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Potential antibacterial and antioxidant activities of ten essential oils from East Kalimantan, Indonesia

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Abstract. *Aryani F, Kusuma IW, Meliana Y, Sari NM, Kuspradini H. 2023. Potential antibacterial and antioxidant activities of ten essential oils from East Kalimantan, Indonesia. Biodiversitas 24: 665-672.* This study aimed to evaluate ten aromatic essential oils' antibacterial and antioxidant activity. Ten aromatic herbs were distilled using the water and steam distillation method. The essential oils obtained was tested for its physical properties, color, refractive index, and specific gravity. The refractive index was measured using a refractometer. On the other hand, specific gravity was measured using a pycnometer. Antioxidant activity testing using the free radical DPPH (2,2-diphenyl-1-picrylhydrazyl) and *ascorbic acid* was used as the control. Antibacterial activity testing using the agar diffusion method and pathogenic bacteria, namely *Salmonella typhi*, *Escherichia coli*, *Propionibacterium acnes*, and *Staphylococcus aureus*. *Chloramphenicol* was used as a positive control and acetone as a negative control. The results obtained essential oils with a slightly yellowish to brownish-yellow color. The highest refractive index and specific gravities were *Syzygium aromaticum* oil, and the smallest was *Litsea elliptica* and *Eucalyptus pellita* oils. The results of the antioxidant activity test carried out at a concentration of 500 µg/mL range from 4.82 % to 85.06 % inhibition and showed that the highest inhibition was *Cymbopogon citratus* and *Syzygium aromaticum* by 55.76 % and 85.06%, respectively. Antibacterial activity of ten essential oils showed a varied inhibition zone (0-60 mm) against *S. typhi*, *E. coli*, *P. acnes*, and *S. aureus*. The oil from *Cymbopogon citratus* and *Citrus hystrix* had the highest activity against *S. typhi* (60 and 21.67 mm, respectively), *E. coli* (60 and 20.33 mm, respectively), *P. acnes* (60 and 31.11 mm, respectively) and *S. aureus* (60 mm and 17.89 mm, respectively). The research results showed that the essential oils in this study had the potency for development as a natural antibacterial and antioxidants agents.

Keywords: Antioxidant, antibacterial, DPPH, essential oils

INTRODUCTION

Essential oils is a low molecular weight compound produced by plants. Essential oils plays an important role as a regulator of metabolism against environmental stress and pathogen attack. In addition, it is considered a therapeutic drug for treating animal and human infectious diseases (Rhimi et al. 2022). Essential oils is also a compound with the lowest molecular weight produced by the plant. The composition of essential oils includes complex mixtures of several compounds. The main group consists of terpenes, terpenoids, and other aromatic and aliphatic constituents, all characterized by low molecular weights (Mihai and Popa 2013).

Essential oils and their respective volatile constituents have been integral to human civilization for thousands of years. Essential oils is widely used as a fragrance in perfumes and cosmetics, contributes to a healthy diet, and acts as an active ingredient in pharmaceutical products. The antibacterial, antiviral and anti-inflammatory properties of essential oils are used for causal and symptomatic therapy of several diseases and prevention. It was obtained from nature, mostly plant materials. Essential oils is a multi-component mixture of more than one constituent substance to several hundred specific compounds. The use of essential oils integrative will play an important role in the world of human and veterinary medicine now and in the future. Essential oils have been widely used globally, for example, in aromatherapy, psychosomatic complaints, and inhalation (Bunse et al. 2022).

Essential oils with antifungal, antibacterial and antiviral properties have been screened worldwide as potential sources of natural antimicrobial compounds. Essential oils can be used as an alternative to treat infectious diseases and are promoted as food preservatives (Solórzano-Santos and Miranda-Novales 2012; Naeem et al. 2018). Cancer disease had the highest death among other conditions worldwide. The treatment of conventional synthetic is widely used to have side effects (Sharma et al. 2022).

Research related to antioxidant activity in search of anti-inflammatory activity in essential oils has also increased over the last few years (Miguel 2010). Essential oils antioxidants must be developed on their potential as preservatives, cosmeceuticals, or nutraceuticals in the food and cosmetic industry. The antioxidant activity of essential oils may prevent lipid peroxidation, free radical scavenging, and in some cases, chelating metal ions. In addition, the antioxidant effect of aromatic plants caused

the presence of a hydroxyl group from the phenolic compound of essential oils.

Phenylpropanoid compounds were synthesized phenolic compounds biochemically from the Shikimic acid. This compound could donate hydrogen to highly reactive radicals as antioxidants, thereby preventing radical formation (Aidi Wannes et al. 2010). East Kalimantan is renowned for its biodiversity and natural resources, including aromatic plants and medicinal plants. The potential of East Kalimantan's medicinal plants and essential oils has been extensively researched, including its potential to be developed as antioxidants, antibacterials, anticancer agents, and other substances (Kuspradini et al. 2019; Ramadhan et al. 2022). However, using plants as essential oils that originate from East Kalimantan has been widely practiced. Conversely, studies on the antioxidant and antibacterial activity of essential oils such as *Litsea angulata*, *Litsea elliptica*, *Cymbopogon nardus*, *Cymbopogon citratus*, *Pogostemon heyneatus*, *Citrus hystrix*, *Syzygium aromaticum*, *Eucalyptus pellita*, *Cananga odorata*, and *Melaleuca leucadendra* have not been observed. This study aimed to investigate and compare the antibacterial activity of ten aromatic essential oils found in East Kalimantan against pathogen bacteria, as well as to identify the most effective essential oils obtained from these plants.

MATERIALS AND METHODS

Study area

Aromatic plant samples were collected from several areas in East Kalimantan. *Litsea angulata*, *Litsea elliptica*, *Cymbopogon nardus*, *Cymbopogon citratus*, *Pogostemon heyneatus*, *Citrus hystrix*, and *Syzygium aromaticum* from Samarinda. *Eucalyptus pellita* from Sebulu, *Cananga odorata* from Tenggarong, and *Melaleuca leucadendra* from Samboja.

Water and steam distillation method

The essential oils were collected by the water and steam distillation method with slight modification (Kartiko et al. 2021). First, the samples were distilled for 4 hours, and then the oils were collected and separated using a separatory funnel. Finally, $MgSO_4$ was added to the oils. The collected essential oils were sealed in a vial bottle and presented in the percentage of yield.

Physico-chemical characteristics

The Physicochemical properties of ten essential oils were characterized by color, specific gravity, and refractive index. The color of ten essential oils was determined visually (Kartiko et al. 2021). The specific gravity was determined using a pycnometer (Boukhatem et al. 2014); and the refractive index was determined using a hand refractometer (Kartiko et al. 2021).

Gass Chromatography-Mass Spectrometry analysis

The volatile composition of *Cymbopogon citratus*, *Citrus hystrix*, and *Syzygium aromaticum* essential oils was analyzed by GC-MS (Ultra Shimadzu-QP-2010), with an RTX5 column (30 m x 250 µm ID) with slight modification (Sohilait and Kainama 2016), with the following program: from 70ºC to 250ºC at 25.71ºC.minute^{-1}, injection temperature 250ºC, the detector temperature was 250ºC, split ratio 200, the inlet pressure was 98.3 kpa, carries gas helium, and flow rate of 3ml.minute^{-1}. The composition was reported as the peak area percentage and identified by comparing mass spectra with the reference mass spectra in NIST databases and literature.

DPPH antioxidant activity testing

The antioxidant test using the Shimizu et al. (2001) method was carried out using a solution of DPPH (2,2-diphenyl-1-picrylhydrazyl) as a free radical, and a UV-VIS spectrophotometer at a wavelength of 517 nm and ascorbic acid was used as a positive control. The test of antioxidants using five concentrations was grouped into 31.25 µg/mL, 62.5 µg/mL, 125 µg/mL, 250 µg/mL, and 500 µg/mL. About 33 µL sample was mixed in a glass tube with 467 µL of ethanol added and 500 µL of 2,2-diphenyl-1-picryhydrazyl (DPPH) radical scavenging activity (0.1 mM DPPH solution). The samples were incubated for 25 minutes in a low-light room at room temperature after the absorbance was measured. The antioxidant activity of the sample extracts was determined based on the percentage of inhibition relative to the control using this equation:

$$\% \text{ inhibition} = \frac{A.Control - A.Sample}{A.Control} \; x \; 100$$

Antibacterial activity testing

Antibacterial activity testing in vitro was carried out (Arung et al. 2017), modified in vitro testing of antibacterial activity using pathogenic test bacteria, namely *Staphylococcus aureus*, *Propionibacterium acne*, *Salmonella typhi*, and *Escherichia coli*. *Nutrient Agar* (NA) was sterilized using an autoclave at 121°C for 15 minutes. After that, under aseptic conditions (in laminar flow), the media was poured into sterile Petri dishes and allowed to solidify. Bacterial suspension with a transmittance value of 70-75% at a wavelength of 600 nm was whitewashed on the surface of the media and leveled using a leveling glass. Then a hole was made in the media with a diameter of 7 mm. The positive control was chloramphenicol with a dose of 10 µg in one well, and the negative control was acetone. The bright area around the well showed inhibition.

RESULTS AND DISCUSSION

The results of the refractive index and specific gravity tests for ten types of essential oils of *L. elliptica* and *E. pelita* oils (Figure 1) with the lowest refractive index was 1.434 and specific gravity of 0.8396 and 0.8982, respectively (Table 1). The highest yield was *S.aromaticum*, with a refractive index of 1.526 and a specific gravity of 1.0155. The index of refraction is the ratio between the speed of light in air and oil.

The ingredient components influence the refractive index value of essential oils. It is suspected that *L. elliptica* and *E. pellita* contain compounds with low molecular weights so that it has low refraction index. The longer the chain components, such as the sesquiterpene components, the higher the specific gravity of the essential oils. As a result, the incident light will be more difficult to refract. This factor can cause a greater refractive index of oil (Dewi et al. 2018). The ingredient components of essential oils, such as *Syzygium aromaticum*, *Cymbopogon citratus*, and *Cytrus hystrix*, were detected by GC-MS analysis (Table 2). The tests of three potential essential oils were recorded in this study. As a result, there were three dominant compounds found.

The essential oils of *S. aromaticum* had a major compound 3-Allyl-6-methoxyphenol (eugenol) by 71.97%; this result is higher compared to similiar studies by 63.56%, 49.16%, 52.68% and 52.46% of eugenol (Kurniasari 2013; Putri et al. 2014). Other studies also reported a high concentration of eugenol at 84.63% (Uchôa Lopes et al. 2020), which is a content higher than those obtained in the present study. The essential oils of *C. citratus* had a major compound E-Citral (geranial) by 50.65%, while other studies reported geranial compound of 1.14%, 5.35%, and 25.03% (Ajayi et al. 2016; Nikhil et al. 2021; Guntarti et al. 2022). Finally, the essential oils of *C. hystrix* had a major compound 6-Octenal, 3,7-dimethyl-, (R)- (citronella) by 74.83%. Other studies reported citronella compound by 56.99% and 85.4% (Hien et al. 2020; Astuti et al. 2022). The differences in a major compound in essential oils according to the place where samples were collected, variety, sample preparation, and distillation method (Amelia et al. 2017; Teles et al. 2021).

Figure 1. Color variations of essential oils from 10 plants. 1. *Litsea angulata,* 2. *Litsea elliptica,* 3. *Cymbopogon nardus,* 4. *Eucalyptus pellita,* 5. *Melaleuca leucadendra,* 6. *Syzygium aromaticum,* 7. *Cananga odorata, 8. Pogostemon heyneatus,* 9. *Cymbopogon citratus,* 10. *Citrus hystrix*

Table 1. Observation of color, refractive index, and specific gravity of essential oils

Plant	Color	Refractive index (20°C)	Spesific gravity (20°C)
Litsea angulata	Yellowish	1.474	0.8645
Litsea elliptica	Yellowish	1.434	0.8396
Cymbopogon nardus	Slightly yellow	1.478	0.9248
Eucalyptus pellita	Yellow	1.434	0.8982
Melaleuca leucadendra	Greenish yellow	1.496	0.9245
Syzygium aromaticum	Light yellow	1.526	1.0155
Cananga odorata (Flower)	Yellow	1.494	0.9106
Pogostemon heyneatus	Yellow	1.501	0.8713
Cymbopogon citratus	Brownish-yellow	1.484	0.9028
Citrus hystrix	yellow	1.449	0.8554

Table 2. Chemical compositions of *Syzygium aromaticum*, *Cymbopogon citratus,* and *Citrus hystrix* essential oils by GC-MS analysis

Plant	Peak	R.Time[a]	Compounds	Synonym	MF[b]	MW[c] (g/mol)	% area
Syzygium aromaticum	1	10.033	3-Allyl-6-methoxyphenol	Eugenol	$C_{10}H_{12}O_2$	164	71.97
	2	10.849	trans-β-Caryophyllene	-	$C_{15}H_{24}$	204	23.48
	3	11.294	*α-humulene*	-	$C_{15}H_{24}$	204	2.38
Cymbopogon citratus	1	8.637	E-Citral	Geranial	$C_{10}H_{16}O$	152	50.65
	2	8.208	Z-Citral	Neral	$C_{10}H_{16}O$	152	35.63
	3	4.572	β-Myrcene	-	$C_{10}H_{16}$	136	6.24
Citrus hystrix	1	6.910	6-Octenal, 3,7-dimethyl-, (R)-	Citronella	$C_{10}H_{18}O$	154	74.83
	2	9.696	6-Octen-1-ol, 3,7-dimethyl-, acetate	Citronellyl acetate	$C_{12}H_{22}O_2$	198	11.77
	3	8.100	β-Citronellol	Citronellol	$C_{10}H_{20}O$	156	2.56

Note: [a]R. Time (Retention Time), [b]MF (Molecular Formula), [c]MW (Molecular Weight).

Antioxidant activity was determined using the DPPH (2,2-diphenyl--picrylhydrazyl) for ten essential oils at a concentration of 500 µg/mL, with the results shown in Figure 2. The highest value with DPPH radical inhibition was *S. aromaticum* and *C. citratus* oils by 85.06% and 55.76%, respectively. The lowest inhibition value was *L. Elliptica* by 4.82%. This difference occurs due to differences in the content of compounds in each essential oils. Eugenol is the largest compound component in *S. aromaticum* oil, with 73.2% (Alawiyah et al. 2019) and 58.6% (Hasim et al. 2016). The high eugenol in *S. aromaticum* oil causes high antioxidant activity (Hassine et al. 2021). According to many scientific reports, the high eugenol content proves that eugenol is a strong chelator with an IC_{50} value ranging from 4.4 to 130.5 g/mL. In another report, Ogata et al. (2000) stated that eugenol could inhibit lipid peroxidation by trapping active oxygen species such as O or hydroxyl radicals by breaking free radical chain reactions. This study's antioxidant activity of *C. citratus* oil obtained an IC_{50} value of 441.29 µg/mL. This value was smaller than the results of Anggraeni et al. (2018), with an IC_{50} value of 15914.0 µg/mL. Several studies have been conducted on *C. citratus* oil, summarized by Zahra et al. (2020), stating that the main compound in *C. citratus* oil is citral, which acts as an antioxidant. Besides, *C. citratus* also contains linalool, which can reduce oxidative stress.

According to Radünz et al. (2021), *S. aromaticum* oil showed activity against free radicals such as hydroxyl and nitric oxide, also precursors of Chronic Non-Communicable Diseases (CNCDs). *S. aromaticum* oil also could inhibit α-amylase and α-glucosidase as antihyperglycemic agents. Several reports about *S. aromaticum* oil suggest the oils contain the highest amount of phenolic compounds with biological activities such as antibacterial, antioxidant, insecticidal, and antifungal (Golmakani et al. 2017; Hatami et al. 2019; Batiha et al. 2020; Haro-González et al. 2021).

Several concentration variations were carried out, such as 0.25%. 0.5%, 1%, 2%, 4%, and 8%, to determine the Minimum Inhibitory Concentration (MIC). Of the four bacteria tested, *C. citratus* can inhibit bacterial growth to the smallest concentration in the test at 0.25%. The MIC C.citratus <0.25%, even at 4% and 8% concentrations, had no bacterial growth. *C. citratus* oil proved effective against all test bacteria. On the other hand, antibacterial activity increased with increasing oil concentration. Several studies by Mukarram et al. (2022) and Gao et al. (2020) stated that the major component of the citral compound acts as an antimicrobial in *Cymbopogon citratus* oil. These results are similar to those of Naik et al. (2010), who stated that *C. citratus* oil was effective against drug-resistant organisms and recommended using *C. citratus* to help treat infections caused by drug-resistant organisms.

In addition to *Cymbopogon citratus* oil as a strong antibacterial, Table 3, 4, 5, and 6 also presents the antibacterial activity of the essential oils of *L. angulata, L. elliptica, C. nardus, E. pellita, M. leucadendra, S. aromaticum C. odorata, P. heyneatus,* and *C. hystrix*. From the comprehensive information obtained that all essential oils have the potential as antibacterial agents with varying MIC. A study by Masyita et al. (2022) reported that the main components of essential oils compounds, namely terpenes and terpenoids with great potential as antimicrobials. Studies about *Cymbopogon citratus* oil have shown antioxidant and antimicrobial activities in vivo and in vitro (Dangkulwanich and Charaslertrangsi 2020; Loko et al. 2021). Furthermore, several studies of the genus *Cymbopogon* proved the effectivity against bacteria, including *Candida albicans, Streptococcus mutans, Escherichia coli, Staphylococcus aureus,* and *Salmonella enterica* (Abdel-Gwad et al. 2021; Martins et al. 2021).

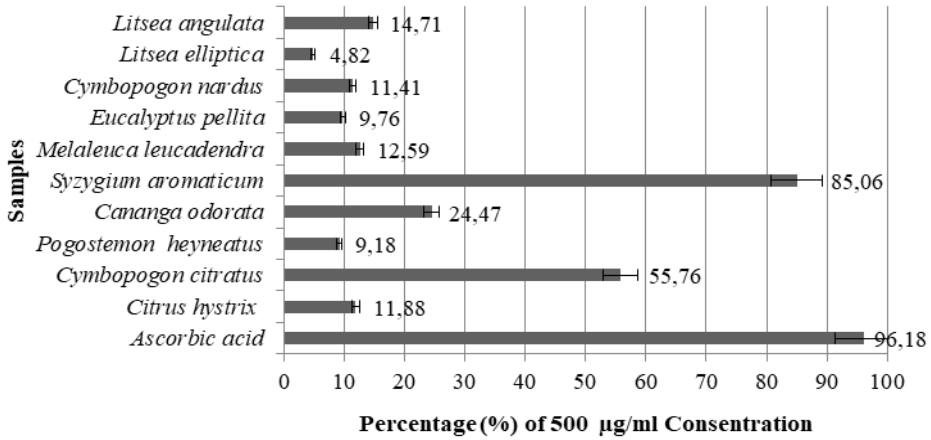

Figure 2. Percentage of DPPH radical inhibition of ten essential oils at a concentration of 500 µg/mL

Table 3. Antibacterial of essential oils against *Salmonella typhi*

Samples	Amount of extracts applied											
	0.25%		0.50%		1%		2%		4%		8%	
	IZ (mm)	AI (%)	IZ (mm)	AI (%)	IZ (mm)	AI (%)	IZ (mm)	AI (%)	IZ (mm)	AI (%)	IZ (mm)	AI (%)
Chloramphenicol 0.5%	26.33 ±0.58											
Litsea angulata	0	0	0	0	0	0	0	0	15.56 ±0.69	59	17.22 ±0.51	65
Litsea elliptica	8.56 ±0.38	32	9.22 ±0.19	35	10.11 ±0.38	38	12.44 ±0.19	47	14.89 ±0.51	57	17.78 ±0.77	68
Cymbopogon nardus	10.00 ±0.33	38	11.78 ±0.51	45	12.15 ±0.32	46	12.22 ±0.19	46	12.56 ±0.19	48	13.56 ±19	51
Eucalyptus pellita	0	0	0	0	0	0	12.11 ±1.50	46	13.67 ±0.67	52	14.67 ±0.67	55
Melaleuca leucadendra	0	0	0	0	0	0	0	0	11.56 ±1.95	44	15.44 ±0.51	58
Syzygium aromaticum	9.33 ±0.00	35	10.78 ±0.19	41	12.11 ±0.38	46	14.56 ±0.84	55	17.67 ±0.33	67	21.67 ±0.33	82
Cananga odorata	10.67 ±0.33	40	12.11 ±0.51	46	12.44 ±0.38	47	13.00 ±0.58	49	13.44 ±0.69	51	14.22 ±0.19	54
Pogostemon heyneatus	0	0	0	0	9.00 ±0.33	34	9.56 ±0.19	36	15.33 ±0.33	58	16.89 ±0.69	64
Cymbopogon citratus	12.56 ±0.51	47	16.67 ±0.33	63	20.67 ±0.88	78	28.56 ±0.84	108	60	227	60	227
Citrus hystrix	0	0	0	0	0	0	0	0	60	227	60	227

Note: *IZ: Inhibition Zone; AI: Activity Index.

Table 4. Antibacterial of essential oils against *Escherichia coli*

Samples	Amount of extracts applied											
	0.25%		0.50%		1%		2%		4%		8%	
	IZ (mm)	AI (%)	IZ (mm)	AI (%)	IZ (mm)	AI (%)	IZ (mm)	AI (%)	IZ (mm)	AI (%)	IZ (mm)	AI (%)
Chloramphenicol 0.5%	22.33 ±0.33											
Litsea angulata	0	0	0	0	0	0	0	0	11.78 ±0.69	53	14.89 ±0.19	67
Litsea elliptica	0	0	0	0	8.22 ±0.19	32	8.67 ±0.33	39	13.89 ±1.35	62	15.56 ±0.38	70
Cymbopogon nardus	9.33 ±0.33	42	11.00 ±0.67	49	12.33 ±0.33	55	12.56 ±1.07	56	13.67 ±1.45	61	15.00 ±0.33	67
Eucalyptus pellita	0	0	0	0	0	0	11.89 ±0.51	53	13.56 ±0.84	61	17.33 ±0.33	78
Melaleuca leucadendra	0	0	0	0	10.00 ±0.88	45	11.56 ±0.51	52	12.89 ±0.77	58	16.44 ±0.69	74
Syzygium aromaticum	9.89 ±0.19	44	11.67 ±0.33	52	13.44 ±0.51	60	15.56 ±0.19	70	18.89 ±0.51	85	20.33 ±0.33	91
Cananga odorata	0	0	0	0	13.11 ±0.19	59	13.14 ±0.51	60	14.00 ±0.33	63	15.00 ±0.67	67
Pogostemon heyneatus	8.00 ±0.33	37	9.44 ±0.19	42	10.89 ±0.19	49	12.33 ±0.33	55	15.11 ±0.19	68	15.44 ±0.38	69
Cymbopogon citratus	11.44 ±0.19	51	12.67 ±0.33	57	18.78 ±0.51	84	23.56 ±0.38	105	60	269	60	269
Citrus hystrix	11.44 ±0.19	51	12.67 ±0.33	57	13.56 ±0.19	61	60	269	60	269	60	269

Note: *IZ: Inhibition Zone; AI: Activity Index.

Table 5. Antibacterial of essential oils against *Propionibacterium acnes*

Samples	Amount of extracts applied											
	0.25%		0.50%		1%		2%		4%		8%	
	IZ (mm)	AI (%)	IZ (mm)	AI (%)	IZ (mm)	AI (%)	IZ (mm)	AI (%)	IZ (mm)	AI (%)	IZ (mm)	AI (%)
Chloramphenicol 0.5%	20.78 ±0.51											
Litsea angulata	0	0	9.33 ±0.33	45	10.11 ±0.38	49	10.78 ±0.51	52	14.67 ±0.33	71	17.56 ±0.38	84
Litsea elliptica	0	0	0	0	9.67 ±0.88	47	12.00 ±0.33	58	13.22 ±0.19	64	14.00 ±0.33	67
Cymbopogon nardus	0	0	9.67 ±0.33	47	12.56 ±0.19	60	13.11 ±0.51	63	14.44 ±1.02	70	15.44 ±0.69	74
Eucalyptus pellita	0	0	0	0	9.67 ±0.88	47	15.78 ±84	76	15.89 ±0.51	76	18.89 ±0.84	91
Melaleuca leucadendra	8.33 ±0.33	40	8.78 ±0.19	42	10.22 ±0.19	49	10.56 ±0.19	51	12.67 ±0.33	61	17.44 ±0.19	84
Syzygium aromaticum	0	0	15.33 ±0.88	74	22.33 ±0.67	107	27.22 ±0.69	131	28.56 ±0.51	137	31.11 ±0.84	150
Cananga odorata	9.00 ±0.51	43	12.67 ±0.33	61	13.22 ±0.19	64	13.89 ±0.51	67	14.44 ±0.69	70	16.11 ±0.51	78
Pogostemon heyneatus	9.78 ±0.51	47	10.33 ±0.33	50	10.56 ±0.51	51	11.78 ±0.38	57	12.00 ±0.33	58	13.67 ±0.88	66
Cymbopogon citratus	10.22 ±0.84	49	12.67 ±0.33	61	16.78 ±0.51	81	27.78 ±0.19	134	60	289	60	289
Citrus hystrix	0	0	0	0	0	0	0	0	60	289	60	289

Note: *IZ: Inhibition Zone; AI: Activity Index.

Table 6. Antibacterial of essential oils against *Staphylococcus aureus*

Samples	Amount of extracts applied											
	0.25%		0.50%		1%		2%		4%		8%	
	IZ (mm)	AI (%)	IZ (mm)	AI (%)	IZ (mm)	AI (%)	IZ (mm)	AI (%)	IZ (mm)	AI (%)	IZ (mm)	AI (%)
Chloramphenicol 0.5%	25.78 ±0.51											
Litsea angulata	10.89 ±0.19	42	11.33 ±0.33	44	12.56 ±0.19	49	12.78 ±0.38	50	13.33 ±0.33	52	15.78 ±0.51	61
Litsea elliptica	0	0	0	0	9.67 ±0.88	38	11.78 ±0.19	46	13.22 ±0.19	51	14.11 ±0.38	55
Cymbopogon nardus	9.22 ±0.19	36	11.33 ±0.67	44	11.78 ±0.19	46	13.22 ±0.38	51	13.89 ±0.38	54	15.22 ±0.84	59
Eucalyptus pellita	0	0	0	0	10.78 ±0.51	42	13.67 ±0.58	53	15.56 ±0.69	60	17.89 ±1.19	69
Melaleuca leucadendra	0	0	9.22 ±0.19	36	10.22 ±0.19	40	12.22 ±0.51	47	14.22 ±0.19	55	16.00 ±0.33	62
Syzygium aromaticum	9.11 ±0.19	35	11.00 ±0.67	43	12.44 ±0.51	48	12.78 ±0.51	50	15.56 ±0.38	60	17.78 ±0.51	73
Cananga odorata	9.33 ±0.33	36	10.78 ±0.69	42	11.78 ±0.69	46	11.89 ±0.19	46	14.11 ±0.38	55	14.56 ±1.07	56
Pogostemon heyneatus	11.33 ±0.33	44	12.44 ±0.19	48	12.78 ±0.19	50	13.00 ±0.33	50	13.78 ±0.19	53	14.44 ±0.19	56
Cymbopogon citratus	13.22 ±0.19	51	15.44 ±0.19	60	16.78 ±1.02	65	25.67 ±0.33	100	60	233	60	233
Citrus hystrix	0	0	11.33 ±0.33	44	12.44 ±0.19	48	16.89 ±0.19	66	60	233	60	233

Note: *IZ: Inhibition Zone; AI: Activity Index.

E-Citral (geranial) is a major compound of *C. citratus* oil reported to inhibit the growth of pathogens bacteria. Therefore, the Geranial compound of *C. citratus* oil had significant potential for pharmaceutical application of treatment infections against *Staphylococcus aureus* and *Candida albicans.* Furthermore, a study by Gao et al. (2020) on 6-Octenal, 3,7-dimethyl-, (R)- (citronella) as a major compound of *C. hystrix* reported inhibiting bacteria such as *S. aureus*, *S. mutans*, *E. coli*, and *P. aeruginosa*. Citronella compound is an aldehyde functional group known as an antibacterial (Husni et al. 2021).

Based on the research, all the essential oils used in this study had the potential as antibacterial agents. In addition, *Syzygium aromaticum* and *Cymbopogon citratus* oils had the potential as natural antioxidants.

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REFERENCES

Abdel-Gwad N, Mahmoud E, Al-Askalany S, Hanafy E. 2021. Antioxidant, antibacterial and cytotoxic effect of *Cymbopogon citratus*, *Mentha longifolia*, and *Artemisia absinthium* essential oils. Egypt J Chem 65 (2): 287-296. DOI: 10.21608/ejchem.2021.86171.4174.

Aidi Wannes W, Mhamdi B, Sriti J, ben Jemia M, Ouchikh O, Hamdaoui G, Kchouk ME, Marzouk B. 2010. Antioxidant activities of the essential oils and methanol extracts from myrtle (*Myrtus communis* var. italica L.) leaf, stem and flower. Food Chem Toxicol 48 (5): 1362-1370. DOI: 10.1016/j.fct.2010.03.002.

Ajayi EO, Sadimenko AP, Afolayan AJ. 2016. GC-MS evaluation of *Cymbopogon citratus* (DC) Stapf oil obtained using modified hydrodistillation and microwave extraction methods. Food Chem 209: 262-266. DOI: 10.1016/j.foodchem.2016.04.071.

Alawiyah AL, Senania A, Sari H, Perdana F, Musthafa I. 2019. Antioxidant activity of volatile compounds from *Syzygium aromaticum* (L.) leaves. J Phys: Conf Ser 1402 (5): 55038. doi:10.1088/1742-6596/1402/5/055038.

Amelia B, Saepudin E, Cahyana AH, Rahayu DU, Sulistyoningrum AS, Haib J. 2017. GC-MS analysis of clove (*Syzygium aromaticum*) bud essential oils from Java and Manado. AIP Conf Proc 1862 (1): 030082. DOI: 10.1063/1.4991186.

Anggraeni NI, Hidayat IW, Rachman SD, Ersanda. 2018. Bioactivity of essential oils from lemongrass (*Cymbopogon citratus* Stapf) as antioxidant agent. AIP Conf Proc 1927 (1): 30007. DOI: 10.1063/1.5021200.

Arung E, Pasedan W, Kusuma I, Hendra M, Supriadi M. 2017. Selected medicinal plants in East and North Kalimantan (Indonesia) against *Propionibacterium acnes*. Biodiversitas J Biol Divers 18 (1): 321-325. DOI: 10.13057/biodiv/d180141.

Astuti IP, Palupi KD, Damayanti F. 2022. Essential oils composition of kaffir lime (*Citrus hystrix* DC.) collection of Bogor Botanic Gardens from Central Java and East Sumba. J Trop Biodivers Biotechnol 7 (1): 66061. DOI: 10.22146/jtbb.66061.

Batiha GE-S, Alkazmi LM, Wasef LG, Beshbishy AM, Nadwa EH, Rashwan EK. 2020. *Syzygium aromaticum* L. (Myrtaceae): traditional uses, bioactive chemical constituents, pharmacological and toxicological activities. Biomolecules 10 (2): 202. DOI: 10.3390/biom10020202.

Boukhatem MN, Ferhat MA, Kameli A, Saidi F, Kebir HT. 2014. Lemon grass (*Cymbopogon citratus*) essential oils as a potent anti-inflammatory and antifungal drugs. Libyan J Med 9 (1): 25431. DOI: 10.3402/ljm.v9.25431.

Bunse M, Daniels R, Gründemann C, Heilmann J, Kammerer DR, Keusgen M, Lindequist U, Melzig MF, Morlock GE, Schulz H, Schweiggert R. 2022. Essential oils as Multi-component Mixtures and Their Potential for Human Health and Well-Being. Front Pharmacol 2645. DOI: 10.3389/fphar.2022.956541.

Dangkulwanich M, Charaslertrangsi T. 2020. Hydrodistillation and antimicrobial properties of lemongrass oil (*Cymbopogon citratus* , Stapf): An undergraduate laboratory exercise bridging chemistry and microbiology. J Food Sci Educ 19 (2): 41-48. DOI: 10.1111/1541-4329.12178.

Dewi IA, Prastyo AM, Wijana S. 2018. Extraction of essential oils from baby Java orange (*Citrus sinensis*) solid waste using water and steam distillation. IOP Conf Ser: Earth Environ Sci 131: 12054. DOI: 10.1088/1755-1315/131/1/012054.

Gao S, Liu G, Li J, Chen J, Li L, Li Z, Zhang X, Zhang Shoumin, Thorne RF, Zhang Shuzhen. 2020a. Antimicrobial Activity of lemongrass essential oils (*Cymbopogon flexuosus*) and its active component citral against dual species biofilms of *Staphylococcus aureus* and *Candida* Species. Front Cell Infect Microbiol 10: 603858. DOI: 10.3389/fcimb.2020.603858.

Golmakani M-T, Zare M, Razzaghi S. 2017. Eugenol enrichment of clove bud essential oils using different microwave-assisted distillation methods. Food Sci Technol Res 23 (3): 385-394. DOI: 10.3136/fstr.23.385.

Guntarti A, Pratiwi HK, Nurani LH, Gandjar IG. 2022. Authentication of Lemongrass Oil By Gas Chromatography-Mass Spectroscopy (GC-MS) Combination Chemometrics. Indones J Pharm Sci Technol 9 (3): 174. DOI: 10.24198/ijpst.v9i3.32558.

Haro-González JN, Castillo-Herrera GA, Martínez-Velázquez M, Espinosa-Andrews H. 2021. Clove Essential oils (*Syzygium aromaticum* L. Myrtaceae): Extraction, chemical composition, food applications, and essential bioactivity for human health. Molecules 26 (21): 6387. DOI: 10.3390/molecules26216387.

Hasim F, Batubara I, Suparto I. 2016. The potency of clove (*Syzygium aromaticum*) essential oils as slimming aromatherapy by in vivo assay. Intl J Pharm Biol Sci 7 (1): 110-116.

Hassine D Ben, Kammoun El Euch S, Rahmani R, Nessrine G, Kane R, Abderrabba M, Bouajila J. 2021. Clove buds essential oils: The impact of grinding on the chemical composition and its biological activities involved in consumer's health security. Biomed Res Intl 4: 1-11. DOI: 10.1155/2021/9940591.

Hatami T, Johner JCF, Zabot GL, Meireles MAA. 2019. Supercritical fluid extraction assisted by cold pressing from clove buds: Extraction performance, volatile oil composition, and economic evaluation. J Supercritical Fluids 144: 39-47. DOI: 10.1016/j.supflu.2018.10.003.

Hien TT, Quyen NTC, Truc TT, Quan PM. 2020. Evaluate the chemical composition of Kaffir lime (*Citrus hystrix*) essential oils using the classical method. IOP Conf Ser: Mater Sci Eng 991 (1): 012014. DOI: 10.1088/1757-899X/991/1/012014.

Husni E, Putri US, Dachriyanus. 2021. Chemical content profile of essential oils from kaffir lime (*Citrus hystrix* DC.) in Tanah Datar Regency and antibacterial activity. In: Proceedings of the 2nd International Conference on Contemporary Science and Clinical Pharmacy 2021 (ICCSCP 2021). Atlantis Press International BV. DOI: 10.2991/ahsr.k.211105.025.

Kartiko AB, Putri AS, Rosamah E, Kuspradini H. 2021. Evaluation of antibacterial activity and physico-chemical profiles of *Eucalyptus pellita* essential oils from East Kalimantan. In: Proceedings of the Joint Symposium on Tropical Studies (JSTS-19). Atlantis Press. DOI: 10.2991/absr.k.210408.002.

Kurniasari F. 2013. Penetapan kadar eugenol dan profil GC-MS daun cengkeh. In: Prosiding "Simposium Nasional Peluang dan Tantangan Obat Tradisional dalam Pelayanan Kesehatan Formal." IKAFI, Yogyakarta. [Indonesian]

Kuspradini H, Putri As, Egra S, Yanti Y. 2019. Short Communication: In vitro antibacterial activity of essential oils from twelve aromatic plants from East Kalimantan, Indonesia. Biodiversitas J Biol Divers 20 (7). DOI: 10.13057/biodiv/d200733.

Loko YL, Medegan Fagla S, Kassa P, Ahouansou CA, Toffa J, Glinma B, Dougnon V, Koukoui O, Djogbenou SL, Tamò M, Gbaguidi F. 2021. Bioactivity of essential oils of *Cymbopogon citratus* (DC) Stapf and *Cymbopogon nardus* (L.) W. Watson from Benin against *Dinoderus porcellus* Lesne (Coleoptera: Bostrichidae) infesting yam chips. Intl J Trop Insect Sci 41 (1): 511-524. DOI: 10.1007/s42690-020-00235-3.

Martins W da S, de Araújo JSF, Feitosa BF, Oliveira JR, Kotzebue LRV, Agostini DL da S, de Oliveira DLV, Mazzetto SE, Cavalcanti MT, da Silva AL. 2021. Lemongrass (*Cymbopogon citratus* DC. Stapf) essential oils microparticles: Development, characterization, and antioxidant potential. Food Chem 355: 129644. DOI: 10.1016/j.foodchem.2021.129644.

Masyita A, Mustika Sari R, Dwi Astuti A, Yasir B, Rahma Rumata N, Emran T Bin, Nainu F, Simal-Gandara J. 2022. Terpenes and terpenoids as main bioactive compounds of essential oils, their roles in human health and potential application as natural food preservatives. Food Chem X: 100217. DOI: 10.1016/j.fochx.2022.100217.

Miguel MG. 2010. Antioxidant and anti-inflammatory activities of essential oils: A short review. Molecules (Basel, Switzerland) 15 (12): 9252-9287. DOI: 10.3390/molecules15129252.

Mihai AL, Popa ME. 2013. Essential oils utilization in food industry a literature review. Sci Bull Ser F Biotechnol 17: 187-192.

Mukarram M, Choudhary S, Khan MA, Poltronieri P, Khan MMA, Ali J, Kurjak D, Shahid M. 2022. Lemongrass essential oils components with antimicrobial and anticancer activities. Antioxidants 11 (1): 1-23. DOI: 10.3390/antiox11010020.

Naeem A, Abbas T, Ali TM, Hasnain A. 2018. Essential oils: Brief Background and Uses. Ann Short Rep1 (1).

Naik MI, Fomda BA, Jaykumar E, Bhat JA. 2010. Antibacterial activity of lemongrass (*Cymbopogon citratus*) oil against some selected pathogenic bacterias. Asian Pac J Trop Med 3 (7): 535-538. DOI: 10.1016/S1995-7645(10)60129-0.

Nikhil D. Solanke PPT, Ughade JD. 2021. GC-MS analysis of bioactive compounds in lemongrass (*Cymbopogon citratus*) powder. Intl J Curr Microbiol Appl Sci 10 (10): 283-290. DOI: 10.20546/ijcmas.2021.1010.034.

Ogata M, Hoshi M, Urano S, Endo T. 2000. Antioxidant activity of eugenol and related monomeric and dimeric compounds. Chem Pharm Bull 48 (10): 1467-1469. DOI: 10.1248/cpb.48.1467.

Putri RL, Hidayat N, Rahmah NL. 2014. Pemurnian eugenol dari minyak daun cengkeh dengan reaktan basa kuat koh dan ba(oh)2 (kajian konsentrasi reaktan). Jurnal Industria 3 (1): 1-12. [Indonesian]

Radünz M, Camargo TM, dos Santos Hackbart HC, Alves PIC, Radünz AL, Gandra EA, da Rosa Zavareze E. 2021. Chemical composition and in vitro antioxidant and antihyperglycemic activities of clove, thyme, oregano, and sweet orange essential oils. LWT 138: 110632. DOI: 10.1016/j.lwt.2020.110632.

Ramadhan R, Phuwapraisirisan P, Amirta R, Darmawan Mfb, Ul-Haq K, Kusuma Iw, Suwito H, Abdulgani N, Mukhdlor A, Saparwadi S. 2022. The potency of selected ethnomedicinal plants from East Kalimantan, Indonesia as antidiabetic agents and free-radical scavengers. Biodiversitas J Biol Divers 23 (4). DOI: 10.13057/biodiv/d230458.

Rhimi W, Mohammed MA, Zarea AAK, Greco G, Tempesta M, Otranto D, Cafarchia C. 2022. Antifungal, antioxidant and antibiofilm activities of essential oils of *Cymbopogon* spp. Antibiotics 11 (6): 829. DOI: 10.3390/antibiotics11060829.

Sharma M, Grewal K, Jandrotia R, Batish DR, Singh HP, Kohli RK. 2022. Essential oils as anticancer agents: Potential role in malignancies, drug delivery mechanisms, and immune system enhancement. Biomed Pharmacother 146: 112514. DOI: 10.1016/j.biopha.2021.112514.

Shimizu K, Kondo R, Sakai K, Takeda N, Nagahata T, Oniki T. 2001. Novel vitamin E derivative with 4-substituted resorcinol moiety has both antioxidant and tyrosinase inhibitory properties. Lipids 36 (12): 1321-1326. DOI: 10.1007/s11745-001-0847-9.

Sohilait HJ, Kainama H. 2016. GC/GC-MS analysis, isolation and identification of bark essential oil components from *Cinnamomum culilawan*, Blume. Am J Appl Chem 4 (4): 157-160. DOI: 10.11648/j.ajac.20160404.16.

Solórzano-Santos F, Miranda-Novales MG. 2012. Essential oils from aromatic herbs as antimicrobial agents. Curr Opin Biotechnol 23 (2): 136-141. DOI: 10.1016/j.copbio.2011.08.005.

Teles AM, Silva-Silva JV, Fernandes JMP, Abreu-Silva AL, Calabrese K da S, Mendes Filho NE, Mouchrek AN, Almeida-Souza F. 2021. GC-MS characterization of antibacterial, antioxidant, and antitrypanosomal activity of *Syzygium aromaticum* essential oils and eugenol. Evidence-Based Complement Altern Med 2021: 1-12. DOI: 10.1155/2021/6663255.

Uchôa Lopes CM, Saturnino de Oliveira JR, Holanda VN, Rodrigues AY, Martins da Fonseca CS, Galvão Rodrigues FF, Camilo CJ, Lima VL, Coutinho HD, Kowalski R, da Costa JG. 2020. GC-MS analysis and hemolytic, antipyretic and antidiarrheal potential of *Syzygium aromaticum* (Clove) essential oils. Separations 7 (2): 35. DOI: 10.3390/separations7020035.

Zahra AA, Hartati R, Fidrianny I. 2020. Review of the chemical properties, pharmacological properties, and development studies of *Cymbopogon* sp. Biointerface Res Appl Chem 11 (3): 10341-10350. DOI: 10.33263/BRIAC113.1034110350.