Phytochemical Screening of Bruguiera sp. Mangrove Propagule Extract from the Mangrove Area of Kutawaru Village, Cilacap

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Abstract
Indonesia is a country with the highest biodiversity, especially in mangrove ecosystems, with a total of around 89 species. The mangrove ecosystem includes Rhizophora (mangrove), Avicennia (api-api), Sonneratia (pedada), Bruguiera (tanjang), and Xylocarpus (nyirih). Mangrove propagules are the fruits of mangroves in the form of hypocotyls that function to store food when the propagules germinate and grow. Secondary metabolites from plants have bioactivity, so it is important to identify them using specific reagents known as phytochemical screening. The group of secondary metabolite compounds is classified based on their chemical structure, consisting of flavonoids, tannins, phenols, alkaloids, steroids, and triterpenoids. This study aims to determine the content of flavonoid, tannin, and phenol compounds in Bruguiera sp. propagules. The method for extracting tannins, phenols, and flavonoids from mangrove propagules can be performed using the Microwave Assisted Extraction (MAE) method. The raw material is 3 kg of Bruguiera sp. mangrove propagules from Kutawaru Village, which are then cut into small pieces and dried using an oven at 105°C for 3 hours, subsequently ground, and sieved with a 60 mesh size. The extraction process of the mangrove propagule powder was weighed at 15 grams using 250 ml of ethanol as the solvent. The results of the phytochemical test on the Bruguiera sp. mangrove propagule extract were positive for flavonoids, tannins, and phenols.

***Keywords*:** *Brugiera sp propagules, Extracts, Secondary Metabolite Compounds.*

Abstrak
Indonesia merupakan negara yang memiliki keanekaragaman hayati khususnya pada ekosistem mangrove paling tinggi di dunia dengan total jumlah sekitar 89 spesies. Ekosistem mangrove meliputi Bakau (Rhizophora), Api-api (Avicennia), Pedada (Sonneratia), Tanjang (Bruguiera) dan Nyirih (Xylocarpus). Propagul mangrove merupakan buah mangrove dalam bentuk hipokotil yang berfungsi menyimpan makanan saat propagul tersebut bertunas dan tumbuh. Senyawa metabolisme sekunder dari tanaman memiliki bioaktifitas,

sehingga penting untuk diidentifikasi dengan menggunakan reagen tertentu yang disebut skrinning fitokimia. Golongan senyawa metabolit sekunder diklasifikasikan berdasarkan struktur kimia terdiri dari flavonoid, tannin, fenol, alkaloid, steroid dan triterpenoid. Penelitian ini bertujuan untuk mengetahui kandungan senyawa flavonoid, tannin dan fenol dalam propagul Bruguiera sp.Metode pengambilan tannin, phenol dan flavonoid dalam propagul mangrove dapat dilakukan dengan menggunakan metode Microwave Assisted Extraction (MAE). Bahan baku 3 kg propagule mangrove Bruguiera sp dari Desa Kutawaru, lalu dipotong kecil dan dikeringkan menggunakan oven bersuhu 105^{o}C selama 3 jam, selanjutnya dihaluskan dan diayak dengan ukuran 60 mesh. Proses ekstraksi serbuk propagule mangrove ditimbang sebanyak 15gram menggunakan pelarut etanol 250 ml. Hasil uji fitokimia ekstrak propagule mangrove Bruguiera sp positif mengandung senyawa flavonoid, tannin, dan fenol.

Keywords**:** *Propagul Brugiera sp, Ekstrak, Senyawa Metabolit Sekunder*.

1. Introduction

The mangrove ecosystem in Indonesia has the highest biodiversity in the world, with a total of around 89 species, which includes 35 species of plants, 9 species of shrubs, 9 species of lianas, 29 species of epiphytes, and 2 species of parasites. Some common types found in Indonesia are Mangrove (*Rhizophora*), *Avicennia* (Api-api), *Sonneratia* (Pedada), *Bruguiera* (Tanjang), and *Xylocarpus* (Nyirih) (Paruntu et al., 2016). Mangrove propagules are elongated mangrove fruit (hypocotyl) that store food while the propagules germinate and sow (Tahzani, 2016). Mangroves possess bioactive compounds that exhibit antibacterial characteristics, which can serve as traditional remedies for conditions such as viruses, fungi, cancer, and tumors. Plants' secondary metabolic compounds demonstrate bioactivity, making it essential to identify them through specific reagents known as phytochemical screening. (Akasia et al., 2021).

Phytochemical test methods are useful for analyzing the content of chemical compounds contained in plant or animal samples, both as a whole and in their smallest parts. By conducting this test, useful information can be obtained to identify new compounds that have pharmacological effects so that they can encourage the discovery of new drugs that are antibacterial, antiviral, and various other types (Atomik, 2024). Based on research by Warsinah et al. (2005, in the journal Faoziyah et al.., 2017, mangrove plants contain various chemical compounds, including *phenolics, alkaloids, steroids, saponins, flavonoids,* and t*annins*. The use of this plant in traditional medicine has shown potential as an anti-cancer. For example, the leaves of the *Rhizopora mucronate* species, in in vitro tests, proved that the growth of cancer cells was inhibited by an LC50 result of 582.00 µg/mL (Faoziyah & Kurniawan, 2017). In addition, previous studies have shown that mangrove plants have been used as biopesticides derived from *Rhizophora apiculata* bark extract, which can eradicate the growth of *S.litura*

F.instar II. Meanwhile, *Avicennia sp*. mangrove leaf extract is useful for inhibiting the growth of *Aeromonas hydrophila* bacteria (Avesina et al., 2021).

Phytochemical compounds found in a plant can be obtained through the extraction process. Factors that affect the effectiveness of the extraction are based on the size of the material, extraction method, temperature, duration of time, use of solvents, and concentration. It is important to ensure that the polarity of the solvent used in the extraction process is the same or close to the polarity of the active ingredient to be extracted. This is important so that extraction can take place efficiently, considering the principle of "like dissolves like," which states that not all compounds can dissolve in one solvent liquid (Prayoga et al., 2019).

Phytochemistry studies the nature and interaction of secondary metabolite chemical compounds contained in plants. Secondary metabolites are very much needed by plants to defend themselves from other living things, inviting insects to help the pollination process. Chemical compounds are formed and broken down in living cells so that chemical changes occur called metabolism. All plant metabolic pathways have an important role for plants to be able to continue their lives, this is called primary metabolism. Meanwhile, secondary metabolism is in another metabolic pathway, although it is needed but is not considered important in plant growth(Julianto, 2019).

Secondary metabolite compounds are classified based on chemical structure and consist of flavonoids, tannins, phenols, alkaloids, steroids, and triterpenoids (Anggraeni Putri et al., 2023). The flavonoids and tannins contained in plants are a natural source of antioxidant protein. Flavonoids function as anti-inflammatory, and tannins, which are phenolic compounds, have natural anti-microbial properties (Rosulva et al., 2021).

The method for extracting tannins and flavonoids from mangrove propagules can be done using an extraction process. The extraction method is divided into two ways, namely the conventional extraction process, such as soxhletation, and the microwave-assisted extraction (MAE) method. The two methods have differences in that the soxhletation extraction process requires a long time, and the energy for increasing the temperature is also higher. Meanwhile, MAE is one of the best methods due to the relatively short extraction time, increased extraction yields, and less solvent required (Handayani et al., 2018). MAE, using microwaves, can facilitate the process of separating active compounds from plant samples into a solvent and has electric and magnetic fields that are perpendicular to each other. The resulting electric flow is in the form of dipolar rotational heat and ionic conduction, so the dielectric constant of the solvent increases to produce heat quickly (Julianto, 2019).

Ethanol is one of the solvents often used in the extraction process (Hakim & Saputri, 2020), because it has polar properties that function to penetrate cell walls so that it can carry out cell diffusion and bioactive compounds are attracted more quickly (Yulianti et al., 2021). Ethanol has volatile, flammable and colorless properties. Ethanol solvent is the best solvent when compared to water and methanol solvents, because ethanol has polar properties that can take chemical compounds in plants (Riwanti et al., 2018). Extraction of mangrove propagules (Rhizophora mucronata) with ethanol solvent produced an extract yield of 27.5% using the microwave assisted extraction (MAE) method (Handayani et al., 2018). The purpose of this

study was to determine the content of flavonoid, tannin and phenol compounds from the extract of bruguiera sp mangrove propagules with 95% ethanol p.a solvent.

2. Method

This research was carried out at the Process Engineering Laboratory for Environmental Pollution Control Engineering at the Cilacap State Polytechnic. This research activity will start from July to November 2024, consisting of the raw material preparation stages, the Bruguiera sp. mangrove propagule extraction process, and qualitative testing of flavonoids, tannins, and phenols in the mangrove propagule extract solution.

The tools used in this research are divided into two categories: for the raw material preparation process, a knife, blender, 60-mesh sieve, and oven. Meanwhile, for the extraction process, there are a microwave, a one-neck flask, a set of distillation equipment, an Erlenmeyer flask, a separating funnel, a stand, and clamps. The materials used are Bruguiera sp. mangrove propagules and 96% ethanol solvent. Flavonoid, tannin, and phenol components are the dependent factors; the solvent volume and powder size are the constant variables; and the mass of the Bruguiera sp. mangrove propagule powder are the independent variables.

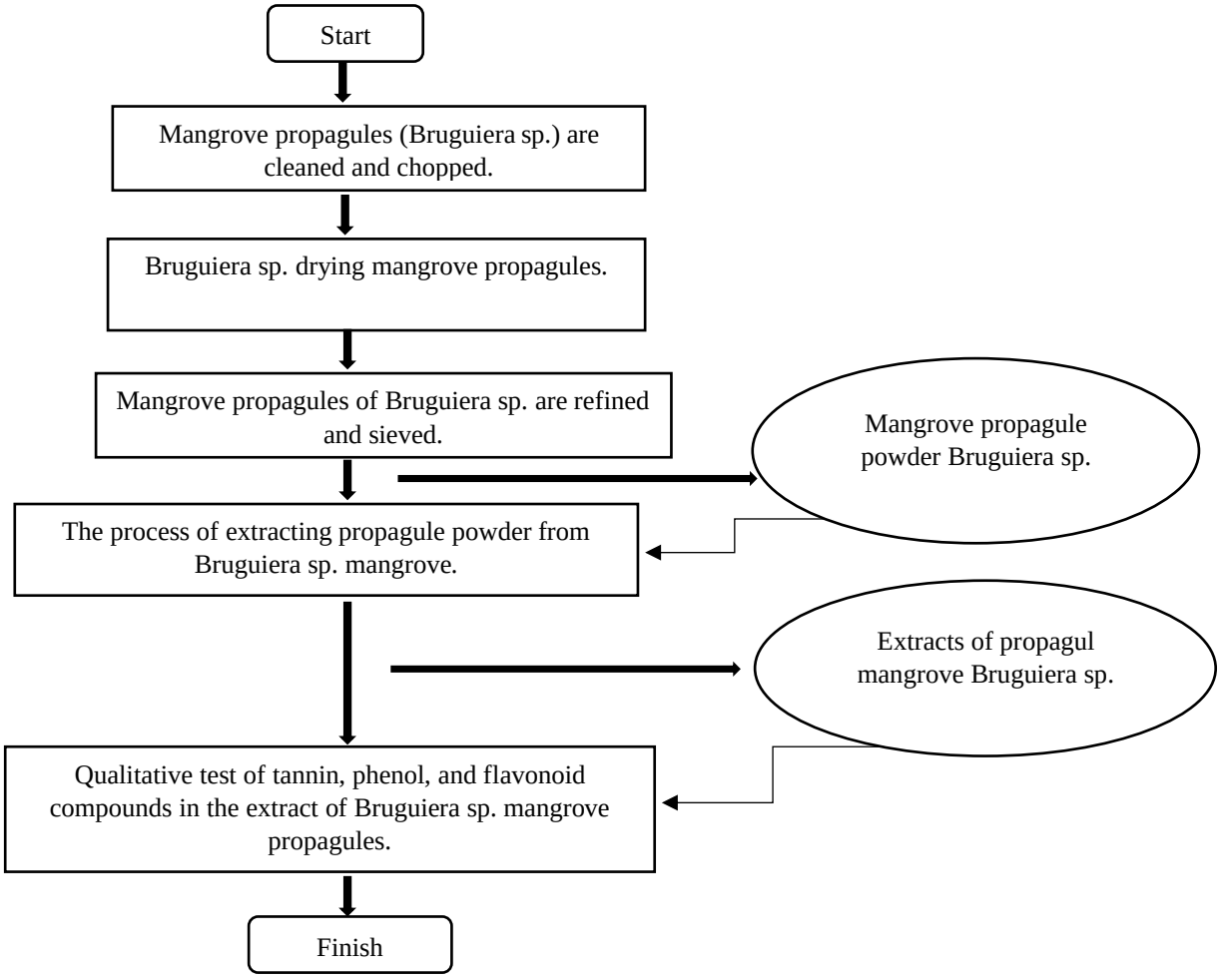


Figure 1. Flow Diagram Process Extraction Propagule Mangrove Bruguiera sp

2.1 Raw Material Preparation Stage

The mangrove propagules used in this research were Bruguiera sp. Take 3 kg of mangrove propagule raw materials from Kutawaru Village, then cut them into pieces and dry them using an oven at 105 ° C for 3 hours until dry. Then, the dried mangrove propagule pieces are subjected to a further grinding process, a sieving process with a 60-mesh sieve.

Source: Researcher, 2024

Figure 2. Bruguiera sp. Mangrove Propagule Powder 60 mesh

2.2 Extraction Process

10 grams and 15 grams of mangrove propagule powder was weighed, and the extraction process was carried out using 250 ml ethanol solvent. The extraction method used is microwave-assisted extraction (MAE). The microwave used has 800 watts of electricity for 3 minutes.

Source: Researcher, 2024

Figure 3. Extraction Process Using the Microwave Assisted Extraction (MAE) Method

2.3 Extraction Test

Test method for extraction of Bruguiera sp. mangrove propagule powder, using a

qualitative phytochemical screening test based on the occurrence of color changes in the extract solution. The following is a test method for flavonoids, tannins, and phenols (Akasia et al., 2021).

a. Flavonoid test

Flavonoid testing was carried out by taking 2 ml of mangrove propagule extract, then adding a few drops of concentrated HCl and weighing 1.5 grams of magnesium. A positive indicator from the flavonoid test is a brownish-red color change.

b. Tannin test

Tannin testing was carried out by taking 2 ml of mangrove propagule extract in $FeCl_3$ and then adding 2 to 3 drops of H_2SO_4. Then, observing the color change to brownish yellow, this shows that the mangrove propagule extract is positive for tannin.

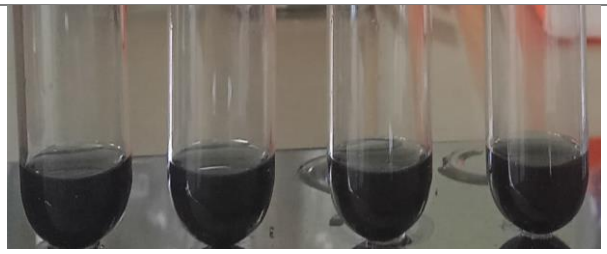
c. Phenolic test

Phenolic testing was carried out by taking 2 ml of mangrove propagule extract and then adding 1% $FeCl_3$. Then, observing the blue-black color change, this shows that the mangrove propagule extract is positive for phenol.

3. Result and Discussion

The results of qualitative phytochemical tests from Bruguiera sp. mangrove propagule extract can be seen in Table 1. A positive result indicates a color change in the mangrove propagule extract that corresponds to the hue of the tested chemical. Conversely, if negative, there is no alteration in color, or it retains the original hue of the extract.

Table 1. Result of Flavonoid, Tannin, and Phenol Content

Content	Mangrove Propagule Extract Bruguiera sp	Indicator
Phenol	Positive	 A blackish blue color is formed
Flavonoid	Positive	 A brownish-red color forms

Content	Mangrove Propagule Extract Bruguiera sp	Indicator
Tannin	Positive	 A brownish yellow color forms

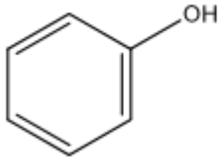
Source: Researcher, 2024

Based on Table 1, the results of the phytochemical test data show that the Bruguiera sp. mangrove propagule extract contains several secondary metabolite compounds, such as phenols, flavonoids, and tannins. The color shift in the extract of mangrove propagules indicates this. Nevertheless, the quantity of mangrove propagule powder utilized had no discernible effect on the color change of the extract. This shows that Bruguiera sp. mangrove propagule extract has potential as an antioxidant and antibacterial.

a) Phenol

Phenolic compounds are natural substances that are characterized by an aromatic ring containing one or more hydroxyl substituents. The polar nature of phenol means it is easily soluble in water (Pangisian et al., 2022). The total phenol in the extract depends on the polarity of the solvent used in the extraction process (Datu et al., 2023).

The content of hydroxyl groups (-OH) found in organic compounds, namely phenol, where the group is directly bound to the carbon atom in the benzene ring. The combination of sulfonic acid salts with sodium hydroxide forms sodium salts and phenol. The bond of phenol and sugar compounds is called glucosides found in plant cell vacuoles. (Antonius et al., 2019).



Source: Antonius et al., 2019

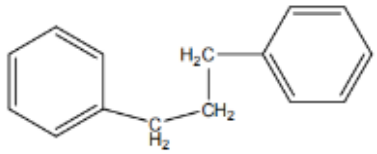
Figure 4. Structure of Phenol Compounds

The way phenol compounds work as antibacterials at low levels can damage the cytoplasmic membrane and cause cell nucleus leakage, while phenol compounds at high levels are coagulated with cellular proteins so that this activity is very effective when bacteria are in the process of dividing in the phospholipid layer surrounding the cell is very thin, as a result phenol compounds can easily damage the cell nucleus (Cahyadi et al., 2018).

b) Flavonoid

One of the secondary metabolite compounds is flavonoids which have a benzene structure substituted with an OH group and are included in the phenol compound group. In addition, flavonoid compounds are also chemical compounds derived from 2-phenyl-benzhyl-γ-pyrone

through the biosynthesis of the phenylpropanoid pathway. The basic structure of flavonoids consists of 15 carbon atoms and there are two benzene rings (C6) bound to a propane chain (C3) (Ningsih et al., 2023).

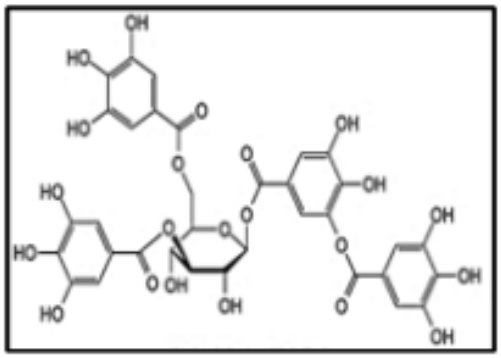


Source: Ningsih et al., 2023
Figure 5. Flavonoid Compound Structure

The role of flavonoids in plants is to provide color, flowers, fruit, aroma and taste to seeds or signaling molecules and act as detoxification agents to protect plants from threats from living and non-living creatures, as well as filtering UV rays (Khafid et al., 2023)(Nabillah & Chatri, 2024). Generally, flavonoids are found in fruit, roots, leaves, stem bark, and almost all plants (Sampepana et al., 2020). Isoflavones contained in flavonoids have a higher ability to fight gram-positive bacteria than gram-negative bacteria and have activity against pathogenic fungi (Luringunusa et al., 2023). The way flavonoid compounds work is as antibacterials that can damage the permeability of microbial cell walls and can bind to functional cell proteins and DNA, thereby inhibiting microbial growth (Cahyadi et al., 2018).

c) Tannin

One of the secondary metabolite compounds found in the plant synthesis process is tannin (Nabillah & Chatri, 2024). Apart from that, tannin is also included in the group of polyphenols, which are polar and have a hydroxyl group (-OH) (Pangisian et al., 2022). In terms of the physical properties of tannin, it is a colloid formed in water, has a distinctive odor and sour taste, is amorphous, and has no melting point. Meanwhile, the chemical properties of tannin are difficult to separate and crystallize, easily soluble in organic solvents, and can be hydrolyzed with acids, bases, and enzymes (Hersia et al., 2023).



Source: Hersia et al., 2023
Figure 6. Structure of Tannin Compounds

Substitutes for chemical pesticides are available in natural ingredients that are easily found in the environment, one of which is in the agricultural sector. Phytochemical compounds

found in plants, such as tannins, saponins, polyphenols, alkaloids, and eugenol, can be used to make vegetable insecticides (Sanjaya et al., 2021).

4. Conclusion

The Bruguiera sp mangrove propagule extract was subjected to a qualitative phytochemical screening test and resulted positive in containing tannins, flavonoids, and phenols.

Appreciation

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