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Effect of Clove Flower Essential Oil (*Syzygium aromaticum*) Addition on the Antibacterial and Antioxidant Activities of Lemongrass Essential Oil (*Cymbopogon citratus*)

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Abstract

Indonesia, as a tropical country, possesses substantial potential for the development and utilization of essential oils, particularly those derived from lemongrass (*Cymbopogon citratus*) and clove flowers (*Syzygium aromaticum*). Lemongrass essential oil is rich in citral and exhibits notable antibacterial activity; however, its antioxidant activity is relatively low. In contrast, clove essential oil is predominantly composed of eugenol, a compound recognized for its strong antibacterial and antioxidant properties. This study aimed to evaluate the effect of clove essential oil addition on the antibacterial and antioxidant activities of lemongrass essential oil. The essential oils were obtained through steam distillation and characterized using gas chromatography–mass spectrometry (GC–MS). Biological activities were assessed by determining total phenolic content, antioxidant activity using the DPPH assay, and antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*. The results demonstrated that the addition of clove essential oil increased the total phenolic content and enhanced both the antioxidant and antibacterial activities of lemongrass essential oil. Among the tested formulations, the 1:2 ratio (lemongrass:clove) exhibited the strongest antioxidant activity, with an IC_{50} value of 14.400 mg/L, which was substantially lower than that of pure lemongrass essential oil (262.28 mg/L). In the antibacterial assay, the 2:1 ratio showed the strongest inhibitory activity against *Staphylococcus aureus*, whereas the 1:1 ratio was more effective against *Escherichia coli*. These findings indicate that the incorporation of clove essential oil can modulate and enhance the biological activities of lemongrass essential oil, highlighting its potential as a natural source of antibacterial and antioxidant agents. Overall, clove essential oil exerted an additive effect on the biological properties of lemongrass essential oil.

1. Introduction

As a tropical country, Indonesia possesses abundant plant biodiversity and a climate that supports the growth of various aromatic plants and spices, including lemongrass (*Cymbopogon citratus*) and clove (*Syzygium aromaticum*). These plants have long been utilized in food products, traditional medicine, and aromatherapy. Such favorable conditions provide Indonesia with significant potential to become a major producer in the global essential oil industry [1].

Lemongrass essential oil is rich in aldehyde compounds, particularly citral, which exhibits antibacterial activity against various Gram-positive and Gram-negative bacterial pathogens. In addition, its antioxidant activity is influenced by its chemical composition. Several studies have demonstrated that lemongrass essential oil can inhibit the growth of certain bacterial species and exhibits relevant minimum inhibitory concentration (MIC) values against *Escherichia coli* and other bacteria in vitro, with inhibition zones varying according to the concentration and testing method employed [2]. Lemongrass essential oil has also been reported to exhibit a DPPH free-radical scavenging activity of 79.96%.

Furthermore, phytochemical constituents such as tannins, steroids, saponins, flavonoids, and alkaloids contribute to its therapeutic potential, particularly in the treatment of digestive disorders [2, 3]. Phenolic compounds, including quercetin, luteolin, and apigenin, play important roles in its antioxidant activity, as reflected by a total phenolic content of 2100 mg GAE/L and an antioxidant IC_{50} value of 199.32 mg/L [4, 5]. Owing to its antioxidant, anti-inflammatory, and antifungal properties, lemongrass essential oil has been widely applied in the food industry and aromatherapy products [6]. However, other studies have reported that although lemongrass essential oil exhibits very strong antibacterial activity at concentrations of 12.5–25%, its antioxidant activity remains relatively limited.

In contrast, clove essential oil (*Syzygium aromaticum*) has been reported to possess more potent biological activity [7]. This essential oil contains eugenol as its major constituent (76.8%), together with eugenyl acetate (1.2%), α-humulene (2.1%), and β-caryophyllene (17.4%) [8]. Eugenol contributes to a wide range of pharmacological properties, including antimicrobial, analgesic, anti-inflammatory, and antioxidant activities [9]. Traditionally, clove oil has been used to treat intestinal infections, as an active ingredient in mouthwashes, and as a component of pain-relieving balms [10, 11]. The bioactive potential of clove oil is further supported by its high phenolic content (185.9 ± 16.0 mg GAE/g) and the presence of flavonoids such as kaempferol, rhamnetin, and eugenitin [12, 13]. Its antioxidant activity has been reported to be very strong, with a DPPH IC_{50} value of 10.87 µg/mL [14], making clove oil a common reference material in studies investigating the bioactivity of essential oils.

A study by Budiati *et al.* [15] demonstrated that both lemongrass and clove essential oils exhibit antibacterial activity against *Salmonella typhimurium*, *Bacillus cereus*, and *Staphylococcus aureus* using the disc diffusion method. The antibacterial effects were further confirmed by MIC determination, which showed that both essential oils exhibit bacteriostatic activity. These findings suggest that both lemongrass and clove essential oils possess antibacterial properties, although the antioxidant activity of lemongrass essential oil remains substantially lower than that of clove essential oil.

Based on these findings, there is an opportunity to explore the combination of lemongrass and clove essential oils as an approach to modify and potentially enhance biological activity, particularly antioxidant activity. The formulation of essential oil mixtures has been reported to influence antioxidant capacity. For example, Yeddes *et al.* [16] reported enhanced antioxidant activity from a combination of clove, lemon, and thyme essential oils, with an IC_{50} of 11.02 µg/mL, compared with the individual essential oils. However, because the composition of the essential oil blend differed from that of lemongrass and clove essential oils, the findings cannot be directly generalized to this combination. To date, scientific information regarding the effect of clove essential oil addition on the antibacterial and antioxidant activities of lemongrass essential oil remains limited.

Therefore, combining lemongrass and clove essential oils may represent a promising strategy for developing natural active ingredients with enhanced antibacterial and antioxidant properties while supporting the formulation of more efficient products with improved sensory characteristics. Accordingly, this study was conducted to quantitatively evaluate the effect of combining lemongrass and clove essential oils on their antibacterial and antioxidant activities.

2. Experimental

2.1. Materials

The materials used in this study included fresh lemongrass (*Cymbopogon citratus*) collected from Ungaran, Semarang Regency, Central Java, Indonesia, and clove flower buds (*Syzygium aromaticum*) obtained from Dieng, Wonosobo Regency, Central Java, Indonesia. The chemicals used were methanol (Merck), distilled water, anhydrous sodium sulfate (Na_2SO_4), Folin–Ciocalteu reagent, sodium carbonate (Na_2CO_3), 1,1-diphenyl-2-picrylhydrazyl (DPPH), dimethyl sulfoxide (DMSO), gallic acid (Merck), and ciprofloxacin as a positive control. Nutrient Broth (NB) and Nutrient Agar (NA) were used for antibacterial assays. *Staphylococcus aureus* and *Escherichia coli* served as the test microorganisms.

2.2. Sample Preparation and Plant Determination

Fresh lemongrass and clove flower buds were thoroughly cleaned to remove adhering impurities, cut into small pieces, and subsequently dried at room temperature until a constant weight was achieved. Botanical identification and taxonomic verification of the plant materials were conducted at the Ecology and Biosystematics Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Semarang, Indonesia, to confirm the authenticity of the species used in this study.

2.3. Isolation of Essential Oils by Steam Distillation

The dried plant materials were subjected to steam distillation to obtain the essential oils. The resulting distillate was allowed to separate into aqueous and oil phases, after which the oil phase was collected. Anhydrous sodium sulfate was added to the essential oil to remove residual moisture. The dehydrated essential oil was subsequently filtered, weighed, and stored in tightly sealed vials until further analysis.

2.4. Determination of the Physical Properties of Essential Oils

The physical properties of the essential oils were evaluated through organoleptic assessment, including color and aroma, as well as measurements of refractive index and specific gravity. The refractive index was determined using a refractometer by placing a drop of essential oil onto the prism surface and directing the instrument toward a light source until a clear light–dark boundary aligned with the crosshair was observed. Specific gravity was determined using a pycnometer. The empty pycnometer was weighed, filled with essential oil to the calibration mark, and weighed again. The specific gravity was then calculated from the mass difference.

2.5. Identification of Chemical Components by GC–MS

The chemical composition of the essential oils was analyzed using Gas Chromatography–Mass Spectrometry (GC–MS) (Agilent Technologies 7890A gas chromatograph coupled with a 5977B mass spectrometer detector) equipped with a DB-5MS capillary column. Helium was used as the carrier gas, and samples were injected using a split ratio of 10:1. The initial oven temperature was set at 50°C and increased at a rate of 4°C/min to a final temperature of 280°C. The total analysis time was 61.5 min.

2.6. Determination of Total Phenolic Content

The total phenolic content of the essential oils was determined using the Folin–Ciocalteu colorimetric method, with gallic acid as the reference standard. A calibration curve was prepared using gallic acid solutions at concentrations ranging from 10 to 60 mg/L, with methanol used as the solvent. Briefly, 0.5 mL of the essential oil sample, dissolved in methanol, was mixed with 10% Folin–Ciocalteu reagent and 7.5% Na_2CO_3 solution. The reaction mixture was then incubated in the dark for 30 min. Absorbance was measured at the maximum absorption wavelength using a UV–Vis spectrophotometer. The total phenolic content was expressed as milligrams of gallic acid equivalents per gram of essential oil (mg GAE/g essential oil) [17].

2.7. Determination of Antioxidant Activity by the DPPH Method

The antioxidant activity of the essential oils was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free-radical scavenging assay. A 0.1 mM DPPH solution was prepared by dissolving 2 mg of DPPH in 50 mL of methanol. Various concentrations of single essential oils and essential oil combinations (lemongrass ratios of 1:1, 1:2, and 2:1, v/v) were prepared using methanol as the solvent according to the desired test concentrations. A total of 1.2 mL of each test solution was mixed with 2.0 mL of 0.1 mM DPPH solution and incubated in the dark for 30 min. Subsequently, the absorbance was measured at the maximum absorption wavelength using a UV–Vis spectrophotometer. The percentage of DPPH radical inhibition was calculated and used to determine the antioxidant activity of the samples [18].

2.8. Antibacterial Activity Assay by the Well Diffusion Method

The antibacterial activity of the essential oils was evaluated against *Staphylococcus aureus* and *Escherichia coli* using the agar well diffusion method. Test solutions were prepared at concentrations of 50, 25, 12.5, and 6.25% using DMSO as the solvent. Bacterial suspensions were standardized to the 0.5 McFarland turbidity standard, corresponding to an optical density (OD) of 0.08–0.10 at 600 nm. A volume of 50 µL of the bacterial suspension was evenly spread onto NA plates, after which wells with a diameter of 3 mm were prepared in the agar medium. Subsequently, 20 µL of the essential oil sample was introduced into each well. The plates were incubated, and inhibition zone diameters were measured at 12, 24, 36, and 48 h. Ciprofloxacin was used as the positive control. Each treatment was performed in duplicate for every concentration and formulation tested.

3. Results and Discussion

3.1. Identification of Lemongrass and Clove Plant Materials

Plant identification was conducted at the Integrated Laboratory of the Faculty of Mathematics and Natural Sciences, Universitas Negeri Semarang. The results confirmed that the lemongrass sample was *Cymbopogon citratus* (DC.) Stapf., while the clove flower sample was identified as *Syzygium aromaticum* (L.) Merr. & L.M. Perry. These results verified the authenticity of the plant materials used in this study and ensured the reliability of subsequent analyses.

3.2. Isolation of Essential Oils

Essential oils were obtained from dried plant materials using the steam distillation method. The yield of lemongrass essential oil was 0.09%, whereas clove essential oil yielded 0.73% (Table 1). These values were lower than those reported in previous studies. Kanza *et al.* [19] reported a lemongrass essential oil yield of approximately 0.1882%, while Chilev and Peshev [20] obtained a clove essential oil yield of approximately 5%. Variations in essential oil yield may be attributed to several factors, including differences in plant origin, environmental conditions, harvest time, raw material quality, moisture content, and extraction parameters such as distillation duration and operating conditions.

3.3. Physical Properties of Essential Oils

The physical properties of lemongrass and clove essential oils are presented in Table 2. The refractive indices of lemongrass oil (1.484) and clove oil (1.529) were within the ranges specified by the International Organization for Standardization (ISO, 1974; ISO, 1997), indicating acceptable purity and quality. The specific gravity of lemongrass oil (0.893 g/mL) also complied with the ISO standard (1974), whereas the specific gravity of clove oil (1.038 g/mL) was consistent with the Indonesian National Standard (SNI, 2006) and remained within the range specified by ISO (1997). These findings indicate that the essential oils produced in this study met the relevant national and international quality standards.

Table 1. Yield of lemongrass and clove essential oils obtained by steam distillation

Sample	Sample weight (g)	Oil volume (mL)	Yield (%)
Lemongrass	8000	8	0.09
Clove flower	1000	7	0.73

Table 2. Physical properties of lemongrass and clove essential oils

Parameter	Lemongrass	ISO standard	Clove	ISO/SNI standard
Refractive index (25°C)	1.484	1.483–1.489	1.529	1.528–1.535
Specific gravity (25°C) (g/mL)	0.893	0.872-0.897	1.038	1.025-1.049
Water content (%)	7.5	-	8.3	-
Aroma	Characteristic lemon-like aroma	Characteristic citral aroma	Strong clove aroma	Characteristic spicy and eugenol aroma
Color	Clear pale yellow	Pale yellow to orange-yellow	Clear light yellow	Yellow to light brown

3.4. Analysis of Essential Oil Components by GC–MS

The GC chromatogram of lemongrass essential oil revealed the presence of 21 chemical components, the composition of which is presented in Table 3. Among the identified compounds, peak 10 exhibited the highest relative abundance, accounting for 36.59% of the total peak area, and was identified as neral. The chemical composition of lemongrass essential oil was dominated by neral (>30%) and geranial (>40%) [21]. These compounds are geometric isomers collectively known as citral, an acyclic monoterpenoid aldehyde that contributes significantly to the characteristic aroma and biological activity of lemongrass essential oil. The predominance of citral is considered responsible for the antioxidant and antibacterial properties of the oil [22].

Figure 1 shows the mass spectrum corresponding to peak 10 at a retention time of 19.344 min. Based on GC–MS analysis, this peak was identified as neral, with a molecular weight of 152.23 g/mol (Figure 2). This finding is consistent with the results reported by Wallace and Moorthy [23], who identified neral as one of the major constituents of lemongrass essential oil.

Table 3. Chemical components identified in lemongrass essential oil

Peak	Compound	Molecular weight (g/mol)	Retention time (min)	Area (%)
1	β-Myrcene	136.23	8.954	8.79
2	trans-β-Ocimene	136.23	10.482	0.59
3	(Z)-3-Undecene-5-yne	150.26	12.482	0.47
4	Linalool	154.25	12.851	1.87
5	6-Octenal. 7-methyl-3-methylene	152.23	14.390	1.10
6	6-Octenal. 3.7-dimethyl-. (R)-	154.25	14.708	0.76
7	Isoneral	152.23	15.077	3.16
8	3.6-Octadienal. 3.7-dimethyl-	152.23	15.764	4.15
9	Citral	152.23	18.175	31.96
10	Neral	152.23	19.344	36.59
11	Dimethyl neral acetal	200.32	19.405	1.28
12	2-Isopropenyl-5-methylhex-4-enal	152.23	19.477	0.43
13	Dimethyl neral acetal	200.32	20.441	1.00
14	Neric acid	168.23	20.964	0.39
15	1-Heptadecyne	236.43	21.180	1.41
16	Neric acid	168.23	22.010	0.96
17	Neric acid	168.23	22.277	1.10
18	(E)-3.6-Heptadien-2-ol. 2.5.5-trimethyl	154.25	22.390	1.80
19	Geranyl acetate	196.29	22.472	1.08
20	β-Selinene	204.35	24.093	0.45

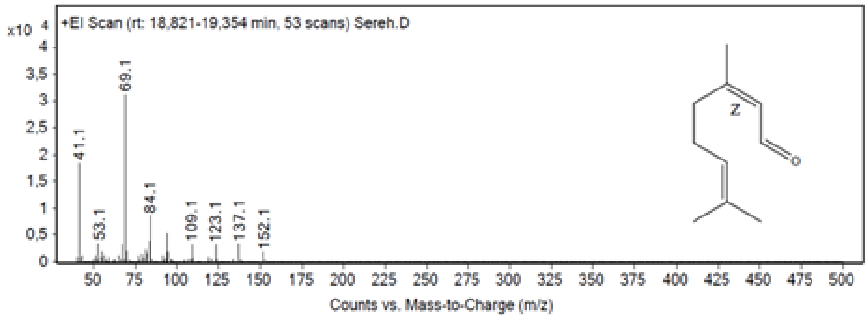

Figure 1. Mass spectrum of Peak 10 (Tr = 19.344 min)

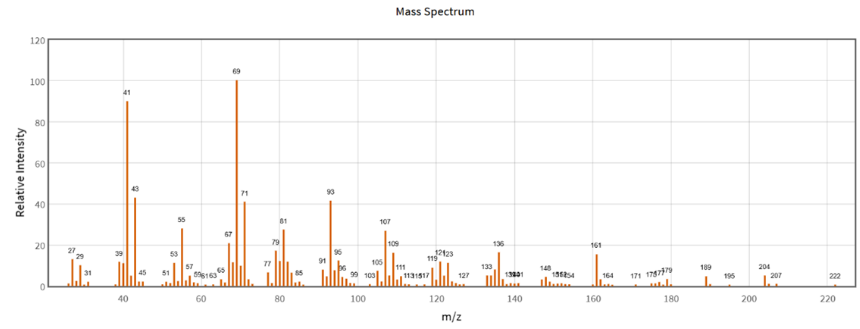

Figure 2. Mass spectrum of neral

The GC–MS analysis indicated that Peak 10 was identified as neral, a compound with a molecular weight of 152.23 g/mol belonging to the class of acyclic monoterpenoid aldehydes. Comparison of the obtained mass spectrum with reference data reported by Wallace and Moorthy [23] confirmed that the spectral pattern of Peak 10 was consistent with that of neral.

Based on the GC–MS data presented in Table 4, seven chemical components were identified in clove essential oil. Among these compounds, eugenol was the predominant constituent, accounting for 75.22% of the total peak area. This finding is consistent with the characteristic chemical profile of clove essential oil, in which eugenol is typically the major component. According to El-Saber Batiha *et al.* [24], eugenol (a phenylpropanoid), eugenyl acetate (a phenylpropanoid ester), and β-caryophyllene (a sesquiterpene) are the principal bioactive constituents of clove essential oil and are responsible for its significant antioxidant and antibacterial activities. The high abundance of eugenol observed in the present study is therefore likely to contribute substantially to the biological activities of the clove essential oil.

The GC–MS analysis indicated that Peak 3 was identified as eugenol, a phenolic compound with a molecular weight of 164.20 g/mol. Comparison of the obtained mass spectrum with the reference spectrum reported by Adams [25] showed a high degree of similarity, confirming that Peak 3 corresponded to eugenol. The predominance of eugenol in the clove essential oil is consistent with the characteristic chemical profile of *Syzygium aromaticum* essential oil.

Table 4. Chemical components identified in clove essential oil

Peak	Compound	Molecular weight (g/mol)	Retention time (min)	Area (%)
1	Methyl salicylate	152.15	16.113	0.76
2	α-Cubebene	204.36	21.282	0.99
3	Eugenol	164.20	22.092	75.22
4	β-Caryophyllene	204.36	23.805	8.98
5	α-Humulene	204.36	24.841	1.50
6	Eugenyl acetate	206.24	26.933	11.75
7	2’,3’,4’-Trimethoxyacetophenone	210.23	31.662	0.79

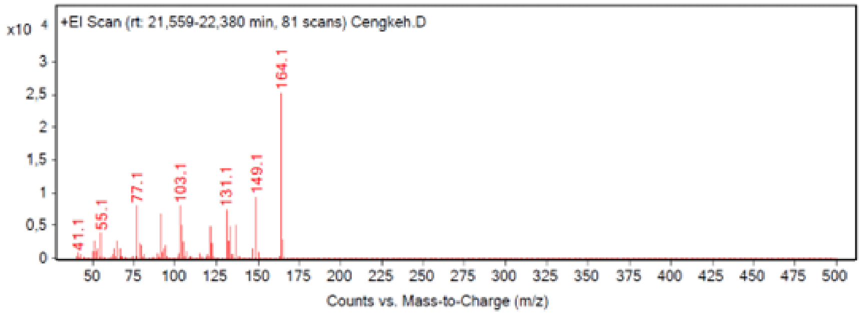

Figure 3. Mass spectrum of Peak 3 (Tr = 22.092 min)

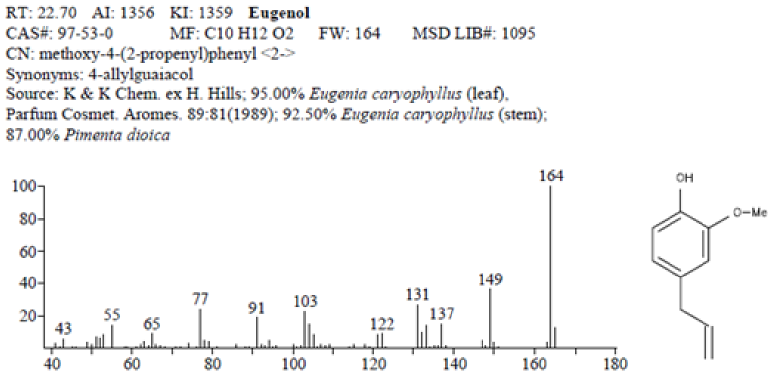

Figure 4. Mass spectrum of eugenol

3.5. Total Phenolic Content

The total phenolic content of lemongrass and clove essential oils was determined using the Folin–Ciocalteu method, which is based on the reaction between phenolic compounds and a mixture of phosphomolybdic and phosphotungstic acids to produce a blue-colored complex. The analysis involved determining the maximum absorption wavelength (400–800 nm) and constructing a gallic acid calibration curve using standard solutions with concentrations ranging from 10 to 60 ppm.

The maximum absorption wavelength was found to be 764 nm. The gallic acid calibration curve exhibited good linearity, with a regression equation of $y = 0.0061x + 0.1177$ and a coefficient of determination (R^2) of 0.9933 (Figure 5). The total phenolic content of the samples was calculated by substituting the absorbance values into the regression equation and expressing the results as gallic acid equivalents (GAE). The results are presented in Table 5.

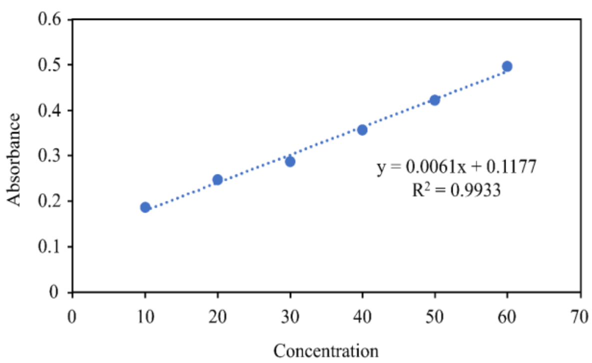

Figure 5. Gallic acid calibration curve at λ = 764 nm

The results demonstrated that the total phenolic content of clove essential oil was substantially higher than that of lemongrass essential oil. This finding is consistent with the GC–MS analysis, which identified eugenol as the predominant constituent of clove essential oil. Eugenol is a phenolic compound that contributes significantly to the total phenolic content. In contrast, the major constituents of lemongrass essential oil were identified as neral and citral, which belong to the aldehyde class of compounds rather than phenolic compounds. Consequently, the lower phenolic content observed in lemongrass essential oil is consistent with its chemical composition.

The differences between the total phenolic contents obtained in the present study and the reference values may be attributed to variations in chemical composition resulting from geographical origin, plant variety or genotype, environmental conditions, and harvest time. In lemongrass, variations in citral content can influence essential oil yield and composition, whereas in clove flower buds, eugenol levels are strongly affected by geographical origin and cultivation conditions, leading to considerable differences in total phenolic content among studies [26].

Table 5. Total phenolic content of essential oils and comparison with reference values [27, 28]

Sample	Results of total reducing capacity (Folin-reactive substances) (mg GAE/g)	Reference (mg GAE/g)
Lemongrass essential oil	27.010 ± 0.023	15.09 ± 0.88
Clove essential oil	956.721 ± 2.318	345.95 ± 7.85

3.6. Antioxidant Activity

The antioxidant activity of the essential oils was evaluated using the DPPH free-radical scavenging assay. The results showed that clove essential oil exhibited very strong antioxidant activity, with an IC_{50} value of 7.387 mg/L, which was comparable to that of gallic acid (IC_{50} = 4.623 mg/L). In contrast, lemongrass essential oil demonstrated relatively weak antioxidant activity, with an IC_{50} value of 262.28 mg/L. All combinations of lemongrass and clove essential oils (1:1, 1:2, and 2:1) exhibited very strong antioxidant activity, with IC_{50} values ranging from 14.400 to 19.885 mg/L (Table 6). The enhanced antioxidant activity observed in the combined formulations is presumed to be associated with the interaction between citral and eugenol, which has been reported to exhibit synergistic antioxidant effects [28].

Based on the results presented in Table 6, increasing the proportion of clove essential oil in the mixture tended to enhance antioxidant activity, as indicated by lower IC_{50} values. Among the combinations tested, the 1:2 (lemongrass) ratio exhibited the strongest antioxidant activity. However, the antioxidant activity of the combined formulations remained lower than that of clove essential oil alone. The strong antioxidant activity of clove essential oil is closely associated with its high eugenol content, which acts as a phenolic antioxidant capable of donating hydrogen atoms to stabilize free radicals. Phenolic compounds generally possess greater antioxidant capacity than non-phenolic monoterpenes due to the resonance stabilization of the phenoxyl radicals formed after hydrogen donation [29].

In contrast, the major constituent of lemongrass essential oil, citral, has been reported to exhibit relatively limited DPPH radical scavenging activity because it lacks an aromatic hydroxyl group, which plays a crucial role in proton donation and free-radical stabilization [30]. This difference in chemical composition explains the substantially lower antioxidant activity of lemongrass essential oil compared with clove essential oil. The improved antioxidant activity observed in the combined formulations is therefore likely attributable to the contribution of eugenol from clove essential oil, which increased the overall phenolic content and antioxidant capacity of the mixtures.

Table 6. IC_{50} value of gallic acid and essential oil samples

Sample	IC_{50} (mg/L)	Activity category
Gallic acid	4.623	Very strong
Lemongrass essential oil	262.28	Weak
Clove essential oil	7.387	Very strong
1:1 combination (L:C)	15.756	Very strong
1:2 (L:C) Combination	14.400	Very strong
2:1 (L:C) Combination	19.885	Very strong

3.7. Antibacterial Activity

The antibacterial activities of lemongrass essential oil, clove essential oil, and their combinations were evaluated using the agar well diffusion method against *Escherichia coli* (Gram-negative) and *Staphylococcus aureus* (Gram-positive). Ciprofloxacin (500 ppm) was used as the positive control, while DMSO served as the negative control. The results demonstrated that ciprofloxacin produced clear inhibition zones against both bacterial species throughout the incubation period. The largest inhibition zone against *Escherichia coli* was observed after 48 h of incubation, with a diameter of 30.00 mm, whereas the maximum inhibition zone against *Staphylococcus aureus* was 24.00 mm and was maintained at 12, 24, and 36 h of incubation. In contrast, DMSO did not produce any inhibition zone against either bacterial species, confirming that it acted solely as a solvent and exhibited no antibacterial activity (Table 7).

The effectiveness of ciprofloxacin against both test bacteria is consistent with its broad-spectrum antibacterial activity. The larger inhibition zones observed for ciprofloxacin compared with the essential oil treatments indicate its high efficacy in suppressing bacterial growth. Meanwhile, the absence of inhibition zones in the DMSO treatment confirms that the observed antibacterial effects in the essential oil samples were attributable to the bioactive compounds present in the oils rather than to the solvent used in the assay (Table 8).

Based on the results presented in Table 8, all concentrations of lemongrass essential oil, clove essential oil, and their combinations produced inhibition zones against *Staphylococcus aureus*, indicating antibacterial activity. Among the tested samples, clove essential oil exhibited the strongest antibacterial effect, as demonstrated by the largest and most consistent inhibition zone diameters across all concentrations and incubation periods. At a concentration of 50%, clove essential oil produced inhibition zones ranging from 19.00 to 23.00 mm during the 48 h incubation period.

The strong antibacterial activity of clove essential oil is closely associated with its high eugenol content. Eugenol is a phenolic compound capable of damaging bacterial cell walls and membranes, disrupting enzyme activity, and inhibiting DNA synthesis [31]. The phenolic hydroxyl group of eugenol is highly reactive and can interact with membrane lipids and cellular proteins, resulting in increased membrane permeability and leakage of intracellular constituents [32]. This effectiveness may also be attributed to the lipophilic nature of eugenol, which facilitates its penetration into the phospholipid bilayer of bacterial membranes. In Gram-positive bacteria such as *Staphylococcus aureus*, the absence of an outer membrane allows phenolic compounds to interact more directly with the peptidoglycan layer and cytoplasmic membrane, thereby enhancing their antibacterial activity [33].

Table 7. Antibacterial activity of positive and negative controls

Test solution	Concentration (ppm)	Incubation time (h)	Inhibition zone diameter (mm)	
			Escherichia coli	*Staphylococcus aureus*
Ciprofloxacin	500	12	29.50	24.00
		24	29.50	24.00
		36	29.50	24.00
		48	30.00	23.50
DMSO	-	12	0	0
		24	0	0
		36	0	0
		48	0	0

Table 8. Inhibition zone diameters of essential oils against *Staphylococcus aureus*

Concentration (%)	Incubation time (h)	Inhibition zone diameter (mm)				
		Lemongrass	Clove	1:1	1:2	2:1
50	12	10.50	23.00	14.50	17.00	19.50
	24	9.00	22.00	14.00	16.00	19.00
	36	6.50	20.50	14.00	14.00	17.00
	48	6.50	19.00	14.00	12.00	16.50
25	12	9.50	22.50	14.50	15.50	17.50
	24	2.50	20.50	13.50	15.50	17.00
	36	2.00	20.50	12.50	15.50	11.00
	48	1.50	18.00	12.50	13.00	10.00
12.5	12	9.00	23.50	13.50	15.50	16.50
	24	6.50	17.00	13.00	15.50	16.50
	36	5.50	16.00	13.00	15.00	11.50
	48	5.00	16.00	13.00	15.00	11.50
6.25	12	8.00	21.50	11.50	15.50	15.50
	24	5.00	15.00	11.00	15.50	15.50
	36	3.50	13.50	10.50	15.00	10.50
	48	2.50	12.00	10.50	15.00	10.50

In contrast, lemongrass essential oil exhibited comparatively weaker antibacterial activity. The antibacterial effect of lemongrass essential oil is primarily associated with citral, a mixture of neral and geranial belonging to the aldehyde terpenoid group. Although citral can interact with bacterial proteins and enzymes, aldehyde compounds are generally more volatile and less stable in agar diffusion systems. Consequently, their antibacterial effectiveness may decrease over extended incubation periods, as reflected by the gradual reduction in inhibition zone diameters observed in this study [34].

The combinations of lemongrass and clove essential oils produced larger inhibition zones than lemongrass essential oil alone, but generally remained less effective than clove essential oil alone. These findings suggest that the interaction between citral and eugenol in the mixed formulations is additive rather than strongly synergistic. From a mechanistic perspective, this additive effect may arise from the different modes of action of the two compounds. Eugenol acts primarily as a membrane-disrupting agent, whereas citral may contribute as a supporting antimicrobial compound that influences diffusion properties or the initial interaction with the bacterial cell surface [35].

Overall, the results indicate that clove essential oil possesses the highest antibacterial potential against *Staphylococcus aureus*, primarily due to its high eugenol content. Although lemongrass essential oil exhibited antibacterial activity, its effectiveness was lower than that of clove essential oil. The combination of the two essential oils enhanced antibacterial activity relative to lemongrass essential oil alone; however, no evidence of strong synergistic interaction was observed.

Table 9. Inhibition zone diameters of essential oils against *Escherichia coli*

Concentration (%)	Incubation time (h)	Inhibition zone diameter (mm)				
		Lemongrass	Clove	1:1	1:2	2:1
50	12	20.50	23.50	23.00	20.50	19.50
	24	19.50	24.00	22.50	17.50	18.00
	36	16.50	24.00	22.50	17.50	18.00
	48	15.00	24.00	22.50	17.50	16.00
25	12	12.50	21.50	21.50	19.00	19.00
	24	11.50	22.00	21.50	17.50	18.00
	36	7.50	22.00	21.50	17.50	18.00
	48	6.50	22.00	21.50	17.50	13.00
12.5	12	9.00	20.00	21.00	18.50	18.50
	24	8.50	20.50	21.00	18.50	11.50
	36	4.50	20.50	21.00	18.50	11.50
	48	4.00	20.50	21.00	18.50	11.50
6.25	12	8.00	19.50	18.50	18.50	15.50
	24	8.00	20.00	18.00	17.50	12.50
	36	4.00	20.00	17.50	17.50	12.50
	48	1.00	20.00	17.50	17.50	12.50

Furthermore, all tested concentrations of lemongrass essential oil, clove essential oil, and their combinations produced inhibition zones, confirming their antibacterial activity. Clove essential oil consistently exhibited stable antibacterial activity, which can be attributed to its high concentration of eugenol. In comparison, lemongrass essential oil demonstrated appreciable antibacterial activity at higher concentrations, but its effectiveness declined with decreasing concentration and prolonged incubation. This reduction may be associated with the volatile nature of essential oil constituents, which can diminish their persistence within the agar medium over time. Additionally, redistribution of antimicrobial compounds within the agar matrix and the potential regrowth of bacterial cells in previously inhibited areas may contribute to the observed decrease in inhibition zone diameters.

The antibacterial activity of clove essential oil is strongly related to the predominance of eugenol, a lipophilic phenolic compound capable of penetrating bacterial membranes and disrupting membrane integrity [33, 36]. The antibacterial activity of lemongrass essential oil, clove essential oil, and their combinations against *Escherichia coli* is presented in Table 9. All tested samples produced measurable inhibition zones, indicating antibacterial activity against the Gram-negative bacterium. Among the individual essential oils, clove essential oil exhibited the strongest and most stable antibacterial activity, with inhibition zone diameters ranging from 19.50 to 24.00 mm across all concentrations and incubation periods. In contrast, lemongrass essential oil showed moderate antibacterial activity at higher concentrations but experienced a marked reduction in inhibition zone diameter with decreasing concentration and prolonged incubation.

The antibacterial activity of clove essential oil against *Escherichia coli* is primarily attributed to its high eugenol content. Although Gram-negative bacteria possess an outer membrane rich in lipopolysaccharides that generally limits the penetration of antimicrobial agents, eugenol is capable of interacting with membrane lipids through hydrophobic interactions. This interaction increases membrane permeability, resulting in leakage of ions and intracellular macromolecules and ultimately leading to bacterial cell damage [37]. This mechanism may explain the substantial inhibition zones observed for clove essential oil throughout the study.

Lemongrass essential oil exhibited lower antibacterial activity than clove essential oil, particularly at lower concentrations and longer incubation periods. This observation may be related to the predominance of citral, which, although possessing antibacterial properties, is relatively volatile and may gradually dissipate from the agar medium. Consequently, its inhibitory effect tends to decrease over time.

The combinations of lemongrass and clove essential oils produced inhibition zone diameters that generally fell between those observed for the individual oils. Among the combinations tested, the 1:1 ratio exhibited the strongest antibacterial activity against *Escherichia coli*, producing inhibition zones of up to 23.00 mm at a concentration of 50%. However, the antibacterial activity of the combined formulations remained lower than that of clove essential oil alone. These findings suggest that the interaction between citral and eugenol is primarily additive, with eugenol serving as the principal antibacterial constituent while citral contributes to a lesser extent as a supporting antimicrobial agent. The absence of antibacterial activity exceeding that of clove essential oil alone indicates that the combinations cannot be considered to exhibit strong synergistic effects [38].

The combined formulations also demonstrated antibacterial and antioxidant activities that were generally greater than those of lemongrass essential oil alone but lower than those of clove essential oil alone. This pattern suggests that the interaction between eugenol and citral is additive or modulatory rather than synergistic. Eugenol remains the dominant bioactive component responsible for the observed biological activities, whereas citral may contribute indirectly by influencing diffusion characteristics or the physicochemical properties of the system. Furthermore, increasing the proportion of non-phenolic compounds in the mixtures may reduce the overall antioxidant capacity by decreasing the relative abundance of phenolic constituents, particularly eugenol, which is the primary antioxidant component of clove essential oil [35].

4. Conclusion

Steam distillation yielded 0.09% lemongrass essential oil and 0.73% clove essential oil. GC–MS analysis showed that lemongrass oil was dominated by neral (36.59%), whereas clove oil contained eugenol as the major constituent (75.22%). Clove essential oil exhibited a substantially higher total phenolic content (956.721 ± 2.318 mg GAE/g) than lemongrass essential oil (27.010 ± 0.023 mg GAE/g), resulting in stronger antioxidant activity (IC_{50} = 7.387 mg/L vs. 262.28 mg/L). The addition of clove essential oil improved the antioxidant activity of lemongrass essential oil, with the 1:2 (lemongrass:clove) combination showing the lowest IC_{50} value among the mixtures (14.400 mg/L). In antibacterial assays, the 2:1 combination exhibited the highest activity against *Staphylococcus aureus* (19.50 mm), while the 1:1 combination showed the greatest inhibition against *Escherichia coli* (23.00 mm). Overall, the addition of clove essential oil enhanced the biological activity of lemongrass essential oil through predominantly additive effects attributed to its high eugenol content.

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