Qualitative and Quantitative Test of Total Flavonoid Buni Fruit (Antidesma bunius (L.) Spreng) with UV-Vis Spectrophotometry Method

La Hamidu, Aktsar Roskiana Ahmad, Ahmad Najib

ABSTRACT

The aim of this research is to determine of total flavonoid content in the Buni fruit (Antidesma bunius L. Spreng) extract. The extract was produced by stratified maceration method with the different solvent, i.e n-Hexane, Ethyl acetate and ethanol. The analysis of chemical compound using chemical reagent and Thin Layer Chromatography (TLC) method. The method is used to determines total flavonoid contains Buni fruit (Antidesma bunius L.) extract was based on the amount of Rutin Equivalent (RE) were used. The result shows that the flavonoid content higher in the n-Hexane extract is 10.72 %, then ethyl acetate extract is 7.9 % and 3.56 % ethanol extract was counted to or as a Rutin.

Key words: Antidesma bunius L. Spreng, Flavonoid content, Spectrophotometry UV-VIS.

EXPERIMENTAL METHOD

Sample Preparation

Buni fruit (A. bunius L.) sample obtained in Enrekang district. The sample is then cleaned of dirt by using running water and then dried with aerated. After dried, the powered samples, then it was maceration gradual method with ethanol as much as 1 L for 3 × 24 hours while stirring periodically. The mixture was filtered and maceration again with 900 mL of ethyl acetate as much as 1 L for 3 × 24 hours. The mixture is then filtered and maceration again with 700 mL of 900 mL of ethyl acetate as much as 1 L for 3 × 24 hours. The mixture is then filtered and maceration again with 700 mL of ethyl acetate as much as 1 L for 3 × 24 hours. The mixture is then filtered and maceration again with 500 mL of ethyl acetate as much as 1 L for 3 × 24 hours.

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concentrated by evaporator simple method to obtain the n-hexane, ethyl acetate and ethanol viscous extract.

**Qualitative Flavonoids Test**

The Thin Layer Chromatography (TLC) method chosen because it has several excesses such as speed and sensitivity. Spots were obtained detected under UV nm light and using spray reagent. Reagents used for flavonoids is AlCl₃ and citroborat. Then observed again under UV nm light. AlCl₃ gives yellow color whereas citroborat reagent gives something positive will fluoresce yellow-green. Each n-hexane, ethyl acetate and ethanol extract of buni fruit diluted with methanol and then spotted on the TLC plate. The plate take in the chamber that contains the eluent chloroform: acetone (1 : 1). Spotted observed under UV nm. Then sprayed with a specific reagent. Reagent commonly used for identification of flavonoids as reagents spray in the TLC is AlCl₃ and citroborat which will give a yellow color.

**Determination of Total Flavonoids**

**Preparation of Rutin Solution**

In the study, total flavonoid content was determined using a modified method based on the procedure of Chang et al. Weighed 10 mg of rutin and dissolved in 10 mL of methanol p.a (1000 ppm). Taken 5 mL stock solution, then added volume to 50 mL with methanol PA (100 ppm). To the stock solution of 100 ppm rutin standard then created a series of concentration is 20 ppm, 30 ppm, 40 ppm, 50 ppm and 60 ppm. The each standard solution (1 mL) was added with 0.2 mL AlCl₃ 10%, 0.2 mL potassium acetate 1M, 3.0 mL methanol p.a and 5.6 mL aqua bidestillata, and then incubated for 30 minutes. Furthermore, absorbance was measured at maximum wavelength 415 nm.

**Preparation of Sample Solution**

Weighed 10 mg of rutin and dissolved in 10 mL of methanol p.a (1000 ppm). The each rutin solution (1 mL) was added with 0.2 mL AlCl₃ 10%, 0.2 mL potassium acetate 1M, 3.0 mL methanol p.a and 5.6 mL aqua bidestillata, and then incubated for 30 minutes. Furthermore, absorbance was measured in 415 nm wavelength. The sample solution made in three replications.

**RESULT AND DISCUSSION**

The sample used in this study is the buni fruit (A. bunius L.). Where the berry fruits containing anthocyanin because of his red to purple (violet) colour. According to research Samappito and Butkhup revealed that the fruit of Mao Luang or buni fruits (A. bunius L.) is a kind of medicinal plants where many villagers in Northeast Thailand use juices of ripe fruits to heal their health problems on diabetes, dysentery, indigestion and constipation and contained some enormous amount of flavonoids chemical compounds, ie, catechin, procyandin B1 and procyandin B2 and also two groups of organic acids, i.e major and minor group where these chemical compounds possess its important role as protective agents against fungus and uv irradiation. Buni fruits are rich in nutritional components such as carbohydrates, sugars, organic acids, proteins, minerals, vitamins. In previous research has shown that methanolic extract buni fruit (A. bunius L.) that grows in India show high antioxidant activity, with an average IC₅₀ value of 100.08 mg/mL, when compared with the other fruits.

The extraction method used in this study is maceration gradual method. Maceration is the process of extracting using solvent with several mixing at room temperatures.

The solvent used is n-hexane, ethyl acetate and ethanol. These solvents in accordance with the level of polarity, started in nonpolar, semi polar and polar. This is intent on to maximize extraction of flavonoid compounds extract of buni fruits (A. bunius L.).

After obtained the yield results percntage, furthermore to identify the class of chemical compounds. The purpose is to identify groups of chemical compounds to determine the flavonoid compounds contained in n-hexane, ethyl acetate and ethanol extracts of buni fruits. Identification of chemical compounds using the TLC method with eluent Chloroform: Acetone (1: 1). Then sprayed with reagent citroborat and AlCl₃.

**Description:**

Stationary phase = Silica gel F (7 × 1 cm)

Mobile phase = Chloroform: Acetone (1 : 1)

- **UV** detection and spray reagent AlCl₃
- **UV** detection and spray reagent citroborat
  1. n-Hexane Extract
  2. Ethyl Acetate Extract
  3. Ethanol Extract

From the results of phytochemical screening on extract of buni fruit showed that each extract of n-hexane, ethyl acetate and ethanol contain secondary metabolites such as flavonoids.

Flavonoids can form a bond in the position of the other with a mixture of boric acid and citric acid on heating, and is known by citroborat reagent. Color/fluorescence that is formed is yellow-green yellow fluorescence under light UV nm. This is a result of the reaction between citroborat with flavonoid group to form a complex between the hydroxyl

<table>
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<th>Table 1: The results of calculations percent yield of n-hexane, ethyl acetate and ethanol extract of buni fruit (A. bunius L.)</th>
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<tbody>
<tr>
<td><strong>Solvent</strong></td>
</tr>
<tr>
<td>n-Hexane</td>
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<td>Ethyl acetate</td>
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<td>Ethanol</td>
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<th>Table 2: Total flavonoid content measurement buni fruit extract (A. bunius L. Spreng)</th>
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<tr>
<td><strong>Sample</strong></td>
</tr>
<tr>
<td>n-Hexan</td>
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<tr>
<td></td>
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<tr>
<td>Ethyl Acetate</td>
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<td></td>
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<td>Ethanol Extract</td>
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Based on the results of the calculation of total flavonoid content of each buni fruit extract that has been carried out, showing that the total flavonoid content of n-hexane extracts by 10.72%, the ethyl acetate extract of 7.9% and the ethanol extract of 3.56%.
and ketone neighboring acid resistant or with groups that are not acid resistant orthohydroxyl.\textsuperscript{11,12} Flavonoids will indicate extinction spots on UV\textsubscript{224} nm, whereas at UV\textsubscript{405} nm spots will fluoresce dark yellow, green or blue.\textsuperscript{4} A portion of phenylalanine ammonia lyase, chalcone synthase, glucosyl transferase, and all of the trans-cinnamate 4-monooxygenase and NADH Cytochrome c reductase (the last an endoplasmic reticulum marker) After being sprayed with AlCl\textsubscript{3}, flavonoid compounds will give yellow.\textsuperscript{4}

The quantitative analysis of flavonoids can use UV-Vis spectrophotometer. Ultraviolet absorption spectra and absorption appear to be a single most useful way to identify the structure of flavonoids.\textsuperscript{12} Flavonoids contain aromatic conjugated system that can show strong absorption band in the UV-Vis.\textsuperscript{8}

Analysis of total flavonoid content using the colorimetric method of Chang et al.\textsuperscript{7} measured the UV-Vis spectrophotometry. Stage of making a standard solution, namely by using a standard solution rutin. Standard solution rutin used because the flavonoids found in plants, most commonly in the form of quercetin glycosides such as 3-rutinosida or rutin compound.\textsuperscript{10}

Quantitative analysis begins with created the series of concentration rutin solutions with modified method based on the procedure of Chang et al.\textsuperscript{7} i.e 20 ppm, 30 ppm, 40 ppm, 50 ppm and 60 ppm. Each standard solution (1 mL) was added with 0.2 mL AlCl\textsubscript{3}, 10\%, which serves to give effect bathochromic by a shift towards a wavelength longer, thus altering the wavelength of a rutin standard to get into range \(\lambda\) ultraviolet and \(\lambda\) visible, and there is also the effect of hyperchromic or increase the intensity of a standard solution regularly produce color thick yellow, so the color reaction which formed can be observed and measured on a UV-Visible spectrophotometer. Then added 0.2 mL potassium acetate 1M, which serves as a stabilizer, so that bathochromic effects that occur can be maintained, added 3.0 mL methanol p.a which serves as a solvent and 5.6 mL aquabidestilata, and then incubated for 30 minutes. It is intended that the reaction between the rutin standard with reagents used can take place perfectly.

In the sample absorbance measurements, weighed 10 mg of rutin standard and dissolved in 10 mL of methanol p.a (1000 ppm). Each standard solution (1 mL) was added with 0.2 mL AlCl\textsubscript{3}, 10\%, 0.2 mL potassium acetate 1M, 3.0 mL methanol p.a and 5.6 mL aqua bidestilata, and then incubated for 30 minutes. Absorbance measurement rutin solution running begins with a wavelength suitable for rutin solution. The running showed that the maximum wave running rutin standards turned away at \(\lambda\) 418 nm. The results of the rutin solution absorbance measurement with several concentrations showed that a linear relationship between the absorbance at a concentration that is equal to 0.9747. The magnitude of this linearity approaching a value of one, so that it can be said that the absorbance is directly proportional to the concentration and follow the linear regression equation is as follows: \(Y = bx + a\). From the calculations, the value of the intercept of 0.0028 and a slope value of 0.0238, so the equation of the standard curve is \(y = 0.0028x + 0.0238\). This equation is used as a comparison in the quantitative analysis on the measurement of the content of rutin flavonoid compounds to \(n\)-hexane, ethyl acetate and ethanol extract of buni fruit.

**CONCLUSION**

Based on research that has been conducted, buni fruit (\textit{A. bunius} (L.) Spreng) contains flavonoids with the percentage of \(n\)-hexane extract concentration by 10.72\%, the ethyl acetate extract of 7.9\% and the ethanol extract of 3.56\% counted towards or as rutin.

**CONFLICT OF INTEREST**

There is no conflict of interest.

**ABBREVIATION USED**

TLC: Thin Layer Chromatography; UV: Ultraviolet; Vis: Visible; AlCl\textsubscript{3}: Aluminium Chloride.

**REFERENCES**

Hamidu et al.: Antidesma bunius (L.) Spreng


- This paper reported content of flavonoid buni fruit (Antidesma bunius L. Spreng)
- Total flavonoid content were determined by reference to the chang method using a UV-VIS spectrophotometer

ABOUT AUTHORS

La Hamidu: Assistant of Laboratory of Natural Products, Faculty of Pharmacy, Indonesia Muslim University, Makasar, Indonesia.

Akstar Roskiana Ahmad: Lecturer of Laboratory of Natural Products, Faculty of Pharmacy, Indonesia Muslim University, Makasar, Indonesia.

Ahmad Najib: Lecturer of Laboratory of Natural Products, Faculty of Pharmacy, Indonesia Muslim University, Makasar, Indonesia.

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