Isolation and Identification of Secondary Metabolic Compounds Acetone Extract from Dutch Eggplant (Cyphomandra betacea)

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ABSTRACT
Isolation and identification of secondary metabolite compound in acetone extract from fruit of terong belanda (Cyphomandra betacea) has been carried out. The aim of this research is to isolate and identify the secondary metabolite compound in acetone extract from fruit terong belanda (Cyphomandra betacea) which obtain from Enrekang. The compound was obtained by isolation process that consist of several stages, including extraction, fractionation, purification and identification. Extraction was carried out using maceration with acetone. The identification was comment by is color test, melt point, solubility and TLC. Base of identification are obtained compound in white powder with melting point 130-131 oC, and positively to the reagent Dragendorf and Wagner gives a brown precipitate that is indicated as a alkaloid group.

Keywords: Alkaloids, Cyphomandra betacea, Isolation

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INTRODUCTION
Distinguishing the meaning between food and food is often difficult. In this case, food is that Indonesian people who live in rural areas or suburbs are accustomed to using traditional medicines which generally come from plants to prevent disease or treat disease. The application of these drugs can be by drinking the extract of the plant or placing the finely ground simplicia on the affected area of the body. The lack of scientific information about the chemical components contained in this plant for traditional medicine results in the economic value of this plant being very low. In addition, its use which is usually in arbitrary doses can cause unwanted effects. Secondary metabolite compounds contained in plants were initially considered as waste from metabolic processes in plant tissues, when in fact these compounds have functions that are very useful for human health, plant life itself as well as for other organisms. Secondary metabolites have been widely used as a source of ingredients for traditional medicines, fragrances, dyes, food additives and as a source of pesticides, so it is necessary to conduct research to determine the chemical compounds that are useful in plants. This situation prompted the author to research plants that are well known by the public as...
traditional medicines, such as the Dutch eggplant (Chyphomandra betacea) whose use is still very limited.

Dutch eggplant (Cyphomandra betacea) belongs to the eggplant family (Solanaceae). This fruit comes from Peru, Latin America and entered Indonesia and was developed in several areas, including Bali, West Java, and Tanah Karo-North Sumatra. The results showed that the Dutch eggplant (Cyphomandra betacea) was rich in provitamin A which is a precursor of vitamin A for eye health, vitamin C for treating canker sores and increasing endurance, fiber which is useful for preventing cancer, constipation and macronutrient content (carbohydrates, water), protein, fat) which are good for consumption as a functional aspect of food. Other components contained in the Dutch eggplant (Cyphomandra betacea) are vitamin E and phenolic compounds (including anthocyanins and other flavonoids) and carotenoids.

Looking at the exposure and information above, it is deemed necessary to conduct further research to determine the content of secondary metabolites contained in the Dutch eggplant (Cyphomandra betacea) because no one has ever specifically examined secondary metabolites in Dutch eggplant (Cyphomandra betacea).

**RESEARCH METHOD**

1. **Research Time and Place**
   The implementation of this research started from February to June 2010 which includes literature study, sampling, sample analysis. Sample preparation was carried out at the Pharmacy Laboratory of UIN Alauddin and sample analysis was carried out at the Chemistry Laboratory of UNM Parang Tambung and the UNHAS basic chemistry laboratory.

2. **Research procedure**
   a. **Tools used**
      The tools used include measuring cups, beakers, dropper pipettes, test tubes, stirring rods, Erlenmeyer flasks of various sizes, evaporator, TLC chamber and plate, vacuum liquid chromatography column, flash chromatography column, analytical balance, water bath, oven, atomizer, electrothermal melting point determination device, porcelain dish.
   b. **Materials used**
      The ingredients used are Dutch eggplant (Cyphomandra betacea), the chemicals used are aquades, some organic solvents such as n-hexane, ethyl-acetate, methanol, chloroform and acetone, some reagents such as Liebermann-Buchard reagent, FeCl₃, Wagner and Dragendorff. Other materials used were H₂SO₄ 10%, acetic acid anhydride, silica gel G 60 (70-230 mesh) and silica gel 60 F₂5₄ for column chromatography, aluminum plate coated with silica gel 60 F₂5₄ for TLC analysis, aluminum foil and filter paper.
   c. **Work procedures**: Extraction, Fractionation, Purification.

**RESULTS AND DISCUSSIONS**

1. **Research result**
   2 kg of Dutch eggplant (Cyphomandra betacea) was macerated with 9 liters of acetone for 3 x 24 hours followed by decantation to produce a brown extract. The thickening process was carried out by the evaporation method to produce a thick extract of 10.506 g. The concentrated extract obtained in TLC to determine the suitable eluent on vacuum liquid column chromatography. From several elution tests conducted, it was found that the eluent of acetone: n-hexane (1 : 5) was good for KKCV which showed the best separation. The separation of the components resulting from the thin layer chromatography analysis can be seen in Figure 1:
The extract obtained was fractionated by vacuum liquid column chromatography with acetone as eluent which was continuously increased in polarity with n-hexane as the mobile phase as shown in the table:

| Fraction | Eluent                  | Fraction characteristics         |
|----------|-------------------------|----------------------------------|
| 1        | n-hexane 100%           | Clear yellow solution            |
| 2        | Acetone : n-hexane 1:9  | Clear yellow solution            |
| 3        | Acetone : n-hexane 3:7  | Chocolate solution (concentrated)|
| 4        | Acetone : n-hexane 4:6  | Yellowish brown solution         |
| 5        | Acetone : n-hexane 5:5  | Yellowish brown solution         |
| 6        | n-hexane : acetone 4:6  | Yellow solution                  |
| 7        | n-hexane : acetone 3:7  | Light yellow solution            |
| 8        | n-hexane : acetone 1:9  | Clear green solution             |
| 9        | 100% acetone            | Yellowish clear solution         |
| 10       | 100% methanol           | Dark brown solution              |

The fractions in TLC to determine the separation of the components and then the fractions with the same stain are combined.

| Fraction code | Merged fractions | Weight (grams) | Fraction characteristics |
|---------------|------------------|----------------|--------------------------|
| A             | 1                | 0.0652         | green solution           |
|               |                  |                | yellowish                |
| B             | 2 – 3            | 0.405          | brown solution           |
|               |                  |                | Chocolate paste          |
| C             | 4 – 6            | 0.1100         | yellow solution          |
|               |                  |                | Chocolate paste          |
| D             | 7 – 10           | 0.2830         | yellow solution          |
|               |                  |                | brown                    |

Fractions with good separation and characterized by the presence of crystals on the walls of the crucible were then subjected to flash column chromatography for further separation.
Table 3. Flash Chromatography Fractionation Results

| Fraction | Fraction characteristics |
|----------|--------------------------|
| 1-3      | Clear solution           |
| 4-6      | Brown solution           |
| 7-11     | Brownish yellow solution |
| 12-25    | Light yellow solution    |

The TLC analysis results for each fraction can be seen in the chromatogram below.

Figure 3. Thin layer chromatography of fraction 1-25 Eluent: acetone : n-hexane (3:7)
Adsorbent: TLC silica gel 60 F254 Stain appearance: H2SO4 10%.

Separation of compound components with one spot (fraction 2 vial no 4-6) was characterized by the presence of crystals on the vial wall purified by repeated recrystallization and continued with TLC analysis which showed one spot was marked by a sharp melting point test (130-131 oC). The crystals were then tested for reagents, purity tests with 3 solvent systems and solubility tests. The solubility test showed that these crystals were insoluble in n-hexane, slightly soluble in chloroform and acetone and well soluble in ethyl acetate.

The results of the identification of the Lieberman-Buchard reagent test showed positive for alkaloids.

Table 4. Results of identification tests on Dutch eggplant crystals

| Test       | Reactor          | Color                  | Note: |
|------------|------------------|------------------------|-------|
| Alkaloids  | Dragendorf f     | Chocolate              | (+)   |
|            | Wagner           | Chocolate precipitate  | (+)   |
|            |                  | Dark green             |       |
| Flavonoids | Concentrated H2SO4 NaOH 10% | Red | Clear | (-) |
|            |                  | Light yellow           | Clear | (-) |
|            | Lieberman-Buchard | green, black blue      | clear | (-) |
| Steroids   | FeCl3 1 N        | Blue black             | Yellow | (-) |
| Tannins    |                  |                        |       |

Figure 5. Thin layer chromatography system 3 eluent (a) Ethyl acetate eluent: chloroform 7: 3 (b) n-hexane eluent: ethyl acetate 7: 3 (b) Ethyl acetate eluent: chloroform 3: 7 Adsorbent: TLC silica gel 60 F254 Stain appearance: H2SO4 10%.
2. Discussion

2.0kg of Dutch eggplant (Cyphomandra betacea) dried samples were macerated with 9 liters of acetone for 3 x 24 hours. Extraction by maceration method was carried out with the aim of extracting secondary metabolites contained in Dutch eggplant fruit. The use of acetone as a solvent here aims to extract the chemical compounds contained in the Dutch eggplant, it is estimated that the chemical compounds, especially the extracted secondary metabolites, are semi-polar metabolites according to the solvent. After that the extract is evaporated which aims to separate the solvent from the extract. The acetone extract was then allowed to stand (evaporated) until all the solvent had evaporated and a dark brown extract of 10.506 g was obtained.

The acetone extract was then subjected to TLC to determine a good eluent for vacuum liquid column chromatography (KKCV). From several eluent tests carried out on acetone extract, namely with eluent; acetone : n-hexane, with various ratios, it was found that the eluent of acetone: n-hexane with a ratio of 1 : 5 was a good eluent for KKCV. This is indicated by the appearance of the stains that are clear and well separated. The chromatogram resulting from the thin layer chromatographic analysis with the eluent revealed 4 components of chemical compounds contained in the acetone extract as shown in Fig.

The extract obtained was then fractionated by vacuum liquid column chromatography using silica gel adsorbent G 60 F254 as the stationary phase and eluent acetone which was continuously increased in polarity with n-hexane as the mobile phase. From the results of the chromatography, 10 fractions were obtained which were then continued with TLC with acetone: n-hexane (1 : 5) as eluent as developer. The TLC results showed that the 2nd and 3rd fractions (Figure 4.20) showed a sharp separation of stains and there were signs of crystals on the phials. Fractions with the same stain are combined to obtain four combined fractions as shown in table 2.

From the results of TLC analysis, fraction B (2nd and 3rd fractions) has the potential to be continued. This is based on the results of TLC analysis which shows that in the chromatogram there is only one spot. In addition, fraction B had more components than the other fractions and showed the presence of a lot of crystals compared to other fractions after being evaporated.

Fraction B in the form of brown paste as much as 0.4015 grams was fractionated by flash column chromatography and continued with TLC analysis until the chromatogram as shown in Figure 3 was obtained using silica G 60 (200-400) mesh adsorbent as the stationary phase and acetone: n-hexane as the stationary phase. mobile phase with various ratios of n-hexane 100%, acetone : n-hexane (1 : 9 ), (2 : 8), (3 : 7), (4 : 6), (5 : 5), (6 : 4), (7 : 3), (8 : 2), (9 : 1), 100% ethyl and 100% methanol, until 25 fractions were obtained. The number of eluent ratios was due to the fact that in the first ratio (1 : 9) the movement of the stain was still not good, so the polarity was continuously increased until a sharp separation was obtained, namely the ratio of acetone: n-hexane (3 : 7). Use of acetone: n-hexane as the eluent at this stage was based on TLC analysis which resulted in a fairly good stain separation. Based on TLC analysis of 25 fractions, the fractions having the same chromatogram were combined to obtain four combined fractions, namely the combined fraction A1 (fraction 1-3), the combined fraction A2 (fraction 4-6), combined fraction A3 (fraction 7-11), the combined fraction A4 (fraction 12-25).

The crystals that were previously found in fractions 4-6 after being combined were in the combined fraction A2 so that they have the potential to be continued. After evaporation, the combined fraction A2 is a yellowish white solid. Since the TLC analysis showed more than one spot, the solid was then recrystallized with n-hexane solvent repeatedly to obtain 0.0046 g of powder and white crystals.

The white powder obtained was then tested for purity by three-eluent TLC system. TLC analysis showed one spot in three eluent systems with respective Rf: (a) 0.06 with n-hexane eluent: chloroform (7:3); (b) 0.7 with n-hexane eluent: ethyl acetate (7:3); and (c) 0.9 with chloroform:ethyacetate (7:3) as eluent, as shown in Figure 5. The melting point of the obtained crystals is 130-131°C with a sharp melting point trajectory (low range). From these data it can be concluded that the compounds obtained can be categorized as pure compounds.
Further identification is done by solubility test, identification by color test. The solubility test showed that these crystals were insoluble in n-hexane, slightly soluble in chloroform and acetone and well soluble in ethyl acetate. In the color test with 10% NaOH and concentrated H$_2$SO$_4$ there was no color change indicating a negative reaction to flavonoids. The Lieberman-Buchard reagent and FeCl$_3$ also showed a negative reaction to steroids and tannins. However, the color test with Dragendorf and Wagner reagents showed a positive reaction to the alkaloids which was indicated by a color change to a brown precipitate. This indicates that the compound is one of the alkaloid compounds with a melting point of 130-131°C.

**CONCLUSION**

Based on the results of the research that has been carried out, it can be concluded as follows: The mass of glucose is very influential on the thickness of nata de rice because it will be directly related to the mass of glucose which will be converted by Acetobacter xylinum into cellulose and based on the results of the study obtained that the mass of glucose is directly proportional to the thickness of nata de rice, i.e. the more glucose mass, the thicker the nata de rice will be. And the most optimum result to obtain the maximum thickness of nata is the addition of 100 grams of glucose with a thickness of 17 mm.

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Based on the results of the study, it can be concluded that the isolation of secondary metabolites from the acetone extract of Dutch eggplant (Chyphomandra Betacea) was obtained in the form of a white powder with a melting point of 130–1310C. The solubility test and color test showed that this compound is one of the alkaloid group compounds that are beneficial for health, for example to treat cancer.

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