence, the main aim of this study was to isolate *C. leptophyllum* essential oil (CSEO), identify its chemical compositions, and finally evaluate its potential antibacterial activity against some pathogenic bacteria responsible for food contamination using ciprofloxacin, an antibiotic drug as a positive control. Antibacterial activities of CSEO were evaluated by using six food-contaminating pathogenic bacteria from three Gram-positive and three Gram-negative strains. The newly reported chemical compositions and antibacterial activity effects of CSEO might provide important information about the bioactivities of phytochemicals of this plant species and its diversified chemical compounds and potential applications.

2. Materials and Methods

2.1. Chemicals and Solvents. In this study, all chemicals and solvents of analytical grade from Sigma-Aldrich were used. The solutions were prepared using sterilized distilled water throughout the antibacterial activity tests.

2.2. Plant Samples' Collection, Authentication, and Preparation. C. leptophyllum, Figure 1, was collected from Tullu Dimtu, Addis Ababa, Central Ethiopia, located at the latitude of 8° 88′ 52″ North and longitude of 38° 80′ 98″ East, in November 2020. Voucher specimens YH21 were authenticated by Mr. Melaku Wondafrash and deposited at the National Herbarium (ETH), Department of Plant Biology and Biodiversity Management, Addis Ababa University, Addis Ababa, Ethiopia. The aerial parts of the plant sample were separated, cleaned carefully, and dried under shade for two weeks. Then, the dried sample was milled using the electric grinder, and the powdered sample was stored in nontransparent plastic bags until the hydrodistillation process.



Figure 1: Images of *C. leptophyllum* plant species.

2.3. Isolation of Essential Oils. Essential oil of *C. leptophyllum* (CSEO) was isolated from a 2 kg powdered sample (dry weight) by using the hydrodistillation technique in Clevenger-type apparatus for 3 hours based on the procedure from European Pharmacopoeia (Phar. Eur. Supplement 7.0). CSEO was separated and dried over anhydrous Na_2SO_4 from the aqueous phase and then stored in brown glass bottles in the refrigerator until further analysis.

2.4. Characterization of Essential Oil

2.4.1. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis. Gas chromatography-mass spectrometry measurements were performed according to the procedure proposed by Freire et al. [39] with some minor modifications. GC-MS analysis of CSEO was carried out using an HP5890 Series II gas chromatograph, HP5972 mass selective detector, and Agilent 6890 Series autosampler (Agilent Technologies, Santa Clara, CA, USA). A Supelco MDN-5S, 30 m × 0.25 mm capillary column with a 0.5 μm film thickness was used with helium as the carrier gas at a 1.0 mL/min flow rate. CSEO was diluted in n-hexane (1 : 10), and GC-MS results were obtained using the following conditions: split 1 : 20; injection volume 0.1 μl; injection temperature 250°C; oven temperature progress from 60 to 130°C at 1°C/min, from 130–200°C at 2°C/min, from 200–250°C at 4°C/min and held at 250°C for 40 min; the ionization model used was an electronic impact at 70 eV. The chemical composition of CSEO was identified from their Kovats retention indices (KIs) on the capillary column. The chemical constituents of

CSEO were identified based on a homologous series of C_7-C_{25} n-alkanes, and we compared their mass spectral fragmentation patterns with those stored in the NIST spectral database and literature reports [39, 40].

2.4.2. Fourier Transform Infrared (FTIR) Analysis. Fourier transform infrared (FTIR) was performed based on the procedure reported by Getahun et al. [41] with some modifications. FTIR spectra of CSEO were recorded using an FTIR spectrophotometer (IS50 ABX, Thermo Scientific, USA) to identify the major functional groups in the CSEO sample. A few drops of CSEO were used with a resolution of 4 cm^{-1}, a spectral range of 400–4000 cm^{-1}, and several scans of 32.

2.5. Antibacterial Activities of Essential Oil

2.5.1. Bacterial Strains. Six food-related pathogenic bacterial strains, three from Gram-positive and three from Gram-negative, were used to study the antibacterial activities of CSEO. Gram-positive bacterial strains were *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (ATCC 12228), and *Streptococcus agalactiae* (ATCC 12386), and Gram-negative bacterial strains were *Escherichia coli* (ATCC 25922), *Proteus mirabilis* (ATCC 35659), and *Pseudomonas aeruginosa* (ATCC 27853). The standard bacterial strains were obtained from the Traditional and Modern Medicine Research Directorate Laboratory, Ethiopian Public Health Institute, Addis Ababa, Ethiopia.

2.5.2. Preparation of Test Solutions. The concentrations of solution of CSEO, positive control, and negative control were prepared as follows: mixing 1.20 ml CSEO with 0.80 ml of 5% (v/v) Tween-80 to obtain 60% of CSEO solution. 60% CSEO was used as stock solution from which 30% CSEO solution was prepared. The solvent is 5% Tween-80 solution. All the remaining lower concentrations of test solutions such as 15%, 7.5% and 3.75% of CSEO solution were prepared from their preceding concentration of CSEO solution based on the same procedure. 5% (v/v) Tween-80 solution was prepared by mixing 5 ml of Tween-80 (purity 99.99%) with 95 ml of sterilized distilled water. 5 μg/ml (5 gm in 1000 ml) of ciprofloxacin and 5% (v/v) Tween-80 were prepared and used as a positive and negative control, respectively.

2.5.3. Agar Diffusion Method. The antibacterial activity test was performed according to the protocol described by Mungole and Chaturvedi [42] with some minor modifications. Cultures of bacterial strains were prepared in the Luria–Bertani (LB) media for assays. Muller–Hinton Agar (MHA) (Oxoid Ltd., Hampshire, UK) culture media were used in Petri dish plates to grow microorganisms. The culture media were boiled in the sterilized distilled water to dissolve the media and autoclaved at 121°C for 50 min. After cooling, 20 ml of MHA media were poured into the Petri dish plates (90 mm in diameter) using the pipette. The solidified culture media in plates were seeded with bacterial suspension using cotton swabs. For the standardization of test organisms, bacterial suspensions were diluted and adjusted to reach 0.5 McFarland (1.5×10^7 CFU/ml) turbidity at 625 nm using a UV spectrophotometer (Evolution 60S Thermo Scientific, USA), with an optical density of 0.08–0.1.

Wells with 8 mm diameter were punctured into the agar plates with a Cork borer. The wells were filled with 120 μl of EO and control solutions using a micropipette. Sterility and growth control plates were used in parallel to ensure the sterility of nutrient media and microorganism growth ability on media, respectively. All equipment and materials used in all activities were sterilized before use. The Petri dish plates were incubated for 24 hours at 37°C. The inhibition of bacteria growth was evaluated by measuring the diameter (in mm) of the clear zone around the wells [43]. Ciprofloxacin (5 μg/ml) and 5% (v/v) Tween-80 and 5 ml of 99.99% Tween-80 to 95 ml of sterilized distilled water were used as a positive and negative control. The zone of inhibition diameter (ZID, mm) was the mean of three replicates, and all values were expressed as mean ± standard deviation (SD).

2.6. Statistical Analysis. All antibacterial activity tests were performed in triplicate. All data were analyzed using IBM SPSS Package (Version 26.0) for statistical analysis. The experimental results were expressed as mean ± standard deviation (SD). The statistically significant differences between concentrations of CSEO solution were compared using a one-way ANOVA. Differences were considered significant when $p \leq 0.05$. Post hoc analysis was also carried out with Tukey's test.

3. Results

3.1. Essential Oil Yield. EO obtained from *C. leptophyllum* (CSEO) was light yellow in colour. The total percentage yield (% v/w) of CSEO obtained from the dry weight of the plant sample by using the hydrodistillation process was 0.84%.

3.2. Characterization of Essential Oil

3.2.1. GC-MS Analysis. GC-MS analysis of CSEO resulted in the identification of 16 chemical constituents (for relative peak area % ≥ 0.11), which represent 96.86% of the relative area percentage of the total EO compositions. CSEO has oxygenated monoterpenes (92.34%), oxygenated sesquiterpenes (0.57%), and nonoxygenated sesquiterpenes (3.95%). The identified chemical constituents of CSEO were 2,5-dimethoxy-*p*-cymene (87.09%), 2-methoxy-1-methyl-4-(1-methylethyl) benzene (3.09%), 2-methoxy-4-methyl-1-(1-methyl ethyl)-benzene (1.71%), humulene (1.15%), α-curcumene (0.91%), E-caryophyllene (0.90%), α-zingiberene (0.42%), humulene-1,2-epoxide (0.31%), δ-cadinene (0.23%), β-bisabolene (0.21%), 4-(1-methylethyl)-benzaldehyde (0.18%), carvacrol (0.16%), (+)-spathulenol (0.14%), α-amorphene (0.13%), eudesma-4(15),7-dien-1β-ol (0.12%), and *p*-cymene (0.11%). Chemical structures and all the related GC-MS results of chemical constituents of CSEO have been displayed in Figure 2 and Table 1. The result of

GC-MS analysis of CSEO regarding its chemical constituents' retention time (min), experimental Kovats retention indices ($KI_{exp.}$), literature Kovats retention indices ($KI_{lit.}$) chemical name, and relative peak area percentage is summarized and presented in Table 1.

3.2.2. Fourier Transform Infrared (FTIR) Analysis. FTIR spectra of CSEO are shown in Figure 3. CSEO displayed FTIR peaks at 2962 cm^{-1}, 1506 cm^{-1}, 1465 cm^{-1}, 1402 cm^{-1}, 1207 cm^{-1}, 1047 cm^{-1}, and 811 cm^{-1}.

3.2.3. Assessment of Antibacterial Activities. Antibacterial activity effects of various concentrations of CSEO solution were evaluated against six food-related pathogenic bacterial strains such as *S. aureus*, *S. epidermidis*, *S. agalactiae*, *E. coli*, *P. mirabilis*, and *P. aeruginosa*. The results of all zone of inhibition diameter (ZID, mm) are the mean ± SD of three replicates and are provided in Table 2. Values of $p \leq 0.05$ were considered significant.

In comparison with the control groups, antibacterial activities of different concentrations of CSEO solution against some bacterial strains were determined based on their relative zone of inhibition diameter percentage (% RZID) calculation as given in (Eq.(1)) [44, 45] and presented in Table 3.

$$\%\ \%\text{RZID} = \frac{\text{ZID sample} - \text{ZID negative control}}{\text{ZID positive control} - \text{ZID negative control}} \times 100, \quad (1)$$

where % RZID is the percentage relative zone of inhibition diameter, the ZID sample is the zone of inhibition diameter of CSEO (mm), the ZID positive control is the zone of inhibition diameter of ciprofloxacin (mm), and the ZID negative control is the zone of inhibition diameter of 5% Tween-80 (mm).

4. Discussion

In this study, the percentage yield and relative area percentage of the total composition of the isolated CSEO were 0.84% and 96.86%, respectively. In the earlier reports, 0.3–1.1% yields of EOs were reported from different parts of *C. leptophyllum*. Helal et al. [36] reported that 1.1% EO yield was obtained from the fruit part of the plant. Helal et al. [38] also determined the percentage yield of EOs of *C. leptophyllum* from its roots, green aerial part, unripe fruit, and ripe fruit as 0.1%, 0.4%, 0.8%, and 1.1%, respectively. Verma et al. [35] also demonstrated that the percentage yield of EO from the fresh aerial part of *C. leptophyllum* at the seed setting stage was 1.0%. In our study, as shown in Table 1, about 16 identified compounds such as 2,5-dimethoxy-*p*-cymene (87.09%), 2-methoxy-1-methyl-4-(1-methylethyl) benzene (3.09%), 2-methoxy-4-methyl-1-(1-methylethyl)-benzene (1.71%), humulene (1.15%), α-curcumene (0.91%), E-caryophyllene (0.90%), α-zingiberene (0.42%), humulene-1,2-epoxide (0.31%), δ-cadinene (0.23%), β-bisabolene (0.21%), 4-(1-methylethyl)-benzaldehyde (0.18%), carvacrol (0.16%), (+)-spathulenol (0.14%), α-amorphene (0.13%), eudesma-4(15), 7-dien-1β-ol (0.12%), and *p*-cymene (0.11%) which account for 96.86% were determined from GC-MS analysis (peak area % ≥ 0.11%). CSEO is a rich source of oxygenated terpenes (92.91%). 2,5-dimethoxy-*p*-cymene (87.09%) was reported as the major component of CSEO of the aerial part of *C. leptophyllum*. The highest relative area percentage of major chemical constituents (87.09%) is reported for this specific plant species. Some previous reports showed that 2,5-dimethoxy-*p*-cymene (46.8%), methyl ether thymol (14.6%), *p*-cymene (13.9%), γ-terpinene (8.9%), carvacrol methyl ether (7.5%), and γ-gurjunene (1.1%) were the first six major components reported from EO of aerial part of *C. leptophyllum,* and the relative area percentage of the total chemical composition of EO of the plant species was 97.7% (for peak area % > 0.05) [35]. Singh et al. [46] also revealed that the EO of *C. leptophyllum* contained 2,5-dimethoxy-*p*-cymene (50.7%) as a major chemical constituent. In this research work, some unidentified compounds with greater peak area percentages were obtained from the GC-MS analysis of CSEO, and even though their experimental Kovats retention indices (KI_{Expt}) were determined, the corresponding chemical compounds could not be identified from the NIST spectral database or literature survey.

For FTIR peak bands of CSEO, in Figure 3, the peak that appeared at 2962 cm^{-1} represents sp^3C-H symmetric stretching bond vibration. The absence of band absorption in the 1850–1600 cm^{-1} region indicates that a carbonyl group is not likely present. FTIR peaks at 1506 cm^{-1}, 1465 cm^{-1}, and 1402 cm^{-1} show the aromatic $C=C$ bond stretching. The strong peak at 1207 cm^{-1} and the medium peak at 1047 cm^{-1} describe an aromatic C-H bond in-plane bending and C-O bond stretching. The weak peak absorbed at 811 cm^{-1} is due to the aromatic C-H bond out-of-plane bending [47–49]. The FTIR results further supported the functional groups present in the major chemical constituents of CSEO.

The major components of CSEO with diversified functional groups are the most responsible organic compounds for potential antibacterial activities against most food-related pathogenic bacterial species. In the present study, as summarized in Tables 2 and 3, the variation of the zone of inhibition diameter (ZID, mm) and the relative zone of inhibition diameter percentage (% RZID) values depend on the type and concentrations of CSEO solution and bacterial strains used for evaluation. The treatments were compared by analysis of ANOVA on the various concentrations of CSEO solution and on the antibacterial activities measured. This analysis was followed by the post hoc Tukey HSD test (95% confidence level) to compare the effects of different conditions on the measured parameters. The concentrations of CSEO solution at lower concentrations revealed weak antibacterial activities against *S. agalactiae, P. mirabilis,* and *P. aeruginosa.*

The most potent antibacterial activity effects were observed for different concentrations of CSEO solution against *S. aureus* for both minimum and maximum concentrations of solution used (ZID at 3.75% CSEO

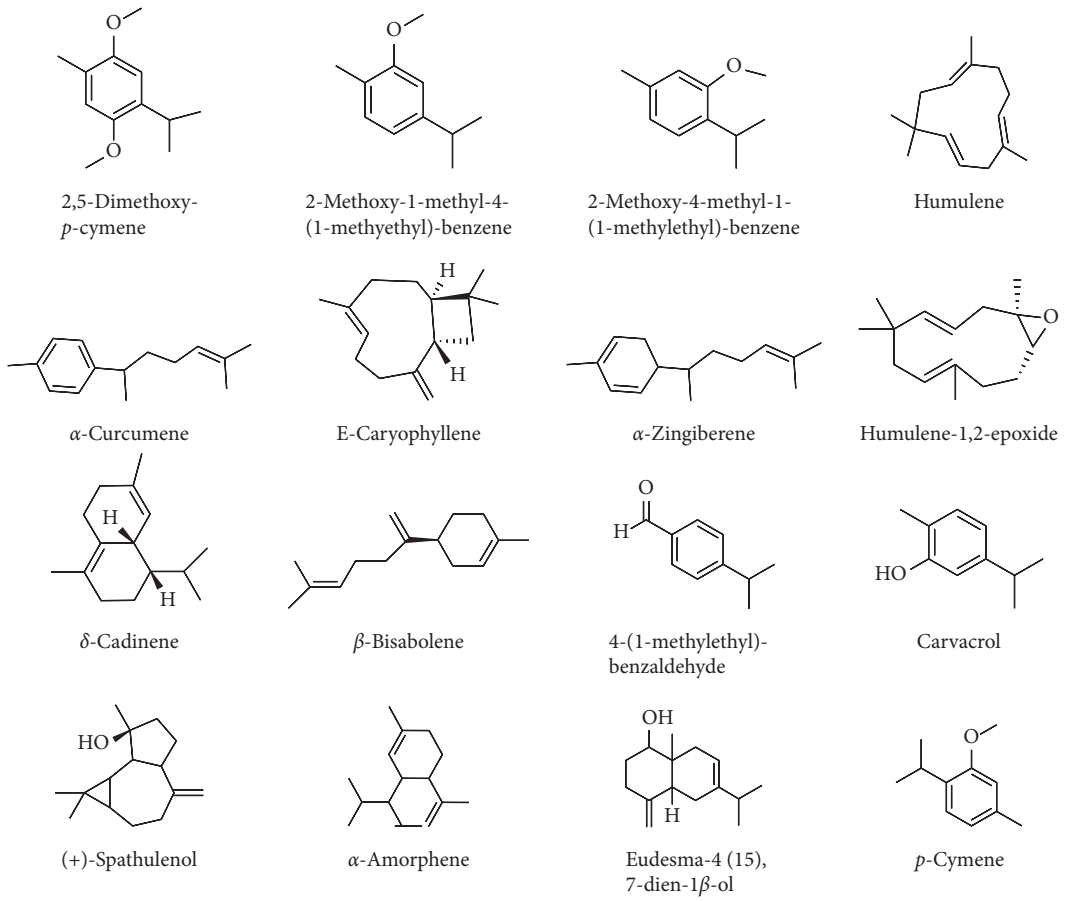


Figure 2: Chemical structures of the first 16 major components of CSEO. The chemical compounds with peak area percentage ≥0.11%.

Table 1: Chemical constituents of CSEO isolated from aerial parts of *C. leptophyllum*.

RT (min)	$KI_{exp.}$	$KI_{lit.}$	Chemical name	Peak area %
15.101	1025	1025	*p*-cymene	0.11
22.749	1224	1224	2-Methoxy-1-methyl-4-(1-methylethyl) benzene	3.09
22.935	1229	1229	2-Methoxy-4-methyl-1-(1-methylethyl) benzene	1.71
23.387	1242	1242	4-(1-methylethyl)-benzaldehyde	0.18
24.744	1280	1280	Carvacrol	0.16
29.297	1416	1415	2,5-Dimethoxy-*p*-cymene	87.09
29.411	1419	1419	E-caryophyllene	0.90
30.554	1456	1456	Humulene	1.15
31.135	1474	1474	α-Amorphene	0.13
31.325	1480	1479	α-Curcumene	0.91
31.749	1493	1493	α-Zingiberene	0.42
32.149	1506	1506	β-Bisabolene	0.21
32.454	1517	1517	δ-Cadinene	0.23
34.201	1576	1576	(+)-spathulenol	0.14
35.178	1608	1608	Humulene-1,2-epoxide	0.31
37.449	1689	1690	Eudesma-4(15), 7-dien-1β-ol	0.12
Class of compounds				% composition
Oxygenated monoterpenes				92.34%
Sesquiterpene compounds				4.52%
Oxygenated sesquiterpenes				0.57%
Sesquiterpene hydrocarbons				3.95%
Total				96.86%

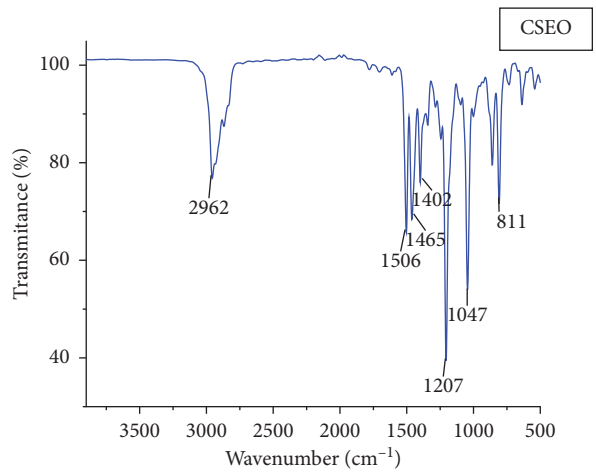


Figure 3: Fourier transform infrared (FTIR) spectra of CSEO.

Table 2: Antibacterial activity of various concentrations of CSEO solution against test microorganisms by agar well diffusion.

S.No.	Test microorganisms	Zone of inhibition diameter (ZID)* mm					Cipro.
		60% CSEO	30% CSEO	15% CSEO	7.5% CSEO	3.75% CSEO	5 μg/ml
1	*S. aureus*	30.67 ± 0.58	24.00 ± 0.00	20.00 ± 0.00	17.67 ± 0.58	14.33 ± 0.58	32.00 ± 0.00
2	*S. epidermidis*	26.67 ± 0.58	23.00 ± 0.00	16.67 ± 0.58	12.00 ± 0.00	9.00 ± 0.00	38.67 ± 1.15
3	*S. agalactiae*	17.00 ± 1.00	8.00 ± 0.00	8.00 ± 0.00	8.00 ± 0.00	8.00 ± 0.00	32.00 ± 1.73
4	*E. coli*	20.00 ± 1.00	14.67 ± 0.58	9.00 ± 0.00	9.00 ± 0.00	9.00 ± 0.00	34.00 ± 1.00
5	*P. mirabilis*	20.33 ± 0.58	11.67 ± 0.58	8.67 ± 0.58	8.00 ± 0.00	8.00 ± 0.00	39.00 ± 1.00
6	*P. aeruginosa*	16.33 ± 0.58	15.00 ± 0.00	12.67 ± 0.58	9.00 ± 0.00	8.00 ± 0.00	32.00 ± 1.00

*ZID values are expressed as the mean ± SD of three replicates. Cipro: ciprofloxacin (positive control), Tween-80 (negative control), and ZID values including well diameter, 8 mm; $p \leq 0.05$ significant as compared to the control.

Table 3: Relative zone of inhibition diameter percentage (% RZID) of different concentrations of CSEO solution against test microorganisms.

Test microorganism	% RZID					
	60% CSEO	30% CSEO	15% CSEO	7.5% CSEO	3.75% CSEO	Cipro.
S. aureus	94.44 ± 2.40	66.67 ± 0	50.00 ± 0	40.28 ± 2.41	26.39 ± 2.41	100.00 ± 0.00
S. epidermidis	60.97 ± 4.09	48.96 ± 1.80	28.27 ± 1.67	13.05 ± 0.48	3.26 ± 0.12	100.00 ± 0.00
S. agalactiae	47.64 ± 4.90	0.00 + 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	100.00 ± 0.00
E. coli	46.23 ± 5.63	25.62 ± 1.49	3.85 ± 0.15	3.85 ± 0.15	3.85 ± 0.15	100.00 ± 0.00
P. mirabilis	39.81 ± 2.23	11.80 ± 1.57	2.18 + 1.89	0.00 ± 0.00	0.00 ± 0.00	100.00 ± 0.00
P. aeruginosa	34.82 ± 3.79	29.20 ± 1.22	19.52 ± 3.09	4.17 ± 0.18	0.00 ± 0.00	100.00 ± 0.00

solution = 14.33 ± 0.58 mm and at 60% CSEO solution = 30.67 ± 0.58 mm as compared to ciprofloxacin = 32.00 ± 0 mm), respectively. Generally, Gram-negative bacterial strains showed more resistance to various concentrations of CSEO solution than Gram-positive strains. Some previous reports displayed that some Gram-negative bacteria strains, including *E. coli*, were more resistant, and Gram-positive bacteria including *S. aureus* were more susceptible microorganisms to various concentrations of EO solution of *C. leptophyllum* [35, 38]. Studies of EO solution of *C. leptophyllum* also revealed stronger inhibitory activity against Gram-positive and Gram-negative bacterial strains such as *S. aureus* and *P. aeruginosa,* respectively, as compared to kanamycin and gentamicin antibiotic drug standards [46].

The antibacterial activity effects of CSEO solution on bacterial test strains were further expressed by the calculated values of the relative zone of inhibition diameter percentage (% RZID). The calculated results of % RZID exhibited higher values at higher concentrations of CSEO solution due to the presence of bioactive compounds in the solution. For instance, as shown in Table 3, 60%, 30%, and 15% test solutions of CSEO inhibited the growth of *S. aureus* bacterial species by 94.44%, 66.67%, and 50.00%, respectively, as compared to ciprofloxacin. Similarly, the growth of *S. epidermidis* was inhibited by 60.97% and 48.96% using 60% and 30% CSEO solutions. 60% CSEO solution also showed the relative zone of inhibition diameters of 47.67% and 46.23% against *S. agalactiae* and *E. coli* compared to the positive control. The significant difference ($p \leq 0.05$) of antibacterial activity of different concentrations of CSEO solution against the test

bacterial strains has been more supported by post hoc multiple comparisons between zones of inhibition diameter percentage values.

Besides antibacterial activity tests, the mechanism of action of bioactive compounds of EOs was also the subject of numerous studies [35, 50]. Even though the mechanisms of action of chemical components are not fully answered, actions of chemical compounds of CSEO against bacterial species most probably follow the same mechanisms reported in the previous related research findings [51, 52]. Hydrophobic properties of major chemical components are mainly responsible for the antibacterial action of EO. Even though the action of EO is due to its constituent's synergic effect, the antibacterial activity of CSEO more probably relies on the highly dominant component CSEO, 2,5-dimethoxy-*p*-cymene, which accounts for 87.09% of the total composition. After 2, 5-dimethoxy-*p*-cymene penetrates through the cell membrane; it destroys the cytoplasm membrane and changes membrane permeability and integrity of bacterial cells. Thus, these phenomena make bacteria leak components necessary for their existence and finally cause the death of bacteria. This proposed action mechanism of CSEO is supported by some literature reviews [34]. Some previous reports displayed that Gram-negative bacterial strains are more resistant to EO than Gram-positive bacteria because the former species have an outer membrane surrounding the cell wall, preventing diffusion of hydrophobic chemical components through their lipopolysaccharide layer [35, 50, 53]. The components in the EOs were reported for alteration of structure, functionality, blockage of energy metabolism system, disruption of whole-cell protein, and DNA of the bacterial strains. This was reported as the frequently proposed mechanism of action of EOs for antibacterial activities [54, 55].

5. Conclusions

GC-MS analysis of CSEO identified the dominant chemical constituents in oxygenated terpenes and diversified functional groups. Their chemical compositions are also known to contain different aromatic compounds. The maximum ever reported total composition percentages of CSEO were obtained as 96.86%. This study reported the major compound, 2,5-dimethoxy-*p*-cymene, with a greater relative area percentage, 87.09%, for the first time from the EO of the aerial part of *C. leptophyllum*. Compared to ciprofloxacin, the relative zone of inhibition diameter percentage (% RZID) of 15–60% of CSEO solution displayed average or above average growth inhibition activities against *S. aureus*. Similarly, 30% and 60% of CSEO solution inhibited the above average percentage of growth of S. epidermidis. *S. agalactiae* exhibited the strongest resistant effect towards all test solutions except 60% CSEO solution. Generally, stronger inhibition activities were observed for Gram-positive bacterial strains than Gram-negative bacterial strains. Bioactive compounds in CSEO potentially inhibit the growth of some food spoilage bacterial strains. Therefore, this study contributes to the scientific evidence required for supporting the traditional medicinal practices exercised by some communities using natural products of *C. leptophyllum*. This research work is also used to strengthen the current efforts of scientific investigation regarding the application of EOs of various medicinal plants from the ecology of Ethiopia as natural antibacterial agents. To evaluate the effectiveness of the antibacterial activities of CSEO, it needs more evaluation of CSEO against a vast number of pathogenic bacterial strains responsible for food contamination.

Abbreviations

EOs: Essential oils
CSEO: *Cyclospermum leptophyllum* essential oil
FTIR: Fourier transform infrared
GC-MS: Gas chromatography-mass spectrometry
ATCC: American Type Culture Collection
RZID: Relative zone of inhibition diameter
KI_{Lit}: Literature-based Kovats retention index
KI_{Exp}: Experimental-based Kovats retention index
MHA: Muller–Hinton agar
LB: Luria–Bertani
NIST: National Institute Standard and Technology.

Data Availability

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare that they have no conflicts of interests.

Authors' Contributions

YHG conceived, designed, performed, and analyzed the experiments and prepared the original manuscript. AAG performed all the in vitro antibacterial activities. SDG carried out the reading of the results of incubated plates. FBT and AB helped revise the prepared manuscript and adjust the tables and figures. MGT and RKB supervised the research work. All authors read and agreed to the published version of the manuscript.

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