

## Research Article

## Ultrasonic-Assisted Flavonoid Extraction from Ant Nest

*Ekstraksi Flavonoid Sarang Semut Berbantu Gelombang Ultrasonik*

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### Abstract

The extraction of active substances from ant nests can be affected by various extraction methods, whether conventional or sonication. Sample extraction was carried out by maceration and ultrasonic methods with the use of solvents to yield flavonoid compounds. The solvents used were aquadest, 70% ethanol and n-hexane, with a material-to-solvent ratio of 1:50 (w/v). The highest flavonoid content of 14% was obtained by ultrasonic-assisted extraction method, with aquadest as the solvent and at operating conditions of 24 minutes at a temperature of 40°C. Due to their high aquadest solubility and the presence of one or more sulphate ions that are bound to the hydroxyl phenol or sugar, the flavonoids produced are classified as flavonoid sulphate.

**Keywords:** ant nest; extraction; flavonoid; ultrasonic; maceration

### Abstrak

Berbagai macam metode ekstraksi baik konvensional maupun sonikasi dapat memberikan pengaruh yang berbeda pada ekstraksi zat aktif pada sarang semut. Penelitian ini mencoba berbagai macam metode ekstraksi dan penggunaan pelarut untuk mengekstraksi senyawa flavonoid sarang semut. Ekstraksi sampel dilakukan dengan metode maserasi dan ultrasonik. Adapun pelarut yang digunakan aquadest, etanol 70%, dan n-hexane. Rasio bahan dengan pelarut 1:50 (b/v). Kadar flavonoid tertinggi diperoleh dengan metode ekstraksi berbantu gelombang ultrasonik, yaitu 14% pada kondisi operasi 24 menit, suhu 40°C dengan pelarut air. Flavonoid yang dihasilkan termasuk pada golongan flavonoid sulfat. Senyawa flavonoid sulfat memiliki sifat mudah larut dalam air dan memuat satu ion sulfat atau lebih, yang terikat pada hidrosil fenol atau gula.

**Kata Kunci:** ekstraksi; flavonoid; maserasi; sarang semut; ultrasonik,

### 1. Introduction

Ant nest is a tuber plant native to Papua. It is a typical Indonesian plant with a high content of flavonoids and tannins [1]. Previous research on *Myrmecodia pendans* reported that ant nests have

antibacterial properties, and the extract is effective against gram-positive and gram-negative bacteria [2].

Ant nest Plants contain chemical compounds from the flavonoid and tannin groups known to cure various diseases.

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Flavonoids have antibacterial, antiviral and anticancer properties [3]. Previous research also reported that ant nests contain many antioxidants and immunostimulants that help increase immunity. Furthermore, immunostimulants protect and assist the body's cells to perform their functions properly [2].

The mechanism for the extraction of bioactive substances from plants involves organic solvents penetrating the plant cell walls and dissolving the bioactive substances. This results in a concentration difference between organic solvents outside the cell and bioactive substances in cells. Therefore, the solution with a higher concentration diffuses out of the cell, and the process continues until there is a balance between the concentration of the active substance inside and outside the cell. [4].

One of the extraction methods that can be used is sonication extraction (ultrasonic), which involves the extraction of bioactive substances into the solvent using ultrasonic waves [5]. In the ultrasonic reactor (sonicator), ultrasonic waves are used to form cavitation bubbles in the solution. The cavitation bubbles that burst close to the cell wall produce shock waves and liquid jets, causing the cell walls of bioactive substances to rupture. This rupture causes the cell components to leak out and mix with the solution.

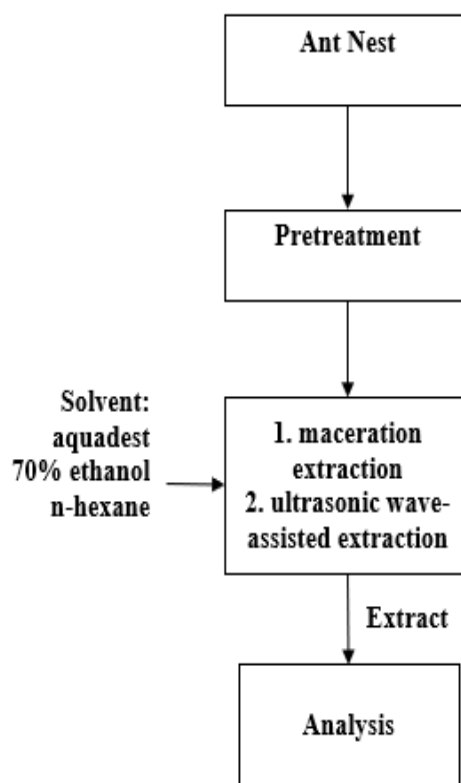
Various extraction methods, including conventional methods and sonication, can have different effects on the extraction of active substances in ant nests. Crisnaningtyas & Rachmadi reported that antibacterial substances extracted from ant nests by leaching method and ethanol solvent did not produce an inhibitory reaction, which could be attributed to the use of volatile ethanol [6]. This research

aimed to determine and compare the effect of ultrasonic-assisted extraction and conventional extraction (maceration). Furthermore, the purpose of this research was to determine the effect of the type of solvent on the extraction.

## 2. Research Methods

### 2.1 Tools and Materials

The materials used in this research include ant nest from Maibo Village, 70% ethanol, n-hexane, and aquadest. The tools used include a digital ultrasonic sonicator CD-2840A Krisbow, beaker, measuring flask, cooler, thermometer, analytical balance, and stirrer.



**Figure 1.** Process Flow Chart

### 2.2 Extraction

#### 2.2.1 Maceration Method

A total of 2 grams of ant nest powder was measured into a beaker and 100 ml of three different solvents (ethanol 70%, n-hexane, aquadest) were then added with a

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sample-solvent ratio of 1:50 (w/v), respectively. The mixture was then stirred and covered. Furthermore, the Immersion process lasted 3 hours at a temperature of 30, 40 and 50°C, respectively. After 3 hours, the filtrate and residue were filtered.

**2.2.2 Ultrasonic Method**

A total of 2 grams of ant nest powder was measured into a beaker and 100 mL of three different solvents (ethanol 70%, n-hexane, aquadest) with a sample-solvent ratio of 1:50 (w/v) were added, respectively. The sample was then inserted into the Krisbow digital ultrasonic sonicator CD-2840A at 50 Hz. The operation was carried out at temperatures of 30, 40 and 50°C with a time of 16, 24, and 32 minutes, respectively.

**3. Results and Discussion****3.1 Chemical Compound Content**

Table 1 shows the phytochemical test results for flavonoid content using ultrasonic-assisted extraction at a temperature of 40°C for 24 minutes. The phytochemical test result using three different solvents shows that the ant nest using aquadest and ethanol as solvents contains flavonoid compounds and tannins. This is consistent with the results by Soeksmanto *et al.* [7] which showed that the phytochemical test results of the water extract of the ant nest *Myrmecodia pendens* type contained flavonoids and tannins, while the aqueous extract of the *Myrmecodia tuberosa* type contained flavonoids and anthocyanidins [3].

**Table 1.** Phytochemical test results

| Group     | Aquadest | Ethanol | n-hexane |
|-----------|----------|---------|----------|
| Alkaloid  | -        | -       | -        |
| Flavonoid | +        | +       | -        |
| Tannin    | +        | +       | -        |

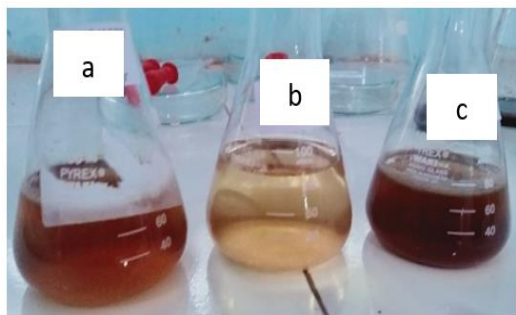
**3.2 Solvent Effect**

The effect of solvents on the yield of flavonoids is shown in Table 2 and Figure 2. Figure 2 is the result of the extraction of ant nests assisted by ultrasonic waves at a temperature of 40°C, for 24 minutes. Flavonoids are plant pigments with red, yellow, and orange-yellow colors. In Table 2 and Figure 2, it can be seen that the extraction result with water solvent shows red color. Based on the results obtained, high flavonoid content is found in ant nest extract with water as a solvent. Therefore, the flavonoid compounds produced are classified as flavonoid sulphate due to their solubility in water and the presence of one or more sulphate ions, which are bound to the hydroxyl phenol or sugar. Structurally, this compound is a bisulphate because it exists as a salt, namely flavone-O-SO<sub>3</sub>K. This bisulphate part is generally bound to the free phenolic hydroxyl or sugar [2] [8]. This compound has a limited distribution, such as in angiosperms that have an ecological relationship with aquatic habitats. Therefore, the ant nest contains sulphate flavonoids because it is an angiosperm.

**Table 2.** Effect of extraction solvent

| Method     | Solvent     | Color           |
|------------|-------------|-----------------|
| Maceration | aquadest    | dark red        |
|            | 70% ethanol | bright red      |
|            | n-hexane    | bright yellow   |
| Ultrasonic | aquadest    | dark red        |
|            | 70% ethanol | pretty dark red |
|            | n-hexane    | bright yellow   |

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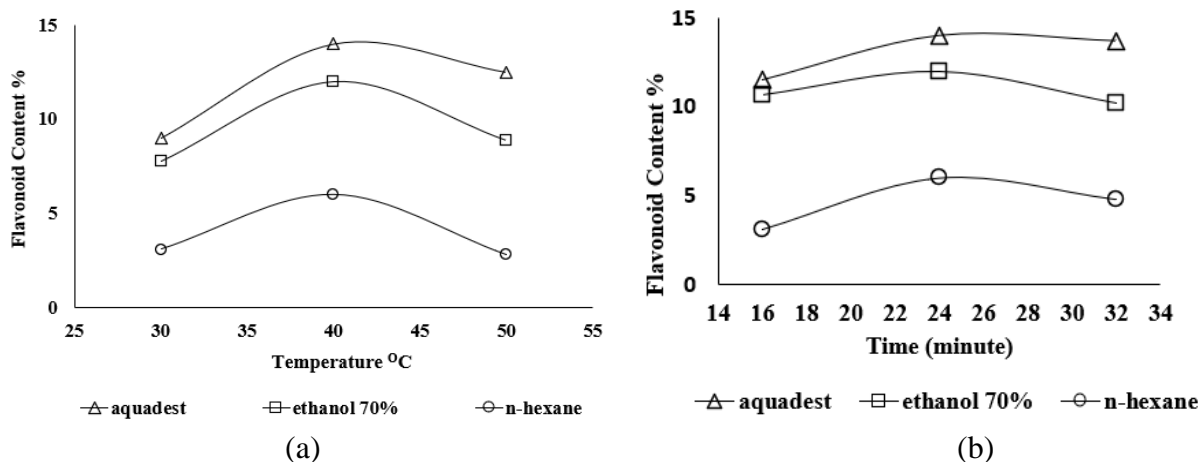


**Figure 2.** Results of ultrasonic-assisted extraction: a) 70% ethanol, b) n-hexane, c) aquadest

**3.3 Effect of Extraction Temperature and Time**

The effect of temperature and extraction time on flavonoid content is shown in Figure 3. Figure 3 shows that the highest flavonoid content of 14% was achieved in 24 minutes and at 40°C. These results are similar to those reported by Handayani *et al.* who stated that the best

extraction time for sour sop leaves using ultrasonic states is 20 minutes [9]. The higher the extraction temperature and time, the lower the flavonoid content produced. The increase in temperature and longer extraction time resulted in lower yields [4] [10]. According to Ibrahim *et al.*, an increase in extraction temperature should be considered because high temperatures, long extraction times, and exceeding the optimum limit can cause compound loss in solution due to oxidation [11]. Flavonoid compounds are not resistant to temperatures above 50°C, resulting in structural changes and low extract. Andriani *et al.* reported that too low temperature and short extraction time resulted in low flavonoid content [12]. This is due to the incomplete flavonoid compounds extracted from the material.



**Figure 3.** Graph of ultrasonic-assisted extraction on flavonoid content: (a) the effect of temperature, (b) the effect of extraction time

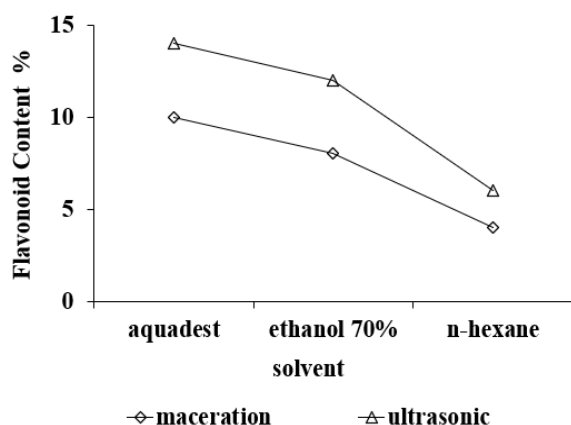
**3.4 Effect of Extraction Method**

Figure 4 shows the effect of the extraction method on the flavonoid content. A high yield of 14% flavonoid content was produced using the ultrasonic method with water as a solvent. The ultrasonic process was carried out in 24 minutes at 40°C. However, the maceration method with water as a solvent and at the

same temperature conditions of 40°C for 3 hours produced a 10% yield. Figure 4 shows that the ultrasonic process produces a high yield compared to the maceration process. This is because ultrasonic waves in water will cause the growth and destruction of microbubbles, resulting in high temperatures and pressures, which trigger the formation of free radicals

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through the thermal dissociation of water and oxygen [5] [13] [14].



**Figure 4.** Comparison of ultrasonic extraction method with maceration

The use of ultrasonic waves creates a cavitation effect that can break down the cell walls of the material, allowing bioactive compounds to be easily extracted with maximum results in a relatively short processing time [15][16]. The advantage of ultrasonic-assisted extraction is that it increases the extract yield compared to

conventional extraction methods such as maceration.

**4. Conclusion**

Based on this research, water as a solvent produces the highest flavonoid content. The flavonoids produced are classified as flavonoid sulphate due to their high solubility in water and the presence of one or more sulphate ions, which are bound to the hydroxyl phenol or sugar. Furthermore, the ultrasonic-assisted extraction method yielded the highest flavonoid content of 14% in 24 minutes and at 40°C temperature.

**5. Acknowledgments**

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