Exploring Antimicrobial Activities of Clove, Tea Tree, Eucalyptus, and Lesser Galangal Essential Oils Against Some Pathogenic Microbes and Their Antioxidant Activities

Dararat Samretwit, PhD[1], Piriyaporn Chongtrakool, PhD[2], Chanwit Tribuddharat, MD[2], Ruxjinda Wattanalai, PhD[1], Ampun Chaikulsareewath, MSc[3], Piyanoot Noiduang, MSc[3], Payud Wongwinyuchon[4], Pawish Wongwinyuchon[4], Tronton Lerttamrab[5], Tanate Suttisaewan, MD[6], Somporn Srifuengfung, PhD[1]

[1] Faculty of Pharmacy, Siam University, Bangkok, Thailand; [2] Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand; [3] Faculty of Science, Siam University, Bangkok, Thailand; [4] High school student, King Mongkut's International Demonstration School, Bangkok, Thailand; [5] High school student, Concordian International School, Samut Prakan, Thailand; [6] CGH Saimai Hospital, Bangkok, Thailand

Abstract

Background: Many essential oils have antimicrobial and antioxidant activities with potential use in medicine. In Thailand, due to a lack of studies on clove, tea tree, lesser galangal, and eucalyptus oils.

Objective: To determine the inhibitory effect against microbial pathogens, antioxidant activity, and evaluate the chemical components of clove, tea tree, lesser galangal, and eucalyptus oils.

Materials and Methods: Antimicrobial activities which were collected of 46 clinical isolates of methicillin-sensitive and resistant *Staphylococcus aureus*, *Acinetobacter baumannii*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Candida albicans* were determined by the disk diffusion method. Antioxidant activity was performed by DPPH (1,1-diphenyl-2-picryl-hydrazyl) assay.

Results: Clove oil had the most antimicrobial activity, followed by tea tree oil, lesser galangal, and eucalyptus oils, respectively. By DPPH assay, clove oil had the most antioxidant activity, followed by tea tree and lesser galangal oils, respectively. The IC_{50} of clove, tea tree, and lesser galangal oils were 0.01±0.00, 15.48±0.00, and 34.27±0.21 mg/mL, respectively. Antioxidant activity of eucalyptus oil was not found. Eugenol, alpha-terpineol, and terpinen-4-ol are components abundant in clove, tea tree, and lesser galangal oils, respectively. Antimicrobial activities of these components are indifferent (by mean ± SD) because inhibition zone diameters were 14.40±5.16, 14.02±5.32, and 12.71±5.59 mm, respectively.

Conclusion: The results suggested that clove, tea tree, lesser galangal oils, and their components may benefit new treatment or prevention against microbial infections. For antioxidant activity, eugenol demonstrated the highest inhibition. Following eugenol, alpha-terpineol, a chemical component in tea tree oil, showed significant antioxidant property, and terpinen-4-ol, found in lesser galangal oil, exhibited the least antioxidant capability.

Keywords: Clove oil; Tea tree oil; Lesser galangal oil; Eucalyptus oil; Essential oil

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Essential oils (EO) are fragrant extracts obtained from various plants. Their composition varies depending on the plant species from which they are extracted. EO can exhibit antimicrobial and antioxidant activities, making it a potential natural alternative

Correspondence to:

Srifuengfung S.

Faculty of Pharmacy, Siam University, 38 Petchkasem Road, Phasicharoen District, Bangkok 10160, Thailand.

Phone & Fax: +66-2-8686665

Email: somporn.sri@mahidol.ac.th

ORCID: 0009-0000-0608-6747

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What is already known about this topic?

According to the previous studies, both antimicrobial and antioxidant activities of EO were reported in clove, lemon, orange, cinnamon, coriander, eucalyptus, rosemary, tea tree, thyme, and parsley. Antibacterial activity was reported in Citrus hystrix (makrut lime) EO in Thailand. Therefore, the study of antimicrobial activity of EO in clinical microbial isolates obtained from Thai patients and antioxidant activity should be investigated.

What does this study add?

Clove oil had the most antimicrobial activity with an inhibition zone diameter (17.04±5.01 mm), followed by TTO (12.19±3.99 mm), lesser galangal (11.63±4.16 mm), and eucalyptus oils (7.54±1.49 mm). Eugenol, alpha-terpineol, and terpinen-4-ol are chemical components abundant in clove, tea tree, and lesser galangal oils, respectively. Antimicrobial activities of these components are indifferent (by mean±SD). For antioxidant activity, clove oil had the most antioxidant activity, followed by tea tree and lesser galangal oils, respectively. Antioxidant activity of eucalyptus oil was not found. Following eugenol (a chemical component in clove oil), alpha-terpineol, a chemical component in TTO, showed significant antioxidant property, and terpinen-4-ol, found in lesser galangal oil, exhibited the least antioxidant capability.

for medicinal applications[1]. The main bioactive compounds of EO are terpenes and terpenoids, which are responsible for the biological activities[1]. The excessive and repeated use of the same drugs used in modern medicine leads to the development of drug-resistant microbes, which make infections difficult or impossible to treat. Using antimicrobial agents may be limited due to the potential for toxicity and allergic reactions[2]. It is interesting in the search for natural antimicrobial alternatives, such as EO, to avoid using chemical agents. EO gained increasing attention due to its therapeutic benefits and generally being considered safe according to the United States Food and Drug Administration[3]. The antimicrobial activity and the minimum inhibitory concentration of EO are assayed by disk-diffusion and broth microdilution methods, respectively. The high phenolic-group-containing compound present in EO is well-known for its antimicrobial activity[3]. The antioxidant effect of EO is assayed by three methods: inhibition of free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH), β-linoleic acid, and ferric reducing antioxidant power methods[3]. Generally, antioxidant activity is the result of interaction between chemical components of EO (alcohols, phenols, terpene, and ketone compounds) acting with synergistic or antagonistic interactions among them. There is an important relationship between the chemical composition of EO and its antioxidant activity. It was reported that the antioxidant activity of EO is related to its chemical composition, particularly the presence of compounds containing a hydroxyl function as well as terpene alcohols and phenolic compounds[3]. Herbs can be used in the form of plant extracts or as their active components[4]. The latter will benefit for lesser dosage intake than plant extracts.

EO derived from clove, tea tree, eucalyptus, and lesser galangal (also known as finger root) are commonly found and used throughout Southeast Asia, with each having a variety of traditional applications. Clove oil, rich in the compound eugenol (85% to 95%)[4], exhibiting both excellent antimicrobial and antioxidant activities, was reported in Saudi Arabia[5], Brazil[6], Mexico[4], and Morocco[7]. Tea tree oil (TTO) exhibits both antimicrobial and antioxidant activities in reports from Australia[8], Portugal[9], Slovakia[10], and Argentina[11]. It is a natural oil derived from the leaves of the *Melaleuca alternifolia* tree, known for its complex composition of approximately 100 compounds, including terpenes and sesquiterpenes like terpinen-4-ol, alpha-terpinene, gamma-terpinene, 1,8-cineole, and terpinolene. With regard to TTO's biological activity, its antimicrobial properties are primarily attributed to terpinen-4-ol, a major component of the oil[8,11]. Furthermore, according to the International Standard Organization (ISO 4730: 2017), the main component of TTO, derived from the Australian native tree *M. alternifolia*, is terpinen-4-ol, and it must be present in a proportion of not less than 40%[11]. The eucalyptus oil exhibits both antimicrobial and antioxidant activities in reports from Ethiopia[12] and Brazil[13]. It contains many compounds[14,15]. The importance of eucalyptus oil in medicine largely depends on the content of one specific constituent, namely, 1,8-cineole (cineole or eucalyptol). The major composition of eucalyptus oil is 1,8-cineole and constitutes more than 70% (v/v) of the total oil, and the other major constituents co-occurring with eucalyptol are limonene and α-terpineol[14]. Lesser galangal (*Alpinia officinarum*), native to China, is an important traditional Chinese medicine for both medicine and food. Up to now, 337 chemical compounds have been extracted, including EO[16]. Lesser galangal is used in many Thai foods for the benefit of a nice aroma and spicy taste. However, there is limited research on the EO regarding its antimicrobial and antioxidant activities. Lesser galangal oil contains a variety of bioactive constituents, with 1,8-cineol/eucalyptol and α-terpineol being among the major ones[17]. This study aimed to determine antimicrobial and antioxidant activities of clove, tea tree, eucalyptus, and lesser galangal oils, and the specific chemical components contributing to these effects.

Materials and Methods

Essential Oils and Chemical Components

Ethical approval for this study was obtained from the Human Research Committee of Siam University with reference code SIAMPY-IRB 2023/006. Clove oil was identified by the product code 171102-20006, with a reference number T230207/2023 and batch number 23031129-1. The oil has a specific gravity of 1.0505 and a refractive index of 1.5360 at 20°C.

TTO was identified by the product code 171102-80012, with a reference number 230404/2023 and batch number 2310009-1. The oil has a specific gravity of 0.8989 and a refractive index of 1.4775 at 20°C.

Lesser galangal oil was identified by the product code 171102-40017, with a reference number RES230021/2023 and batch number 2310009-3. The oil has a specific gravity of 0.8842 and a refractive index of 1.4806 at 20°C.

The eucalyptus oil was identified by the product code: 171102-20014, with a reference number

R230308/2023 and batch number 2310009-2. The oil has a specific gravity of 0.9106 and a refractive index of 1.4595 at 20°C. All EO were obtained from Thai-China Flavours and Fragrances Industry Co., Ltd., and were prepared by steam distillation.

Purified chemical components, such as eugenol, were obtained using a United States Pharmacopeia reference standard (eugenol USP grade). Eugenol (Product Code CA0502-A-GM100-p) was purchased from Chemipan Corporation Co., Ltd. Alpha-terpineol (Product Code: 432628, Batch No. SHBP7968), with a gas chromatography purity (sum of isomers) of 99.6% and an infrared spectrum conforming to standards, was obtained from Sigma-Aldrich, USA. Terpinen-4-ol (Catalog No. 27043, Lot No. A0451606), with a gas chromatography purity (sum of isomers) of 99.5%, was obtained from Thermo Fisher Scientific Co., USA. All EO and purified chemical components were stored in dark bottles at 4°C until use.

Microbial Isolates

A total of 46 clinical isolates used in the antimicrobial activity testing were methicillin-sensitive *Staphylococcus aureus* (MSSA, 8 isolates), methicillin-resistant *S. aureus* (MRSA, 8 isolates), *Acinetobacter baumannii* (12 isolates), *Escherichia coli* (6 isolates), *Pseudomonas aeruginosa* (6 isolates), and *Candida albicans* (6 isolates) (Figure 1). One isolate was collected from each patient to prevent the acquisition of duplicate microbial isolates with the same antimicrobial activities at Siriraj Hospital (the largest university tertiary care center in Thailand) over 6 months (January to June 2024). There were various clinical specimens (sputum, pus, urine, or blood) whose processing, microbial isolation, and identification were performed according to standard microbiological methods[18]. Sputum was considered acceptable for culture if it contained more than 25 polymorphonuclear cells and less than 25 epithelial cells per low-powered field.

Antimicrobial Activity Testing

We carried out testing following the disk diffusion method[19]. A sterile Whatman disc (6 mm) saturated with 10 µL of EO[3,7,20] was put on a lawn of a bacterial inoculum, which has a turbidity in 1% (w/v) tryptone water equated to a McFarland No. 0.5 standard (approximately 10^8 CFU/mL). Each experiment was conducted three times (in triplicate), and the value presented in the table for each data point is the average (mean) of those three measurements. In a microbiology experiment, the final analysis of inhibition zone sizes

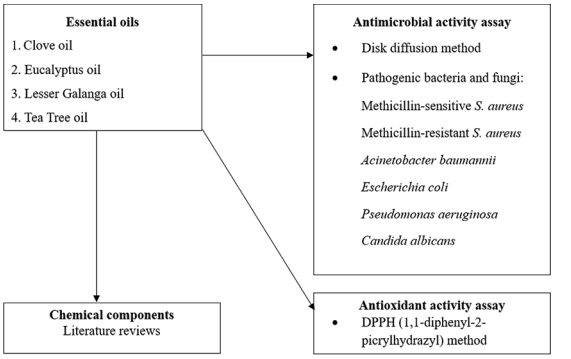

Figure 1. Flow chart of the study.

for all tested microbes was presented as the average (mean) value, along with a measure of its variability (standard deviation). *S. aureus* ATCC 25923 (a representative of gram-positive bacteria) and *E. coli* ATCC 25922 (a representative of gram-negative bacteria) were used as quality controls in antimicrobial activity testing according to Clinical and Laboratory Standards Institute guidelines[19]. For *Candida* testing, *C. parapsilosis* ATCC22019 was used as quality control strain, whereas Mueller-Hinton agar with 2% glucose with 0.5 ug/mL methylene blue dye medium, pH 7.2-7.4, was used as susceptibility testing medium. The inoculum size and other processes are the same as those of bacteria[21].

Antioxidant Activity Testing

The antioxidant assay for all samples was carried out by the Center of Analysis for Product Quality (Natural Products Division), Faculty of Pharmacy, Mahidol University (Bangkok, Thailand). The DPPH assay is a method for measuring antioxidant activity by observing the decolorization of the DPPH radical (1,1-diphenyl-2-picrylhydrazyl) solution in the presence of antioxidants[5,7,8]. DPPH is a stable, deep violet free radical that absorbs light at 520 nm. When mixed with an antioxidant, the antioxidant donates a hydrogen atom, reducing DPPH to a stable diamagnetic molecule and causing the solution to lose its violet color. The antioxidant activity was expressed in IC_{50} (50% inhibitory concentration), calculated by linear regression.

The % inhibition for all samples was also carried out by the Center of Analysis for Product Quality (Natural Products Division), Faculty of Pharmacy, Mahidol University (Bangkok, Thailand), accredited under ISO/IEC 17025: 2017. The use of different concentrations of chemical components in EO to study their antioxidant activities was based on the

Table 1. Inhibition zones of clove, eucalyptus, lesser galangal, and tea tree oils

Microbial isolates	Number	Disk diffusion method (mm)			
		Clove oil	Eucalyptus oil	Lesser galangal oil	Tea tree oil
S. aureus	ATCC 25923	18	7	11	9
E. coli	ATCC 25922	17	8	11	17
MSSA clinical isolates (n=8)					
MSSA	1	17	7	13	14
	2	15	8	12	14
	3	14	8	11	11
	4	17	7	11	8
	5	16	8	13	12
	6	13	7	11	10
	7	16	9	11	12
	8	16	7	12	9
MRSA clinical isolates (n=8)					
MRSA	1	18	7	16	14
	2	17	8	11	10
	3	17	7	12	16
	4	20	8	10	12
	5	19	10	15	14
	6	18	8	16	18
	7	18	8	12	12
	8	18	8	13	14
Acinetobacter baumannii clinical isolates (n=12)					
A. baumannii	1	24	7	11	13
	2	25	7	13	14
	3	29	7	14	19
	4	19	7	9	14
	5	15	7	9	11
	6	22	7	9	16
	7	20	7	12	12
Acinetobacter baumannii clinical isolates (cont.)					
A. baumannii	8	16	7	12	12
	9	20	7	28	28
	10	17	7	10	10
	11	17	7	13	13
	12	18	7	12	12
Escherichia coli clinical isolates (n=6)					
E. coli	1	13	12	9	12
	2	16	14	12	19
	3	18	10	9	16
	4	13	8	8	12
	5	19	8	8	14
	6	14	8	8	15
Pseudomonas aeruginosa clinical isolates (n=6)					
P. aeruginosa	1	6	6	6	6
	2	8	7	7	10
	3	7	6	7	7
	4	7	6	7	8
	5	8	7	8	9
	6	8	6	7	7
Candida albicans clinical isolates (n=6)					
C. albicans	1	20	9	15	7
	2	28	6	20	12
	3	24	6	14	8
	4	19	7	11	7
	5	22	6	14	8
	6	22	6	33	8
Mean of the total		17.04	7.54	11.63	12.19
Standard deviation of the total		5.01	1.49	4.16	3.99

MSSA=methicillin-sensitive *Staphylococcus aureus*; MRSA=methicillin-resistant *Staphylococcus aureus*

determination of the specific concentration of each chemical component, which inhibited DPPH by a two-fold serial dilution method.

Data Analysis

Data were entered and analyzed with IBM SPSS Statistics, version 20.0 (IBM Corp., Armonk, NY, USA) for descriptive analysis. Discrete variables were expressed as percentages, mean, standard deviation, and proportion.

Results

The results of antimicrobial activity testing found that by using the mean ± standard deviation (SD) of the total, clove oil exhibited the strongest activity (17.04±5.01 mm), followed by TTO (12.19±3.99 mm) and lesser galangal oil (11.63±4.16 mm), with no significant difference between the latter two. The eucalyptus oil showed very little antimicrobial activity in the present study (7.54±1.49 mm) (Table 1). The good inhibitory effect of clove oil on drug-resistant MRSA, drug-resistant *A. baumannii*, and *C. albicans* was found. However, all *P. aeruginosa* isolates were resistant to all the EO tested.

In the chemical component testing for antimicrobial activity, we selected clove, TTO, and lesser galangal oils for further testing due to their strong combined antimicrobial and antioxidant properties. Eucalyptus oil was excluded because it exhibited minimal antimicrobial activity (Table 1) and lacked significant antioxidant activity. Eugenol, alpha-terpineol, and terpinen-4-ol are chemical components abundant in clove, tea tree, and lesser galangal oils, respectively. Interestingly, all chemical components, such as

Table 2. Inhibition zones of eugenol, alpha-terpineol and terpinen-4-ol

Microbial isolates	Number	Disk diffusion method (mm)		
		Eugenol	Alpha-terpineol	Terpinen-4-ol
S. aureus	ATCC 25923	12	13	8
E. coli	ATCC 25922	13	17	25
MSSA clinical isolates (n=8)				
MSSA	1	12	12	29
	2	12	15	12
	3	13	8	26
	4	12	12	11
	5	14	9	14
	6	12	7	11
	7	12	10	11
	8	10	12	11
MRSA clinical isolates (n=8)				
MRSA	1	12	10	12
	2	12	12	11
	3	11	11	9
	4	12	9	9
	5	13	11	9
	6	12	11	12
	7	14	12	12
	8	10	11	12
Acinetobacter baumannii clinical isolates (n=12)				
A. baumannii	1	19	14	11
	2	14	13	13
	3	15	15	14
	4	14	14	9
	5	14	15	9
	6	14	15	9
	7	20	18	12
Acinetobacter baumannii clinical isolates (cont.)				
A. baumannii	8	15	17	9
	9	30	28	12
	10	24	20	12
	11	20	20	12
	12	19	17	12
Escherichia coli clinical isolates (n=6)				
E. coli	1	17	20	30
	2	14	12	20
	3	14	13	25
	4	9	13	18
	5	15	20	17
	6	15	15	13
Pseudomonas aeruginosa clinical isolates (n=6)				
P. aeruginosa	1	6	7	8
	2	6	6	8
	3	6	7	10
	4	6	8	6
	5	9	6	8
	6	9	7	8
Candida albicans clinical isolates (n=6)				
C. albicans	1	22	24	10
	2	23	22	11
	3	23	25	10
	4	22	18	9
	5	19	20	10
	6	20	22	11
Mean of the total		14.40	14.02	12.71
Standard deviation of the total		5.16	5.32	5.59

MSSA=methicillin-sensitive *Staphylococcus aureus*; MRSA=methicillin-resistant *Staphylococcus aureus*

eugenol, alpha-terpineol, and terpinen-4-ol, exhibited good antimicrobial activity for all microbes tested with no significant difference (by mean±SD) (Table 2).

Table 3 shows that inhibition zones of eugenol, alpha-terpineol, and terpinen-4-ol, with mean and standard deviation of the diameter of the inhibition zone for each microbe; eugenol and alpha-terpineol exhibited similar high antimicrobial activity against a broad range of bacteria. In contrast, terpinen-4-ol was lower in antimicrobial activity against *A. baumannii* and *C. albicans* than eugenol and alpha-terpineol.

Clove oil exhibited the strongest antioxidant activity based on IC_{50} values determined by using the DPPH method. Tea tree and lesser galangal oils follow, while eucalyptus oil demonstrated no significant antioxidant activity in this context (Table 4).

Eugenol, alpha-terpineol, and terpinen-4-ol are chemical components abundant in clove, tea tree, and lesser galangal oils, respectively. In terms of antioxidant activity, eugenol, a chemical component in clove oil, demonstrated the highest inhibition (65.37%). Following eugenol, alpha-terpineol, a chemical component in TTO, showed significant antioxidant property, and terpinen-4-ol, found in lesser galangal oil, exhibited the least antioxidant capability (Table 5).

Discussion

Aromatic plants are commonly used in traditional medicine. In several countries worldwide, especially in rural areas, aromatic plants are used in primary healthcare[12]. The lack of studies on antimicrobial and antioxidant activities in clove, tea tree, lesser galangal, and eucalyptus oils, along with their chemical

Table 3. Inhibition zones of eugenol, alpha-terpineol and terpinen-4-ol for each microbe

Microbiological isolates		Disk diffusion method (mm)		
		Eugenol	Alpha-terpineol	Terpinen-4-ol
S. aureus	ATCC 25923	12	13	8
E. coli	ATCC 25922	13	17	25
MSSA clinical isolates (n=8)				
MSSA	Mean	12.13	10.63	15.63
	SD	1.13	2.62	7.44
MRSA clinical isolates (n=8)				
MRSA	Mean	12.00	10.88	10.75
	SD	1.20	0.99	1.49
Acinetobacter baumannii clinical isolates (n=12)				
A. baumannii	Mean	18.17	17.17	11.17
	SD	4.10	4.11	1.75
Escherichia coli clinical isolates (n=6)				
E. coli	Mean	14.00	15.50	20.50
	SD	2.68	3.62	6.09
Pseudomonas aeruginosa clinical isolates (n=6)				
P. aeruginosa	Mean	7.00	6.83	8.00
	SD	1.55	0.75	1.26
Candida albicans clinical isolates (n=6)				
C. albicans	Mean	21.50	21.83	10.17
	SD	1.64	2.56	0.75

MSSA=methicillin-sensitive *Staphylococcus aureus*; MRSA=methicillin-resistant *Staphylococcus aureus*; SD=standard deviation

Table 4. IC_{50} (50% inhibitory concentration) of clove, tea tree, lesser galangal and eucalyptus oils

Essential oil	IC_{50} (mg/mL)
Clove oil	0.01±0.00 (n=3, % RSD=2.02)
Tea tree oil	15.48±0.00 (n=3, % RSD=1.68)
Lesser galangal oil	34.27±0.21 (n=3, % RSD=0.60)
Eucalyptus oil	No inhibition by DPPH assay

RSD=relative standard deviation

An IC_{50} value quantifies antioxidant activity, representing the concentration of a substance needed to inhibit 50% of a free radical activity in a given assay, such as the DPPH radical scavenging method. A lower IC_{50} value indicates a higher antioxidant capacity because less of the substance is required to achieve 50% inhibition, signifying stronger antioxidant

Table 5. % Inhibition of eugenol, alpha-terpineol and terpinen-4-ol (n=3)

Results	Eugenol	Alpha-terpineol	Terpinen-4-ol
Concentration (µg/mL)	13.34	207,784.17	230,180.83
% Inhibition	65.37±0.97 (% RSD=1.49)	18.30±0.41 (% RSD=2.25)	9.54±0.42 (% RSD=4.44)

RSD=relative standard deviation

components in Thai research, is a knowledge gap. For the effects against microbial pathogens, the present study found that clove oil, TTO, and lesser galangal oil exhibited good antimicrobial effects, similar to other reports[2,6-9,11], while eucalyptus oil showed a weaker effect. The difference in eucalyptus oil's effectiveness might stem from its composition, as it is a diverse genus within the Myrtaceae family, comprising over 900 species and subspecies[12]. Approximately 300 species have volatile oils[15]. This plant originates in Australia and has spread worldwide, including Thailand, because Eucalyptus is easy to cultivate and grows rapidly. The antioxidant properties are influenced by the specific Eucalyptus species, the chemical composition of the oil, and the methods used for testing. This may be due to the chemical composition of EO, which can vary based on factors like origin and extraction methods, significantly influencing their antimicrobial and antioxidant activities, as different compounds contribute to these properties.

In the present study, terpinen-4-ol is lower in antimicrobial activity significantly by considering the mean±SD against *A. baumannii* and *C. albicans* than eugenol, alpha-terpineol (Table 3). Our results support studies[22,23] which showed that eugenol and alpha-terpineol tend to have stronger antimicrobial effects against certain bacteria (like *A. baumannii*) and fungi (like *C. albicans*) compared to terpinen-4-ol. This suggests that eugenol and alpha-terpineol may be more effective in combating specific microbial infections like multiple drug-resistant *A. baumannii* and *C. albicans*. Clove oil's antimicrobial activity is attributed to eugenol, which can inhibit the growth of both gram-positive and gram-negative bacteria, including *C. albicans*.

In the present study, *P. aeruginosa* was resistant to all EO and all chemical components tested, indicating a high degree of antibiotic resistance in this bacterial species. This suggests that finding effective treatments against *P. aeruginosa* infections can be challenging, as the bacteria have evolved mechanisms to withstand various antimicrobial agents. EO, due to its lipophilic nature, readily passes through bacterial cell membranes, disrupting their integrity and leading to cell death. However, the cell membrane composition of gram-positive and gram-negative bacteria impacts the effectiveness of EO, with gram-negative bacteria being generally more resistant due to their outer membrane and lipopolysaccharides[4].

In recent years, due to the fewer side effects than antimicrobial agents, the use of herbal materials instead of synthetic or chemical drugs is increasing. Herbs can be used in the form of plant extracts or as their active components[2], with the former offering a broader spectrum of benefits and the latter providing

a more concentrated effect. For example, eugenol presents higher oxidant activity than clove oil with an IC_{50} of 12.66 and 78.98 µg/mL, respectively[6]. The antioxidant properties of clove oil and eugenol help protect against damage caused by free radicals[7]. A lower IC_{50} indicates greater antioxidant potential[12]. Antioxidants protect cells by neutralizing free radicals, which are unstable molecules that can cause cellular damage[12].

The distinct strength of this study lies in examining the effects of EO against clinical microbial isolates obtained from Thai patients. There were limitations in the present study. First, the data were from a small sample size of EO. It would be beneficial to further investigate larger sample sizes. Second, the authors did not perform minimal inhibitory concentration (MIC) testing. Therefore, data relating to MIC in ug/mL from each microbe were lacking. Third, we did not know the species of Eucalyptus from which Eucalyptus oil was obtained. Fourth, a larger sample size and a greater variety of microbial isolates would have increased the generalizability of the study findings. Fifth, a wider range of antioxidant methods will be of interest for further study to provide confirmatory results of Eucalyptus oil. This is because different antioxidant assays can measure different aspects of antioxidant activity, and utilizing a wider range can help confirm the overall antioxidant potential of the eucalyptus oil. Sixth, the DPPH assay results, specifically the percentage inhibition and IC_{50} values, were determined based on the concentration of the samples. This suggests that the DPPH assay was conducted without directly comparing the samples to ascorbic acid as a reference standard.

Conclusion

The present study found clove oil to be the best EO for both antimicrobial and antioxidant activities, followed by TTO and lesser galangal oil. For chemical components, eugenol, alpha-terpineol, and terpinen-4-ol exhibited excellent and equal antimicrobial activity. For antioxidant activity, eugenol could be the best antioxidant chemical component, followed by TTO and lesser galangal oil. The present data should support ongoing studies evaluating current trends of EO in antimicrobial and antioxidant activities, so it may be beneficial for alternative natural source medicine to prevent and treat many microbial diseases.

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Authors Contributions

SS, DS, and CT designed the study to obtain financial support; PC, RW, AC, and PN performed laboratory work; PY, PW, and TL performed statistical techniques; TS, SS, and DS prepared the manuscript and discussion of the results. All authors have read and approved the final manuscript and agree to be accountable for all aspects of the work.

Clinical Trial Registration

This study is not a clinical trial; therefore, a clinical trial registration number is not required.

Conflicts of Interest

The authors declare no conflict of interest.

Data Availability Statement

Database for this study was obtained from Siam University.

Ethics Approval and Consent to Participate

Ethical approval for this study was obtained from the Human Research Committee of Siam University with reference code SIAMPY-IRB 2023/006.

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Use of Artificial Intelligence

This study did not use any AI or AI-assisted technologies to prepare this manuscript.

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