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Evaluasi Fitokimia dan Antioksidan dari *Piper hispidum* Sw. yang dikoleksi dari Desa Gunung Malang pada Vegetasi yang Berbeda

(Phytochemical and Antioxidant Evaluation of *Piper hispidum* Sw. Collected from Gunung Malang Village on Different Vegetation)

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Abstrak: Jamaican pepper (*Piper hispidum* Sw.) adalah anggota Piperaceae, tumbuhan liar, dan merupakan jenis tumbuhan invasif yang dilaporkan digunakan untuk menyembuhkan luka dan mengobati gejala leishmaniasis kulit. Penelitian ini bertujuan untuk mengetahui kandungan fitokimia, khususnya kadar fenolik dan aktivitas antioksidan *Piper hispidum* di dua kondisi abiotik yang berbeda di dalam hutan, yaitu hutan terbuka dan hutan ternanungi. Eksplorasi dilakukan di Desa Gunung Malang, Taman Nasional Gunung Halimun Salak (TNGHS). Metode ekstraksi menggunakan ultrasonik, asam galat sebagai pembanding pada pengujian fenol dan metode DPPH untuk pengujian antioksidannya. Hasil uji kandungan total fenol menunjukkan ekstrak etanol 70% daun *P. hispidum* Sw. yang ternaung adalah 17,4775 mg GAE/g dan di hutan terbuka adalah 12,6137 mg GAE/g sampel. Uji aktivitas antioksidan menunjukkan bahwa IC_{50} dari vegetasi tertutup dan terbuka berturut-turut adalah 94,8414 dan 94,3412 ppm dan serta asam galat sebagai pembanding sebesar 7,9817 ppm. Hasil penelitian menunjukkan bahwa perbedaan vegetasi dan faktor biotik dan abiotik dapat mempengaruhi perbedaan kadar senyawa kimia, nilai uji fenol vegetasi terbuka dan terpapar matahari memiliki nilai yang lebih rendah dibanding ternanung tetapi tidak signifikan mempengaruhi kapasitas antioksidan.

Kata kunci: antioksidan, fenol, *Piper hispidum* Sw., vegetasi terbuka, vegetasi tertutup.

Abstract: Jamaican pepper (*Piper hispidum* Sw.) is a wild plant, member of Piperaceae family and invasive plant species reported used to heal wounds and treat symptoms of skin leishmaniasis. This study aims to explore phytochemicals of *Piper hispidum*, especially phenolic content and antioxidant capacity, in two different abiotic condition of the forest, shaded and open area. Exploration of *P. hispidum* was conducted in Gunung Malang Village, Halimun Salak Mountain National Park (TNGHS). The sample was extracted with the ultrasonic method. The phenolic level and antioxidant activity were determined with Folin Ciocalteu and DPPH method where gallic acid was used as a standard. The total phenolic content assay showed 70% ethanol extract of *P. hispidum* Sw. leaves from the shaded and open forest were 17.4775 mg GAE/g and 12.6137 mg GAE/g of sample respectively. The antioxidant activity assay showed that IC_{50} of the sample from shaded and open forest were 94.8414 and 94.3412 ppm and gallic acid was 7.9817 ppm. This study showed that differences in vegetation and environmental abiotic factor may contributed in the amount of chemical compounds where open vegetation exposed to the sunlight had lower values than shaded but not significantly affected the antioxidant value.

Keywords: antioxidant, phenol, *Piper hispidum* Sw., phenol, open forest, shaded forest.

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INTRODUCTION

THE ENVIRONMENTAL abiotic factors in which a plant lives will affect the growth and chemical composition of the plant as well as the pharmacological activity obtained. Temperature, irradiation, soil pH, latitudes were some of the abiotic factors that can affect plant growth and metabolism. In the previous research, we reported that environmental abiotic factors play a role in the differences of phenolic compound level and antioxidant activity of ferns[1].

Based on a survey done in Gunung Malang Village, Halimun Salak Mountain National Park (TNGHS), West Java, *Piper hispidum* Sw. was found abundantly. Interestingly, this species was found in different types of vegetation, in the open and shaded forests.

The genus piper belongs to the family Piperaceae and consists of approximately 1,300 species in the Neotropics and an estimated 700 species in the old worlds tropic[2]. *Piper hispidum* is a swingle, a shrub native to the lowlands of Mexico, is a species of pantropical distribution, commonly found throughout both disturbed and forest sites[3]. Numerous species of piper have been widely used for various purposes in Indonesia, especially for cooking because of the distinctive aroma as well as for socio-cultural activities (rituals, signs of respect, etc.), ingredients for traditional medicinal herbs and potential for economic purposes[4].

Piper hispidum has been traditionally used to treat wounds and symptoms of cutaneous leishmaniasis, skin ailments, and stomach aches[5]. Michel et al. (2010) reported that *P. hispidum* has been used to treat aches and pains in Nicaragua, to regulate menstruation in Peru, and to treat urinary infections in the Amazon. In Peru, the crushed leaves were traditionally applied on the skin to heal wounds and to treat cutaneous leishmaniasis. Various states of America have been used *P. hispidum* for many ethnomedicinal purposes including snakebites, insect bites, head lice, amygdalitis and mouth sores, and as a skin cleansing, diuretic, teeth whitening, and antihemorrhagic agent[6].

In addition, a study about its pharmacological use has been done in various research. Navickiene et al. (2000) reported the antifungal activities of amide isolated from its species[7]. The *P. hispidum* leaf extracts enhanced the expression of estrogen-responsive reporter and endogenous genes in MCF-7 cells, demonstrating estrogen agonist effects[3]. Lipophilic extracts of *Piper hispidum*, proved to be active against both a chloroquine-sensitive and a resistant strain of *Plasmodium falciparum*[8].

Leaves of *P. hispidum* showed antibacterial effect against *Candida albicans* and *Staphylococcus aureus* and gave inhibition of planktonic cells also inhibits biofilm cells. Furthermore, three chalcones compound isolated from leaves extract of *P. hispidum* and reported contains amide compounds, chalcone, phenylpropanoid, lignans, alkaloids, and flavones. In addition, *P. hispidum* Sw. leaves also contain butenolide compounds[9,10].

A large number of utilizations of this plant for various purposes of traditional medicine in different countries shows the importance of cultivating it further by paying attention to environmental abiotic factors that are suitable for its growth. Plants consist of multiple compounds that are related to its pharmacological activities produces. Observing the environmental conditions and nutrients that affect plant physiology is expected to control the pharmacological activities. Therefore, the present study aimed to investigated the effect of abiotic factors on open and closed forest vegetation of *P. hispidum* collected from Gunung Malang village on phenol total levels and antioxidant activity.

This area has high diversity of vegetation, which was around 700 species[11]. In addition, the species have various benefits, including for food and medicinal ingredients, especially for residents around the area, as well as building materials[11,12].

MATERIAL AND METHODS

MATERIAL. Plant collection and authentication. 2 kg of Piper hispidum Sw. leaves each taken based on differences in shaded and open forest vegetation in Gunung Malang Village, Bogor, West Java, and confirmed for its identity with the determination process in LIPI Cibinong.

Chemical reagents. Folin-Ciocalteu, gallic acid, DPPH were purchased from Sigma Aldrich. Sodium carbonate, ethanol was obtained from Merck Chemical Supplies. All chemicals used were analytical grade.

Instrument. Total phenol and antioxidant were measeured with Spectrofotometer UV-Vis (UV-1601 series, Shimidzu, Kyoto, Japan).

METHODS. Determination of Environmental Abiotic Parameter. Environmental abiotic parameter 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 GPS devices.

Extraction. Leaves powders were extracted with ethanol using an ultrasonic method with 70% ethanol at intervals of 30 minutes and repeated several times until the extraction was completely marked by colorless filtrate. The extraction results are filtered and evaporated in vacuo with a rotary evaporator at 50 °C to yield concentrated extract.

Extract Characterization. Characterization parameters of the extract include organoleptic examination, drying loss, and ash content were observed. The extract characterization method was performed by standard procedures in Indonesian Herb Pharmacopoeia[13].

Phytochemical Screening. Phytochemical analysis of this extract was conducted qualitatively to identified the presences of alkaloids, phenol, flavonoid, tannin, triterpenes, steroid, and saponin according to procedures in Indonesian Herb Pharmacopoeia (2008) [13] and Hanani et al. (2018)[14].

Total Phenol Assays. The Analysis was done according to Folin-Ciocalteu method performed by Stankovic et al. (2011) with a few modifications. Ethanol leaves extract 0.5 mL (1000 ppm), 2 mL Folin Ciocalteu and 4 mL Na_2CO_3 1 M reagent were mixed homogeneously. The mixed solution was incubated in an operating time range that has been determined before by scanned the absorbance of gallic acid in the range of 1 to 120 minutes, the time indicating a stable absorbance was determined which is obtained at 100 minutes. 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). The assay was carried out in triplicate and the phenolic level obtained was counted as equivalent to standard gallic acid[15].

Antioxidant Activity Assays. Antioxidant capacity of *P. hispidum* leaves extract was analyzed with DPPH method which is performed by Molyneux (2004). the absorbance of the blank solution was determined first by dissolving two mg of DPPH in 100 mL of MeOH, added 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 with maximum absorbance (λmax). 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). The assay was carried out in triplicate[16].

RESULT AND DISCUSSION

Plant Determination. Plant determination was carried out at the Herbarium Bogoriense, Botanical Center of LIPI Bogor. The results of this determination indicated that the plant used in this study was the *Piper hispidum* Sw. which is included in the Piperaceae family.

Determination of environmental abiotic parameter. The conservation area of Mount Halimun-Salak National Park is located in three districts, Bogor, Sukabumi Regency West Java Province and Lebak Regency in Banten province. The topography of the area is generally hilly and mountainous, located in an altitude range between 500-2,211 m.asl.[12]. A sampling of *Piper hispidum* was taken both in the open and shaded forest in Gunung Malang Village included in Halimun Salak Mountain National Park (TNGHS). The location was chosen purposively considering the population of *P. hispidum* that are abundant.

The survey was conducted in March 2019, and founded *P. hispidum* at two different vegetation in the open and shaded forest. Environmental abiotic factors were analyzed to observed the correlation with chemical compound and antioxidant studied and the result present in Table 1. Besides that, morphology of *P. hispidum* in origin ecosystem also observed and present in Table 2 and Figure 1.

Table 1. Environmental abiotic factor of open and shaded forest vegetation.

Environmental parameters	Open forest	Shaded forest
altitude	868 m. asl	889 m. asl
light intensity (LUX)	4765–20000	538-9202
air humidity (% RH)	48.94-67.77	58.5-74.4
air temperature (ºC)	26.94-35.64	25.8-29.5
soil temperature (ºC)	21.3-23	22-23.3
soil pH	7.5	7.5
soil humidity	Dry	Wet
coordinate	SL 06°40’27.7” BT 106°43’27.7”	SL 06°40’29.4” EL 106°43’28”

Table 2. Morphology of *Piper hispidum* Sw.

Criteria	Albeiro *et al.* (2006), Backer & Brink (1963)	Result
stem	rounded and hairy	rounded and hairy
stalk	short	short
leaf	oblong	ovate oblong
leaf surface	halus	smooth
leaf tip	acuminate	acuminate
leaf base	-	rounded
leaf edge	entire	entire
fin-type leaves	-	pinate
whole leaf	-	petiolate
leaf position	-	alternate
spike flower color	whitish, greenish-white	whitish, greenish-white

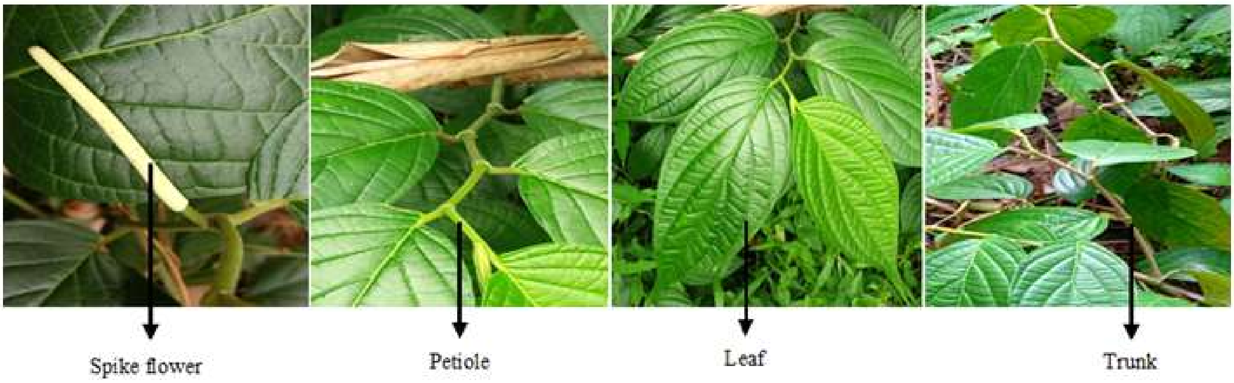

Figure 1. *Piper hispidum* morphology .

Extraction and Characterization of Extract. Extraction of piper leaves with 70% ethanol using the ultrasonic method from two different vegetation, open and shaded forests, obtained extract yields of 14.96 and 14.52%, respectively. The character of the extract studied was ascertained by analyzing the parameters of drying loss and total ash content. The result present in Table 3.

Table 3. The measurement results of the extract standardization parameters.

Parameters	Open forest	Shaded forest
drying loss	5.32%	6.36%
total ash content	7.59%	5.33%

Phytochemical Screening. Phytochemical screening assays showed both concentrated extract of piper leaves gave positive results for phenolic, flavonoid, tannin, alkaloid, and saponin.

Total Phenol Assays. Phenolic level assay carried out to observed the relation of environment abiotic parameter to the chemical compound of sample collected in two different vegetation. The phenolic level was determined with Spectrophotometry Uv-Vis using Follin-Ciocalteu method at a wavelength of 759.5 nm and gallic acid used as a standard. Operating time aims to determine the right time span to read the absorption in a sample of the solution so as to produce a stable absorbance value so that it can maximize the measurement[17]. The absorption obtained was stable at 100 minutes. The calibration curve of a standard solution was analyzed first and the result shown in Figure 2. The total phenol content of each sample is present in Table 4.

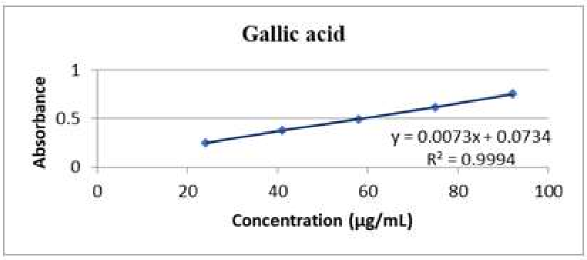

Figure 2. Calibration curve of gallic acid .

Table 4. Total phenol analysis of *Piper hispidum* leaves extract in two vegetation.

Samples	Phenolic content (mgGAE/g)			Mean (mgGAE/g)
	1	2	3	
open forest	12.5367	12.5775	12.7270	12.6137
shaded forest	16.9261	17.6818	17.8248	17.4775

Antioxidant Activity Assays. The antioxidant capacity of each extract was analyzed with the DPPH method and measurement was done at wavelength 516 nm with the absorbance of DPPH was 0.790. the result was shown in Table 5.

Table 5. Antioxidant activity of P. hispidum leaves extract and gallic acid.

Samples	Concentrations (ppm)	Inhibition (%) (mean±SD)	IC_{50} (ppm)
open forest	20	2.5316±0.1266	
	40	16.9619±0.2531	
	60	24.5147±0.4445	94.3412
	80	43.4598±0.3185	
	100	53.0379±0.2531	
shaded forest	20	6.9619±0.5063	
	40	19.5358±0.3185	
	60	30.7172±0.3867	94.8414
	80	42.8691±0.5270	
	100	51.6877±0.3185	
gallic acid	2	17.1307±0.0730	
	4	28.3121±0.9832	
	6	37.1307±0.0730	7.9817
	8	49.8311±0.5982	
	10	62.2380±0.0747	

Mount Haimun Salak is one of the largest mountainous areas with high biodiversity. With a variety of species that grow so that it is widely used for various functions by the community, especially local residents. So that a survey is carried out to explore plant species, especially those that provide benefits for medicinal substances. Interestingly, on the survey trip, the species of piper was found in two different places.

Piper is one of widely distributed plant genera in pantropical regions and interesting genus to study. Piper plants are also known with the common name " Jamaican pepper". According to Salleh et al. (2014), genus piper, which belongs to the Piperaceae family, consists of five subgenera and roughly 1400 species distributed throughout the tropical and subtropical regions(18). The presence of oil cells in the structures of almost all Piper species places them in the group of aromatic plants. Besides their well-known uses as culinary spices, the secondary metabolites isolated from piper plants show wide-ranging human health effects(5).

Plants take advantage of nature for their live, in addition, with ingredients from nature, various secondary metabolite compounds are produced. various studies have proven changes in the environment in which it grows can affect the compounds of plants. Studies showed that changes in environmental factors can greatly affect carbon and nitrogen cycling in terrestrial ecosystems which will be related to the physiology of plants(19,20). Environmental factors certainly will affect plant growth and development, thus also will regulate the biosynthesis of secondary metabolites(1). For most plants, external factors or variables (light, temperature, groundwater, soil fertility, and salinity) can significantly influence several processes associated with plant growth and development, even their ability to synthesize secondary metabolites, which in turn leads to change of the whole phytochemical profile which plays a strategic role in the production of bioactive substances(21,22,23).

Present studies focusing on studying the phenol level of plants and antioxidants as one of the related pharmacological activities produces. The character of the extract studied was confirmed by testing the drying loss and ash content parameters. Determination of drying loss levels aims to measure the amounts of substances lost during drying where this parameter will also be affected by the amount of other volatile substances such as essential oils. The ash content is the amount of inorganic matter remaining in the sample that is reconstructed. Ash content determined by ash or burning the sample in a furnace at a temperature of 500-600 ºC until the sample turns to ash. Ash content testing aims to provide an overview of the internal and external mineral content from the beginning until the extract is formed[13].

Current study showed that even if both samples have similar chemical compositions but the phenol level was different in the open and shaded forests where shaded was higher than open. Irradiation as one of the abiotic factors that are contributed to plant metabolism but its study shows the contribution of other factors that influence phenol levels. Even though the antioxidant was not significantly different, IC_{50} of the shaded forest still higher than the open.

Chemical compounds in plants are the product of metabolism and plant growth, variations in secondary metabolite are one way for plants to interacted and adapted to biotic and abiotic factors even in unfavorable situation[24]. Early studies showed that the duration of light radiation had a predominant role in regulating the amounts of various phenolic phenylpropane derivatives in the *Xanthium species*[25].

Indole alkaloid, camptothecin able to respond the stress condition of the environment and its amount was varied under light irradiation conditions[26,27]. The anthocyanin content of *Daucus carota* cell cultures was significantly enhanced by the UV irradiation[28]. Various phenolic compound gave different respond to the Uv light exposure with different of concentration[29-31]. This phenomenon is expected to be a consideration in the cultivation of *Piper hispidum* in a certain area. so that bioactive compounds that are closely related to their pharmacological effects can be maximally produced.

CONCLUSION

Plants always adapt during their life so that able to survive the conditions in which its grow. Changes in environmental conditions will definitely provide different physiological responses to plant metabolism which have an effect on the compounds produced. Biotic and abiotic factors around the environment where plants grow support the physiological processes that occur as well as the compounds produced. Present study showed that besides light radiation was one of the important factors in photosynthetic process, the contribution of other biotic and abiotic factor may affect the phenolic levels and antioxidant activity of *Piper hispidum* Sw. So that, establish the environmental condition where it grows was the main concern in the cultivation.

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