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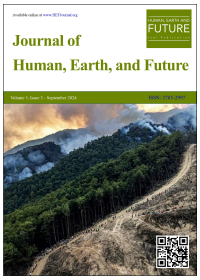

Antimicrobial Potential of *Calotropis gigantea* Leaf Against *Klebsiella pneumoniae* in Ventilator-Associated Pneumonia

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Abstract

Calotropis gigantea or *biduri* has traditional medicinal properties. However, the effect of *C. gigantea* leaves in assisting the function of antibiotic-resistant ventilator-associated pneumonia (VAP), particularly caused by *Klebsiella pneumoniae*, has not been evaluated. The purpose of this study was to identify the active compounds of *C. gigantea* leaf extracts growing in the coastal area of Alue Naga, Banda Aceh, Indonesia, and assess its antimicrobial potential in preventing microbial resistance. *C. gigantea* leaves were extracted using three different solvents: ethanol, ethyl acetate, and *n*-hexane. The three extracts of *C. gigantea* leaves were analyzed for antioxidant activity using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method, and the active compounds of *C. gigantea* were identified using gas chromatography-mass spectroscopy (GC-MS). The dominant bioactive compounds of the *C. gigantea* extract were enrolled for molecular docking analysis. The results of this study showed that the inhibitory concentration 50% (IC50) of the ethanol extract had a higher antioxidant activity (IC50 value of 3.3 ppm) than the ethyl acetate (IC50 value of 22.97 ppm) and *n*-hexane (IC50 value of 32.9 ppm). Bioactive compound identification using GC-MS from the three extracts showed similar dominant compounds, which were α-amyrin and lup-20(29)-en-3-ol, and these compounds belonged to the class of triterpenoid derivative compounds. Molecular docking analysis showed that α-amyrin (-9.6 Kcal/mol), β-amyrin (-9.6 Kcal/mol), and epilupeol (-9.2 Kcal/mol) in *C. gigantea* leaves had higher binding free energy values compared to cefixime (-8.7 Kcal/mol). Thus, it could be concluded that *C. gigantea* leaf extract is assumed to have great potential as an antimicrobial agent and in preventing microbial resistance, particularly in cases of VAP caused by *Klebsiella pneumoniae*.

Keywords: Calotropis gigantea; Biduri; Ventilator-Associated Pneumonia (VAP); α-Amyrin; Alue Naga.

1. Introduction

Ventilator-associated pneumonia (VAP) is one of the most common bacterial infections in patients exposed to invasive mechanical ventilation for 48 hours that commonly acquired lung parenchymal infection in the intensive care unit (ICU) [1]. The most common Gram-positive microbe in VAP is *Staphylococcus aureus*, while common Gram-negative microorganisms include *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Acinetobacter species* [2]. The most popular course of therapy involves giving antibiotics, which are substances that

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both kill and stop the development of germs. However, certain bacterial systems have evolved to become resistant to antibiotics because of the increased use of antibiotics by humans [3–5]. The increasing antibiotic resistance among pathogenic bacteria associated with VAP has made the empirical treatment options for VAP increasingly challenging [6]. The increasing resistance of bacteria to antibiotics has attracted researchers to utilize natural products in various scientific studies [7]. Traditional medicinal plants have long been a target in the search for new drugs [8–11]. According to data from the World Health Organization (WHO), 80% of people worldwide have cured various illnesses with natural medicines [12]. Compounds derived from medicinal plants can offer new approaches to pathogenic bacteria. The development of the use of traditional medicine, especially from plants, to help improve public health has become widespread [13].

According to previous research, the use of antimicrobial agents in combination can produce synergistic effects if each drug targets different mechanisms of action or signaling pathways [14]. Plant antimicrobials have been found to act as synergistic enhancers; although they may not possess antimicrobial properties on their own, they enhance the effects of standard drugs when taken concurrently [15, 16]. *Calotropis gigantea* or known as "*biduri*" is one of the traditional medicines that has properties to treat several illnesses [17]. Numerous studies have reported that *C. gigantea* contains abundant secondary metabolite compounds such as tannins, alkaloids, phenolics, flavonoids, terpenoids, and saponins [18] thus *C. gigantea* has bioactive activities with potential as analgesic, antimicrobial, antioxidant, antipyretic [12, 19], insecticidal, cytotoxic [20], hepatoprotective [21], pregnancy-disrupting [22], procoagulant [23], and wound-healing [24]. The antibacterial potential of *C. gigantea* leaves have been widely studied against various types of pathogenic bacteria including *Klebsella pneumonia*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Escherichia coli*, and *Staphylococcus aureus* [25, 26].

The antibacterial potential of *C. gigantea* leaves has been extensively researched against various pathogenic bacteria, including *P. aeruginosa* and *S. aureus* [25]. However, research regarding the potential of *C. gigantea* leaves in reducing antibiotic-resistant function in patients with VAP, especially *Klebsiella pneumonia*, is still very limited. . Therefore, this study aimed to identify the active compounds of *C. gigantea* leaf extracts growing in the coastal area of Alue Naga Beach location of Banda Aceh city, Aceh-Indonesia and assess the potential of antimicrobial activity as a natural antibiotic agent in preventing microbial resistance (Figure 1).

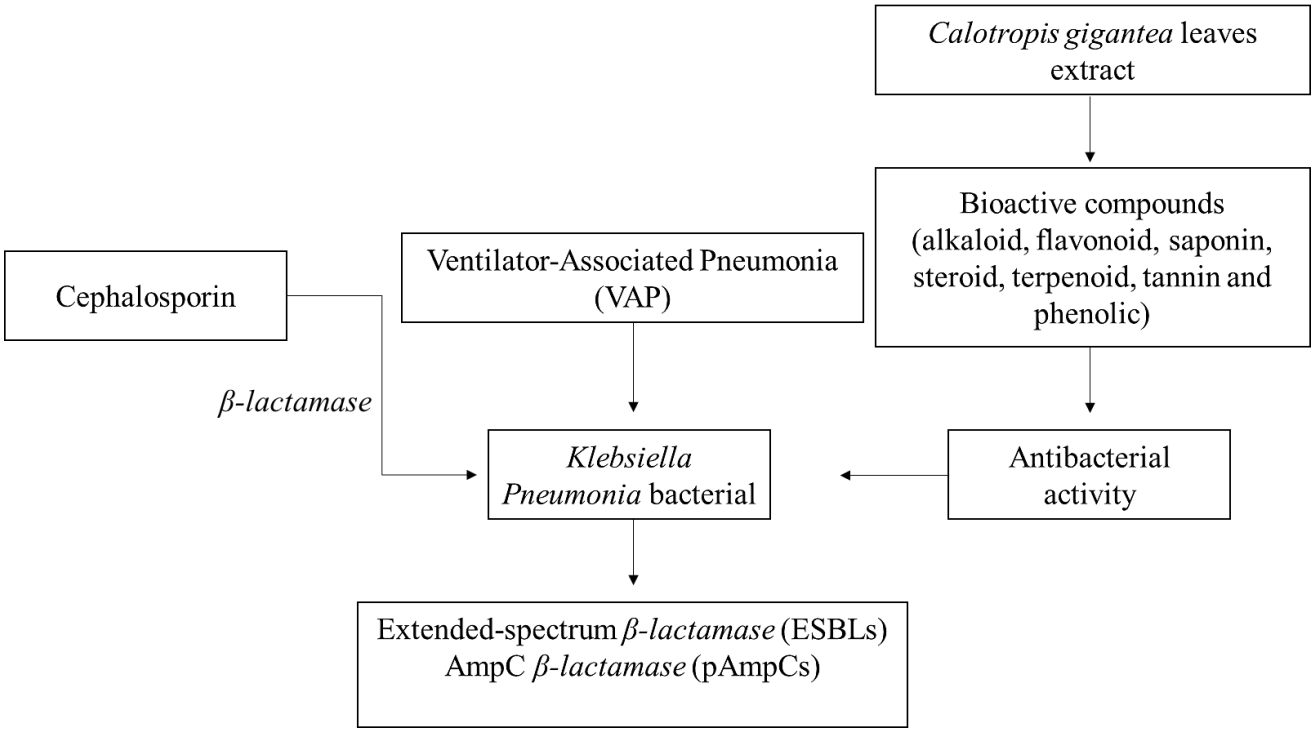


Figure 1. Structure of the article

2. Research Methodology

2.1. Material

The samples used in this study were *C. gigantea* with local name *biduri* leaves. Samples were obtained from coastal area of Alue Naga, Gampong Alue Naga, Banda Aceh City, Indonesia. The coordinates of the sampling location were 5° 21' 25" E - 5° 35' 0" to 5° 20' 25" - 5° 35' 60" and 1 meter above sea level (Figure 2). Samples were identified by botanists at the Department of Biology, Universitas Syiah Kuala, Indonesia.

Figure 2. Research sampling location map (Source: Google Earth)

2.2. Sample Preparation and Extraction Process

Fresh *C. gigantea* leaf samples were collected and dried for seven days. After drying, the leaves were ground to a powder and stored in three containers. The maceration method was used to extract *C. gigantea* for 72 hours in each container, utilizing *n*-hexane, ethyl acetate, and ethanol as solvents. Following the maceration process, the extract was filtered using filter paper before evaporating to get a concentrated extract.

2.3. Antioxidant Analysis

The extract obtained was diluted using methanol solution to obtain concentrations of 20, 40, 60, 80 and 100 ppm. each solution was put into a test tube as much as 5 mL, then added 1 ml of 0.5 mM 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution. The solution was homogenized using a vortex and covered using aluminum foil. The mixture was incubated for 30 minutes at 27°C until the color change of DPPH activity occurred. All extract samples that have been incubated were tested for absorbance values using a UV-Vis spectrophotometer (Kyoto, Japan) at a wavelength of 517 nm. The absorbance value of the DPPH solution on the sample solution was calculated as inhibition percentage (% inhibition) using the Equation below [27].

$$\text{inhibitory concentration (\%)} = \frac{\text{blank absorbance} - \text{sample absorbance}}{\text{blank absorbance}} \times 100 \quad (1)$$

2.4. Identification of Active Compounds using Gas Chromatography-Mass Spectrometry (GC-MS)

GC-MS analysis was conducted to determine the compound content of each *C. gigantea* leaf extract sample (ethanol, *n*-hexane, and ethyl acetate extracts). The instrument used was TRACE 1310 GC-iSQ 7000 MS where 1 µL was injected into GC-MS [28].

2.5. Molecular Docking Analysis

The enzymes/receptors used for molecular docking was *Klebsiella pneumonia* β-*lactamase* (PDB ID: 5OE0) [27]. The molecular docking process was conducted using AutoDock Vina, then the results of molecular docking results was visualized using BIOVIA Discovery Visualizer [28].

2.6. Biological Activity Prediction of Activity Spectra for Substances (PASS)

Further analysis uses PASS approach to determine the most active phytochemical ingredient in extract [29, 30]. The examination findings were expressed as Pa (probability of activity) and Pi (probability of inactivity), with Pa and Pi values ranging from 0.000 to 1000. A molecule's bioactivity is defined by Pa values that exceed Pi and 0.700 [31].

2.7. Antibacterial Activity Test (In vitro)

The antibacterial testing was conducted using the Kirby Bauer disc diffusion method. The samples to be tested were divided into several groups as follows:

P1: Cephalosporin antibiotic (20%; 10%; 5%).

P2: Absolute ethanol solvent.

P3: Extract of *C. gigantea* (60%; 30%; 15%).

P4: Combination of extract and antibiotic (3:1).

All samples were tested for their inhibition zones on colonies of clinically isolated pathogenic bacteria obtained in the previous stage. All bacterial colonies collected were grown on Mueller Hinton Agar (MHA) medium for 24 hours at 37°C. Subsequently, colonies from the liquid medium were spread on petri dishes containing MHA agar medium using a spreader. Sterile disc papers (size 6 mm) were prepared and placed on the petri dishes for inoculation. All sample groups (I to X) were then loaded onto each disc paper. All petri dishes were then incubated for 24 hours at 37°C. The inhibition zones were measured using a ruler in millimeters.

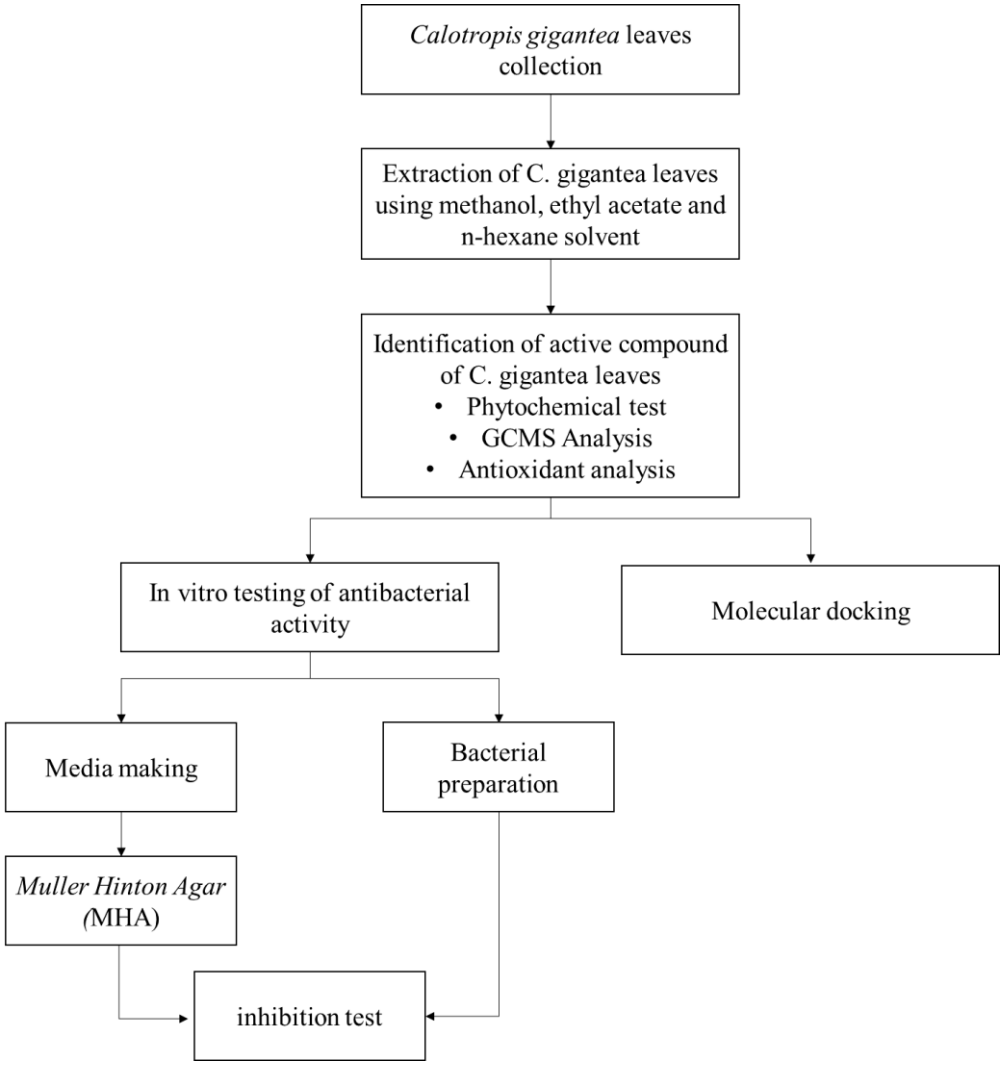


Figure 3. Flowchart of the methodology

3. Results

3.1. Antioxidant Activity of *C. Gigantea* Leaves

Antioxidant activity analysis of *C. gigantea* leaf extract was performed using the DPPH method with UV-VIS spectrophotometry at a wavelength of 517 nm. The antioxidant activity of the fractions was tested at different concentrations of 100, 80, 60, 40 and 20 ppm and reported as IC_{50} value. Some variables, including the solvent and extraction technique for antioxidant molecules and their derivatives, might affect the variation in antioxidant activity that is generated [32]. Based on the results obtained, ethanol solvent proved to be more effective in attracting active compounds that have antioxidant activity. It was assumed that the ethanol solvent has the properties to penetrate the cell wall material so that it can perform cell diffusion and attract bioactive compounds more and faster [33]. However, ethyl acetate and *n*-hexane extract also showed high antioxidant activity, as evidenced by the IC_{50} values of ethyl acetate and *n*-hexane extracts of 22.97 and 32.9 ppm, respectively (<50 ppm). This is because non-polar solvents can only extract compounds with the same polarity [34]. The results of the antioxidant analysis of *C. gigantea* leaves were presented in Table 1**.** Based on the analysis, the antioxidant activity of the ethanol extract of *C. gigantea* leaves was higher than the other extracts, with IC_{50} value reaching 3.3 ppm. Ethyl acetate and *n*-hexane extracts also showed high antioxidant activity, as evidenced by the IC_{50} values of ethyl acetate and *n*-hexane extracts of 22.97 and 32.9 ppm, respectively (<50 ppm). Previous investigations have demonstrated the significant antioxidant activity of *C. gigantea* leaves, with IC_{50} values less than 50 ppm, indicating that *C. gigantea* leaf extract has the potential as a natural antioxidant source [35].

Table 1. Antioxidant activity test results of *C. Gigantea* leaf extract

Concentration (ppm)	Absorbance	Inhibition (%)	Slope	Intercept	IC_{50} (ppm)
Ethyl acetate extract					
100	0.337	66.1			
80	0.353	64.5			
60	0.381	61.7	0.24	44.48	22.97
40	0.443	55.4			
20	0.531	46.6			
Ethanol extract					
100	0.347	69.0			
80	0.355	68.3			
60	0.374	66.6	0.2	49.3	3.3
40	0.447	60.1			
20	0.556	50.4			
***n*-Hexane extract**					
100	0.370	67.1			
80	0.403	64.2			
60	0.497	55.8	0.3	41.1	32.9
40	0.512	54.5			
20	0.619	45.0			

3.2. GC-MS Analysis of *C. Gigantea* Leaves

GC-MS analysis of *C. gigantea* leaves showed that ethanol extract of *C. gigantea* leaves contained 32 active compounds, ethyl acetate extract contained 35 active compounds and *n*-hexane extract contained 44 active compounds. Table 2 shows some compounds with a high percentage (above 5%) in each extract.

Table 2. Metabolite compounds from *C. gigantea* leaf extracts using GC-MS

Retention time (min)	Name of compound	Molecular formula	Molecular weight (g/mol)	Peak area (%)
Ethanol extract				
32.72	Phytol	$C_{20}H_{40}O$	296.5	7.53
53.53	β-amyrin	$C_{30}H_{50}O$	426.72	8.38
54.28	α-amyrin	$C_{30}H_{50}O$	426.72	24.52
55.04	Olean-12-en-3-ol, acetate, (3ß)-	$C_{32}H_{52}O_2$	468.75	6.64
55.76	α-amyrin	$C_{30}H_{50}O$	426.72	7.57
57.68	Lup-20(29)-en-3-ol, acetate, (3ß)-	$C_{30}H_{50}O$	468.75	11.59
Ethyl acetate extract				
33.73	Hexadecanoic acid	$C_{16}H_{32}O$	256.42	5.07
35.34	Phytol	$C_{20}H_{40}O$	296.5	5.04
54.27	α-amyrin	$C_{30}H_{50}O$	426.72	16.42
55.07	Olean-12-en-3-ol, acetate, (3ß)-	$C_{32}H_{52}O_2$	468.75	8.56
57.81	Lup-20(29)-en-3-ol, acetate, (3ß)-	$C_{30}H_{50}O$	468.75	21.34
***n*-Hexane extract**				
31.84	Hexadecanoic acid, methyl ester	$C_{16}H_{32}O$	256.42	6.13
35.20	9,12,15-Octadecatrienoic acid, methyl ester, (9Z,12Z,15Z)-	$C_{19}H_{32}O_2$	292.5	9.86
54.34	α-amyrin	$C_{30}H_{50}O$	426.72	14.11
55.13	Olean-12-en-3-ol, acetate, (3ß)-	$C_{32}H_{52}O_2$	468.75	8.34
57.88	Lup-20(29)-en-3-ol, acetate, (3ß)-	$C_{30}H_{50}O$	468.75	17.32

Based on the results of GC-MS analysis, the compounds that dominate from *C. gigantea* leaves in three solvents were α-amyrin and lup-20(29)-en-3-ol, acetate, (3ß)-. These compounds were the 2 most found compounds in the three extracts analyzed. The main components in the ethanol extract were α-Amyrin (24.52%) and lup-20(29)-en-3-ol (11.59%), while the ethyl acetate and *n*-hexane extracts contained lup-20(29)-en-3-ol (21.34% and 17.32%) and α-Amyrin (16.42% and 14.11%), respectively. Both compounds are triterpenoid-derived compounds [36].

3.3. Biological Activity Prediction

The potential biological activities of *C. gigantea* leaf extracts were predicted using a structure-based prediction technique known as Prediction for Activity Spectra for Substances (PASS) (Table 3). The predicted value was indicated as the probability of active (Pa) and inactive (Pi). A compound biological activity was determined by a probability value ranging from 0 to 1, with higher values indicating more activity [37]. According to the PASS test, *C. gigantea* leaf extract contains secondary metabolite compounds, such as α-amyrin as insulin promoter (Pa: 0.934), β-amyrin as insulin promoter (Pa: 0.977), β-sitosterol as delta 14-sterol reductase inhibitor (Pa: 0.965), phytol as prenyl-diphosphate inhibitor (Pa: 0.911), squalene as prenyl-disphosphate inhibitor (Pa: 0.969), compesterol as Delta 14-sterol reductase inhibitor (Pa: 0.973), epilupeol as caspase stimulant (0.978), eugenol as carminative (Pa: 0.941), and tocopherol as lipid peroxidase inhibitor (Pa: 0.981). Furthermore, many previous studies indicated this kind of greater potential. Thus, the uses and specifics of the observed effects of *C. gigantea* might be attributable to the combined action of multiple phytoconstituents, including those reported here and additional ones yet to be characterized.

Table 3. Biological activity of metabolite compounds

Ethanol Extract		
α-amyrin		
Pa	Pi	Activity Name
0.934	0.002	Insulin promoter
0.926	0.002	Hepatoprotection
0.911	0.004	Apoptosis agonist
0.901	0.005	Antineoplastic
0.897	0.002	Transcription factor NF kappa B stimulant
β-amyrin		
Pa	Pi	Activity Name
0.977	0.001	Insulin promoter
0.976	0.002	Caspase 3 stimulant
0.944	0.001	Transcription factor stimulant
0.944	0.001	Transcription factor NF kappa B stimulant
0.939	0.004	Mucomembranous protector
Eugenol		
Pa	Pi	Activity Name
0.941	0.001	Carminative
0.937	0.004	Aspulvinone dimethylallyltransferase inhibitor
0.902	0.005	Chlordecone reductase inhibitor
0.881	0.005	Feruloyl esterase inhibitor
0.878	0.003	Antimutagenic
Ethyl Acetate Extract		
Phytol		
Pa	Pi	Activity Name
0.911	0.002	Prenyl-diphosphatase inhibitor
0.907	0.001	Retinol dehydrogenase inhibitor
0.905	0.005	Ubiquinol-cytochrome-c reductase inhibitor
0.893	0.007	Phobic disorders treatment
0.885	0.002	Undecaprenyl-phosphate mannosyltransferase inhibitor

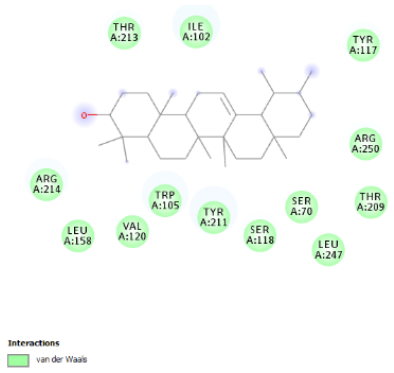
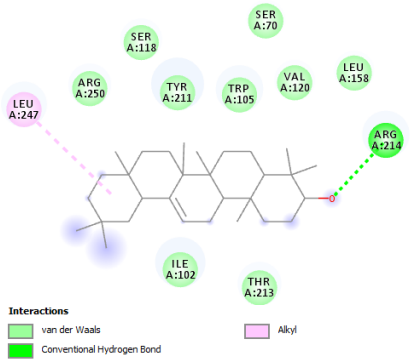
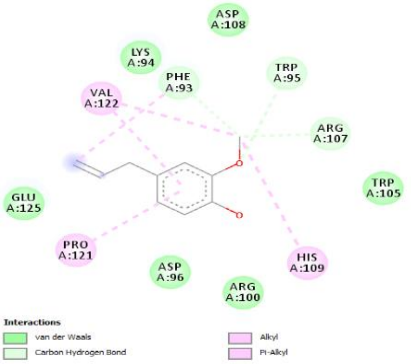
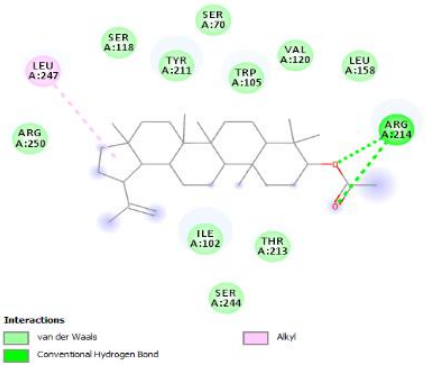
Epilupeol		
Pa	Pi	Activity Name
0.978	0.002	Caspase 3 stimulant
0.947	0.001	Transcription factor NF kappa B stimulant
0.947	0.001	Transcription factor stimulant
0.95	0.004	Antineoplastic
0.907	0.002	Hepatoprotectant
Compesterol		
Pa	Pi	Activity Name
0.973	0	DELTA14-sterol reductase inhibitor
0.962	0.002	Prostaglandin-E2 9-reductase inhibitor
0.962	0.002	Antihypercholesterolemic
0.955	0.001	Cholesterol antagonist
0.955	0.002	Alkenylglycerophosphocholine hydrolase inhibitor
***n*-Hexane Extract**		
Squalene		
Pa	Pi	Activity Name
0.969	0.001	Prenyl-diphosphatase inhibitor
0.959	0.003	Mucomembranous protector
0.956	0.001	Undecaprenyl-phosphate mannosyltransferase inhibitor
0.91	0.001	BRAF expression inhibitor
0.911	0.006	Aspulvinone dimethylallyltransferase inhibitor
β-sitosterol		
Pa	Pi	Activity Name
0.965	0.001	DELTA14-sterol reductase inhibitor
0.96	0.002	Antihypercholesterolemic
0.959	0.002	Prostaglandin-E2 9-reductase inhibitor
0.957	0.001	Cholesterol antagonist
0.952	0.002	Alkenylglycerophosphocholine hydrolase inhibitor
Tocopherol		
Pa	Pi	Activity Name
0.981	0.001	Lipid peroxidase inhibitor
0.956	0.002	Antioxidant
0.949	0.003	TP53 expression enhancer
0.931	0.004	Acute neurologic disorders treatment
0.931	0.005	Antiischemic, cerebral

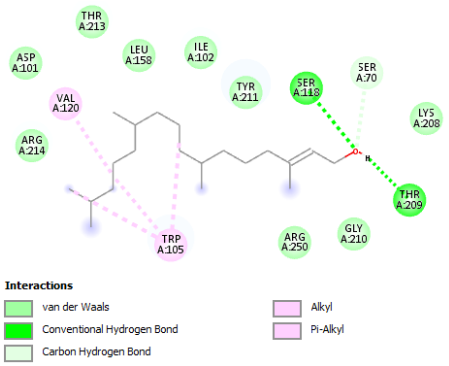
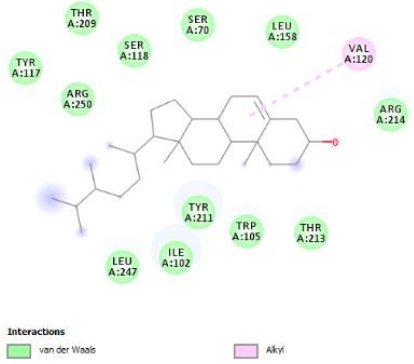
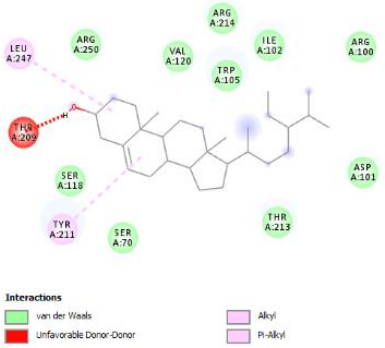
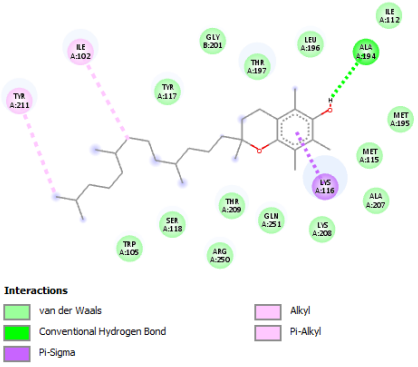
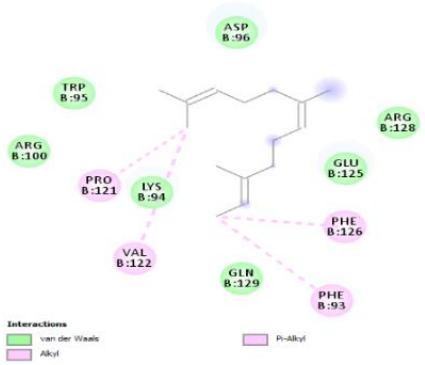
3.4. Molecular Docking

Based on the docking results of *C. gigantea* leaf extract using 3 different solvents, nine main compounds were found from each extract. The result of binding the molecule to the target is the binding affinity and the interaction of the ligand with the target receptor. The result parameter is shown in the inhibitory ability or binding affinity value (Kcal/mol). The result of molecular docking revealed that the α-amyrin molecule had the highest binding affinity for

Klebsiella pneumoniae β-lactamase receptor, resulting from interactions between amino acid residues (Thr:213, Ile:102, Tyr:117, Arg:214, Leu:158, Val:120, Trp:105, Tyr:211, Ser:118, Ser:70, Leu:247, Thr:209, Arg:250). The results of this study can be seen in Table 4.

Table 4. The binding affinity value between receptor *Klebseilla pneumonia β-lactamase* and phytochemical compounds

Compounds	Binding affinity (Kcal/mol)	Interactions	Amino acid residues	Visualizations
Ethanol extract				
α-amyrin	-9.6	Van der Waals	Thr: 213 Ile: 102 Tyr: 117 Arg: 214 Leu: 158 Val: 120 Trp: 105 Tyr: 211 Ser: 118 Ser: 70 Leu: 247 Thr: 209 Arg: 250	
β-amyrin	-9.6	Alkyl Conventional Hydrogen Bond Van der Waals	Leu: 247 Arg: 214 Arg: 250 Ser: 118 Tyr: 211 Trp: 105 Ser: 70 Val: 120 Leu: 158 Ile: 102 Thr: 213	
Eugenol	-5.5	Alkyl Carbon Hydrogen Bond Van der Waals	Val: 122 Pro: 121 His:109 Phe: 93 Trp: 95 Arg: 107 Lys: 94 Asp:108 Glu:125 Asp: 96 Arg:100 Trp: 105	
Ethyl acetate extract				
Epilupeol	-9.2	Alkyl Conventional Hydrogen Bond Van der Waals	Leu: 247 Arg: 214 Arg: 250 Ser: 118 Tyr: 211 Trp: 105 Ser: 70 Val: 120 Leu: 158 Ile: 102 Thr: 213 Ser: 244	

Phytol	-5.6	Alkyl Carbon Hydrogen Bond Conventional Hydrogen Bond Van Der Waals	Val: 120 Trp:105 Ser: 70 Ser: 118 Thr: 209 Arg: 214 Asp:101 Thr: 213 Leu: 158 Ile: 102, Tyr: 211 Lys: 208 Gly: 210 Arg: 250	
Campesterol	-7.8	Alkyl Van der Waals	Val: 120 Tyr: 117 Arg: 250 Thr: 209 Ser: 118 Ser: 70 Leu: 158 Arg: 214 Thr: 213 Trp: 105 Tyr: 211 Ile: 102 Leu: 247	
		***n*-Hexane extract**		
β-sitosterol	-8	Alkyl Van der Waals	Leu: 247 Tyr: 211 Arg: 250 Val: 120 Arg: 214 Trp: 105 Ile: 102 Arg: 100 Ser: 118 Ser: 70 Thr: 213 Asp:101	
Tocopherol	-7.5	Alkyl Conventional Hydrogen Bond Van der Waals	Ile: 102, Tyr: 211 Ala: 194 Tyr: 117 Gly: 201 Thr: 197 Leu: 196 Ile: 112 Met:195 Met:115 Ala:207 Lys: 208 Gln: 251 Thr: 209 Arg: 250 Ser: 118 Trp: 105	
Squalene	-5.7	Alkyl Van der Waals	Pro: 121 Val: 122 Phe: 93 Phe: 126 Asp: 96 Arg: 128 Glu: 125 Gln: 129 Lys: 94 Arg: 100 Trp: 95	

3.5. Antibacterial Activity Test (In vitro)

The antibacterial activity testing of ethanol extract from *C. gigantea* leaves was conducted using the Kirby Bauer disc diffusion method. The results of this testing indicated that the antibiotic testing against *K. pneumonia* bacteria exhibited the highest inhibition zone among various doses administered, with an average of 13.48±2.41. It can be observed that the ethanol extract of *C. gigantea* leaves combined with antibiotics showed a higher inhibition zone compared to the ethanol extract alone. The data are presented in Table 5 and Figure 4.

Table 5. The diameter of the inhibition zone (mm) from the antibacterial activity of ethanol extract of *C. gigantea* against *K. pneumonia*

Treatments	Concentrations of Ethanol Extract			Total
	60%	30%	15%	
P1 (Antibiotic)	15.72±0.76	13.79±0.64	10.93±0.16	13.48±2.41
P2 (Ethanol Absolute)	6.18±0.16	6.06±0.10	6.51±0.36	6.25±0.23
P3 (Extract *C. gigantea*)	7.53±0.46	7.22±0.44	7.78±0.42	7.51±0.28
P4 (combination of extracts and antibiotics)	11.92±0.50	10.58±0.30	7.29±0.38	10.14±2.03

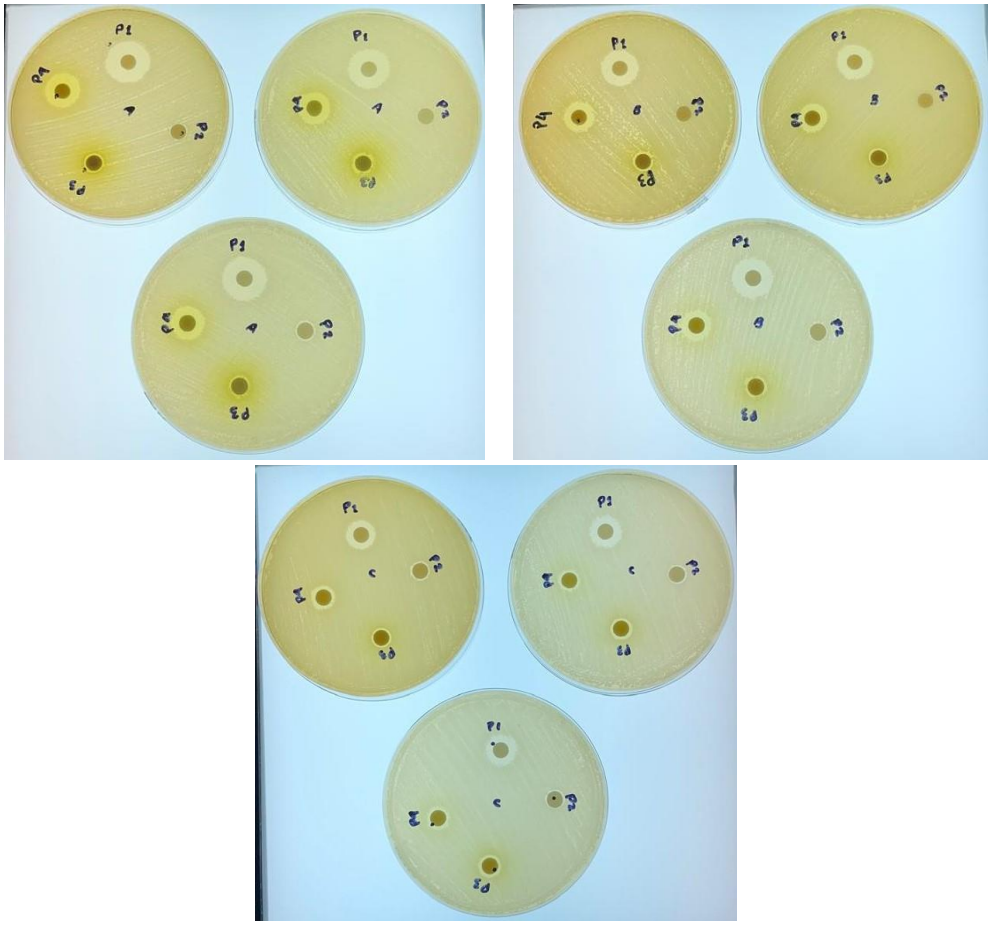


Figure 4. The diameter of the inhibition zone of *K. pneumonia* bacteria. Descriptions: P1 (antibiotic); P2 (ethanol absolute); P3 (*C. gigantea* extract); P4 (combination of extracts and antibiotics)

4. Discussion

The antioxidant analysis conducted using the 2,2-diphenyl picrylhydrazyl (DPPH) method demonstrated the high antioxidant activity of *C. gigantea* leaves. This finding aligned with the research findings of Rehman et al. (2023) [38], which asserted that *C. gigantea* leaves exhibited higher antioxidant activity compared to other parts of the plant. Most plants contained active compounds responsible for antioxidant properties, such as flavonoids, tannins, and various phenolic compounds [39]. The high content of phenolic and flavonoid compounds was associated with the potential for higher antioxidant activity [24]. Additionally, other factors influencing antioxidant properties included the type of solvent used. Among the solvents used, previous studies indicated that ethanol extracts proved more effective in enhancing antioxidant functions [40, 41]. This was based on prior studies stating that ethanol was an effective solvent for extracting a number of bioactive compounds from plants, thereby contributing to antioxidant capacity [42].

GC-MS analysis was used to reveal the profile of bioactive compounds in plants using information from mass spectra and chromatograms. Previous studies reported several major phytochemical groups in the methanol extract of *C. gigantea* leaves, namely flavonoids, steroids, terpenoids, alkaloids, carbohydrates, polyphenols, and glycosides [43]. Another study also reported that the ethanol extract of *C. gigantea* leaves contained 16 secondary metabolite compounds consisting of ethyl ester fatty acid and triterpenoid compound groups [19, 44]. In this study, the main components in the *C. gigantea* leaves extract were α-Amyrin and lup-20(29)-en-3-ol, both compounds are triterpenoid-derived compounds [36, 45]. Some previous studies stated that the compound lup-20(29)-en-3-ol possessed antibacterial, antimicrobial, anti-inflammatory [45] and antioxidant activities [46]. Similarly, α-Amyrin compounds also had antioxidant [47], anti-inflammatory [48], antidepressant, anticonvulsant, hepatoprotective and gastroprotective activities [49]. These results were consistent with previous studies indicating that *C. gigantea* leaves contain lup-20-en-3-ol and α-amyrin compounds that contribute to the antioxidant activity in *C. gigantea* leaves [50].in addition, The GC-MS analysis results indicated the presence of hexadecanoic acid compound in ethanol and *n*-hexane extracts. Lim (2012) stated that hexadecanoic acid compound had high antimicrobial activity and naturally occurred in plants. Hexadecanoic acid compound exhibited antibacterial activity against several microorganisms such as *E. coli, S. aureus, K. pneumoniae*, and others [51, 52].

The pharmacological profile generated from the active compounds contained in a plant could be assessed using a structure-based prediction system with the aim of understanding their biological activities. These predictive values were indicated as the probability of activity (Pa). The biological activity of a compound was determined by the probability values ranging from 0 to 1, with higher values indicating higher activity [37, 53]. The prediction results showed a range of probability values (Pa) from 0.885 to 0.981. These findings were consistent with research conducted by Alam et al. (2020), which stated that the prediction results indicated various activities, including values potentially as anti-diarrheal, antibacterial, and antioxidant agents [54].

In this study, molecular docking was conducted on 9 phytochemical compounds such as α-amyrin, β-amyrin, β-sitosterol, campesterol, tocopherol acetat, squalene, phytol, eugenol, epilupeol and 1 target receptor *Klebseilla pneumonia* β-*lactamase* (PDB ID: 5OE0). Cefixime was used as a control ligand against the target receptor *Klebseilla pneumonia* β-*lactamase* (PDB ID: 5OE0). The compounds with docking activity are strong towards the receptors employed indicate a high docking value [55]. Three compounds obtained from *C. gigantea* leaf extracts namely α-amyrin (-9.6 Kcal/mol), β-amyrin (-9.6 Kcal/mol), and epilupeol (-9.2 Kcal/mol) were known to have higher inhibition or binding affinity values compared to the positive control used which was cefixime (-8.7 Kcal/mol). The bond with the most negative energy value (highest binding energy) is considered the best candidate with maximum binding energy [56, 57].

Based on the docking analysis conducted, it has been proven that the antibacterial activity in *C. gigantea* leaves is mediated by active secondary metabolites detected. This is evidenced by the inhibition zones produced from antibacterial testing using the Kirby Bauer disc diffusion method. The ethanol extract concentrations were differentiated into 60%, 30%, and 15% with the aim of comparing the inhibition zone areas of each concentration. The concentration differences showed various levels of antibacterial activity produced [58]. From the obtained results, there was an increasingly wider inhibition zone with increasing concentration used. These findings were consistent with previous studies showing that antimicrobial activity increased with rising concentrations [59].

The ethanol extract of *C. gigantea* leaves showed a slight inhibition zone against *K. pneumonia* compared to cephalosporin, which was the positive control. Cephalosporin, the antibiotic used in this study, works by inhibiting the cell wall formation process and activating autolytic enzymes to destroy bacterial cell walls [60]. The combination testing of antibiotic and ethanol extracts showed a decrease in the bacterial inhibition zone value. This could be attributed to the bioactive compounds contained in *C. gigantea* leaves, such as carbonyl and phenolic groups [59]. Phenolic compounds work by precipitating proteins and deactivating cellulolytic/hemicellulolytic enzymes [61, 62] Thus, the difference in mechanisms between the ethanol extract of *C. gigantea* leaves and the antibiotic used leads to a non-synergistic effect after combination.

5. Conclusion

Calotropis gigantea (*biduri*) leaves have the potential as a source of bioactive compounds with significant antioxidant activity. Ethanol extract of *C. gigantea* leaves had the highest antioxidant activity (IC_{50} value 3.3 ppm) compared to ethyl acetate (IC_{50} value 22.97 ppm) and *n*-hexane (IC_{50} value 32.9 ppm) extracts. The active compounds identified in *C. gigantea* leaves, such as α-amyrin and lup-20(29)-en-3-ol belong to the class of triterpenoid-derived compounds. In-silico analysis using molecular docking indicates that compounds such as α-amyrin, epilupeol and β-sitosterol from *C. gigantea* leaves showed higher inhibition or binding affinity values compared to cefixime as a positive control. Overall, the results of this study support traditional claims about the efficacy of *C. gigantea* leaves as a traditional medicine. In addition, the antimicrobial potential identified through in-silico analysis provides additional value to the utilization of *C. gigantea* leaves in preventing microbial resistance, particularly in cases of Ventilator-Associated Pneumonia (VAP). From the results of the antibacterial activity testing with three types of extract concentrations, it can be concluded that the increase in the inhibition zone of *K. pneumonia* bacteria is directly proportional to the amount of extract concentration used.

6. Declarations

6.1. Author Contributions

Conceptualization, T.Z., T.N.S., and A.S.; methodology, T.Z. and A.S.; software, T.N.S.; validation, A.R. and T.N.S.; formal analysis, A.S. and T.N.S; investigation, T.Z. and A.R.; resources, T.Z.; data curation, T.Z., A.S., and A.R.; writing—original draft preparation, T.Z., T.N.S., A.R., and A.S.; writing—review and editing, A.S. and T.N.S.; visualization, A.R.; supervision, T.N.S., A.R., and A.S.; project administration, T.Z. and A.S.; funding acquisition, T.Z. All authors have read and agreed to the published version of the manuscript.

6.2. Data Availability Statement

The data presented in this study are available in the article.

6.3. Funding

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6.4. Acknowledgements

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6.5. Institutional Review Board Statement

Not applicable.

6.6. Informed Consent Statement

Not applicable.

6.7. Declaration of Competing Interest

The authors declare that there is no conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancies have been completely observed by the authors.

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