Preliminary Evaluation of Anti Fish Pathogenic Bacteria and Metabolite Profile of Andaliman Fruit (*Zanthoxylum acanthopodium* DC.) Ethanol Extract

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Abstract. Andaliman (*Zanthoxylum acanthopodium*) is an endemic plant commonly utilized by people in North Sumatera as an additional food ingredient regarding its intense flavor. The previous study has proven that the andaliman fruit showed antimicrobial, antioxidant, and anti-inflammatory properties. The purposes of this research were to evaluate the antibacterial activity of andaliman fruit ethanol extract against fish pathogenic bacteria and understand its metabolite profile. The extraction was carried out using the maceration method with agitation (115 r.p.m) for 24 hours in ethanol. Thin Layer Chromatography (TLC) and phytochemical test were carried out to characterized the secondary metabolite. The antibacterial activity evaluation was conducted using the paper disc diffusion method against *Vibrio anguillarum*, *Vibrio alginolyticus*, and *Vibrio harveyi*. Ethanol extract of andaliman fruit gave 16 spots on the TLC plate, while the phytochemical results showed the presence of tannin, flavonoid, terpenoid steroid, and quinone. Besides, the evaluation of antibacterial activity gave negative results against fish pathogenic bacteria.

1. Introduction
Andaliman (*Zanthoxylum acanthopodium* DC.) is a herbal plant that can be found in tropical countries and classified in the Rutaceae family [1]. In Indonesia, this plant can be found in Dairi, North...
Tapanuli, Sidikalang, Simalungun, Sumbul, and around Lake Toba [2-5]. Due to its distinctive flavour and aroma, people in North Sumatra utilize andaliman fruit as an additional food ingredient [6-7]. Wijaya et al. [8] reported that the distinctive aroma of andaliman (Z. acanthopodium DC.) comes from aromatic compounds, such as geranyl acetate and limonene. Based on its ethnobiological function, many people in North Sumatra have utilized andaliman as a cure for diarrhea, stomachache, and toothache [9-11].

Previous studies have reported that andaliman contained various biological activities, such as antioxidant, antidiabetic, anticancer, antibacterial, and anti-inflammation [12-17]. However, there is still a lack of studies regarding andaliman potential for aquaculture, especially as an antibacterial agent against fish pathogenic bacteria. Therefore, the purposes of this study were to obtain andaliman (Zanthoxylum acanthopodium DC.) fruit ethanol extract, evaluate its potential as a biological agent against fish pathogenic bacteria, and analyze its metabolite profile.

2. Materials and Methods

2.1. Sample Preparation

Andaliman fruit 1 kg was obtained from local farmers in Parsoburan Village, North Sumatera, in September 2018. The sample was wrapped using styrofoam to maintain sample quality then brought to the Laboratory of Natural Product, Diponegoro University, Semarang, Indonesia, for further step.

2.2. Extraction

Andaliman fruit 240 g was dried and resized using mortar and pestle, then the sample was extracted using ethanol 96% by maceration method with agitation (115 r.p.m) at room temperature (27ºC ) for 24 h. The sample was separated to obtain the solvent phase then concentrated using a rotary evaporator (30-35ºC) [17]. The water content found in the sample caused the extract to be not appropriately evaporated. The partition method was carried out by adding ethyl acetate manually then separated using a separatory funnel.

2.3. Phytochemical Test

According to Sibero et al. [18], a phytochemical test was performed to screen several bioactive groups such as alkaloid, flavonoid, quinone, saponin, steroid terpenoid, and tannin.

2.4. Thin Layer Chromatography

A TLC glass plate (Merk, F254) was prepared for the stationary phase, while n-hexane and ethyl acetate with a ratio of 7:3 were prepared as the eluent system.

2.5. Antibacterial Test

Antibacterial activity assay against fish pathogenic bacteria such as Vibrio alginolyticus, Vibrio anguillarum, and Vibrio harveyi) was carried out using paper disk-diffusion according to Sibero et al. [19]. The fish pathogenic bacteria were re-cultured on marine agar (ZMA 2216, Himedia) for 48 h. The bioassay was started by re-culturing the bacteria in marine broth (ZMB 2216, Himedia) for 24 h then the bacteria were inoculated on marine agar by using a sterile cotton swab.

The ethanol extracts were diluted in dimethyl sulfoxide (DMSO) became several concentrations, which were 2 mg/L, 1.5 mg/L, 1 mg/L, and 0.5 mg/L, DMSO was used as negative control while Ampicillin was used as the positive control. The positive result was indicated by the formation of the inhibitory zone on media.

3. Results and Discussion

Metabolite studies of andaliman (Zanthoxylum acanthopodium DC.) fruit are quite often studied [12-17]. The secondary metabolites of andaliman (Zanthoxylum acanthopodium DC.) fruit ethanol extract was characterized using a phytochemical test. The result of the phytochemical test is shown in Table 1.
Table 1. Phytochemical content in andaliman (Z. acanthopodium DC.) fruit

| Bioactive Groups       | Indicator                                                                 | Figure       | Result |
|------------------------|---------------------------------------------------------------------------|--------------|--------|
| Alkaloid               | An arrangement of yellow to orange precipitates after addition of Dragendorff reagent | -            |        |
| Flavonoid              | A formation of yellow/orange/reddish color in amyl alcohol layer           | +            |        |
| Quinone                | A formation of yellow or orange color in sodium hydroxide layer             | +            |        |
| Saponin                | A formation of stable foam after 30 min and addition of 2N HCl             | -            |        |
| Steroid/Triterpenoid   | An arrangement of green coloration of the upper layer and red coloration of the lower layer | +            |        |
| Tannin                 | A formation of dark blue to blackish-green color after the addition of FeCl₃ reagent | +            |        |

(+: indicates the presence of the substance; -: indicates the absence of the substance)

Four of six bioactive groups of andaliman fruit ethanol extract were successfully detected through the phytochemical test. On the other hand, Sibero et al. [17] has proven that the andaliman fruit methanolic extract contained five bioactive groups, such as alkaloid, flavonoid, saponin, steroid/triterpenoid, and tannin. Anggraeni [20] stated that the andaliman (Z. acanthopodium DC.) extract also contained a glycoside compound. Various types of compounds in the andaliman (Z. acanthopodium DC.) fruit extracts become evidence of the diversity of andaliman fruit biological activity [4]. Analysis of further metabolite profile was performed using Thin Layer Chromatography (Figure 1).
According to the TLC profile, the andaliman (Z. acanthopodium DC.) fruit gave 16 spots on the TLC plate with 0.93 as the highest retention factor.

The result of the antibacterial assay was shown in Table 3.

### Table 2. The retention factor ($R_f$) of the andaliman (Z. acanthopodium DC.) fruit ethanol extract

| Notation | $R_f$ | Notation | $R_f$ |
|----------|------|----------|------|
| A        | 0.09 | I        | 0.58 |
| B        | 0.19 | J        | 0.61 |
| C        | 0.22 | K        | 0.64 |
| D        | 0.27 | L        | 0.67 |
| E        | 0.31 | M        | 0.71 |
| F        | 0.39 | N        | 0.78 |
| G        | 0.43 | O        | 0.85 |
| H        | 0.48 | P        | 0.93 |

This research has proven that the andaliman (Z. acanthopodium DC.) fruit ethanol extract showed no activity against three fish pathogenic bacteria. However, the antibacterial activity of andaliman fruit has been reported in previous studies. Sibero et al. [17] reported that the andaliman fruit methanol extract had shown no activity against four pathogenic bacteria, such as *Tenacibaculum maritimum*, *V. alginolyticus*, *V. anguillarum*, and *V. harveyi*. On the other hand, Muzafri et al. [21] the andaliman fruit methanol extract showed activity against *E. coli*, *Salmonella typhimurium*, and *Staphylococcus aureus*. A study conducted by Julistiono et al. [22] reported that the andaliman fruit n-hexane showed antibacterial properties against *Mycobacterium smegmatis*. Moreover, Amelia et al. [23] reported that the andaliman fruit water extract could inhibit bacterial contaminant growth in *Cyprinus caprio* fish. This study showed that the andaliman fruit ethanol extract could not be used as an antibacterial agent. This result also proved that ethanol is not an excellent organic solvent to extract the antibacterial compound from andaliman (Z. acanthopodium DC.) fruit.
4. Conclusion
Flavonoids, quinone, steroid terpenoid, and tannin were bioactive groups contained in andaliman fruit ethanol extracts. According to the antibacterial activity, the ethanol extract of andaliman fruit showed no activity against fish pathogenic bacteria.

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