

Exploration, phenolic content determination, and antioxidant activity of dominant pteridophytes in Gunung Malang Village, Mount Halimun Salak National Park, Indonesia

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Abstract. Rindita, Anggia V, Rahmaesa E, Devi RK, Alawiyah LF. 2020. Exploration, phenolic content determination, and antioxidant activity of dominant pteridophytes in Gunung Malang Village, Mount Halimun Salak National Park, Indonesia. *Biodiversitas* 21: 3676-3682. Pteridophytes have been known to have pharmacological activities as an antioxidant, anti-inflammation, antimicrobials, and anticancer, such as *Selaginella* sp. In this research, exploration was conducted in Gunung Malang Village, Mount Halimun Salak National Park (TNGHS), West Java. Samples were collected by purposive sampling method, related to its abundant populations, *Histiopteris incisa*, *Nephrolepis biserrata*, and *Selaginella willdenowii* were collected based on different height and light intensity. Abiotic factors were measured to know their ecological requirements. Samples were extracted using ultrasonic method. Organoleptic test, yield, and phytochemical screening were done for the crudes extract. Total phenolic level was determined with Folin-Ciocalteu method and antioxidant activity test with DPPH. Phytochemical screening showed that *H. incisa* contains phenolics, saponins, tannins, and steroids; *N. biserrata* contains phenolics, flavonoids, and saponins while *S. willdenowii* contains phenolics, alkaloids, flavonoids, saponins, and tannins. Phenolic content of *H. incisa* and *N. biserrata* from 700 m. asl. was 9.8523 mg GAE/g \pm 0.9694 and 17.5399 mg GAE/g \pm 0.5350 respectively, from 1000 m. asl. was 18.1231 mg GAE/g \pm 2.1535 and 8.8468 mg GAE/g \pm 0.1437. *S. willdenowii* extract collected from shaded canopy forest was 19.2324 mg GAE/g \pm 0.6041 and opened canopy forest was 38.7087 mg GAE/g \pm 1.484. Antioxidant IC₅₀ of *H. incisa* and *N. biserrata* from 700 m. asl. respectively was 96.4271 ppm and 85.1907 ppm, while from 1000 m. asl. was 75.6381 ppm and 95.0678 ppm, *S. willdenowii* from the closed canopy is 101.7326 ppm, while from the opened canopy was 92.0998 ppm. This study shows that all ferns tested gave significant antioxidant activity and was influenced by environmental factors.

Keywords: Antioxidant activity, *Histiopteris incisa*, *Nephrolepis biserrata*, phenolic content, *Selaginella willdenowii*

INTRODUCTION

Indonesia is one of the ASEAN countries that have the greatest biological wealth and has the second-highest number of indigenous medicinal plants, after the Amazon rain forests (Elfahmi et al. 2014). Its biodiversity is a potential source in the exploration of beneficial compounds. Exploration of medicinal plants in their natural habitat is an alternative search for medicinal plants which is useful for finding medicinal raw materials. Based on a survey done in Mount Halimun Salak National Park (Taman Nasional Gunung Halimun Salak/TNHGS), it is known that there are many large groups of plants, one of which is ferns. Only very few explorations of beneficial natural compounds done on Indonesian ferns even though the amount is very abundant in nature. Approximately 12000-15000 kinds of ferns spread across the world (Roos 1996). However, ferns not only abundantly found inside the forest but also in damaged sites, since its presence may be regarded as an alert of ecological disturbances (Dai et al. 2020).

Ferns have been uses from ancient times to treat various illnesses throughout the world (Raimana et al. 2011).

Several studies have been carried out to explore the pharmacological activities of ferns. Various secondary metabolites were reported as active components mostly belonging to the terpenoid group (triterpenoids, diterpenoids, sesquiterpenoids), phenolic group (phenylpropanoid derivatives and others), flavonoid, and alkaloid (Keller and Prance 2015). Ferns contain various secondary metabolites with therapeutically-relevant bioactivities, including anti-cancer, antioxidant, and anti-inflammatory activities (Raimana et al. 2011). Some active compounds from ferns have been shown to provide beneficial pharmacological activities. Angiopteriside from *Angiopteris evecta* showed active against *Bacillus subtilis* and significant activity to inhibit the growth of HIV-1 Reverse transcriptase (Anggia et al. 2015). Phenolic compounds from *Trichomanes chinense* are shown to provide significant antibacterial activity (Syafni et al. 2012). The water extract of *Cheilanthes farinosa* was found to have antiproliferative and apoptotic activity in human liver cancer cells (Radhika 2010).

Ferns tend to live in humid tropical areas and Indonesia is one of the places to find various types of ferns. Based on the surveys carried out in Mount Halimun Salak National Park, western Java, Indonesia it was observed that there are

several dominant populations species of ferns occupied the area i.e *Selaginella willdenowii*, *Histiopteris incisa*, and *Nephrolepis biserrata*. *S. willdenowii* extract was reported to contain flavonoids 4', 7"-di-O-metilamentoflavone, isocriptomerin, and 7"-O-methylrobusta-flavone which were significantly cytotoxic against various cancer cells (Silva et al. 1995). The traditional use of its ferns is not to be a novelty in different countries. One of the subtribes in Ifugao, Philippines used crushed leaves of *H. incisa* to treat burns (Balangcod 2011). Besides consumed as a vegetable, *N. biserrata* has been used traditionally to treat boils, abscesses, and blisters (Rani 2010). and in the African region, this fern is commonly used to treat malaria (Koudouvo 2011). *Selaginella* is useful for traditional medicine, especially for treating wounds, postpartum, menstrual disorders and body fit improvement (tonics) (Setyawan 2016). Unfortunately, not many references discuss the constituents of *N. biserrata* and *H. incisa*, so it will be interesting to study bioactive compounds and their pharmacological activities.

Phenolic compounds are natural products that are widely used today. Their biological activities provide a major role in human interests. There some report on the activity of phenolic compounds and its derivates for health benefit especially to inhibit free radicals, peroxide decomposition, metal inactivation or oxygen scavenging in biological systems, and prevent oxidative disease (Babbar 2015). Various studies have proven that both biotic and abiotic factor from the environment in which plants grow will affect their growth and the chemical compounds produced. Sulasmi et al. (2019) found that four fern species in Baluran National Park have different types and content of flavonoids. The environmental factors, such as light intensity and altitude, irradiation factors will affect the growth and development of plants, thereby regulating the biosynthesis of secondary metabolites. In this study, phenolic content levels and antioxidant activity of *S. willdenowii*, *H. incisa*, and *N. biserrata* found in different places, altitude and sun exposure in Gunung Halimun National Park (TNGHS) will be reported.

MATERIALS AND METHODS

Material

Plant collection and authentication

Histiopteris incisa, *N. biserrata*, and *S. willdenowii* collected from Gunung Malang Village, Gunung Halimun Salak National Park (TNGHS) in August 2020 and subjected to determination process at Research Center for Biology, Indonesian Institute of Sciences, Cibinong, Bogor,

Indonesia. Samples were taken based on differences in forest vegetation that is shaded and open forests and differences altitude between 700 and 1,000 m. asl. Administratively, Gunung Malang Village is included in Bogor District, West Java Province, Indonesia

Chemical and reagents

Folin-Ciocalteu, 2,2-diphenylpicrylhydrazyl (DPPH), gallic acid, were purchased from Sigma Aldrich and other chemical reagents used in this research were analytical grade.

Methods

Determination of environmental parameters

Environmental parameters of the location are determined i.e light intensity, air humidity, air temperature using 4-in-1 digital weather meter; soil pH, and moisture using soil tester digital and determination of coordinates using a GPS device.

Extraction

150 g of powdered leaves of each fern were extracted with ethanol using ultrasonic method with 15 L of 70% ethanol at intervals of 30 minutes and repeated several times until the extraction was completely marked by colorless filtrate. Each resulting extracts were filtered and evaporated *in vacuo* with rotary evaporator at 50°C to yield concentrated extract of ferns (Table 1).

Phytochemical screening

Concentrated extracts were diluted using ethanol and identified qualitatively for the presence of alkaloid, flavonoid, tannin, terpene, steroid, and saponin (Tiwari et al. 2011).

Determination of phenolic compound level

Determination of total phenolic levels of ferns extracts was done using Folin-Ciocalteu method performed by Stankovic et al. (2011) with a few of modification. Ferns leaves extract 0.5 ml (1000 ppm), 2 ml Folin Ciocalteu and 4 ml Na₂CO₃ 1 M reagent were mixed homogeneously. The mixed solution was incubated in operating time range that has been determined before by observed the absorbance of gallic acid in the range of 1 to 120 minutes and determined the time that provides stable absorbance which is obtained at 100 minutes. All determination was carried out in triplicate and the phenolic level obtained was counted as equivalent to gallic acid. The standard calibration curve determined from the linear regression equation between series concentrations of gallic acid (x) and the absorbance obtained from reaction with Folin-Ciocalteu reagent (y) (Stankovic et al. 2011).

Table 1. Extraction yield of each fern extract

	<i>Histiopteris incisa</i>		<i>Nephrolepis biserrata</i>		<i>Selaginella willdenowii</i>	
	700 m. asl	1000 m. asl	700 m. asl	1000 m. asl	Open forest	Shaded forest
Extraction yield	19.58%	21.91%	23.97%	19.71%	25.90%	26.90%

Determination of antioxidant activity

Determination of antioxidant activity was carried out according to the method of Molyneux (2004). Two mg of DPPH dissolved in 100 ml of MeOH, then 3.8 ml of DPPH solution and 0.2 mL of MeOH was added and left for 30 minutes in the darkroom. The absorbance was measured with a UV-Vis spectrophotometer (at 400-800 nm) to obtain the wavelength of maximum absorbance (λ_{max}). Each sample was diluted with MeOH to prepare extracts with concentrations of 20, 40, 60, 80, and 100 ppm. 1 ml of each concentration was pipetted into a test tube, then 3 ml of DPPH solution of 0.887 mM was added, shaken, and allowed to stand for 30 minutes. Absorbance was measured at 516 nm. Gallic acid was used as a positive control (Molyneux 2004).

RESULTS AND DISCUSSION

Botanical determination is the first step in research to ensure the truth of plant species. Verification of specimen identity was carried out at the Indonesian Institute of Sciences (LIPI) Biology Research Center, Cibinong, Bogor. The results of verification of these specimens were *H. incisa*, *N. biserrata*, and *S. willdenowii*.

Determination of environmental parameters

Based on the data of survey obtained it was found that *H. incisa* and *N. biserrata* were grown at a different altitude between 700 and 1000 m. asl, meanwhile *S. willdenowii* was found both in shaded and open forest areas in Gunung Malang Village, Gunung Halimun Salak

National Park. The aim of pursuing the environmental parameters is to determine the relationship between environmental factors with their chemical constituents and antioxidant activity. Environmental parameters measured were light intensity, air humidity, air temperature, soil pH, moisture, and coordinates of each found ferns species. The result can be seen in Tables 2 and 3.

Organoleptic

Histiopteris incisa has large, black rhizomes and root fibers spread along the rhizomes, upright stem, brownish yellow, rounded, shiny and covered with fine hairs along the stem. The leaves have different shapes, sizes and arrangements. Generally, the leaves are arranged in double triple pinnate compounds (tripinnate), dark green, rough-textured, and stiff. Pina facing and having the same distance, sporangium is located in the abaxial part of the leaf. Young plants that curl can come out of the roots or are at the ends of the stems of adult plants. Young leaves that are curled light green and covered with fine white hairs (Figure 1) (Purnawati 2014).

Nephrolepis biserrata is an epiphyte plant that has a narrow and thinly spread scale morphology, the edges of the scales have fine hair. When young, pale green scales, when old, brown scales with pale brown edges, while scales that are on greedily scattered and are very small in size. The distinctive feature of the *N. biserrata* fern is located on the scales on the stip and the location of the sorus close to the edge of the medial pinna (Figure 2) (De Winter and Amoroso 2003).

Table 2. Environmental parameter of *Histiopteris incisa* and *Nephrolepis biserrata* in difference height

Parameters	700 m.asl		1000 m.asl	
	<i>H. incisa</i>	<i>N. biserrata</i>	<i>H. incisa</i>	<i>N. biserrata</i>
Light intensity (LUX)	112-1,851	914-7,980	2,390-4,920	1,526-8,456
Air humidity (% RH)	58.2-68.5	58.1-71.3	55.4-69.4	55.4-69.4
Air temperature (°C)	25.0-28.6	26.9-29.2	24.1-29.8	24.1-29.8
Soil temperature (°C)	21-23	22-23	22-23	21-23
Soil pH	7.0	7.0	7.0	7.0
Soil humidity (%)	20-30	40	25-40	25-40
Coordinate	SL: 06°40'10,7" EL: 106°43'22,1"	SL 06°40'10.7" EL 106°43'22.1"	SL: 40°28'9" EL: 43°31'5"	SL : 06°28' 9" EL: 106°31' 5"

Table 3. Environmental parameter of *Selaginella willdenowii* vegetation in shaded and exposed forest

Environmental parameters	Shaded	Exposed
Altitude	908 m. asl	1,050 m. asl
Light intensity (LUX)	670-4,280	5,490-20,000
Air humidity (% RH)	57.9-67.3	38.9-65.0
Air temperature (°C)	25.0-27.6	28.2-37.5
Soil temperature (°C)	20-21	22-25
Soil pH	7	6.0-6.7
Soil humidity (%)	15-35	10-30
Coordinate	S 06°40'50,6"; E 106°41'11,3"	S 06°40'57"; E 106°43'32"

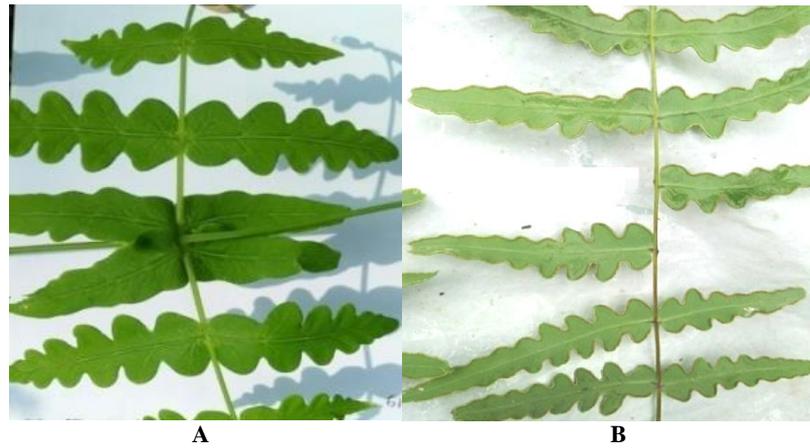


Figure 1. Leaf shape of *Histiopteris incisa*

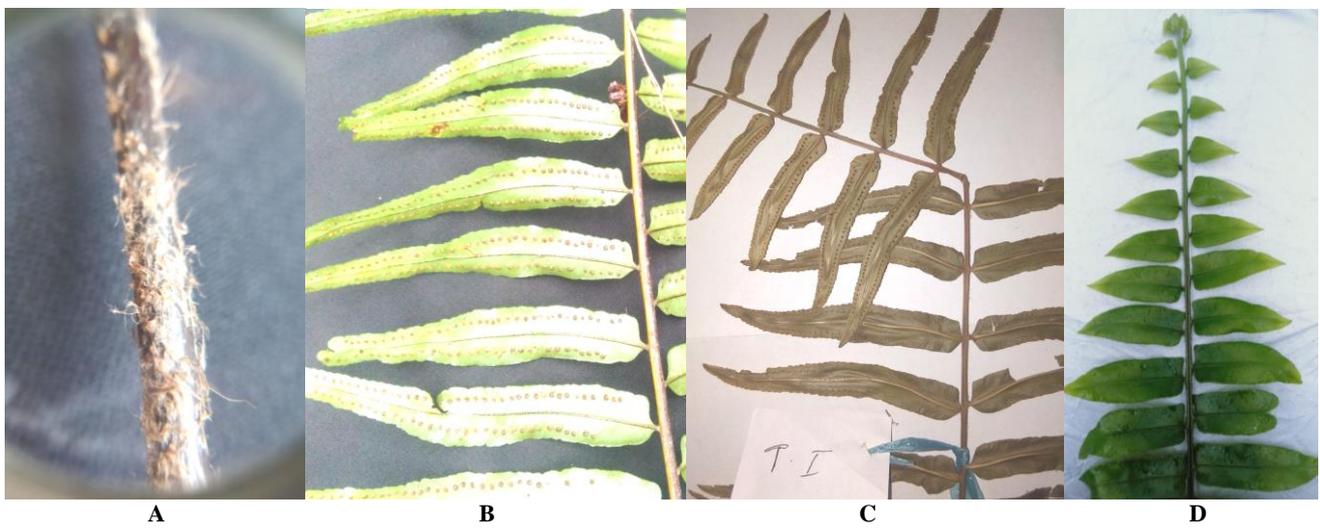


Figure 2. Plant morphology of *Nephrolepis biserrata*. A. Scales, B. Sorus, C. Lamina, D. Rachis

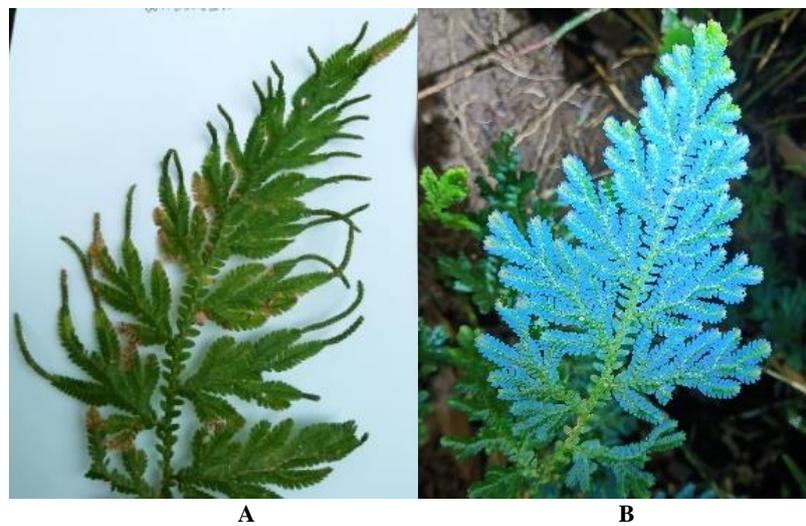


Figure 3. Plant morphology of *Selaginella willdenowii* A. Leaves in open forest; B. Leaves in shaded forest

Selaginella willdenowii is a climbing herb plant, 1-5 m long. The stems, at the bottom of the leaves line 4, the distance from one another is far apart. Leaves from the front row are very small, attached to the stem, leaves from both sides are larger, spaced wide, easily fall out, form a weak sickle. Fertile ovary leaves are wide, with a short pointed tip, tightly crammed into ears with a length of 0.5-2.5 cm (Steenis 2013) (Figure 3).

Phytochemical screening

The preliminary phytochemical screening of the ethanolic extract of the leaves of *H. incisa*, *N. biserrata*, and *S. willdenowii* leaves extract were presented in Table 4.

Determination of phenolic content level

Levels of phenolic content of *H. incisa*, *N. biserrata*, and *S. willdenowii* leaves extract obtained from different ecological factors were determined to analyze the influence of environmental factors on their chemical content. Phenolic content was determined with Uv-Vis spectrophotometry method at wavelength of 759.5 nm using Follin Ciocalteu reagent and gallic acid as a standard. Phenolic content of each fern extract is shown in Table 5.

Determination of antioxidant activity

Antioxidants activity of *H. incisa*, *N. biserrata*, and *S. willdenowii* leaves extract were determined by using DPPH following the method of Molyneux (2004); and experiment was done in triplicate. The samples were measured at 516 nm with absorbance of DPPH was 0.790. Antioxidant properties of each sample are shown in Table 6.

Table 4. Phytochemical screening of *Histiopteris incisa*, *Nephrolepis biserrata* and *Selaginella willdenowii* leaves extract

Chemical analysis	<i>H. incisa</i>		<i>N. biserrata</i>		<i>S. willdenowii</i>	
	700 m. asl	1000 m. asl	700 m. asl	1000 m. asl	Open forest	Shaded forest
Alkaloid	-	-	-	-	+	+
Phenol	+	+	+	+	+	+
Flavonoid	-	-	+	+	+	+
Tanin	+	+	-	-	+	+
Saponin	+	+	+	+	+	+
Triterpene	+	+	-	-	-	-
Steroid	+	+	-	-	+	+

Table 6. Antioxidant properties of *Histiopteris incisa*, *Nephrolepis biserrata* and *Selaginella willdenowii* leaves extract

Samples	Conc. (ppm)	Inhibition (%) (Mean ± SD)	IC ₅₀ (ppm)
<i>H. incisa</i> 700 m. asl	20	0.2352 ± 0.0002	96.4271
	40	22.0253 ± 0.1266	
	60	26.2023 ± 0.0002	
	80	37.5105 ± 0.0730	
	100	53.2911 ± 0.6698	
	1000 m. asl	20	
40		25.5695 ± 0.0004	
60		38.5232 ± 0.0731	
80		54.3461 ± 0.0734	
100		65.1477 ± 0.0731	
<i>N. biserrata</i> 700 m. asl	20	0.3795 ± 0.0004	85.1907
	40	11.0267 ± 0.1289	
	60	32.5738 ± 0.7309	
	80	45.8649 ± 0.3654	
	100	61.3922 ± 0.0002	
	1000 m. asl	20	
40		14.8100 ± 1.4598	
60		25.8649 ± 0.5269	
80		40.4219 ± 0.3867	
100		54.0084 ± 2.266	
<i>S. willdenowii</i> Open forest		20	2.4052 ± 0.0003
	40	12.7004 ± 0.0731	
	60	30.8860 ± 0.0127	
	80	43.9240 ± 1.2929	
	100	53.1645 ± 0.1934	
	Shaded forest	20	2.3004 ± 0.0003
40		11.7722 ± 0.0003	
60		24.9363 ± 0.0004	
80		34.1773 ± 0.0004	
100		51.2657 ± 0.2657	
Gallic acid		2	17.0885 ± 0.1266
	4	28.3121 ± 0.9832	
	6	37.1308 ± 0.0730	
	8	49.8312 ± 0.5981	
	10	62.2363 ± 0.0730	

Note: Conc.: Concentrations

Table 5. Phenolic compound analysis of *Histiopteris incisa*, *Nephrolepis biserrata*, and *Selaginella willdenowii* leaves extract

Sample	Phenolic content (mg GAE/g)			Mean ± SD
	1	2	3	
<i>H. incisa</i> 700 m.asl	8.7709	10.1430	10.6432	9.8523 mg GAE/g ± 0.9694
	16.1390	17.8173	20.4132	
1000 m.asl				18.1231 mg GAE/g ± 2.1535
<i>N. biserrata</i> 700 m.asl	18.0805	17.5288	17.0106	17.5399 mg GAE/g ± 0.5350
	8.7984	8.7336	9.0085	
1000 m.asl				8.8468 mg GAE/g ± 0.1437
<i>S. willdenowii</i> Open canopy forest	36.9999	39.6766	39.4498	38.7087 mg GAE/g ± 1.484
	18.5348	19.5823	19.5800	
Shaded canopy forest				19.2324 mg GAE/g ± 0.6041

Discussion

Indonesia, having a humid tropical climate is a good place to live for various ferns, one of the locations is Gunung Halimun Salak National Park. Based on observational data in the field it appears that all of the ferns studied can grow well in there. It was observed that the different ecological sites of ferns gave different physicochemical parameters. It was also noticed that *H. incisa* and *N. biserrata* found in open and in shaded forest locations with different altitudes between 700 and 1000 m. asl and *S. willdenowii* appeared to be two different vegetation ecotypes.

The presence and amount of phytochemical constituents are influenced by several factors both internal factors such as genes and external factors such as light, temperature, humidity, pH, nutrient content in the soil and altitude. According to Hoshizaki and Moran (2001), ferns in the tropics generally require a temperature range between 21-27°C to grow. Laily (2012) stated that the height of the place is one of the factors that influence the growth of ferns. So, it is suspected that the growth and development of the plants at different altitudes, will be different. As a result, a series of processes of metabolism in these plants will also be disrupted and the compounds produced will also be different.

Histiopteris incisa belongs to Dennstaedtiaceae family which is also known as the batwing ferns and is typical primary colonizer of disturbed ground such as in land clearings caused by tree falls, or in a forest that has been seriously damaged by browsing animals (De Lange PJ. 2020). The family Dennstaedtiaceae consists of 10 genera with about 265 pantropical and, occasionally, boreal or temperate regions species (PPG I 2016). Based on research results in its natural habitat, *H. incisa* was spread well in different locations with different height but found more in open areas. The temperatures at an altitude of 700 m. asl range between was 25.0-28.6°C, and at 1,000 m. asl was 24.1-29.8°C. From the results obtained states that environmental parameters at an altitude of 700 m. asl with 1,000 m. asl is not much different and the range of abiotic factors is suitable as *H. incisa* habitat. It was observed that the phenol content at an altitude of 1,000 m. asl. is higher. This may be caused by the presence of abiotic environmental factors such as altitude, light intensity, soil pH, humidity, and soil temperature. Environmental factors certainly will affect plant growth and development, thus also will regulate the biosynthesis of secondary metabolites. Phenolic constituents as one of the secondary metabolites induced antioxidant activity that affects IC₅₀ from *H. incisa* leaves extract. Both extracts were classified as having strong antioxidants.

Praptosuwiryo et al. (2019) found that *N. biserrata* tends to grow abundantly on palm trees plantation especially on upper zone of trees. Besides, it useful to maintain the land humidity and keep the soil from lack of water (Ariyanti 2016). In Mount Halimun Salak we found *N. biserrata* at two different heights of 700 and 1000 m.asl. Characteristics of the *N. biserrata* are located on the scales on the stip and the location of the sorus close to the edge of

the medial pinna. It has a narrow and thinly spread shell morphology, the edges of shell have fine hair (De winter 2003). The measurement results of one of the environmental factors, humidity show differences at two heights, this is because at 700 m. asl. was covered by pine tree vegetation which causes the temperature to decrease due to lack of light intensity so that the humidity increases, while at an altitude of 1,000 m asl. the measurement parameter is more open which causes the plant to be directly exposed to sunlight which will affect the humidity value. The determination of phenolic levels of *N. biserrata* showed that an altitude of 700 m. asl. higher than 1000 m altitude and affected to antioxidant activity and IC₅₀.

Selaginella willdenowii grows both in shaded and open forest areas in Gunung Malang Village, Gunung Halimun Salak National Park. However, basically, most of *Selaginella* grow under the forest canopy and is protected from direct sunlight, spreads well in tropical forest areas and thrives on the forest surface. In terms of morphology, the leaves of *S. willdenowii* that grow in shaded areas have a bright blue color, while those that grow in open areas are brownish-yellow (Figure 3); it could possibly because it can modify chloroplasts to encourage the exploitation of sunlight by forming non-optical structures which are schemochromic tydall blue colored, so that *S. willdenowii* have a green color with a blue flame when it grows in shaded place (Fox and Wells 1971). Based on adequate population, the range of environmental factors obtained indicates conditions suitable for *S. willdenowii* growth. The phenol content in the shaded forest is lower than the phenol content in the open forest. Irradiation factors will affect plant growth and development, thereby regulating the biosynthesis of secondary metabolites (Hyun et al. 2017). Antioxidant activity also shows results that are in line with phenol levels.

In conclusion, this research shows that all ferns tested gave significant antioxidant activity and was influenced by environmental factors. This research is also expected to be the initial data in the search for sources of compounds that are pharmacologically efficacious.

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