Screening phytochemical compound of *Alstonia scholaris* R.Br in different sites in Indonesia

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Abstract. Indonesia’s tropical forest is widely used as a source of raw materials for traditional medicine. Pulai (*Alstonia scholaris* R.Br) is one of forest medicinal plants widely spread in the Indonesia forest to treat various diseases. Phytochemical compounds play an important role in the pharmacology effect depending on many factors such as age, geographical site, solvents, and extraction methods. This research aimed to investigate the phytochemical content of *A. scholaris*’ bark from a different region in Indonesia and to study the effect of the maceration and reflux extraction method on the yield of crude extract. *A. scholaris* barks were extracted by maceration and reflux method to get the yield. Then they were tested for phytochemical content for alkaloids, flavonoids, tannins, steroid saponins, and triterpenoids. The results showed that pulai bark extracted by maceration was bigger in yield than that of the reflux method, as stated follows: Palembang, Cifor, Banjar Baru, and Ngebel, which were 5.2%, 7.92%, 3.35%, 4.19, respectively. The phytochemical test showed that all samples of positive flavonoids and alkaloids. *A. scholaris*’ bark from Cifor-Bogor had the strongest flavonoids content of all, and it had potential activity as an antioxidant.

1. Introduction

Indonesian traditional medicine as a cultural heritage has been known to have activities against various diseases. Nowadays, many researchers have proved their scientific research based medicine for human health. Plants containing chemical compounds are often referred to as secondary metabolites, produced by plants to defend themselves from unfavorable environments, such as temperature, climate, and pest and disease disorders [1]. Indonesia is the third country with the greatest biodiversity, and there are about 7500 plant species types, but only 940 species are used as medicinal plants [2].

*Alstonia scholaris* R. Br (Pulai) is an *Apocynaceae* family, which consists of 200 genus and 2000 species, and spreads in the tropics and subtropics. *A. scholaris* R. Br is one of the most widely distributed species in Asia, including Indonesia. It grows in teak, mixed, and small forests in the countryside in the lowlands to 900 meters above sea level. It has a different name according to areas such as Pule (Java, Bali), Lame (Sunda), Kay Kuleh (Sumba), Kompanga (Sulawesi), Aliag (Papua) [3]. This plant has been widely cultivated and used for treating many kinds of diseases. Plant parts, which are frequently used, are leaves, bark, and flowers.

This species is used as medicine for fever, malaria, cough, hyperlipidemia, ulcers, diabetes, asthma, and rheumatism [4]. According to [5], pulai plant extracts can be used as antioxidants agents to...
minimize free radical reactivity in free radical reactions. According to [6], ethanol extract of pulai leaves can act as an antioxidant in vivo by preventing lipid peroxidation.

Secondary metabolites content in plants depends on many factors such as species, geographical location, temperature, climate, soil fertility, season, solvent, and extraction method. Plants in the same species have different phytochemical content, if they are grown in different regions [7-9] Compound composition features may differ in the same species depending on age, way cultivated, environment, time of harvest, and post-harvest processing or storage [10, 11]. This variation could cause inconsistencies in the safety, efficacy, and stability of herbal medicinal products.

A lot of researches on pulai leaves had been done. On the other hand, research on pulai bark is still limited. According to this situation, it is needed to investigate pulai plants from different areas in Indonesia to analyze their phytochemical content. This study used A. scholaris from different sites, Cifor-Bogor, Palembang, Banjar Baru, Ngebel, and Carita-Banten, to examine their phytochemical content.

2. Materials and methods

2.1. Materials

Plant material used was A. scholaris R.Br bark. Samples were taken from several research locations, namely, Cifor (Bogor), Banjarbaru (South Kalimantan), Ngebel (East Java), Palembang (South Sumatra), and Carita (Banten). Chemicals used in this research were 70% ethanol, amyl alcohol, magnesium powder, Meyer reagent, Wagner reagent, Dragendorf reagent, NH₄OH, H₂SO₄, FeCl₃, ether, and anhydrous acetic acid. The instruments used in this research were HITACHI UV/VIS spectrophotometer U2800 BRUKER, and EPOCH microplate spectrophotometer, vortex, evaporator, water bath, analytical balance, and glassware.

2.2. Methods

2.2.1. Sample Extraction. Sample of 500 gr of A. scholaris R.Br bark was dried, and grounded using a hammer mill, then filtered to obtain 60 mesh simplicia powder. The extraction used two techniques, namely maceration (3x 24 hours in room temperature) and reflux (three hours, 60 °C).

2.2.2. Maceration. 10 gr of simplicia of A. scholaris R. Br bark was soaked with 70% ethanol and filtered 3x 24 hours to obtain filtrate [12].

2.2.3. Reflux. 25 g of Simplicia of A. scholaris R. Br bark in 70% ethanol was refluxed for three hours, in 60 °C, then concentrated with a rotary vacuum evaporator to obtain crude and their phytochemical content. The rendemen was calculated using the equation:

\[
Yield = \frac{\text{concentrated extract weights (gr)}}{\text{simpilia weight (gr)}} \times 100\%
\]  

(1)

2.2.4. Phytochemical Test. The phytochemical compound of pulai bark was tested qualitatively by the standard method, Harbone. The phytochemical compound of A. scholaris R.Br bark was tested for alkaloid, flavonoid, tannin, saponin, steroid, and triterpenoid [13].

3. Results and discussion

3.1. Extraction of pulai bark

The simplicia of pulai bark from the different regions was extracted using two techniques, namely maceration and reflux techniques. Plant extraction aims to get components of secondary metabolites in pulai bark. Both extraction techniques use the same solvent, 70% ethanol. This solvent was chosen because ethanol 70% is a safe solvent and an option for extracting unknown structure and initial
screening secondary metabolites. It has a board power extraction because many secondary metabolites could be dissolved in 70% ethanol [14].

Pulai bark obtained from several regions in Indonesia produced variable extraction yields (Figure 1). Pulai bark from Palembang, extracted by the maceration method, had the highest yield (19.78%). On the other hand, by the reflux method, samples from Banjar Baru and Palembang had almost the same yield, 11.64%, and 11.26%, respectively. The lowest yield is shown by extracts from the Carita-Banten region for both extraction methods.

Geography is one factor affecting the yield extract. Pulai bark from Palembang had the best yield value compared to other samples. Differences in plant growth areas will affect differences in temperature, climate, nutrition, and intensity of sunlight obtained by plants [7]. The process of photosynthesis produces various compounds that can be precursors in the synthesis of secondary metabolites and affect the content of secondary metabolites of plants that are soluble in ethanol [15].

The extraction method affects the yield of pulai bark extract. Figure 1 shows that the maceration technique resulted in higher yields than the reflux technique, except for pulai bark from Carita-Banten. Palembang, Cifor, Banjar Baru, and Ngebel, which were 5.2%, 7.92%, 3.35%, 4.19, and 0%, respectively. The maceration technique is the immersion of plant samples in solvents. Optimal maceration lasts for three days [16]. Maceration is widely used as a choice extraction technique, especially in preliminary research, because it is simple and easy to do. Maceration does not involve heat in the extraction process, so it is the best used to extract secondary metabolites that are not heat resistant [16]. Reflux is an extraction process by boiling the mixture between the sample and the appropriate solvent at a certain temperature and time. Heat treatment aims to increase interactions between solvent and solute. However, this technique can only be used for secondary metabolites that are resistant to heat [17].

The reflux technique produced lower yield than maceration did in this study. Extraction time for three hours might not be optimal to homogenize the temperature to all parts of the extraction. Uneven temperature resulted in an interaction between solvent and Simplicia is not optimal so that the secondary metabolites dissolved are less. According to [18], in the reflux method, the heat is delivered in the conviction process so that the parts, which are closest to the plate, will heat up first. Therefore, the reflux method required a longer time to homogenize the temperature to all parts of the extraction. Literature reported that the reflux technique with six hours extraction was more optimal for dissolving secondary metabolites in bitter plants with higher yields than that of maceration techniques [19].

![Figure 1. Pulai bark yield extraction from different sites using maceration and reflux method.](image-url)
3.2. The phytochemical compound of pulai bark

In this study, qualitatively phytochemical screening was carried out by color testing to detect secondary metabolite content in pulai bark. Pulai bark from different sites Ngebel, Palembang, Cifor, Banjar Baru, and Carita-Banten. According to the phytochemical test, all pulai barks contained flavonoid compounds (Table 1). Flavonoids were detected very strong from Cifor and Palembang. Different plant growth areas show different flavonoid contents. It is related to the environmental conditions in which plants grow, especially soil fertility and sunlight intensity.

The presence of flavonoids was shown by the orange color in the amyl alcohol layer of the Wilsnater method flavonoid test. According to [20], the color changes occur due to the reductive reaction of the benzopiron nucleus to the flavonoid structure. It is because of the addition of Mg and HCl metals. The results of these reactions produce flavilium salts that can be red or orange. Flavonoid compounds belong to the largest group of secondary metabolites found in nature. Most of the flavonoid compounds in nature are found in the form of glycosides, which are flavonoid units bound to a carbohydrate [21].

Tannin was detected on samples from Cifor and Palembang, while samples from Ngebel, Banjar Baru, and Carita-Banten were not detected. The positive results of the tannin test were shown by a blackish green color. The blackish green complex was due to the formation of complex compounds resulting from the reaction of tannins in samples with Fe\(^{3+}\) ions from the addition of 1% FeCl\(_3\).

Flavonoids and tannins are polyphenol compounds that have antioxidant activity [15]. According to [22], phenolic groups, especially flavonoids, can act as antioxidants. The antioxidant activity of flavonoids can be obtained structurally, namely the presence of hydroxy groups that can donate hydrogen atoms to free radicals so that they can mitigate free radical reactions. According to [23], flavonoids have many biological activities, including inhibitors of HMG-CoA reductase, hepatoprotective, antibacterial, anti-inflammatory, anticancer, and antiviral.

All barks of A. scholaris from different locations contained alkaloid compounds. Alkaloids are organic bases that contain secondary, tertiary, or cyclic amines. Nitrogen atoms in alkaloids have lone pairs that can form bonds with logan ion ligands and explain the alkaline nature of the alkaloids [15]. Alkaloids are produced by many organisms, ranging from bacteria, fungi (fungi), plants, and animals. Alkaloids derived from both plants and animals showed a variety of pharmacological activities.

Commonly alkaloids include basic compounds containing one or more nitrogen atoms, usually as part of a cyclic system. Chemically, alkaloids are a very heterogeneous group ranging from simple compounds, such as conine to the strychnine pentacyclic structure. Many alkaloids are terpenoids in nature, and some are steroids. Other alkaloids are aromatic compounds, for example, colchicine [13]. Some of the effects of alkaloids are triggering the nervous system, raising blood pressure, reducing pain, antimicrobials, sedatives, medications for heart disease, and antidiabetic [15].

Alkaloids are classified as large secondary metabolites so that using three reagents (Meyer, Dragendorf and Wagner reagents) for a qualitative test that represents alkaloid content in plants. All reagents are based on the principle of precipitation due to ligand replacement. The presence of alkaloid compounds in the experimental results was characterized by the presence of white precipitate by Meyer reagents in the form of potassium-alkaloid complexes. Meyer’s reagent contains potassium iodide and mercury (II) chloride, which will react to form red deposits of mercurium (II) iodide. The addition of excess potassium iodide will produce potassium tetraiodomercurate [13]. Pairs of free electrons in the alkaloid nitrogen atom can form coordinate covalent bonds with metal ions. Potassium-alkaloid deposits are thought to form from the reaction of nitrogen atoms in alkaloids with K\(^+\) ions from potassium tetraiodomercurate (II) [23].

Alkaloids with Dragendorf reagents will produce light brown to yellow deposits. The formed precipitate is a potassium alkaloid complex. Dragendorf reagent contains bismuth nitrate salt. Bi\(^{3+}\) ions from bismuth nitrate will react with potassium iodide to form bismuth (III) iodide, which will dissolve in excess potassium iodide to form potassium methethodobismutate. The nitrogen atom in the alkaloids will then react with potassium ions to form a potassium-alkaloid complex [23]. Wagner reagents with alkaloids will produce orange deposits. The nitrogen atom in the alkaloid compound has a free electron pair that can replace the iodo (I\(^-\)) ion in Wagner reagents [13].
Table 1. Phytochemical screening of A. scholaris bark.

| No | Alstonia sp.   | District       | Phytochemical Test          |
|----|----------------|----------------|----------------------------|
|    |                |                | Sap. | Flav. | Tan. | Alk. | Triterpenoid/Steroid |
| 1  | A. scholaris   | Cifor-Bogor    | -    | +++   | +    | +    | + / -                  |
| 2  | A. scholaris   | Ngebel         | -    | +     | -    | +    | - / +                  |
| 3  | A. scholaris   | Banjar Baru    | -    | +     | -    | +    | + / -                  |
| 4  | A. scholaris   | Palembang      | -    | +     | ++   | +    | - / +                  |
| 5  | A. scholaris   | Carita-Banten  | -    | +     | -    | +    | - / +                  |

Remark: Sap.: Saponin; Flav.: Flavonoid; Tan.: Tanin; Alk.: Alkaloid; (+++): very strong detected; (++): strong; (+): weak; (-): not detected.

All pulai barks tested in this study contained secondary metabolites of terpenoids. The types of terpenoids identified were triterpenoids and steroids. Both of these terpenoids were detected weakly in all Pulai barks. Pulai bark species of A. scholaris from Cifor-Bogor and Banjar contained triterpenoids. Meanwhile, A. scholaris from Ngebel, Palembang, and Carita-Banten contained steroids. Terpenoids are classified as large secondary metabolites and composed of isoprene (C5) molecules. Terpenoids are classified according to their isoprene building units, which are 2-8 isoprene units. Terpenoids are mainly used for essential oils, which can be classified as mono and volatile sesquiterpenes (C10 and C15), slightly volatile penes (C20), and non-volatile triterpenoids and sterols (C30) and carotenoid pigments [12]. Identification of steroids and triterpenoids in studies used the Lieberman-Burchard test producing a green-blue color. The color is formed due to the oxidation of steroid/terpenoids compounds through the formation of conjugated double bonds [21]. All pulai barks tested in this study contained secondary metabolites of terpenoids.

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4. Conclusion

Extraction techniques affected the yield value of pulai bark extract, and the maceration technique produced more yield than the reflux did. All A. scholaris and Alstonia pneumathopora samples contained alkaloids, and the strongest flavonoid was detected from Cifor-Bogor. Flavonoid and alkaloids are potential secondary metabolites as antioxidants. So, it is a chance for A. scholaris and Alstonia pneumathopora to be natural medicine in the future.

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