

Article



Chemical Composition of the *Cinnamomum malabatrum* Leaf Essential Oil and Analysis of Its Antioxidant, Enzyme Inhibitory and Antibacterial Activities

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Abstract: Cinnamomum species are a group of plants belonging to the Lauraceae family. These plants are predominantly used as spices in various food preparations and other culinary purposes. Furthermore, these plants are attributed to having cosmetic and pharmacological potential. Cinnamomum malabatrum (Burm. f.) J. Presl is an underexplored plant in the Cinnamomum genus. The present study evaluated the chemical composition by a GC-MS analysis and antioxidant properties of the essential oil from C. malabatrum (CMEO). Further, the pharmacological effects were determined as radical quenching, enzyme inhibition and antibacterial activity. The results of the GC-MS analysis indicated the presence of 38.26 % of linalool and 12.43% of caryophyllene in the essential oil. Furthermore, the benzyl benzoate (9.60%), eugenol (8.75%), cinnamaldehyde (7.01%) and humulene (5.32%) were also present in the essential oil. The antioxidant activity was indicated by radical quenching properties, ferric-reducing potential and lipid peroxidation inhibition ex vivo. Further, the enzyme-inhibitory potential was confirmed against the enzymes involved in diabetes and diabetic complications. The results also indicated the antibacterial activity of these essential oils against different Gram-positive and Gram-negative bacteria. The disc diffusion method and minimum inhibitory concentration analysis revealed a higher antibacterial potential for C. malabatrum essential oil. Overall, the results identified the predominant chemical compounds of C. malabatrum essential oil and its biological and pharmacological effects.

Keywords: *Cinnamomum malabatrum;* GC-MS analysis; essential oil; antioxidant activity; antibacterial activity; enzyme inhibitory activity

1. Introduction

Spices are an important class of plants and have been traditionally used all over the world. The different plant species belonging to the *Zingiberaceae*, *Lauraceae*, *Myrtaceae* and *Schisandraceae* families are reputed spice products [1,2]. The source of spices includes the leaves, roots, stem bark, buds and flowers [3]. The spices are predominantly used as dietary ingredients or supplements that enable the flavoring of the cuisines [4,5]. The spices are traditionally used in medicinal systems including Ayurveda and Chinese medicines for the management of various illnesses [6,7]. In addition, the plants are well-known for



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Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). their cosmetic potential as well as pharmaceutical activities. The prevention of infectious diseases by controlling the microbial communities and multidrug resistance is an integral function of the spices. In addition, the novel "spiceceuticals", the pharmacologically active spice products, are also emerging against numerous degenerative disorders including metabolic syndromes and cancers [8,9].

The *Cinnamomum* spp. are important spices that are used for various purposes in different parts of the world. The genus Cinnamomum comprises approximately 250 species that are distributed in the Asian and Australian continents [10]. Among these, the C. zeylanicum and C. cassia are the prominent representatives of the genus Cinnamomum. The C. zeylanicum (now known as C. verum) is the "true cinnamon", which is also known as "Ceylon cinnamon" [11]; the *C. cassia* (previously *C. aromaticum*) is known as "Chinese cinnamon" [12]. The most important cinnamon oils in the world trade are those from *Cinnamonum zeylanicum* (or C. verum), C. cassia and C. camphora [13]. Among the different plants, C. zeylanicum is well-studied; the antibacterial properties are also attributed to C. zeylanicum leaves and their bioactive compounds against clinically drug-resistant bacteria [14]. Further, a study by Assaran et al. [15] indicated the protective effect of C. zeylanicum extract on pentylenetetrazole-induced seizure. It was also effective in preventing the doxorubicinmediated oxidative damage to the heart tissue and subsequent cardiomyopathy [16]. The bark extract of the plant was protective against gentamicin-induced renal toxicity by preventing inflammatory insults [17]. It was also found to protect against formaldehydemediated inflammation and apoptosis in neurons [18]. The extracts of *C. zeylanicum* were also known to have antidepressant properties in murine models [19]. Furthermore, the anticancer activities were also evident for *C. zeylanicum* extract by modulating various cellular signaling cascades [20]. C. zeylanicum extract was also an effective antimicrobial agent against the infection of *Toxoplasma gondii* in murine models [21]. The *C. cassia* is another important plant belonging to the family; it was effective against the gastrointestinal toxicities in animal models [22]. C. burmannii is also attributed to having pharmacological effects; the administration of the extract improved hepatic redox balance and subsequently protected against high-fat diet-mediated liver toxicity [23]. C. burmanii was also effective against bacterial pathogens by inhibiting bacterial proliferation and blocking biofilm formation [24].

The essential oils isolated from different *Cinnamomum* spp. are another important extract with potential insecticidal and pharmacological properties. The C. camphora essential oil was found to be effective against bacterial forms and dust mites [25]. The essential oil was also effective against bacterial strains that are antibiotic-resistant [26]. It was also found to be useful in the management of mosquitoes by killing the larval forms of Anopheles stephensi [27]. Besides their insecticidal and antimicrobial properties, pharmacological properties are also attributed to C. camphora essential oil. The essential oil had anti-inflammatory properties in cultured cells and animal models [28,29]. Further, the essential oil had analgesic properties in animal models [30]. The essential oil was also effective in preventing learning impairment and memory loss in mice [31]. Apart from the plant, C. burmannii was shown to have radical quenching anticancer properties [32]. The C. zeylanicum essential oil was also shown to have antibacterial and antineoplastic properties [33]. Likewise, the essential oil of *C. verum* was reported to have protective efficacy against CCl4-induced hepato-renal toxicities in rats [34]. The essential oil-based nanoemulsions of *C. litseifolium* were shown to have antioxidant and hypoglycemic activities [35]. The C. glanduliferum essential oil was shown to protect against ethanol-induced inflammation and gastritis in rats [36]. The essential oil of *C. osmophloeum* was reported to have lipid-lowering properties in mice, and the efficacy was comparable to the bioactive compounds such as linalool [37]. The essential oil was also found to be effective against pancreas toxicity [38] and endotoxin-induced intestinal damage [39].

Among the different species of the *Cinnamomum* genus, the *C. malabatrum* (Burm.f.) J.Presl is an endemic medicinal plant that belongs to the Western Ghats, Kerala, India. Limited studies are available on the essential oil of the plant; Leela et al. [40] indicated the

chemical composition of the essential oil of *C. malabatrum* where (E)-Caryophyllene (28.6%), (E)-Cinnamyl acetate (15.1%) and Bicyclogermacrene (14.4%) were the predominant compounds. Later, a study by Sriramavaratharajan and Murugan [41] reported the predominant compounds as β -Phellandrene (12.0%) and linalool (15.4%). The present study, therefore, aimed to extract the essential oil from the *C. malabatrum* leaves and analyze its chemical composition. Further, the radical quenching properties of the essential oil and its enzyme-inhibitory properties were evaluated using in vitro models. The enzyme-inhibition activity was assessed in terms of diabetes-associated enzymes; the α -amylase and α -glucosidase are major enzymes associated with carbohydrate metabolism and thereby contribute to type 2 diabetes mellitus [42,43], and are a prominent target for antidiabetic drugs [44,45]. The activation of polyol pathway enzymes aldose reductase and sorbitol dehydrogenase plays a crucial role in the microvascular complications of diabetes [46–48]. The antibacterial activity was also determined using two methods: the disc diffusion method and minimum inhibitory concentrations.

2. Results

2.1. C. malabatrum Essential Oil Yield and Chemical Contents

The yield of *C. malabatrum* leaf essential oil was $0.72 \pm 0.13\%$ using the hydrodistillation method. The GC-MS chromatogram of the essential oil is shown in Figure 1. There were eleven main peaks observed in the chromatogram.

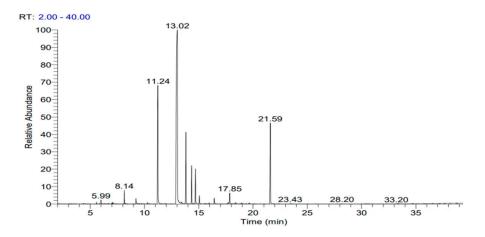


Figure 1. The chromatograms of GC-MS analysis of C. malabatrum leaf essential oil.

The results of the GC-MS analysis indicated the presence of $38.26 \pm 0.41\%$ of linalool, $12.01 \pm 0.54\%$ of cinnamaldehyde and $11.43 \pm 0.52\%$ of caryophyllene in the essential oil. In addition, the benzyl benzoate ($9.60 \pm 0.05\%$), eugenol ($8.75 \pm 0.23\%$) and humulene ($5.32 \pm 0.12\%$) were also present in the essential oil (Table 1).

Table 1. Predominant compounds of *C. malabatrum* leaf essential oil (the complete list is given in Supplementary Table S1).

Retention Time	Component	Percentage Composition	
13.02	Linalool	38.26 ± 0.41	
11.24	Cinnamaldehyde	12.01 ± 0.54	
14.34	Caryophyllene	11.43 ± 0.52	
21.59	Benzyl Benzoate	9.60 ± 0.05	
16.43	Eugenol	8.75 ± 0.23	
15.06	Humulene	5.32 ± 0.12	

2.2. Antioxidant Effects of C. malabatrum Essential Oil

We observed a dose-dependent scavenging of various free radicals in *C. malabatrum* essential oil treatments (Table 2). The IC₅₀ values were found to be less than 100 μ g/mL in the entire radical quenching assay for the essential oil. Further, among the different radicals analyzed, the DPPH was more sensitive to the essential oil treatment. However, the radical quenching properties of the CMEO were significantly lower than those of ascorbic acid (*p* < 0.001). On the contrary, the CMEO was having a higher antioxidant potential in terms of DPPH and ABTS radical scavenging (*p* < 0.001). The peroxide scavenging and lipid peroxidation potential of the linalool were higher than the CMEO (*p* < 0.001). The reducing potential (FRAP) of *C. malabatrum* essential oil was significantly lower than the ascorbic acid, whereas it was significantly higher than the linalool (*p* < 0.001).

Table 2. Radical quenching abilities of the essential oil extracted from *C. malabatrum* leaves. The values expressed are half-maximal inhibition concentration-IC₅₀ (μ g/mL).

	CMEO	Linalool	Ascorbic Acid
DPPH radical scavenging	21.50 ± 0.17	35.22 ± 0.11	8.13 ± 0.09
ABTS radical scavenging	36.91 ± 0.41	40.01 ± 1.33	12.82 ± 0.40
H_2O_2 radical scavenging	42.77 ± 0.34	38.09 ± 2.45	19.11 ± 0.26
Ferric-reducing potential	12.38 ± 0.11	35.93 ± 0.24	15.38 ± 0.66
Lipid peroxidation inhibition	85.83 ± 0.47	78.49 ± 3.07	63.02 ± 0.33

(Statistical comparison has been detailed in Supplementary Table S2).

2.3. Enzyme-Inhibitory Activities of C. malabatrum Leaf Essential Oil

The enzyme-inhibitory activities of the essential oil were evaluated using different enzymes associated with diabetes and diabetic complications. The *C. malabatrum* was found to inhibit the enzymes such as α -amylase and α -glucosidase (Table 3); however, the bioactive compounds linalool and ascorbic acid were found to be more potent inhibitors of these enzymes. The inhibition of aldose reductase and sorbitol dehydrogenase was also observed in CMEO treatment with the respective IC₅₀ values 82.90 ± 0.67 and 98.61 ± 3.18 µg/mL. However, a more significant inhibition in the ascorbic acid treatment 28.70 ± 2.14 and 60.09 ± 1.32 µg/mL (*p* < 0.001) was observed. Likewise, the linalool also showed significant inhibition but to a lesser extent than the ascorbic acid (*p* < 0.001).

Table 3.	Enzyme-i	nhibitory	abilities	(IC_{50})	in µg,	/mL) c	of C.	malabatrum	leaf essential oil.
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Enzyme Inhibition	CMEO	Linalool	Ascorbic Acid
α-Amylase	74.19 ± 1.55	62.34 ± 2.91	45.17 ± 2.36
α-Glucosidase	47.07 ± 3.14	30.93 ± 3.41	36.03 ± 1.98
Aldose reductase	82.90 ± 0.67	59.04 ± 2.26	28.70 ± 2.14
Sorbitol dehydrogenase	98.61 ± 3.18	88.37 ± 3.75	60.09 ± 1.32

(Statistical comparison has been detailed in Supplementary Table S3).

2.4. Antibacterial Effects of C. malabatrum Essential Oil

The antibacterial potential of the *C. malabatrum* essential oil was tested against both Gram-positive and Gram-negative organisms using the disc diffusion method (Table 4), and also in terms of minimum inhibitory concentration (Table 5). The antibacterial activity was found to be similar in CMEO to that of GM in the *Pseudomonas aeruginosa* strain (p = 0.3150). Likewise, the CMEO was effective as that of linalool against *Escherichia coli* and *Salmonella enterica*. However, in other strains, a significantly higher antibacterial activity was observed for CMEO than linalool.

	Zone of Inhibition (mm)				
Bacteria	СМЕО	Linalool	GM		
Staphylococcus aureus	16.2 ± 0.3	18.1 ± 0.2	18.5 ± 0.5		
Bacillus cereus	14.8 ± 0.4	17.6 ± 0.3	21.3 ± 0.5		
Streptococcus pyogenes	16.7 ± 0.3	17.9 ± 0.1	19.2 ± 0.7		
Escherichia coli	18.1 ± 0.2	17.8 ± 0.1	21.3 ± 0.3		
Pseudomonas aeruginosa	20.8 ± 0.5	19.3 ± 0.2	21.6 ± 0.4		
Salmonella enterica	17.4 ± 0.2	16.8 ± 0.3	19.9 ± 0.3		

Table 4. Antibacterial properties of *C. malabatrum* by disc diffusion method.

(Statistical comparison has been detailed in Supplementary Table S4).

Table 5. Minimum inhibitory concentrations (mg/mL) of *C. malabatrum* essential oil and antibiotic gentamicin.

D estants	MIC Value				
Bacteria	CMEO	Linalool	GM		
Staphylococcus aureus	1.25 ± 0.05	0.325 ± 0.00	0.325 ± 0.00		
Bacillus cereus	0.75 ± 0.05	0.625 ± 0.10	0.325 ± 0.00		
Streptococcus pyogenes	0.625 ± 0.10	0.325 ± 0.00	0.167 ± 0.00		
Escherichia coli	1.00 ± 0.10	0.625 ± 0.05	0.325 ± 0.00		
Pseudomonas aeruginosa	0.625 ± 0.15	0.325 ± 0.10	0.167 ± 0.00		
Salmonella enterica	0.625 ± 0.05	0.325 ± 0.00	0.167 ± 0.00		

(Statistical comparison has been detailed in Supplementary Table S5).

The minimum inhibitory concentrations of CMEO were comparable for the *C. malabatrum* essential oil and linalool in the *Bacillus cereus* (p = 0.0028). Likewise, the MIC values of linalool and GM were similar in *P. aeruginosa*, *S. aureus*, *S. pyogenes* and *S. enterica* (p > 0.05). The antibacterial activity of CMEO was significantly lower than that of gentamicin (p < 0.001).

3. Discussion

Cinnamomum spp. is well-known for its culinary uses in different parts of the world. In addition, the essential oil extracted from the spice is of cosmetic and pharmacological uses. Among these, the *C. verum*, *C. zeylanicum* and *C. tamala* are widely evaluated. The *C. malabatrum* is an endemic plant which is less explored for its biological and pharmacological properties. The present study evaluated the chemical components of the plant essential oil by a GC-MS analysis.

The results of the GC-MS analysis indicated the presence of 38.26% of linalool, 12.01% of cinnamaldehyde and 11.43% of caryophyllene in the essential oil. Furthermore, the benzyl benzoate (9.60%), eugenol (8.75%) and humulene (5.32%) were also present in the essential oil. A previous study by Leela, Vipin, Shafeekh, Priyanka and Rema [40] indicated that (E)-Caryophyllene (28.6%), (E)-Cinnamyl acetate (15.1%) and Bicyclogermacrene (14.4%) were the predominant compounds. Further, Benzyl benzoate (8.5%), α -Humulene (4.7%), Globulol (2.7%) and β -Phellandrene (2.2%) were other minor compounds present in the leaf essential oil according to their study. On the contrary, another study by Sriramavaratharajan and Murugan [41] indicated the presence of β -Phellandrene (3.5–12.0%), linalool (13.1–15.4%), (E)-Caryophyllene 8.4–31.4%) and Bicyclogermacrene (12.9–20.0%) in the essential oil.

The dose-dependent scavenging of various free radicals in *C. malabatrum* essential oil treatments was observed. The IC_{50} values were found to be less than 100 µg/mL in the entire radical quenching assay for the essential oil. Further, among the different radicals analyzed, the DPPH was more sensitive to the CMEO treatment. In addition, it was interesting to note that the ferric-reducing potential of the CMEO was comparable to that of the standard ascorbic acid. The free radicals are important agents associated with oxidative stress and inflammation [49]. Hence, the radical quenching is important to

prevent the oxidative damage to cellular macromolecules, and thereby prevent various degenerative diseases [50,51]. Hence, the inhibition of the radicals by *C. malabatrum* indicates the potential of the essential oil in preventing chronic diseases. Further, the compounds such as linalool [52], caryophyllene [53] and cinnamaldehyde [54,55] are shown to prevent oxidative damage in various conditions of animal models and clinical studies. Hence, it must be possible that the bioactive stress volatiles of the *C. malabatrum* might be responsible for the antioxidant potentials.

The enzyme inhibitory activities of the essential oil were evaluated using different enzymes associated with diabetes and diabetic complications. The *C. malabatrum* was found to inhibit the enzymes such as α -amylase and α -glucosidase. The α -amylase and α -glucosidase are two enzymes associated with type 2 diabetes mellitus [42,43]. Several synthetic drugs are known to inhibit these enzymes as a preventive measure to diabetes, and thereby making these enzymes an antidiabetic drug target [44,45]. Likewise, the secondary diabetic complications including retinopathy, nephropathy and cardiomyopathy are another important concern of diabetic patients [56,57]. The activation of polyol pathway enzymes aldose reductase and sorbitol dehydrogenase plays a crucial role in the microvascular complications of diabetes [46–48]. Numerous plant products and bioactives are reported to interfere with polyol enzymes and are thereby found to be protective against the microvascular complications of diabetes [58,59]. Hence, the *C. malabatrum* essential oil may prove beneficial against diabetes and associated microvascular complications.

The antibacterial potential of the *C. malabatrum* essential oil was tested against both Gram-positive and Gram-negative organisms using the disc diffusion method, and also in terms of minimum inhibitory concentration. The selected microorganisms are known to be associated with various diseases in humans, animals and poultry. The E. coli is reported to cause infections in urinary and respiratory tracts [60]; whereas, the *P. aeruginosa* is associated with wound infections during surgery and transplantations [61]. Staphylococcus and *Streptococcus* are associated with cutaneous and genital infections in humans causing various diseases [62,63]. The Bacillus cereus is an important pathogen which is known to produce toxins and is subsequently associated with food poisonings, and it is often fatal [64,65]. Hence, the inhibition of the growth of these organisms by the *C. malabatrum* essential oil may be indicative of its antibacterial potential. Further, the bioactive compounds present in the essential oil such as linalool, caryophyllene and cinnamaldehyde are known for their antimicrobial properties [66–68]. It is therefore possible that the antibacterial properties exhibited by the C. malabatrum essential oil may be attributed to the bioactive metabolites present in it. Hence, the present study confirms the chemical components as well as the antibacterial and antidiabetic properties of the leaf essential oil of C. malabatrum.

4. Materials and Methods

4.1. Collection of C. malabatrum Leaves and Extraction of Essential Oil

The leaves of *Cinnamonum malabatrum* (voucher specimen number KFRI-26/2020 was deposited in KFRI, Peechi, India) were obtained from the cultivation area of Kerala Agricultural University (10.54544° N, 76.28830° E), Thrissur, India. The extraction of the essential oil was by a Clevenger-type apparatus using the hydro-distillation method. The essential oil was dehydrated using sodium sulfate (anhydrous). and stored in the dark during cooling.

4.2. Chemical Component Analysis by GC-MS Analysis

The characterization of the essential oil extracted from *C. malabatrum* was carried out using the TSQ 8000 Evo GC-MS instrument (Thermo Scientific, Waltham, Massachusetts, USA) with an autosampling unit. The TG-5MS chromatographic column ($30 \text{ mm} \times 0.25 \text{ mm} \times 0.25 \text{ }\mu\text{m}$) with helium (1 mL/min) as the carrier gas was used in the analysis. The oven temperature of the system was set at 50 °C with a ramp temperature of 10 °C/min until 120 °C; later, the ramp was 5 °C per minute, and finally fixed at 270 °C. The chemical composition was analyzed by the matching of MS spectra with the NIST library, and the retention index (RI)

values were estimated by calibrating their instrument with a homologous series of alkenes $(C_7-C_{30} \text{ n-alkene})$ under the same conditions [69].

4.3. Antioxidant Activities of C. malabatrum Leaf Essential Oil

The concentration of essential oil used was a different series from 0 to 100 μ g/mL for each radical quenching assay. The quenching of DPPH radicals was analyzed using the methods of [70]. The ABTS radicals scavenging activity was analyzed according to the methods of Li et al. [71]. The hydrogen peroxide quenching potential of the essential oil was following the methods of Munteanu and Apetrei [72]. The ferric-reducing abilities of the essential oil were estimated according to the methods described by He et al. [73]. The methods described by Okoh et al. [74] were followed for the lipid peroxidation inhibition assay.

4.4. Enzyme-Inhibitory Properties of C. malabatrum Leaf Essential Oil

The enzyme-inhibitory potential of the essential oil was evaluated by mixing different concentrations (0–100 μ g/mL) against the respective enzymes and their substrates. The enzyme activities will be estimated in terms of the substrate utilized after incubation. The inhibition of α -amylase [75], α -glucosidase [76], aldose reductase [77] and sorbitol dehydrogenase [78] was carried out according to the methods previously described.

4.5. Antibacterial Activity Analysis

4.5.1. Bacterial Strains Used

The bacteria were procured from Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh and maintained under standard conditions as prescribed by Bonnet, et al. [79]. The bacterial strains used include *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus cereus*, *Streptococcus pyogenes* and *Salmonella enterica*.

4.5.2. Disc Diffusion Method

Initially, the bacteria cultured were completed in Luria-Bertani broth; for the antibacterial study, the inoculum of the bacteria was made on a Mueller Hinton Agar (MHA) agar plate (Himedia, Mumbai, Maharashtra, India) at a thickness of 5 mm. Later, a filter paper disc (8 mm in diameter) containing the leaf essential oil of *C. malabatrum* (10 μ L) was placed in the agar plate at a distance of 50 mm. At the end of 24 h, the formation of the growth inhibition zone was estimated [80].

4.5.3. Minimum Inhibitory Concentration (MIC)

The determination of the MIC value was made according to the methods described by Campana, et al. [81]. Before beginning, the density of the inoculum was spectrophotometrically set to 5×10^5 CFU/mL. From this, about 50 µL was transferred to individual wells of a 96-well plate containing different concentrations of *C. malabatrum* essential oil. Later, 2,3,5-triphenyltetrazolium chloride (10 µL) was added to each well; the pink color of the 2,3,5-triphenyltetrazolium chloride was lost in the absence of bacterial growth. The MIC value was considered as the lowest concentration without a detectable pink color.

4.6. Statistical Analysis

The results are presented as the mean± standard deviation value of three independent experiments. The statistical analysis comparison was made between the standard compounds used, linalool and essential oil by a one-way analysis of variance using GraphPad prism ver. 7.0 (San Diego, CA, USA).

5. Conclusions

The present study confirms the pharmacological potential of the *Cinnamomum malabatrum*, an endemic plant of Western Ghats, India. *C. malabatrum* leaf essential oil was found to have significant radical quenching abilities against different free radical sources. Likewise, the essential oil was also capable of inhibiting enzymes associated with diabetes and asso-

ciated secondary complications. The strong antibacterial potential for the *C. malabatrum* essential oil was observed for both Gram-positive and Gram-negative bacteria. Hence, based on the signification of the results, the *C. malabatrum* essential oil may be a useful pharmacological agent.

Supplementary Materials: The following supporting information can be downloaded at: https: //www.mdpi.com/article/10.3390/antibiotics12050940/s1. Table S1. Chemical composition of *C. malabatrum* leaf essential oil. Table S2. Statistical comparison of the antioxidant activities between the CMEO, linalool and ascorbic acid. Table S3. Statistical comparison of the enzyme-inhibitory potentials between the CMEO, linalool and ascorbic acid. Table S4. Statistical comparison of the antibacterial activity in terms of disc diffusion assay between the CMEO, linalool and gentamicin. Table S5. Statistical comparison of the antibacterial activity in terms of MIC value between the CMEO, linalool and gentamicin.

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