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Comparison Of Ethanol, N-Hexane and Ethyl Acetate Extract in Andaliman Leaves (*Zanthoxylum Acanthopodium DC)* Anti-Bacterial Test for *Staphylococcus Aureus*

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ABSTRACT

Skin infections caused by this bacterium remain common, and antibiotic resistance continues to increase, creating a need for natural antibacterial sources. Andaliman leaves are one plant that can be used as a natural antibacterial active ingredient due to their content of flavonoids, tannins, saponins, terpenoids, and essential oils. This study aimed to determine whether n-hexane, ethyl acetate, and ethanol extracts of andaliman leaves exhibit antibacterial activity against Staphylococcus aureus. The study was conducted experimentally, beginning with successive extraction of andaliman leaves using n-hexane, ethyl acetate, and ethanol, followed by antibacterial activity testing using the disc diffusion method at concentrations of 20%, 30%, and 40%. The results showed that the n-hexane extract produced inhibition zones of 8.3±0.12 mm at 20%, 8.8±0.16 mm at 30%, and 9.3±0.04 mm at 40%. The ethyl acetate extract showed increased inhibitory activity with inhibition zones of 9.4 ± 0.28 mm at 20%, 12.7 ± 1.52 mm at 30%, and 16.0 ± 1.31 mm at 40%. The ethanol extract exhibited the highest antibacterial activity, with inhibition zones of 13.0±1.66 mm at 20%, 15.0±1.81 mm at 30%, and 19.4±1.12mmat40%.

INTRODUCTION

Infectious diseases are a frequent health problem in tropical countries like Indonesia. The warm temperatures and high humidity of tropical environments create conditions that favor the growth of various infection-causing microbes (Ramadhani et al., 2020) .

Infectious diseases in Indonesia are increasing every year due to various factors. These factors include low public awareness of hygiene, limited trained healthcare workers, high population density, and a lack of knowledge and application of basic infection prevention principles. Furthermore, unsafe procedures and suboptimal government guidelines and policies contribute to the increasing incidence of infectious diseases (Ginting et al., 2025) .

Skin infections are usually caused by bacteria such as *Staphylococcus aureus*, *Staphylococcus epidermidis, Propionibacterium acnes, Streptococcus pyogenes, Pseudomonas aeruginosa, Streptococcus β hemoliticus* (Afriani, 2022) Bacteria that cause infections such as *Staphylococcus aureus* are Gram-positive bacteria that are round in shape with a size of 0.7 to 1.2 micrometers, forming irregular groups that look like grapes, are facultative anaerobes, do not form spores, and do not have the ability to move, growing optimally at a temperature of 37 ^{o} C (Gaol et al., 2025)

Bacterial attacks can usually be prevented by administering antibacterials. Antibacterials are metabolites produced by various types of microorganisms. These compounds, at low concentrations, can inhibit the growth of other microorganisms. Antibacterial activity is divided into two categories: bactericidal, which kills microorganisms, and bacteriostatic, which inhibits the growth of microorganisms (Nazar, 2023) . However, the incorrect use of antibiotics can cause resistance, resulting in treatment failure (Mubarak et al., 2022) .

One of the Indonesian plants included in the endemic plant category is andaliman (*Zanthoxylum acanthopodium* DC.). Andaliman is a spice plant typical of North Sumatra which belongs to the Rutaceae family. This plant is a shrub or small tree with a thorny stem and branches and pinnate compound leaves. The leaves are dark green and emit a distinctive aroma when crushed, while the fruit is small and has a bitter, spicy, and numbing taste. Andaliman grows primarily in mountainous areas at elevations of around 800 to 2,000 meters above sea level and is commonly found in the Toba region and its surroundings (Ompusunggu & Irawati, 2021) .

The parts of the andaliman plant that are often used include the leaves and fruit. Chemically, andaliman contains various bioactive compounds such as alkaloids, flavonoids, terpenoids, and essential oils. Traditionally, andaliman is used as a culinary spice and is also used to help treat digestive disorders, as an antibacterial, antioxidant, and anti-inflammatory (Hutapea et al., 2024) .

Research by (Sepriani et al., 2020) reported that the results of the antibacterial activity test of andaliman bark and leaf extract against *Staphylococcus aureus bacteria* with an inhibition zone of 11 mm at a concentration of 5x104 ^{ppm} which is included in the strong category, while the andaliman leaf extract with an inhibition zone of 10 mm at a concentration of 5x104 ^{which} is included in the moderate category.

Another researcher by (Muzafri, 2019) reported the results of testing the antibacterial activity of methanol extract of andaliman fruit against *Staphylococcus epidermidis bacteria* which was carried out using the disc paper diffusion method, showing the average diameter of the zone. inhibition for concentrations of 25%; 7 mm, 50%; 7.4 mm, 75%; 7.6 mm, and 100%; 9.4 mm are in the medium category.

Based on the description above, the researcher is interested in conducting research on andaliman leaves (*Zanthoxylum acanthopodium* DC.) by extracting them in stages based on polarity, namely n-hexane, ethyl acetate and ethanol and testing their antibacterial activity against *Staphylococcus aureus*.

METHOD

This research is an in vitro laboratory experimental study with the aim of comparing the antibacterial activity of n-hexane, ethyl acetate, and ethanol extracts of andaliman leaves (*Zanthoxylum acanthopodium DC*) against Staphylococcus aureus bacteria. The research was conducted at the Integrated Laboratory of Prima Indonesia University, Medan, from October to December 2025. The research stages include sample preparation, multistage extraction, bacterial suspension preparation, antibacterial activity testing using the disc diffusion method, and inhibition zone diameter measurement.

Tools and materials

The tools used in this study include analytical scales, blenders, a set of laboratory glassware, beakers, Erlenmeyer flasks, measuring cylinders, filter paper, rotary vacuum evaporators, water baths, autoclaves, incubators, ovens, micropipettes, sterile cotton swabs, petri dishes, test tubes, tweezers, paper discs, vernier calipers, and Laminar Air Flow.

The materials used in this study were fresh andaliman leaves (*Zanthoxylum acanthopodium DC*.), n-hexane solvent, ethyl acetate, 96% ethanol, distilled water, Staphylococcus aureus bacterial culture, Mueller Hinton Agar (MHA) media, Nutrient Agar (NA) media, 0.9% NaCl solution, 0.5 McFarland standard, chloramphenicol as a positive control, and dimethyl sulfoxide (DMSO) or an appropriate solvent as a negative control.

Sample Preparation

Fresh andaliman leaves were collected, cleaned of any dirt, and then washed under running water. The leaves were then air-dried at room temperature until completely dry. The dried samples were then ground using a blender to obtain a powdered herbal medicine. The powdered herbal medicine was stored in a closed, dry container until used for the extraction process.

Multilevel Extraction of Andaliman Leaves

Andaliman leaf powder extraction was carried out using a multilevel maceration method based on the solvent polarity level. The powdered simplicia was first macerated using n-hexane solvent for 3 x 24 hours at room temperature with occasional stirring. After the maceration process was complete, the filtrate was separated by filtration, while the dregs were macerated again until the solvent appeared clear. The entire n-hexane filtrate obtained was then evaporated using a rotary vacuum evaporator to obtain a thick n-hexane extract.

The n-hexane extraction residue was then dried and re-extracted using ethyl acetate solvent using the same procedure. After that, the remaining residue was re-extracted using 96% ethanol using the same method. Each extraction filtrate was then evaporated using a rotary vacuum evaporator and a water bath to obtain a thick extract of n-hexane, ethyl acetate, and ethanol.

Preparation of Extract Concentration

Each extract was made in three concentration variations, namely 20%, 30%, and 40% (w/v). A certain amount of extract is weighed as needed, then dissolved using a suitable solvent such as DMSO until a homogeneous test solution is obtained. This concentration is used for antibacterial activity testing.

Making Bacterial Suspension

The test bacteria used in this study was Staphylococcus aureus. The bacterial culture was first rejuvenated on Nutrient Agar (NA) media and incubated at 37°C for 24 hours. Several bacterial colonies that had grown were then aseptically collected and suspended in a sterile 0.9 % NaCl solution. The turbidity of the bacterial suspension was adjusted to the McFarland 0.5 standard, which is equivalent to approximately 1.5×10^8 CFU/mL.

Antibacterial Activity Test

Antibacterial activity testing was carried out using the disc diffusion (Kirby-Bauer) method. Sterile Mueller Hinton Agar (MHA) media is poured into sterile petri dishes and allowed to solidify. The standardized Staphylococcus aureus bacterial suspension was then inoculated evenly onto the surface of the MHA media using sterile cotton swabs.

Sterile paper discs were then dripped with each extract at concentrations of 20%, 30%, and 40% of n-hexane, ethyl acetate, and ethanol extracts, respectively, and then placed on the surface of the media that had been inoculated with bacteria. Discs containing chloramphenicol were used as positive controls, while discs containing DMSO or solvents were used as negative controls.

All petri dishes were then incubated at 37°C for 24 hours. After the incubation period is complete, the clear zone formed around the disc is observed as the zone of bacterial growth inhibition.

Measurement of Inhibition Zone Diameter

The diameter of the inhibition zone formed around the disc was measured using a vernier caliper in millimeters (mm). Measurements were carried out three times for each treatment, then the results were expressed in the form of mean ± standard deviation (SD).

Data analysis

The data from the antibacterial activity test were analyzed descriptively based on the diameter of the inhibition zone formed against Staphylococcus aureus bacteria. The research results are presented in tabular form and compared between n-hexane, ethyl acetate, and ethanol extracts at concentrations of 20%, 30%, and 40%. The extract that showed the largest inhibition zone diameter was declared to have the strongest antibacterial activity.

RESULTS AND DISCUSSION

Results of Processing Plant Materials

Fresh andaliman leaves (*Zanthoxylum acanthopodium* DC) were taken as much as 3 kg and then dried andaliman leaves were obtained as much as 1,500 grams. From this result, andaliman leaf (*Zanthoxylum acanthopodium* DC) simplicia powder was then made into a fine, green, bitter and astringent leaf powder with a drying loss of 50%. Drying loss is the result of subtracting the weight of the wet sample from the weight of the dry sample after drying to determine the water content contained in the simple substance during storage and to avoid contamination by fungi or mold. (Ningrat et al., 2025).

The drying shrinkage is expressed in percentage value. in the form of 67.29 grams of thick n-hexane extract, 107.38 grams of thick ethyl acetate extract and 138.51 grams of thick ethanol extract with a brownish yellow color and yields of 13.45%, 21.47% and 46.17%, respectively. Based on the results obtained, it shows that the yield value of each solvent is different. The lowest yield was shown by n-hexane, and the highest by ethanol. This may occur because ethanol's dielectric constant is much higher than ethyl acetate and n-hexane, causing ethanol to be more polar than ethyl acetate and n-hexane. Therefore, extraction using ethanol can dissolve more secondary metabolites in the polar andaliman leaves (Nawaz et al., 2020).

The yield of extracts using n-hexane was lower than that using ethanol and ethyl acetate. This is due to the lower dielectric constant of n-hexane, which makes it difficult to dissolve polar secondary metabolites. Furthermore, it is suspected that the number of non-polar compounds in andaliman leaves is smaller than the number of polar compounds (Afriani, 2022) .

Phytochemical Screening Results

Phytochemical screening examination of n-hexane, ethyl acetate, and ethanol extracts of andaliman leaves (Zanthoxylum acanthopodium DC) included examination of alkaloids, flavonoids, tannins, saponins, and steroids/triterpenoids. The results of phytochemical screening of n-hexane, ethyl acetate, and ethanol extracts of andaliman leaves (Zanthoxylum acanthopodium DC) can be seen in the following table:

Table 1. Phytochemical Screening Results of n-Hexane Extract of Andaliman Leaves (*Zanthoxylum acanthopodium DC*)

No	Inspection	Reagent	Results Obtained	Conclusion
1	Alkaloid	Mayer's reaction	There is a yellowish sediment	Positive
		Bouchardat's reagent	Brown Sediment	Positive
		Dragendorff's reagent	Brown sediment	Positive
2	Flavonoid	Mg + HCl Powder (P)	No yellow solution is formed	Negative
3	Saponin	Hot distilled water, shaken vigorously + HCl	No Foam Formed	Negative
4	Steroids/ Triterpenoid	Lieberman-Burchard	Red Ring	Positive
5	Tannin	1 % FeCl3 solution	No blackish green solution is formed	Negative

Table 2. Phytochemical Screening Results of Ethyl Acetate Extract of Andaliman Leaves (*Zanthoxylum acanthopodium DC*)

No	Inspection	Reagent	Results Obtained h	Conclusion
1	Alkaloid	Mayer's reaction	There is a yellowish sediment	Positive
		Bouchardat's reagent	Brown Sediment	Positive
		Dragendorff's reagent	Brown sediment	Positive
2	Flavonoid	Mg + HCl Powder (P)	A yellow solution is formed	Positive
3	Saponin	Hot distilled water, shaken vigorously + HCl	Foam Formed	Positive
4	Steroids/ Triterpenoid	Lieberman-Burchard	Red Ring	Positive
5	Tannin	1 % FeCl3 solution	A blackish green solution is formed.	Positive

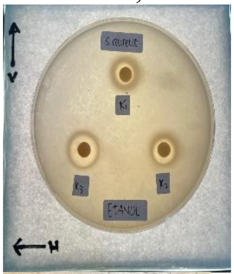
Table 3. Phytochemical Screening Results of Ethanol Extract of Andalima Leaves (*Zanthoxylum acanthopodium DC*)

No	Inspection	Reagent	Results Obtained	Conclusion
1	Alkaloid	Mayer's reaction	yellowish sediment	Positive
		Bouchardat's reagent	Brown Sediment	Positive
		Dragendorff's reagent	Brown sediment	Positive
2	Flavonoid	Mg + HCl Powder (P)	A yellow solution is formed	Positive
3	Saponin	Hot distilled water, shaken vigorously + HCl	Foam Formed	Positive
4	Steroids/ Triterpenoid	Lieberman-Burchard	Red Ring	Positive
5	Tannin	1 % FeCl3 solution	A blackish green solution is formed.	Positive

Based on tables 1, 2 and 3 above, it shows that the results of phytochemical screening on n-hexane extract showed positive results containing alkaloids and steroids, while the ethyl acetate and ethanol extracts of andaliman leaves were positive for alkaloids, flavonoids, tannins, steroids, and saponins. These results are also supported by the explanation (Butar-Butar et al., 2023) . that the type of solvent with different polarity can affect the profile of the detected phytochemical compounds. (Ningsih et al., 2025) . also added that the content of active ingredients contained in the same type of plant can vary and certain solvents can extract one or more compounds.

1. Antibacterial Activity Test Results of n-Hexane, Ethyl Acetate and Ethanol Extracts of Andaliman Leaves (*Zanthoxylum acanthopodium DC*) Against Staphylococcus aureus Bacteria.

The results of measuring the diameter of the inhibition zone against *Staphylococcus aureus bacteria* can be seen in tables 4, 5 and 6 below:



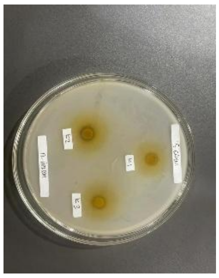


Table 4. Diameter of the Growth Inhibition Zone of *Staphylococcus aureus Bacteria* by n-hexane extract of andaliman leaves

Formula	Antibacterial Inhibitory Power against *Staphylococcus aureus* (mm)			Mean ±SD	Category
	P1	P2	P3		
K0	0	0	0	0	There isn't any
K1	8.2	8.3	8.5	8.3 ±0.12	Currently
K2	9.0	8.7	8.6	8.8 ±0.16	Currently
K3	9.2	9.3	9.3	9.3 ±0.04	Currently
K+	20.4	20.8	20.6	20.6 ±0.16	Very strong

Table 5. Diameter of the Growth Inhibition Zone of *Staphylococcus aureus Bacteria* by Ethyl Acetate Extract of Andaliman Leaves

Formula	Antibacterial Inhibitory Power to *Staphylococcus aureus* (mm)			Mean ±SD	Category
	P1	P2	P3		
K0	0	0	0	0	There isn't any
K1	9.4	9.0	9.7	9.4 ±0.28	Currently
K2	14.5	12.7	10.8	12.7 ±1.52	Strong
K3	15.7	17.7	14.5	16.0 ±1.31	Strong
K+	20.4	20.8	20.6	20.6 ±0.16	Very strong

Table 6. Diameter of the Growth Inhibition Zone of *Staphylococcus aureus Bacteria* by the ethanol extract of andaliman leaves

Formula	Antibacterial Inhibitory Power to *Staphylococcus aureus* (mm)			Mean ±SD	Category
	P1	P2	P3		
K0	0	0	0	0	There isn't any
K1	14.6	13.7	10.7	13.0 ±1.66	Strong
K2	16.8	15.6	12.5	15.0 ±1.81	Strong
K3	19.9	20.4	17.8	19.4 ±1.12	Strong
K+	20.4	20.8	20.6	20.6 ±0.16	Very strong

Information:
K0 : (blank)
K1 : Ethanol extract of andaliman leaves, 20% concentration
K2 : Ethanol extract of andaliman leaves, concentration 30%
K3 : Ethanol extract of andaliman leaves, concentration 40%
K+ : Gentamicin ® Ointment

Based on the results of research using n-hexane, ethyl acetate and ethanol extracts of andaliman leaves (*Zanthoxylum acanthopodium* DC) With 5 treatment groups and 3 repetitions, the inhibition zone diameters in each group differed in effectiveness. N-hexane, ethyl acetate, and ethanol extracts of Andaliman leaves (*Zanthoxylum acanthopodium* DC) with concentration variations of 20%, 30%, 40% affecting the diameter of the inhibition of *Staphylococcus aureus bacterial growth.* A material is said to have antibacterial activity if the diameter of the inhibition zone formed is greater than or equal to 6 mm. The criteria for antibacterial power are as follows:

1. The diameter of the inhibition zone is 5 mm or less and is categorized as weak.
2. The diameter of the inhibition zone is 5-10 mm and is categorized as moderate.
3. The diameter of the inhibition zone is 11-20 mm and is categorized as strong.
4. The diameter of the inhibition zone is 20 mm or more and is categorized as very strong.

Based on these categories, the n-hexane extract of andaliman leaves (Zanthoxylum acanthopodium DC) with concentration variations of 20%, 30%, 40% has moderate inhibitory power, the ethyl acetate extract of andaliman leaves (*Zanthoxylum acanthopodium DC*) with concentration variations of 20% is classified as moderate, and concentrations of 30% and 40% are classified as strong, and the ethanol extract of andaliman leaves (*Zanthoxylum acanthopodium* DC) with concentration variations of 20%, 30% and 40% is classified as strong in inhibiting the growth of Staphylococcus aureus bacteria (Ningrat et al., 2025) . This is in line with the findings that extracts with polar solvents such as ethanol and ethyl acetate generally show higher antibacterial activity against Gram-positive bacteria compared to non-polar solvents such as *n*- hexane, and this antibacterial activity often increases with increasing extract concentration (Nugroho & Wardani, 2022) . The results of the same study reported that the ethanol extract of Andaliman leaves (Sepriani et al., 2020) (Zanthoxylum acanthopodium DC) at a concentration of 50,000 ppm gave an inhibition zone diameter of 10 mm in the strong category. Meanwhile, in a study by (Rizqoh et al., 2024) the optimum andaliman leaf extract gave an inhibition zone diameter at a concentration of 15%, namely 6.36 mm in the moderate category.

The presence of antibacterial activity is due to the andaliman leaves (*Zanthoxylum acanthopodium* DC) Contains phytochemical compounds, namely saponins, polyphenols, saponins, tannins and flavonoids which can act as antibacterials. (Afriani, 2022) . Flavonoids are antimicrobials that work by disrupting the function of the cytoplasmic membrane. Flavonoids are readily soluble in polar solvents such as ethanol, butanol, and acetone (Afriani, 2022) . Flavonoids are the largest group of phenolic compounds, and they are effective in inhibiting the growth of viruses, bacteria, and fungi. (Afriani, 2022)

Flavonoids work by denaturing proteins, thereby increasing the permeability of cell membranes, so that protein denaturation causes disruption in cell formation, thereby changing the composition of protein components (Yan et al., 2024). Saponins are a group of plant glycosides that are soluble in water and can attach to lipophilic steroids (C_{27}) or triterpenoids (C_{30}). The mechanism of action of saponins is included in the antibacterial group that disrupts the permeability of bacterial cell membranes, resulting in damage to cell membranes and causing the release of various important components from within bacterial cells, namely proteins, nucleic acids and nucleotides (Wei et al., 2021). This ultimately results in bacterial cells experiencing lysis.

Tannins are organic substances found in plant extracts that are soluble in water, are polyphenol compounds (C6-C3-C6) that precipitate proteins and form complexes with polysaccharides, and consist of a very diverse group of oligomers and polymers (Nabil & Kafesa, 2024) . The antimicrobial mechanism of tannins is related to the ability of tannins to form complexes with polypeptide proteins of bacterial cell walls, resulting in disruption of the bacterial wall and bacterial lysis. (Gaol et al., 2025) . Alkaloids are organic compounds found in plants, are basic, and their chemical structure has a heterocyclic ring with nitrogen as its hetero atom (Yan et al., 2021) . Alkaloids have antibacterial properties, their mechanism of action is by disrupting the peptidoglycan components in bacterial cells so that the cell wall layer is not formed completely, disrupting peptidoglycan synthesis so that cell formation is imperfect because it does not contain peptidoglycan and the cell wall only covers the cell membrane. (Thawabteh et al., 2024) .

Damage to the cell wall will inhibit bacterial cell growth and ultimately the bacteria will die (Gaol et al., 2025) . The comparator used in this study was Gentamicin ointment. Gentamicin is an aminoglycoside antibiotic that works quickly on aerobic bacteria. It has a strong spectrum against Gram-negative bacteria such as E. coli, Klebsiella, Enterobacter, and Pseudomonas. Activity against Gram-positive bacteria is more limited. (Adaszyńska-Skwirzyńska et al., 2023) . This drug only helps (Kishk et al., 2021) . when combined with beta-lactams in germs such as Staphylococcus aureus. because beta-lactams can damage the bacterial cell wall so that gentamicin can more easily enter the cytoplasm and reach its target. After entering, the drug binds to the 30S ribosomal subunit. This bond causes the bacteria to misread mRNA codons (Webster & Shepherd, 2023) . The resulting protein becomes malformed and accumulates in the cell. This protein buildup damages the membrane, the membrane becomes leaky, and the bacteria cannot maintain their life functions (Krause et al., 2016) et al., 2024) This process makes gentamicin bactericidal, this effect appears maximally in aerobic conditions with a neutral pH. (Wei et al., 2021)

CONCLUSIONS AND RECOMMENDATIONS

Based on the results of the research that has been done, it can be concluded that the n-hexane, ethyl acetate, and ethanol extracts of andaliman leaves (*Zanthoxylum acanthopodium* DC.) have antibacterial activity against *Staphylococcus aureus bacteria*. This is evidenced by the formation of inhibition zones in all extract treatments with concentration variations of 20%, 30%, and 40%, where all the diameters of the inhibition zones produced are above 6 mm so that they meet the criteria as materials that have antibacterial activity. Although all extracts show the ability to inhibit bacterial growth, the level of inhibition produced varies between the types of solvents used.

Differences in solvent polarity levels were shown to produce differences in antibacterial activity against *Staphylococcus aureus*. The more polar ethanol extract showed the highest antibacterial activity with a strong category at all concentrations, and at a concentration of 40% produced the largest inhibition zone diameter approaching the positive control of gentamicin. The ethyl acetate extract showed moderate to strong antibacterial activity, while the n-hexane extract only showed moderate antibacterial activity. This difference is related to the ability of the solvent to extract secondary metabolite compounds, where ethanol and ethyl acetate extracts contain more complete phytochemical compounds such as alkaloids, flavonoids, tannins, saponins, and steroids, which are known to act as antibacterials. Thus, it can be concluded that the higher the polarity level of the solvent used, the greater the antibacterial activity of andaliman leaf extract against *Staphylococcus aureus.*

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