Effect of Extraction Time on *Averrhoa bilimbi* Leaf Ethanolic Extracts Using Soxhlet Apparatus

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Abstract. *Averrhoa bilimbi* leaf extract is used as herbal medicine for various disorders treatment by communities. The causes of coronary disease such as cholesterols and triglycerides are widely established by *Averrhoa bilimbi* leaf extracts due to the presence of bioactive compounds such as glycoside, tannins, flavonoids and phenolic compounds. This study extracted *Averrhoa bilimbi* leaf using Soxhlet apparatus and 500 mL ethanol 96 % as a solvent extraction. Ethanol was used as solvent due to the solubility of bioactive compounds, non-toxic and biodegradable. The leaves were collected, chopped and dried at 60 °C until reached a constant weight. The moisture content of leaf was obtained 48.67 % at 24 hours of drying. The objective was to examined extraction time effect on the yield of leaf ethanol extracts. Extraction was carried out at constant temperature 70 °C and the time was varied at 60, 120, 240, 360 and 480 minutes. The highest yield and total flavanoid obtained for 13.77 % 13132.52 mg/L at 240 minutes. The yield of extraction was reduced at 360 minutes due to the optimum extraction time had been reached. In spite of that, this work indicate that the longer of extraction time, the more bioactive compound was extracted.

1. Introduction

Plants are the source of herbal medicine that has been used by civilization since a long time ago, before the discovery of chemicals drugs. The example of using plants as medicine is the treatment of *Diabetes mellitus* disease. *Diabetes mellitus* is a metabolic disease characterized by hyperglycemia and it is caused by insulin secretion damage, insulin action, or both. Chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and the failure of different organs, especially the eyes, kidneys, nerves, heart and blood vessels [1]. Prior to the discovery of insulin, various herbs were used as a cure for diabetes. After the introduction of insulin as a diabetes drug, the used of herbal medicine is increasingly abandoned or even rejected by some communities, but others still continue to utilize herbal plants as a conventional treatment solution [2].

The Indonesian people used herbs as a medicine since a long time ago even though it was not based on the knowledge about the content of any compounds in plant [3], for example is the use of *Averrhoa bilimbi* fruit and leaves to overcome various dangerous diseases such as *Diabetes mellitus*, hypertension, inflammation and bacterial infections [4]. The result of phytochemical analysis show that *Averrhoa bilimbi* fruit extracts contain phenol, flavonoid and tannin which are useful as antioxidants. Fruit extract containing total phenolic compound around 50.23-68.67 mg GAE/g extract was obtained by fifteenth days of maceration using methanol as solvent [5]. The previous research by Pushparaj et al. [6] show...
that *Averrhoa bilimbi* leaves extract successfully reduced 50 % of blood glucose levels in diabetic rats and 130 % of triglyceride levels in rats bloods. Many researchers developed the utilization of *Averrhoa bilimbi* leaf extract in Indonesia. Most of them studied about the benefits of extracts for human health e.g. measuring of *Averrhoa bilimbi* extract in vitro effectivity to against *Escherichia coli* and *Staphylococcus aureus* growth [7], evaluating of *Averrhoa bilimbi* leaves and *Catharanthus roseus* combination as hypoglycemic agent [8], evaluating of *Averrhoa bilimbi* extract to mice physical ability against cigarette smoke-induced [9] and measuring of *Averrhoa bilimbi* leaf extract antibacterial activity [10]. Ethanol and water were used as solvent in their extraction process to attracted bioactive compound. However, water is only effective to extract polar and hydrophilic bioactive compounds but less effective to extract non-polar and hydrophobic ones [11].

Nair et al. [12] studied about the anticancer activity of *Averrhoa bilimbi* leaf and fruit extract. Extraction was processed in soxhlet apparatus using several solvents i.e. petroleum ether, chloroform, ethyl acetate, ethanol 80 % and water. The result show that ethanol 80 % was the optimum solvent by producing only 47 ± 1.2 μg/g plant of total flavonoids. *Averrhoa bilimbi* leaves extract contain flavonoids, phenolic compound and glycosides which are not only reduced blood glucose levels, but also reduced the levels of triglyceride. Triglyceride is a substance which cause coronary heart disease in human body. Therefore, ethanol 96 % was used as extraction solvent in this work due to the result of previous research which reported that ethanol is best solvent for flavonoid isolation. This study aims to determine the effect of extraction time on the yield of *Averrhoa bilimbi* extract and the total content of flavonoids extract.

2. Methods

2.1 Materials

*Averrhoa bilimbi* leaf was collected from Penajam, East Borneo, Indonesia. Ethanol 96 % was purchased from One Med, AlCl₃ and Quarcetin standard were purchased from Sigma Aldrich. All other chemicals used were obtained from commercial sources.

2.2 *Averrhoa bilimbi* leaf extraction

*Averrhoa bilimbi* leaf was extracted using soxhlet apparatus and 500 mL of ethanol 96 % as solvent. Before the extraction process, the leaf was dried at 60 °C. Dry leaf sample was chopped and crushed until the coarsely powdered formed. 25 gram of *Averrhoa bilimbi* leaves powder were placed in filter paper and inserted into soxhlet apparatus. The operating temperature at 70 °C under atmospheric pressure and the extraction time were varied at 60, 120, 240, 360 and 480 minutes. The extract was purified using distillation apparatus at 80 °C for removing ethanol content. Then, the extract was analyzed by spectrophotometer to measure the total flavonoid content. The moisture content and extraction yield also calculated by using equation that can be seen below:

\[
\text{Moisture content (\%)} = \frac{\text{Mass of fresh leaves} - \text{Mass of dry leaves}}{\text{Mass of fresh leaves}} \times 100 \% \quad (1)
\]

\[
\text{Extract yield (\%)} = \frac{\text{mass of extract (g)}}{\text{mass of dry leaves sample (g)}} \times 100 \% \quad (2)
\]

2.3 Total flavanoid analysis

Total flavonoids content in this work was determined using UV-Vis Spectrophotometer V-550. The calibration curve was obtained by using AlCl₃ reagents with quarcetin standard 95 % (Sigma Aldrich). A straight line passing through the origin was obtained at a wavelength of 416 nm (R² = 0.9666). The *Averrhoa bilimbi* extract (0.1 gram) was dissolved in 1 mL of distilled watert to make a solution. Then, 0.5 mL of extract solution was dissolved into the mixture of 1.5 mL methanol, 0.1 mL AlCl₃, and water.
(10 %) solution, 0.1 mL CH$_3$COONa 1 M, and 2.8 mL distilled water. The mixture of solution was placed at room temperature (± 30 °C) for 30 minutes. After 30 minutes, the solution was dilute in distilled water. Sample was placed in 1 cm cuvette and the spectrophotometer was operated at 1 nm bandwidth and 1 nm data pitch. The blank solution consist of 1.5 mL methanol, 0.1 mL AlCl$_3$, 0.1 mL CH$_3$COONa, and 2.8 mL distilled water. The calibration curve of quarcetin standard shown in Figure 1.

![Calibration curve of quarcetin standard](image)

**Figure 1.** Calibration curve of quarcetin standard

### 3. Results and Discussion

#### 3.1 Extraction time effect on the yield of Averrhoa bilimbi extract

*Averrhoa bilimbi* leaf contain flavonoid compounds which is useful as natural antioxidants. Moisture content of *Averrhoa bilimbi* leaf was obtained for 48.66 %. This work used ethanol as a solvent to isolate flavonoids from the leaf. Flavonoid compound was well dissolved in ethanol due to its polarity. Ethanol is composed of a polar -OH group and a non-polar CH$_2$-CH$_3$ group, the non-polar property made flavonoid is optimally extracted by ethanol from *Averrhoa bilimbi* leaf. In spite of solvent selection, extraction time also influence the result of extraction process. The longer of extraction time will provide the longer of contacting time between solvent and the substance to be dissolved. The results of this study indicated that the extraction time affects the yield of the extract. **Figure 2** is show the relationship between extraction time and extract yield at the end of extraction process.

![Extraction time effect on the yield of extraction](image)

**Figure 2.** Extraction time effect on the yield of extraction
Figure 2 shows that the longer of extraction time resulted the higher yield of Averrhoa bilimbi extract. The highest extract yield was 13.77 % at 240 minutes of extraction time. However, the result of extract yield at 360 and 480 minutes of extraction time was decreased, it was due to the natural antioxidants may be substantially loss during the longer of heating in soxhlet extraction process. The fact that most of the bioactive compounds are relatively unstable when exposed to heat. The previous research by Rawson et al. [13] found that most of the bioactive compound in plants for example flavonoid, phenolic, antioxidant, carotenoid and vitamin C will change their chemical and physical structure also loss the bioavailability due to the thermal degradation process when exposed to high temperature. Another research by Arancibia-Avalia et al. [14] reported that heating process caused the dropped in flavonoids content.

3.2 Extraction time effect on the total flavonoids content
Flavonoids can be found as a group of polyphenol compounds. In general, flavonoids are divided into 4 groups namely flavones, flavonols, flavanones and flavanons. Averrhoa bilimbi based on previous studies contain flavone and flavonol [15]. Quercetin and kaempferol are included in the flavonol class, hence the flavonoid analysis using the quarcetin standard. It is well dissolved in an ethanol solvent due to these class of flavonoids belong to a less polar species.

Based on Figure 3, it was found that total flavonoid content at 60, 120, 240, 360 and 480 minutes are 8419.89, 12064.32, 13132.52, 12629.84, 927.3 mg/L, respectively. The highest total flavonoids concentration was obtained at 240 minutes for 13132.52 mg/L. Overall, the total flavonoid content increased due to the longer of extraction time, but when extraction time running at 360 and 480 minutes the total flavonoid was decreased. This is in accordance with the results obtained on the effect of extraction time on the yield of Averrhoa bilimbi extract.

4. Conclusion
The highest yield of extraction and total flavonoid content were obtained at 240 minutes extraction time using ethanol 96 % as a solvent. The result of this research represent the effect of extraction time on total flavonoid and the yield of extraction. Despite of that, this work also shows that Averrhoa bilimbi leaves contain flavonoid as bioactive compound that can be used as raw material for medicine production.
Acknowledgements

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