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PHYTOCHEMICAL ANALYSIS OF BITUNG TREE SEEDS (*Baringtonia asiatica* L.)

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Abstract

This study aims to obtain data on the phytochemical content of the methanol extract of *Baringtonia asiatica*. Obtain data on the phytochemical content of the *B. asiatica* clorform extract. This research was carried out in the Biology laboratory and Chemistry Laboratory, State University of Manado. The study consisted of extraction and phytochemical analysis using the Harborne method, the results showed that from the extraction process the highest yield was obtained in extracts with n-hexane solvent, then chloroform and methanol. The results of phytochemical analysis show that saponins are the phytochemical group with the strongest intensity found compared to other phytochemical groups. Furthermore, flavonoids were found in high intensity also in the analyzed samples.

Keywords: phytochemical, seeds, *Baringtonia asiatica L.*

INTRODUCTION

Keben (*Barringtonia asiatica* L. Kurz) belongs to the Lecythidaceae family. This plant is often found around the coast, along rivers or in mangrove forests at an altitude of 350 m above sea level. In some areas, this plant is often referred to as a poisonous plant (poisonous plant), because in some areas the fruit is used as a fish poison. Papuan people use keben seeds to catch fish. The seeds are grated and then spread on the surface of the ditch which reaches 1 meter deep so that the fish will faint and be easily caught on the surface of the water (Lemmes & Bunyapraphatsara 2003; Samah 1988)

The use of this plant varies in each country and region. The plant parts used are seeds, fruit and leaves. In the Philippines the leaves are used as a remedy for stomach aches. The people of Indonesia and Indo China use the fruit or seeds as fish poison. Aboriginal tribes in Australia use this plant as a fish poison and as a remedy for headaches (Lemmes & Bunyapraphatsara 2003; Samah 1988; Duryatmo 2006). Keben contains saponins, terpenes, alkaloids, triterpenoids, phenolics and tannins (Duryatmo 2006). It is suspected that most of the medicinal properties in keben seeds come from the saponins in the plant. Herlt, (2002), showed that keben seeds contain saponin compounds which are efficacious as fish

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poison.

One of the plants that has the potential to be developed as a source of botanical insecticides is *Barringtonia asiatica* (L.) Kurz (Lecythidaceae) which is commonly known as sea poison tree (Tan, 2001; Anonymous, 2006). All parts of this tree are known to contain saponins which can inhibit insect feeding activity (Kardinan, 2002). The methanol extract of *B. asiatica* seeds had insecticidal activity on C. pavonana larvae with an LC50 of 0.75%. Seed extract is the most active part compared to extracts from the leaves and bark (Dono & Sujana 2007). This information is the first report on the biological activity of *B. asiatica* on C. pavonana. Herlt *et al*. (2002) reported having isolated compounds from the saponin group from *B. asiatica* seed extract which were antifeedant against Ephilachna larvae. The active compound contained in B. asitica that can cause poisoning in fish is a group of saponin compounds. Saponins are glycosides, namely secondary metabolites that are widely found in nature, consisting of sugar groups linked to aglycones or sapogenins. These compounds are also toxic to reptiles and invertebrates (Prihatman, 2001). The methanolic extracts of the leaves, fruits, seeds, and bark and seeds of B asiatica showed broad spectrum antibacterial activity. A number of its fractions showed antifungal activity (Khan & Omoloso, 2002).

In some places, *B. asiatica* is used as a traditional medicine and fish poison (The Cook Islands Natural Heritage Trust, 2005). In the Philippines, heated *B. asiatica* leaves are used to treat stomach aches and rheumatism (EEBG, 2006). In the Pacific Islands, the seeds of *B. asiatica* are used to poison fish by draining the wash water of the seeds into the water (Cox, 1979 cit. Cannon *et al*., 2004). The active compound contained in B. asitica that can cause poisoning in fish is a group of saponin compounds. These compounds can be found in all parts of the plant (Tan, 2002; EEBG, 2006). In general, saponins are toxic to cold-blooded animals (Vickery & Vickery, 1981 cit. Cannon *et al*., 2004). One of the most active saponin compounds as fish poison from *B. asiatica* extract is Ranuncoside VIII (Burton *et al*., 2003). Two other major saponins isolated from the methanolic extract of *B. asiatica* seeds were antifeedant against Epilachna larvae (Herlt *et al*., 2002). In addition, the methanol extract of the leaves, fruit, seeds, and bark and seeds of B asiatica showed broad-spectrum antibacterial activity. A number of its fractions showed antifungal activity (Khan & Omoloso, 2002). A search of the literature shows that there is little information reporting the insecticidal activity of the compounds extracted from *B. asiatica*. Therefore, efforts to use *B. asiatica* as a botanical insecticide still need to be developed. In this study, the insecticidal activity of extracts of several parts of the plant *B. asiatica* was investigated for its effect on Crocidolomia pavonana F. (Lepidoptera: Pyralidae) which is the main pest of Brassicaceae vegetable crops.

Although there have been several studies reporting the phytochemical content of *B. asiatica*, studies of the phytochemical content of *B. asiatica* originating from North Sulawesi have not been widely reported. The phytochemical content of a plant is highly dependent on the habitat, the available nutrient content, interactions with microorganisms or plant-eating organisms (phytophages) and the ecophysiological conditions in which the plant grows. Because of the phytochemical potential of this plant, it is necessary to carry out research on phytochemical analysis of *B. asiatica* to be developed as a source

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of plant chemical compounds to be applied as bioinsecticides and pharmacological potentials. This study aims to: Obtain data on the phytochemical content of the methanol extract of *B. asiatica*. Obtain data on the phytochemical content of the *B. asiatica* clorform extract. The results of this study are expected to provide data on the phytochemical profiles (main phytochemical groups) of methanol extract and *B. asiatica* chloroform extract as the basis for further research.

MATERIALS AND METHODS

Tools and materials

The tools used in this study include: Philips Blender, Kern brand analytical balance, Rotavapor Merel Bunchi for extract evaporation process, Glass funnel, Beaker, Separating funnel, Empty vials, Test tubes, Test tube rack, Hot plate, Micro pipette 1000-100 l, Measuring cup, Lamp, Whatman 04 filter paper, Stationery, Camera, and others.

While the materials used are Bitung tree seeds, methanol PA, chloroform PA, ion-free water, Dragendorff reagent, Mayer's reaction, Wagner's reaction, HCL, metal Mg, Na2CO3, FeCl3, H2SO4, acetic anhydride.

Research methods

1. Content Analysis of Main Phytochemical Groups

Extracts of leaves and stems of purple lemongrass were tested for their chemical composition. The composition test was carried out to determine the content of alkaloids, saponins, flavonoids, tannins, steroids or terpenoids.

Phytochemical Analysis (Harbourne Method, 1996)

Alkaloid test. A total of 0.1 grams of extract was added 3 mL of chloroform and 3 drops of ammonia. The chloroform fraction was separated and acidified with 10 drops of 2 M H2SO4. The acid fraction was taken, then Meyer and Wagner's reagent was added. The presence of alkaloids was indicated by the formation of a white precipitate by the Meyer reaction and a brown precipitate by the Wegner reaction. As a comparison, use the blood footprint.

Saponin and Flavonoid Test. A total of 1 gram of extract was put in a beaker then added 100 ml of hot water and boiled for 5 minutes, after that it was filtered and the filtrate was used for testing. The saponin test was carried out by shaking 10 ml of the filtrate in a closed test tube for 10 seconds and then left for 10 minutes. The presence of saponins is indicated by the formation of stable foam. Another 10 ml of the filtrate was added with 0.5 grams of magnesium powder, 2 ml of carbohydrate alcohol (a mixture of 37% HCL and 95% ethanol in a ratio of 1:1) and 20 ml of amyl alcohol then shaken vigorously. The formation of red, yellow and orange colors on the amyl alcohol layer indicates the presence of flavonoids.

Tannin Test. A total of 0.1 grams of extract was added to 2 mL of water and then boiled for several minutes. Then filtered and the filtrate was added with 1 drop of 1% FeCl3 (w/v). Dark blue or greenish black color indicates the presence of tannins.

Triterpenoid and Steroid Test. A total of 0.1 grams of extract added 2 mL of 30% ethanol then

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heated and filtered. The filtrate was evaporated and then 1:1 ether was added. The ether layer was added with Lieberman Burchard's reaction (3 drops of acetic anhydride and 1 drop of concentrated H2SO4). Red and green colors indicate the presence of triterpenoids and green colors indicate the presence of steroids.

RESULTS AND DISCUSSION

Bitung tree seeds used were obtained from Likupang, North Minahasa Regency. Bitung tree seeds after drying, milled and sieved on a sieve with a sieve diameter of 100 mesh, in order to obtain simplicia for extraction in the form of flour. Bitung tree seed flour powder is brownish white with a sharp odor. Extraction was carried out using the maceration method, namely the simplicia immersion of bitung tree seeds in the planned solvent. Extraction was carried out at room temperature using a shaker at 40 rpm. Extraction was carried out for 3 x 24 hours. The ratio of the solvent used is 1 (one) part of simplicia dissolved in 4 (four) parts of solvent or as much as 100 g of bitung tree seed powder macerated with 400 ml of solvent. The solvents used were methanol pan n-hexane and technical chloroform.

Extraction was carried out separately where each solvent was used in different simplicia and carried out 2 replications. The yields obtained varied, the highest yield was obtained from the n-hexane extract, which was 5.65 gr, while the lowest yield was obtained from the methanol extract, which was 3.25 gr (Table 1).

Table 1 Bitung Tree Seed Extract Yield

Simplicity	Test	% yield
Methanol Extract pa	1	3,25
	2	3,75
Chloroform Extract	1	4,35
	2	4,97
Extract n-hexane	1	5,65
	2	5.25

Phytochemical Analysis

The purpose of the phytochemical analysis is to determine the class of phytochemical compounds contained in the crude extract. The phytochemical analysis technique used is the Harborne Method (1996). The compounds analyzed included alkaloids, saponins, flavonoids, tannins, steroids and triterpenoids. This phytochemical analysis is qualitative in nature so that the presence of compounds in simplicia is determined based on the intensity of the color, foam or precipitate formed according to the phytochemical analysis procedure for each group of compounds. The results of the phytochemical analysis showed that the content of saponins, flavonoids and alkaloids was relatively high in Bitung tree seeds (Table 2).

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Table 2. Content of Phytochemical Compounds in Bitung Tree Seeds

Coarse Extract Type	Compound Group					
	Alkaloids	Flavonoids	Saponins	Tannins	Steroids	Triterpenoids
Methanol Extract	++	++	+++	+	-	-
Chloroform Extract	+	++	+++	+/-	-	-
Extract n-hexane	+	+++	+++	+	-	+

From the extraction process, the highest yield was obtained in the extract with n-hexane solvent, then chloroform and methanol. N-hexane is a non-polar solvent, thus this solvent will attract non-polar plant active compounds. Non-polar plant active compounds are generally phytochemical compounds composed of fatty acids or fatty derivatives as basic ingredients. Chloroform is a semi-polar solvent and thus can attract polar and semi-polar phytochemical compounds. Methanol is a polar solvent so it attracts polar phytochemical compounds.

From the results of phytochemical analysis, it is known that saponins are the phytochemical group with the strongest intensity found compared to other phytochemical groups. Furthermore, flavonoids were found in high intensity also in the analyzed samples. Saponins are complex glycosides with high molecular weight produced mainly by plants, lower marine animals and some bacteria. The term saponin is derived from the Latin 'SAPO' which means soap, taken from the word Saponaria vaccaria, a plant that contains saponins used as soap for washing. Saponins are soluble in water but insoluble in ether. Saponins contain sugar groups, especially glucose, galactose, xylose, rhamnose or methylpentose which are linked to a hydrophobic aglycone (Sapogenin) in the form of triterpenoids, steroids or steroidal alkaloids. Aglycones can contain one or more unsaturated C-C bonds. The oligosaccharide chains are generally bound at the C3 position (monodesmosidic), but some saponins have an additional sugar group at C26 or C28 (bidesmosidic). The very complex structure of saponins occurs due to the variation of the aglycone structure, the nature of the chain and the position of attachment of the sugar group to the aglycone.

Steroid saponins are composed of a steroid core (C27) with a carbohydrate molecule. Hydrolysis of steroidal saponins will give an aglycone known as sarsaponin. Some examples of steroidal saponins are Asparagosides, Avenocosides, Disogenin ($C_{23}H_{22}O_6$), Ecdysterone ($C_{27}H_{44}O_7$), Tigogenin ($C_{27}H_{44}O_3$). Triterpenoid saponins are composed of a triterpene (C30) with a carbohydrate molecule. Hydrolysis of triterpenoid saponins will give aglycones known as sapogenins. This type of saponin is a derivative of -amyrine. Some examples of triterpenoid saponins are Asiaticoside ($C_{48}H_{78}O_{18}$), Bacoside Cyclamin ($C_{58}H_{94}O_{27}$), Glychyrhizin ($C_{42}H_{62}O_{16}$), Panaxadiol and panaxatriol. Saponins are found in various plant species, both wild plants and cultivated plants. In cultivated plants, triterpenoid saponins are the main type, while steroidal saponins are commonly found in plants used as medicinal plants. Triterpenoid saponins are found in other legumes such as soybeans, chickpeas, peas, lucerne, as well as tea, spinach, sugar beet, sunflower and ginseng. Steroidal saponins are found in oats, capsicum pepper,

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aubergine, tomato seeds, asparagus, vines, yucca and ginseng. Several factors such as physiological age, agronomic and environmental conditions can affect the content of saponins in plants. Young plants in a species have higher saponin content than mature plants. The steps of saponin biosynthesis have not been elucidated at the molecular level. Triterpenoid saponins, such as sterols, are synthesized from mevalonic acid via the isoprenoid pathway.

Potential pharmacological bioactivity of saponins

Antifungal Activity. Saponins have a high level of toxicity against fungi. Fungicidal activity against Trichoderma viride has been used as a method to identify saponins. The mechanism of action of saponins as antifungals is related to the interaction of saponins with membrane sterols.

Antivirus activity. Several saponins and sapogenins have shown the ability to inactivate viruses. The triterpenoid sapogenin oleanolic acid inhibits the multiplication of the HIV-1 virus by inhibiting the activity of the HIV-1 protease.

Antioxidant. Oxidation reactions have adverse biological effects. The saponin group produced by legumes, especially group B soyasaponin, contains a group antioxidants attached to the C23 atom (Yoshiki *et al*. 1998). This characteristic sugar residue allows the saponins to cleave the superoxide through the formation of hydroperoxide intermediates, thereby preventing damage to biomolecules by free radicals.

Effect on Nervous System Function. Ginseng extract showed neurotrophic and neuroprotective effects (Rudakewich *et al*. 2001). Ginseng was able to improve learning ability and cognitive function in brain-damaged mice and improve the appearance of normal mice. This effect is carried out through membrane stabilization such as blocking Na+ and Ca+ channels.

Cholesterol Metabolism. Saponins can reduce blood and tissue cholesterol levels in poultry and mammals but have not been able to successfully reduce egg cholesterol. The main source of egg cholesterol is endogenous synthesized cholesterol in the ovaries, so that a decrease in blood cholesterol levels in laying hens does not cause a decrease in egg cholesterol. Saponins are able to reduce blood serum cholesterol concentrations by binding and preventing cholesterol absorption because the saponin-cholesterol interaction is an insoluble complex. Low cholesterol absorption lowers blood serum cholesterol concentrations and forces increased cholesterol metabolism in the liver. Saponins can also deplete blood cholesterol by limiting reabsorption and increasing excretion. However, it should be noted that a decrease in blood serum cholesterol concentration can only occur if there is hypercholesterolemia in the diet

CONCLUSION

1. From the extraction process, the highest yield was obtained in the extract with n-hexane solvent, then chloroform and methanol.
2. From the results of phytochemical analysis, it is known that saponins are a phytochemical group with the strongest intensity found compared to other phytochemical groups. Furthermore, flavonoids were

found in high intensity also in the analyzed samples.

REFFERENCES

Bustanussalam dan Simanjuntak, P. 2008. Uji Bioaktivitas Senyawa Glikosida dari Biji Keben (*Barringtonia asiatica* L. Kurz). Jurnal Natur Indonesia 12(1), Oktober 2009: 9-14 ISSN 1410-9379, Keputusan Akreditasi No 65a/DIKTI/Kep./2008

Burdick and Jackson, 2010. Dielectric Constants. http:// macro. lsu. edu /howto/solvents. Dielectric Constant.htm. Diakses Tanggal Juli 2012.

Octavia, D,R. 2009. Uji Aktivitas Penangkap Radikal Ekstrak Petroleum Eter, Etil Asetat dan Etanol Daun Binahong (Anredera Corfolia (Tenore) Steen) dengan metode DPPH (2,2-difenil-1-pikrihidrasil.). Skripsi Diterbitkan. Surakarta: Fakultas Farmasi Universitas Muhamadiyah

Musri Musman*, Sofyatuddin Karina, Kavinta Melanie. Uji selektivitas ekstrak etil asetat (EtOAc) biji putat air (Barringtonia racemosa) terhadap keong mas (Pomacea canaliculata) dan ikan lele lokal (Clarias batrachus). Depik, 1(1): 27-31 ISSN 2089-7790

Sudarmadji, S., B. Haryono dan Suhardi. 2007. Prosedur Analisa Bahan Makanan dan Pertanian. Yogyakarta: Liberty.

Wagner, H and Bladt, S. 2001. Plant Drugs Analysis A Thin Layer Chromatography Atlas second edition. New York: Springer Verlag Berlin Heidenberg

Vogel. 1978. Text Book Of Practical Organic Chemistry, 4th Edition. London: Longman Group Limited.