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Antibacterial Activity of Combined Essential Oils of Cinnamon (*Cinnamomum verum*) and Tamanu (*Calophyllum inophyllum*) Against Acne-Causing Bacteria

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Abstract: Antibacterial agents are microbial metabolism inhibitors that are present in essential oils produced by Cinnamon (*Cinnamomum verum*) and Tamanu (*Calophyllum inophyllum*). The objective of this research is to investigate the secondary metabolites and the antibacterial activity of a combination of cinnamon and tamanu essential oils (CTE) against Staphylococcus aureus ATCC 6538 and Staphylococcus epidermidis ATCC 12228. The essential oils of cinnamon and tamanu were analyzed by GC–MS. The combination of the two essential oils was prepared in three different concentrations (1.25, 2.5, and 5%) with five ratios (1:1, 1:2, 1:3, 2:1, and 3:1) for each concentration. The antibacterial test was conducted using the disk diffusion method. The results of the GC–MS analysis showed that the essential oil of cinnamon contained cinnamaldehyde as the main compound (82.59%), while the essential oil of tamanu contained linoleic acid (48.29%). The antibacterial test demonstrated that the CTE at a concentration of 5% in a 3:1 ratio exhibited the highest inhibitory effect against S. aureus ATCC 6538 with an inhibition diameter of 11.25 mm and S. epidermidis ATCC 12228 with an inhibition diameter of 11.25 mm.

Keywords: Antibacterial activity combination; Cinnamon; *Staphylococcus epidermidis*; Tamanu

Introduction

Acne vulgaris is a common dermatological condition among people living in tropical areas (Yang et al., 2020). This condition affects the sebaceous glands and hair follicles of the skin, which explains its predominant occurrence in areas of the body that contain a large number of sebaceous glands, such as the face and back (Setiawan et al., 2025). Acne vulgaris is typically manifested by skin eruptions accompanied by redness and blackheads. It is caused by the proliferation of bacteria, namely S. aureus and S. epidermidis, within the hair follicles and sebaceous glands. S. epidermidis was previously considered a harmless microorganism on human skin (Abdulatif et al., 2023). Currently, however, this bacterium is responsible for chronic infections at a prevalence that is comparable to that of *S. aureus* (Thomas, 2023).

The long-term use of antibiotics to treat acne is no longer a viable option due to the alarming rise in antibiotic resistance. The emergence of antibiotic resistance is the result of interactions among several genetic and environmental factors, including the interaction between bacteria and specific antibiotics, the

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host species, environmental influences, and the manner in which antibiotics are utilized (Issusilaningtyas et al., 2025). Medicinal plants have been extensively investigated as potential alternatives for the treatment of diseases, particularly to address the challenge of antibiotic resistance (Dessinioti & Katsambas, 2022). This research evaluated the antibacterial activity of the essential oils of cinnamon (Cinnamomum verum) and tamanu (*Calophyllum inophyllum*) that have traditionally been used as antimicrobial agents.

Essential oils have been widely employed as therapeutic and medicinal agents. The antibacterial activity of essential oils is exhibited not only by several compounds they contain, including terpenes, alcohols, aldehydes, ketones, and aromatic hydrocarbons. The antibacterial activity of essential oils can target a range of cellular components simultaneously, leading to the death of bacteria (Angane et al., 2022). Essential oils that have been proven to have the potential to treat acne vulgaris are the essential oil of cinnamon (*Cinnamomum verum*) and the essential oil of tamanu (*Calophyllum inophyllum*). The secondary metabolites present in the essential oils derived from these two plant species have been shown to possess antibacterial properties. Secondary metabolites are protective compounds that are produced by plants to defend themselves against competitors and pathogens. These compounds include cinnamaldehyde, cinnamic acid, cinnamate, and volatile oil components, such as eugenol, cinnamyl acetate, trans-cinnamaldehyde, l-borneol, camphor, β-caryophyllene, caryophyllene oxide, l-bornyl acetate, α-cubebene, α-terpineol, e-nerolidol, α-thujene, and terpinolene (Vasconcelos et al., 2018).

One volatile oil component cinnamaldehyde, is an unsaturated aldehyde with acrolein groups (alpha, beta-unsaturated carbonyl groups) that exhibits antibacterial activity. Trans-cinnamaldehyde is susceptible to instability and volatility upon exposure to air. This is due to the reactive nature of the unsaturated aldehyde, which is prone to oxidation to cinnamic acid. Such instability may also occur in vivo prior to the exertion of its bactericidal effect, as the absorbed trans-cinnamaldehyde can be rapidly and irreversibly converted to cinnamic acid through enzyme-catalysed process, thus rendering it unstable in the blood (Vasconcelos et al., 2018).

Cinnamon substances with low antibacterial activity include benzaldehyde, cinnamyl acetate, and coumarins. Given that mixtures of different components can exert effects even in small quantities or exhibit antibacterial activity only when combined, these small components may be able to improve the effect of trans-cinnamaldehyde or serve other functions within bacterial cells (Atmanto, 2019). Alternatively, tamanu essential oil triglycerides are distinguished by their fatty acid composition, which includes palmitoleic acid, stearic acid, palmitic acid, linoleic acid, oleic acid, arachidonic acid, alpha-linoleic acid, gadoleic acid, dihomo-gamma-linolenic acid, docosadienoic acid and behenic acid (Tran et al., 2018).

Tamanu essential oil is notable for its relatively high proportion of stearic acid, with saturated fatty acids (SFA) representing the primary components. Unsaturated fatty acids, including polyunsaturated fatty acids (PUFAs) and monounsaturated fatty acids (MUFAs), are present in substantial quantities, particularly in the forms of oleic acid and linoleic acid. Tamanu essential oil has been purported to possess beneficial properties for addressing skin-related pathogens, attributed to its antibacterial and antifungal properties. Tamanu oil has shown high antibacterial activity against bacteria associated with skin diseases. The excellent antibacterial activity was evidenced by the oil's efficacy against a range of aerobic gram-positive bacterial strains, including *S. aureus*, a multidrug-resistant bacterium linked to hospital and skin infections, *S. epidermidis*, a causative agent of catheter-related infections, and *C. minutissimum,* a bacterium associated with catheter-related infections (Raharivelomanana et al., 2018).

Both cinnamon and tamanu essential oils have been demonstrated to possess inhibitory activity against bacteria that are etiological agents of acne. This research was conducted to evaluate test the antibacterial activity of a combination of essential oils from cinnamon (*Cinnamomum verum*) and tamanu (*Calophyllum inophyllum*) against bacteria associated with acne vulgaris. The results are expected to inform the development of various facial care formulations in future research.

Method

Preparations of Cinnamon Essential Oils

Cinnamon oil was obtained from bark through steam distillation (Do et al., 2024). An amount of 3 kg of cinnamon sticks was cut into small pieces with a size of 1–2 cm and then put into a distillation flask containing water, then the distillation flask was connected to a generator and condenser. The distillation process was carried out at a temperature of 100°C for 5 hours. The mixture obtained was collected in a container and allowed to settle and was separated from the oil using a separating funnel. The essential oil settled in the lower layer of the separating funnel and was separated several times until the essential oil was obtained (Hariroh & Marzuki, 2021).

Preparations of Tamanu Essential Oils

Tamanu seeds were extracted using the cold-pressed method. The dried tamanu seeds were then pressed until the oil was released. This oil was used in research.

Identification of Essential Oil Compounds

The compounds of cinnamon and tamanu essential oils were identified using gas chromatography–mass spectrometry (GCMS), which was conducted using the Agilent Technologies 7890 gas chromatograph with an autosampler and a 5975-mass selective detector, as well as the Chemstation Data System instrument, HP Ultra 2. The capillary column length was 30 m with an internal diameter of 0.20 mm and a film thickness of 0.11 µm. The temperature increased from 80 to 150°C at a rate of 3°C/min, and then from 150 to 280°C over the course of 26 minutes. The process was conducted for a duration of 10 minutes at a temperature of 280°C, with helium serving as the carrier gas at a flow rate of 1.2 mL/min. The identification process, using GC-MS equipment, revealed the presence of bioactive compounds, as evidenced by the chromatogram peaks, which served as the basis for chromatographic identification, and the mass spectrum, which provided the molecular weight of each bioactive compound, thus establishing mass spectrometry (MS) identification (Fachriyah et al., 2023).

Preparation of Samples Essential Oil with Varied Concentrations

The essential oils of cinnamon and tamanu were prepared in three variations of concentration: 1.25, 2.5, and 5%v/v with the solvent being DMSO. Essential oil combinations were also prepared using cinnamon and tamanu essential oils with a ratio of 1:1, 1:2, 1:3, 2:1, and 3:1. Clindamycin antibiotics at 5% w/v were used as positive control, and DMSO was used as a negative control.

Preparation for Bacterial Suspension

The preparation of the bacterial suspension was done by mixing 0.9% NaCl into the bacterial culture in a test tube until the turbidity was the same as the McFarland 0.5 standard. The absorbance of the bacterial suspension was measured using a spectrophotometer with a wavelength (λ) of 625 nm and absorbance of 0.08-0.10, which, was equivalent to the 1.5 x10^8 CFU/ml (Fachriyah et al., 2023).

Antibacterial Activity

The antibacterial activity test was conducted using the disc diffusion method and repeated 3 times. The bacterial test suspension was equated to the McFarland 0,5 standard (1.5x10^8 CFU/ml) (Dayamrita, 2021). To prepare the solid medium, Mueller Hinton agar (MHA) was dissolved using distilled water and sterilized in an autoclave at 121°C for 15 minutes. After sterilization, MHA medium was solidified in a petri dish, and then the bacterial suspension was spread on the surface of the MHA medium using a sterile cotton swab (Harahap et al., 2025). After inoculation, the plates were left to allow the bacteria to be absorbed into the solid media. Then, paper disks were dripped with 20 µL of each test sample and the positive control (1% clindamycin antibiotic) and the negative control (DMSO). The bacteria on the MHA were incubated for 24 hours at 37°C (Alvionita et al., 2020). Antibacterial activity was observed by the presence of an inhibition zone (a clear zone). The inhibition zone was categorized as weak with a diameter of less than 5 mm, a diameter of 6–10 mm was categorized as medium, a diameter of 11–20 mm was categorized as strong, and a very strong category of 20 mm (Dafale et al., 2016).

Results and Discussion

GCMS

GCMS is used to perform accurate qualitative analysis of the content of various aromatic compounds and essential oils (Mutlu et al., 2023). The compounds in cinnamon and tamanu essential oils were analyzed using GCMS with a 30 m column filled with helium. Based on the GCMS results, cinnamon essential oil showed 4 peaks, and tamanu essential oil showed 9 peaks, which can be seen in Figure 1.

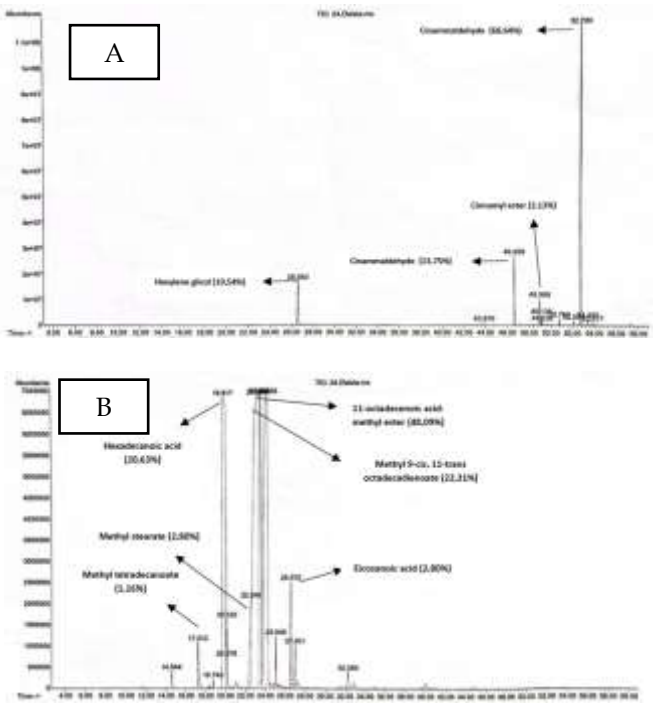

Figure 1. Chromatogram of cinnamon essential oil (A) and tamanu essential oil (B)

The compounds of cinnamon essential oil included coumarin (1.07%), n-hexadecanoic acid (1.12%), hexylene glycol (10.54%), cinnamyl ester (2.13%), and cinnamaldehyde (82.59%). According to the SNI, the requirements for cinnamaldehyde in cinnamon essential oils are not less than 50% (Badan Standardisasi Nasional, 2006). Cinnamaldehyde is the main component of cinnamon essential oil and is a natural compound that gives a sweet, spicy taste and a distinctive aroma. Cinnamaldehyde has various biological properties, such as antitumor, antimicrobial, antifungal, antimutagenic effects and cytotoxic effects (Do et al., 2024). The cinnamaldehyde compound has been shown to have antibacterial activity on *S. aureus* and *E. Coli* by damaging the microbial cell membranes, thereby affecting permeability and ultimately leading to cell damage (Kim et al., 2022). Components of tamanu essential oil included methyl tetradecanoate (1.16%), methyl stearate (2.80%), hexadecanoic acid (20.63%), eicosanoic acid (2.08%), vaccinic acid (22.31%), and linoleic acid (48.09%).

Antibacterial Activity

The antibacterial activity of the essential oils against *S. aureus* ATCC 6538 and *S. epidermidis* ATCC 12228 was assessed using the disk diffusion assay. The results are presented in Table 1.

Table 1. Antibacterial activities of Cinnamon and Tamanu essential oils

Bacterial	Sample	Diameter of Inhibition Zone (mm)		
		1.25%	2.5%	5%
S. aureus ATCC 6538	DMSO (-)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Clindamycin (+)	14.50 ± 0.25	14.50 ± 0.25	14.50 ± 0.25
	Cinnamon oil	5.50 ± 0.25	6.25 ± 0,25	8.25 ± 0,25
	Tamanu oil	2.50 ± 0.25	5.25 ± 0.25	6.25 ± 0.35
	CTE (1:1)	2.38 ± 0.18	6.25 ± 0.25	8.42 ± 0.38
	CTE (1:2)	1.88 ± 0.14	5.13 ± 0.18	7.42 ± 0.38
	CTE (1:3)	3.25 ± 0.25	5.25 ± 0.35	6.65 ± 0.13
	CTE (2:1)	5.42 ± 0.38	6.42 ± 0.38	9.42 ± 0.38
	CTE (3:1)	5.88 ± 0.18	7.50 ± 0.25	11.25 ± 0.25
S. epidermidis ATCC 12228	DMSO (-)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Clindamycin (+)	14.75 ± 0.25	14.75 ± 0.25	14.75 ± 0.25
	Cinnamon oil	5.50 ± 0,25	7.50 ± 0.25	8.25 ± 0.25
	Tamanu oil	3.50 ± 0.25	4.42 ± 0.38	7.25 ± 0.25
	CTE (1:1)	3.25 ± 0.25	6.25 ± 0.25	9.25 ± 0.25
	CTE (1:2)	1.25 ± 0.25	5.25 ± 0.25	7.25 ± 0.25
	CTE (1:3)	2.25 ± 0.25	6.50 ± 0.25	7.75 ± 0.25
	CTE (2:1)	3.33 ± 0.14	7.25 ± 0.25	9.42 ± 0.38
	CTE (3:1)	4.50 ± 0.25	8.00 ± 0.25	11.25 ± 0.25

CTE: cinnamon and tamanu essential oils

The results of the antibacterial activity test, as presented in Table 1, showed that the combination of cinnamon and tamanu essential oils (CTE) provided a larger clear zone compared to the oils used individually against S. aureus ATCC 6538 and *S. epidermidis* ATCC 12228. The most effective combination of cinnamon and tamanu essential oils (CTE) against *S. aureus* ATCC 6538 and S. epidermidis ATCC 12228 was at a concentration of 5% and a ratio of 3:1 with an inhibition zone diameter of 11.25 mm. The inhibition zone category consists of four categories, namely weak if the diameter is less than 5 mm, medium if the diameter is 6–10 mm, strong if the diameter is 11–20 mm, and very strong if the diameter is greater than 20 mm (Dafale et al., 2016). Therefore, the combination of CTE was included in the strong antibacterial group, which means that the combination of cinnamon and tamanu essential oils is effective in inhibiting the growth of *S. aureus* ATCC 6538 and *S. epidermidis* ATCC 12228 bacteria as an anti-acne agent.

The positive control (clindamycin) showed a strong antibacterial effect with an inhibition zone diameter 14.50 mm on *S. aureus* ATCC 6538 and 14.75 on and *S. epidermidis* ATCC 12228. The antibiotic clindamycin is one of the most common and effective antibiotics used for acne vulgaris (Leonita et al., 2022), whereas the negative control (DMSO) showed no inhibition zone, because DMSO does not have antibacterial properties and only functions as a solvent for essential oils. Therefore, it did not affect the inhibition zone of essential oils.

Cinnamaldehyde in cinnamon essential oil and linoleic acid in tamanu essential oil can work synergistically in inhibiting the growth of *S. aureus* ATCC 6538 and *S. epidermidis* ATCC 12228. Cinnamaldehyde in cinnamon essential oil is an

electronegative molecule that interferes with cellular biological processes containing nitrogen, such as proteins and nucleic acids. Cinnamon essential oil kills bacteria by reducing cell division, ATPase, porin membrane biofilm formation and mobility, and changing the lipid profile of bacteria (Khasanah et al., 2021). Linoleic acid in tamanu essential oil works to maintain the permeability of the stratum corneum which can reduce the occurrence of acne caused by dirt, toxins, or damaged skin. Linoleic acid can accelerate the production and release of β-defensin 2 from macrophages. β-Defensin 2 acts as a natural antibiotic of the immune system, damaging gram negative bacterial membranes and inhibiting mitosis of *S. aureus* ATCC 6538 and *S. epidermidis* ATCC 12228, and slowing the synthesis of nucleic acids and proteins in bacterial cells (Marciano et al., 2024).

Conclusion

The results of the GCMS compound analysis showed that the essential oil of cinnamon contained the main compound cinnamaldehyde (82.59%), while the essential oil of tamanu contained linoleic acid (48.29%). The antibacterial test demonstrated that the CTE at a concentration of 5% in a 3:1 ratio exhibited the most inhibitory effect against *S. aureus* ATCC 6538 and *S. epidermidis* ATCC 12228 with an inhibition zone diameter of 11.25 mm (strong antibacterial activity). Based on the results of the antibacterial activity test, CTE has the potential to be developed into an anti-acne cream formulation for further research.

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Author Contributions
Conceptualization, S.M., Z.H., M.S., and D.S.; methodology, Z.H. and M.S.; contributed in analyzing data, S.M., Z.H., and M.S.; contributed in writing, reviewing, and editing the article, S.M. and D.S.

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Conflicts of Interest
The authors declare that there is no conflict of interest regarding the publication of this article.

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