Phytochemical Analysis by FTIR of Zanthoxylum Acanthopodium, DC Fruit Ethanol Extract, N-hexan, Ethyl Acetate and Water Fraction

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ABSTRACT

Background: Andaliman fruit is used as spice in Batak cuisine, North Sumatera, Indonesia. The potency of andaliman fruit extract as herbal medicine is widely studied. Many studies elaborate the benefits of andaliman fruit extract as an antioxidant, antibacterial or anti-cancer. Objective: The aim of this study was to identify the phytochemical compounds of andaliman fruit extract and its fractions. Methods: The andaliman fruit was originated from Baliage city, Tobasa Regency, North Sumatera. The extract was made by maceration within ethanol and followed by fractionation with n-hexan, ethyl acetate and water. The phytochemical screening by chemical reactions, thin layer chromatography, and Fourier Transform Infrared Spectrophotometer were performed. Results: This study found andaliman fruit ethanol extract consists of alkaloids, flavonoids, glycosides, tannins, triterpens, and steroids. The n-hexan fraction consists of triterpens and steroids, ethyl acetate fraction consists of flavonoids and glycosides, and water fraction consists of alkaloid, flavonoid, and glycosides. Andaliman fruit ethanol extract had eight color spots, n-hexan and ethyl acetate fraction had five color spots and water fraction had two color spots. Andaliman fruit ethanol extract had functional group of C-H alkanes, =C-H alkenes (aliphatic), O-H, C=C C=C aromatics, C=C alkynes, C-O, C=O, and NO2. The n-hexan fraction had C-H alkanes, =C-H alkenes (aliphatic), C-O, C=O, and NO2. The ethyl acetate fraction had C-H alkanes, =C-H alkenes (aliphatic), O-H, C=C C=C aromatics, C=C C=C alkynes, and NO2. The water fraction had C-H alkanes, =C-H alkenes (aliphatic), O-H, C=C C=C aromatics, C=C C=C alkynes, and NO2. The Conclusion: Phytochemical screening found that andaliman fruit ethanol extract, n-hexan, ethyl acetate and water fraction positive of phytochemical compounds. The FTIR of andaliman fruit ethanol extract, n-hexan fraction, ethyl acetate fraction, and water fraction showed the absorbance of C-H alkanes, O-H, C=O, and C=C alkenes indicating the presence of alkaloids, flavonoids, and triterpenoid saponin.

Keywords: Andaliman, Extract, Fraction, Functional groups, Zanthoxylum acacontinentum.
on placenta (7) proliferation, differentiation and embryonic processes in the human placenta are NOTCH1 and Hes1. The aim of this research was to analyze the expression of NOTCH1 and Hes1 genes after administering nano herbal andaliman to the trophoblast cells of the human placenta. Materials and Methods: HTR8 trophoblast cells were divided into two groups, namely, control and treatment (nano herbal andaliman. Study of andaliman fruit n-hexan fraction on TD47 breast cancer cell culture found its potency as anti-cancer trough cell cycle inhibition, apoptosis induction, and decreasing cyclin D1 expression (8), and also have ability to halt 4T1 breast cancer cell migration (9). Either andaliman fruit ethanol extract or andaliman fruit alkaloid extract have cytotoxicity on T47D breast cancer cell (10,11). Then, ethanol extract or andaliman fruit alkaloid extract have breast cancer cell migration (9). Either andaliman fruit or andaliman fruit alkaloid extract have clin D1 expression (8), and also have ability to halt 4T1 cycle inhibition, apoptosis induction, and decreasing cytotoxicity on MCF-7 breast cancer cell, HeLa cervical cancer cell, and Raji lymphoma cell (11).

2. OBJECTIVE
The aim of this study was to identify the phytochemical compounds of andaliman (Zanthoxylum acanthopodium, DC) fruit extract and its fractions.

3. MATERIALS AND METHODS

Materials
The andaliman fruit (Zanthoxylum acanthopodium, DC) was obtained from Balige city, North Tapanuli Regency, North Sumatera Province, Indonesia with coordinate 2.335661°N 99.065271°E. Plant identification was conducted in Herbarium Medanense, Universitas Sumatera Utara, Kampus USU Medan. The extraction process was made in Pharmacy Biology Laboratory, Faculty of Pharmacy, Universitas Sumatera Utara, Medan. Chemicals used in this study ethanol 96% solution, n-hexan solution, ethyl acetate solution, Dragendroff reagent, Bouchardat, Meyer, Powder Mg + amyl alcohol, hydrogen chloride, H2SO4, FeCl3, and Lieberman-Bourchat.

Extraction Process
The Zanthoxylum acanthopodium, DC fruit was wet sorted, washed on running water, and dried on 40°C oven. After being grinded, the one kg fine powder was macerated with 10 L of 96% ethanol for three days in room temperature. The extract was separated from the residue by filtering the macerate. The filtrate was evaporated with a vacuum evaporator and obtained 110 g crude ethanol extract with product yield 11%. As much as 30 g of the andaliman ethanol extract was added with 120 ml water and put in separated funnel then added with n-hexan solution repeatedly until a clear n-hexan solution was obtained. The clear color marked the end of n-hexan fractionation process and validated with Liebermann-Bourchart reaction. Then the rotary evaporated was performed to separate the extract compounds from its solution and obtained 3 g of n-hexan fraction with product yield 10%. The fractionation was continued with ethyl acetate solution by putting the water result of n-hexan fraction process in a separate funnel until clear ethyl acetate solution was obtained and validated by FeCl3 reaction, if the color is clear or nothing change, the ethyl acetate fractionation was ended. Then the ethyl acetate fraction was rotary evaporated and obtained 5 g of ethyl acetate fraction with product yield 16.7%. The left of fractionation process was 2 g water fraction with product yield 6.7%.

Characteristic identification
The characteristics of simpilisia and extract was conducted by evaluating the water content, soluble substance in water, soluble substance in ethanol, ash content and insoluble ash in acid.

Phytochemical Screening
To identify the phytochemical compounds contained in the extract and fractions was by using some chemical reactions. The alkaloids identification was by adding the Dragendorff, Bouchardat and Meyer reagents to the test tube. The formation of orange to reddish, brown and white to yellowish was the sign of alkaloids presence, respectively (12,13). The flavonoids identification was by adding 0.1 mg of magnesium powder, 1 ml of amyl alcohol and hydrogen chloride to the test tube. The formation of yellow or orange to reddish color indicated the presence of flavonoids (13). The glycosides identification was by mixing 5 ml of extract and 25 ml of H2SO4, then heated to boiling points for 15 minutes then cooled and added NaOH, next with 5 ml Fehling solution. The formation of red brick indicated the presence of glycosides (12). Saponins identification by heating the extract to its boiling point then shake until the formation of foam layer that indicated the presence of saponins (13). The tannins identification was by adding the two drops of 1% FeCl3 to the test tube, the formation of greenish to black color indicated the presence of tannins (13). The steroids and triterpens identification was Liebermen-Bourchar test by adding chloroform, acetic anhydride, and H2SO4 to the test tube. The formation of dark pink or red color or reddish brown in the lower layer indicated the presence of steroids and green color in the upper layer for triterpens (13,14).

Thin Layer Chromatography (TLC)
The spot color of phytochemical compound in the extract and its fractions was evaluated using thin layer chromatography (E. MERCK KGaA). The stationary phase was High Performance Thin-Layer Chromatography (HPLTC) plates size 2.0x1.0 cm precoated with silica gel 60 F 254 as the absorbance, the mobile phase was n-hexan:ethyl acetate (93:0.7), and spotted visual using vanillin – sulphate acid with ultraviolet 366 nm.

The Fourier Transform Infrared Spectrophotometer (FTIR)
The functional groups contained in andaliman fruit ethanol extract, n-hexan, ethyl acetate and water fractions were identified by the Fourier Transform Infrared Spectrophotometer (FTIR).

4. RESULTS

Extract Characteristics
Table 1 shows the characteristics of both simpilisia and extract. The water content in simpilisia was 7.3% and in extract was 24.56%. The ash content in simpilisia was 5.40% and in extract was 0.95%. The insoluble ash
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in acid in extract was 0.73% and in extract was 0.54%.

The Phytochemical Screening
The andaliman fruit ethanol extract was positive of alkaloids, flavonoids, glycosides, tannins, triterpenes, and steroids. The n-hexan fraction was positive of triterpenes and steroids, the ethyl acetate fraction was positive of flavonoids and glycosides, and the water fraction was positive of alkaloids, flavonoids, and glycosides (Table 2).

The Thin Layer Chromatography (TLC)
The TLC showed that andaliman fruit ethanol extract contained eight color spots, n-hexan and ethyl acetate fraction contained five color spots and water fraction contained two color spots with each Retention factor (Rf) value. The color spots that was identified in the ethanol extract was purple (0.81). In the ethyl acetate were green (0.50), red (0.64 and 0.86), and orange (0.71). In water fraction was blue (0.15) and yellow (0.23).

The Fourier Transform Infrared Spectrophotometer (FTIR)
The FTIR showed that andaliman fruit extract and its fraction had some functional groups. The andaliman fruit ethanol extract showed absorbance of C-H alkanes at wavenumber 2858.51, 2949.09 and 1450.47 cm⁻¹, =C-H alkenes (aliphatic) at wavenumber 725.23, 837.11, and 987.55 cm⁻¹, O-H at wavenumber 3309.85 cm⁻¹, C=C aromatics at wavenumber 1546.91 cm⁻¹, C≡C alkynes at wavenumber 2152.56 cm⁻¹, C-O at wavenumber 1091.71, 1168.86 and 1238.3 cm⁻¹, C=O at wavenumber 1735.93 cm⁻¹, and NO₂ at wavenumber 1373.32 cm⁻¹.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Percentage</th>
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<td>Simplisia</td>
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<tr>
<td>Soluble substance in water</td>
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<tr>
<td>Insoluble substance in ethanol</td>
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<tr>
<td>Ash content</td>
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<tr>
<td>Insoluble ash in acid</td>
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<tr>
<td>Extract</td>
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<tr>
<td>Ash content</td>
<td>0.95</td>
</tr>
<tr>
<td>Insoluble ash in acid</td>
<td>0.54</td>
</tr>
<tr>
<td>Water content</td>
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Table 1. The characteristics of andaliman fruit simplisia and extract

<table>
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<tr>
<th>Phytochemical compounds</th>
<th>Reagents</th>
<th>Ethanol extract</th>
<th>n-hexan fraction</th>
<th>Ethyl acetate fraction</th>
<th>Water fraction</th>
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<tbody>
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<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Bouchardat (+)</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Meyer (+)</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Powder Mg+ amyl Alcohol + HCl (+)</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>Molish+H₂SO₄ (+)</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>Hot water/ shaken (-)</td>
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<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Tannins</td>
<td>FeCl₃ (+)</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Triterpenes</td>
<td>Lieberman-Bourchardat (+)</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Steroids</td>
<td>Lieberman-Bourchardat (+)</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
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</tbody>
</table>

Table 2. Phytochemical compounds of andaliman fruit extract and its fractions
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1, O-H at wavenumber 3305.99 cm⁻¹, C=C alkenes at wavenumber peak value 1631.78 and 1666.5 cm⁻¹, C-O at wavenumber 109943, 1168.86 and 1238.3 cm⁻¹, C=O at wavenumber 1735.93 cm⁻¹, and NO₂ at wavenumber 1546.91 cm⁻¹. The ethyl acetate fraction showed absorbance of C-H alkanes at wavenumber 2862.36, 2924.09 and 1450.47 cm⁻¹, =C-H alkenes (aliphatic) at wavenumber 3098.82, 833.25 and 99.4 cm⁻¹, O-H at wavenumber 3309.85 cm⁻¹, C=C alkenes at wavenumber 1620.21 cm⁻¹, C=C aromatics at wavenumber 1550.77 cm⁻¹, C-O at wavenumber 1076.28, 1168.86 and 1253.73 cm⁻¹, C=O at wavenumber 1369.46 cm⁻¹. The water fraction showed absorbance of C-H alkanes at wavenumber 2889.37, 2951.09 and 1392.61 cm⁻¹, C=C aromatics at wavenumber 1600.92 cm⁻¹, C≡C alkanes (aliphatic) at wavenumber 825.53 and 921.97 cm⁻¹, O-H at wavenumber 3340.71 cm⁻¹, C=C aromatics at wavenumber 1600.92 cm⁻¹, C≡C alkenes at wavenumber 2129.41 cm⁻¹, and C-O at wavenumber 1242.16 cm⁻¹ (Figure 2a, 2b, 2c, and 2d).

5. DISCUSSION

The water content in simplisia in this study was 7.3%, it was met the criteria made by Indonesia Health Ministry that water content in simplisia must less than 10% (15). Water content is important in determining the quality of simplisia because water content may influence its stability (16). If a simplisia are less dried, toxigenic fungi and insects will damage and lessen the quality. This study found the water content in the extract is 24.56%. The standard profile of andaliman fruit extract has not been listed in the Monografi Farmakope Herbal Indonesia (17). Meanwhile, the water content of various extracts that are listed in Monografi Farmakope Herbal Indonesia are varies such as the water content of cardamom fruit extract is not more than 27.3% and the jibeling leaves extract is not more than 30% (17). The ash content shows the amount of mineral and foreign materials contained. The insoluble ash in acid shows the amount of soil and fine particles of sand contained in the extract that have relation in the amount of heavy metal (18). The ash content and the insoluble ash in acid content in the simplisia and extract in this study was low less than 16% (Table 1).

The phytochemical screening showed that andaliman fruit ethanol extract was positive alkaloids, flavonoids, glycosides, tannins, triterpens and steroids (Table 2). These results are similar with previous study that found andaliman fruit extract was positive of alkaloids, flavonoids, tannins, triterpens, steroid, saponins, and phenols (19,20). The n-hexan fraction was positive of triterpens and steroids (Table 2), meanwhile previous study found andaliman fruit n-hexan fraction was positive of triterpens and alkaloids (21). The ethyl acetate fraction was positive of flavonoids and glycosides (Table 2), previous study found that andaliman fruit ethyl acetate fraction was positive flavonoids, alkaloids, glycosides, saponins, tannins, and steroid (19,21). The water fraction on this study was positive of alkaloids, flavonoids, and glycosides (Table 2), meanwhile previous study found that andaliman fruit water fraction was positive of flavonoids, glycosides, and tannins (21).

The TLC showed the spot colors in extract and fractions. The mobile phase in this study was n-hexan:ethyl acetate (93:0.7), this solution was non polar so they can attract compounds like alkaloid, flavonoids and steroids. The TLC found that andaliman fruit ethanol extract had
eight spot colors, orange (Rf 0.64) for alkaloids, yellow (0.43) for flavonoids, red (0.57 and 0.71) for glycosides, green (0.28) for tannins and triterpenes, and purple or dark pink (0.07, 0.35 and 0.93) for sterols. The spot colors in n-hexan fraction were green (0.38) for triterpenes and tannins, red (0.56 and 0.75) for glycosides, orange (0.63) for alkaloids, and purple or dark pink (0.81) for steroids. The spot colors in ethyl acetate fraction were green (0.50) for triterpenes and tannins, red (0.64 and 0.86) for glycosides, orange (0.71) for alkaloids, purple (0.93) for steroids. The spot colors in fraction were blue (0.15) for tannins, and yellow (0.23) for flavonoids and alkaloids (Figure 1).

FTIR is a recommended tool to identify functional groups in compounds (22). The functional groups of alkaloids contain C-H and C-N, N-H, and C=O; flavonoids contain C=O and O-H; terpenoids and steroids contain aromatic C-H groups (23,24), and triterpenoid saponins contain –OH, -C=O, C-H, and C=C (25).

The FTIR showed that andaliman fruit ethanol extract had characteristics functional groups of alkaloids by the absorbance of C-H alkanes at wavenumber 2858.51, 2949.09 and 1450.47 cm⁻¹, flavonoids by the absorbance of C=O at wavenumber 1392.61 cm⁻¹ and O-H at wavenumber 3309.85 cm⁻¹, and triterpenoid saponins by the absorbance of C=C alkenes at wavenumber 1631.78 and 1666.5 cm⁻¹ indicating the presence of triterpenoid saponins. The ethyl acetate fraction showed the absorbance of C-H alkanes at wavenumber 2862.36, 2924.09 and 1450.47 cm⁻¹ indicating the presence of alkaloids, O-H at wavenumber 3309.85 cm⁻¹ and C-O at wavenumber 1735.93 cm⁻¹ indicating the presence of flavonoids, and C=C alkenes at wavenumber 1631.78 and 1666.5 cm⁻¹ indicating the presence of triterpenoid saponins. The n-hexan fraction showed the absorbance of C-H alkanes at wavenumber 2858.51 and 2924.09 cm⁻¹ indicating the presence of alkaloids, O-H at wavenumber 3305.99 cm⁻¹ and C-O at wavenumber 1735.93 cm⁻¹ indicating the presence of flavonoids, and C=C alkenes at wavenumber 1627.92 and 1662.62 cm⁻¹ and C=C aromatics at wavenumber 1546.91 cm⁻¹.

five color spots and water fraction had two color spots. The FTIR results of andaliman fruit ethanol extract, n-hexan fraction, ethyl acetate fraction, and water fraction showed the absorbance of C-H alkanes, O-H, C=O, and C=C alkenes functional groups indicating the presence of alkaloids, flavonoids, and triterpenoid saponin.

6. CONCLUSION
The phytochemical screening showed that andaliman fruit ethanol extract was positive of alkaloids, flavonoids, glycosides, tannins, triterpenes, and steriods. The n-hexan fraction was positive of triterpenes and steroids. The ethyl acetate fraction was positive of flavonoids and glycosides. The water fraction was positive of alkaloids, flavonoids, and glycosides. The thin layer chromatography showed that the andaliman fruit ethanol extract had eight color spots, n-hexan and ethyl acetate fraction had five color spots and water fraction had two color spots.

REFERENCES
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