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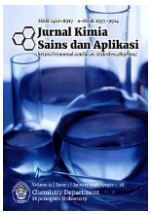

Combination of Nutmeg Seed (*Myristica fragrans Houtt.*) and Lemongrass (*Cymbopogon citratus*) Essential Oils: Evaluation of Antioxidant and Antibacterial Activities

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Abstract

Essential oils are secondary metabolites that exhibit various biological activities, such as antioxidants and antibacterial activities. This study aimed to evaluate the antioxidant and antibacterial activities of nutmeg seed (*Myristica fragrans* Houtt.) and lemongrass (*Cymbopogon citratus*) essential oils, as well as their combination. The essential oils were obtained by steam distillation, physically characterized, and their chemical components analyzed using GC-MS. Antioxidant activity was determined using the DPPH method, and antibacterial activity was evaluated using the well-diffusion method against *E. coli* and *S. aureus*. The results showed that nutmeg seed essential oil contained α-pinene (18.49%) as the major compound with moderate antioxidant activity (IC_{50} = 139.49 mg/L), whereas lemongrass essential oil was dominated by α-pinene (29.19%) with lower antioxidant activity (IC_{50} = 263.54 mg/L). In antibacterial assays, nutmeg essential oil showed moderate to strong activity, while lemongrass essential oil exhibited weak to moderate activity. Among the combinations, the ratio of 2:1 (nutmeg:lemongrass) demonstrated the highest antibacterial activity, with inhibition zones of 13.50 mm against *E. coli* and 14.00 mm against *S. aureus* at 50% concentration, indicating improved antibacterial effects compared to other combinations. The combined essential oils of nutmeg and lemongrass exhibited bacteriostatic activity and showed improved antibacterial effects; however, no enhancement in antioxidant activity was observed.

1. Introduction

Indonesia is one of the world's megabiodiversity countries, with approximately 30,000 species of flowering plants, of which around 7,000 have potential as medicinal resources [1]. Among these, nutmeg (*Myristica fragrans*) and lemongrass (*Cymbopogon citratus*) are widely utilized not only as culinary spices but also as sources of bioactive compounds. Nutmeg essential oil is known to contain major constituents, such as α-pinene (22.06–22.50%) and β-myrcene (4.70–6.05%), which contribute to its characteristic aroma and biological activity [2]. Additionally, compounds such as myristicin and trimyristin have been reported to exhibit antimicrobial properties [3].

On the other hand, lemongrass essential oil is dominated by citral components, namely geranial (39.53%) and neral (33.31%), along with minor monoterpenes such as α-pinene and myrcene, which also influence its biological activities [4]. Lemongrass is further reported to contain various secondary metabolites, including phenols, flavonoids, tannins, alkaloids, and saponins, which are associated with antioxidant and antibacterial activities [5, 6]. Although both nutmeg and lemongrass essential oils have been individually reported to possess significant antioxidant and antibacterial activities, studies investigating their combined use remain limited and have not been systematically explored, particularly in terms of potential synergistic effects at the chemical and bioactivity levels. Previous research has shown that combinations of

essential oils, such as nutmeg and basil, can enhance antibacterial activity against *Staphylococcus aureus* [7]; however, no comprehensive study has evaluated the interaction between nutmeg and lemongrass essential oils, particularly in relation to their chemical composition and combined antioxidant and antibacterial effects.

Therefore, this study aims to isolate and characterize the essential oils of nutmeg and lemongrass, and to evaluate their combined antioxidant and antibacterial activities. The novelty of this research lies in (i) the investigation of the synergistic potential between nutmeg and lemongrass essential oils, (ii) the comparative analysis between single and combined applications, and (iii) the correlation of their chemical profiles with enhanced biological activities. This approach is expected to provide new insights into the development of natural product-based antioxidants and antibacterial agents.

2. Experimental

2.1. Materials

The materials used in this study were nutmeg seeds (*Myristica fragrans Houtt.*), lemongrass (*Cymbopogon citratus*), methanol (analytical grade, SmartLab), anhydrous sodium sulfate, distilled water, gallic acid (analytical grade, Sigma Aldrich), Follin ciocalteu reagent (Merck), DPPH (1,1-diphenyl-2-picryhydrazyl) (Merck), Agarpac, nutrient broth, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, ciprofloxacin (Merck), and dimethyl sulfoxide (DMSO, Merck).

2.2. Instrumentation

The tools used in this study consisted of a set of steam distillation tools, a set of analytical glassware, analytical balance, porcelain exchanger, micropipette (Eppendorf), refractometer (ATAGO), pycnometer (Pyrex), dropper pipette, stirring rod, electric bath, petri dish, loop needle, spreader, autoclave, laminar air flow, incubator, volume pipette, Genesys 10s UV-Vis spectrophotometer (Thermo Fisher Scientific), and gas chromatography-mass spectrometry (Agilent Technologies).

2.3. Sample Preparation

Nutmeg and lemongrass plants were identified at the Integrated Laboratory of the Faculty of Mathematics and Natural Sciences, Semarang State University. A total of 3500 g of nutmeg seeds were peeled and crushed using a traditional mortar until coarse particles (simplicia) were obtained. Meanwhile, 8000 g of lemongrass were cut into small pieces and subsequently dried to obtain simplicia. All prepared simplicia were weighed and stored in airtight containers until further use.

2.4. Isolation of Essential Oils by Steam Distillation

Essential oils were isolated by steam distillation using a steam distillation apparatus equipped with a heating plate. A total of 3500 g of nutmeg and 8000 g of lemongrass were subjected to distillation for 4 hours. In this method, water is heated to generate steam, which passes through the plant material and facilitates the release of volatile compounds. These volatile components evaporate together with the steam and are subsequently condensed into a liquid in a cooling system. The resulting distillate separates into two layers due to differences in polarity and density, consisting of essential oil and water, allowing the essential oil to be collected [8]. The essential oils obtained were tested for physical properties, including color, odor, and specific gravity, using a pycnometer, and their refractive index was determined with a refractometer. The components of the compounds in the nutmeg and lemongrass essential oils were analyzed using GC-MS. This analysis was conducted at the Laboratory of the Faculty of Mathematics and Natural Sciences, Semarang State University.

2.5. Total Phenol Determination

Total phenol content was determined using the Folin-Ciocalteu method [8]. A standard gallic acid solution was prepared by dissolving 10 mg of gallic acid in 10 mL of methanol to obtain a stock solution of 1000 mg/L. This was then diluted to 100 mg/L and varied in concentrations from 10–60 mg/L. A 0.5 mL standard solution was mixed with 2.5 mL of 10% Folin-Ciocalteu reagent. After 4–7 minutes, 2 mL of 7.5% Na_2CO_3 was added, and the mixture was incubated in the dark for 30 minutes. Absorbance was measured at a maximum wavelength of 764 nm to obtain a standard curve. Nutmeg and lemongrass essential oil samples were analyzed using the same procedure, and the results were expressed as mg GAE/g sample.

2.6. Antioxidant Activity Testing Using the DPPH Method

Antioxidant activity was determined using the DPPH method [9]. A 0.1 mM DPPH solution was prepared by dissolving 2 mg of DPPH in 50 mL of methanol, whereas a standard gallic acid solution was prepared by dissolving 1 mg of gallic acid in 10 mL of methanol. The maximum wavelength was determined in the range of 400–800 nm, with a λ_{max} of 515 nm. A standard curve of gallic acid was prepared from various concentrations of 1–5 mg/L, each reacted with 2 mL of 0.1 mM DPPH, incubated for 30 minutes in the dark, and the absorbance was measured at 515 nm.

Samples comprising nutmeg and lemongrass essential oils and their combinations were prepared and evaluated for antioxidant activity using the DPPH method. Nutmeg essential oil was tested at concentrations of 40, 80, 120, 160, and 200 mg/L, while lemongrass essential oil was tested at concentrations of 100, 200, 300, 400, and 500 mg/L. The combination samples were prepared at ratios of 1:1, 1:2, and 2:1 (v/v), each evaluated at concentrations of 50, 100, 150, 200, and 250 mg/L. All samples were reacted with 0.1 mM DPPH solution prior to measurement, incubated in the dark for 30 minutes, and then their absorbance was measured at 515 nm using a UV-Vis spectrophotometer.

2.7. Antibacterial Activity Testing Using the Well Diffusion Method

Antibacterial activity was determined using the well diffusion method [10]. The test bacteria, *S. aureus* and *E. coli*, were cultured in nutrient broth media and

incubated at 37°C for 24 hours. The positive control used was a 5% (w/v) ciprofloxacin solution. Essential oil solutions of nutmeg, lemongrass, and a combination of both were prepared in various concentrations of 50, 25, 12.5, and 6.25% using DMSO as the solvent.

Microbial suspensions were prepared to achieve a turbidity equivalent to 0.5 McFarland standards (absorbance 0.08–0.1 at 600 nm). Solid media were prepared from a mixture of agar, nutrient broth, and distilled water and then sterilized by autoclaving at 121°C for 55 minutes. After the media solidified, 50 µL of the bacterial suspension was evenly inoculated, and wells with a diameter of 3 mm were made. A total of 20 µL of essential oil samples was added to each well, then incubated for 48 hours. Antibacterial activity was determined by measuring the diameter of the clear zone formed around the well. All experiments were performed in duplicate, and the results were presented as mean values.

3. Results and Discussion

3.1. Essential Oil Isolation

Nutmeg plant identification was conducted at the Integrated Laboratory of the Faculty of Mathematics and Natural Sciences, Semarang State University. The nutmeg plant was identified as *Myristica fragrans Houtt*. Lemongrass was also identified at the Integrated Laboratory of the Faculty of Mathematics and Natural Sciences, Semarang State University, and the lemongrass plant was identified as *Cymbopogon citratus*. The distillation results for nutmeg and lemongrass essential oils are shown in Table 1.

Table 1. The results of the distillation of essential oils from nutmeg and lemongrass

Sample	Sample weight (g)	Oil volume (mL)	Yield (%)
Nutmeg	3500	9.5	0.24
Lemongrass	8000	8	0.09

The yield of nutmeg essential oil in this study was 0.24%. Research conducted by Wulandari [7] obtained a yield of nutmeg essential oil of 0.34%. The yield of lemongrass essential oil in this study was 0.09%, while [11] reported a yield of 0.399%. The difference in the yield of essential oils in this study compared to those by Wulandari [7] and Zaituni *et al.* [11] may be attributed to differences in plant habitat, plant age, and sample storage [12]. According to the Indonesian National Standard [13] and the International Organization for Standardization [14], the physical properties of nutmeg and lemongrass essential oils include color and odor, specific gravity, refractive index, water content, and solubility. The results of the physical property tests on nutmeg and lemongrass essential oils are listed in Table 2.

Table 3 presents the results of gas chromatography (GC) of nutmeg essential oil, which contained approximately 23 peaks or 23 compounds. Based on research conducted by Matulyte *et al.* [15], approximately 24 peaks were observed, with the major compounds being sabinene (61.42%) and limonene (5.62%). The results of the identification of 23 GC chromatogram peaks are presented in Table 3. In Table 3, the largest area percentage is peak 1, which is estimated to be an α-pinene compound (18.49%) with a retention time of 3.538 min.

Table 2. Results of physical properties tests of nutmeg and lemongrass essential oils

Parameter	Nutmeg essential oil	SNI (nutmeg)	Lemongrass essential oil	ISO (lemongrass)
Color	Colorless	Colorless – pale yellow	Clear yellow	Pale yellow – yellow
Odor	Characteristic nutmeg, spicy	Characteristic aromatic, spicy	Characteristic lemongrass, spicy	Characteristic lemon-like
Density (25°C) (g/mL)	0.876	0.860 – 0.910	0.893	0.880 – 0.905
Refractive Index (25°C)	1.478	1.470 – 1.490	1.484	1.483 – 1.489
Distilled water	–	–	–	–
n-hexane	+	+	+	+
Chloroform	+	+	+	+
Ethanol	+	+	+	+
Methanol	+	+	+	+

Note: (+) soluble; (-) insoluble in the solvent used

Table 3. Chemical content of nutmeg essential oil

Peak No.	Retention time (min)	Area (%)	Compound name
1	3.538	18.49	α-Pinene
2	4.583	16.95	β-Myrcene
3	4.731	9.97	β-Phellandrene
4	5.102	1.50	Δ-3-Carene
5	5.218	2.39	β-Myrcene
6	5.340	1.28	1-Phellandrene
7	5.611	3.83	α-Terpinene
8	6.055	4.40	Bornylene
9	6.220	3.36	β-Phellandrene
10	7.031	5.32	γ-Terpinene
11	7.408	2.51	1-Methyl-4-(1-methylethyl)benzene
12	7.828	1.97	α-Terpinolene
13	18.264	8.24	3-Cyclohexan-1-ol, 4-methyl-1-(1-methylethyl)
14	21.533	1.49	3-Cyclohexane-1-methanol, α,α,4-trimethyl
15	27.255	2.63	1,3-Benzodioxole, 5-(2-propenyl)
16	31.978	1.00	Benzene, 1,2-dimethoxy-4-(2-propenyl)
17	34.633	0.38	2-Methoxy-4-(2-propenyl)phenol
18	35.887	0.91	cis-Asarone
19	36.347	11.00	1,3-Benzodioxole, 4-methoxy
20	37.284	0.63	1,2-Dimethoxy-4-(2-propenyl)benzene
21	39.287	0.59	β-Elemene
22	40.831	0.90	D-Germacrene
23	43.133	0.26	Palmitic acid

Table 4. Chemical content of lemongrass essential oil

Peak No.	Retention time (min)	Area (%)	Compound name
1	3.626	29.19	α-Pinene
2	3.920	0.82	Camphene
3	4.391	0.88	β-Pinene
4	5.086	7.07	Δ-3-Carene
5	6.062	8.90	Limonene
6	13.798	1.17	Citronellal
7	15.938	1.03	Linalool
8	21.539	16.75	Z-Citral
9	21.695	0.37	3-Cyclohexane-1-methanol, α,α,4-trimethyl
10	23.533	21.34	E-Citral
11	24.111	3.40	β-Citronellol
12	26.935	7.95	Geraniol
13	30.169	0.42	1,7-Octadiene, 2,3,3-trimethyl
14	31.710	0.71	Bicyclo[3,1,1]-heptan-3-one, 2,6,6-trimethyl

Table 4 shows the results of gas chromatography (GC) of lemongrass essential oil, which produced approximately 14 peaks or 14 compounds contained in lemongrass essential oil. Based on research conducted by Cortes-Torres *et al.* [16], 48 peaks were detected, with the major compounds being citral (38.62%), neral (27.58%), and β-myrcene (8.76%). The results of the identification of 14 GC chromatogram peaks are presented in Table 4.

From Table 4, the largest area percentage is peak 1, which is estimated to be an α-pinene compound (29.19%) with a retention time of 3.626 min. The GC analysis revealed that α-pinene (29.19%) was the major compound in lemongrass essential oil, which differs from commonly reported profiles where citral predominates. This discrepancy may be attributed to variations in geographical origin, environmental conditions, plant maturity, and extraction methods, which are known to significantly influence the chemical composition of essential oils. Such variability has been widely reported in previous studies, indicating that the composition of lemongrass essential oil is not always consistent across different samples.

3.2. Total Phenol Test of Nutmeg and Lemongrass Essential Oils

In this study, λ_{max} was obtained at 764 nm. The standard curves for total phenols and gallic acid are shown in Figure 1. As shown in Figure 1, the regression equation y = 0.0061x + 0.1175 and the coefficient of determination R^2 = 0.9933 were obtained. Total phenolics were determined using the regression equation obtained by distributing the sample absorbance values to the regression equation.

The total phenol contents of nutmeg and lemongrass essential oils are listed in Table 5. The total phenol content of nutmeg essential oils in this study was 54.524 mg GAE/g, and the total phenol content of nutmeg essential oils in a previous study by Feninlambir *et al.* [17] was 25.91 mg GAE/g. The total phenol yield of lemongrass essential oil in this study was 27.010 mg GAE/g, and the total phenol yield of lemongrass essential oil in a study by Godwin *et al.* [18] was 7.3 mg GAE/g. The difference in total phenol yield between this study and those by other researchers may be due to differences in growth habitat, plant species, and harvest time [19]. These results indicate that the total phenol content of nutmeg essential oil is higher than that of lemongrass essential oil. The total phenol yield in essential oils is related to antioxidant activity. This is because phenol compounds can act as free radical scavengers. The higher the total phenol content in essential oils, the stronger the antioxidant activity tends to be [20].

Table 5. Total phenol yield of essential oil

Sample	Total phenol
Nutmeg essential oil	54.524 ± 0.464 mg GAE/g
Lemongrass essential oil	27.010 ± 0.023 mg GAE/g

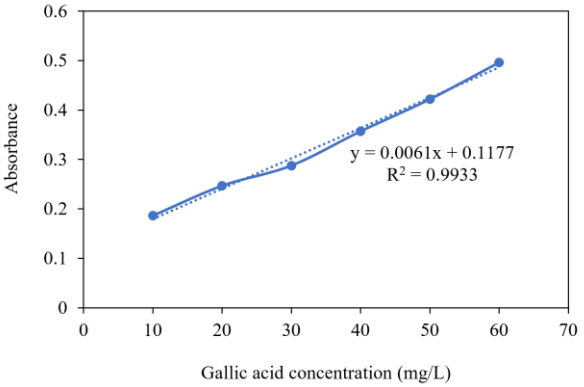

Figure 1. Standard curve of gallic acid in the determination of total phenol

3.3. Antioxidant Activity Test

In this study, the DPPH λ_{max} was 515 nm. The IC_{50} values of gallic acid standards and essential oil samples were obtained through antioxidant calculations and can be seen in Table 6. Based on the data in Table 6, the IC_{50} value of gallic acid was 4.622 mg/L, and that of nutmeg essential oil was 139.488 mg/L. This study differs from that conducted by Feninlambir *et al.* [17], who obtained the IC_{50} of nutmeg essential oil of 116.47 mg/L, which has stronger antioxidant activity than this study. The study conducted by Khairan *et al.* [21] reported an IC_{50} value of 216.69 mg/L, indicating that the IC_{50} value in this study has stronger antioxidant activity. The IC_{50} value of lemongrass essential oil in this study was 263.544 mg/L.

The study conducted by P. *et al.* [22] on lemongrass essential oil had an IC_{50} of 22.57 mg/L. The difference in the IC_{50} value of the antioxidant activity of essential oils in this study compared to the studies conducted by Feninlambir *et al.* [17], Khairan *et al.* [21], and P. *et al.* [22] can be caused by several factors, such as the concentration of active compounds and storage conditions of raw materials. The difference in IC_{50} values can also be influenced by the 30-min incubation conditions before measurement, when exposure to light and room temperature can reduce the stability of DPPH and volatile compounds of essential oils, resulting in variations in antioxidant activity.

The IC_{50} values indicate differences in antioxidant activity among the tested samples. Nutmeg essential oil exhibited a lower IC_{50} value (139.488 mg/L) compared to lemongrass essential oil (263.544 mg/L), indicating relatively higher antioxidant activity. Among the combination samples, the ratio of nutmeg to lemongrass essential oil (2:1) showed the lowest IC_{50} value (172.807 mg/L) compared to the other combinations (1:1 = 190.899 mg/L; 1:2 = 204.622 mg/L), indicating comparatively better antioxidant activity among the mixtures. However, all combination samples exhibited higher IC_{50} values than nutmeg essential oil alone, suggesting that the combination did not enhance antioxidant activity beyond that of the individual nutmeg essential oil. Therefore, the results indicate a comparative effect rather than a synergistic effect, as the combination does not result in a lower IC_{50} value than the most active single component.

Table 6. IC_{50} values of gallic acid and essential oil standards

Sample	IC_{50} (mg/L)	Category
Gallic acid	4.622	Very strong
Nutmeg essential oil	139.488	Moderate
Lemongrass essential oil	263.544	Very weak
Combination of essential oils (N:L) (1:1)	190.899	Weak
Combination of essential oils (N:L) (1:2)	204.622	Very weak
Combination of essential oils (N:L) (2:1)	172.807	Weak

N: Nutmeg, L: Lemongrass

3.4. Antibacterial Activity Test

Two bacteria, *E. coli* (Gram-negative) and *S. aureus* (Gram-positive), were used in this antibacterial activity test. Ciprofloxacin (500 mg/L) and DMSO served as positive and negative controls, respectively. The positive control was used to compare the antibacterial activity of the samples based on the pattern and size of the inhibition zones, while the negative control ensured that DMSO had no antibacterial activity. Antibacterial activity was determined by the presence of a clear zone around the well, indicating inhibition of bacterial growth. The antibacterial activity results for ciprofloxacin and DMSO are presented in Table 7. Higher essential oil concentrations produced stronger antibacterial effects. In contrast, longer incubation times tended to reduce the inhibition zone because the volatile active compounds in essential oils can evaporate or degrade. Consequently, the oil is more likely to inhibit bacterial metabolism than directly kill the bacteria.

The clear zone diameter of nutmeg essential oil was larger than that of lemongrass essential oil. The combination of nutmeg and lemongrass essential oils with a higher nutmeg (N(2):L(1) ratio produced a larger clear zone diameter than the essential oil combinations (1:1 and 1:2). This indicates that the antibacterial activity of the N(2):L(1) essential oil combination works synergistically. In nutmeg essential oil, the compound α- pinene is known to damage bacterial cell membranes by increasing lipid permeability [23, 24], whereas β- myrcene, as a non-polar monoterpene, can facilitate the diffusion of active compounds into the membrane, thereby strengthening the antibacterial effect [25].

In lemongrass essential oil, the citral compound plays a role through its reactive aldehyde properties, allowing it to interact with lipids and bacterial membrane proteins [26], and the presence of α-pinene also increases the membrane permeabilization effect [24]. The combination of the active components of these two essential oils provides a synergistic effect, such that the antibacterial activity becomes more optimal [27]. This study made similar observations regarding the antibacterial activity test on *S. aureus* bacteria, as shown in Table 8.

Table 7. Results of antibacterial activity tests of nutmeg essential oil, lemongrass, and combinations (1:1; 1:2; 2:1) against *E. coli*

Sample	Concentration (%)	Inhibition zone diameter (mm)	Category
Nutmeg EO	50	10.00	Strong
	25	8.00	Moderate
	12.5	6.00	Moderate
	6.25	5.25	Moderate
Lemongrass EO	50	10.00	Strong
	25	4.00	Weak
	12.5	3.00	Weak
	6.25	1.50	Weak
(N:L) 1:1	50	7.00	Moderate
	25	6.75	Moderate
	12.5	5.75	Moderate
	6.25	4.50	Weak
(N:L) 1:2	50	7.25	Moderate
	25	6.25	Moderate
	12.5	5.00	Weak
	6.25	4.00	Weak
(N:L) 2:1	50	13.50	Strong
	25	11.00	Strong
	12.5	7.50	Weak
	6.25	7.00	Weak
Ciprofloxacin	0.05	26.00	Strong
DMSO	0.05	0.00	Weak

EO: Essential oil, N: Nutmeg, and L: Lemongrass

Based on observations in Table 8, the higher the concentration of essential oil used, the greater its antibacterial activity. This is consistent with the characteristics of essential oils, which depend on the number of active compounds used to disrupt bacterial cell structure [27].

At the highest concentration of 50%, lemongrass essential oil produced a larger clear zone diameter than nutmeg essential oil. The combination of nutmeg and lemongrass essential oils in a ratio of N (2): L (1) produced a larger clear zone diameter (15.25 mm) after a 48-hour incubation compared to the essential oil combinations (1:1 and 1:2). This indicates that nutmeg essential oil provides an optimal synergistic effect in inhibiting the growth of *S. aureus*. This effectiveness is due to compounds such as α-pinene and β-myrcene in nutmeg, which are known to increase membrane permeability and damage bacterial cell walls [23, 25].

Table 8. Results of antibacterial activity tests of nutmeg essential oil, lemongrass, and combinations (1:1; 1:2; 2:1) against *S. aureus*

Sample	Concentration (%)	Inhibition zone diameter (mm)	Category
Nutmeg EO	50	9.50	Strong
	25	7.00	Moderate
	12.5	5.00	Moderate
	6.25	4.00	Weak
Lemongrass EO	50	11.00	Strong
	25	6.00	Moderate
	12.5	5.50	Moderate
	6.25	5.50	Moderate
(N:L) 1:1	50	11.00	Strong
	25	8.50	Moderate
	12.5	7.50	Moderate
	6.25	6.00	Moderate
(N:L) 1:2	50	9.00	Moderate
	25	8.00	Moderate
	12.5	5.25	Moderate
	6.25	4.75	Weak
(N:L) 2:1	50	14.00	Strong
	25	11.00	Strong
	12.5	9.00	Moderate
	6.25	3.00	Weak
Ciprofloxacin	5	26.00	Strong
DMSO	5	0.00	Weak

EO: Essential oil, N: Nutmeg, and L: Lemongrass

At a concentration of 6.25%, all treatments showed a significant decrease in the diameter of the clear zone, with the N(2):L(1) ratio reaching only 3.00 mm at 48 hours. This decrease may be due to the volatile nature of essential oils and their easy degradation, especially over long incubation periods, thus reducing their effectiveness in damaging bacterial cell walls [24].

Essential oils of nutmeg and lemongrass exhibit bacteriostatic properties, with the primary mechanism being membrane damage, which leads to changes in permeability and the release of ions and important metabolites [26, 27]. The α-pinene compound found in both essential oils disrupts the membrane's lipid structure, thereby increasing permeability and facilitating the entry of other active compounds [24]. β- Myrsene from nutmeg essential oil, as a non-polar monoterpene, enhances this effect by interacting directly with the membrane's lipid layer, making the membrane more permeable [25].

Citral, present in lemongrass essential oil, acts as a reactive aldehyde that can interact with membrane proteins and lipids, thereby inhibiting membrane enzyme activity and reducing bacterial cell metabolism [28].The different chemical interactions of α-pinene, β-myrcene, and citral produce a synergistic effect that accelerates bacterial membrane damage [23]. The antibacterial activity of the N(2):L(1) combination is highest against *S aureus* because the gram-positive structure lacks an outer membrane, allowing hydrophobic compounds to more easily enter the cell [25, 29]. In contrast, *E. coli* has a lipopolysaccharide layer that inhibits the diffusion of hydrophobic compounds, resulting in a smaller inhibition zone [29, 30].

4. Conclusion

The oil contents of nutmeg and lemongrass essential oils were 0.24% and 0.09%, respectively, and the main components of nutmeg and lemongrass oils were α- pinene. The total phenol content of nutmeg essential oil was 54.524 mg GAE/g, and the total phenol content of lemongrass essential oil was 27.010 mg GAE/g. The antioxidant activity of nutmeg essential oil was stronger than that of lemongrass essential oil and the combination of both essential oils. The combination of N(2):L(1) essential oils exhibited better antioxidant activity than the other essential oil combination ratios. The antibacterial activity of nutmeg essential oil was better than that of lemongrass essential oil. The combination of the N(2):L(1) ratio exhibited better antibacterial activity than the other combination ratios or both.

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