Journal of TROPICAL BIODIVERSITY E-ISSN : 2774-4116

Phytochemical Screening on Some Leaves and Fruits Consumed by Javan Gibbons (*Hylobates Moloch*) from Cikaniki Area, Mount Halimun Salak National Park, West Java

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Abstract

Javan gibbon (*Hylobates moloch*) consumes different species of food, including fruits, leaves, flowers and insects. The food eaten by *Hylobates moloch* contains beneficial chemical compounds. One type of chemical compound found in *Hylobates moloch* food plants is secondary metabolite compounds that can affect also to feeding behavior. Secondary metabolites are chemical compounds in a plant that do not play a direct role in the needs of the plant's life but play a direct role in its environment. Several groups of secondary metabolites found in *Hylobates moloch* food plants include alkaloids, flavonoids, saponins, and tannins. The secondary metabolite compounds in these plants have potential as medicines. A sampling of *Hylobates moloch* food plant was taken from the Cikaniki area, Mount Halimun Salak National Park, West Java, based on the results of interviews with local communities and the research was continued with phytochemical tests. The results obtained 23 samples from 22 species of *Hylobates moloch* food which are included in 13 plant families, consisting of 18 types of leaves and 5 types of fruit. The results of qualitative phytochemical tests carried out on 23 samples of *Hylobates moloch* food plants obtained alkaloids, flavonoids, saponins, and tannins. The results of interviews with communities and literature studies show that *Hylobates moloch* food plants that are often used as medicine to cure certain diseases include: *Dysoxylum parasiticum*, *Euodia latifolia,* and *Cinnamomum parthenoxylon*

Keyword: *Hylobates moloch* food plants, Medicine, Phytochemical screening

INTRODUCTION

Various kinds of chemical compounds in nature are mostly found in plants. One type of chemical compound found in plants is secondary metabolites. Secondary metabolites are chemical compounds in a plant that do not play a direct role in the needs of the plant's life but play a direct role in its environment (Pagare, Bhatia, Tripathi, Pagare, & Bansal, 2015). Secondary metabolites consist of small molecules, are specific to each plant species, have various structures, and have different functions or roles. Several groups of secondary metabolites found in plants include alkaloids, flavonoids, saponins, and tannins (Harborne, 1998). In plants, secondary metabolites generally function to defend themselves in their habitat (Ergina, Nuryanti, & Pursitasari, 2014). Another function of secondary metabolites for plants is as an attractant. Attractants are compounds in the form of aromas produced by plants to attract insects so that they can help the pollination process (Kardinan, 2005).

Secondary metabolite compounds can be used directly by primates to help the digestive process and drugs for injure healing. The ability of animals to self-medicate is called zoopharmacognosy, where these animals utilize secondary metabolites (nonnutritive) from plants. An example of a primate species that utilizes secondary metabolites is the Orangutan Kalimantan (*Pongo pygmaeus wurmbii*). According to the research by Panda and Gunawan (2018), female orangutans are known to consume food plants containing secondary metabolites. This is done by female orangutans as an increase in stamina to fatigue conditions when carrying a baby every time.

The Javan gibbon (*Hylobates moloch*) is an endemic primate species found only on Java island. This primate species is only limited in its distribution in the forests of West Java, especially in protected areas (Supriatna & Wahyono, 2000). The Cikaniki area, Mount Halimun Salak National Park / Taman Nasional Gunung Halimun Salak (TNGHS), West Java has a tropical rain forest ecosystem that is still very good and represents an elevation zone, starting from the collin zone (500 – 1000 m asl), submontane (1000 – 1500 m asl) and montane (1500 – 1700 m asl). The tropical forest area in Cikaniki is still inhabited by various wild animals, including primates. Various species of primates found in this area include: *Hylobates moloch*, grizzled surili (*Presbytis comata*), javan langur (*Trachypithecus auratus*), and slow loris (*Nycticebus javanicus*) (Basalamah et al., 2010).

Hylobates moloch is frugivorous primates, eating fruit as their main diet. In addition, *Hylobates moloch* also eats leaves and flowers as alternative food (Surono, Mustari, & Rinaldi, 2015). The pattern of feeding behavior in primates has a close relationship with food quality. This depends on the nutrient content that compounds in the food (Harrison, 1986). Knowledge of the content of phytochemical compounds from *Hylobates moloch* food in the Cikaniki area has never been done while several types of this *Hylobates moloch* food are also known used by local people as medicine.

Based on this background, it is necessary to conduct research on the analysis of the content of secondary metabolites in *Hylobates moloch* food. The purpose of this study was to determine the content of secondary metabolites contained in *Hylobates moloch* food by using phytochemical screening methods. The results of this study are expected to be able to know the content of compounds in *Hylobates moloch* food that have the potential to be utilized and processed as medicinal ingredients. The results of this study are also expected to be a source of information for the development of science in the community so that the utilization by the community can be in accordance with the content of compounds found in plants.

METHOD

Sampling and phytochemicals test of *Hylobates moloch* food was doing at Cikaniki area, TNGHS, West Java (Figure 1). This research was conducted in September 2019.

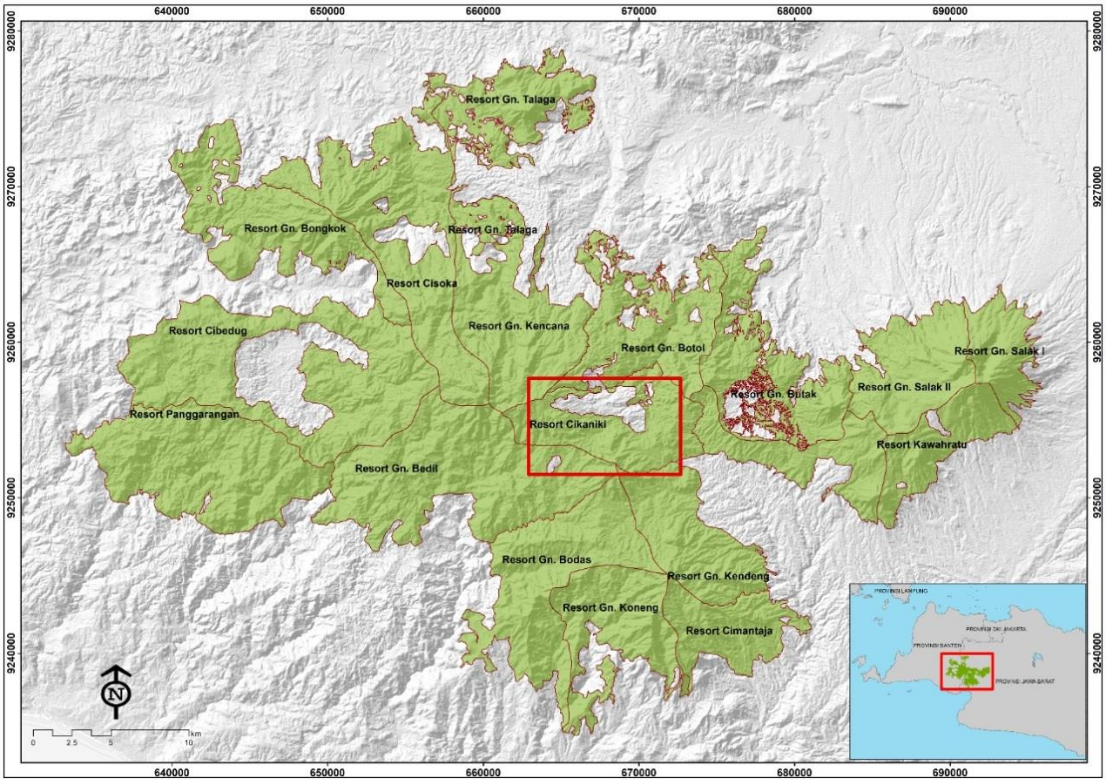


Figure 1. Location of Cikaniki area, Taman Nasional Gunung Halimun Salak, West Java (TNGHS, 2021)

The tools used in this study were plastic, plastic cups, knives, digital scales, blenders, electric stoves, labels, newspapers, containers (trays), strainer, small plastic spoons, pipette droppers, filter paper, test tube, test tube racks, funnels, beaker glass, graduated cylinder and stationery.

The materials used in this study were plant samples (leaves and fruit) at the research site, distilled water, concentrated HCl, 1N HCl, 2N HCl, Mg powder, 5% $FeCl_3$, and Bouchardat reagent.

1. Sampling of food plants for *Hylobates moloch*

A sampling of food plants for *Hylobates moloch* was conducted based on interviews with local communities in the Cikaniki area. Samples of forage plants were taken directly from the tree. The samples were brought to the camp to be made into dry sample powder and test the phytochemical screening.

2. Preparation of dry sample powder

Parts of fresh plant samples (leaves and fruits) were weighed as much as 10 – 20 g using a digital scale. The sample is then cut into small pieces using a knife. Samples were dried directly under the sun. After the sample is dry, the sample is then mashed using a blender. The sample that has been refined is then filtered through a 60 mess strainer so that it becomes a simplicia powder.

3. Preparation of Bouchardat reagent

Bouchardat's reagent was made by dissolving 4 g of KI with 50 mL of distilled water, then adding 2 g of Iodine crystals until dissolved. The solution is then diluted with distilled water to a volume of 100 mL (Rivai, Meliyana, & Handayani, 2016).

4. Phytochemical screening

Phytochemicals are chemical compounds found in plants, including secondary metabolites. Groups of secondary metabolites commonly found in plants include alkaloids, flavonoids, saponins, and tannins (Harborne, 1998). In this research, a qualitative analysis of the content of active substances was carried out to identify the presence of secondary metabolites in samples of *Hylobates moloch* food plants.

a. Alkaloid test

The dry sample was weighed as much as 0.5 g and put into a beaker glass. The sample was given the addition of 2 N HCl solution and distilled water in a ratio of 1:9. Then the sample was heated using an electric stove for 5 minutes. After heating, the sample is cooled for a while. Then the sample was filtered using filter paper to take the filtrate.

1 mL of sample filtrate was taken and put into a test tube. The filtrate was added with 10 drops of Bourchardat reagent. If the Bourchardat reagent forms a reddish-brown to black precipitate, it indicates that the sample contains alkaloid compounds (Tiwari, Kaur, & Kaur, 2011).

b. Flavonoid test

The sample was weighed as much as 0.5 g and put into a beaker glass. The sample was given the addition of 50 mL of hot water. Then the sample was heated for 5 minutes using an electric stove. After that, the sample was cooled for a while. Then the sample was filtered using filter paper to take the filtrate.

The sample filtrate was taken as much as 2.5 mL and put into a test tube. The filtrate was added with 0.03 g of Mg powder and 0.5 mL of concentrated HCl solution. If there is a color change to red, yellow, or orange, it indicates that the sample contains flavonoid compounds (Bandiola, 2018; Tiwari et al., 2011).

c. Saponin test

The sample was weighed as much as 0.5 g and put into a test tube. Then added 10 mL of distilled water and 5 drops of 1 N HCl solution. The solution was shaken vigorously vertically for 10 seconds. If a 1 cm foam is formed which is stable for 10 minutes and does not disappear when 1 drop of 2 N HCl is added, then the sample contains saponin compounds (Tiwari et al., 2011).

d. Tanin test

The sample was weighed as much as 0.5 g and put into a beaker glass. The sample was added with 10 mL of distilled water. Then the sample is heated using an electric stove until it boils, then the sample is filtered using filter paper to take the filtrate.

1 mL of sample filtrate was taken and 10 drops of 5% $FeCl_3$ solution were added. If there is a blue-black or dark green color change, the sample contains tannin compounds (Bandiola, 2018; Sangi, Runtuwene, Simbala, & Makang, 2019).

RESULT

Hylobates moloch food plants

Based on the results of interviews with local communities and searching in the Cikaniki forest, 23 samples were obtained from 22 species belong to 13 families from *Hylobates moloch* food plant. The results obtained 23 types of *Hylobates moloch* food samples, consisting of 18 leaves and five fruits. When the research was conducted, the fruit season in the forest was declining. This resulted in a small sample of fruit obtained. Samples of *Hylobates moloch* food plants can be seen in table 1.

Tabel 1. Samples of *Hylobates moloch* food plants from Cikaniki forest

No	Local Name	Famili	Species	Part Collect
1.	Lolo	Apocynaceae	*Scindapsus marantaefolius*	Leaves
2.	Sarai	Arecaceae	*Caryota mitis*	Leaves
3.	Rotan liana	Arecaceae	*Calamus manan*	Pulp
4.	Bingbin	Arecaceae	*Pinanga coronata*	Pulp
5.	Ganitri	Elaeocarpaceae	*Elaeocarpus ganitrus*	Leaves
6.	Tokbray	Euphorbiaceae	*Blumeodendron tokbrai*	Leaves
7.	Kisereh	Lauraceae	*Cinnamomum parthenoxylon*	Leaves
8.	Kilimo	Lauraceae	*Litsea cubeba*	Leaves
9.	Huru gemblung	Lauraceae	*Litsea robusta*	Leaves
10.	Kecapi	Meliaceae	*Sandoricum koetjape*	Leaves
11. 12.	Kihaji	Meliaceae	*Dysoxylum parasiticum*	Leaves Pulp
13.	Ficus pohon	Moraceae	*Artocarpus nitidus*	Pulp
14.	Kondang kecil	Moraceae	*Ficus glandulifera*	Leaves
15.	Beunying	Moraceae	*Ficus fistulosa*	Leaves
16.	Hamerang	Moraceae	*Ficus padana*	Leaves
17.	Ficus kisigung	Moraceae	*Ficus villosa*	Leaves
18.	Ficus kecil	Moraceae	Not identified	Pulp
19.	Kimokla	Myristicaceae	*Knema cinerea*	Leaves
20.	Kopi dengkung	Nysaceaea	*Nyssa javanica*	Leaves
21.	Pakis keras	Polypodiaceae	*Oleandra pistillaris*	Leaves
22.	Kisampang	Rutaceae	*Euodia latifolia*	Leaves
23.	Kihujan	Sapindaceae	*Pometia sp*	Leaves

Hylobates moloch are included to frugivorus primates (fruit eaters). The plant parts commonly eaten by *Hylobates moloch* are fruit, leaves, and flowers (Kappeler, 1984). The results of the latest research from Oktaviani, Kim, Cahyana, and Choe (2018) show that eating activity is one of the most important factors for the life of the *Hylobates moloch*. *Hylobates moloch* usually eats fruit, mature leaves, young leaves, flowers, stem and insects. The composition of *Hylobates moloch* food consisted of ripe fruit (74.2%), young leaves (14.5%), and the rest various types of flowers, stem and insects.

Phytochemical screening

Qualitative phytochemical tests from the group of alkaloids, flavonoids, saponins, and tannins were carried out on 23 samples from 22 species of *Hylobates moloch* food plants. The results of phytochemical screening can be seen in table 2 also figure 2 and 3.

Tabel 2. Result of phytochemical screening from of *Hylobates moloch* food plants

Species	Famili	Part eaten	Screening phytochemical			
			Alkaloid	Flavonoid	Saponin	Tannin
Scindapsus marantaefolius	Apocynaceae	Leaves	+	+	-	-
Caryota mitis	Arecaceae	Leaves	+	-	-	-
Calamus manan	Arecaceae	Pulp	+	+	+	+
Pinanga coronata	Arecaceae	Pulp	+	+	+	+
Elaeocarpus ganitrus	Elaeocarpaceae	Leaves	+	+	-	+
Blumeodendron tokbrai	Euphorbiaceae	Leaves	+	+	-	+
Cinnamomum parthenoxylon	Lauraceae	Leaves	+	+	+	+
Litsea cubeba	Lauraceae	Leaves	+	+	+	+
Litsea robusta	Lauraceae	Leaves	+	+	-	+
Dysoxylum parasiticum	Meliaceae	Leaves	+	+	+	+
		Pulp	-	+	-	-
Sandoricum koetjape	Meliaceae	Leaves	-	-	-	-
Artocarpus nitidus	Moraceae	Pulp	+	+	+	+
Ficus glandulifera	Moraceae	Leaves	+	+	+	-
Ficus fistulosa	Moraceae	Leaves	+	+	-	+
Ficus padana	Moraceae	Leaves	+	+	+	+
Ficus villosa	Moraceae	Leaves	+	+	+	+
Not identified	Moraceae	Pulp	-	+	-	+
Knema cinerea	Myristicaceae	Leaves	+	-	-	+
Nyssa javanica	Nysaceaea	Leaves	+	+	+	+
Oleandra pistillaris	Polypodiaceae	Leaves	-	+	-	+
Euodia latifolia	Rutaceae	Leaves	+	+	+	+
Pometia sp	Sapindaceae	Leaves	+	+	+	-

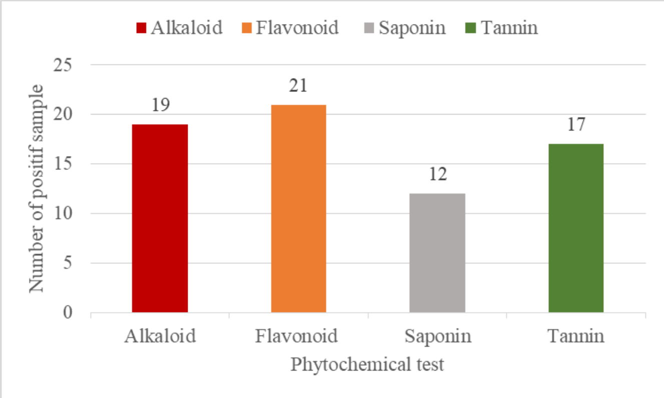


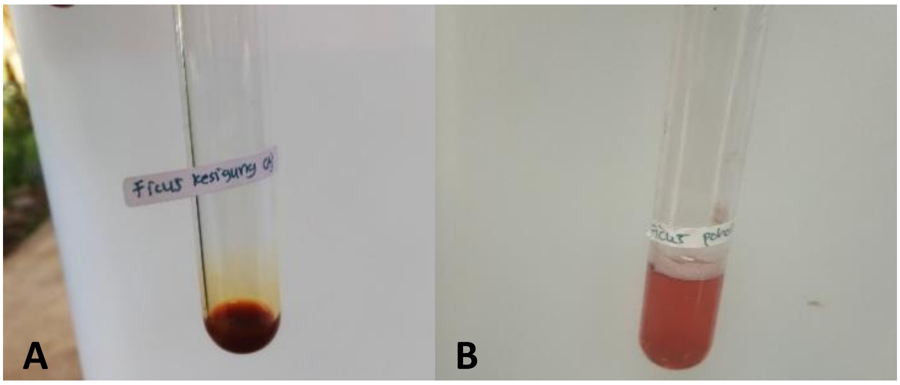
Figure 3. The result of phytochemical screening on secondary metabolite

Based on the results of phytochemical tests, 19 samples of *Hylobates moloch* food plants were found that were positive for alkaloid compounds. There were 17 leaves samples positive containing alkaloids, while three fruit samples that were positive for alkaloids. This can be seen by the formation of a reddish-brown precipitate in the sample after being given Bouchardat reagent.

The results of the phytochemical test, obtained 21 samples of *Hylobates moloch* food plants that were positive for flavonoid compounds. There were 16 leaves samples that positive containing flavonoids, while five fruit samples that were positive for flavonoids. The test results are indicated by the presence of a red, yellow, or orange precipitate.

Based on the results of the phytochemical test, 12 samples were obtained positive for saponin compounds. There were nine leaves samples that positive containing saponins, while three fruit samples that were positive saponin. It could be visible through the formation of foam in the plant sample.

Based on the results of phytochemical tests, 17 samples of *Hylobates moloch* foodd plants were found positive for tannin compounds. The leaves samples positive for tannin contained 13 samples, while the fruit samples that were positive for tannins were four samples. It could be observed from the formation of a color change to blue-black or dark green.



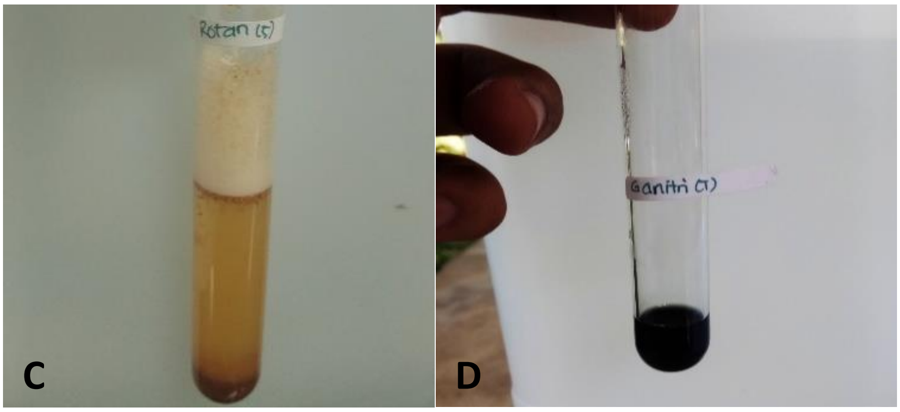


Figure 3. The result of changing the color of the solution in the phytochemical screening (A. Alkaloid; B. Flavonoid; C. Saponin; D. Tannin)

Hylobates moloch food plants that have the potential as medicine

Based on the results of interviews with the local communities around the Cikaniki area, TNGHS, West Java, it was found that people often use the *Dysoxylum parasiticum* as a medicinal plant which is believed to have efficacy to cure stomach pain and other stomach problems. The part that is used from the *Dysoxylum parasiticum* is the leaves. From the results we have obtained, the leaves of the *Dysoxylum parasiticum* had positive results when tested for alkaloids, flavonoids, saponins, and tannins. Other types of food plants for *Hylobates moloch* that are commonly used by the community as medicine are *Cinnamomum parthenoxylon* and *Euodia latifolia*. Those two types of plants that are used by local communities are the leaves and the utilization is done by boiling with water. *Euodia latifolia* plant is used by local people as a stomach ache medicine. Based on the phytochemical test, *Euodia latifolia* positive contained alkaloids, flavonoids, saponins, and tannins. *Cinnamomum parthenoxylon* is also used by local people as a medicine for shortness of breath. The results of the phytochemical test on this *Cinnamomum parthenoxylon* plant were positive for containing alkaloids, flavonoids, saponins, and tannins.

DISCUSSION

Alkaloids test with Bouchardat reagent will result in a reaction between iodine and I^- ions from potassium iodide to produce I^{3-} ions which will form coordinate covalent bonds with nitrogen, thus forming a precipitated potassium alkaloid complex (Marliana, Suryanti, & Suyono, 2005). Alkaloid compounds in general from several species of plants are known to have medical functions in the health sector, including siamine. Siamine is an alkaloid compound found in the johar plant (*Cassia siamea*). Siamine has a function as an antioxidant (Minarti, Kardono, & Wahyudi, 2002). Alkaloid compounds also have effects in the form of triggering the nervous system, raising blood pressure, reducing pain, antimicrobial, sedative, heart disease and antidiabetic activity (Agustina, Ruslan., & Wiraningtyas, 2016).

The color change in the flavonoid test was caused by the reduction of flavonoids by Mg and concentrated HCl. Some samples of *Hylobates moloch* food plants at the time of testing there were only a few sediment. This shows that the flavonoid compounds contained in one species of *Hylobates moloch* food plant are not many. Flavonoids are secondary metabolites of polyphenols, found widely in plants. In the world of medicine, flavonoid compounds have a role in various bioactive effects such as antioxidant, anti-viral and anti-inflammatory (Wang et al., 2016).

The formation of foam in the saponin test results indicates the presence of glycosides which have the ability to make a foam in water which is hydrolyzed into glucose and other compounds. This foam-like state indicates the presence of saponins in the sample. Saponins have a glycosyl that functions as a polar group and a terpenoid/steroid group as a nonpolar group. Compounds that have polar and nonpolar groups have surface active properties, so when shaken with water, saponins can form a foam (Marliana et al., 2005). In the medical sector, saponin compounds have properties to reduce the risk of atherosclerosis, because of their ability to bind cholesterol. Saponins are also efficacious as antimicrobials and external wound drugs because they can stop blood from the skin (Agustina et al., 2016).

In the tannin test, $FeCl_3$ reagent was used to identify the presence of tannins in the sample. The color change to blue-black or dark green occurs due to the formation of complex compounds between tannins and $FeCl_3$. Tannins are polyphenol compounds that can be distinguished from other phenols because of their ability to precipitate proteins (Ikalinus, Widyastuti, & Setiasih, 2015). In plants, tannins function as self-defense from bacteria, fungi, virus, herbivorous insects and herbivorous vertebrates. In addition, tannins are also important to prevent excessive degradation of nutrients in the soil. In the health sector, tannins have potential as antibiotics. The working principle of tannins as antibiotics is by forming complexes with extracellular enzymes produced by pathogens or by interfering with the metabolic processes of these pathogens (Agustina et al., 2016).

Based on the results of previous studies, it appears that a few species of *Hylobates moloch* food plant contain bioactive compounds that can be used as medicine. According to research from Susilo and Denny (2016) stated that the roots and bark of *Artocarpus nitidus* are usually chewed together with *Piper betle* used as medicine. *Blumeodendron tokbrai* is an ingredient in HIV antiviral drugs (Denny & Kalima, 2016). Based on the research of Silalahi, Purba, and Mustaqim (2018) which states that various compounds such as phytosterols, triterpenes, alkaloids, flavonoids and saponins have been isolated from *Caryota mitis* seed oil. Although not yet eligible for clinical trials, these compounds are known to have good antimicrobial effects. According to research results from Sanusi (2014) the leaves of *Cinnamomum parthenoxylon*, methanol extract contains flavonoid compounds, saponins, triterpenoids, steroids and phenols. The n-hexane fraction contains triterpenoids and steroids, the ethyl acetate fraction contains flavonoids, saponins, steroids, and phenols. The methanol-water fraction contains flavonoids, saponins, triterpenoids, and phenols. According to research Mayanti et al. (2017) seen that the leaves of *Dysoxylum parasiticum* contain flavonoid compounds that have antioxidant activity. Research on the ethanolic extract of *Elaeocarpus ganitrus* leaves showed the presence of active compounds in the form of phenols, flavonoids, tannins, saponins, terpenoids and glycosides and had antibacterial effects (Rahmatulloh, Kiromah, & Rahayu, 2020). Goh, Chung, Sha, and Mak (1990) stated that *Euodia latifolia* leaves mainly contain coumarin compounds.The result from Nugroho and Manurung (2015) show that extract ethanol of *Litsea cubeba* leaf containing secondary metabolites compounds such as alkaloids, flavonoids, phenolic and steroid which have antibacterial activities.

CONCLUSION

The conclusions that can be drawn from this research are as follows:

1. The results obtained 23 samples from 22 species of *Hylobates moloch* food plants in Cikaniki area, Mount Halimun Salak National Park.
2. The results of the phytochemical screening test of the 23 samples proved to contain secondary metabolites. The most common compounds found in the sample were flavonoids (21 samples) and alkaloids (19 sample).
3. Several types of food plants for *Hylobates moloch* obtained are proven to be used as medicine by the community at Cikaniki area to cure diseases, these species are: *Dysoxylum parasiticum*, *Euodia latifolia*, and *Cinnamomum parthenoxylon*.

ACKNOWLEDGMENT

Many thanks to Balai Taman Nasional Gunung Halimun Salak that given permission to conduct this research. The authors thank the Faculty of Biology for supporting research activities in the field. Also thank you for local assistant that helping on identifying the plant sample.

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