

Potential Use of Jerangau Rhizome (*Acorus calamus* L.) as an Antibacterial Agent for *Streptococcus pneumoniae*

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Abstract

Pneumonia, an infectious disease affecting lung tissue, is primarily caused by bacteria, viruses, and fungi. *Streptococcus pneumoniae* is a common bacterium responsible for pneumonia, residing in the upper respiratory tract and infecting many individuals globally. Traditional remedies for lung inflammation include various plants, among which *Acorus calamus* (L.), known for its respiratory benefits, has been used across cultures. The rhizome of *Acorus calamus* contains asarone compounds, which have antispasmodic and anti-inflammatory properties, aiding in respiratory relief.

Aims: The objective of this study is to provide scientific validation of the antibacterial potential of a jerangau rhizome extract against *Streptococcus pneumoniae*. Previous empirical uses and studies have demonstrated promising results, which this study aims to replicate.

Study design: The experimental use of *Streptococcus pneumoniae* (SP) bacteria and jerangau (*Acorus calamus* L.) rhizome extract (JRE).

Place Study: Pharmacy Department, Poltekkes Kemenkes Makassar, Indonesia.

Methodology: Antibacterial activity was assessed using liquid dilution and agar diffusion methods. The study utilized *Streptococcus pneumoniae* cultures obtained from clinical isolates. Jerangau rhizome simplicia was prepared, dried, and macerated using hexane, ethanol, and ethyl acetate solvents. The antibacterial activity was tested using the agar diffusion method with Mueller Hinton Agar (MHA) media and the liquid dilution method to determine MIC and MKC values. Observations were made after 24 and 48 hours of incubation to measure the inhibition zones and turbidity levels, respectively, to evaluate the antibacterial effectiveness of the extracts. Data were analyzed using SPSS for significant differences in antibacterial activity between various concentrations of extracts and controls.

Results: The antibacterial activity of JRE against SP was determined to be effective at concentrations of 0.5%, 1%, 2%, 4%, and 8% w/v. The minimum inhibitory concentration (MIC) and minimum kill concentration (MKC) were found to be 0.5% and 1% in HJ, >2.5% at EJ, and 0.25% and 0.5% at EAJ.

Conclusion: JRE has demonstrated antibacterial properties against SP in vitro.

Keywords: Activity herbal, antipneumonia, bacterial growth, MIC/MKC.

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Introduction

Pneumonia is an infectious disease of lung tissue caused by bacteria, viruses and fungi. Typical signs of pneumonia include a persistent cough, high fever, chills, and shortness of breath. Pneumonia caused by pneumococcal bacteria

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is generally caused by *Streptococcus pneumoniae* which typically resides in the upper respiratory tract and infects over 900,000 individuals in the United States annually [1].

The findings from Ristoja 2017 [2] identified 10 plant species commonly included in remedies for lung inflammation, namely: turmeric, miana, ginger, jerangau, coconut, andong, patikan kebo, bandotan, garlic and bitter. *Acorus calamus* (L) has been used in traditional medicine in various cultures for centuries for digestive treatment, stress relief, and respiratory problems such as coughs and shortness of breath. The rhizome of *Acorus calamus* (L) contains compounds called asarone, which have antispasmodic and anti-inflammatory properties. The substances found in this plant are believed to assist in clearing the respiratory passages. The chemical constituents of *Acorus calamus* (L) rhizomes have been identified as flavonoids, phenols, saponins, triterpenoids and asaron [3-8]. The antioxidant potential of jerangau rhizome (*Acorus calamus* (L)), hexane extract 5.57 mg/ml, ethanol extract 0.72mg/ml and ethyl acetate extract 0.9mg/ml [4]. The potential of the Jerangau *Acorus calamus* (L) herbal has been previously studied by [5] who identified flavonoid compounds, fatty acids and antioxidant potential. The potential for prevention and treatment of cardiovascular disease in preclinical animal models [6]. The antifungal, antitumor and anti-inflammatory potential is proven by the empirical use of decoction of Jarangau leaves and rhizomes [7]. The potential for reducing blood sugar levels [8]; alternative therapy for E. Coli and Staphylococcus aureus infections [3, 13]; *Helicobacter pylori* [10]; *Streptococcus pyogenes* [11]. The potential of a-asarone for nephroprotective, neurodegenerative therapy [16, 17]. Increasing the effectiveness of jerangau rhizome extract has also been developed as *Acorus calamus* ZnONPs [14]; extraction method using sonication [15].

Research Problem

Previous research has identified the potential therapeutic benefits of jerangau rhizomes for treating various illnesses, including their antimicrobial properties. Moreover, several studies have shown promising results in enhancing the effectiveness of jerangau rhizomes through methods like sonication and ionization. The scientific proof of a material as an antibacterial is based on the mechanism of action that occurs when the test material and bacteria interact both in vitro and in vivo. The development of the potential of jerangau rhizomes based on the empirical use [2] as a preventative, complementary medicine and treatment of pneumonia needs to be scientifically proven. This study aimed to bolster confidence in the practical application and future advancement of products derived from jerangau rhizome extract through various solvent extraction methods, including polar, semi-polar, and non-polar solvents.

Research Focus

This study aimed to test the antibacterial activity of *Acorus calamus* (L) against the growth of *Streptococcus pneumoniae* in vitro using agar diffusion and liquid dilution methods. This research was focused on scientifically proving the results of the interaction of pneumonia-causing bacteria and jerangau rhizome extract which was extracted with polar, semi-polar and non-polar solvents.

Research Aim and Research Questions

How is the effectiveness of jerangau rhizome extract compared to standard treatment for *Streptococcus pneumoniae* based on its ability to inhibit bacterial growth? What concentration of hexane, ethanol and ethyl acetate extract of jerangau rhizome is effective as an antibacterial against *Streptococcus pneumoniae* based on the Minimum Inhibitory Concentration and Minimum Killing Concentration?

Research Methodology

General Background

The rhizome of jerangau (*Acorus calamus* (L)) has long been utilised by the community, particularly in South Sulawesi, for treating respiratory issues in children. The traditional practice involves consuming the juice of the rhizome as a cough medicine or applying it to the chest as a remedy for shortness of breath. Previous research has proven the potential of *Acorus calamus* (L) as an anti-inflammatory and contains asarone. Based on this, scientific evidence is needed to determine the active substances of the plant and determine the antibacterial potential that causes pneumonia (manifestation of symptoms of cough and shortness of breath).

Sample / Participants / Group

The bacteria tested as samples in this study were *Streptococcus pneumoniae*, culture results from clinical sample isolates that were sensitive to Amoxicillin, obtained from the Microbiology Laboratory, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia.

Instrument and Procedures

Jerangau rhizome simplicia is prepared by: fresh jerangau rhizome obtained from Luwu Regency, South Sulawesi, Indonesia is cleaned (wet sorting) from all types of dirt and removing damaged or young parts of the rhizome. Once

cleaned, the rhizomes are first cut into coarse pieces, and then further chopped into simplicia powder. This powder is then ready for drying, which is done in an oven set at a temperature between 40-50°C [16]. The dry simplicia test material of jerangau rhizome was extracted using a separate maceration method using hexane, ethanol and ethyl acetate solvents. Maceration was carried out at room temperature and stirred regularly with a spatula. During the maceration process, the maceration container was tightly closed in order to prevent evaporation of the solvent, contamination from outside the container and stored in a room protected from sunlight. The solvent replacement was carried out periodically according to the procedures of the Indonesian Herbal Pharmacopoeia [16]. Next, the macerated extract was concentrated gradually using a rotary evaporator at 60 °C until all the liquid extract became thick to be used as a test material. The obtained extract was diluted into a concentration series as a test material. To test the agar diffusion method, each extract (hexane, ethanol and ethyl acetate) used 6 concentrations, namely: 0.25%w/v; 0.5%w/v; 1%w/v; 2%w/v; 4%w/v and 8%w/v. For testing the liquid dilution method, a concentration of 0.1% w/v is used; 0.25%w/v; 0.5%w/v; 0.75%w/v; 1%w/v; 1.25%w/v; 1.5%w/v; 1.75%w/v; 2%w/v; 2.25%w/v; 2.5%w/v. This process ensured that the concentration chosen for the liquid dilution test was effective in inhibiting the growth of the bacteria. By starting with a higher concentration in the agar diffusion test and gradually diluting it in the liquid dilution test, it was possible determining the minimum concentration needed to achieve the desired result. This approach helped optimising the testing process and ensures accurate and reliable results.

The antibacterial activity to determine the growth inhibition zone for *Streptococcus pneumoniae* was carried out using the agar diffusion method using Mueller Hinton Agar (MHA) media. The test bacteria were prepared by suspending samples of *Streptococcus pneumoniae* culture in sterile water until a turbidity level equivalent to the Mc Farland standard of 0.5 was obtained. The test materials for 6 concentrations of each extract (hexane, ethanol and ethyl acetate) of jerangau rhizomes were prepared separately in sterile petri dishes. Next, blank paper discs were immersed in each extract for 1 hour and then allowed to dry. The test bacteria were evenly inoculated onto each plate containing MHA media. Subsequently, the paper discs were symmetrically placed on the surface of the media, which had been inoculated with the test bacteria, for each concentration of the test material. The treatment was made in 3 replications and incubated in an incubator at 37°C for 1x24 hours and 2x24 hours. Observations were made by measuring the inhibition zone that occurs around the paper disc.

The antibacterial effectiveness was determined by the turbidity parameters that occur in the Nutrient Broth (NB) media. Test materials were inserted into tubes containing NB media and the same amount of test bacteria was inoculated for each tube. There were 4 media tubes prepared for each concentration of test material (triplicate for the concentration of test material extract inoculated with *Streptococcus pneumoniae* suspension and one for media control + test material, without test bacteria). The same treatment was made for a total of 18 concentrations of 3 extracts (hexane, ethanol and ethyl acetate) and incubated in an incubator at 37°C for 1x24 hours and 2x24 hours. Turbidity levels were observed by comparing the readings from tubes in series 1-3 (containing both test material and test bacteria) with those from series 4 (containing only test material).

Data Analysis

The observation of the agar diffusion method produced a table of diameters of the inhibition zone for the growth of the *Streptococcus pneumoniae* test bacteria. The data was processed using SPSS in order to determine test materials that have the potential to act as *Streptococcus pneumoniae* antibacterials. Data analysis used SPSS to show significant differences in inhibition zone data from test materials with negative controls, positive controls and other test materials (differences between concentrations of test materials). Data analysis used a significance confidence level of $\alpha=0.05$. The observation of the liquid dilution method produces a table of bacterial growth in each treatment tube to determine the smallest concentration of the test material that can inhibit the growth of *Streptococcus pneumoniae* bacteria. Minimum inhibitory concentration (MIC) was determined after 1x24 hours of incubation and Minimum Killing Concentration (MKC) is determined after 2x24 hours of incubation.

Research Results

The research results are listed in tables 1 to table 6.

Table 1. Mann Whitney test results data on differences in bacteriostatic and bacteriocidal activity of jerangau rhizome hexane extract against *Streptococcus pneumoniae*

Treatment group	n	Bacterial growth inhibition zone (mm) bacteriostatic activity of jerangau rhizome hexane extract				
		Mean	SD	Median	Min	Maks
1	3	12.00	2.64	11.00 ^a	10.00	15.00
2	3	13.50	1,80	14.00 ^{a,b}	11.50	15.00
3	3	15.50	1.32	16.00 ^{a,b,c}	14.00	16.00

4	3	17.16	1.04	17.50 ^{a,b,c,d}	16.00	18.00
5	3	18.83	3.05	19.50 ^{a,b,c,d,e}	15.50	21.50
6	3	16.83	1.44	16.00 ^{c,d,e}	16.00	18.00
7	3	6.00	0.00	6.00	6.00	6.00
Treatment group	n	Bacterial growth inhibition zone (mm) bacteriocidal activity of jerangau rhizome hexane extract				
		Mean	SD	Median	Min	Maks
1	3	11.33	0.57	11.00	11.00	12.00
2	3	13.16	0,76	13.00 ^p	12.50	14.00
3	3	15.83	1.04	15.50 ^q	15.00	17.00
4	3	16.33	1.15	17.00 ^{q,r}	15.00	17.00
5	3	17.33	2.08	18.00 ^{q,r,s}	15.00	19.00
6	3	13.66	1.15	13.00 ^{p,q,r,s}	13.00	15.00
7	3	6.00	0.00	6.00	6.00	6.00

Information :

a,b,c the same superscript indicates there is no difference between treatment groups (based on the Mann Whitney test)

p,q,r same superscript indicates no difference between treatment groups (based on Mann Whitney test)

1: group 1 treated with test material, hexane extract of jerangau rhizomes, concentration of 0.5% w/v

2: group 2 treated with the test material hexane extract of jerangau rhizome at a concentration of 1% w/v

3: group 3 treated the test material with hexane extract of jerangau rhizomes at a concentration of 2% w/v

4: group 4 treated the test material with hexane extract of jerangau rhizomes at a concentration of 4% w/v

5: group 5 treated the test material with hexane extract of jerangau rhizomes at a concentration of 8% w/v

6: group 6 treated with positive control test material Amoxicilin with a concentration of 50 ppm

7: group 7 treated with negative control test material

Table 1 demonstrates that hexane extract is effective as an antibacterial agent against *Streptococcus pneumoniae* at a concentration of 2% w/v, outperforming the 50 ppm amoxicillin antibiotic treatment. This conclusion is supported by the results of the Mann Whitney follow-up analysis. This analysis provides significant differences between treatments at a significant level of $p = 0.05$. The data in table 1 states the potential to inhibit bacterial growth from hexane extract of jerangau rhizomes at a treatment concentration of 0.5% w/v, however the inhibitory activity is shown starting from a concentration of 2% w/v because it is not significantly different from a concentration of 8% w/v and the amoxicillin control. However, in killing bacteria, it turns out that optimal effectiveness is shown by a concentration of 4%w/v which is not significantly different from a concentration of 8%w/v and amoxicillin 50 ppm.

Table 2. Mann Whitney test results data on differences in bacteriostatic and bacteriocidal activity of ethanol extract of jerangau rhizomes against *Streptococcus pneumoniae*

Treatment group	N	Bacterial growth inhibition zone (mm) bacteriostatic activity of ethanol extract of jerangau rhizomes				
		Mean	SD	Median	Min	Maks
1	3	13.00	1.73	12.00 ^a	12.00	15.00
2	3	15.66	3,88	14.50 ^{a,b}	12.50	20.00
3	3	15.33	3.21	14.00 ^{a,b,c}	13.00	19.00
4	3	13.83	1.04	13.50 ^{a,b,c,d}	13.00	15.00
5	3	17.50	0.50	17.50 ^{a,c,d,e}	17.00	18.00

6	3	17.66	0.28	17.50 ^{c,d,e}	17.50	18.00
7	3	6.00	0.00	6.00	6.00	6.00
Treatment group	N	Bacterial growth inhibition zone (mm) bacteriocidal activity of ethanol extract of jerangau rhizomes				
		Mean	SD	Median	Min	Maks
1	3	6.00	0.00	6.00 ^p	6.00	6.00
2	3	6.00	0.00	6.00 ^{p,q}	6.00	6.00
3	3	6.00	0.00	6.00 ^{p,q,r}	6.00	6.00
4	3	11.50	0.50	11.50 ^s	11.00	12.00
5	3	13.16	0.76	13.00 ^{s,t}	12.50	14.00
6	3	12.16	0.76	12.00 ^t	11.50	13.00
7	3	6.00	0.00	6.00 ^{p,q,r}	6.00	6.00

Information :

a,b,c the same superscript indicates there is no difference between treatment groups (based on the Mann Whitney test)

p,q,r same superscript indicates no difference between treatment groups (based on Mann Whitney test)

1: Group 1 treated the test material with ethanol extract of jerangau rhizomes at a concentration of 0.5% w/v

2: group 2 treated with ethanol extract of jerangau rhizomes at a concentration of 1% w/v

3: group 3 treated the test material with ethanol extract of jerangau rhizomes at a concentration of 2% w/v

4: group 4 treated the test material with ethanol extract of jerangau rhizomes at a concentration of 4% w/v

5: group 5 treated the test material with ethanol extract of jerangau rhizomes with a concentration of 8% w/v

6: group 6 treated with positive control test material Amoxicilin with a concentration of 50 ppm

7: group 7 treated with negative control test material

Table 2 shows the data from the Mann Whitney analysis at a significant level of $p=0.05$ which states that the ethanol extract of jerangau has potential as an antibacterial for *Streptococcus pneumonia* starting from 2%w/v. Table 1 states the potential to inhibit the growth of *Streptococcus pneumoniae* from the ethanol extract of jerangau rhizomes starting from a treatment concentration of 2%w/v which is not significantly different from the concentration of 8%w/v and the amoxicillin control. However, in killing bacteria, it turns out that optimal effectiveness is shown by a concentration of 8%w/v which is not significantly different from 50ppm amoxicillin. Comparing the efficacy of the antibiotic amoxicillin 50 ppm, commonly used in treating pneumonia infections, to the potential activity of an extract concentration with bacteriostatic and bacteriocidal properties will determine the extract's effectiveness in fighting bacteria.

Table 3. Mann Whitney test results data on differences in bacteriostatic and bacteriocidal activity of ethyl acetate extract of jerangau rhizomes against *Streptococcus pneumoniae*

Treatment group	N	Bacterial growth inhibition zone (mm) bacteriostatic activity of ethyl acetate extract of jerangau rhizomes				
		Mean	SD	Median	Min	Maks
1	3	12.16	1.04	12.50 ^a	11.00	13.00
2	3	13.66	1.52	14.00 ^{a,b}	12.00	15.00
3	3	14.00	0.86	13.50 ^{b,c}	13.50	15.00
4	3	15.83	1.75	16.00 ^{a,b,c,d}	14.00	17.50
5	3	20.66	2.75	22.00 ^{a,b,d}	17.50	22.50
6	3	16.00	0.00	16.00 ^d	16.00	16.00
7	3	6.00	0.00	6.00	6.00	6.00
Treatment group	n	Bacterial growth inhibition zone (mm) bacteriocidal activity of ethyl acetate extract of jerangau rhizomes				

		Mean	SD	Median	Min	Maks
1	3	9.00	1.00	9.00 ^p	8.00	10.00
2	3	10.50	0.50	10.50 ^{p,q}	10.00	11.00
3	3	11.50	1.32	12.00 ^r	10.00	12.50
4	3	12.33	0.76	12.50 ^{r,s}	11.50	13.00
5	3	14.50	0.50	14.50 ^{r,s}	14.00	15.00
6	3	11.16	1.25	11.00 ^q	10.00	12.50
7	3	6.00	0.00	6.00	6.00	6.00

Information :

a,b,c the same superscript indicates there is no difference between treatment groups (based on the Mann Whitney test)

p,q,r same superscript indicates no difference between treatment groups (based on Mann Whitney test)

1: Group 1 treated the test material with ethyl acetate extract of jerangau rhizomes at a concentration of 0.5% w/v

2: group 2 treated with ethyl acetate extract of jerangau rhizomes at a concentration of 1% w/v

3: group 3 treated the test material with ethyl acetate extract of jerangau rhizomes at a concentration of 2% w/v

4: group 4 treated the test material with ethyl acetate extract of jerangau rhizomes at a concentration of 4% w/v

5: group 5 treated the test material with ethyl acetate extract of jerangau rhizomes with a concentration of 8% w/v

6: group 6 treated with positive control test material Amoxicilin with a concentration of 50 ppm

7: group 7 treated with negative control test material

Table 3 states that the antibacterial potential of *Streptococcus pneumoniae* from ethyl acetate extract of jerangau rhizomes is effective at a treatment concentration of 4% w/v. Data shows that the treatment with a concentration of 4%w/v is not significantly different in killing bacteria with a concentration of 8%w/v and is bacteriostatic compared to the amoxicillin control. Some data shows that the analysis results are not significantly different for treatments with a concentration of 0.5%w/v and concentrations of 1%w/v, 2%w/v, 4%w/v or even 8%w/v. This analysis suggests that there is no significant difference in antibacterial potency among the concentrations tested, indicating that their potency levels are similar. This applies to inhibitory zone data from hexane extract treatment (table 1), ethanol extract (table 2) and ethyl acetate extract (table 3).

Table 4. Results of Testing the Effectiveness of Jerangau Rhizome Hexane Extract as an Antibacterial against *Streptococcus pneumoniae*

Observation time		Concentration of test material for jerangau rhizome hexane extract											Control	
	Tube												(+) (+)	(-) (-)
		0,1 %	0,25 %	0,5 %	0,75 %	1 %	1,25 %	1,5 %	1,75 %	2 %	2,25 %	2,5 %		
1 x 24 hours	1-3	3+	2+	-	-	-	-	-	-	-	-	-	-	+
2 x 24 hours	1-3	3+	3+	3+	+	-	-	-	-	-	-	-	-	+

Description

Control (+): Cefadroxil

Control (-): Na CMC

+: There is turbidity in the liquid media which indicates the growth of *Streptococcus pneumoniae*

- : No differences were found in turbidity in the media compared to the control tube

Table 4 shows the test data of the liquid dilution method to determine the effective concentration as MIC and MKC. The hexane extract treatment of jerangau rhizomes in inhibiting and killing *Streptococcus pneumoniae* bacteria was effective at MIC (0.5%w/v) and MKC (1%w/v). This data is determined based on the parameter Level of turbidity that occurs after incubation of bacteria in medical products containing the test material.

Table 5. Results of Testing the Effectiveness of Jerangau Rhizome Ethanol Extract as an Antibacterial against *Streptococcus pneumoniae*

Observation time	Tube	Concentration of test material for jerangau rhizome ethanol extract											Control	
		0,1	0,25	0,5	0,75	1	1,25	1,5	1,75	2	2,25	2,5	(+)	(-)
		%	%	%	%	%	%	%	%	%	%	%		
1 x 24 hours	1-3	2+	1+	-	-	-	-	-	-	-	-	-	-	+
2 x 24 hours	1-3	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	-	+

Description

Control (+): Cefadroxil

Control (-): Na CMC

+: There is turbidity in the liquid media which indicates the growth of *Streptococcus pneumoniae*

- : No differences were found in turbidity in the media compared to the control tube

Table 5 shows that the ethanol extract treatment of jerangau rhizomes in inhibiting and killing *Streptococcus pneumoniae* bacteria was effective at MIC (0.5%w/v) but MKC > 2.5%w/v. Table 5 shows that the ethanol extract treatment of jerangau rhizomes in inhibiting and killing *Streptococcus pneumoniae* bacteria was effective at MIC (0.5%w/v) but MKC > 2.5%w/v.

Table 6. Results of Testing the Effectiveness of Jerangau Rhizome Ethyl Acetate Extract as an Antibacterial against *Streptococcus pneumoniae*

Observation time	Tube	Concentration of test material for jerangau rhizome ethyl acetate extract											Control	
		0,1	0,25	0,5	0,75	1	1,25	1,5	1,75	2	2,25	2,5	(+)	(-)
		%	%	%	%	%	%	%	%	%	%	%		
1 x 24 hours	1-3	3+	-	-	-	-	-	-	-	-	-	-	-	+
2 x 24 hours	1-3	3+	3+	-	-	-	-	-	-	-	-	-	-	+

Description

Control (+): Cefadroxil

Control (-): Na CMC

+: There is turbidity in the liquid media which indicates the growth of *Streptococcus pneumoniae*

- : No differences were found in turbidity in the media compared to the control tube

Table 6 shows the test data of the liquid dilution method to determine the effective concentration as MIC and MKC. Treatment of ethyl acetate extract of jerangau rhizomes in inhibiting and killing *Streptococcus pneumoniae* bacteria was effective at MIC (0.25%w/v) and MKC (0.5%w/v). Data tables 4, 5, and 6 display the maximum potential of the ethyl acetate extract from jerangau rhizomes as an antibacterial agent against *Streptococcus pneumoniae*.

Discussion

The antibacterial activity of *Streptococcus pneumoniae* from jerangau rhizomes was observed based on the bacterial growth inhibition zone after 1 x 24 hour and 2 x 24 hour incubation. The results of statistical analysis showed that the hexane extract of jerangau rhizomes was bacteriostatic at all extract concentrations tested. However, the bacteriocidal properties started at a concentration of 1% and are optimal at a concentration of 8% w/v. The bacteriostatic and bacteriocidal properties were produced by the active compound content extracted from jerangau rhizomes using hexane solvent. Alkaloid, tannin, steroid and terpenoid compounds supported the antibacterial potential of hexane extract from jerangau rhizomes.

The results of statistical analysis showed that the ethanol extract of jerangau rhizomes was bacteriostatic at all extract concentrations tested. However, the bacteriocidal properties started at a concentration of 4% and were optimal at a concentration of 8% w/v. The bacteriostatic and bacteriocidal properties were produced by the active compound content extracted from jerangau rhizomes using ethanol solvent. Alkaloid compounds, saponins, flavonoids, steroids and phlobatin supported the antibacterial potential of the ethanol extract of jerangau rhizomes. This active substance has also been found in other herbs which have the potential to act as anti-pneumonia [17-19].

The results of statistical analysis showed that the ethyl acetate extract of jerangau rhizomes was bacteriostatic at all extract concentrations tested. However, the bacteriocidal properties started at a concentration of 0.5% and were optimal at a concentration of 4% w/v. The bacteriostatic and bacteriocidal properties were produced by the active compound content extracted from jerangau rhizomes using ethyl acetate solvent. Alkaloid, flavonoid and steroid compounds supported the antibacterial potential of the ethanol extract of jerangau rhizomes. The results of this research found that the compounds from semipolar extracts (ethyl acetate extract) were more effective in inhibiting bacterial growth. This is in line with similar research which used ethyl acetate solvent to extract phytochemical compounds [20-22].

Paper discs are a convenient medium for use, however, they have limited absorption of active substances which may result in a reduced inhibiting power. Despite this, the homogeneity of the disc's number and diffusion mechanism ensures consistent and reliable data replication results. It should be noted that when using paper discs, after immersing the paper disc in the test material, the paper disc should be drained until there is no excess liquid in the test material. The excess fluid on the paper disc will cause the paper disc plate to move during the incubation period, so that the resulting inhibition zone does not form a circle. The absorption capacity of a paper disc is 0.2 ml (4 standard drops). However, soaking for a long time will give the paper disc the opportunity to absorb more so that it contains more active substances. However, replicating the same treatment for all test materials will still provide homogeneous treatment.

Antibacterial activity testing uses the diffusion method so that it can be carried out with the help of paper discs, supports and wells as containers to accommodate the test material. The test material will diffuse out of the container into the surrounding area. If the active substance contained in the test material is sufficient in quantity and is antibacterial, a growth inhibition zone will form around it in the form of a clear area where bacteria do not grow. The testing of herbal extracts against *Streptococcus pneumoniae* has also been tested using miana leaf extract either alone or in combination [23-26]; bitter melon [27]; tamarind fruit [28]; basil [29] and bay leaf [30]. All the results of this research used the same method as this research, namely agar diffusion using the paper disc or well technique.

The determination of the MIC and MKC values of an extract indicates the smallest concentration used to inhibit or kill *Streptococcus pneumoniae*. Observations were observed with the level of turbidity after incubation for 1x24 hours or 2x24 hours. The study findings revealed that the hexane extract from jerangau rhizome exhibited inhibitory activity at a minimum concentration of 0.5%w/v and lethal effects at a minimum concentration of 1%w/v. Furthermore, ethanol extract of jerangau rhizome can inhibit at a minimum level of 0.5% w/v and can kill at levels > 2.5% w/v. Jerangau rhizome ethyl acetate extract can inhibit at a minimum level of 0.25%w/v and can kill at a minimum level of 1%w/v.

The advantage of the method used for testing using liquid dilution was that it could obtain effective concentration data either as a minimum barrier or to kill bacteria. Konsentrasi hambat minimal (KHM) atau minimal inhibitor concentration (MIC) adalah konsentrasi minimal dari suatu bahan antibakteri untuk menghambat pertumbuhan bakteri uji setelah diinkubasi hingga 24 jam. Minimum killing concentration (KBM) or minimum killing concentration (MIC) was the minimum concentration of an antibacterial agent to kill test bacteria after incubation for up to 48 hours.

Previously, a liquid dilution method was developed for testing herbal extracts or colored test materials. The results obtained from this method can provide reliable observation data by comparing the interaction of extract colors as a color control, in addition to media control and test bacteria control [31].

The minimum dose was observed using a liquid dilution method. Sterile media was prepared for each concentration in a series of four. Series 1-3 were replications, while series 4 served as the control. The control was important for determining color standards when the media only contained extracts or test materials. In this particular experiment, the test material being used was colored or alters the original color of the media. The observation

parameters for this method were changes that occurred in the media mixture, the test material and exposure to the test bacteria/fungi. Changes could take the form of turbidity, viscosity, color and other physical forms that were visible to the eye. Organoleptic observation was the main parameter for this test which can be done manually with the observer/researcher's eyes or using spectrophotometry. The media used for this method was enrichment media for bacteria or fungi depending on the type of test microorganism. Liquid media such as nutrient broth (NB), lactose broth (LB), potato dextrose broth (PDB) and others could be used to test this liquid dilution method. The advantage of the liquid dilution method was that it could estimate the minimum concentration that had the potential to inhibit and/or kill bacteria based on the level of turbidity changes that occurred in the media due to bacterial growth. Observations have shown that the more bacteria grew, the more cloudy the media became and was accompanied by color and physical changes such as viscosity and clumping.

This research was successful in obtaining accurate data on color changes because it used a 4th series tube comparison in accordance with previous research which had developed methods for determining MIC and MKC for colored extract test materials [31]. The methods for determining MIC and MKC for plant extracts and antibacterial controls have been previously carried out on Miana leaf extract [32] and 26 types of herbs that have potential for antituberculosis [33]. This will help differentiate this study from existing studies and underscore its contribution to the field of natural antibacterial agents.

This research has proven the potential of jerangau rhizomes as an antibacterial for *Streptococcus pneumoniae* in vitro based on agar diffusion and liquid dilution methods. These results were in line with research that proved the potential of jerangau as an antibacterial for *Staphylococcus aureus* and *Escherichia coli* [34], [3], [9], *Helicobacter pylori* [10]; *Streptococcus pyogenes* [11]. Increasing the potential of jerangau rhizome extract has also been carried out by making nanoparticle formulations [34], [15] and extraction methods using ultrasonics [35], [36] which apparently increased the potential of jerangau rhizome extract as an antibacterial. These results added to the scientific evidence of jerangau rhizome as an antibacterial.

The potential of jerangau rhizome as an antioxidant, antibacterial, anti-inflammatory, antitumor, diabetic, cardiovascular, immunomodulator and others cannot be separated from the active compound content it contains. Research has identified the content of flavonoid and fatty acid compounds [5], flavonoids, phenols [37], [4], phenylpropanoids, sesquiterpenes and monoterpenes [38], α -asaronol, β -asaronol compounds, γ -asaronol [39]. The findings resulting from several studies have concluded a relationship between the content of active compounds in jerangau rhizomes and the medicinal activity that has been empirically used. The safety of using jerangau rhizomes has been proven by toxicity tests [36]. This evidence can be recommended in conventional medicine as a complement to infection therapy with immunomodulatory mechanisms (modulation of CD4 expression, TNF- α) and antioxidants [5]. This evidence also had positive implications for the public to use jerangau rhizomes as an alternative anti-respiratory tract infection treatment.

Apart from the antibacterial potential of jerangau rhizomes, especially against *Streptococcus pneumoniae*, this research has limitations in terms of the bacterial samples used in testing and the method of preparing the extract as test material. It is known that several external factors can influence the quality of the active substances from simplicia/natural extracts such as the source of simplicia (concerning plant profile), extraction method, type of solvent used in extraction. To minimise this diversity, simplicia is taken from the same planting location. In connection with this, the simplicia used in this research was taken directly from Luwu Regency as the location for empirical data on the use of simplicia. However, extract standardisation has been carried out to ensure the quality of the jerangau rhizome extract meets the standards of the Indonesian Herbal Pharmacopoeia to eliminate doubts regarding the suitability of the extract test material used as test material [4]. The *Streptococcus pneumoniae* test bacteria used in this study were bacterial isolates resulting from isolation and purification from clinical bacterial samples. The bacterial isolate has been found to be sensitive to amoxicillin, making it suitable for use as a positive control in this test. However, it is important to note the limitations of using unlicensed test bacterial isolates, particularly when testing for other bacteria that cause pneumonia. These limitations should be taken into consideration in future tests.

Conclusions and Implications

The potential bacteriocidal effectiveness of jerangau rhizomes compared to 50 ppm amoxicillin occurred at concentrations of 2%w/v (hexane extract), 8%w/v (ethanol) and 4%w/v (ethyl acetate). Based on the liquid dilution method: MIC obtained value = 0.5%; and MKC = 1% in HJ; MIC value = 0.5% and MKC > 2.5% at EJ; MIC value = 0.25% and MKC = 0.5% at EAJ.

Demonstrating the antibacterial efficacy of jerangau rhizomes extracted using hexane, ethanol, and ethyl acetate

solvents against *Streptococcus pneumoniae* in vitro has confirmed their potential as a natural antibacterial agent. These findings support the traditional use of jerangau rhizome as a treatment for pneumonia and suggest its viability as an herbal remedy. This research is ready for publication and the development of jerangau rhizome-based products for pneumonia management.

Suggestions for further research

Further research will provide in vivo evidence of various mechanisms that support the function of curing pneumonia, such as antibacterials, immunomodulators, dietary supplements, inhalation or other appropriate mechanisms. Further research into the potential benefits of jerangau rhizome products in the treatment of pneumonia has the potential to develop user-friendly herbal products that can be easily prepared at home by the general public. Empirically, jerangau rhizomes can be used by making rhizome juice for drinking and drying it for inhalation. Based on empirical use, balsam formulations can be made, or herbal drink products in powdered or chopped form. In addition, future studies could explore the integration of jerangau rhizome extracts with modern pharmaceutical methods to improve their efficacy and stability.

Research into the optimal dosage and long-term effects of jerangau rhizome products will further validate their safety and efficacy. Finally, assessing the economic viability and market acceptance of these herbal products may pave the way for their widespread adoption and use in traditional and modern healthcare practices.

Declarations

Author Contributions

Conceptualisation, Sesilia Rante Pakadang and Ida Adhayanti; methodology, Sesilia Rante Pakadang and Ida Adhayanti; software, Sesilia Rante Pakadang and Ida Adhayanti; validation, Sesilia Rante Pakadang and Ida Adhayanti; formal analysis, Sesilia Rante Pakadang and Ida Adhayanti; investigation, Sesilia Rante Pakadang and Ida Adhayanti; resources, Sesilia Rante Pakadang and Ida Adhayanti; data curation, Sesilia Rante Pakadang and Ida Adhayanti; writing-original draft preparation, Sesilia Rante Pakadang; writing -review and editing, Ida Adhayanti; visualization, Sesilia Rante Pakadang and Ida Adhayanti; supervision, Sesilia Rante Pakadang and Ida Adhayanti; project administration, Sesilia Rante Pakadang and Ida Adhayanti; funding acquisition, Sesilia Rante Pakadang and Ida Adhayanti; All authors have read and agreed to the published version of the manuscript.

Data Availability Statement

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

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Institutional Review Board Statement

This research data is part of a research project that is protected by an ethical suitability statement "ETHICAL EXEMPTION" No.: 0229/O/KEPK-PTKMS/III/2023 " dengan judul "Characteristics and Potency of *Acorus calamus* Linn., *Coleus scutellarioides* (L) Benth and *Andrographis paniculata* Ness extracts on CD4+/CD8+ T-cell modulation and hematological tests on *Rattus norvegicus* infected with *Streptococcus pneumoniae*".

Informed Consent Statement

Informed Consent Statement is not applied in this research.

Conflicts of Interest

The authors state that there is no conflict of interest in this research, whether in the form of data or published articles, with any party.

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