Research Article

In vitro and *In silico* Antibacterial Potency of *Eucalyptus* Leaf Oil from Hybrid Clones of *E. grandis* with *E. urophylla* and *E. pellita*

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Abstract
Hybridization can introduce beneficial traits from different species, such as increased disease resistance, yield, or stress tolerance. This study aimed to describe the effect of hybridization on the essential oil yield, phytochemical components, *in vitro* and *in silico* antibacterial activity of essential oil extracted from *Eucalyptus* leaves from various clones against 4 different types of bacteria. The *E. grandis-E. pellita* clones were characterized by higher abundances of α-pinene and α-terpineol. Meanwhile, the *E. grandis-E. urophylla* clones were characterized by higher abundances of γ-terpinene, phellandrene, terpinen-4-ol, and caryophyllene. Clone from *E. grandis-E. pellita* showed the highest antibacterial against *Klebsiella pneumoniae*, *Bacillus subtilis*, and *Salmonella typhi*. Meanwhile, clones from *E. grandis-E. urophylla* showed the highest antibacterial against *Staphylococcus aureus*. The *in silico* analysis predicted two compounds, namely spathulenone and α-terpineol as the most potent bioactive compounds against four different antibacterial protein targets. This finding demonstrates the potential of *Eucalyptus* leaf essential oil as an antibiotic against both Gram-positive and Gram-negative bacteria, and it is expected that in the future, its activity can be enhanced by focusing on increasing the content of active compounds predicted from the *in silico* analysis.

Keywords: Antibacterial, Essential oils, *Eucalyptus*, Hybridization, Leaf, Molecular docking

1 Introduction

The *Eucalyptus* (Family: Myrtaceae) includes over 700 species worldwide and is native to Australia and Indonesia [1]. Its wood is used in construction, furniture, fiber, pulp, and as fuel. *Eucalyptus* leaf essential oil exhibits various biological activities, including antimicrobial, antiseptic, antioxidant properties, and is used for treating respiratory and gastrointestinal disorders [2]. *Eucalyptus* trees are fast-growing and show excellent environmental adaptability. They were primarily introduced to Taiwan in the 1980s as a source of pulp for paper production [3]. Due to variations in wood properties across clones, *Eucalyptus* stands out for its high productivity and environmental adaptability, making it suitable for various sectors of the timber industry [4]. If the differences in wood qualities among clones are well

understood, specific clones of *Eucalyptus* can be matched to particular markets, such as the lumber and furniture industries, the pulp and paper industries, or charcoal production in the steel industry.

Plants are crossbred or hybridized for various reasons, primarily, to increase genetic diversity and adapt to changing environments [5]. Hybridization can introduce beneficial traits from different species, such as improved disease resistance, increased yield, or enhanced stress tolerance. For instance, hybrids often exhibit "heterosis" or hybrid vigor, resulting in better performance than either parent due to the combination of advantageous genes [6]–[9]. Moreover, hybrids can tolerate diverse environmental conditions, such as drought, heat, cold, and soil salinity, ensuring stable crop production across different agroecological zones and mitigating the impacts of climate change [10]. Advances in genetic research have greatly expanded our understanding of *Eucalyptus* trees, particularly through the genome sequencing of *Eucalyptus grandis*. This research has provided valuable insights into the genetic control of growth, wood formation, and adaptation to environmental changes [11]. The *E. grandis* genome sequence serves as a key resource for comparative genomic studies within woody plants and has significant implications for breeding programs aimed at improving wood quality and biomass productivity [12]. Another study demonstrated that hybridization can significantly increase the yield of essential oil compared to non-hybrid eucalyptus varieties, as well as the bioactivity against cosmopolitan parasites [13].

Several studies have shown that *E. pellita*, particularly its leaves, offers a wide range of potential biological applications. The leaf extract exhibits allelopathic properties, indicating its potential as a bioherbicide [14]. High concentrations of phenolic and flavonoid compounds in the stem wood and bark suggest that the plant may possess antioxidant qualities [15]. Additionally, its essential oil has demonstrated antibacterial activity against *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Staphylococcus aureus* [16].

Through controlled pollination, *E. grandis* × *E. urophylla* has been successfully hybridized, resulting in hybrids with rapid growth and strong stress resistance. This hybridization has made *E. grandis* × *E. urophylla* the most widely cultivated artificial *Eucalyptus* variety in China. However, most studies on *E. grandis* × E. *urophylla* have focused on wood cultivation and processing, with limited research on its bioactive compounds. Recent findings have shown that the leaves and branches of *E. grandis* × *E. urophylla* are rich in essential oils [17].

Eucalyptus essential oils have long been used in traditional medicine to treat ailments such as fever, bronchitis, and colds [18], [19]. Due to its antiviral and antibacterial properties, *Eucalyptus* oil has also been employed to treat respiratory disorders [20]. When inhaled through steam or a diffuser, it is effective at clearing airways and reducing symptoms of respiratory infections. The effectiveness of *Eucalyptus* essential oils in treating respiratory conditions is widely recognized [21], primarily due to their active ingredient, 1,8-cineole (eucalyptol), which exhibits strong antiviral and antibacterial properties [22]. *Eucalyptus* oil helps relieve cough by loosening mucus in the chest, making it easier to expel [23]. Its expectorant and anti-inflammatory qualities make it an effective treatment for respiratory infections such as bronchitis.

The effectiveness of eucalyptus essential oils as antibacterial agents has been widely studied, including the activity of their constituent compounds. The essential oils from the fruits and leaves of *E. globulus*, *E. citriodora*, and *E. radiata* have demonstrated potent antibacterial activities against resistant bacteria [24]. Further studies using single compounds revealed an antibacterial activity trend, ranked from high to low as follows: aromadendrene, citronellol, citronellal, and 1,8-cineole. Another study explored the antibacterial mechanism of *E. grandis* essential oil, highlighting its membrane-damaging effects against resistant bacteria [25]. This study identified α-Pinene (29.69%), p-Cymene (19.89%), 1,8-cineole (12.80%), α-terpineol (6.48%), borneol (3.48%), and D-limonene (3.14%) as the major phytochemical constituents of the essential oil.

Thus, *Eucalyptus* essential oil has the potential to be further explored as an antibacterial agent by optimizing its phytochemical components and bioactivity through hybridization. This study aims to evaluate the antibacterial activity of *Eucalyptus* leaf essential oil from various clones against *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Bacillus subtilis*, and *Salmonella typhi*, and to analyze the relationship between this antibacterial activity and the variations in the phytochemical profile of compounds from each clone. Moreover, the bioactive compounds of *Eucalyptus* essential oil are predicted by molecular docking against 4 types of proteins.

2 Materials and Methods

2.1 *Plant materials*

Eucalyptus leaves were obtained from stands of mature *Eucalyptus* trees cut at the industrial plantation forest of Toba Pulp Lestari, Ltd. Aek Nauli sector, North Sumatra, Indonesia. The trees used in this research consisted of four types of clones resulting from the hybridization of *E. grandis* with *E. urophylla* and *E. pellita* with different gene arrangements and parents. The list of clones and the composition of the parents are listed in Table 1.

Table 1: Clone type from crossing *Eucalyptus grandis* with *E. urophylla* and *E. pellita*.

Code Number of Clone	Parent	
	Female	Male
71	*E. grandis*	*E. pellita*
72	*E. grandis*	*E. pellita*
106	*E. grandis*	*E. urophylla*
120	*E. grandis*	*E. urophylla*

2.2 *Extraction*

The *Eucalyptus* leaves were distilled in the Aek Nauli Sector (Toba Pulp Lestari, Ltd) to keep the leaves fresh. The leaves were picked and aired for one day; then, the leaves were distilled using water and steam distillation. A total of 10-11 kg of leaves were distilled for 1.5 h after the oil was released from the condenser. The resulting oil was then determined for its refining yield.

2.3 *Antibacterial assay*

Staphylococcus aureus, *Klebsiella pneumoniae*, *Bacillus subtilis*, and *Salmonella typhi* were utilized in antibacterial tests. Bacterial inoculants were cultured on Nutrient Agar media, and then transferred into 10 mL of Nutrient Broth media. The cultures were incubated for 24 h at 37 °C. *Eucalyptus* essential oil (EEO) at concentrations of 25% and 50% was used in the tests. The bacterial cultures at a concentration of 10^8 CFU/mL were spread onto solid Nutrient Agar. The Whatman no. 1 filter papers were immersed and soaked, and then placed on the dried bacterial cultures. The bacteria and sample were incubated at 37 °C for 18–24 h. The clear zones that appeared around the bacteria were measured with a caliper to determine the antibacterial effect. Antibacterial activities were categorized as shown in Table 2.

Table 2: Bacterial inhibition categories are based on the diameter of the inhibition zone [26].

Resistance Diameter	Category
< 5 mm	Weak
5–10 mm	Medium
10–19 mm	Strong
> 20 mm	Very strong

2.4 *Phytochemical analysis*

The phytochemical profile of EEO was analyzed using gas chromatography-mass spectrometry (GCMS) Agilent Technologies 7890 (Agilent Inc, United State) with a 5975 mass selective detector. The separation column used was an RTX 5MS with a length of 30 m, a pressure of 0.75 kPa, and a diameter of 2.25 mm. The injection volume was 0.5 µL, total flow was 35.2 mL/minute, and column flow was 1.53 mL/minute using the electrospray ionization method. The initial injection temperature was 200 °C. The initial column temperature was 70 °C, which occurred for 2 min. The temperature was increased by 20 °C/min, so the temperature change was 180 °C for 3 min. When the process ends, the temperature is at 250 °C at the 27th minute. Compound identification/compound annotation compares the mass spectrum obtained from analysis with that in the National Institute of Standards and Technology database. Clustering to see similarities based on the composition of phytochemical compounds was carried out using heatmap analysis. Analyze using MetaboAnalyst 6.0 (https://www.metaboanalyst.ca/).

2.5 *Molecular docking study*

The phytochemical compounds identified based on GCMS with high abundance in each of the essential oils of different clones of *Eucalyptus* were analyzed for their binding mechanism to the antibacterial target protein. Four protein targets related to antibacterial activity were FabI (PDB ID: 6AH9), FtsZ (PDB ID: 2VXY), DNA gyrase (PDB ID: 4URO), and peptide deformylase (PDB ID: 4JE7). The 3D structures of the protein were obtained from a protein data bank (https://www.rcsb.org/). The grid center values for each receptor are shown in Table 3. Molecular docking was carried out using the AutoDockVina algorithm [27], [28]. Visualization of molecular docking results was carried out using Discovery Studio Visualizer v21.1.0.20298 (Biovia, United State).

Table 3: Grid center for molecular docking analysis.

Receptor	Grid Center			Ref.
	x	y	z	
6AH9	19.270	12.174	12.016	nf*
2VXY	–1.157	15.204	8.651	[29]
4URO	–1.346	0.221	–12.885	[30]
4JE7	8.770	–6.144	–17.487	[31]

*Reference was not found in https://www.rcsb.org/

3 Results and Discussion

3.1 *Effect of clone differences on yield and phytochemical components Text area*

The EEO derived from the cross between *E. grandis* and *E. pellita* is higher than that from the cross between *E. grandis* and *E. urophylla*. The EEO yield, ranging from 0.24% to 0.30%, showed significant variations depending on the type and variety of species used (Table 4). Nadhilah and Ilhamisari [32] reported that the essential oil yield of *E. grandis* ranges from 0.165% to 0.220%, with a maximum yield of 0.220% achieved after three days of storage and four hours of distillation.

Table 4: Yield value of 4 EEO's clone

Clone Code	Yield (%)
71	0.29
72	0.30
106	0.24
120	0.24

Crossing different species or varieties, such as *E. grandis* and *E. pellita*, can increase essential oil yields due to genetic recombination. This process creates new genetic combinations that can incorporate superior traits from both parent species. During meiosis, genetic material is exchanged between homologous chromosomes through crossing over, resulting in offspring with unique allele combinations [33]. This genetic variation can lead to improved traits, such as enhanced essential oil production. Toloza *et al*., [13] reported that species genetically affected the EEO production with higher EEO yield were resulted from *E. grandis* × *E. tereticornis* and *E. grandis* × *E. camaldulensis* than *E. grandis*, *E. tereticornis*, and *E. camaldulensis*.

In addition, factors such as growing conditions, climate, and extraction techniques also play a role in determining the yield and quality of the essential oil produced from each *Eucalyptus* variety. Increased resistance to disease and pests and resistance to less-than-ideal environmental conditions can influence the increase in essential oil production. Healthier and stronger plants will produce more biomass and important chemical components, which can increase the amount of essential oil extracted [34]. Hybrid plants developed using advanced biotechnological approaches can show better resistance to adverse environmental conditions [35].

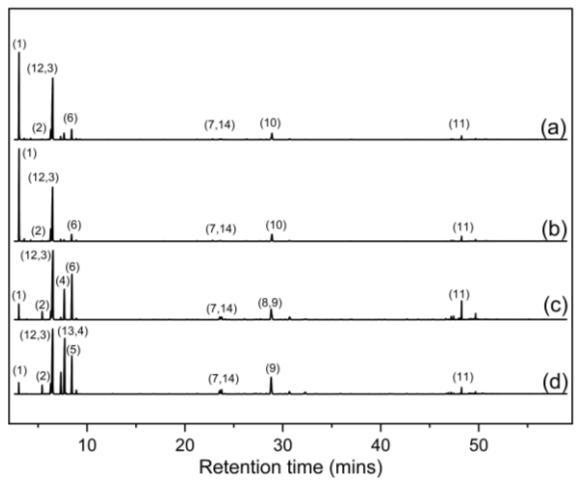

Figure 1: Total ion chromatogram of EEO from the leaves of clone 71(a), 72 (b), 106 (c), and 120 (d). Peak numbers 1–13 were related to Table 5.

Hybridization also affects the chemical profile of EEO in the four clones. The chromatogram profiles of the clones revealed distinct differences in retention times within the first 10 min (Figure 1). Clones resulting from the hybridization with *E. pellita* exhibited high-intensity peaks at retention times under 5 min (Figure 1(a) and (b)). In contrast, clones from the hybridization with *E. urophylla* displayed several high-intensity peaks between 5 and 10 min (Figure 1(c) and (d)). These variations in peak intensity across the chromatograms indicate differences in the abundance or concentration of compounds, which can serve as markers unique to each hybrid clone and specific to particular *Eucalyptus* species.

The genetic recombinant of four clones shows different relative abundances of phytochemical compounds (Table 5). Clones 71 and 72, which were a cross of *E. grandis* and *E. pellita*, had a higher relative abundance of α-pinene than clones 106 and 120, which were a cross of *E. grandis* and *E. urophylla*. According to several studies, *E. urophylla* generally had a higher α-pinene content than *E. pellita*. This content can reach around 30–40% of the essential oil, depending on the extraction method and growth conditions [36], [37].

Table 5: The chemical components of EEO in four clones.

Retention Time (mins)	Compound Name (peak number)	Similatiry Indeks (%)	Relative Abundance (%)			
			71	72	106	120
3.04	α-pinene (1)	95	21.52 ± 0.52	42.44 ± 5.16	2.46 ± 0.38	1.73 ± 0.39
5.42	δ-Phellandrene (2)	91	0.14 ± 0.10	0.10 ± 0.13	2.84 ± 0.41	2.12 ± 0.10
6.52	Eucalyptol (3)	98	40.77 ± 0.35	33.54 ± 2.09	41.16 ± 1.94	31.07 ± 1.47
7.67	γ-terpinene (4)	97	2.42 ± 0.35	0.48 ± 0.34	11.53 ± 1.02	22.39 ± 1.30
8.47	m-Cymene (5)	95	nd	0.58 ± 0.82	5.61 ± 7.93	7.76 ± 5.48
8.44	o-Cymene (6)	97	2.88 ± 2.07	2.62 ± 1.91	11.00 ± 7.78	nd
23.58	Caryophyllene (7)	99	0.40 ± 0.29	0.30 ± 0.21	1.46 ± 0.17	1.88 ± 0.10
28.83	α-Terpinyl acetate (8)	91	nd	nd	2.38 ± 3.37	3.03 ± 4.28
28.83	α-terpinene (9)	91	nd	nd	nd	5.79 ± 4.11
28.89	α-terpineol (10)	95	4.83 ± 1.03	5.37 ± 0.86	nd	nd
48.26	Spathulenol (11)	99	0.82 ± 0.58	0.55 ± 0.78	2.83 ± 2.01	0.86 ± 0.61
6.31	D-Limonene (12)	97	5.84 ± 0.09	7.00 ± 0.21	4.06 ± 0.21	4.72 ± 0.88
7.32	trans-β-Ocimene (13)	96	0.82 ± 0.59	0.53 ± 0.39	0.50 ± 0.35	4.28 ± 3.06
23.76	Terpinen-4-ol (14)	97	0.29 ± 0.21	0.11 ± 0.16	1.39 ± 0.11	2.06 ± 0.24

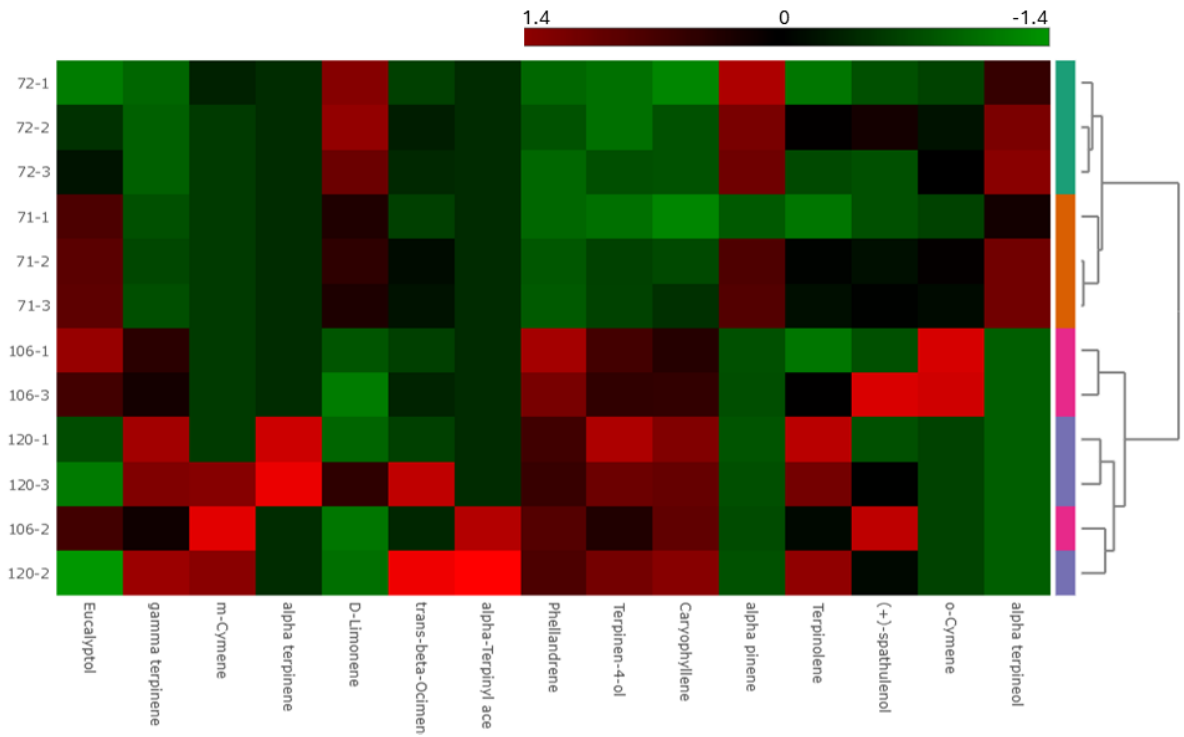

Figure 2: Heatmap of phytochemical composition across different *Eucalyptus* clones. The colors on the right side represent Clone 72 (green), 71 (orange), 106 (magenta), and 120 (purple).

Interactions between genetic and environmental factors can produce significant differences in essential oil composition. For example, certain environmental conditions may favor the activation of phytohormones and other biochemical processes that increase α-pinene biosynthesis in *E. grandis* × *E. pellita* hybrids compared with *E. grandis* × *E. urophylla* hybrids. Research has shown that factors such as soil acidity and geographic location can significantly impact the concentration of essential oil compounds due to their influence on plant metabolism and stress response [38], [39].

The different phytochemical components in clones 71 and 72 compared to 106 and 120 are well illustrated through cluster analysis depicted in a heatmap (Figure 2). Clones 71 and 72, as well as clones 106 and 120, each formed their own clusters. The intensity of color ranging from green – black – red

represents compound abundance values greater than the average abundance (indicated by green, positive values) and abundance values lower than the average abundance (indicated by red, negative values). The compounds that distinguish clones 71 and 72 from clones 106 and 120 were α-pinene, α-terpineol, γ-terpinene, phellandrene, terpinen-4-ol, and caryophyllene. These six compounds can be used to differentiate the clones derived from the *E. pellita* and *E. urophylla*. The *E. grandis-E. pellita* clones were characterized by higher abundances of α-pinene and α-terpineol (positive values on the heatmap). Meanwhile, the *E. grandis-E. urophylla* clones were characterized by higher abundances of γ-terpinene, phellandrene, terpinen-4-ol, and caryophyllene (positive values on the heatmap).

3.2 *Antibacterial activity*

Antibacterials are substances that can inhibit or kill bacteria that cause infection. This infection is caused by pathogenic microorganisms, where these microbes enter the body's tissues and multiply in them. EEO has antibacterial properties that vary according to its terpenoid content, with 1,8-cineole being the most common component [40]. However, the dominant constituents in the oil, such as 1,8-cineole, γ-terpinene, p-cymene, α-pinene, spathulenol, and citronellal, vary according to the species and may cause the oil to have different biological activities [41], [42]. Differences in parents will affect the content of the chemical component, which will affect antibacterial activity. Two concentrations tested for bacterial activity inhibition were 25% and 50% of EEO concentration.

In general, clones derived from *E. grandis-E. pellita* (71 and 72) show the better antibacterial potential compared to clones from *E. grandis-E. urophylla* (106 and 120) (Figure 3). Clone 72 exhibited the highest antibacterial activity against *B. subtilis* and *S. typhi* at a 25% concentration (Figure 3(b) and (d)). Clone 71 exhibited the highest antibacterial activity against *K. pneumoniae* at a 25% concentration (Figure 3(b) and (d)). Meanwhile, the EEO with the best antibacterial activity against *S. aureus* at a concentration of 25% concentration was clone 106. However, some clones showed no activity at a concentration of 25%, specifically clone 120 against all bacteria. Additionally, clones 106 and 72 also showed no activity against *B. subtilis* and *K. pneumoniae* at a 25% concentration, respectively. A unique phenomenon occurred with clone 106. A significant increase in antibacterial activity of clone 106 was observed with the concentration increase to 50% against *S. aureus* bacteria. The phenomenon of a significant increase in antibacterial activity from clone 106 was also observed against *K. pneumoniae*.

The antibacterial properties against both Gram-positive and Gram-negative bacteria highlight the two best EEOs. Clone 106 (*E. grandis* × *E. urophylla*) showed the best antibacterial activity against the gram-positive bacteria *S. aureus* at concentrations of 25% and 50% (Figure 3(a)). However, the activity of this clone did not show a clear effect against another gram-positive bacterium (*B. subtilis*) because this clone did not exhibit the best activity at a 25% concentration (Figure 3(b)). Meanwhile, Clone 72 from the *E. grandis-E. pellita* hybrid demonstrated good potential compared to other clones against Gram-positive bacteria (*B. subtilis*) and Gram-negative bacteria (S. *typhi*). This was observed through the highest inhibition values of Clone 72 compared to other clones at concentrations of 25% and 50% (Figure 3(b) and (d)).

The abundance of α-pinene and α-terpineol in the EEO from *E. grandis* x *E. pellita*, clones 71 and 72, improves antibacterial activity. The α-pinene and α-terpineol contained higher in clones 71 and 72 than in clone 106 and 120 (Table 5), as well as identified as marker compounds for clones 71 and 72 (Figure 2). In addition, the higher abundance of these two compounds in Clone 72 resulted in better activity compared to Clone 71. Ben Akacha *et al.*, [43] reported that a mixture of α-pinene and α-terpineol in a 1:1 ratio shows the best antibacterial activity against *Salmonella enterica* and *Escherichia coli* (gram-negative bacteria) compared to mixtures containing 1,8-cineole, as well as α-pinene and α-terpineol that are not mixed.

All clone types have a high content of eucalyptol (Table 5). Eucalyptol has demonstrated efficacy against both Gram-positive and Gram-negative bacteria. For example, this drug is effective against *S. aureus* and *E. coli*, with the ability to prolong the slow phase of bacterial growth and inhibit cell proliferation [44]. The antibacterial activity of EEO is due to its hydrophobic nature, which allows it to interact with bacterial cell membranes, causing changes in cell permeability and disruption of cell function [45]. This disruption can inhibit essential processes within the bacteria, ultimately causing cell death. Clones 106 and 120, crosses of *E. grandis* and *E. urophylla*, have higher relative concentrations of γ-terpinene, o-cymene,

and m-cymene than crosses of *E. grandis* and *E. pellita*. Studies have shown that γ-terpinene can disrupt bacterial cell membranes, causing cell death [46], [47]. Its effectiveness is known against several bacteria, making it a valuable component in antimicrobial formulations.

O-cymene is another monoterpene with strong antibacterial properties. Research shows that o-cymene can cause structural damage to bacterial cell membranes, inhibiting their growth and survival [48]. Similar to o-cymene, m-cymene exhibits strong antibacterial effects. It has been shown to work synergistically with other bioactive compounds in essential oils, increasing overall antimicrobial efficacy. This synergy helps to fight a broad spectrum of pathogenic bacteria [47]. The high relative concentration of active antibacterial compounds in clone 106 causes this type of clone to have more potent antibacterial activity than other clones of *S. aureus* and *K. pneumoniae* (Figure 3(a) and c)).

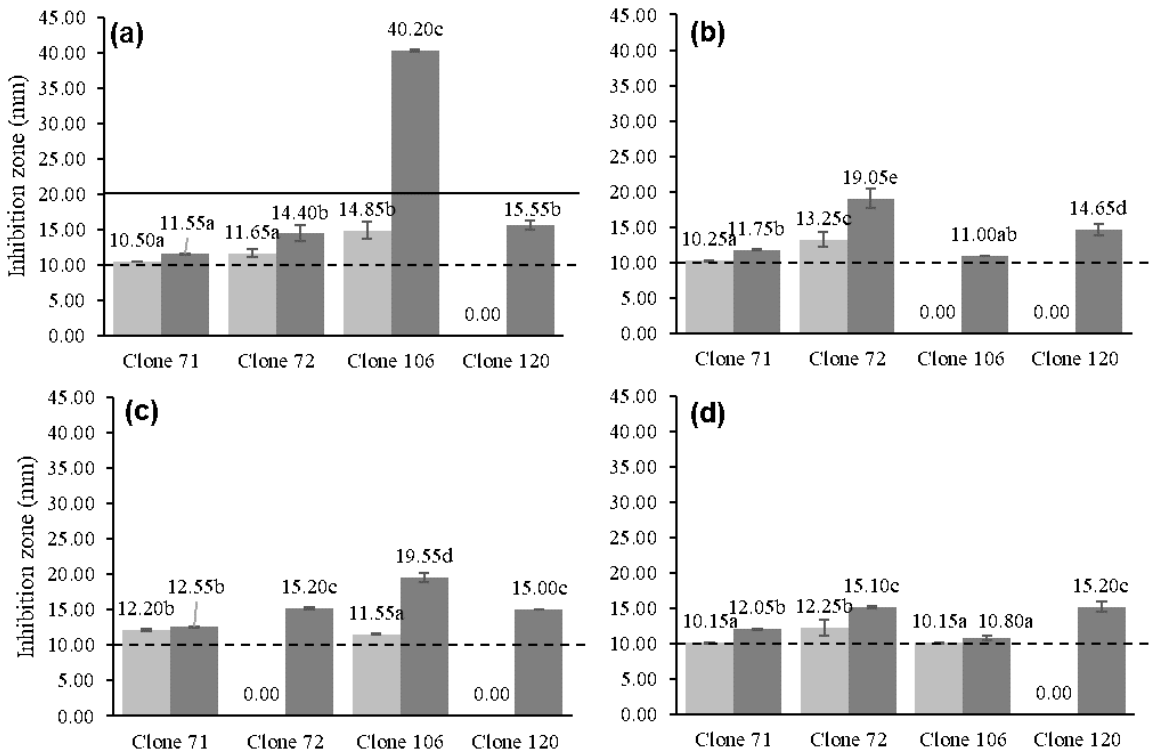

Figure 3: Inhibition diameter zone of EEO at concentrations of 25% (light gray) and 50% (gray) against *S. aureus* (a), *B. subtilis* (b), *K. pneumoniae* (c), and *S. typhi* (d). Strong inhibition (above dashed line); very strong inhibition (above non-dashed line). Number followed by different letters showed significant differences (*p*-value < 0.05).

3.3 *Bioactive compound predictions*

Molecular docking analysis of four target proteins with different mechanisms or pathways shows promising potential for the compounds spathulenol and α-terpineol. According to Table 6, spathulenol demonstrated the best activity against the proteins peptide deformylase, FabI, and DNA gyrase. Meanwhile, α-terpineol exhibited the best activity against FtsZ. Clones 71 and 72 contain α-terpineol, a compound that is not found in clones 106 and 120 (Table 5). This compound is suspected to be one of the factors contributing to the antibacterial activity of clones 71 and 72 compared to clones 106 and 120. It indicates that the inhibition of the FtsZ protein is one of the key mechanisms underlying the antibacterial activity of clones 71 and 72. Meanwhile, spathulenol was present in relatively low abundance in the essential oils of all four different clones. In vitro testing reports that the antibacterial activity of the

spathulenol shows a minimum inhibitory concentration value greater than 200 µg/mL against *S. aureus* and *K. pneumoniae* [49]. No specific information is available regarding the inhibition of spathulenol on proteins (peptide deformylase, FabI, and DNA gyrase). In addition, the antibacterial potential of α-terpineol through the inhibition of the FtsZ protein is also not yet known.

Table 6: Binding affinity of phytochemical compounds against four antibacterial targeted proteins.

Ligand/Chemical Component	Binding Affinity (kcal/mol)			
	Peptide Deformylase	FabI	FtsZ	DNA Gyrase
α-pinene	–5.379 ± 0.005	–6.291 ± 0.015	–4.422 ± 0.004	–4.585 ± 0.02
δ-phellandrene	–5.476 ± 0.020	–6.251 ± 0.050	–5.359 ± 0.023	–5.29 ± 0.009
Eucalyptol	–5.145 ± 0.018	–6.190 ± 0.005	–4.614 ± 0.003	–4.731 ± 0.012
γ-terpinene	–5.354 ± 0.011	–6.443 ± 0.004	–5.521 ± 0.012	–5.128 ± 0.007
m-cymene	–5.428 ± 0.007	–6.202 ± 0.011	–5.606 ± 0.016	–5.450 ± 0.012
o-cymene	–5.385 ± 0.015	–6.202 ± 0.017	–5.463 ± 0.005	–5.176 ± 0.007
Caryophyllene	–6.192 ± 0.013	–7.687 ± 0.008	–5.394 ± 0.013	–5.423 ± 0.003
α-terpinil asetat	–5.890 ± 0.119	–6.726 ± 0.035	–5.709 ± 0.190	–5.972 ± 0.038
α-terpinene	–5.265 ± 0.004	–6.300 ± 0.008	–5.480 ± 0.038	–5.210 ± 0.000
α-terpineol	–6.157 ± 0.020	–6.270 ± 0.005	–6.075 ± 0.026	–5.202 ± 0.016
Spathulenol	–6.749 ± 0.005	–8.032 ± 0.009	–5.803 ± 0.011	–7.644 ± 0.022
D-limonene	–5.296 ± 0.010	–6.315 ± 0.004	–5.601 ± 0.007	–5.018 ± 0.004
Trans beta ocimene	–4.892 ± 0.132	–5.422 ± 0.026	–4.888 ± 0.012	–4.740 ± 0.013
Terpinene-4-ol	–5.395 ± 0.023	–6.145 ± 0.002	–4.941 ± 0.031	–5.783 ± 0.028

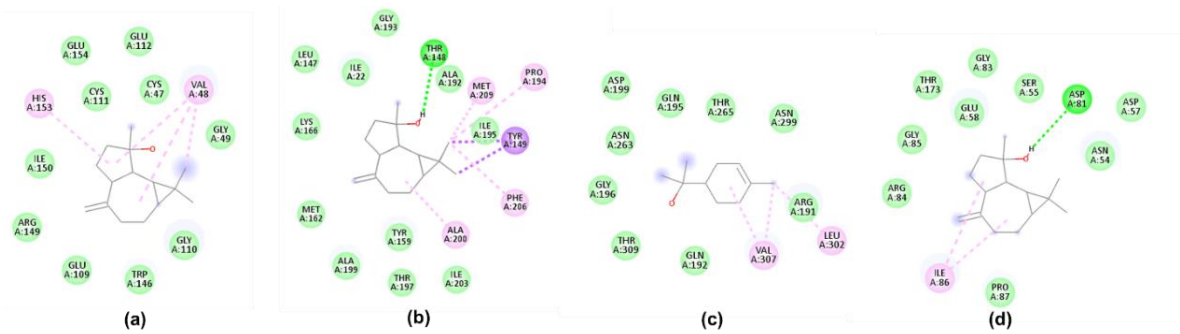

Figure 4: Ligan-receptor interaction of spathulenol-peptide deformylase (a), spathulenol-FabI (b), α-terpineol-FtsZ (c), and spathulenol-DNA gyrase (d). Note: light green (Van der Waals), light purple (alkyl, pi-alkyl), green (conventional hydrogen bond), and purple (pi-sigma).

The four proteins used in this molecular docking study have different antibacterial mechanisms. Belete *et al*., [50] reported that peptide deformylase, FtsZ, and FabI are target proteins in the development of antibacterial agents, each with a distinct mechanism. Inhibition of peptide deformylase and FabI is associated with the inhibition of protein and fatty acid synthesis in bacterial cells, respectively. Meanwhile, inhibition of FtsZ can hinder the cell division process. DNA gyrase is a target protein in antibacterial activity that can be inhibited through two mechanisms: inhibition of the enzymatic activity of gyrase, and disruption of the stabilization of the covalent enzyme–DNA complex, or gyrase poisoning [51]. The different mechanisms of these proteins indicate that the phytochemical compounds from EEO also have different modes of action, as these compounds exhibit varying binding affinities and trends in activity for each protein.

A molecular docking analysis of the interactions between ligands (spathulenol and α-terpineol) and the proteins peptide deformylase, FabI, FtsZ, and DNA gyrase shows that these interactions involve more than one type of intermolecular interaction (Figure 4). The interactions of spathulenol and α-terpineol with peptide deformylase and FtsZ proteins, respectively, exhibited similar types of interactions, specifically Van der Waals and alkyl interactions. Meanwhile, the interactions of spathulenol with FabI and DNA gyrase demonstrated additional interactions beyond Van der Waals and alkyl interactions, namely hydrogen bonds and pi-sigma interactions. The relationship between

the type of interaction and binding affinity was observed by comparing the spathulenol-peptide deformylase and α-terpineol-FtsZ complexes with the spathulenol-FabI and spathulenol-DNA gyrase complexes. The presence of hydrogen bonds and pi-sigma interactions resulted in higher binding affinities, leading to better complex formation compared to complexes that only exhibit Van der Waals and alkyl interactions. The key amino acids involved in forming hydrogen bonds with spathulenol in FabI and DNA gyrase are THR148 and ASP81, respectively.

4 Conclusions

The hybridization of *E. grandis* with *E. pellita* results in a higher yield of essential oil in the leaves compared to its hybridization with *E. urophylla*. This higher essential oil production in *E. grandis*-*E. pellita* leads to a greater abundance of α-pinene and α-terpineol compared to the essential oil from *E. grandis*-*E. urophylla*. This difference also impacts the antibacterial activity, with the essential oil from *E. grandis*-*E. pellita* showing superior efficacy against *B. subtilis*, *K. pneumoniae*, and *S. typhi* compared to the essential oil from *E. grandis*-*E. urophylla*. Meanwhile, the essential oil from *E. grandis*-*E. urophylla* exhibits the best activity against *S. aureus*, particularly in clones with higher levels of o-cymene and eucalyptol. Docking studies identified α-terpineol and spathulenol as the best-performing compounds. Notably, α-terpineol was exclusively identified in the essential oil of *E. grandis*-*E. pellita*, suggesting that this compound may contribute to its strong antibacterial activity through the inhibition of the FtsZ protein. This finding suggests that the hybridization process not only enhances the quantity of essential oil produced but may also influence its chemical composition. By selecting parent species with desirable traits, hybridization could optimize the production of key bioactive compounds, further improving the oil's antibacterial efficacy and broadening its potential applications in various industries. Moreover, the α-pinene and α-terpineol can be used further for quality control-compound for the development of antibacterial Eucalyptus essential oil.

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Author Contributions

R.K.S., A.P., and G.P.: Conceptualization and Project administration; R.K.S., A.P., and E.T.M.: Resources; R.K.S., Y.H.P., and A.C.: Methodology; R.K.S., A.C., and Y.H.P.: Software; R.K.S. and Y.H.P.: Validation, R.K.S. and Y.H.P.: Formal analysis; R.K.S., A.P., G.P., and E.T.M.: Investigation; R.K.S. and Y.H.P.: Data curation; Y.H.P., R.K.S., A.W.A., and A.C.: Writing original draft preparation; Y.H.P. and R.K.S.: Writing review and editing; R.K.S. and Y.H.P.: Visualization; R.K.S., A.P., and G.P.: Funding acquisition. All authors have read and agreed to the published version of the manuscript. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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