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# PHYTOCHEMICAL ANALYSIS, ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF EXTRACT AND FRACTION OF *Z. Acanthopodium* DC. FRUITS

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**ABSTRACT**

Free radicals must be considered to prevent the increasing incidence of degenerative diseases. Antibiotic resistance is of particular concern in preventing the development of superbacteria. Andaliman (*Z. acanthopodium* DC.) can be developed to treat degenerative and infectious diseases. This research analyses chemical compounds in Andaliman fruit, which is essential to determine what compounds contribute to its pharmacological activity. The extract was made using reflux extraction with methanol followed by liquid-liquid fractionation to obtain the n-hexane fraction and analyzed for phytochemical content using GC-MS and antioxidant activity using the 1,1-diphenyl-2-picrylhydrazy method and antibacterial activity using agar diffusion. The analysis results showed that the methanol extract and n-hexane fraction of Andaliman fruit contained geranyl acetate (6.48% and 2.38%) and had antioxidant activity with $IC_{50}$ values of 76.27 ± 2.82 µg/mL and 83.81 ± 6.02 µg/mL. Antibacterial activity of methanol extract with vigorous activity at concentrations of 75 mg/mL and 300 mg/mL against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, and *Salmonella typhi*; and has the potential to be developed as an antioxidant and antibacterial.

**Keywords:** Antioxidants, Antibacterial, Methanol Extract, N-Hexane Fraction, *Z. Acanthopodium.*
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## INTRODUCTION

The escalating prevalence of degenerative diseases, instigated by free radicals, necessitates costly treatments.[1] Free radicals induce tissue and cell damage, as evidenced by their reactions with proteins, fatty acids, and DNA.[2] Free radicals are generated during the oxidation of other molecules, a process that can be averted or decelerated by antioxidants. Antioxidants are pivotal in inhibiting or halting free radical oxidation, thereby preventing cell damage.[4] Bacterial infections are a form of exogenous free radicals that can cause disease.[6] Using antibacterials is a way to fight this infection. However, bacterial resistance that often arises when treating infectious diseases brings problems that can thwart therapy. Improper use of antibiotics causes resistance in various bacteria, resulting in more extended hospital stays, higher costs, and more.[9] This resistance represents the greatest threat to human health. The exploration of antioxidants and antibacterial properties in natural ingredients is crucial due to their rich secondary metabolites and relative safety.[15] Andaliman, scientifically known as *Z. acanthopodium*, is a plant commonly used as a cooking spice for the Batak tribe in North Sumatra, Indonesia.[16-18] Traditionally used to cure stomach aches and toothaches and stimulate appetite. Several studies demonstrate that Andaliman fruit exhibits anticancer activity against breast cancer cells and anti-inflammatory, anti-free radical, and cardioprotective activity.[19-21] The pharmacological potential of Andaliman fruit is significant in treating degenerative and infectious diseases.[22,23] In this study, we analyzed the chemical compounds in Andaliman fruit using methanol


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extraction and n-hexane fractionation and analyzed the phytochemical composition. Understanding the compounds that contribute to its pharmacological activity is crucial. The DPPH (1,1-diphenyl-2-picrylhydrazy) method was used for the antioxidant test, and the agar diffusion method was used for antibacterial.

## EXPERIMENTAL

### Material

The Andaliman fruits, a local specialty from the Medan market in Sumatera Utara, Indonesia, were used in this study. The chemicals employed included n-hexane (SmartLab), methanol (Merck), distilled water, Mueller Hinton Agar (Himedia), Nutrient broth (Himedia), dimethylsulfoxide (Merck), and 1,1-diphenyl-2-picrylhydrazil (TCI).

### Preparation of Extract and Fraction

Andaliman fruit powder (1 Kg) was extracted for 5 hours by reflux method with methanol (1:10). The collected filtrate was evaporated at low pressure and dried in a water bath to produce a thick extract. The methanol extract (10 g) was then fractionated with n-hexane (3 x 100 mL) using the liquid-liquid fractionation method.[23]

### Chemical Constituent Analysis

The phytochemicals of methanol extract and n-hexane fraction were examined using a GC-MS with column (TG-5MS, 30 m x 0.25 mm, film 0.25 m). Helium was used as the mobile gas at a 1.02 mL/min flow rate. The temperature was programmed to increase from 70ºC to 280ºC at a rate of 5ºC/min over 5 mins. The temperature of the injector was adjusted to 280ºC for detection using a mass spectrometer with an ionization impact of 70eV. The results were identified using the NIST 14 Mass Spectra library and Search software based on the similarity in the fragmentation of compounds.[23]

### Antioxidant Assay

A 0.2 mM DPPH solution in methanol was prepared and applied 100 µl to extracts and fractions of different concentrations. Absorption was measured at a wavelength of 516 nm after 60 minutes.[24]

### Antibacterial Test

The antibacterial test was carried out using the agar diffusion method, with different methanol extract and n-hexane fraction concentrations (300, 150, 75, 37.5 mg/mL) against *S. aureus*, *S. epidermidis*, *E. coli*, and *S. typhi*.[25]

## RESULTS AND DISCUSSION

### Phytochemicals of Extract and Fraction

A phytochemical analysis of methanol extract and n-hexane by GC-MS produced ten main compounds, as shown in Table 1. The chromatogram results are shown in Fig.-1. The compounds most commonly found in the methanol extract of Andaliman fruit include Cyclohexyl-(3a,6-dimethyl-3a,4,5,7a-tetrahydro-3H-benzofuran) (19.28%) and geranyl acetate (6.48%). Andaliman's n-hexane fraction mainly contains geranyl acetate (3.5%) and 12,15-Octadecatrienoic methyl ester (Z, Z, Z) (2.38%).

The fractionation process with n-hexane may affect the content of Andaliman fruit. Fractionation aims to separate compounds based on their polarity level, where nonpolar compounds will first come out of the chromatography column. The solvent used can affect the compounds contained therein. Polar substances tend to dissolve in solvents that are also polar, while nonpolar compounds will dissolve in nonpolar solvents.[26] The geranyl acetate contained in the fruit of this plant has an exogenous antioxidant effect, which helps increase superoxidase dismutase. Geranyl acetate has been reported to have potent antioxidant activity and can ward off free radicals through an electron or hydrogen donor mechanism.[27,28] It is a crucial monoterpene found in the essential oils of several plants. It has been shown to have extraordinary pharmacological potential, such as anti-inflammatory, antioxidant, antimicrobial, antifungal, hepatoprotective, cardioprotective, and neuroprotective effects.[29,30] This compound has been reported to have cytotoxic properties on cancer cells.[31,32]

### Antioxidant Result

The antioxidant test of the extracts and fractions was analyzed, where DPPH changed from purple to yellow, indicating that the extracts and fractions had antioxidant activity. Polar materials tend to dissolve in solvents

that are also polar, while nonpolar compounds will dissolve in nonpolar solvents. The $IC_{50}$ values (Fig.-2) for methanol extract were 76.27 ± 2.82 µg/mL and n-hexane fraction 83.81 ± 6.02 µg/mL and has the strong activity category ($IC_{50}$ 50-100 µg/mL).

Table-3: Phytochemical Analysis Result

| Methanol Extract | % | n-hexane Fraction | % |
|---|---|---|---|
| Cyclohexyl-(3a,6-dimethyl-3a,4,5,7a-tetrahydro-3H-benzofuran | 19.28 | Geranyl acetate | 3.15 |
| Geranyl acetate | 6.48 | 9,12,15-Octadecatrienoic acid, methyl ester, (Z, Z, Z)- | 2.38 |
| 1-Heptatriacotanol | 5.69 | 9,12-Octadecadienoic acid (Z, Z)-, methyl ester | 2.11 |
| 3-Isopropylidene-tricyclo[4.3.1.1(2,5)]undecan-10-one | 5.36 | Farnesol, acetate | 1.69 |
| Phthalic acid, di(2-propylpentyl) ester | 4.85 | 6-Octen-1-ol, 3,7-dimethyl-, acetate | 1.55 |
| (E)-3,7-Dimethylocta-2,6-dien-1-yl palmitate | 4.79 | E)-3,7-Dimethylocta-2,6-dien-1-yl palmitate | 1.49 |
| Geranyl oleate | 4.65 | Geraniol | 1.30 |
| 6,9,12,15-Docosatetraenoic acid, methyl ester | 3.86 | Diisooctyl phthalate | 1.25 |
| Ethyl iso-allocholate | 3.06 | Hexadeca-2,6,10,14-tetraen-1-ol, 3,7,11,16-tetramethyl- | 1.11 |
| Geranyl vinyl ether | 2.78 | 2,3,7-Trimethyl-3-vinyl-oct-6-enoic acid | 1.10 |

(a)

(b)

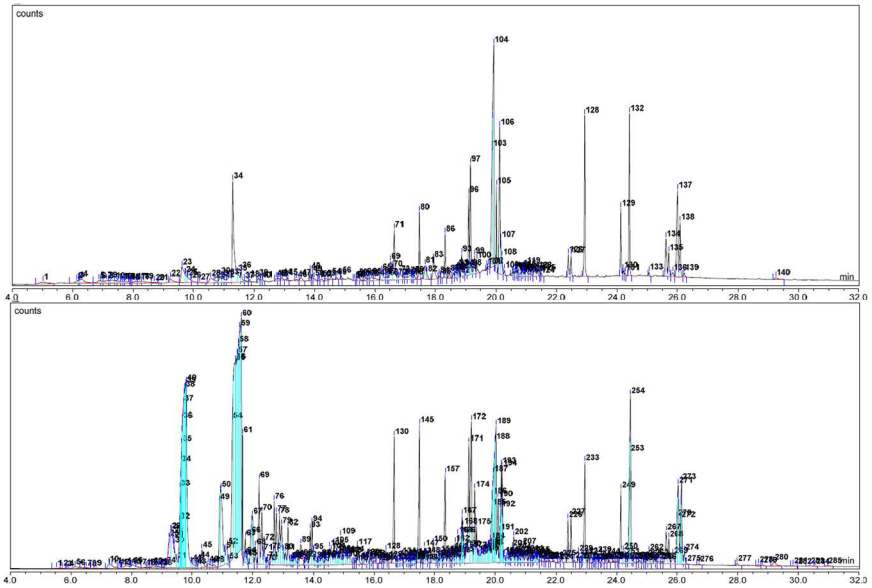


Fig.-1: The Results of Chromatogram Analysis of Methanol Extract (a) and n-Hexane Fraction (b) of Andaliman Fruit

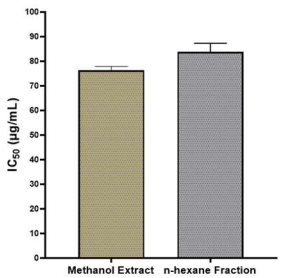


Fig.-2: Results of $IC_{50}$ analysis

There is no significant difference between the two ($p>0.05$). This activity is caused by phytochemicals containing terpenoid compounds with many double bonds in their structure that can donate hydrogen to

radicals. The DPPH radical's unpaired electron absorbs light significantly at 517 nm, resulting in a purple. When an unpaired electron combines with another electron, the original hue slowly fades to a light yellow. A few plants have antioxidant activity from multiple mechanisms, such as preventing initiation, decomposing peroxides, ion catalysts for transition metal binding, scavenging radicals, and preventing hydrogen abstraction.[33,34]

**Antibacterial Result**

Analysis of antibacterial activity using the disc diffusion method on *S. aureus*, *S. epidermidis*, *E. coli*, and *S. typhi* bacteria were shown in Fig.-3 and Fig.-4. Antibacterial strength criteria: inhibition zone diameter ≤ 5mm = weak, 5-10mm = moderate, 10-20 mm = strong, ≥ 20mm = very strong.[35] There was a significant difference in the antibacterial power of the two types of solvents used ($p<0.05$). Methanol extract's inhibitory potency is considered strong due to an inhibition zone over 10 mm at all concentrations on all tested bacteria. The inhibitory power of the n-hexane fraction against *S. aureus* is strong, while against *S. epidermidis*, it is classified as moderate. However, *E. coli* and *S. typhi* did not show antibacterial inhibition. The difference in the bacterial inhibitory power of the two types of solvents is influenced by the compounds dissolved in them. The results of the content analysis of methanol extract show that the compounds contained are more significant than the n-hexane fraction. Higher concentrations of extracts and fractions also produce larger zones of inhibition. The main compound found in Andaliman fruit is geranyl acetate. In methanol extract, the percentage is known to be higher, namely 6.48%, compared to the n-hexane fraction, which is only 3.15%. This compound shows antibacterial activity against *B. cereus*, *S. aureus*, and *P. aeruginosa*.[36,37] In terms of clinical implications, the fruit of this plant has the potential to be used to be formulated as a topical or oral antibacterial for treating infections in the future.

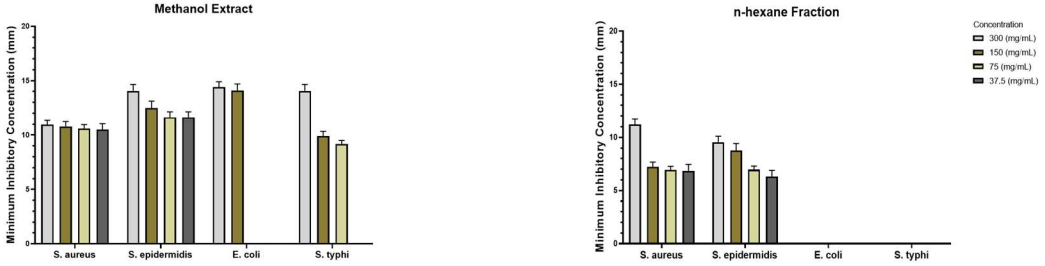


Fig.-3: Results of Bacterial Inhibition Analysis

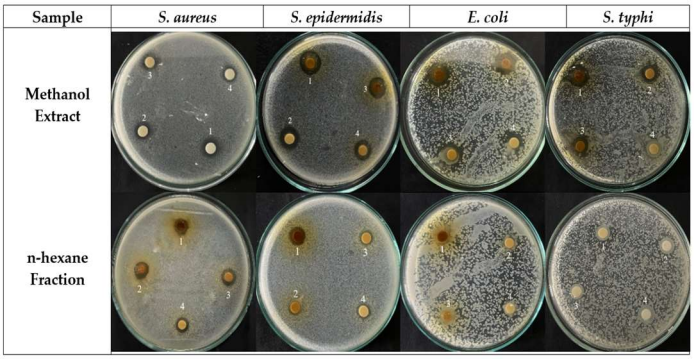


Fig.-4: Results of Bacterial Inhibition Analysis, (1) 300 mg/mL; (2) 150 mg/mL; (3) 75 mg/mL; (4) 37.5 mg/mL.

## CONCLUSION

Based on the antioxidant and antibacterial analysis, the methanol extract of Andaliman fruit is identified as the most effective sample for inhibiting free radicals and bacterial growth (*S. aureus*, *S. epidermidis*, *E. coli*, and *S. typhi*). However, further research is crucial to understand the mechanism of action of the Andaliman fruit as an antioxidant and antibacterial.

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## CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

## AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing, and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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