International Journal of Multidisciplinary Research and Publications
ISSN (Online): 2581-6187

The Antioxidant Activity Test with DPPH and Antibacterial Method with Minimum Inhibitory Concentration Method of Andaliman Fruit (Zanthoxylum acanthopodium DC.)

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Abstract**—***The utilization of bioavailability, according to the World Health Organization (WHO) records, is extensive; it is estimated that nearly 80% of humanity, especially in developing countries, still uses plants as medicinal materials to maintain their health. This experimental research method includes collecting and processing andaliman fruit, making ethanol extracts, and examining antioxidant activity testing using the DPPH (1,1-diphenyl-2-picrylhydrazil) free radical capture method and antibacterial activity using the minimum inhibitory concentration method. The research was conducted at the Pharmacognosy Laboratory and Research Laboratory, Faculty of Pharmacy, University of North Sumatra. The study was conducted in November - December 2021 for sampling and examination. Ethanol extract from andaliman fruit is a sample with extreme antioxidant activity because it has an IC50 value with a concentration of 12.5-100µg/mL and potent antioxidant activity with an IC50 value with a concentration of 25-100 µg/mL. Ethanol extract from andaliman fruit can inhibit Staphylococcus aureus and Staphylococcus epidermidis, effective at a concentration of 300 mg/ml with an inhibition diameter of 10.17 mm and 10.80 mm.*

Keywords**—** *Antioxidant, antibacterial, Zanthoxylum acanthopodium DC.*

I. INTRODUCTION

The utilization of biological availability, according to the World Health Organization (WHO) records, is extensive; it is estimated that nearly 80% of humanity, especially in developing countries, still uses plants as medicinal materials to maintain their health (1).The andaliman plant contains terpenoids, phenols, and steroid compounds. Phenol compounds are bioactive components that are toxic to predatory animals. However, some studies show that andaliman fruit extract has anti-inflammatory and immunostimulatory effects, and antioxidant compounds in spices can also trigger the immune system, especially anticancer activity. Several studies on medicinal plants have reported that many medicinal plants contain large amounts of antioxidants (2). The antioxidant effect is mainly due to phenol compounds such as flavonoids and phenolic acids. Usually, compounds with antioxidant activity are phenol compounds with hydroxyl groups distributed in ortho and para positions against -OH and -OR groups (3).

Antioxidants function to overcome or neutralize free radicals, so it is expected that by giving antioxidants, the aging process is inhibited and can prevent damage to the body from the onset of degenerative diseases. The role of antioxidants is vital in neutralizing and destroying free radicals that can cause cell damage and also damage biomolecules in the body, which can eventually trigger degenerative diseases. Antibacterial is a natural or synthetic compound that suppresses or inhibits the growth of bacteria. Nevertheless, bacteria can enter, multiply and cause infectious diseases. The digestive tract is one of the most infected parts of the body by bacteria. Cholera, diarrhea, and gastroenteritis are digestive tract infections that are widely experienced by the broader community due to bacterial contamination of food and lack of sanitation (4).

Andaliman contains phenol compounds, monoterpenes, sesquiterpenes, nones, and essential oils, which of terpenoid compounds. Based on its chemical content and physiological activity, the utilization of andaliman can be increased; no longer just a seasoning but also a preservative, medicinal material and supplement, and vegetable pesticide (5). Several studies have reported the potential of andaliman as an antimicrobial, antioxidant, anti-inflammatory, xanthine oxidase inhibitor, and cytotoxic. Other studies have also reported the antibacterial activity of andaliman extract against food-pathogenic bacteria such as Bacillus cereus, Escherichia coli, Staphylococcus aureus, and Salmonella typhimurium (6); (7). Based on the description above and some related literature, researchers are interested in researching antioxidant activity with the DPPH method and antibacterial with the Minimum Inhibitory Concentration (KHM) method from Andaliman fruit (Zanthoxylum acanthopodium DC.).

II. RESEARCH METHODS

The method used in this research is experimental research. The research includes collecting and processing andaliman fruit, making simplistic ethanol extracts, and examining antioxidant activity testing using the DPPH (1,1-diphenyl-2-picrylhydrazil) free radical scavenging method and antibacterial activity using the minimum inhibitory concentration method. The research was conducted at the Pharmacognosy Laboratory and Research Laboratory, Faculty of Pharmacy, University of North Sumatra. The research was conducted in November - December 2021 for sampling and examination. The tools used in this study include laboratory

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glassware, aluminum foil, blender (National), drying cabinet, electric oven, coarse balance (O'haus), digital balance (Vibra), desiccator, stopwatch, porcelain cup, autoclave (Webeco), stirring rod, beaker, Laminar Air Flow Cabinet (Astec HLF I200L), blender (Miyako), bunsen, petri dish, porcelain cup, erlenmeyer, measuring cup, incubator (Memmert), jigsaw, ose needle, sterile cotton and knife, rotary evaporator (Heidolph VV-300), UV/Vis spectrophotometer (Shimadzu UV-1800).

The materials used in this study were peridot leaves (Saurauia vulcani), ethylacetate, distilled water, n-hexane, ethanol, methanol, and DPPH (1,1-diphenyl-2-picrylhydrazil), dimethylsulfoxide (DMSO), nutrient agar (Oxoid), nutrient broth (Oxoid), Staphylococcus aureus ATCC 25923, Staphylococcus epidermidis ATCC 12228. Sampling was done purposively without comparing with the same material from other regions. The samples used in this study were andaliman fruit (Zanthoxylum acanthopodium DC.) purchased 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.

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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 qualitative analysis of the active components of andaliman extract extracted with ethylacetate solvent above shows that andaliman fruit extract with ethyl acetate solvent contains almost all secondary metabolites, except steroids/triterpenoids. Secondary metabolite compounds in andaliman extract that act as antimicrobials are alkaloids, flavonoids, glycosides, saponins, and tannins. In previous studies on andaliman extraction (8), the solvents used were three types; hexane, methanol, and ethyl acetate. The screening test results showed that andaliman extract using ethylacetate solvent contained almost all secondary metabolite compounds of alkaloids, flavonoids, glycosides, saponins, and steroids, except tannins. While research conducted by Sihotang et al. (2016), the screening test results showed that andaliman extract using ethylacetate solvent contained almost all secondary metabolite compounds of alkaloids, flavonoids, glycosides, tannins, saponins, except steroids (9).

Another metabolite compound found in andaliman extract is tannin. Although, according to research by Karlina et al. (2013), tannins are phenol compounds that cause damage to cell wall polypeptides; the mechanism of tannin inhibition occurs using bacterial walls that have been lysed due to saponin and flavonoid compounds, thus causing tannin compounds to enter bacterial cells and coagulate bacterial cell protoplasm easily. In addition, according to Robinson (1995), the mechanism of action of tannins as antibacterial is to inhibit the enzymes reverse transcriptase and DNA topoisomerase so that bacterial cells cannot form.

Antioxidant activity testing on an ethanol extract of andaliman fruit with DPPH method using UV-VIS spectrophotometer with a wavelength of 516 nm. Here are the results of the antioxidant activity test, which can be seen in Table 2 below:

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

No	Concentration (ppm)	Absorbance	% Damping
1.	Blanko	0,778	0
2.	100 mg/mL	0,102	78,2221
3.	50 mg/mL	0,112	74.428
4.	25 mg/mL	0,233	47,1180
5.	12,5 mg/mL	0,462	31,3801

The antioxidant activity test was conducted with the DPPH method using a UV-Visible spectrophotometer. Butanol extract from andaliman fruit contains a class of phenolic compounds that are antioxidants (Harborne, 1996). The results show a decrease in DPPH absorbance with the addition of ethanol extract of andaliman fruit compared to the blank solution without the addition of ethanol extract of andaliman fruit. The reduction in absorbance value occurs because the test solution damps DPPH, and damping occurs due to the electron transfer of antioxidant hydrogen atoms to DPPH.

Table 3. Strength Level of Antioxidant Compounds with DPPH Method

Intensity	Nilai IC_{50} (mg/mL)
Very strong	< 50
Strong	50 – 100
Currently	101 – 150
Weak	> 150

Table 4. Antibacterial Activity Test Results of Ethanol Extract of Andaliman Fruit for Stapylococcus aureus bacteria

Concentration (mg/mL)	P1	P2	P3	X	SEM
300	11,2	11,0	11,0	10,28	0,12
200	9,1	9,2	9,2	9,41	0,15
100	8,6	8,2	8,1	8,41	0,13
50	7,8	8,4	7,4	7,25	0,11
25	7,2	6,2	6,4	6,22	0,13
12,5	6,2	6,2	6,4	6,34	0,11
6,25	6	6	6	6,00	0,00
K-	6	6	6	6,00	0,00

Table 5. Antibacterial Activity Test Results of Ethanol Extract of Andaliman Fruit for Stapylococcus epidermidis bacteria

Concentration (mg/mL)	P1	P2	P3	X	SEM
300	11,1	11,4	10,3	10,46	0,20
200	9,2	6,2	9,1	8,34	0,32
100	8	8,4	8,2	8,18	0,32
50	7,2	7,4	8,1	7,25	0,51
25	6,6	6,4	6,5	6,46	0,11
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

Based on Tables 4 and 5, antibacterial activity testing aims to determine the potential of bacterial activity in the test sample. The antibacterial activity test method used in this study is the agar diffusion method; in this method, antibacterial activity against test samples is indicated by forming an inhibition zone around the disc paper. Disc paper containing antimicrobial agents is placed on agar media planted with microorganisms. The clear area suggests the inhibition of microorganism growth by antimicrobial agents on the surface of agar media (7). In this study used pathogenic bacteria, namely Staphylococcus aureus and Staphylococcus epidermidis. The results of the antibacterial activity test of the ethanol extract of andaliman fruit showed that the section had moderate antibacterial activity at a concentration of 300 mg/ml with an inhibition zone of 10.28 mm on Staphylococcus aureus bacteria and an intermediate category for Staphylococcus epidermidis bacteria with an inhibition zone of 10.46 mm. The difference in the diameter of the inhibition zone in the two bacteria indicates differences in the sensitivity of the extract to the test microbes. Antimicrobial compounds can cause damage to the cell wall and damage to the cell membrane in the form of the denaturation of proteins and fats that make up the cell membrane (5). 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

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influenced by several factors, namely the concentration of the extract and the type of bacteria inhibited (7).

Based on the table obtained, pathogenic bacterial strains were used for antibacterial screening of ethanol extract of andaliman fruit from the maceration extraction process: 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; no known bacterial colonies grow by observing the number of bacterial colonies that grow (Tortora et al., 2010). An inhibition zone diameter of 5 mm or less is categorized as weak, an inhibition zone diameter of 5-10 mm is classified as moderate, an inhibition zone diameter of 10-20 mm is categorized as vital, and an inhibition zone of 20 mm or more is classed as very strong. This study shows that the higher the concentration of the extract, the greater the number of antibacterial compounds released, thus facilitating the penetration of these compounds into the cell. In other words, the higher the concentration of the extract and the longer the 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 (10).

The cell wall of gram-negative bacteria contains much less peptidoglycan than gram-positive, so the permeability of gram-positive bacteria is lower than that of gram-negative bacteria. With low permeability, the active substances from the methanol extract of longing plant leaves will have difficulty penetrating the cell membrane of gram-positive bacteria so that the bacterial effect is less than optimal on growing bacterial cells and causes cell death. In addition, Flavonoid compounds can form complexes with bacterial cell proteins through hydrogen bonds. The structure of the cell wall and cytoplasmic membrane of bacteria-containing proteins becomes unstable because the structure of bacterial cell proteins becomes damaged due to hydrogen bonds with flavonoids, so bacterial cell proteins lose their biological activity. As a result, the function of bacterial cell permeability is disrupted, and bacterial cells will experience lysis, resulting in the death of bacterial cells (8).

Flavonoid compounds are thought to have a mechanism of action that denatures bacterial cell proteins and damages cell membranes beyond repair. Flavonoids are also lipophilic, which will damage microbial membranes because flavonoids contain a phenol compound. The growth of Staphylococcus aureus and epidermidis bacteria can be disrupted due to phenol compounds. Where this compound is acidic alcohol; besides that, phenol also can denature proteins and damage cell membranes. Therefore, acidic conditions with phenol can affect the growth of Staphylococcus aureus and Staphylococcus epidermidis bacteria (11); (12).

IV. CONCLUSION

Based on the research results, it can be concluded that the andaliman fruit ethanol extract sample has antioxidant activity with an IC50 value of 17.9745 mg/mL. The ethanol extract of andaliman fruit is a sample with very strong antioxidant activity because it has an IC50 value with a concentration of 12.5-100μg/mL and potent antioxidant activity with an IC50 value with a concentration of 25-100 μg/mL. Ethanol extract from andaliman fruit can inhibit Staphylococcus aureus and Staphylococcus epidermidis, effective at a concentration of 300 mg/ml with an inhibition diameter of 10.17 mm and 10.80 mm.

REFERENCES

1. Yunarto N, Aini N, Sulistyowati I, Oktoberia IS. Aktivitas Antioksidan serta Penghambatan HMG CoA dan Lipase Kombinasi Ekstrak Daun Binahong-Rimpang Temulawak Antioxidant Activity along with Inhibition of HMG CoA Reductase and Lipase from Anredera cordifolia Leaf - Curcuma xanthorrhiza Rhizome Combinati. 2019;9(2):89–96.
2. Muzafri A. Uji Aktivitas ANtimikroba Ekstrak Andaliman (Zanthoxylum acanthopodium DC.) pada Staphylococcus aureus. J Sungkai. 2019;7(1):122–6.
3. Winarti W, Simanjuntak P, Syahidin MF. Identifikasi Senyawa Kimia Aktif Antioksidan Dari Ekstrak Etil Asetat Buah Andaliman (Zanthoxylum acanthopodium DC). In: Talenta Conference Series: Tropical Medicine (TM). Medan: Talenta Publisher; 2018. p. 162–6.
4. Purba ST, Sinaga DP. Evaluasi Potensi Ekstrak Tumbuhan Andaliman (Zanthoxylum acanthopodium) sebagai Potensi Imunostimulan pada Tikus (Rattus norvegicus L.). In: Prosiding Seminar Nasional III Biologi dan Pembelajarannya. Universitas Negeri Medan; 2017.
5. Natasutedja AO, Lumbantobing E, Josephine E, Carol L, Junaedi I, Normasiwi S, et al. Botanical Aspects , Phytochemicals and Health Benefits of Andaliman (Zanthoxylum acanthopodium). Indones J Life Sci. 2020;02(01):8–15.
6. Rahmawati F. Potensi Antijamur Ekstrak Andaliman (Zanthoxylum acanthopodium DC) terhadap Candida albicans. J Pro-Life. 2022;9(November):610–20.
7. Sitanggang FMC, Duniaji AS, Pratiwi IDPK. Daya Hambat Ekstrak Buah Andaliman (Zanthoxylum acanthopodium DC) dalam Etil Asetat terhadap Pertumbuhan Escherichia coli. J Ilmu dan Teknol Pangan. 2019;8(3):257–66.
8. Adolf J. N. Parhusip. Kajian Mekanisme Antibakteri Ekstrak Andaliman (Zanthoxylum acanthopodium) terhadap Bakteri Patogen Pangan. Sekolah Pascasarjana Institut Pertanian Bogor; 2006.
9. Rosidah, Hasibuan PAZ, Haro G, Masri P, Satria D. Antioxidant activity of alkaloid fractions of zanthoxylum acanthopodium dc. Fruits with 1,1-diphenyl-2-picrylhydrazyl assay. Asian J Pharm Clin Res. 2018;11(Special Issue 1):33–4.
10. Sibero MT, Siswanto AP, Murwani R, Frederick EH, Wijaya AP, Syafitri E, et al. Antibacterial, cytotoxicity and metabolite profiling of crude methanolic extract from andaliman (Zanthoxylum acanthopodium) fruit. Biodiversitas. 2020;21(9):4147–54.
11. Sepriani O. Potensi Ekstrak Tumbuhan Andaliman (Zanthoxylum acanthopodium DC.) Sebagai Antibakteri Staphylococcus aureus. J Pendidik dan Ilmu Kim [Internet]. 2020;21(1):1–9. Available from: http://journal.um-surabaya.ac.id/index.php/JKM/article/view/2203
12. Sitanggang FMC, Duniaji AS, Pratiwi IDPK. Daya Hambat Ekstrak Buah Andaliman (Zanthoxylum acanthopodium DC) Dalam Etil Asetat Terhadap Pertumbuhan Escherichia coli. J Ilmu dan Teknol Pangan. 2019;8(3):257.