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Research article

Antibacterial efficacy of essential oils from *Eugenia javanica* Lam. and *Eugenia paniala* Roxb. leaves against *Staphylococcus chromogenes* and *Streptococcus uberis* for dairy mastitis

Banthita Saengsitthisak [1], **Wasana Chaisri** [2], **Sukolrat Boonyayatra**[3], **Surachai Pikulkaew**[2],
Noppason Pangprasit[4] **and Nuttakorn Baisaeng**[5,*]

[1]Department of Pharmaceutical Technology, Faculty of Pharmacy, Payap University, Chiang Mai 50000, Thailand.
[2] Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai 50100, Thailand.
[3] Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Long Island University, Brookville, NY 11548, United States.
[4]Akkhraratchakumari Veterinary College, Walailak University, Nakhon Si Thammarat 80160, Thailand.
[5] Department of Pharmaceutical Sciences, School of Pharmaceutical Sciences, University of Phayao, Phayao 56000, Thailand.

Abstract

The aim of this research was to explore the antibacterial properties of essential oils derived from the leaves of *Eugenia javanica* Lam. (*E. javanica* Lam.) and *Eugenia paniala* Roxb. (*E. paniala* Roxb.) against prevalent mastitis-causing bacteria such as *Staphylococcus chromogenes* (*S. chromogenes*) and *Streptococcus uberis* (*S. uberis*). These oils were extracted from the leaves of *E. javanica* Lam. and *E. paniala* Roxb. using hydrodistillation with a Clevenger-type apparatus. Subsequently, the chemical compositions of the crude oils were determined using gas chromatography-mass spectrometry. The yield of the essential oil was found to be 0.05 ± 0.01% (w/w) and 0.12 ± 0.01% (w/w) on a fresh weight basis. The primary compounds identified consisted of γ-Terpinene (30.78%), p-Cymene (12.28%), (E)-Caryophyllene (8.84%), and β-Pinene (8.18%) for *E. javanica* Lam., while trans-β-ocimene (57.29%), trans-caryophyllene (7.42%), linalool (4.60%), and caryophyllene oxide (3.85%) were the predominant constituents for *E. paniala* Roxb. The antibacterial activity of each essential oil was assessed through minimum inhibitory concentration (MIC) and minimum bactericidal concentrations (MBC) using the broth microdilution method. The MIC and MBC values of the essential oils from *E. javanica* Lam. and *E. paniala* Roxb. leaves against each pathogen were compared using the Mann-Whitney U test for pairwise analysis. The results revealed that the essential oil from *E. paniala* Roxb. leaves exhibited superior inhibitory and bactericidal effects against *S. chromogenes* with lower MIC and MBC values ($P < 0.05$). In summary, essential oils extracted from *E. javanica* Lam. and *E. paniala* Roxb. leaves demonstrated antibacterial activity against major mastitis pathogens, including *S. chromogenes* and *S. uberis*.

Keywords: Antibacterial properties, Essential oil, *Eugenia javanica* Lam., *Eugenia paniala* Roxb., Mastitis pathogens.

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[*] **Corresponding author:** Nuttakorn Baisaeng, Department of Pharmaceutical Sciences, School of Pharmaceutical Sciences, University of Phayao, Phayao 56000, Thailand. E-mail: patchateeya@yahoo.com.

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INTRODUCTION

Inflammation of the udder in cows, known as mastitis, is a significant concern in dairy farming due to its direct impact on milk production (Abera et al., 2012). This condition leads to increased expenses for farmers in terms of mastitis treatment and loss of income, including the cost of treating the raw milk that cannot be sold due to contamination with antibiotic residues and discarded milk from cows unresponsive to treatment (Batavani et al., 2007). The occurrence of mastitis in cows is primarily due to bacterial infections by *Staphylococcus* spp. and *Streptococcus* spp. (Boonyayatra et al., 2005; Pangprasit et al., 2021). Conventional treatment for mastitis typically involves the use of antibiotics. However, this approach can lead to issues related to antibiotic residues in milk, posing health risks to consumers, as well as the development of antibiotic resistance in animals (Desissa et al., 2012).

To address this issue, some research groups have focused on the antibacterial properties of herbal extracts or essential oils obtained from various plants, such as basil and coriander essential oils, with the aim of providing an alternative treatment for mastitis in cows (Lopes et al., 2020), lemongrass essential oil (Suwannapakdee et al., 2012), guava (Sakorn Thonglao, 2012), lychee, blueberry, pomegranate, sea almond, jujube, and buffalo thorn extracts (Thancharoen et al., 2007). Many of these substances are considered safer than conventional antibiotics since they are less likely to cause residue issues when used in the treatment of subclinical mastitis in cows. Plant-based essential oils and extracts, particularly those from the Myrtaceae family, have demonstrated promising antibacterial properties (Singh et al., 2012). Common sources of such essential oils include eucalyptus and *Eugenia aromatic* L. (*E. aromatic* L.) (Aliaa Saad, 2012).

Previous research has reported the antibacterial effects of *Eugenia* spp. plants, apart from *E. aromatic* L. Other plants within this genus, such as *E. javanica* Lam. (Tina et al., 2011), *Eugenia malaccensis var. purpurea* (Figueirôa Ede et al., 2013), *Eugenia jambolana* (Jasmine et al., 2010), and *E. paniala* Roxb. (Niorn et al., 2012), have sparked interest from researchers for their potential antibacterial properties in preventing subclinical mastitis in cows. No antibacterial property of the essential oils from *E. javanica* Lam. and *E. paniala* Roxb. leaves have been reported.

Therefore, the aim of this research is to investigate the antibacterial properties of essential oils extracted from the leaves of *E. javanica* Lam. and *E. paniala* Roxb. within the same genus as *E. aromatic* L. against major dairy mastitis pathogens, including *S. chromogenes* and *S. uberis*. In this study, their potential is explored as an alternative treatment or protection for subclinical mastitis in cows, ultimately reducing the use of antibiotics and chemical substances that have adverse effects on dairy farming, consumers, and the environment. The findings will provide valuable information and guidelines for the future development of oil extracts from these plants, which can be utilized as a formula in the future.

MATERIALS AND METHODS

Plant material

Fresh leaves of *E. javanica* Lam. and *E. paniala* Roxb. were collected from San Sai, Chiang Mai, Thailand and Mae Lao, Chiang Rai, Thailand. The voucher specimen was deposited as B.saeng-002 and B.saeng-003 in the forest herbarium of the Queen Sirikit Botanic Garden in Mae Rim, Chiang Mai, Thailand.

Extraction and chemical identification of essential oils

Following the British Pharmacopoeia guidelines, fresh leaves from *E. javanica* Lam. and *E. paniala* Roxb. were finely chopped into small pieces. These pieces underwent hydrodistillation in a Clevenger apparatus for a duration of two hours. The resulting leaf oils from *E. javanica* Lam. and *E. paniala* Roxb. were concentrated using a rotary evaporator. To remove water from the concentrated mixture, anhydrous sodium sulfate was added and allowed to stand for 30 minutes. The crude oils were then filtered and stored in a refrigerator at a temperature between 2 and 4°C.

The chemical composition of the oils was analyzed using gas chromatography-mass spectrometry (GC-MS) with an Agilent 6890 gas chromatograph equipped with a Hewlett Packard 5973 mass-selective detector in electron impact mode (70 eV) with full-scan capabilities. The capillary column employed was an HP 19091S-433 linked capillary column (30 × 0.25 mm i.d.; 0.25 µM) with HP-5MS fused 5% phenyl methyl siloxane. The oven temperature programme was initiated at 70°C with a 3°C/min ramp-up. The injector and detector temperatures were set at 280 and 325°C, respectively. A 1 µl volume of a diluted sample in dichloromethane (1:1000, v/v) was injected in splitless mode, and helium served as the carrier gas at a flow rate of 1 ml/min. Identification of individual compounds involved matching the mass spectra to Kováts retention indices (RI) in relation to a mixture of n-alkanes (C8-C20). All compounds were identified by comparing their obtained mass spectra with the Wiley & NIST databases. Compounds were selected based on their peak areas in the gas chromatogram, with a minimum threshold of 90%.

Preparation of bacterial pathogens

The bacterial isolates employed in this method consisted of field strains, specifically *Staphylococcus Coagulase* Negatives, including *S. chromogenes* (SC-1), *S. chromogenes* (CNS11), *S. chromogenes* (CNS32), and *Streptococcus* species, comprising *S. uberis* (SU-1: KM8.4), *S. uberis* (SU-2: KM9), and *S. uberis* (SU-3: KD13.2). All these strains were originally isolated from cases of bovine mastitis in Chiang Mai, Thailand. They were cultured on non-selective agar media (5% bovine blood agar, Nutrient agar, Tryptic Soy agar) and then incubated at 37°C for 24 hours. Subsequently, the inoculum was suspended in 9 ml of 0.85% Normal Saline Solution (NSS) to achieve a final concentration of 10^7-10^8 CFU/ml, with a McFarland density of 0.5.

Antibacterial assays

The essential oils were diluted from a stock solution (37.5% v/v) using various solvents (i.e., Tween20; 19.0% v/v, DMSO; 6.0% v/v, TSB; 37.5% v/v). This dilution resulted in a macroemulsion with an opaque appearance. The suspension was further diluted with broth to reach concentrations ranging from 37.5% to 0.15% v/v in 96-well plates. The mixture of Tween 20; 19.0% v/v, DMSO; 6.0% v/v, TSB; 37.5% v/v was used as vehicle control. Each well was then inoculated with 20 µl of bacterial suspension, yielding a final inoculum size of 10^5 CFU/ml, followed by incubation at 37°C for 24 hours. The MIC and the MBC evaluations were carried out in triplicate for each bacterial strain and antimicrobial agent. The MIC was defined as the lowest concentration of herb extraction oil that visibly inhibited bacterial growth in the broth. For the MBC, 10 µl of transparent medium was streaked on TSA/bovine blood agar (for *Streptococcus* spp.) and incubated at 37°C for 24 hours. The MBC was determined as the lowest concentration of herb extraction oil that inhibited bacterial growth on the agar medium, as shown in Figure 1.

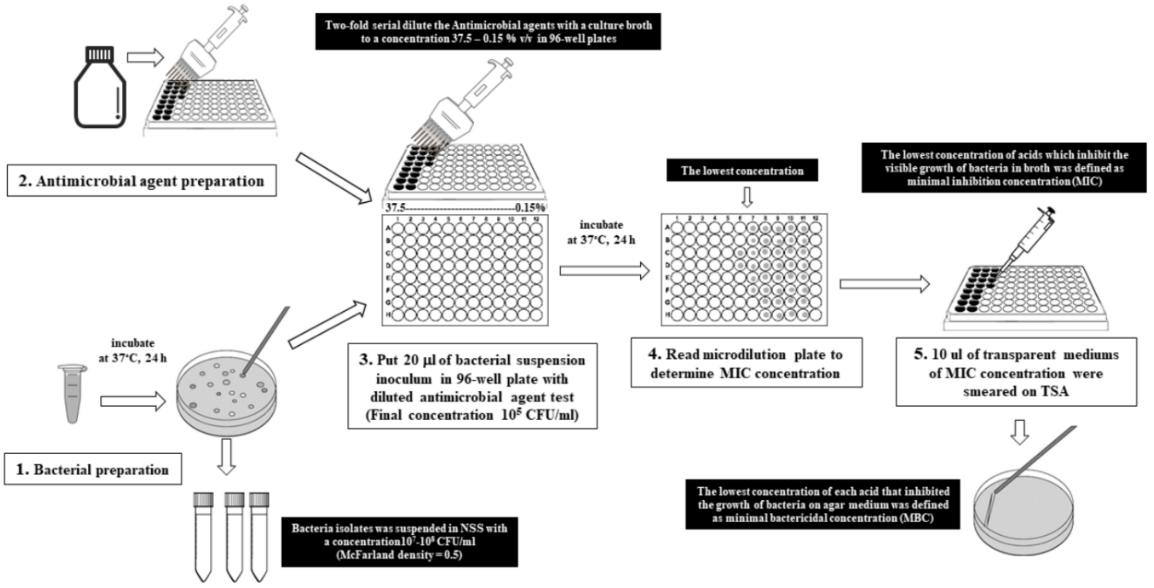

Figure 1 MIC and MBC determination of the essential oils of *E. javanica* Lam. and *E. paniala* Roxb. leaves by broth microdilution method

Statistical analysis

The MIC and MBC distributions were described using Mean ± standard error (SE). Statistical analysis of two distinct samples was carried out using the Mann-Whitney U test. A statistical significance level of 0.05 was considered.

RESULTS

Plant material

E. javanica Lam. and *E. paniala* Roxb. leaves used in this study were collected from San Sai, Chiang Mai and Mae Lao, Chiang Rai, Thailand. Preliminary examination through organoleptic testing, which involves sensory inspection, revealed the following characteristics. The *E. javanica* Lam. leaves are arranged in an opposite pattern and thick with dark green glossy topside (Figure 2a) and a light green underside (Figure 2b). Fresh leaves have a unique essence of *E. javanica* Lam., while *E. paniala* Roxb. leaves are oblong with parallel edges and a dark green glossy topside (Figure 2c) and a light green underside (Figure 2d). Fresh leaves have a unique scent of *E. paniala* Roxb., and *E. paniala* Roxb leaves are oiler than *E.javanica* Lam. leaves. Both of specimens collected for this study are being preserved as voucher specimens under the code B.Saeng-002 and B.Saeng-003 at the Royal Botanic Garden, Queen Sirikit Botanic Garden, Mae Rim District, Chiang Mai, Thailand.

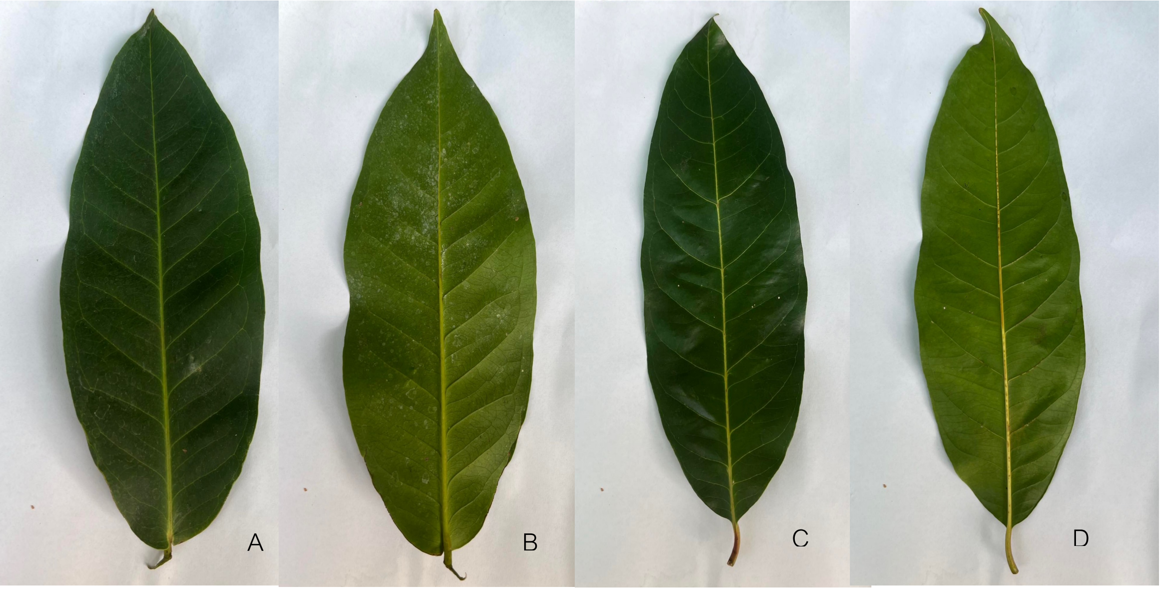

Figure 2 Topside (A) and underside (B) of *E. javanica* Lam. leaves and Topside (C) and underside (D) of *E. paniala* Roxb. leaves

Extraction and chemical identification of essential oils

Through the hydrodistillation extraction process, the crude oils yielded 0.05% ± 0.01% (w/w) from *E. javanica* Lam. leaves and 0.12% ± 0.01% (w/w) from *E. paniala* Roxb. leaves based on fresh weight. Both of essential oils exhibited a light-yellow hue and a unique aroma. The yield of these essential oils is related to the morphology of their leaves, with *E. paniala* Roxb. leaves being oiler than those of *E. javanica* Lam. Using the elution order on the HP-5MS column, a total of 30 significant peaks were identified, and the compounds characterized for 21 peaks in the essential oil of of *E. javanica* Lam. leaves through GC-MS analysis. The height of each peak varied when comparing the mass spectrum of each peak, along with comparing the KI value and comparing it to the mass spectrum of standard substances in the NIST and WILEY databases. The GC-MS database was used to determine the type of substance and the relative peak area (%) utilized to compare the quantities of each type of compound found in the essential oil. The findings revealed that in the essential oil from *E. javanica* Lam. leaves, γ-Terpinene was the main component, accounting for 30.78%. Following this, p-Cymene (12.28%), (E)-Caryophyllene (8.84%), β-Pinene (8.18%), Bicyclo (4,4,0)-dec-1-ene, 2-isopropyl-5-methyl-9-methylene (7.19%), Terpinolene (4.78%), Terpinene-4-ol (4.12%), alpha-Cadinol (3.45%), δ-cadinene (2.46%), caryophyllene oxide (1.86%), α-Terpinene (1.55%), α-Phelladrene (1.23%), α-Amorphene (1.14%), α-Copaene (0.98%), Limonene (0.73%), α-Terpineol (0.71%), β-Selinene (0.68%), Linalool (0.50%), trans-β-Ocimene (0.45%), and (+)-Aromadendrene (0.44%), as shown in Table 1.

Additionally, a total of 21 significant peaks were identified, and the compounds were characterized for 16 peaks from the analysis of GC-MS of the crude oil of *E. paniala* Roxb. leaves. The height of each peak varied when comparing the mass spectrum of each peak, and the KI value, along with comparing it to the mass spectrum of standard substances in the NIST and WILEY databases. The GC-MS database was used to determine the type of substance, and the relative peak area (%) utilized to compare the quantities of each type of compound found in the essential oil. According to the findings, the essential oil from *E. paniala* Roxb. leaves, trans-β-ocimene was the main component, accounting for up to 57.29%. Following this, trans-caryophyllene (7.42%), linalool (4.60%), caryophyllene oxide (3.85%), β-selinene (1.71%), (+)-aromadendrene

(1.48%), β-myrcene (1.39%), α-cadinol (1.23%), (Z)-9-octadecenamide (0.76%), and para-cymene (0.60%) were identified in descending order, as shown in Table 2.

Table 1 Chemical composition of the essential oil of *E. javanica* Lam. Leaves

Peak number	Retention time	KI^a	KI^b	Compounds	Area (%)	QA (%)
1	3.97	941	941	α-Pinene	2.97	94
2	4.12	978	978	β-Pinene	8.18	97
3	5.09	-	-	Unidentified	0.36	96
4	5.40	-	-	Unidentified	0.55	96
5	5.79	1009	1003	β-Phelladrene	1.23	97
6	6.14	1022	1017	α-Terpinene	1.55	98
7	6.40	1032	1030	p-Cymene	12.28	95
8	6.50	1035	1029	Limonene	0.73	97
9	6.76	1043	1050	trans-β-Ocimene	0.45	97
10	7.48	1066	1060	γ-Terpinene	30.78	97
11	8.43	1092	1089	Terpinolene	4.78	98
12	9.00	1108	1097	Linalool	0.49	95
13	11.93	1185	1177	Terpinene-4-ol	4.12	98
14	12.55	1198	1189	α-Terpineol	0.71	91
15	21.61	1420	1420	(E)-Caryophyllene	8.84	99
16	22.97	1454	1490	β-Selinene	0.68	98
17	24.65	1494	-	Unidentified	0.22	< 80
18	25.43	1515	1485	α-Amorphene	1.14	98
19	25.82	1526	1523	δ-Cadinene	2.46	98
20	28.08	1584	1583	Caryophyllene oxide	1.86	95
21	28.21	1588	1580	(+)-Aromadendrene	0.44	91
22	29.37	1619	-	Unidentified	0.98	89
23	29.86	1632	-	Unidentified	1.17	< 80
24	30.43	1648	1648	Bicyclo (4,4,0)-dec-1-ene, 2-isopropyl-5-methyl-9-methylene	7.19	93
25	30.62	1653	1377	α-Copaene	0.98	97
26	30.94	1662	1640	α-Cadinol	3.45	98
27	46.57	-	-	Unidentified	0.32	93
28	47.89	-	-	Unidentified	0.79	< 80
29	47.99	-	-	Unidentified	0.17	< 80
30	48.31	-	-	Unidentified	0.12	< 80
				Total identified	99.99	

KI^a	Kovat retention index from NIST and WILEY database
KI^b	Kovat retention index from experiment
QA	Matching quality in NIST and WILEY database

Table 2 Chemical composition of the essential oil of *E. paniala* Roxb. Leaves

Peak number	Retention time	KI^a	KI^b	Compounds	Area (%)	QA (%)
1	3.34	-	-	Unidentified	0.30	< 80
2	5.40	988	989	β-Myrcene	1.39	96
3	6.39	1031	1025	para-Cymene	0.60	95
4	6.49	1034	1034	Benzocyclopentadiene	0.49	<80
5	6.77	1043	1042	m-Diethylbenzene	6.50	97
6	7.13	1055	1050	trans-β-Ocimene	57.29	97
7	8.43	1092	1093	Undec-1-ene	0.86	98
8	8.84	1103	1102	n-Undecane	0.58	< 80
9	9.00	1108	1097	Linalool	4.61	91
10	13.12	1214	1214	Triethylbenzene	0.75	91
11	13.41	1221	1227	Citronellol	7.76	95
12	16.15	1287	1287	Pentamethylbenzene	1.19	< 80
13	21.60	1419	1419	trans-Caryophyllene	7.42	99
14	22.98	1454	1490	β-Selinene	1.71	98
15	28.08	1584	1583	Caryophylline oxide	3.84	93
16	28.21	1587	1490	(+)-Aromadendrene	1.48	84
17	30.40	1647	1640	α-Cadinol	1.24	91
18	44.46	-	-	Unidentified	0.49	< 80
19	46.57	-	-	Unidentified	0.76	< 80
20	46.71	-	-	Unidentified	0.21	< 80
21	47.74	-	-	Unidentified	0.53	< 80
				Total identified	100.00	

KI^a	Kovat retention index from NIST and WILEY database
KI^b	Kovat retention index from experiment
QA	Matching quality in NIST and WILEY database

Antibacterial assays

Regarding the evaluation of antibacterial activity assay, the essential oils of *E. javanica* Lam. leaves and of *E. paniala* Roxb. leaves showed strong inhibitory properties against *S. chromogenes* and *S. uberis* for mastitis in cows, as shown in Table 3. Bacterial growth was considerably inhibited with concentrations between 0.15 and 37.5 %v/v. Regardless of *S. chromogenes* and *S. uberis* for mastitis in cows, the MIC values (0.78 and 4.68 %v/v) were not statistically significant ($p > 0.05$) from the MBC values (9.37 and 37.50 %v/v) for the essential oils of *E. javanica* Lam. leaves, and the MIC value (2.34 %v/v) was also not statistically significant ($p > 0.05$) from the MBC value (37.50 %v/v) for the essential oils of *E. paniala* Roxb. leaves. Therefore, MIC values were almost at biostatic levels against *S. chromogenes* and *S. uberis*. Otherwise, the MIC values (1.56 %v/v) was significantly different ($p < 0.05$) from the MBC value (3.12 %v/v) for the essential oils of *E. paniala* Roxb. leaves. This means the MIC values were almost at biocidal levels against *S. chromogenes*.

Table 3 MICs and MBCs values of the essential oils of *E. javanica* Lam. and *E. paniala* Roxb. Leaves

Bacteria (n = 3)	**Essential oil of *Eugenia javanica* L. leaves**		**Essential oil of *Eugenia paniala Roxb* leaves**	
	MIC (%v/v)	**MBC (%v/v)**	**MIC (%v/v)**	**MBC (%v/v)**
Staphylococcus chromogenes	0.78 ± 0.34	9.37 ± 0.00	1.56 ± 0.68	3.12 ± 1.35
Streptococcus uberis	4.68 ± 0.00	37.50 ± 0.00	2.34 ± 0.00	37.50 ± 0.00

Data are represented as mean ± standard error

DISCUSSION

Numerous medicinal plants have garnered considerable attention as a valuable source of natural bioactive compound for the discovery of new medicine to cure or prevent disease in both humans and animals, especially for treating infection. Secondary metabolites extracted from plants, such as terpenes, aldehydes, phenolic, terpenoids, have demonstrated the therapeutic properties and pharmacological activities (Chassagne et al., 2021; Murugaiyan et al., 2022). In addition, the type and amount of chemical substance and extract yield have exhibited considerable variability, influenced by many factors such as type and part of plants, environmental conditions (climate, pressure, humidity, and edaphic), and bioclimatic conditions of the region (Labhar et al.2022; Mkaddem et al. 2022). The present study reveals the chemical components in the extracted oil of *E. javanica* Lam. and *E. paniala* Roxb. leaves, collected from Northern part of Thailand.

The percentage yield obtained from the essential oil of *E. javanica* Lam. leaves was lower than the 0.63% previously reported in Nigeria (Moses et al., 2013) but higher than another study in Thailand of 0.03% (Suksamrarn, 1987). This may be due to growing habitats and seasonal variation (Matias et al., 2016; Dhouioui et al., 2016). The essential oil found in *E. javanica* Lam. leaves from Nigeria had a completely different composition, comprised largely of α-terpineol (14.1%), terpinen-4-ol (7.2%), (E)-caryophyllene (6.6%), α-cadinol (12.2%), caryophyllene oxide (9.6) and 1-epicubenol (6.0%). (Suksamrarn et al., 1987). Otherwise, the major component of this essential oil correlated with a previous study from Thailand that explored largely component of γ-terpinene (28.5%) in the essential oil of *E. javanica* Lam. leaves (Suksamrarn et al., 1987). The variations in quantitative results compared to previous studies may stem from differences in geographic or environmental conditions, genetic diversity, analytical techniques, and extraction methods of *E. javanica* Lam. leaves. Notably, this study contributes new data on the essential oil composition of *E. paniala* Roxb. leaves. Trans-β-ocimene emerges as a predominant component, consistent across various species within the genus (Alencar et al., 1996; Maia et al., 2002; Rodriguez-Burbano et al., 2010). Moreover, in the present study, the *in vitro* antibacterial activity screening of oil derived from *E. javanica* Lam. and *E. paniala* Roxb. leaves were investigated isolated mastitis pathogens. Based on the results, this is the first evidence to support the antibacterial activity against *S. chromogenes* and *S. uberis* mastitis-associated pathogens. The MIC and MBC values demonstrated that the extracted oils have antibacterial activity against bovine mastitis isolates. Otherwise, all vehicles showed no antibacterial activity as the negative control (data not shown). Regarding the positive control, this study represents the initial investigation into the chemical composition and antibacterial properties of essential oils from the leaves of *E. javanica* Lam. and *E. paniala* Roxb., collected from Northern Thailand. The preliminary antibacterial study focused on evaluating the antibacterial effects of these two oils. Consequently, there has yet to be a comparative study between the antibacterial properties of the oils and a positive control. Typically, substances used to combat mastitis infections include antiseptics like iodine and povidone-iodine (PVP) (Skowron et al., 2019; Sharun et al., 2021). In future, the researchers plan to test the efficacy of these essential oils against positive controls to provide essential information for determining the appropriate dosage of the oils extracted from the leaves of *E. javanica* Lam. and *E. paniala* Roxb., sourced from Northern Thailand, for treating mastitis caused by *Staphylococcus* and *Streptococcus* infections. From these antibacterial results, the correlation between composition and activity suggests that the antimicrobial properties may be attributed to the main monoterpene constituents: γ-Terpinene (30.78%), p-Cymene (12.28%), (E)-Caryophyllene (8.84%), and β-Pinene (8.18%) for *Eugenia javanica* L., and trans-β-ocimene (57.29%), trans-caryophyllene (7.42%), and linalool (4.60%) for *E. paniala* Roxb. These findings imply that the combined action of various bioactive compounds in the essential oils may contribute to their antimicrobial effects, potentially through synergistic interactions.

Previous studies have demonstrated the effectiveness of medicinal plants against isolates of bacterial causing bovine mastitis. The current observation agrees with previous studies detecting the extracted oil against the isolated pathogens from bovines (Caneschi et al., 2023). Tomani´c et al. (2022) in the Republic of Serbia reported the antibacterial activity against bovine mastitis pathogens of the essential oil from *Origanum vulgare* L., *Thymus serpyllum* L., and *Thymus vulgaris* L. from the Lamiaceae family, the dominant components being carvacrol (78.94%), thymol (46.37%), and thymol (55.11%), respectively. Furthermore, the results of this present research are similar to those obtained by Corona-Gómez et al. (2022), who reported that tea tree (*Melaleuca alternifolia)* oil also performed microbicidal activity against field isolates of *Staphylococcus* spp. *Streptococcus* spp. from cases of bovine clinical mastitis. Similarly, Montironi et al. (2016) demonstrated that the essential oil of *Minthostachys verticillata* and one of its compounds, limonene, inhibited the growth of *S. uberis,* causing bovine mastitis in Argentina (Montironi et al., 2016). Further research is warranted to evaluate the antibacterial activity of these compounds individually or in combination and to elucidate their mechanism of action against bovine pathogens. This includes investigating potential mechanisms such as the disruption of bacterial cell membranes, inhibition of metabolic pathways, or modulation of gene expression.

Based on the results of this present work, reaffirming the role of natural products, the bioactive substances in the extract from medicinal plants performed antibacterial activity against bovine mastitis, opening a new opportunity for their application in the treatment and control of bovine mastitis. This approach aims to minimize reliance on antibiotics and harmful chemicals, thereby mitigating their adverse impacts on dairy farming, consumers, and the environment. However, an appropriate dose of the essential oils should be determined to avoid adverse effects.

CONCLUSIONS

The present research revealed that the oil extracted from *E. javanica* Lam. and *E. paniala* Roxb. leaves, collected from Northern part of Thailand, as well as terpenoids, one of their main components, performed antibacterial efficacy against *S. chromogenes* and *S. uberis*. These results support the importance of bioactive compounds isolated from medicinal plants and their influence on the elimination of microorganisms. Utilizing these essential oils may reduce reliance on antibiotics and harmful chemicals. Further research is required to evaluate the antibacterial efficacy of these compounds individually or in combination and to elucidate the potential mechanisms of action against bovine pathogens and the toxic effects of the active compounds of these extracted oils. Moreover, a pharmaceutical preparation of the extracted oil could be generated with the accurate dosage calculated to make its application convenient in the administered clinical treatment of livestock.

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AUTHOR CONTRIBUTIONS

Details of each author with his/her contribution to this paper are mentioned as follows;

Conceptualization: Banthita Saengsitthisak (BS), Wasana Chaisri (WC), Sukolrat Boonyayatra (SB)

Methodology: Banthita Saengsitthisak (BS), Wasana Chaisri (WC), Noppason Pangprasit (NP), Surachai Pikulkaew (SP), Nuttakorn Baisaeng (NB)

Writing—original draft preparation: Nuttakorn Baisaeng (NB)

Writing—review and editing: Banthita Saengsitthisak (BS), Wasana Chaisri (WC), Sukolrat Boonyayatra (SB), Noppason Pangprasit (NP), Surachai Pikulkaew (SP), Nuttakorn Baisaeng (NB)

Visualization: Wasana Chaisri (WC), Sukolrat Boonyayatra (SB), Noppason Pangprasit (NP), Surachai Pikulkaew (SP), Nuttakorn Baisaeng (NB)

Supervision: Banthita Saengsitthisak (BS)

Project administration: Banthita Saengsitthisak (BS), Wasana Chaisri (WC)

All authors have read and agreed to the published version of the manuscript

CONFLICT OF INTEREST

The authors assert that there are no conflicts of interest.

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