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Evaluation of Antioxidant and Antibacterial Activities in Andaliman Fruit (*Zanthoxylum acanthopodium* DC.): An Approach to Bioactive Compound Analysis and Use of *In Vitro* Test Methods

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Abstract: Andaliman fruit extract has been shown to exhibit anti-inflammatory activity, immunostimulant effects, and anticancer activity. It contains antioxidant compounds, which can stimulate the immune system. The purpose of the study was to determine the antioxidant activity of andaliman fruit (*Zanthoxylum acanthopodium* DC.) using the DPPH method (2,2-diphenyl-1-picrylhydrazyl). The experimental research was conducted in January 2024. The sample used in this study was andaliman fruit (*Zanthoxylum acanthopodium* DC.) purchased from Onan Rungu village, Samosir Regency, North Sumatra Province. Ethanol extract of andaliman fruit showed significant antibacterial activity against *Staphylococcus aureus* and *Staphylococcus epidermidis* at various concentrations, with the largest zone of inhibition at a concentration of 300 mg/mL. These results demonstrate the potential of andaliman as a source of natural antibacterial agents that can be used in the development of health products.

Keywords: Andaliman fruit extract; Antioxidant activity; Antibacterial properties; Natural health products; Experimental research

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1. Introduction

Andaliman (*Zanthoxylum acanthopodium* DC.), a member of the Rutaceae family, is a plant familiar to the Batak community and is considered a wild plant native to North Sumatra Province. The utilization of natural resources

is widespread, with approximately 80% of the global population—particularly in developing nations—relying on plants as medicinal resources to maintain their health, as documented by the World Health Organization (WHO) [1]. The antioxidant compounds present in spices including andaliman can stimulate the immune system and particularly demonstrate anticancer activity. Numerous studies focusing on medicinal plants have highlighted the substantial antioxidant content found in many of these plants [2]. The antioxidant properties of compounds are primarily attributed to phenol compounds, including flavonoids and phenolic acids. Compounds exhibiting antioxidant activity typically feature hydroxyl groups positioned in ortho and para positions relative to –OH and –OR groups [3]. The function is essential in neutralizing and eliminating free radicals in degenerative conditions. The digestive tract is particularly susceptible to bacterial infections, with common ailments such as cholera, diarrhea, and gastroenteritis affecting many individuals due to bacterial contamination in food and inadequate sanitation practices [4].

Andaliman can also serve as a preservative, medicinal ingredient and supplement, and even as a vegetable pesticide [5]. Other research has indicated the antibacterial potential of andaliman extract against food-pathogenic bacteria such as *Bacillus cereus*, *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella typhimurium* [6,7]. Based on the information provided by relevant literature, this study aims to investigate the antioxidant activity of andaliman fruit (*Zanthoxylum acanthopodium* DC.) using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method, as well as evaluating its antibacterial properties through the minimum inhibitory concentration (MIC) method.

2. Research methods

The research methodology employed in this study is experimental research. The subsequent analysis of antioxidant activity used the DPPH free radical scavenging method. The study was conducted in January 2024. The equipment used included laboratory glassware, aluminum foil, and a laminar airflow cabinet (Astec HLF I200L), a rotary evaporator (Heidolph VV-300), and a UV-visible spectrophotometer (Shimadzu UV-1800).

The materials utilized in this study were Peridot leaves (*Saurauia vulcani*), ethyl acetate, distilled water, n-hexane, ethanol, methanol, DPPH, dimethyl sulfoxide (DMSO), nutrient agar (Oxoid), nutrient broth (Oxoid), *Staphylococcus aureus* ATCC 25923, and *Staphylococcus epidermidis* ATCC 12228. The sampling approach was purposive, without comparison with the same material from other regions. The andaliman fruit samples used in the study were obtained from Onan Rungu village, Samosir Regency, North Sumatra Province.

2.1. Preparation of extract

The andaliman fruit (*Zanthoxylum acanthopodium* DC.) was dried in a cabinet at a temperature of ± 40°C before pulverizing with a blender. The powder was weighed and stored in a plastic container to prevent the influence of moisture and other impurities.

Extraction was carried out by maceration using 96% ethanol solvent. First, 500 g of andaliman fruit powder with a suitable acceptable degree was poured into a vessel, mixed with 75 parts of 96% ethanol, then closed and left for five days, protected from light while stirring once a day. After five days, it was filtered, and the pulp was squeezed out. The dregs were washed with enough solvent, mixed, and filtered to obtain 100 parts. The macerate was collected into a closed vessel, and left protected from light for two days. The solvent was evaporated with a rotary evaporator at 50°C, and concentrated in a water bath until a thick extract was obtained (Directorate General of POM RI, 1979).

2.2. Antioxidant activity testing using DPPH method

The DPPH method measures the ability of the test sample to reduce the oxidation process of DPPH free radicals in methanol solution (resulting in a change in the color of DPPH from purple to yellow) with IC50 value (concentration of the test sample that can reduce 50% free radicals) as a parameter to determine the antioxidant activity of the model.

For the preparation of blank solution, 5 ml DPPH 0.5 mM solution (200 ppm concentration) was pipetted and put into a 25 ml volumetric flask, and sufficed with methanol until the marked line (40 ppm concentration). The 40 ppm concentration of DPPH was homogenized and measured its absorption at a wavelength of 400–800 nm, which is the wavelength of visible light.

2.3. Preparation of standard solution

A total of 3 mg of andaliman ethanol extract was weighed and put into a 3 ml volumetric flask dissolved with methanol; then, the volume was filled with methanol until the marked line (concentration of 1000 ppm).

2.4. Preparation of quercetin standard solution

A total of 1 mg of quercetin powder was weighed, put into a 10 ml volumetric flask, dissolved with methanol, and then the volume was filled with methanol to the marked line (concentration 100 ppm).

The concentration was determined after several orientations. First, the standard liquid was pipetted as much as 0.125 ml, 0.25 ml, 0.5 ml, and 1 ml into a 10 ml volumetric flask; into each volumetric flask was added 1 ml of 0.5 mM DPPH solution (concentration 200 ppm), then methanol was added to the marked line. It was left to stand for 60 minutes, and then the absorbance was measured using a UV-visible spectrophotometer at the wavelength of maximum absorption obtained.

2.5. Preparation of quercetin test solution

The standard liquid was pipetted as much as 0.3125 ml, 0.625 ml, 1.25 ml, and 2.5 ml, and put into 25 ml volumetric flasks to obtain test solution concentrations of 1.25 ppm, 2.5 ppm, 5 ppm, and 10 ppm into each volumetric flask was added 5 ml of 0.5 mM DPPH solution (concentration 200 ppm), and methanol was added to the marked line. It was left to stand for 60 minutes, then the absorbance was measured using a UV-visible spectrophotometer at the wavelength obtained.

A total of 0.1 ml of the inoculum was put in a sterile petri dish, and 15 ml of nutrient agar medium was poured before placing at a temperature of 40–50°C. Petri dishes were shaken on a table surface so that the medium and bacterial suspensions were evenly mixed and allowed to solidify. Antibacterial activity was tested using the agar diffusion method using paper discs. Paper discs that had been dripped 0.1 ml with several concentrations of andaliman ethanol extract test solution were placed on top of the solid media that had been inoculated with bacteria and left for 15 minutes, then incubated in an incubator at 36 ± 1°C for 18 hours, after which the diameter of the growth inhibition area (clear zone) around the disc was measured using a caliper.

3. Result and discussion

Phytochemical screening tests are carried out to determine and identify the components of bioactive compounds contained in andaliman fruit extract. Some components of active compounds identified include alkaloids, steroids/triterpenes, saponins, tannins, flavonoids, and glycosides. The screening results of andaliman fruit extract extracted

using ethyl acetate solvent can be seen in **Table 1**.

Table 1. Phytochemical screening test results of andaliman fruit extracts

Bioactive compounds	Andaliman fruit extract
Alkaloid	+
Flavonoid	+
Saponin	+
Tannin	+
Steroid/triterpenoid glycoside	-
	+

Description:

(+) = contains compounds

(-) = does not contain compounds

The screening test results revealed that the andaliman extract, when using ethyl acetate as the solvent, contained nearly all secondary metabolite compounds such as alkaloids, flavonoids, glycosides, saponins, and steroids, with the exception of tannins. However, in another study, the screening test results indicated that the andaliman extract, also prepared using ethyl acetate as the solvent, contained almost all secondary metabolite compounds including alkaloids, flavonoids, glycosides, tannins, and saponins, but did not contain steroids [8]. **Table 2** shows the absorbance results of ethanol extract of andaliman fruit, while **Tables 3** and **4** present the antibacterial activity test results of ethanol extract of andaliman fruit against *Staphylococcus aureus* and *Staphylococcus epidermidis*.

Table 2. Absorbance measurement results of ethanol extract of andaliman fruit

Concentration (ppm)	Absorbance	Percentage reduction
Blank	0.778	0
100 mg/mL	0.104	78.4441
50 mg/mL	0.114	74.448
25 mg/mL	0.433	47.1180
12.5 mg/mL	0.464	31.3801

Table 3. Antibacterial activity test results of ethanol extract of andaliman fruit against *Staphylococcus aureus*

Concentration (mg/mL)	P1	P2	P3	X	SEM
300	11.3	11.0	11.0	10.38	0.13
200	9.1	9.3	9.3	9.41	0.15
100	8.6	8.3	8.1	8.41	0.13
50	7.8	8.4	7.4	7.35	0.11
25	7.3	6.3	6.4	6.33	0.13
12.5	6.3	6.3	6.4	6.34	0.11
K-	6	6	6	6.00	0.00

Table 4. Results of antibacterial activity test of ethanol extract of andaliman fruit against *Staphylococcus epidermidis*

Concentration (mg/mL)	P1	P2	P3	X	SEM
300	13.3	13.4	10.3	10.46	0.20
200	9.2	6.2	9.3	8.34	0.32
100	8	8.4	8.2	8.38	0.32
50	7.2	7.4	8.3	7.25	0.53
25	6.6	6.4	6.5	6.46	0.33
12.5	6.9	6.2	6.6	6.22	0.04
6.25	6	6	6	6.00	0.00
K-	6	6	6	6.00	0.00

Antimicrobial compounds can cause damage to cell walls and cell membranes through the denaturation of proteins and fats that make up the cell membrane. Ethanol extract of andaliman fruit effectively inhibits the growth of Gram-positive bacteria *Staphylococcus aureus* and *Staphylococcus epidermidis*, this is likely due to antibacterial activity influenced by several factors, namely the concentration of the extract and the type of bacteria inhibited [7]. MIC is the minimum concentration of antimicrobial substances that can inhibit bacterial growth after 24 hours of incubation and no known bacterial colonies grow by observing the number of bacterial colonies. An inhibition zone diameter of 5 mm or less is categorized as weak, an inhibition zone diameter of 5–10 mm is categorized as moderate, an inhibition zone diameter of 10–20 mm is categorized as strong, and an inhibition zone of 20 mm or more is categorized as very strong. This study shows that the higher the concentration of extract, the greater the amount of antibacterial compounds released, thus facilitating the penetration of these compounds into cells; in other words, the higher the concentration of extracts and the length of contact time, the more active the antibacterial activity, it is stated that Gram-positive bacteria whose outer membrane consists of more peptidoglycan layers than Gram-negative whose outer membrane consists of lipopolysaccharides namely lipids, polysaccharides, and proteins [5]. The cell wall of Gram-negative bacteria contains much less peptidoglycan than Gram-positive so the permeability of Gram-positive bacteria is lower than the permeability of Gram-negative bacteria. With low permeability, the active substance from the methanol extract of plant leaves will have difficulty penetrating the cell membrane of Gram-positive bacteria so the antibacterial effect is less optimal. Flavonoid compounds have the ability to form complexes with bacterial cell proteins through hydrogen bonds. As a result, the permeability function of bacterial cells is disrupted and bacterial cells undergo lysis and cell death [7].

Flavonoid compounds are thought to have a mechanism of action that denatures bacterial cell proteins and irreparably damages cell membranes. Flavonoids are also lipophilic, which will damage microbial membranes because flavonoids contain phenol compounds [9]. The growth of bacteria *Staphylococcus aureus* and *Staphylococcus epidermidis* can be disrupted due to acidic phenol compounds. Besides, phenol also has the ability to denature proteins and damage cell membranes. Acidic conditions in the presence of phenols can affect the growth of *Staphylococcus aureus* and *Staphylococcus epidermidis* [10,11].

4. Conclusion

Based on the results, it can be concluded that the ethanol extract of andaliman fruit shows significant antibacterial

activity against *Staphylococcus aureus* and *Staphylococcus epidermidis* at various concentrations, with the largest zone of inhibition at a concentration of 300 mg/mL. These results demonstrate the potential of andaliman as a source of natural antibacterial agents that can be used in the development of health products. However, more research is needed to understand its mechanism of action and potential side effects. Ethanol extract of andaliman fruit has potential as an interesting alternative in the development of natural antibacterial agents.

Disclosure statement

The authors declare no conflict of interest.

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