Microwave-assisted extraction (MAE) of bioactive saponin from mahogany seed (*Swietenia mahogany* Jacq)

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Abstract. Mahogany is frequently used for medicines for cancer, tumor, and diabetes, as it contains saponin and flavonoid. Saponin is a complex glycosydic compound consisted of triterpenoids or steroids. Saponin can be extracted from a plant by using a solvent extraction. Microwave Assisted Extraction (MAE) is a non-conventional extraction method that use microwave waves in the process. This research was conducted by a Complete Random Design with two factors which were extraction time (120, 150, and 180 seconds) and solvent ratio (10:1, 15:1, and 20:1 v/w). The best treatment of MAE were the solvent ratio 15:1 (v/w) for 180 seconds. The best treatment resulting crude saponin extract yield of 41.46%, containing 11.53% total saponins, and 49.17% of antioxidant activity. Meanwhile, the treatment of maceration method were the solvent ratio 20:1 (v/w) for 48 hours resulting 39.86% yield of saponin crude extract, 9.26% total saponins and 56.23% of antioxidant activity. The results showed MAE was more efficient (less time of extraction and solvent amount) than maceration method.

1. Introduction

Mahogany (*Swietenia mahogany* Jacq) is one of the widely grown plants in Indonesia and is widely used as medicine for several diseases. Habsah et al. [1] reported that mahogany seeds have anti-inflammatory, anti-cancer, anti-viral, anti-infective, anti-tumor and anti-diabetic effects. Mahogany seeds contain saponins and flavonoids, therefore often used to cure various diseases [2]. Saponin is a complex glycoside compound formed from triterpenoids or alkaloid steroids, non-volatile and amphiphilic [3]. Steroid saponin are common type of saponin playing a role as health-promoting properties.

Saponin can be separated from other components in a material by extraction using a solvent. Commonly, saponin is extracted by using a solvent like water, ethanol, and methanol. Conventional methods such as reflux and maceration are common method to extract saponin. The conventional methods have negative effect on saponin extract as time consuming tended to extract degradation. Recently, non-conventional methods are used to overcome the negative effect of conventional method, such as Microwave-assisted extraction [4].

Microwaves are non-ionizing electromagnetic waves with a frequency range from 0.3 to 300 GHz [4]. The use of MAE to extract saponin in green tea showed that the yield was increased by increasing the process length [5], while the yield of the extract was determined by the ratio of the material and the solvent [5]. Using low ratio of solvent will make the solvent quickly saturated by the extracted solid
Therefore, this research uses the Microwave-Assisted Extraction to extract of saponin of mahogany seeds.

2. Materials and Method
2.1. Materials
Mahogany seeds are obtained from mahogany plantations in Tuban area of East Java. The chemicals were vanillin (Sigma-Aldrich), 70-72% perchloric acid (Sigmaco), oleanolic acid (Sigma-Aldrich), 0.02 mM DPPH reagent having grade pa except 96% ethanol (Smart-Lab Factory) concentrated sulfate (Merck), petroleum ether (Smart-Lab Factory) and acetic acid (Smart-Lab Factory).

2.2 Apparatus
The equipments were cabinet dryer (OVG-12), blender (Miyako), 40 mesh sieve (ATE-126), analytical balance (Denver M-310), plastic basin, ultrasonic bath (Elmasonic S40) 45 kHz, rotary vacuum evaporator (Buchi B-490), spectrophotometer (Labomed Inc.), Oven (Memmert), UV-Vis spectrophotometer (Jenway 6305), and FTIR spectroscopy F108 wavelength 500-4000 cm⁻¹ (Shimadzu).

2.3 Extraction by MAE [7]
Defatted mahogany seeds extracted with ethanol solvent (boiling point 78.37 °C) using microwave with predetermined variables. The variables were solvent ratio of 10:1 (v/b), 15:1 (v/b), and 20:1 (v/b) and time of 120s, 150s and 180s. The extracts were filtered and concentrated using rotary vacuum evaporator at 50 rpm and 50°C for 15 min.

2.4 Extraction by Maceration [8]
The maseration method was used as a comparison. The extraction of mahogany seed by maceration method was done by using solvent ratio of 10:1 (v/b), 15:1 (v/b), and 20:1 (v/b) with 48 hours extraction time. The extracts were filtered and concentrated using rotary vacuum evaporator at 50 rpm and 50°C for 15 min.

2.5 Chemical Analysis
Saponin extract from mahogany seeds was analyzed for saponin compound [9], type of saponin using Liebermann-Burchard method [10], saponin content [11], and antioxidant activity [12]. The extraction process yield was determined by [13]. The best treatment was analyzed by its functional group with FTIR (Fourier Transmitter Infra-Red).

3. Results and Discussion
3.1 Qualitative Properties of Mahogany Ethanol Extracts
3.1.1 Identification of Saponin Compound
The saponin compound can be seen from its ability to form foam when shaking. Both extract obtained by MAE and maceration can form a stable foam 1-2 cm for 30 minutes.

3.1.2 Determination of Saponin Types
The identification of the type of saponin whether terpenoid or steroidal agitote in saponin compounds can be done by Liebermann-Burchard method [14]. In this study, brown color was formed on Liebermann-Burchard test, which indicated the triterpenoid type saponins in mahogany seed extracts. The color changes occurred due to the oxidation of terpenoid compounds through the formation of the conjugated double bond.
3.1.3 Identification of Functional Groups of Mahogany Ethanol Extract with FTIR (Fourier Transmitter Infra-Red)

[15] reported that the C = H (Alkene) group that read at the peak of 1340-1470 (cm\(^{-1}\)), the C = O group was read at the peak of 1690-1760 (cm\(^{-1}\)), and the C = C group was read peak 2100-2260 (cm\(^{-1}\)), indicates the presence of a trisaccharide (sugar group) in the triterpene. OH group was read at the peak of 3200-3600 (cm\(^{-1}\)), C = H group was read at the peak of 675-995 (cm\(^{-1}\)), C = C group was read at peak 2100-2260 (cm\(^{-1}\)), cluster C = O which is read at the peak of 1690-1760 (cm\(^{-1}\)), and the CO group read at the peak of 1050-1300 (cm\(^{-1}\)), indicating the presence of triterpenoidoleanane which is read on mahogany seed extract.

The FTIR (Figure 1) showed O-C = O group indicating a carboxylic acid (oleanolic acid) group or an ester group [16]. Crude extract contains many impurities of protein (amine/amide) was read at the point of 1180-1360 cm\(^{-1}\) and other alkaloids indicated by the number of peaks was read on functional group reading with FTIR, but the saponins compound is still read according to the standard of quillaja saponin.

![Figure 1. FTIR result of saponin extract from defatted mahogany seed with MAE](image)

3.2 Quantitative Properties of Ethanol Extract of Mahogany Seed

3.2.1 Yield of extract

The yield of ethanol extract of mahogany seeds was 30.83% - 41.46%. Extending the extraction time resulted in greater yield of the extract, but the solvent ratio had a limit so that the increase in the solvent ratio leads to a decrease in the yield. The addition of solvent to MAE can lower the yield of the extracted compound [17]. This was due to a decrease in microwave radiation energy. The energy was absorbed by the solvent and decreased the energy received by the sample. Its energy capability to dissolve and pull the solute out of the sample was also smaller so that the solvent ratio needs to be limited.

In contrast to the yield obtained from the MAE method, the highest yield on the extraction by maceration was obtained on the use of solvent ratio of 20: 1 for 48 hours. The yield tended to increase with the addition of the ratio solvent: sample above 20: 1.
3.2.2 Saponin content

The saponin content of ethanolic extract from mahogany seeds was 8.69% - 11.53% (Figure 2). Based on analysis of variance (α=0.05), the highest saponin content was obtained at solvent ratio 15:1 for 180 seconds, while in the greater amount of solvent, the lower the saponin content. The addition of an excessive portion of the solvent may result in the reduction of the extracted compound [18]. More solvents will reduce the microwave energy received by the solute to be extracted so it will not dissolve. More time is needed for the extraction process when the likelihood of degradation of the compound being extracted is greater.

Based on Figure 2, the extraction with a longer time will result in the decrease of saponin content. Yulianti et al. [19] reported that at certain extraction times, saponins increased and then reached their peak point. After that stage, the addition of extraction time will not increase the saponin content produced.

Different results on saponin content were obtained by maceration for 48 hours. Based on analysis of variance (α=0.05), the highest saponin content yielded by the solvent:material ratio of 20:1 (v/b) resulting in 9.26% of saponin. There was a tendency that saponin levels can still increase when it used a larger portion of solvent. In this study, maceration was done with a mild shaking, different with MAE. Microwave exposure helps the process of cell tissue destruction, as well as rotation of dipolar that occurs in the system will accelerate the occurrence of collisions between molecules of solute and solvent causing solute expenditure occurs much faster [17].

3.2.3 Analysis of Antioxidant Activity

Antioxidant activity of ethanol extract of mahogany seed showed DPPH inhibition ability of 39.62% - 49.17% (Figure 3). The condition showed an indication that DPPH inhibitor compound was dominated by saponin compounds contained in the ethanol extract of mahogany seeds. The lower the saponin extract, the antioxidant ability to capture free radical DPPH was decreasing [6] (Figure 3). Based on analysis of variance (α=0.05), the highest antioxidant activity was obtained at solvent ratio 15:1 for 150 seconds. Kusumawati [20] reported that antioxidant activity can be affected by saponin and also phenolic compounds or other flavonoid compounds.
3.3 The Best Treatment and Comparative Effectiveness of MAE Extraction Process with Maseration

3.3.1 Best Treatment

The selection of the best treatment was done using Multiple Attribute. Assessment included yield of extract, saponin content and also antioxidant activity. The best treatment was obtained at solvent ratio of 15:1 (v/b) for 180 seconds. The best treatment parameter values of MAE can be seen on Table 1.

| Ratio of Solvent:Sample (v/b) | Extraction Time | Average of Yield Extract (%) | Average of Saponin Content (%) | Percentage of Inhibition (%) |
|-----------------------------|-----------------|------------------------------|-------------------------------|-----------------------------|
| 15:1                        | 180 s           | 41.46                        | 11.3                          | 49.17                       |

3.3.2 The Effectiveness of Extraction

The total antioxidant capacity of ethanol extract of mahogany seeds can be expressed as the multiplication of the quantity value of the extracted compound with its antioxidant activity. In this study, the antioxidant capacity of the extract obtained was represented by the value of the effectiveness of the extraction process of antioxidant compounds from mahogany seeds with ethanol solvent.

| Methods              | Ratio of Solvent:Sample(v/b) | Time | Yield (%) (A) | Saponin Content (%) | Antioxidant activity (%) (B) | Process Effectiveness (A x B) |
|----------------------|------------------------------|------|---------------|---------------------|-------------------------------|-------------------------------|
| MAE                  | 1:15                         | 180 s| 41.46         | 11.53               | 49.17                         | 1940.07                       |
| Maceration           | 1:20                         | 48 h | 39.86         | 9.26                | 56.23                         | 2241.14                       |
| T Test               | -                            | -    | 0.024         | 0.004               | 0.0034                        | 0.018                         |

Extraction of mahogany seeds using microwave was more effective in terms of the length of extraction time and the amount of solvent needed compared to maceration. Zhou [4] reported that the need for fewer solvents was caused by some energy generated in extraction with microwaves such as electrical energy and magnetic energy that can produce thermal energy causing the process of dissolving the cell and diffusion of compounds into the solvent are better with fewer solvents.
4. Conclusion

The best treatment of microwave-assisted saponin extraction using ratio of solvent:sample was 15:1 (v/b) and extraction time of 180 seconds. The yield of extract was 41.46%, the inhibition percentage was 46.83%, the saponin content was 11.53% and the effectiveness of extraction was 1940 units. The extraction results were significantly different compared to the maceration extraction method for 48 hours with the solvent: sample ratio of 20:1 (v/b). Thus it can be said that the microwave-assisted extraction method for mahogany saponin with 96% ethanol solvent was more efficiently used than the maceration method.

Acknowledgments

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