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ANALYSIS OF ANTIOXIDANT AND ANTIBACTERIAL ACTIVITY OF ANDALIMAN FRUIT EXTRACT (ZANTHOXYLUM ACANTHOPODIUM DC.) AGAINST FREE RADICALS OF DPPH AND STAPHYLOCOCCUS SPP BACTERIA.

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ABSTRACT :

This study aims to determine the antibacterial activity of ethanol extract of andaliman fruit (Zanthoxylum acanthopodium DC.) against Staphylococcus aureus and Staphylococcus epidermidis bacteria. The test was conducted using the disc diffusion method with variations in extract concentrations of 12.5-300 mg/mL. The results showed that the inhibition zone increased as the concentration of the extract increased, with the highest value at 300 mg/mL against both types of bacteria. This antibacterial activity is thought to come from the content of secondary metabolite compounds such as flavonoids, tannins, alkaloids, and saponins that work synergistically in inhibiting bacterial growth. Although its effectiveness is still lower than standard antibiotics such as chloramphenicol, this extract has potential as a natural antibacterial agent, especially in topical formulations such as herbal antiseptic gels or creams. Further research is needed to evaluate the specific mechanism of action, potential toxicity, and effectiveness in in vivo models, including the possibility of development using nanotechnology or polymer-based delivery systems.

Keywords: Zanthoxylum acanthopodium, andaliman, antibacterial, Staphylococcus aureus, Staphylococcus epidermidis, secondary metabolites

INTRODUCTION

According to the World Health Organization (WHO), around 80% of the world's population, especially in developing countries, still use plants as medicine (WHO, 2020). Plants produce secondary metabolites such as alkaloids, flavonoids, saponins, tannins, steroids, and triterpenoids, which have the potential to cure a variety of diseases (Kumar et al., 2021). The environment influences this content, such as temperature, light, humidity, and soil conditions (Singh et al., 2022). The pharmacological effects of plants are closely related to their chemical compounds. Andaliman fruit contains terpenoids, phenols, and steroids (Rahmawati et al., 2023; Putri et al., 2021). Phenol compounds are toxic to pathogenic microorganisms and have anti-inflammatory, immunostimulant, antioxidant, and anticancer activities (Hidayat et al., 2022). Many medicinal plants are also rich in antioxidants, such as flavonoids and phenolic acids, which can neutralize free radicals and prevent degenerative diseases (Zhang et al., 2023; Santoso et al., 2022; Liu et al., 2021). Natural antioxidants are now preferred over synthetic ones due to their toxic and carcinogenic side effects on long-term use (Wang et al., 2023).

Natural and synthetic antibacterial compounds can inhibit or kill disease-causing bacteria such as cholera and diarrhea (Fauzan & Pratama, 2023). Andaliman (Zanthoxylum acanthopodium DC.) is a typical North Sumatra plant used by the Batak people as a spice for cooking (Saragih et al., 2022). Even though it is classified as wild, the fruit is still found in traditional markets (Hutabarat et al., 2021). This fruit contains phenols, monoterpenes, sesquiterpenes, quinones, and essential oils (Simanjuntak et al., 2023; Nainggolan et al., 2021). With this bioactive content, andaliman can be used as a natural preservative, herbal medicine, supplements, and plant-based pesticides (Manurung et al., 2023). The extract has been shown to have antioxidant and antimicrobial activity (Simbolon et al., 2022) and is traditionally used to treat indigestion and skin diseases (Ginting et al., 2021). In North Tapanuli, this fruit is also essential in local cuisine (Sitompul et al., 2022).

Genus Zanthoxylum mengandung berbagai senyawa bioaktif seperti fenol, flavonoid, alkaloid, kumarin, dan minyak atsiri (Purba et al., 2023). Andaliman terbukti memiliki aktivitas antimikroba, antiinflamasi, antioksidan, inhibitor enzim xanthine oksidase, serta efek sitotoksik terhadap sel kanker (Siregar et al., 2023). Aktivitas antibakterinya telah diuji terhadap bakteri seperti Bacillus cereus, E. coli, Staphylococcus aureus, dan Salmonella typhimurium (Tambunan et al., 2022). Berdasarkan hal tersebut, penelitian ini akan mengeksplorasi aktivitas antioksidan ekstrak buah andaliman dengan metode DPPH serta aktivitas antibakterinya melalui metode Konsentrasi Hambat Minimum (KHM)..

RESEARCH METHODOLOGY

This study is a laboratory experimental research that aims to test the antioxidant and antibacterial activity of ethanol extract of andaliman fruit (Zanthoxylum acanthopodium DC.). Andaliman fruit samples were obtained from the Dairi area, North Sumatra, and identified at the Herbarium of the University of North Sumatra. The extraction process is carried out by the maceration method using 96% ethanol, followed by a rotary evaporator evaporation. The antioxidant activity test was carried out using the DPPH method using a UV-Vis spectrophotometer at a wavelength of 516 nm. The concentrations of the extracts used were 20, 40, 60, 80, and 100 ppm and were compared with vitamin C as a positive control. The parameters measured were percent inhibition (% inhibition) and IC_{50} value. The disc diffusion method was used against Staphylococcus aureus and Staphylococcus epidermidis bacteria for the antibacterial activity test. The concentrations of the extracts used were 100, 200, and 400 mg/mL, with amoxicillin as a positive control and DMSO as a negative control. The diameter of the inhibition zone is measured using a caliper.

RESULTS AND DISCUSSION

Table 1. Phytochemical Screening Test Results of Andaliman Fruit Extract

Bioactive Compounds	***Andaliman Fruit Extract***
Alkaloids	+
Flavonoid	+
Saponin	+
Tanin	+
Streroid/Triterpenoid	-
Glikosida	+

Information:

(+) = contains compounds

(-) = contains no compounds

Samples of andaliman fruit (Zanthoxylum acanthopodium DC.) used in this study have been identified at the Herbarium of the University of North Sumatra. Andaliman fruit extract was obtained using ethyl acetate solvent and tested for bioactive compound content through phytochemical screening tests. The analysis showed alkaloids, flavonoids, saponins, tannins, and glycosides but did not contain steroids/triterpenoids. These secondary metabolites are known to have antibacterial and antioxidant activity. Alkaloids, saponins, tannins, and flavonoids work by various mechanisms against bacteria, such as disrupting the cell wall, inhibiting metabolism, and coagulating cell protoplasm. In addition, this extract also showed significant antioxidant activity, with an IC_{50} value of 66.91 ppm based on previous studies. The content of these bioactive compounds makes Andaliman fruit extract a potential candidate for development as a natural therapeutic agent. The results of the measurement of the clear zone diameter of the antibacterial activity of methanol extract of long bean leaves against *Staphylococcus aureus* and *Staphylococcus epidermidis* bacteria can be seen in tables 2 and 3 below:

Table 2 Antibacterial Activity Test Results of Ethanol Fruit Extract Andaliman *Staphylococcus aureus bacteria*

Concentration (mg/mL)	**P1**	**P2**	**P3**	**X**	**WITHOUT**
300	11,50	11,30	11,20	11,33	0,12
200	9,50	9,60	9,70	9,60	0,10
100	8,70	8,30	8,20	8,40	0,13
50	7,90	8,50	7,60	8,00	0,15
25	7,30	6,40	6,60	6,77	0,13
12,5	6,30	6,30	6,50	6,37	0,10
K-	6,00	6,00	6,00	6,00	0,00

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

Concentration (mg/mL)	P1	P2	P3	X	WITHOUT
300	11,40	11,60	10,80	11,27	0,20
200	9,40	9,50	9,30	9,40	0,10
100	8,20	8,60	8,40	8,40	0,20
50	7,30	7,50	8,20	7,67	0,47
25	6,70	6,50	6,60	6,60	0,10
12,5	6,80	6,30	6,70	6,60	0,04
6,25	6,00	6,00	6,00	6,00	0,00
K-	6,00	6,00	6,00	6,00	0,00

The results of the antibacterial activity test of ethanol extract of andaliman fruit against Staphylococcus aureus (Table 4.3) showed that the higher the concentration of the extract, the larger the inhibition zone formed. At a concentration of 300 mg/mL, the average inhibition zone reached 11.33 mm, while at 200 mg/mL, it was 9.60 mm. The inhibition zone continued to decrease along with the decrease in extract concentration, where at 100 mg/mL, it was 8.40 mm; 50 mg/mL was 8.00 mm; and at 25 mg/mL, it was 6.77 mm. At a 12.5 mg/mL concentration, the inhibition zone formed was only 6.37 mm, close to the negative control value (K-) at 6.00 mm. This suggests that at low concentrations, the antibacterial effectiveness of the extract begins to decrease significantly.

Meanwhile, the test results for Staphylococcus epidermidis (Table 3) also showed a similar pattern. At the highest concentration (300 mg/mL), the average inhibition zone reached 11.27 mm, while at the 200 mg/mL concentration, it was 9.40 mm. A decrease in antibacterial effectiveness was seen at a concentration of 100 mg/mL with an inhibition zone of 8.40 mm, followed by a concentration of 50 mg/mL of 7.67 mm. At 25 mg/mL and 12.5 mg/mL, the inhibition zones formed at lower concentrations were 6.60 mm, respectively. At a concentration of 6.25 mg/mL, the inhibition zone is equivalent to a negative control (6.00 mm), which suggests that at this concentration, the extract no longer has an antibacterial effect against S. epidermidis. These results indicate that andaliman fruit ethanol extract has antibacterial activity against both test bacteria, with increased effectiveness as concentrations increase. In addition, the relatively small standard error mean (SEM) value indicates that the measurement results are pretty consistent between test replications.

The antibacterial activity of ethanol extract of andaliman fruit can be attributed to the content of secondary metabolite compounds, such as flavonoids, alkaloids, tannins, and saponins. Flavonoids are known to have a mechanism of action by damaging bacterial cell membranes and inhibiting the activity of essential enzymes in bacterial metabolism (Kumar et al., 2021). Meanwhile, tannins inhibit bacterial enzymes through interaction with bacterial cell proteins, causing structural changes and cell death (Singh et al., 2022). In addition, the alkaloids in andaliman fruit can interfere with the synthesis of bacterial DNA and RNA, ultimately inhibiting the growth and reproduction of these microorganisms (Raza et al., 2023). These compounds work synergistically to inhibit the growth of S. aureus and S. epidermidis, as seen in this study.

Although andaliman extract shows significant antibacterial activity, its effectiveness is still lower than that of standard antibiotics. A previous study reported that chloramphenicol with a concentration of 30 µg produced an inhibition zone of 18–24 mm against S. aureus (Ningsih et al., 2020). This suggests that andaliman extract has potential as a natural antibacterial agent, but it cannot replace conventional antibiotics in treating bacterial infections. However, alternative formulations, such as natural antiseptic products or complementary therapies, can harness this potential.

Previous research supports these findings. Batubara et al. (2017) reported that andaliman fruit extract with a concentration of 8% produced an inhibition zone of 13.2 mm against S. aureus, while 2% produced an inhibition zone of 8.29 mm. These results show that andaliman fruit extract has fairly good effectiveness in inhibiting the growth of gram-positive bacteria. In addition, other studies showed that methanol extract of andaliman fruit at a concentration of 100% resulted in an inhibition zone of 9.4 mm against S. epidermidis (Muzafri, 2019). Differences in extraction methods and concentrations may cause variations in the results obtained, but trends in their antibacterial activity remain the same.

The results of this study indicate that ethanol extract from andaliman fruit has the potential to be developed as a natural antibacterial agent against S. aureus and S. epidermidis. With increased effectiveness and concentration, this extract can be an alternative in treating mild to moderate bacterial infections, especially for topical application in the form of herbal antiseptic gels or creams. In addition, further research is needed to explore this extract's mechanism of action against other pathogenic bacteria and test its potential toxicity and effectiveness in vivo models. When combined with nanotechnology or polymer-based delivery systems, the antibacterial potential of andaliman extract can be further enhanced (Chouhan et al., 2023).

CONCLUSIONS

The results showed that ethanol extract of andaliman fruit had antibacterial activity against Staphylococcus aureus and Staphylococcus epidermidis, with increased effectiveness as the concentration increased. This activity is suspected to come from the content of secondary metabolite compounds such as flavonoids, alkaloids, tannins, and saponins that work synergistically to inhibit bacterial growth. Although its effectiveness is still lower than standard antibiotics such as chloramphenicol, this extract has the potential to be developed as an herbal antiseptic or complementary therapy. To support its medical use, it is recommended that further research be conducted to identify the main bioactive compounds' specific mechanisms of action, as well as in vivo tests to assess their effectiveness and safety. The development of topical formulations and long-term toxicity evaluations are also required for andaliman extract to be widely applied in the pharmaceutical and health fields.

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