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Chemical Composition and Antibacterial Activity of Plant Essential Oils against Human Pathogenic Bacteria

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Abstract

In this study, the chemical composition and antibacterial properties of plant essential oils (*Citrus hystrix* leaves, *Citrus maxima* peels, *Cymbopogon citratus* (DC) Stapf leaves, *Illicium verum* Hooker seeds, and *Syzygium aromaticum* L. flower buds) were evaluated against human pathogenic bacteria. GC-MS was used to determine the composition of the essential oils, which were produced using hydrodistillation. The main constituents of *Citrus hystrix* and *Citrus maxima* essential oils were citronellal, while *Cymbopogon citratus* (DC) Stapf, *Illicium verum* Hooker, and *Syzygium aromaticum* L. essential oils were geraniol, anethole, and eugenol, respectively. Based on the results of the disc diffusion method, the essential oil of *Syzygium aromaticum* L. inhibited all three bacterial strains (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Staphylococcus aureus* ATCC 25923) with MICs of 0.234 to 0.938 μg/μl and MBCs of 0.469 to 1.875 μg/μl, which is less than gentamicin (MICs and MBCs of 0.117-0.938 μg/μl). *Syzygium aromaticum* L. essential oil exhibited the lowest MIC against *E. coli* ATCC 25922 (0.234 μg/μl), while *Cymbopogon citratus* essential oil showed the lowest MIC against *S. aureus* ATCC 25923 (0.117 μg/μl), the same MIC as gentamicin. All essential oils exhibited a bactericidal effect with an MBC/MIC ratio ranging from 1.0 to 2.0.

Keywords: antibacterial activity, essential oil, GC-MS analysis

Introduction

Staphylococcus aureus, *Pseudomonas aeruginosa*, and *Escherichia coli* are important human pathogens that cause a wide range of illnesses and dramatically raise morbidity and mortality associated with healthcare. These bacteria continue to be a problem for healthcare systems around the world, especially as multidrug-resistant types become more common[1,2].

E. coli is typically harmless inhabitants of our gut, but certain strains, like O157:H7, can cause severe gastrointestinal issues with bloody diarrhea due to toxins, primarily spread through contaminated food/water or poor hygiene[3]. *P. aeruginosa* is an environmental bacterium that primarily infects immunocompromised individuals, causing various infections such as pneumonia, urinary tract infections, and wound infections, which are often transmitted through contact with contaminated surfaces or medical equipment[4]. While *S. aureus* commonly resides on human skin and nostrils, causing a range of infections from common skin boils and impetigo to more serious conditions like food poisoning, pneumonia, or even life-threatening sepsis, it is primarily transmitted through direct contact or contaminated objects[5].

Antimicrobial resistance is a severe public health crisis[6,7], characterized by complex resistance mechanisms such as extended-spectrum beta-lactamase production in *E. coli*, multidrug resistance in *P. aeruginosa*, and methicillin resistance in *S. aureus*. In response to this crisis, scientific interest in natural compounds, including essential oils, as potential antimicrobial agents have significantly surged. Essential oils, which are volatile aromatic compounds extracted from plants, are particularly captivating due to their complex chemical compositions and established *in vitro* antimicrobial activity against various bacterial pathogens, including *E. coli*, *P. aeruginosa*, and *S. aureus*[8,9]. Their mechanisms of action include disrupting bacterial cell membranes, inhibiting enzyme activity, interfering with metabolic processes, and preventing biofilm formation[10]. This multi-target approach may make it harder for bacteria to develop resistance compared to single-target antibiotics[11]. Moreover, most essential oils are categorized as Generally Recognized as Safe (GRAS) by the United States Food and Drug Administration[12]. Therefore, this study aimed to determine the inhibitory effect of essential oils from five plants (*Citrus hystrix*, *Citrus maxima*, *Cymbopogon citratus* (DC) Stapf, *Illicium verum* Hooker, and *Syzygium aromaticum* L.)

against the pathogenic bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Staphylococcus aureus* ATCC 25923) and to evaluate the chemical composition of essential oils using GC-MS.

Material and method

1. Plants material and extraction

Five plants (*Citrus hystrix* leaves, *Citrus maxima* peels, *Cymbopogon citratus* (DC) Stapf leaves, *Illicium verum* Hooker seeds and *Syzygium aromaticum* L. flower buds) were obtained from a local vegetable and fruit market in Bangkok, Thailand, in February, 2024, and were thoroughly washed with distilled water, air-dried at room temperature, and subsequently at 40°C until constant weight. The dried plants were homogenized into a fine powder. Esssential oil were extracted by hydrodistillation using a Clevenger-style apparatus[13] for three hours. After being extracted from 100 g of each plant, the essential oil was dried over anhydrous sodium sulfate and kept in a dark, tightly sealed vial at 4°C until further use. The essential oil yields were calculated in percentage (%v/w) relative to the starting dry plant material in three replications.

2. Preparation of bacterial organisms

The three bacterial strains, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *and S. aureus* ATCC 25923, stored at -20°C, were subcultured onto Sheep Blood Agar (SBA) . The plates of bacterial strains were then incubated at 35°C for 24 hours. Subsequently, the bacterial cultures were transferred to Tryptic Soy Broth (TSB) and incubated at 35°C for 24 hours.

3. Disc diffusion method

The efficacy of essential oils in inhibiting bacterial growth was assessed using the disc diffusion method. Bacterial suspensions were prepared by culturing from SBA and adjusted to a turbidity equivalent to a 0.5 McFarland standard (approximately 1.5×10^8 CFU/ml) using sterile 0.85% saline solution. A sterile cotton swab was dipped into the bacterial suspension, and excess liquid was removed by pressing the swab. The bacterial suspension was then evenly streaked across the surface of Mueller-Hinton Agar (MHA) plates using a three plane streaking technique, with each plane at a 60-degree angle to ensure uniform distribution[14]. Sterile paper discs (6 mm diameter) were placed onto the inoculated MHA plates, and 3 µl of the essential oil being studied was dispensed onto each disc using an automatic pipette. For controls, a positive control used a 10 µg gentamicin antibiotic disc, while a negative control consisted of an untreated disc.

The inoculated plates were then incubated at 35°C for 24 hours. After incubation, the diameters of the clear zones (inhibitory zones) around each disc were measured using a vernier caliper in millimeters. The experiment was performed in triplicate, and the results were reported as the mean ± standard deviation. The inhibition zone diameter (IZD) of essential oil was measured and interpreted using the following criteria: no activity, IZD = 6 mm; weak activity, 6 mm < IZD < 10 mm; moderate activity, 10 mm < IZD < 20 mm; and strong activity, IZD > 20 mm.

4. Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) by micro-broth dilution method

The MIC was determined using the micro-broth dilution method in a 96-well microtiter plate, following the Clinical and Laboratory Standards Institute (CLSI) guidelines[15] with few modifications. Serial two-fold dilutions of the essential oils, dissolved in 95% absolute ethanol, were prepared in Mueller-Hinton Broth (MHB) to achieve the final concentrations ranging from 0.029 to 7.500 µg/ µl in each well of the 96-well plate. The three bacterial strains were prepared by fresh sub-culture and diluted to a turbidity equivalent to a 0.5 McFarland standard (1.5 x 10^8 CFU/ml), and 20 µl of each suspension was added to each well. Positive controls contained only the bacterial test strains in MHB, while solvent controls included bacterial test strains in MHB with 2% ethanol. Wells with antibiotic (gentamicin) for comparison contained concentrations ranging from 0.029 to 3.750 µg/µl. Additionally, wells containing only MHB were included as negative controls. Each 96-well plate was shaken horizontally and then incubated at 35°C for 24 hours. The results were recorded, and the MIC was determined as the lowest concentration of essential oil at which no visible bacterial growth was observed, indicated by the absence of turbidity. The experiment was performed in triplicate.

Samples from the MIC testing, which showed inhibition of bacterial growth, were streaked onto Sheep Blood Agar (SBA) plates using the streak plate technique. The plates were then incubated at 35°C for 24 hours. The results were recorded. If the essential oil concentration was bactericidal (able to kill bacteria) , no bacterial growth would be observed on the agar plate. The concentration at which no growth was observed was reported as the MBC. The estimation of the MBC/MIC ratio describes the bactericidal effect (MBC/MIC < 4) or bacteriostatic effect (MBC/MIC ≥ 4) of the test essential oil. Each experiment was repeated three times.

5. GC-MS analysis of essential oil

GC-MS was used to establish the essential oil's composition. An Agilent 6890 gas chromatograph operating in electron impact mode (70 eV) was utilized for this investigation. It was connected to an Agilent 5973 mass selective detector and has a fused silica capillary column (HP-5MS; 30.0 m x 0.25 mm i.d. and 0.25 m film thickness). The carrier gas, helium, flowed at a rate of 1.0 ml/min. The oven temperature was set to rise from 100°C at a rate of 3°C/min to 188°C, then to 280°C at a rate of 20°C/min, with a final hold period of three minutes. The temperatures of the injector and detector were kept at 280°C. The ionization source temperature was set to 200°C, and chromatograms were screened in scan mode from m/z 50 to 500 at a rate of 3.25 scan/s. In split mode, 0.2 µl of diluted samples were injected at a ratio of 1: 50. By comparing their mass spectra with those from the MS database (National Institute of Standards and Technology, NIST 98 and Wiley 7n.1 Libraries), the components were identified. The relative percentages of essential oil constituents were expressed as a percentage by peak area normalization.

6. Statistical analysis

An analysis of variance (ANOVA) was used to determine statistical significance based on two factors: (1) essential oil type and (2) essential oil concentration. The three replicates' means plus standard deviations were used to express the data using SPSS for Windows. Tukey's post hoc test, which uses multiple comparison tests to identify significant differences between mean values, was used to conclude that a difference of $p < 0.05$ was significant.

Results

1. Yield of essential oil

By hydrodistillation, five plants with varying amounts of essential oil were identified from 100 g of dried plants. Essential oil yields on dry weight ranged from 0.25% to 4.50% (v/w). *Cymbopogon citratus* (DC) Stapf produced the highest yield (4.50±0.00%), followed by *Syzygium aromaticum* L. (4.13±0.15%), *Citrus hystrix* (1.82±0.05%), *Illicium verum* Hooker (0.80±0.00%), and *Citrus maxima* (0.25±0.05%).

2. GC-MS analysis of plant essential oils

The chemical composition of five plant essential oils was analyzed by GC-MS. Table 1 shows the total essential oil compositions and the main constituents with concentrations more than 10%. A total of 10 compounds were identified in *Citrus hystrix* essential oil, with citronellal (84.22%) being the main compound. *Citrus maxima* essential oil contained citronellal (15.86%) and germacrene-D (13.92%) as its main compounds. Twenty-five compounds were identified in the *Cymbopogon citratus*

(DC.) Stapf essential oil, including geraniol (37.29%) and neral (24.63%) as the main compounds. Trans-anethole (92.65%) was the main compound in *Illicium verum* Hooker essential oil. For *Syzyglum aromaticum* L. essential oil, the main compounds were eugenol (74.64%), eugenol acetate (13.18%), and trans-caryophyllene (10.64%).

Table 1. The total composition and major components of essential oils

Essential oil	Total Compound	Main Composition[a]
Citrus hystrix	10	Citronellal (84.22%)
Citrus maxima	10	Citronellal (15.86%), Germacrene-D (13.92%)
Cymbopogon citratus (DC.) Stapf	25	Geraniol (37.29%), Neral (24.63%), Selina-6-en-4-ol (21.86%)
Illicium verum Hooker	12	Trans-anethole (92.65%)
Syzyglum aromaticum L.	9	Eugenol (74.64%), Eugenol acetate (13.18%), Trans-Caryophyllene (10.64%)

[a] the main composition found are more than 10%

3. Disc diffusion method

The antimicrobial activity of five essential oils was evaluated against three bacterial strains: *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, and *S. aureus* ATCC 25923, using the disc diffusion method. Table 2 presents the inhibition zone diameters (IZD) in millimeters (mm) with standard deviations (SD). For the disc diffusion method in this study, *Syzygium aromaticum* L. essential oil showed the broadest inhibition of activity, inhibiting all three bacterial strains. The highest IZD for this oil was significantly ($p < 0.05$) inhibited against *E. coli* ATCC 25922 (10.6±0.13 mm) with moderate activity. It also showed moderate activity against *S. aureus* ATCC 25923 (11.5±0.05 mm) and weak activity inhibition against *P. aeruginosa* ATCC 27853 (6.25±0.05 mm). *Cymbopogon citratus* (DC) Stapf and *Citrus hystrix* essential oils showed weak inhibition against *E. coli* ATCC 25922 (9.55±0.20 mm and 9.37±0.19 mm, respectively) and moderate inhibition against *S. aureus* ATCC 25923 (14.37±0.08 mm and 12.43±0.12 mm, respectively). *Citrus maxima*

and *Illicium verum* Hooker essential oils displayed weak activity against *E. coli* ATCC 25922 and *S. aureus*, ATCC 25923, with lower IZD values. *P. aeruginosa* ATCC 27853 exhibited significant resistance to all tested essential oils except for *Syzygium aromaticum* L. essential oil, which showing some inhibition, with weak activity (6. 25±0. 05 mm) . Gentamicin, as a standard antibiotic, exhibited significantly ($p < 0.05$) higher activity than the essential oils. The results indicate that *Syzygium aromaticum* L. , *Cymbopogon citratus* (DC) Stapf, and *Citrus hystrix* essential oils possess promising antimicrobial properties. The broad-spectrum activity of *Syzygium aromaticum* L. , especially against *P. aeruginosa* ATCC 27853.

Table 2. Inhibition zone diameters (IZD) of essential oils.

Essential oil	Inhibition zone diameter (IZD; mm ± SD)		
	Escherichia coli ATCC 25922	*Pseudomonas aeruginosa* ATCC 27853	*Staphylococcus aureus* ATCC 25923
Citrus hystrix	9.37±0.19	NI	12.43±0.12
Citrus maxima	6.22±0.10	NI	8.02±0.13
Cymbopogon citratus (DC.)Stapf	9.55±0.20	NI	14.37±0.08
Illicium verum Hooker	7.07±0.03	NI	6.33 ± 0.03
Syzygium aromaticum L.	10.6 ±0.13	6.25 ± 0.05	11.5 ± 0.05
Gentamicin (10µg)	19.25±0.13	22.23±0.38	21.39±0.30
Negative control	NI	NI	NI

NI = No Inhibition zone

4. Determining the MIC and MBC of the essential oils by micro-broth dilution method

The antibacterial activity of five essential oils was quantified in terms of MIC and MBC values. The results were shown in Table 3. Table 3 presents the MIC and MBC that were obtained using the micro-broth dilution method in order to confirm and quantify its antibacterial potency. The more sensitive *E. coli* ATCC 25922 had an MIC between 0. 234 and 3. 750 µg/ µl. The resistance of *P. aeruginosa* ATCC 27853 was higher in the range of 0. 938 to 7.500 µg/µl compared to *E. coli* ATCC 25922

and *S. aureus* ATCC 25923. *Syzygium aromaticum* L. essential oil exhibited the lowest MIC against *E. coli* ATCC 25922 (0. 234 μg/ μl) , while *Cymbopogon citratus* (DC.) Stapf essential oil showed the lowest MIC against *S. aureus* ATCC 25923 (0. 117 μg/ μl) , the same MIC as gentamicin. *Illicium verum* Hooker essential oil showed the highest MIC and MBC values across all tested bacteria, indicating comparatively lower activity. While gentamicin generally exhibited lower MIC and MBC values, the essential oils demonstrated notable antimicrobial activity, particularly *Syzygium aromaticum* L. and *Cymbopogon citratus* (DC) Stapf essential oil.

Table 3. Antibacterial activity of plant essential oils by micro-broth dilution.

Essential oil	*Escherichia coli* ATCC 25922		*Pseudomonas aeruginosa* ATCC 27853		*Staphylococcus aureus* ATCC 25923	
	MIC (μg/μl)	MBC (μg/μl)	MIC (μg/μl)	MBC (μg/μl)	MIC (μg/μl)	MBC (μg/μl)
Citrus hystrix	0.469b	0.938b	3.750a	3.750a	0.938a	1.875a
Citrus maxima	1.875a	1.875a	3.750a	3.750a	0.938a	0.938b
Cymbopogon citratus (DC.)Stapf	0.469b	0.469c	7.500b	7.500b	0.117b	0.234c
Illicium verum Hooker	3.750a	3.750a	7.500b	7.500b	7.500a	7.500a
Syzygium aromaticum L.	0.234c	0.469c	0.938c	1.875c	0.938a	1.875a
Gentamicin	0.234c	0.234d	0.938c	0.938c	0.117b	0.117d
Negative control	-	-	-	-	-	-

Differences in superscript letters in the column indicate significant difference ($p<0.05$).

Discussion

Previous studies have shown the different yields and chemical compositions of essential oils produced from various plant materials. In this study, *Citrus maxima* essential oil was found to have citronella (15.86%) as the main compound. This result is in contrast to other research that has been published, which showed that D-limonene (86.4%) was the main component in 0.31% of the yield[16]. However, the results from the other essential oil that was examined in this study were similar to previous research.

Citrus hystrix essential oil yield was about 1%, with citronellal of 85.4%[17]. *Cymbopogon citratus* essential oil yields was around 1.4%, and is characterized by geraniol

(37.1%)[18]. *Illicium verum* seeds offer a substantial essential oil yield of 4.13%, with trans-anethole (83.46%) being the main constituent[19]. *Syzygium aromaticum* generally yields a high amount of essential oil, averaging 11.6% (w/w)[20]. Its main compound is eugenol (78.72%), followed by β-caryophyllene (8.82%) and eugenyl acetate (8.74%). It's important to note that variations in the chemical composition reported in different literature can be attributed to numerous factors. These include genetic variety, geographic location, agronomic and environmental conditions, and the extraction methods used[21].

Previous research on the antimicrobial activity of essential oils from *Citrus hystrix* leaves, *Citrus maxima* peels, *Cymbopogon citratus* leaves, *Illicium verum* seeds, and *Syzygium aromaticum* flower buds frequently reports their MIC and MBC against various pathogenic microorganisms. These values provide insights into their potential as antimicrobial agents, while the MBC/MIC ratio helps classify their mode of action *Syzygium aromaticum* flower bud essential oil is widely recognized as one of the most potent natural antimicrobial agents among essential oils, primarily due to its exceptionally high eugenol content. It exhibitsvery low MIC and MBC values, demonstrating strong bactericidal activities. Specifically, MICs ranging from 1.36 to 2.72 mg/ml and MBCs varying from 5.45 to 10.9 mg/ml have been reported against enteropathogenic bacteria[20]. Furthermore, an MIC of 0.23 mg/mL against *E. coli* ATCC 35218[22] and 0.2 mg/ml[23] against *E. coli* ATCC 35218, with an MBC of 3.12 mg/ml[23], have also been noted.

Cymbopogon citratus essential oil is widely known for its broad-spectrum antimicrobial activity, include serveral multidrug-resistant Gram-negative bacterial strains such as *P. aeruginosa*, *E. coli*, *Enterobacter cloaceae*, *Morganella morganii*, *Proteus mirabilis* or *Burkholderia cepacian*[24]. Antibacterial activity of the essential oil from *Cymbopogon citratus* reported its potent activity against Gram-positive bacteria compared to Gram-negative bacteria[25]. Essential oil from *Cymbopogon citratus* showed no inhibition activity against *P. aeruginosa* CRBIP 19.249 and more than 80 mg/ml of MIC. MIC of *S aurus* ATCC 9144 and *E.coli* CIP 105182 showed 2.5 and 10 mg/ml, respectivelty, as a bactericidal effect[26]. *P. aeruginosa* demonstrated the highest resistance *via* disc diffusion and also exhibited the highest MIC and MBC values when compared to other tested microorganisms[26]. Geraniol is the main

compound of *Cymbopogon citra*tus essential oil, causes perturbation in the lipid fraction of the plasma membrane of the microorganism, which results in alterations of the permeability of the membrane and consequently in cellular death by plasmolysis[27].

Trans-anethole dominates the essential oil of *Illicium verum*, which has strong antibacterial activity, particularly against bacteria and fungi. However, it is generally expected that the sensitivity of Gram-positive bacteria is higher than that of Gram-negative bacteria. Gram-positive bacteria (*S. aureus* and *S. epidermidis*) are sensitive compared to Gram-negative bacteria (*Enterobacter cloacae*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, and *E. coli*)[19]. The essential oil of *Illicium verum* has shown efficacy against *Salmonella enterica* CECT 443, *Listeria monocytogenes* CECT 933, *S. aureus* ATCC 6538, *B. subtilis* CIP 5265, and *E. coli* ATCC 35218 with an inhibitory zone of more than 9 mm. *Illicium verum* essential oil and trans-anethole both is showed bacteriostatic efficacy against eight tested Gram-positive and Gram-negative food pathogenic bacteria, according to their MIC/MBC ratios[28]. This study showed that the essential oil of *Illicium verum* Hooker had bacterial activity on all test pathogenic bacteria. It was more efficient against the *E. coli* strain, with an inhibitory zone diameter of 7.07 mm and an MIC and MBC of 3.70 μg/μl.

Citrus hystrix essential oil has been shown in experiments to inhibit the growth of bacteria. *P. eruginosa* ATCC 27853 (22.17 mm inhibition diameter), *B. cereus* ATCC 11778 (16.18 mm inhibition diameter), and *S. aureus* ATCC 25923 (22 mm inhibition diameter) had the strongest inhibitory effects. Additionally, the essential oil demonstrated bactericidal activity against a range of respiratory and resistance to multiple drugs. Gram-positive bacteria (MIC, 1.3 to 5.3 mg/ml) were more susceptible to the effects of *Citrus hystrix* essential oil than Gram-negative bacteria (MIC, 1.2 to 16.0 mg/ml). Nevertheless, neither the broth microdilution nor the agar disk showed any action against *P. aeruginosa*[29]. This research study indicates that the essential oils examined are more effective at inhibiting *E. coli*. ATTC 25922 and *S. aureus* ATCC 25923 than *P. aeruginosa* ATCC 27853. This observation aligns consistently with other scientific investigations, which frequently highlight *P. aeruginosa* as a particularly challenging Gram-negative bacterium, notorious for its inherent resistance to a wide spectrum of antibiotics, various antimicrobials, and even essential oils[10].

The essential oil of *Citrus maxima* exhibited antibacterial activity against *E. coli*, *P. aeruginosa*, *B. subtilis*, *S. aureus*, *B. licheniformis*, and *B. altitudinis*[16]. The antibacterial activity of *Citrus maxima* essential oil was against Gram-positive bacteria (*S. aureus* ATCC 25923 and *S. epidermidis* ATCC 12228) by inhibition diameter of 19.3 mm and Gram-negative bacteria (*E. coli* ATCC 25922 and *S. dysenteriae* ATCC 13313) by inhibition diameter of 8.3 and 11.7 mm, respectively. While the inhibition zones of *Citrus maxima* essential oil against *S. aureus* ATCC 25923, *S. aureus* ATCC 43300, and MRSA isolates were 10.0, 9.0, and 9.9 mm, respectively, indicating a weak activity. The MBC/MIC value against *S. aureus* ATCC 43300, and *S. aureus* ATCC 25923 was 0.8 and 1.3, respectively, as a bactericidal effect[30].

Essential oils contain numerous active components, including citronellal, geraniol, anethole, and eugenol. It is highly probable that their mode of action involves multiple targets within the bacterial cell[31,32]. A key aspect of their antibacterial mechanism is the hydrophobicity of essential oils. This property allows them to partition into the bacterial cell membrane and mitochondria, rendering them permeable and leading to leakage of cell contents[33,34].

Citronella and geraniol as major components in these essential oils, a characteristic supported by previous research on their antimicrobial properties[32,34]. Anethole demonstrates inhibitory effects against various bacteria, including *E. coli*, *P.aeruginosa*, and *S. aureus*, through multiple mechanisms such as disrupting bacterial cell membranes and inhibiting biofilm formation, as demonstrated in previous research[35,36]. Eugenol effectively inhibits both *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 through a critical mechanism to damages the structure and disrupts the vital functions of the bacterial outer cell membrane. In the case of *E. coli* ATCC 25922, this leads to the leakage of essential intracellular components, ultimately causing cell death[31]. For *S. aureus* ATCC 25923, eugenol not only inhibits growth but also prevents the formation of biofilms, which are protective layers that make bacteria more resistant to treatment[37]. Furthermore, the significance of eugenol extends to its reported ability to inhibit the growth and biofilm formation of *P. aeruginosa*. This is a crucial finding because biofilm formation is a primary mechanism by which *P. aeruginosa* establishes chronic infections and develops its formidable drug resistance. Biofilms act as a physical barrier, reducing antibiotic penetration and promoting the survival of

resistant bacterial strains within the protective matrix[38]. The ability of eugenol to disrupt this mechanism suggests a potential avenue for combating this highly problematic pathogen[39]. Despite the inherent resistance of *P. aeruginosa* ATCC 27853, it is important to note that the experiments in this study found that the essential oils examined, including those less effective against *P. aeruginosa* ATCC 27853 than against *E. coli* ATCC 25922 and *S. aureus* ATCC 25923. In this study, all essential oils exhibited a bactericidal effect against *E.coli* ATCC 25922, *P. aeruginosa* ATCC 27853 and *S. aureus* ATCC 25923, with an MBC/MIC ratio ranging from 1.0 to 2.0.

Conclusion

This research provides valuable quantitative data for *Syzygium aromaticum* L. and *Cymbopogon citratus* essential oils, which possess strong antimicrobial potential. *P. aeruginosa* ATCC 27853 of resistance is consistent with its known resilience. Further research should explore these essential oils mechanisms and applications as natural antimicrobials, particularly against resistant bacteria.

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