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To cite this article: Zuraida 2019 *IOP Conf. Ser.: Earth Environ. Sci.* **308** 012027

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Phytochemical compounds of different species of *Alstonia* sp. from Palembang- South Sumatera

Zuraida

Forest Research and Development Centre, Environment & Forestry Research Development and Innovation Agency (FORDA), Ministry of Environment and Forestry, Bogor 16610, Indonesia. Jalan Gunung Batu No. 5 Bogor, Jawa Barat, Indonesia. Telp. (0251) 8633234; Fax (02518638111)

Email: zuraidaus21@gmail.com

Abstract. *Alstonia* sp is one of Indonesia tropical forest plant widely used for various benefits such as wood used for furniture, blackboards, wooden sandals, while barks and leaves used for traditional medicine for various diseases. Phytochemical content is influenced by various factors including species, age, and geographical location. This study aimed to identify phytochemical compounds of *Alstonia* sp. from various species, namely *Alstonia scholaris*, *Alstonia pneumatophora*, and *Alstonia angustiloba* from Palembang South Sumatera. Phytochemical analyzed flavonoids, tannins, saponins, alkaloids, steroids, and triterpenoids qualitatively. The result showed that all *Alstonia* sp. contained flavonoid and steroid compounds. Alkaloids and tannins were only detected in *Alstonia Scholaris*. Saponins were detected in *Alstonia scholaris* and *Alstonia pneumatophora*. Phytochemical content in *Alstonia* indicated that they had potency to be utilized as a source of medicinal plants. *Alstonia* sp. with the same genus but different species had different phytochemical content.

1. Introduction

Hypertension, diet, obesity, lack of exercise, and smoking can cause degenerative diseases. Degenerative diseases are diseases due to decrease in cellular function because of tissue or cell damage. Some degenerative diseases are atherosclerosis, cancer, diabetes mellitus, gout and hypercholesterolemia. Nowadays, plants are often used as source of medicine to treat various degenerative diseases [1].

Plants contains chemical compounds both primary metabolic chemicals such as carbohydrates, proteins, fats that are used by plants themselves for growth, as well as a source of secondary metabolites such as alkaloids, flavonoids, steroids, terpenoids, saponins and tannins [2]. Secondary metabolite compounds act to defend them from unfavorable environments such as temperature, climate, pest and plant diseases. Secondary metabolite can be used as medicine [3,4]

Pulai (*Alstonia* sp.) is a genus of forest plants in Indonesia. Some of their species are *Alstonia scholaris*, *Alstonia pneumatophora*, and *Alstonia angustiloba*. This plant mainly used as a source of wood, and in Palembang *Alstonia scholaris* is also used for materials in pencil industry [5]. On the other hand, pulai have been used traditionally as medicine in India, China, and Indonesia to treat various disease. It could be used to treat swelling, ulcers, diabetes, asthma and rheumatism [6].



Several studies had explored about biological activity of Pulai. According to Swastiratu [7], ethanol extract pulai leaves contained alkaloids, flavonoids, saponins, phenolics, tannins, steroids and triterpenoids. Flavonoids and other polyphenols play important role as antioxidants [8,9]. Purnama [10] reported that the ethanol extract of pulai leaves from Bogor acted antioxidant activity with IC_{50} 55.49 $\mu\text{g/mL}$, and Arulmozhi et al., [11] showed that ethanol extract of pulai leaves act as an antioxidant *in vivo* by preventing lipid peroxidation.

Alstonia scholaris often used as object reserarch, while studied about *Alstonia pneumatophora*, and *Alstonia angustiloba* were limited. The aimed of this study was to investigate phytochemical content of *Alstonia scholaris*, *Alstonia pneumatophora*, and *Alstonia angustiloba* from Palembang South Sumatera.

2. Materials and Methods

Material used in this research were leaves of *Alstonia scholaris*, *Alstonia pneumatophoa* dan *Alstonia angustilloba* with 100 mesh in size obtained from Palembang, South Sumatera. Other materials used were ethanol, amyl alcohol, magnesium, Meyer, Wagner, and Dragendorf reagent, NH_4OH , H_2SO_4 , FeCl_3 , ether, and acetic acid. Main tools used were vortex, evaporator, water bath, Scale AND GR-200 series analytical balance, glassware, BRANSON B1510 sonicator.

2.1. Phytochemical extraction

Pulai leaves were dried, grounded using a hammer mill, then filtered using 100 mesh sieve to obtain 100 mesh simplicia powder, then extracted by maceration in ethanol 96%, 3x24 hours to get filtrate. The filtrate was concentrated with vacuum rotary evaporator to obtain crude extract. Then, it tested for their phytochemical content.

2.2. Phytochemical test

Phytochemical compound of leaves *Alstonia* sp. (*Alstonia scholaris*, *Alstonia pneumatophoa* dan *Alstonia angustilloba*) were tested qualitatively by Harbone metode [2]. Secondary metabolite of *Alstonia* sp. leaves tested for flavonoid, alkaloid, saponin, tannin, steroid, and triterpenoid.

2.3. Flavonoid test

5 grams of simplicia from various regions were added with 15 mL of distilled water and then heated for about 5 minutes to boil, then filtered. Filtrate was added with magnesium powder, HCl: ethanol (1:1), and 1 mL amyl alcohol. Positive results were indicated by orange color layer at the upper layer of amyl alcohol.

2.4. Alkaloid test

5 grams simplicia were dissolved in 5 mL of chloroform, then added NH_4OH . Chloroform fraction seperated by H_2SO_4 . H_2SO_4 fraction was added by Dragendorf, Meyer and Wagner reagents to three different samples on the drop plate. The presence of alkaloids is indicated by formation of white sediment by Meyer's reagent, red sediment by Dragendorf reagent and chocolate deposits by Wagner reagents.

2.5. Tanin test

5 grams simplicia from various species were added to 15 mL of distilled water, boiled for 15 minutes then filtered. Then filtrate added with 1% FeCl_3 . Positive results indicated by the formation of a blackish green color.

2.6. Saponin test

5 grams simplicia from various species were added to 15 mL aquades, boiled for 5 minutes then filtered. Filtrate was shaken vigorously in a closed tube for 10 minutes. Positive results shown by formation stable foam.

2.7. Triterpenoid and steroid tests

5 grams simplicia from different species, each added 10 mL ethanol, and boiled, then filtered until dried. Then 1 mL diethyl ether was added and homogenized with vortex. On a porcelain dish, 5 drops of filtrate, 1 drop of anhydrous acetic acid, and 1 drop of H₂SO₄. Positive triterpenoid shown by the formation of red or purple, while steroid in green.

3. Results and Discussion

3.1. Extraction

Fresh simplicia dried under sun light to reduce water content in the sample. This treatment can minimize microbial growth, and it won't influence chemical composition of pulai leaves. Dried simplicia then mashed to produce powder to increase the effectiveness of extraction due to increased interaction between solvents and samples during extraction [12].

3.2. Phytochemical compounds of *Alstonia* sp. leaves

Pulai (*Alstonia* sp.) leaves were different species i.e. *Alstonia scholaris*, *Alstonia pneumatophora*, and *Alstonia angustiloba*. Phytochemical screening was carried out by qualitatively based on the color complex produced. Based on phytochemical screening all *Alstonia* species with the same genus, but different species have different phytochemical content. All species positive for flavonoids and steroids, while tannin and alkaloid were only detected in *Alstonia scholaris* (Table 1). This result showed that different species had different secondary metabolites. It could be caused by several factors such as: biotic (plant interactions with microorganisms or physiological aspects of plants), and abiotic (light, uv, radiation, water, and soil composition) [13]. Similar results were also reported by [14] which proved that secondary metabolites in several species from genus *Senecio* L were also different.

Table 1. Phytochemical compound of *Alstonia* sp. from Palembang.

No	<i>Alstonia</i> sp.	Phytochemical Test				
		Saponin	Flavonoid	Tanin	Alkaloid	Triterpenoid/Steroid
1	<i>Alstonia scholaris</i>	++	+	+	+	- / +
2	<i>Alstonia pneumatophora</i>	++	+	-	-	- / +
3	<i>Alstonia angustiloba</i>	-	+	-	-	- / +

Remarks: (+++): very strong; (++): strong; (+): weak; (-): not detected

Flavonoids are phenol group compounds, mostly found in nature in red, purple or yellow plants. High concentrations of flavonoids usually at the epidermis of the leaves and fruit peels. There are six main classes of flavonoids, namely flavones, flavonols, flavan-3-ol, isoflavones, flavanones and anthocyanidins [15]. The presence of flavonoids in plants indicate that its potential to use as antioxidants [16]. Antioxidant activity could be used for degenerative diseases. According to Kumar & Pandey [17], flavonoids have biological activities such as inhibiting enzyme HMG-CoA reductase in cholesterol biosynthesis, hepatoprotective, antibacterial, anti-inflammatory, anticancer and antiviral. Identification of flavonoids using the Wilstater test indicated by orange in the amyl alcohol layer. According to Tiwari *et al.* [18] the color changes because of reduction benzopyrone nuclei on flavonoids by adding Mg and HCl, and result in red or orange flavilium salt.

Alstonia scholaris leaves in this research contained flavonoids, tannins, saponins, alkaloids and steroids. This study was different from Emilia [19] which reported that the leaves of *Alstonia scholaris* from Palembang didn't contain flavonoid, alkaloid, and terpenoid compounds. The difference was likely due to differences in the age of the plants used, solvent, and site.

Alkaloid was only detected in *Alstonia scholaris* and it wasn't detected in other species. Alkaloid was detected for three reagents such as, Meyer, Dragendorf and Wagner. The principle of tree types of reagents is the ligand replacement. Metals in the reagents will form coordinative covalent bonds with nitrogen in the alkaloids. The result was a white precipitate in Meyer's reagent, young brown to yellow in Dragendorf reagent and orange deposits on Wagner's reagents [2].

Alkaloid is the main secondary metabolites in *Alstonia scholaris* [20,21]. *A. scholaris* leaves contained alkaloids, namely pikrin, picralinal, narelin and striktamin [22]. Alkaloid is secondary metabolite that contain nitrogen atom in the form of secondary, tertiary or cyclic amines. Nitrogen atoms can form bonds with metal ion ligands and determine alkaloid alkaline properties. Alkaloid can trigger nervous system, increase blood pressure, reduce pain, antimicrobials, sedatives, medications for heart disease and antidiabetic drugs [15].

Several secondary metabolites of alkaloids had been isolated, including etichamine chloride and Alstonine. Etichamine chloride is an alkaloid indole, have pharmaceutical effect on anticancer activity and cytotoxic activity. Etichamine chloride can also increase the activity of endogenous antioxidants such as reduced glutathione, superoxide dismutase and glutathione peroxidase. Alstonine is alkaloid indole isolated from *Alstonia scholaris* showed biological activity as antimalarial, anticancer, and limiting DNA synthesis in cancer cells [21].

4. Conclusion

Phytochemical content of *Alstonia scholaris* was more divers than other species. Flavonoid, alkaloid, and tannin in *Alstonia scholaris* played important role in antioxidant activity. It indicated that it had more potency as antioxidant than those of other species as medicinal plants for degenerative disease. *Alstonia* sp. with the same genus but different species had different phytochemical content.

5. References

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