International Journal of Research in Engineering and Science (IJRES)
ISSN (Online): 2320-9364, ISSN (Print): 2320-9356
www.ijres.org Volume 12 Issue 4 ǁ April 2024 ǁ PP. 201-206

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
Some studies show that andaliman fruit extract exhibits anti-inflammatory activity, immunostimulant effects, and antioxidant compounds, which can stimulate the immune system, especially anticancer activity. The purpose of the study was to determine the antioxidant activity with the DPPH method (2,2-diphenyl-1-picrylhydrazil) from andaliman fruit (Zanthoxylum acanthopodium DC.). The method used in this study is experimental research, 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.*

Date of Submission: 02-04-2024 Date of acceptance: 14-04-2024

I. 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, as documented by the World Health Organization (WHO), is widespread, with approximately 80% of the global population, particularly in developing nations, relying on plants as medicinal resources to preserve their health (Yunarto et al., 2019). The andaliman plant is rich in terpenoids, phenols, and steroid compounds. Phenol compounds, known for their bioactivity, can be toxic to predatory animals. Despite this, certain studies indicate that the extract from andaliman fruit exhibits anti-inflammatory and immunostimulatory effects. Additionally, the antioxidant compounds present in spices, including andaliman, can stimulate the immune system, particularly demonstrating anticancer activity. Numerous studies focusing on medicinal plants have highlighted the substantial antioxidant content found in many of these plants (Muzafri, 2019). 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 (Winarti et al., 2018). Numerous studies have demonstrated that extracts from andaliman fruit possess anti-inflammatory properties, immunostimulatory effects, and contain antioxidant compounds that can activate the immune system, particularly showing potential in anticancer activity. Antioxidants play a crucial role in combating or neutralizing free radicals, thereby potentially inhibiting the aging process and protecting the body from degenerative diseases. Their function is essential in neutralizing and eliminating free radicals, which can otherwise cause cell damage and harm biomolecules in the body, ultimately leading to degenerative conditions. On the other hand, antibacterial agents, whether natural or synthetic, work to suppress or inhibit bacterial growth. However, despite these measures, bacteria can still invade, proliferate, and lead to infectious diseases. 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 (Purba & Sinaga, 2017).

Andaliman is rich in various terpenoid compounds, including phenol compounds, monoterpenes, sesquiterpenes, nones, and essential oils. Due to its chemical composition and physiological effects, the utilization of andaliman can be expanded beyond being just a seasoning. It can also serve as a preservative, medicinal ingredient and supplement, and even as a vegetable pesticide (Natasutedja et al., 2020). Numerous studies have highlighted the potential of andaliman as a versatile compound with various beneficial properties. These include its antimicrobial, antioxidant, anti-inflammatory, xanthine oxidase inhibitor, and cytotoxic activities. Additionally, 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 (Rahmawati, 2022); (Sitanggang et al., 2019). Based on the information provided and relevant literature, researchers have shown interest in investigating the antioxidant activity of Andaliman fruit (Zanthoxylum acanthopodium DC.) using the DPPH method, as well as evaluating its antibacterial properties through the Minimum Inhibitory Concentration (MIC) method. These methods are commonly employed in scientific research to assess the antioxidant potential and antimicrobial efficacy of natural compounds like Andaliman.

II. RESEARCH METHODS

The research methodology employed in this study is experimental research. The study involves several stages, including the collection and processing of Andaliman fruit, preparation of simple ethanol extracts, and subsequent analysis of antioxidant activity using the DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging method, as well as evaluation of antibacterial activity using the minimum inhibitory concentration (MIC) method. The study was conducted in January 2024. The equipment used in the study includes laboratory glassware, aluminum foil, a blender (National brand), a drying cabinet, an electric oven, a coarse balance (O'haus), a digital balance (Vibra), a desiccator, a stopwatch, a porcelain cup, an autoclave (Webeco), a stirring rod, a beaker, a Laminar Air Flow Cabinet (Astec HLF I200L), a blender (Miyako brand), a Bunsen burner, petri dishes, erlenmeyer flasks, a measuring cup, an incubator (Memmert), a jigsaw, an ose needle, sterile cotton, a knife, a rotary evaporator (Heidolph VV-300), and a UV/Vis spectrophotometer (Shimadzu UV-1800).

The materials utilized in the study include Peridot leaves (Saurauia vulcani), ethyl acetate, distilled water, n-hexane, ethanol, methanol, DPPH (1,1-diphenyl-2-picrylhydrazyl), 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.

Preparation of simplistic

The andaliman fruit (Zanthoxylum acanthopodium DC.) was cleaned from adhering dirt, washed with clean water, drained, and then weighed the wet weight. The andaliman fruit is then dried in a cabinet at a temperature of ± 40 ° C until dry, dry sorting, and then weighed as dry weight. Finally, the dried samples were pulverized with a blender, weighed the weight of the powder, and stored in a plastic container to prevent the influence of moisture and other impurities.

Preparation of Ethanol Extract from Andaliman Fruit

Extraction was carried out by maceration using 96% ethanol solvent. First, 500 g of andaliman fruit simplistic powder with a suitable acceptable degree was poured into a vessel, 96% ethanol, as much as 75 parts, 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. Collect the macerate into a closed vessel, left relaxed and protected from light for two days, then pour. The solvent is evaporated with a rotary evaporator at 40-50□C, then concentrated in a water bath until a thick extract is obtained (Directorate General of POM RI, 1979).

Antioxidant Activity Testing Using Free Radical Capture Method

DPPH 3.13.1 Principle of Free Radical Capture Method (DPPH) 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.

Preparation of Blank Solution

DPPH 0.5 mM solution (200 ppm concentration) was pipetted as much as 5 ml, put into a 25 ml volumetric flask, and sufficed with methanol until the marked line (40 ppm concentration). 3.13.3 Measurement of Maximum Absorption Wavelength of DPPH Solution of DPPH concentration of 40 ppm was homogenized and measured its absorption at a wavelength of 400-800 nm, which is the wavelength of visible light (Gandjar and Rohman, 2007).

Preparation of Master Solution
Test Sample As much as 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).
Preparation of Quercetin Master 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).
Preparation of Extract Test Solution
The concentration was determined after several orientations. First, the mother liquor was pipetted as much as 0.125ml; 0.25 ml; 0.5 ml; 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 the volume was sufficient with methanol to the marked line. Let stand for 60 minutes, then measure the absorbance using a UV-Visible spectrophotometer at the wavelength of maximum absorption obtained.
Preparation of Quercetin Test Solution
The mother liquor was pipetted as much as 0.3125 ml; 0.625 ml; 1.25 ml; 2.5 ml; into 25 ml volumetric flasks to obtain test solution concentrations of 1.25 ppm, 2.5 ppm, five ppm, and ten ppm into each volumetric flask was added 5 ml of 0.5 mM DPPH solution (concentration 200 ppm) then the volume was sufficed with methanol to the marked line. Let stand for 60 minutes, then measure the absorbance 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, then 15 ml of nutrient agar medium was poured was placed at a temperature of 40o -50oC. Petri dishes were shaken on a table surface so that the press 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 tetanus leaf fraction 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 ± 1oC for 18 hours, after which the diameter of the growth inhibition area (clear zone) around the disc was measured using a caliper.

III. RESULT AND DISCUSSION

Phytochemical screening tests are carried out to determine and identify the components of bioactive compounds contained in andaliman fruit extract. As for some components of active compounds identified include: alkaloids, steroids / triterpenes, saponins, tannins, flavonoids and glycosides. The screening results of andaliman fruit extract extracted using ethylacetate solvent can be seen in Table 1.

Table 1. Phytochemical Screening Test Results of Andaliman Fruit Extracts

Bioactive Compounds	Andaliman Fruit Extract
Alkoloid	+
Flavonoid	+
Saponin	+
Tannin	+
Streroid/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 a study conducted by Sihotang et al. (2016), 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 (Rosidah et al., 2018).

Table 1. Absorbance Measurement Results of Ethanol Extract of Andaliman Fruit

No	Concentration (ppm)	Absorbance	% Reduction
1.	Blanko	0,778	0
2.	100 mg/mL	0,104	78,4441
3.	50 mg/mL	0,114	74.448
4.	25 mg/mL	0,433	47,1180
5.	12,5 mg/mL	0,464	31,3801

Antioxidant activity test was conducted with DPPH method using UV-Visible spectrophotometer. The butanol extract of andaliman fruit contains a class of phenolic compounds that are antioxidants. Table 1. shows the absorbance measurement results of Andaliman fruit ethanol extract at various concentrations. Absorbance is measured in ppm, and the percentage of quenching (% Quenching) indicates the relative decrease in absorbance compared to the blank value (0.778), which represents the absorbance without the extract. From these measurement results, it can be observed that as the concentration of Andaliman ethanol extract increases, the absorbance value significantly decreases, as indicated by the higher percentage of quenching. This suggests the potential of Andaliman fruit ethanol extract in exhibiting free radical quenching activity, which is an indication of strong antioxidant activity in the extract..

Table 2. Antibacterial Activity Test Results of Ethanol Extract of Andaliman Fruit Stapylococcus aureus bacteria

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 3. Results of Antibacterial Activity Test of Ethanol Extract of Andaliman Fruit for Stapylococcus epidermidis bacteria

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

Table 2 presents the results of the antibacterial activity test of andaliman fruit ethanol extract against Staphylococcus aureus bacteria at various concentrations. The test data showed that at a concentration of 300 mg/mL, the ethanol extract of andaliman fruit had a significant growth inhibition value, with an inhibition zone value of 11.3 mm (P1), 11.0 mm (P2), and 11.0 mm (P3). At a concentration of 200 mg/mL, the inhibition zones were 9.1 mm (P1), 9.3 mm (P2), and 9.3 mm (P3), respectively. Similarly, at concentrations of 100 mg/mL, 50 mg/mL, and 25 mg/mL, each showed a decreasing inhibitory effect. In the negative control (K-), which did not use the extract, the bacterial growth zone was 6.0 mm. These results provide an indication that the ethanol extract of andaliman fruit has potential antibacterial activity, especially at the highest concentration, which can be the basis for further research related to the utilization of andaliman in the development of natural antibacterial agents.

Table 3 illustrates the results of the antibacterial activity test of ethanol extract of andaliman fruit against Staphylococcus epidermidis bacteria at various concentrations. At a concentration of 300 mg/mL, the ethanol extract showed significant inhibition zone values, namely 13.3 mm (P1), 13.4 mm (P2), and 10.3 mm (P3). At a concentration of 200 mg/mL, the zone of inhibition decreased to 9.2 mm (P1), 6.2 mm (P2), and 9.3 mm (P3). Concentration of 100 mg/mL produced an inhibition zone of 8 mm (P1), 8.4 mm (P2), and 8.2 mm (P3). Similarly, at concentrations of 50 mg/mL, 25 mg/mL, and 12.5 mg/mL, the antibacterial activity decreased as the concentration decreased. In the negative control (K-), which did not use the extract, the bacterial growth zone was 6.0 mm. These results indicate that the ethanol extract of andaliman fruit has potential antibacterial activity against Staphylococcus epidermidis, with the highest concentration providing a stronger effect, which supports its potential use as a natural antibacterial source.

Compounds that are antimicrobial can cause damage to cell walls and damage to cell membranes in the form of denaturation of proteins and fats that make up the cell membrane. Ethanol extract of andaliman fruit effectively inhibits the growth of gram-positive bacteria Stapylococcus aureus and Stapylococcus epidermidis, this is likely due to antibacterial activity influenced by several factors, namely the concentration of the extract and the type of bacteria inhibited (Sitanggang et al., 2019). Based on the table obtained pathogenic bacterial strains used for antibacterial screening of ethanol extract of andaliman fruit from maceration extraction process, namely: Staphylococcus aureus and Staphylococcus epidermidis. inhibit bacterial growth. growth and the number of bacteria < 10 colonies. KHM 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 that grow. Inhibition zone diameter of 5 mm or less is categorized as weak, inhibition zone diameter of 5-10 mm is categorized as moderate, inhibition zone diameter of 10-20 mm is categorized as strong and 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 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 (Natasutedja et al., 2020). The cell wall of gram-negative bacteria contains much less peptidoglycan than gram-positive so that 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 loning plant leaves will have difficulty penetrating the cell membrane of gram-positive bacteria so that the bacterial effect is less optimal on growing bacterial cells and causes cell death. Flavonoid compounds have the ability to form complexes with bacterial cell proteins through hydrogen bonds. The structure of the cell wall and membrane of the bacterial cytoplasm containing proteins becomes unstable because the protein structure of bacterial cells becomes damaged due to hydrogen bonds with flavonoids, so that bacterial cell proteins lose their biological activity. As a result, the permeability function of bacterial cells is disrupted and bacterial cells will undergo lysis which results in bacterial cell death (Sitanggang et al., 2019).

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 (Sepriani, 2020). The growth of bacteria Staphylococcus aureus and Staphylococcus epidermidis can be disrupted due to phenol compounds where this compound is an acidic alcohol 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 bacteria (Sibero et al., 2020); (Adolf J. N. Parhusip, 2006).

IV. CONCLUSION

Based on the results of research that has been carried out it can be concluded that e 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.

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