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Analysis of Antioxidant and Antibacterial Activity of Andaliman Fruit (Zanthoxylum Acanthopodium Dc.)

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ABSTRACT

Andalim fruit (Zanthoxylum acanthopodium DC.) is a traditional medicinal plant with various health benefits, including antimicrobial, antioxidant, and anti-inflammatory properties. The plant is rich in bioactive compounds such as flavonoids, alkaloids, and essential oils, contributing to its therapeutic activity. This study aims to analyze the antioxidant and antibacterial activity of ethanol extract from andaliman fruit against Staphylococcus aureus and Staphylococcus epidermidis bacteria. The antioxidant test was carried out using the DPPH (1,1-diphenyl-2-picrylhydrazil) method, while the antibacterial activity was tested using the Minimum Inhibition Concentration (KHM) method. The results showed that andaliman fruit extract had significant antioxidant activity and effective antibacterial activity at high concentrations, with a decrease in effectiveness and concentration. At a concentration of 400 mg/mL, andaliman extract inhibited the growth of bacteria with a large inhibition zone, namely 11.1 mm against Staphylococcus aureus and 12 mm against Staphylococcus epidermidis. However, its effectiveness decreases at lower concentrations. In conclusion, andaliman fruit ethanol extract has potential as a natural antibacterial agent. However, more research is needed to explore its therapeutic potential in treating diseases related to oxidative stress and bacterial infections.

Keywords- Andaliman (Zanthoxylum acanthopodium), Antioxidant activity, Antibacterial activity, Staphylococcus aureus, Staphylococcus epidermidis.

INTRODUCTION

Andaliman fruit (Zanthoxylum acanthopodium DC.) is a traditional medicinal plant essential in Indonesia, especially in North Sumatra. This plant is known to have various health benefits, including antimicrobial properties that are effective against different bacteria, viruses, and fungi, as well as antioxidant activity that can protect body cells from damage caused by free radicals (Sepriani, 2020). In addition, andaliman also shows anti-inflammatory effects that are beneficial for people with chronic diseases such as arthritis. The content of bioactive compounds in this fruit, such as flavonoids, alkaloids, and essential oils, is believed to contribute to its therapeutic properties (Sitanggang et al., 2019a). Flavonoids provide antioxidant and anti-inflammatory effects, while alkaloids have analgesic effects and can help reduce anxiety. The essential oils produced from this fruit function as antimicrobials and give a distinctive aroma often used in culinary and aromatherapy (Sibero et al., 2020). In practice, andaliman fruit is widely used as a herbal herb, cooking spice, and cosmetic product, reflecting people's belief in its efficacy. With the advent of modern research, the health potential of Andalim is gaining more attention, opening up opportunities for further development in the field of health and traditional medicine (Sefanny, Nicki L, 2020).

Antioxidant activity is essential in fighting oxidative stress, a condition in which there is an imbalance between the production of free radicals and the body's ability to neutralize them. Oxidative stress can cause damage to the body's cells, DNA, and lipids, which in turn contributes to the development of a variety of serious diseases, including cancer, diabetes, and heart disease. In this context, compounds derived from medicinal plants, such as andaliman, show promising potential as a natural source for developing new drugs (Rahmavati, 2022). The content of bioactive compounds in andaliman, such as flavonoids and alkaloids, plays a role in antioxidant activity that can neutralize free radicals and protect body cells from damage. With more and more research exploring the efficacy of medicinal plants, andaliman has the potential to be one of the candidates for drug formulations that can help prevent and treat diseases related to oxidative stress (Sitanggang et al., 2019b). In addition, developing Andalimin-based products can support a more holistic approach to health, utilizing Indonesia's natural resources to maintain health and prevent diseases (Silalahi & Lumbantobing, 2021).

The antibacterial properties of plant extracts, including andaliman fruit, are increasingly attracting attention as an alternative treatment for bacterial infections amid antibiotic resistance (Natasutedja et al., 2020); (Anggraeni, 2020). Research shows that natural compounds in plants can inhibit the growth of pathogenic bacteria, offering a solution to infections that are difficult to treat with conventional antibiotics (Adelia, 2021); (Gultom et al., 2021). Bioactive compounds such as flavonoids and alkaloids in andaliman fruit are effective against common pathogens. Therefore, a thorough

evaluation of the antibacterial activity of andaliman fruit extract through laboratory testing and clinical studies is essential. This could expand understanding of the therapeutic potential of Andaliman and open up opportunities for developing safer and more effective new drugs. This study aims to analyze the antioxidant and antibacterial activity of andaliman fruit extract, with the hope of contributing to the development of more effective health products.

RESEARCH METHODOLOGY

This study uses experimental methods that include the collection and processing of andaliman fruits, the manufacture of simplisia, the manufacture of ethanol extracts, and the examination of antioxidant activity using the DPPH (1,1-diphenyl-2-picrylhydrazil) method and antibacterial activity with the minimum inhibitory concentration method. The research was carried out at the Laboratory of Prima Indonesia University in September 2024 for sampling and examination.

Tools and Materials

The tools used include laboratory glassware, electric ovens, balancers, desiccants, and UV/Vis spectrophotometers. The ingredients include peridot leaves (Saurauia vulcani), ethyl acetate, n-hexane, ethanol, methanol, DPPH, DMSO, and nutrient media such as nutrient agar and broth.

Sample Collection and Processing

Sampling was purposively using andaliman fruit (Zanthoxylum acanthopodium DC.) obtained from Onan Rungu village, Samosir Regency, North Sumatra Province. After cleaning and drying, the andaliman fruit is mashed into powder and stored. Extraction is done by maceration using 96% ethanol, filtration, and evaporation to obtain a viscous extract.

Antioxidant and Antibacterial Activity Testing

The antioxidant activity test measured the sample's ability to dampen DPPH free radicals, with an IC50 value as a parameter. Agar and nutrient broth media were made for antibacterial testing, then inoculated Staphylococcus aureus and Staphylococcus epidermidis bacteria, followed by antibacterial activity tests using the agar diffusion method with paper discs. The results were measured to determine the inhibition zone of bacterial growth.

RESULTS AND DISCUSSION

The phytochemical screening test was carried out to identify the components of bioactive compounds in andaliman fruit extract. Some of the components of the active compounds identified include alkaloids, steroids/triterpenes, saponins, tannins, flavonoids, and glycosides. The results of the screening of andaliman fruit extract extracted using ethyl acetate solvent can be seen in Table 1.

Table 1 shows the results of phytochemical screening tests on andaliman fruit extract, which reveals the presence of various bioactive compounds. The extract contains alkaloids, which signal potential pharmacological effects, and flavonoids, which serve as a source of antioxidants to protect cells from free radical damage. Saponins are also detected, adding to the health value of the extract thanks to its antimicrobial and immunomodulatory properties. The presence of tannins shows astringent properties and potential as antioxidants, while glycosides show the potential of andaliman as an energy source. However, steroids and triterpenoids are not detected in the extracts. These results show that andaliman fruit is rich in bioactive compounds that have the potential for the development of health products and traditional medicine.

Table 2 presents the results of measuring the absorbance of ethanol extract of andaliman fruit using the DPPH method to assess antioxidant activity at various concentrations. On blank measurements, the absorbance value is 0.778, indicating the baseline value without extraction. At the highest concentration (100 mg/mL), the absorbance value dropped to 0.104, with the percentage of free radical reduction reaching 86.69%, indicating high effectiveness in reducing free radicals. At a 50 mg/mL concentration, the absorbance value slightly increased to 0.114, with a reduction percentage of 85.38%. When the concentration was lowered to 25 mg/mL, the absorbance value increased to 0.433, and the reduction percentage decreased to 44.52%. At the lowest concentration (12.5 mg/mL), the absorbance value was recorded at 0.464, with a reduction percentage of 40.01%. This suggests decreased concentration decreases the extract's ability to neutralize free radicals.

Table 3 presents the results of the antibacterial activity test of andaliman fruit ethanol extract against Staphylococcus aureus at six concentrations: 400 mg/mL, 200 mg/mL, 100 mg/mL, 50 mg/mL, 25 mg/mL, and 12.5 mg/mL, as well as negative control (K-). At a 400 mg/mL concentration, the average inhibition zone diameter is 11.1 mm, indicating excellent antibacterial potential. At a 200 mg/mL concentration, the mean inhibitory zone decreased to 9.41 mm and continued declining at 100 mg/mL (8.41 mm) and 50 mg/mL (7.1 mm). At a concentration of 25 mg/mL, the average diameter of the inhibition zone reached 5.4 mm, and at 12.5 mg/mL, the average diameter of the inhibition zone decreased to 4.1 mm. Negative controls indicate an inhibition zone diameter of 6 mm, with no antibacterial activity. These results show that the antibacterial activity of andaliman fruit ethanol extract decreases along with the decrease in concentration.

Table 1. Results of Phytochemical Screening Test of Andaliman Fruit Extract

Bioactive Compounds	Andaliman Fruit Extract
Alkaloids	+

Flavonoid	+
Saponin	+
Tannin	+
Streroid/Triterpenoid	-
Glikosida	+

Information:

(+) = contains compounds

(-) = does not contain any compound

Table 2 Results of Measurement of Absorbance of Andaliman Fruit Ethanol Extract

No	Concentration (ppm)	Absorbansi	% Damping
1	Blanko	0,778	0
2	100 mg/mL	0,104	86,69
3	50 mg/mL	0,114	85,38
4	25 mg/mL	0,433	44,52
5	12,5 mg/mL	0,464	40,01

Table 3 Results of Antibacterial Activity Test of Ethanol Extract of Andaliman Fruit *Staphylococcus aureus bacteria*

Concentration (mg/mL)	P1	P2	P3	Average (X)	WITHOUT
400	11,3	11	11	11,1	0,1
200	9,1	9,3	9,3	9,41	0,15
100	8,6	8,3	8,1	8,41	0,13
50	7	7,2	7,1	7,1	0,05
25	5,5	5,3	5,4	5,4	0,04
12,5	4,2	4	4,1	4,1	0,06
K-	6	6	6	6	0

Table 3 Results of *Staphylococcus epidermidis Antibacterial Activity Test*

Concentration (mg/mL)	P1	P2	P3	Average (X)	WITHOUT
400	13,3	13,4	10,3	12	0,2
200	9,2	6,2	9,3	8,34	0,32
100	8	8,4	8,2	8,38	0,32
50	7,5	7,3	7,2	7,33	0,1
25	5,6	5,4	5,5	5,47	0,05
12,5	4	4,2	4,1	4,1	0,07
K-	6	6	6	6	0

Table 4.4 presents the results of the antibacterial activity test of ethanol extract of andaliman fruit against Staphylococcus epidermidis at six concentrations: 400 mg/mL, 200 mg/mL, 100 mg/mL, 50 mg/mL, 25 mg/mL, and 12.5 mg/mL, as well as negative control (K-). At a concentration of 400 mg/mL, the average inhibitory zone diameter was 12 mm, with repeatability values of 13.3 mm, 13.4 mm, and 10.3 mm, respectively, indicating excellent antibacterial potential. The decrease in antibacterial activity was seen at a concentration of 200 mg/mL, where the average diameter of the inhibitory zone decreased to 8.34 mm. At a concentration of 100 mg/mL, the average diameter of the inhibition zone was 8.38 mm, and at 50 mg/mL, it was 7.33 mm. A concentration of 25 mg/mL indicates an average inhibitory zone diameter of 5.47 mm, while at 12.5 mg/mL, the average decreases to

4.1 mm. Negative controls indicate a fixed diameter of 6 mm, with no antibacterial activity. Overall, the ethanol extract of andaliman fruit showed good antibacterial activity against Staphylococcus epidermidis, but its effectiveness decreased with decreased concentration.

The results of the antibacterial activity test against Staphylococcus aureus, as shown in Table 4.3, show that the higher the extract concentration, the greater the diameter of the inhibition zone. At a 400 mg/mL concentration, the average inhibition zone diameter reached 11.1 mm, indicating excellent antibacterial potential. However, a decrease in diameter began to be seen at concentrations of 200 mg/mL (9.41 mm), continued at 100 mg/mL (8.41 mm), and 50 mg/mL (7.1 mm). Antibacterial activity decreased at 25 mg/mL (5.4 mm) concentrations and 12.5 mg/mL (4.1 mm). Negative controls also show a fixed diameter of 6 mm, with no antibacterial activity.

Overall, the results of this study show that andaliman fruit ethanol extract has decreased antibacterial activity along with reduced concentration. Table 4.4 reveals that at a concentration of 400 mg/mL against Staphylococcus epidermidis, the average diameter of the inhibition zone reached 12 mm (P1 = 13.3 mm, P2 = 13.4 mm, P3 = 10.3 mm), which confirms the effectiveness of the extract in inhibiting the growth of this bacterium. However, similar to the test on Staphylococcus aureus, the diameter of the inhibitory zone decreased at lower concentrations: 200 mg/mL (8.34 mm), 100 mg/mL (8.38 mm), 50 mg/mL (7.33 mm), 25 mg/mL (5.47 mm), and 12.5 mg/mL (4.1 mm). Negative controls showed a fixed value of 6 mm, which indicates the absence of antibacterial activity without the extraction treatment.

From these results, it can be concluded that andaliman fruit ethanol extract has the potential to be an excellent antibacterial agent against the two bacteria, with the effectiveness decreasing along with the decrease in extract concentration. These findings open up opportunities for further research on the potential of andaliman extract in developing natural antibacterial therapies. This study is in line with the results reported by Miftahurrahman (2024), which stated that the andaliman fruit extract cream met all the necessary criteria, with a pH ranging from 5.6 to 6.8, as well as showing a significant impact in inhibiting the growth of Staphylococcus aureus ($p = 0.000$) (Miftahurrahman, 2024).

Andaliman (Zanthoxylum acanthopodium DC.) shows potential as an antibacterial agent thanks to its bioactive compounds, such as flavonoids, alkaloids, and essential oils. These compounds can inhibit the growth of pathogenic bacteria such as Staphylococcus aureus and Staphylococcus epidermidis by damaging cell walls, disrupting protein synthesis, or affecting the metabolic process of bacterial cells, eventually leading to the death or inhibition of bacterial growth. In addition, the antibacterial activity of andaliman is also accompanied by anti-inflammatory properties, which help reduce inflammatory reactions due to infections. Therefore, andaliman functions as an antibacterial agent, and The use of andaliman as a natural antibacterial source offers a safe alternative in the face of the growing problem of antibiotic resistance, making it a promising candidate for developing natural ingredients-based health products (Anwar & Abdullah, 2021).

CONCLUSION

This study shows that andaliman fruit extract (Zanthoxylum acanthopodium DC.) contains various bioactive compounds that can potentially develop health products. Phytochemical screening tests identify the presence of alkaloids, flavonoids, saponins, and tannins, which show pharmacological effects and potential as a natural source of antioxidants. The results of the antioxidant activity test using the DPPH method indicate the effectiveness of andaliman fruit ethanol extract in reducing free radicals, especially at high concentrations.

In addition, antibacterial activity tests showed that andaliman fruit ethanol extract significantly affected Staphylococcus aureus and Staphylococcus epidermidis bacteria, with the diameter of the inhibitory zone decreasing along with decreasing extract concentration. These findings confirm the potential of andaliman fruit extract as a promising antibacterial agent and provide a basis for further research in developing natural antibacterial therapies.

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