

Potent bioactivity of Andaliman (*Zanthoxylum acanthopodium* DC.)

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Abstract

Introduction: Andaliman (*Zanthoxylum acanthopodium* DC.) is a plant originating from North Sumatra, Indonesia which has traditionally been used as a treatment of toothache, cough, rheumatism, lumbago, stomach colic, asthma, fever. Various kinds of secondary metabolites such as alkaloids, flavonoids, tannins, saponins, triterpenoids, steroids, and phenols in andaliman are known to have pharmacological activity.

Method: This review discusses pharmacological activities of andaliman by using libraries from Google Scholar, ResearchGate, and Pubmed with a maximum publication year of the last 10 years, namely from 2013–2023.

Results and discussions: The results showed that plant parts in the form of fruits, leaves, bark, and roots of andaliman proved to have pharmacological activity of antibacterial, anti-inflammatory, anticancer, antioxidant, and immunomodulatory properties.

Keywords

Andaliman, Bioactivity, *Zanthoxylum acanthopodium* DC.

Introduction

Almost all parts of the plant such as fruits, roots, seeds, leaves, rhizome, stems, and tubers can be utilized as traditional medicine. Plant activity in treating various diseases is based on secondary metabolites contained therein. Based on data to date, from 30,000 species plants that are known to have pharmacological efficacy and activity, 9,600 species spread in Indonesia. However, only about 300 species have been utilized as ingredients in traditional medicine (Emilda et al. 2017). Basic Health Research (RISKESDAS) data in 2018, reported that 48% of Indonesians have used finished herbs from traditional medicines and as many as 31.8% have used traditional medicines

with their own herbs as alternative treatments. There is an increase in the number of those who take advantage of the use of traditional medicine (Ministry of Health of the Republic of Indonesia 2018).

Secondary metabolites are chemical compounds produced by the plants and do not play a role in the process of growth and development. Instead, their functionality is to defend themselves from many negatives effect of the environment. Secondary metabolite compounds include flavonoids, alkaloids, tannins, saponins, polyphenols, terpenoids, quinones, coumarins, steroids (Julianto 2019).

Andaliman belongs to the family Rutaceae. One of the main characteristics of Rutaceae is that its leaves contain

oil glands such as those of Andaliman. Several studies on the content of this plant have been carried out, and proved that Andaliman contains secondary metabolite class compounds in the form of alkaloids, glycosides, carbohydrates, tannins, phenols, flavonoids, steroids, oils and fats. Andaliman Fruit Extract has various benefits and these have been studied scientifically. Study results proved that andaliman has pharmacological activity as an antibacterial, anti-inflammatory, anticancer, immunomodulator, antioxidant. The aim of this study is to review the scientific data about the pharmacological activity of andaliman plant which can be used as a source of information in medicine and pharmaceutical and research.

Andaliman (*Zanthoxylum acanthopodium* DC) is a wild plant from North Sumatra also known as Intir-intir, Tuba, and Syarnar (Dewana et al. 2022). Andaliman is often used in traditional medicine such as toothache, cough, rheumatism, lumbago, stomach colic, asthma, and fever (Negi et al. 2011; Silalahi et al. 2011; Majumder et al. 2014; Silalahi et al. 2018). Andaliman is known to contain flavonoid compounds, alkaloids, tannins, saponins, quinones, glycosides, steroids, as well as essential oils such as linalool, cineol, geraniol, and citronellal (Wijaya et al. 2001; Muzafri and Karno 2022).

Method

The method used for the review was literature study with an online search from E-sources. The secondary data sources used are available on Google Scholar, ResearchGate, and Pubmed database using the keywords *Zanthoxylum acanthopodium* DC, antibacterial effectiveness of *Zanthoxylum acanthopodium* DC extract, anticancer effectiveness of *Zanthoxylum acanthopodium* DC extract, anti-inflammatory effectiveness of *Zanthoxylum acanthopodium* DC extract, antioxidant effectiveness of *Zanthoxylum acanthopodium* DC extract, immunomodulatory effectiveness of *Zanthoxylum acanthopodium* DC extract, dosage form *Zanthoxylum acanthopodium* DC. References are obtained based on inclusion and exclusion criteria. Inclusion criteria are references related to the effectiveness of *Zanthoxylum acanthopodium* DC based on in vitro and in vivo tests published in the last 10 years. The exclusion criteria are references that are not available in full text form, containing information about andaliman other than the keywords above, as well as reference journals with publications under 2013.

Results and discussion

The antibacterial activity of andaliman plant has been widely reported against gram-positive and gram-negative bacteria. Antibacterial activity was evaluated from the inhibitory power in the form of a clear zone in the media. The inhibition zone categories were weak (<5 mm), medium (5–10 mm), strong (>10–20 mm), and very strong

(>20–30 mm) (Muzafri and Karno 2022). The following are search results from references that meet the criteria related to the activity and dosage forms of andaliman plant (*Zanthoxylum acanthopodium* DC).

Antibacterial activity

The antibacterial activity of andaliman leaves has been tested against *Escherichia coli* bacteria with MHA (Mueller Hinton Agar). Various solvent samples were used including ethyl acetate, methanol, water, and hexane extract. The concentrations used were 25, 50, 75 and 100%. Results obtained from ethyl acetate, methanol, water, and hexane extracts at a concentration of 25% were 9.5 ; 8.2; 8; and 5.2 mm (medium). At a concentration of 50% were 13.8 and 13.8 mm (strong); as well as 9.5 ; and 6.5 mm (medium). At a concentration of 75% were 15.1 ; 15 ; 12 mm (strong); and 8.2 mm (medium). At a concentration of 100% were 19.15 ; 16.5 ; 14 ; and 11.8 mm (strong).

Secondary metabolites contained in Andaliman leaves are flavonoids, alkaloids, tannins, saponins, glycosides, triterpenes/steroids, and anthraquinone glycosides (Muzafri et al. 2022). According to Rijayanti and Rika (2014), secondary metabolites have activity as antibacterial agents in the form of flavonoids, alkaloids, tannins, and saponins. Flavonoids have a mechanism by inhibiting the growth of *E. coli* by offloading the cell wall, resulting in cell lysis. Alkaloids can cause an imperfectly formed bacterial cell layer to cell death because the peptidoglycan component of bacteria is disturbed by alkaloids. The cell wall acts as a defense and protection of bacteria from the environment and controls compounds that enter and exit the bacteria (Rijayanti and Rika 2014).

The bark contains secondary metabolites in the form of alkaloids, flavonoids, triterpenoids, tannins, and saponins with greater levels than the metabolites contained in the leaves. Saponins can cause cell death due to leakage of cell membranes due to the presence of saponins on the cell surface. Leakage occurs because saponins have a mechanism of action by lowering the surface tension of the cell. Triterpenoids can inhibit bacterial cell walls so that their growth is disrupted. The mechanism of action of tannins is by denaturing and coagulating proteins from bacterial cells (Sepriani et al. 2020).

Many antibacterial studies have been conducted on andaliman fruit, one of which was conducted by Sitanggang et al. (2019) on *Escherichia coli* bacteria with NA (Nutrient Agar) media and extract solvents in the form of ethyl acetate. The concentrations used are 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100%. The results obtained at concentrations of 10 and 20% fall into the weak category, concentrations of 30–50% fall into the medium category, and concentrations of 60–100% fall into the strong category. Tetracycline was used as a comparison control resulting in an inhibitory zone of 26.51 mm which falls into the strong category. The resulting MIC (Minimum Inhibitory Concentration) is indicated by a concentration of 60% (7.20 mm) because it has a strong inhibitory activity with the smallest concentration

Table 1. Antibacterial Activity of Andaliman plant.

| Plant parts | Types of extracts | Compounds | Bacteri | Research methods | Reference |
|----------------|-------------------------|---|--------------------------------------|--|---------------------------|
| Leaf | Ethyl acetate | Flavonoid, alkaloid, saponin, tannin, glycoside | <i>Escherichia coli</i> | In vitro by disc paper method | (Muzafri and Karno 2022) |
| | Methanol | Flavonoid, alkaloid, saponin, tannin, glycoside, glycoside anthraquinone, triterpene/ steroid | | | |
| | Watering | Flavonoid, tannin, glycosides | | | |
| | Hexane | Alkaloid, triterpene/ steroid | | | |
| Leaf Cortex | Ethanol 96% | Alkaloid, flavonoid, tannin, saponin, triterpene | <i>Staphylococcus aureus</i> | In vitro by disc diffusion method | (Sepriani 2020) |
| Fruit | Ethyl acetate | Not mentioned | <i>Escherichia coli</i> | In vitro by agar diffusion method (well) | Sitanggang et al. 2019) |
| Fruit | water | Flavonoid, tannin, glycoside | <i>Escherichia coli</i> | In vitro by agar diffusion method (well) | (Muzafri et al. 2018) |
| | | | <i>Staphylococcus aureus</i> | | |
| | | | <i>Salmonella typhimurium</i> | | |
| | Methanol | Alkaloid, flavonoid, saponin, tanin, glycoside, triterpen/ steroid, glycoside anthraquinon | <i>Escherichia coli</i> | | |
| | | | <i>Staphylococcus aureus</i> | | |
| | | | <i>Salmonella typhimurium</i> | | |
| | Ethyl acetate | Alkaloid, flavonoid, saponin, tanin, glycoside, | <i>Escherichia coli</i> | | |
| | | | <i>Staphylococcus aureus</i> | | |
| | | | <i>Salmonella typhimurium</i> | | |
| | Hexane | Alkaloid, triterpene/ steroid | <i>Escherichia coli</i> | | |
| | | | <i>Staphylococcus aureus</i> | | |
| | | | <i>Salmonella typhimurium</i> | | |
| Fruit | Methanol | Flavonoid, alkaloid, tanin, saponin, terpenoid, phenol | <i>Staphylococcus epider-between</i> | In vitro by disc paper diffusion method | (Djuang et al. 2022) |
| Fruit | water | Flavonoid, taninn, glycoside | <i>Staphylococcus aureus</i> | In vitro by disc paper diffusion method | (Muzafri et al. 2019) |
| | Methanol | Alkaloid, flavonoid, tanin, saponin, glycoside, glycoside anthracuionon, triterpen/ Steroid | | | |
| | Ethyl acetate | Alkaloid, flavonoid, tanin, saponin, glycoside | | | |
| | Hexane | Alkaloid, triterpene/ steroid | | | |
| Fruit | Ethanol | Tannin, saponin, alkaloid, steroids | <i>Bacillus subtilis</i> | In vitro by disc paper diffusion method | (Sihombing et al. 2018) |
| | | | <i>Salmonella typhi</i> | | |
| Fruit | Hexane | Alkaloid, flavonoid, saponin | <i>Bacillus subtilis</i> | In vitro by disc paper diffusion method | (Susanti et al. 2020) |
| | | | <i>Salmonella typhi</i> | | |
| | | | <i>Staphylococcus aureus</i> | | |
| | Ethyl acetate | | <i>Bacillus subtilis</i> | | |
| | <i>Salmonella typhi</i> | | | | |
| Fruit | Ethanol 96%% | Flavonoid, alkaloid, saponin, tannin, glycoside | <i>Staphylococcus aureus</i> | In vitro by disc paper diffusion method | (Syaputri et al. 2022) |
| | | | <i>Staphylococcus epidermidis</i> | | |
| | | | | | |
| Fruit | Ethanol | Saponin, flavonoid, alkaloid, taninn, glycoside | <i>Escherichia coli</i> | In vitro by disc paper diffusion method | (Rijayanti and Rika 2014) |
| | | | <i>Pseudomonas aeruginosa</i> | | |

compared to concentrations that have strong inhibitory power. The concentration of the extract used is directly proportional to the inhibitory ability against bacteria. Where the greater the concentration, the greater the resistance generated (Sitanggang et al. 2019).

In other studies, water; methanol; ethyl acetate; and hexane extracts of andaliman fruit have also been tested against *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella typhimurium* bacteria with MHA (Mueller Hinton

Agar). The concentrations used were 25, 50, 75, and 100%. Based on the resulting inhibitory zone, the inhibitory strength of the extract falls into the categories of medium, strong, and very strong (Muzafri et al. 2018).

The antibacterial activity of andaliman fruit methanol extract has been tested against acne-causing bacteria, namely *Staphylococcus epidermidis* which was seen for its inhibitory power using the disc method. The concentrations used were 25, 50, 75, and 100% with the result of an

inhibitory diameter of 7 mm each; 7.4; 7.6; and 9.4mm which all fall into the medium category. Clindamycin was used as a comparison control and resulted in an inhibitory diameter of 58.4 mm which is included in the very strong category (Djuang et al. 2022).

There are other studies related to antibacterial activity tests of andaliman fruit with water solvents, methanol, ethyl acetate, and hexane against *Staphylococcus aureus* bacteria using the disc method. The greatest yield was obtained on ethyl acetate extract and the smallest result on hexane extract. Such results can be influenced by the content of secondary metabolites. Based on phytochemical screening results, ethyl acetate and methanol extracts contain almost all metabolites while water and hexane extracts contain only a few (Muzafri 2019). Differences in solvent polarity can affect the number of secondary metabolites attracted because the principle of compound separation is based on like dissolved like, that is, a compound will dissolve in a solvent that has the same polarity (Pratiwi 2010).

Ethanol extract of andaliman fruit has been tested for activity against gram-positive and gram-negative bacteria, namely *Bacillus subtilis* and *Salmonella typhi*. The concentrations used were 25, 50, and 75%. The largest inhibitory zone in *Bacillus subtilis* bacteria was found at a concentration of 75%, which was 14.5 mm (strong), while the smallest zone was at 25%, (10 mm, which was classified as medium). Ethanol extract at 70% concentration against *Salmonella typhi* revealed inhibitory zone of 19.5 mm (strong) while that at 25% showed smallest inhibitory zone of 8.5 mm (medium). Chloramphenicol, which was used as standard, showed inhibitory of 26 mm (very strong) against *Bacillus subtilis* and 30 mm (very strong) against *Salmonella typhi* (Sihombing et al. 2018).

In another study, 96% ethanol extract of andaliman fruit was tested against *Staphylococcus aureus* and *Staphylococcus epidermidis* by using the disc diffusion method. Variations in concentration ranging from 6.25 mg/mL to 300 mg/mL were used. The results obtained from *Staphylococcus aureus* and *Staphylococcus epidermidis* fell into the moderate category. The sensitivity of the extract to the two bacteria was different so that the inhibitory power produced was different also at the same concentration (Syaputri 2022).

The antibacterial activity of andaliman fruit ethanol extract has been tested against *Pseudomonas aeruginosa* and *Escherichia coli* bacteria by disc diffusion method using NA (Nutrient Agar) media. The concentration used varies from the smallest 6.25 mg/mL to the largest 300 mg/mL. In *Pseudomonas aeruginosa* bacteria obtained strong antibacterial activity (10.03 mm) with a concentration of 300 mg/mL while in *Escherichia coli* bacteria was found at concentrations of 200 mg/mL (10.13 mm) and 300 mg/mL (10.87 mm). Other concentrations tested produced moderate category antibacterial activity (Dewana et al. 2022).

Research was conducted by Susanti et al. (2020) on andaliman fruit with hexane and ethyl acetate extract

solvents with concentrations of 12.5, 25, 50, and 75%. The bacteria used were *Bacillus subtilis*, *Salmonella typhi*, and *Staphylococcus aureus* with MHA media (Mueller Hinton Agar). A clear zone was obtained on each bacterium that showed antibacterial activity from the extract used. Chloramphenicol was used as a comparison control that produced a larger clear zone than the extract (Susanti et al. 2020).

Andaliman fruit contains secondary metabolites in the form of alkaloids, flavonoids, saponins, glycosides, tannins, triterpenes / steroids, anthraquinone glycosides. Polyphenols have also been shown to be antibacterial agents in addition to flavonoids, alkaloids, and saponins. Polyphenols have a mechanism of action by preventing bacterial growth by damaging cell wall growth. Based on the research of Scazzocchio et al. (2001) alkaloid compounds that act as antibacterial agents in the form of canadine, berberine, canadine, and beta-hydrastine (Scazzocchio et al. 2001). Terpenoids are constituent components of essential oils that have antibacterial activity. Triterpenoid compounds that act as antibacterial such as linalool, merediol, cadinen, and indole (Kubo et al. 1993). Another mechanism of tannins is to inhibit the formation of bacterial cells by preventing reverse transcriptase enzymes and DNA topoisomerase (Karlina et al. 2013).

Based on some of the results of the research above, andaliman plants are proven to have activity as antibacterial agents because they can produce clear zones which indicate that bacterial growth can be inhibited by adherence. Such antibacterial activity comes from secondary metabolites contained in it. Where each compound has its own mechanism as an antibacterial agent.

Anticancer activity

Andaliman roots, bark, leaves, and fruits have been tested for toxicity which is a preliminary test of anticancer activity with the BSLT (Brine Shrimp Lethality Test) method. Where a compound will be toxic or has potential as an anticancer agent if it produces an LC value of 50 (Lethal Concentration 50%) less than 1000 ppm. LC₅₀ is a concentration that can kill shrimp larvae as much as 50% (Karlina et al. 2013). According to Meyer et al. (1982) toxicity is categorized based on LC 50 values namely very strong (<10 ppm), strong (10–100 ppm), medium (100–500 ppm), and weak (500–1000 ppm) (Panggabean et al. 2020).

The LC₅₀ values from ethanolic extracts of andaliman roots, bark, leaves, and fruits were 65.313 (strong), 57.677 (strong), 77.983 (strong), and 191.426 ppm (medium) respectively. The LC value of 50 bark was the smallest compared to other plant parts, due to less secondary metabolites contained. The results obtained indicated that andaliman has toxic properties and potential as an anticancer agent (Rosidah et al. 2019).

Study on anticancer activity of Andaliman's fruit ethanolic extract has been conducted on 4T1 cells in breast cancer using the MTT method (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). The

Table 2. Anticancer activity of Andaliman plant.

| Plant parts | Types of extracts | Compounds | Methods | Culture cell | Reference |
|-------------|------------------------|--|--------------------|--------------|--------------------------|
| Root | Ethanol | Saponin, alkaloid, flavonoid, terpenoid | In vitro with BSLT | – | (Rosidah et al. 2019) |
| Cortex | | Saponin, alkaloid, flavonoid, tannin, | | | |
| Leaf | | terpenoid | | | |
| Fruit | | | | | |
| Fruit | Ethanol 96% | Saponin, alkaloid, flavonoid, tannin, triterpenoid | In vitro with MTT | 4T1 | (Arsita et al. 2019) |
| Seed | Ethanol 96% | Flavonoid, alkaloid, tannin, saponin | In vitro with MTT | MCF-7 | (Satria et al. 2019) |
| Fruit | Ethyl acetate fraction | Flavonoid, alkaloid, tannin, saponin | In vitro with MTT | T47D | (Tala and Siregar 2022) |
| Fruit | Ethyl acetate | Flavonoid, alkaloid, saponin | In vitro with MTT | HepG2 | (Napitupulu et al. 2022) |
| Fruit | Ethanol | Not mentioned | In vitro with MTT | HCT-116 | (Pratiwi et al. 2015) |
| | | | | WiDr | |

mechanism of the MTT method is that formazan crystals will be formed from the reaction between living cells and MTT solution. Formazan crystals are dissolved with the addition of 10% Sodium lauril sulphate and then the cells are further incubated and shaken. Next, the absorbance was measured at a wavelength of 595 nm. IC_{50} value of the andaliman extract was 54.48 $\mu\text{g} / \text{mL}$ and was categorized as active. Doxorubicin was used as a comparison and an IC_{50} value of 0.80 $\mu\text{g} / \text{mL}$ which is classified as a very active anticancer agent. The mechanism of action of Doxorubicin is by prevention of the RNA and DNA topoisomerase II formation (Arsita et al. 2019). A cancer activity is divided into three categories based on IC_{50} values namely very active ($IC_{50} < 10 \mu\text{g}/\text{mL}$), active ($IC_{50} 10\text{--}100 \mu\text{g}/\text{mL}$), and sufficiently active ($100\text{--}500 \mu\text{g}/\text{mL}$) [26].

A study on ethyl acetate fraction of andaliman's fruit against breast cancer cell models T47D cells has also been conducted. IC_{50} value of the fraction was 48.94 $\mu\text{g} / \text{mL}$ which is classified as active category (Satria et al. 2019). In addition, ethyl acetate extract of andaliman fruit can inhibit cell growth, prevent the rate of proliferation, and cause HepG2 liver cancer cells to experience apoptosis. IC_{50} value of the ethyl acetate extract was 122.656 $\mu\text{g}/\text{mL}$ which was categorized as moderately active activity. The activity comes from the metabolite compounds contained in the extract such as alkaloids, tannins, saponins, and flavonoids (Tala and Siregar 2022). Ethanolic extract of Andaliman's fruit has also been tested against HCT-16 cells and Wi Dr in colon cancer. It was proved to have anticancer activity because it was able to prevent the HCT-16 and Wi Dr cells growth. The IC_{50} values of the extract were 96.64 $\mu\text{g}/\text{mL}$ and 95.61 $\mu\text{g}/\text{mL}$ (Napitupulu et al. 2022) respectively.

Andaliman fruit has second metabolites as active compounds including saponin, alkaloids, flavonoids, tannins, and triterpenoids. The mechanism of action of alkaloids, saponins, and flavonoids is to prevent the process of mitosis so that cell division is inhibited and can trigger apoptosis (Meyer et al. 1982; Pratiwi et al. 2015; Sirait et al. 2019). Triterpenoids are able to trigger apoptosis and prevent the process of mitosis because the G2/M phase cell cycle is blocked by the compound (Batra and Sharma 2013). Tannins can prevent the proliferation of cancer cells by

preventing the activation of tyrosine kinase in the form of receptors that play a role in the growth of cancer cells (Putram et al. 2017).

In another study, ethanolic extract of andaliman seed was tested on MCF-7 cells which is one of the breast cancer cell models by using MTT method. The IC_{50} value of this experiment was 221.31 mg / mL which is included in the category of quite active in preventing the proliferation of MCF-7 cells. Secondary metabolites in ethanolic extract of andaliman seeds are phenols, saponins, tannins, flavonoids, alkaloids, and triterpenes (Arsita et al. 2019). The mechanism of action of flavonoids as anticancer agents is to reduce the expression of Bcl-2 and Bcl-xl genes by preventing the activation of DNA topoisomerase I or DNA topoisomerase II (Putram et al. 2017). Steroids have sulfatase inhibitor enzymes and aromatase enzymes which are inhibitory enzymes for breast cancer cells (Setiawati et al. 2007; Sisodiya 2013). Another mechanism of steroids is by killing the cells, thus necrosis occurs by destroying mitochondrial permeability (Setiawati et al. 2007; Marwati et al. 2020). In addition, saponins also act as anticancer agents by inhibiting the proliferation of cancer cells and are involved in the DNA replication process due to the presence of hydrophilic glycoside groups (Setiawati et al. 2007). Based on some of the results of the above research it can be shown that andaliman plants have activity as anticancer agents because they can inhibit cell proliferation.

Antioxidant activity

The activity of free radicals capable of damaging cells can be inhibited by the work of antioxidants. Research has been conducted related to the test of antioxidant activity of andaliman extract (Table 3) using 2,2-diphenyl-1-picrylhydrazyl (DPPH) method, xanthine oxidase inhibition, and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) method. In the DPPH method, free radical capture activity will occur which can be seen from the reduced intensity of the purple color of the DPPH solution (Ren et al. 2003; Zakaria et al. 2011; Reinoviar et al. 2019). The potential for antioxidant activity is determined by an IC_{50} value of 50, which is the concentration of a test compound that can capture DPPH free radicals by 50%. A small value of

Table 3. Antioxidant activity of Andaliman plant.

| Plant parts | Types of extracts | Compounds | Method | IC ₅₀ | Reference | |
|-------------|---------------------------|---|---|-------------------------------|-----------------------|------------------------|
| Fruit | Acetone | Alkaloid, flavonoid, steroid | In vitro with DPPH method | 857.71 ppm | (Effendi 2020) | |
| | Ethyl acetate | Alkaloid, flavonoid, steroid, tannin | | 359.99 ppm | | |
| | Ethanol | Alkaloid, flavonoid, steroids, tannin, saponin | | 344.75 ppm | | |
| Fruit | Ethanol | Steroid/ triterpenoid, alkaloid, saponin, flavonoid, glycoside | In vitro with DPPH method | 239.061 ppm | (Rosidah et al. 2018) | |
| Fruit | n-hexane | Not mentioned | In vitro with DPPH method | 237.20 ppm | (Kristanty 2013) | |
| | Chloroform fraction pH 3 | Alkaloid | | 116.62 ppm | | |
| | Chloroform fraction pH 7 | | | 61.10 ppm | | |
| | Chloroform fraction pH 9 | | | 23.15 ppm | | |
| | Chloroform fraction pH 11 | | | 112.40 ppm | | |
| | Water fraction | Not mentioned | | 172.45 ppm | | |
| Fruit | Petroleum ether | Not mentioned | In vitro with DPPH method | 220.67 µg/mL | (Farida et al. 2021) | |
| | Dichloro Methane | | | 88.26 µg/mL | | |
| | Ethyl acetate | | | 83.50 µg/mL | | |
| | n-butanol | Alkaloid, flavonoid, glycoside tanin, anthracuinon, terpenoid | In vitro with method inhibition of xanthine oxidase | 53.51 µg/mL | | |
| | Methanol | | | 26.39 µg/mL | | |
| | Petroleum ether | Not mentioned | | 9.9 µg/mL | | |
| | Dichloro Methane | | | 3.9 µg/mL | | |
| | Ethyl acetate | | | 9.54 µg/mL | | |
| | n-butanol | Alkaloid, flavonoid, glycoside tanin, anthracuinon, terpenoid | | 3.69 µg/mL | | |
| Methanol | 4.03 µg/mL | | | | | |
| Fruit | Ethanol | Phenol | | In vitro with DPPH method | 17.97 mg/mL | (Syaputri et al. 2022) |
| Fruit | Ethanol | Flavonoid | | In vitro with the ABTS method | 64.46 mg/mL | (Dewana et al. 2022) |
| Fruit | n-hexan | Steroid/ triterpenoids, essential oils | In vitro with method DPPH | 494.9 µg/mL | (Farida et al. 2021) | |
| | Ethyl acetate | Alkaloid, flavonoid, saponin, steroid/ triterpenoid, minyak atsiri, kumarin | | 108.5 µg/mL | | |
| | Ethanol 70% | Flavonoid, saponin, tanin, steroid, kumarin | | 84.1 µg/mL | | |
| | Dry extract of ethanol | Not mentioned | | 71.7 µg/mL | | |

IC₅₀ indicates that the compound's ability to capture free radicals by 50% is getting bigger. The antioxidant categories based on IC₅₀ values are IC₅₀ <50 ppm very strong, IC₅₀ 50–100 ppm strong, IC₅₀ 101–150 ppm moderate, IC₅₀ 151–200 ppm weak, and IC₅₀ >200 ppm very weak (Ren et al. 2003; Reinoviar et al. 2019; Effendi 2020).

In the research of Rienoviar et al. (2019), three solvents were used for fruit extraction, namely acetone, ethyl acetate, and ethanol. The results showed that the IC value of 50 ethanol extract (344.75 ppm) was smaller than that of IC₅₀ value of acetone extract (857.71 ppm) and ethyl acetate (359.99 ppm). This suggests that the antioxidant activity of ethanol extract is greater despite belonging to the category of very weak antioxidants (Rienoviar et al. 2019).

In another study, ethanol extract tests of andaliman fruit were also conducted. Results showed that the antioxidant activity of vitamin C in capturing free radicals was greater (IC₅₀ = 16.92 ppm) compared to samples of

andaliman fruit ethanol extract (IC₅₀ = 239.06 ppm) (Effendi 2020).

Research related to antioxidant activity was conducted by Rosidah et al. (2018) with samples in the form of andaliman fruit as ethanolic extract, water fraction, and also chloroform fraction in various pH, namely pH 3, 7, 9, and 11. The results showed that the antioxidant activity of chloroform fractions (IC₅₀ = 23.15 (pH 9); 61.10 (pH 7); 112.40 (pH 11); and 116.62 (pH 3)) was greater than that of fractions (IC₅₀ = 172.45) of water and also n-hexane extracts (IC₅₀ = 273.20) (Rosidah et al. 2018).

In another study, the antioxidant activity test of andaliman fruit was also carried out with two test methods, namely the DPPH method and the xanthine oxidase inhibition method. The samples used were the extracts with various solvents such as petroleum ether, dichloromethane, ethyl acetate, n-butanol, and methanol. In the DPPH method, the results showed that the antioxidant activity

of *butylated hydroxytoluene* (BHT) ($IC_{50} = 5.52 \mu\text{g/mL}$) as blanko was greater than that of the extract samples. The principle of the xanthine oxidase inhibition method was to measure the absorption of uric acid as the end product of the reaction of xanthine oxidase catalysis with xanthine at maximum wavelength of 284 nm. The results showed that the activity of alopurinol ($IC_{50} = 0.02 \mu\text{g/mL}$) was greater compared to the sample. The strong antioxidant activity of the sample was shown by n-butanol extract which was $3.69 \mu\text{g/mL}$ (IC_{50}) (Kristanty et al. 2013).

In the research of Dewana et al. (2022), the ABTS method was used to test the antioxidant activity of andaliman fruit ethanol extract. In the ABTS method, there will be a reduction in the intensity of the blue color of the ABTS solution which shows the antioxidant activity obtained from the oxidation of potassium persulfate. IC value of 50 samples of 64.46 mg / mL was obtained which is included in the category of strong antioxidants (Dewana et al. (2022).

The antioxidant activity of andaliman fruit has been tested in the form of viscous extracts, dry extracts, and also in dosage forms as effervescent granules using the DPPH method. The solvents used were n-hexane, ethyl acetate, and 70% ethanol. IC_{50} values from extract using n-hexane, ethyl acetate, 70% ethanol viscous, ethanol dry extract were respectively $494.9 \mu\text{g/mL}$ (very weak); $108.5 \mu\text{g/mL}$ (medium) and $84.1 \mu\text{g/mL}$ (strong). Ethanol extract after being formulated into FI, FII, and FIII of effervescent granules revealed IC_{50} values from 71.7 – $91.9 \mu\text{g/mL}$ (strong). Vitamin C is used as a standard with an IC_{50} value of $3.37 \mu\text{g / mL}$ which is included as very strong category. Vitamin C is an antioxidant agent with mechanism of action to capture oxygen so that oxidation reactions do not occur (Farida et al. 2021).

The antioxidant activity produced in the study above came from secondary metabolites contained in samples such as alkaloids, flavonoids, tannins, saponins, phenols, and triterpeneoids. Flavonoids can block cell damage due to oxidative stress because flavonoids act as exogenous antioxidant agents with phenolic group content. Flavonoids also play a role in inhibiting free radicals directly (Saija 1995; Nijveldt et al. 2001). In addition, flavonoids can stabilize reactive free radicals by donating hydrogen ions (Saija 1995; Arora 1998; Nijveldt et al. 2001). Another mechanism of flavonoids is to increase the production of SOD (*superoxide dismutase*) which is a gene for the formation of endogenous antioxidant enzymes. Alkaloids have lone pairs of electrons on nitrogen atoms that can capture free radical activity. Triterpenoids can stabilize reduced free radicals by donating hydrogen atoms. Saponins have a mechanism of action by inhibiting superoxide which makes biomolecular damage inhibited (Saija 1995; Sumardika and Jawi 2012; Hasan et al. 2022).

Anti-inflammatory activity

Andaliman fruit extract can reduce levels of IL-6 and TNF- α which are proinflammatory cytokines that play a role in defense and the immune system when there is potential

infection or danger in mice induced with lipopolysaccharide (LPS) as endotoxin substance derived from gram-negative bacteria that will activate macrophages in the body. Macrophages will trigger the release of proinflammatory cytokines such as interleukin-1 (IL-1), IL-6, *atumor necrosis factor*- α (TNF- α) (Nieman et al. 2007). IL-6 and TNF- α levels in LPS-induced mice without treatment were 67.5 and 109.7, respectively. However, after being sampled, these levels decreased almost close to IL-6 and TNF- α levels in mice without LPS induction. The results showed that Andaliman fruit extract has activity as an anti-inflammatory agent by lowering IL-6 and TNF- α levels (Barus et al. 2020).

The activity produced may be andaliman fruit extract can be caused by the role of secondary metabolites contained in it. The mechanism of action of flavonoids as anti-inflammatory agents is to inhibit cyclooxygenase (COX) and lipooxygenase enzymes which can result in reduced secretion of proinflammatory cytokines due to inhibited leukocyte accumulation (Nieman et al. 2007; Gardi et al. 2015; Ullah et al. 2020). Flavonoids can also inhibit NF- κ B (Nuclear Factor kappa B) which plays a role in regulating the expression of proinflammatory cytokines, causing TNF- α levels to decrease. Other metabolites such as alkaloids, tannins, triterpenes, and glycosides contained in andaliman can inhibit cyclooxygenase (COX) enzymes both 1 and 2, inhibiting the formation of PGE₂, proinflammatory cytokines (IL-6, IL-1 β), as well as inhibiting the formation of TNF- α (Nieman et al. 2007; Mohammed et al. 2014; Sartika et al. 2021).

Secondary metabolites that act as anti-inflammatory agents have other mechanisms of action besides lowering IL-6 and TNF- α levels, namely alkaloids can reduce IL-1 secretion, preventing prostaglandin E₂ synthesis and prevents the release of histamine which is a mediator of proinflammation. Tannins act as anti-inflammatory agents by preventing the expression of proinflammatory mediators (Mohammed et al. 2014).

Immunomodulator activity

Immunomodulators are substances used in regulating immune system including *innate* immune and *adaptive* immune by regulating immune cells such as cytokines, adhesion molecules, nitric oxide, hormones, neurotransmitters, and other peptides (Spelman et al. 2006; Kumar et al. 2012; Purba and Sinaga 2017). Immunomodulators work by suppressing or normalizing abnormal immune reactions (immunosuppressants) or by improving the immune system (immunostimulants). Immunostimulants are substances that can increase or stimulate the immune system by increasing the activity of immune system components to fight diseases and infections. White blood cells are one of the body's defense systems to fight infection and play a role in the immune response. White blood cells consist of granulocytes (eosinophil, basophils, neutrophil) and agranulocytes (lymphocytes and monocytes) (Hoffbrand and Pettit 1996; Suhirman and Winarti 2010; Hashemi and Davoodi 2012; Sutoyo et al. 2018).

Ethanol extract of adaliman fruit has been tested as immunomodulators in rats as animal and the levels of granulocytes consisting of basophils, neutrophils, and eosinophils in white blood cells had been evaluated. Basophils play a role in histamine secretion and hypersensitivity reactions. Neutrophils play a role in the process of phagocytosis and eosinophils play a role in the production of antibodies and the process of phagocytosis (Hoffbrand and Pettit 1996; Hashemi and Davoodi 2012; Sutoyo et al. 2018). There was an increase in granulocytes in the group given andaliman extract compared with the control group. It has been revealed that andaliman extract has immunomodulatory activity as an immunostimulant because it can affect the immune system by maintaining white blood cell counts despite the slight effect observed (Sutoyo et al. 2018).

Immunomodulatory activity in the form of immunostimulants of andaliman fruit is contributed from the secondary metabolites of the fruit. Flavonoids can affect the proliferation of lymphocytes that have an important role in the immune system (Astuya 2017). Flavonoids are also able to improve the body's immune system by increasing the proliferation of T cells lymphocytes, B cells, and the release of cytokines TNF- α , IL-4, and IFN- γ . Accordingly, it can stimulate the release of nitric oxide by macrophages, lysosomal enzyme activity, as well as macrophage phagocytosis activity (Hariyanti et al. 2015; Astuya 2017; Grigore 2017). In addition, flavonoids also play a role in multiplying IL-2 activity (Astuya 2017).

Polyphenols can affect nonspecific immune responses primarily through increased phagocytosis and lymphocytes as well as neutrophil proliferation. Epigallocatechin gallate (EGCG) is one of the polyphenolic compounds

that can stimulate the production of interleukin-1 alpha (IL-1 α), interleukin-1 beta (IL-1 β), tumor necrosis factor alpha (TNF- α). EGCG can also aid the phagocytosis process, increase lymphocyte resistance, lymphocyte proliferation, IL-12 macrophage secretion, increase IFN- γ , and inhibit histamine production (Grigore 2017; Shakoor et al. 2021). Polyphenolic compounds can also act as immunosuppressant agents with mechanisms such as inhibiting T cell proliferation and reducing the production of IL-1, IL-6 and IFN- γ which acts as a cytokine proinflammatory (Han et al. 2021; Shakoor et al. 2021; Renda et al. 2022). Triterpenes in andaliman fruit act as immunostimulants which can stimulate TNF- α , IL-6, IL-2, help the phagocytosis process, and increase the formation of antibody cells (Renda et al. 2022). Another mechanism of flavonoids as immunostimulants is to increase the production of IL-4. In addition, flavonoids also act as immunosuppressant agents by increasing IL-10 where IL-10 is an anti-inflammatory mediator so that the presence of IL-10 can inhibit or decrease the production of IL-2 and IFN- γ which function as the immune system (Han et al. 2021; Renda et al. 2022).

Conclusion

The results of some of the studies above show that plant parts in the form of fruits, leaves, bark, and roots of andaliman are proven to have a pharmacological activity, containing as they do antibacterial, anti-inflammatory, anticancer, antioxidant, and immunomodulatory properties. The activity comes from active compounds in it such as alkaloids, tannins, saponins, flavonoids, steroids, triterpenes, glycosides, and phenols.

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