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Comparison of Secondary Metabolite Content of *Pteris vittata L.* in Baluran National Park and Malang and Its Effect on Environment

Eko Sri Sulasmi[1], Uun Rohmawati[1], Achmad Makin Amin[1]

[1]*Department of Biology, Universitas Negeri Malang, Semarang Street 5, Malang, Indonesia*

[*]*Corresponding author*
Email: : eko.sri.fmipa@um.ac.id
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Abstract

Fern is cosmopolitan plants which are almost scattered in all parts of the world, one of which is found in Baluran National Park and in Malang. The potential and benefits of these ferns are quite important for agriculture and medicine because of the chemical compounds they have, especially in Pteris vitatta L. This study aimed to analyze secondary metabolites contained in P. vittata L. in Baluran National Park, Situbondo and in Malang. The samples used were leaves and rhizome P. vittata extracted using methanol 96%, followed by a qualitative test of the content of alkaloids, flavonoids, terpenoids, polyphenols, tannins, saponins, using phytochemical screening methods with several reagents. The results showed that the leaves and Rhizome P. vittata L.. in Baluran National Park, Situbondo were positively containing secondary metabolites of terpenoids, polyphenols, tannins, saponins, and alkaloids (Dragendorf and Bouchardat reagents), whereas flavonoids were not present in all samples. However, the results of P. vitatta L. phytochemical screening around Malang State University positively contained flavonoids, polyphenols, terpenoids, alkaloids (Wagner and Dragendorf reagents). The difference in results from these two places is because the secondary metabolite content in plants is affected by stressful environmental conditions such as soil texture where it is grown or is affected by the precursors of the secondary metabolites of the metabolites.

1. INTRODUCTION

Indonesia is a tropical country with a biodiversity of flora, one of which is P. *vittata* L.. P. *vittata* L.. is a cosmopolitan plant that is very widely distributed ranging from tropical and subtropical ecosystems. P. *vittata* L.. commonly grows along the roadside, in almost all calcareous substrates such as rocks,

sidewalks, and building gaps, but it can also live on savanna ecosystems, mixed tropical forest ecosystems and mountains to an altitude of 1800 m dpl (Mumpuni, 2016). The widespread of P. *vittata* L.. has the potential to be used as a plant that can increase economic value, used as antimicrobial activity, antioxidant and arsenic hyperaccumulator.

Compounds that have activities as antimicrobials, antioxidants in P. *vittata* L. plants are secondary metabolites. These secondary metabolites are only produced under certain conditions. This difference in the place of growing P. *vittata* L.. will affect the content of the compound and its pharmacological activity. According to Salim (2016), the secondary metabolites are produced in certain organisms in specific conditions.

Based on the above reasons, the researchers intend to compare the two types of plants P. *vittata* L.. namely P. *vittata* L.. which comes from Baluran National Park and P. *vittata* L. which grow in the Biology environment of Universitas Negeri Malang. The condition of Baluran National Park is extreme, which often changes according to the global conditions that affect it. The environmental conditions of the National Park Baluran temperature between 27 - 32 ^{0}C, air humidity 77%, while at the Universitas Negeri Malang the temperature between 27-28 ^{0}C and air humidity 55%. This study aims to see the relationship of environmental conditions to secondary metabolism in P. *vittata* L.

2. MATERIALS AND METHODS

Sample Preparation

The sample of leaves and rhizome P. *vittata* L. used in this research were collected randomly from three spots of the Baluran National Park. Collection of plant samples was carried out on 12-15 April 2018. The samples obtained were then identified in the Malangensis herbarium of the State University of Malang. The fresh sample was washed and then air dried and mashed to get powder using the blender or grinder. The powder is stored for the extraction stage.

Extraction

In the extraction of leaves and rhizome P. *vittata* L.. this was done by maceration method using 96% methanol. Maceration is done 3 times, where the results of the filtrate obtained are evaporated for 3 hours to get the pure extract.

Phytochemical Screening

Phytochemical screening in this research follows the standard method Harborne (1984). Phytochemical screening includes tests of flavonoids, terpenoids, polyphenols, gallate tannins, catechol tannins, saponins, and alkaloids.

Flavonoid Test

Take 2 mL of sample extract and add 8 mL of heated aquades. Then filtered and added concentrated HCl a few drops and some Mg powder. Positive results are indicated by changes in dark red or pink.

Terpenoid Test

2 ml of sample extract was added 8 ml of heated aquadest and 3 drops of bouchardat were added. If the result is positive for steroid terpenoids, the color changes to bluish green, and if the brownish color contains triterpenes.

Polyphenol Test

The methanol extract was put into a test tube as much as 2 mL and added 8 mL of heated aquadest. Then filtered and added 3 drops of $FeCl_3$. Positive results are blackish brown, blue or green.

Tanin Test

The sample extract was put into two test tubes, where each test tube was filled with 2 g of powder samples, then for the test of gallic tannin extract, the sample was added 20 mL of heated aquadest for ± 10 minutes. Then filtered and then added 1% sodium acetate and

$FeCl_3$. Positive results are blue, purple or black. Whereas procedur catechol tanin test is 2 g sample extract was added 8 mL of heated aquadest for ± 10 minutes. Then added 3% formaldehyde and concentrated HCl (4: 2). Positive results are red sediment.

Saponin Test

2 mL of methanol extract added 8 mL of heated water for ± 10 minutes and filtered. Then add 2 mL of hot water after it is shaken. Positive Results: permanent foam forms for no less than 10 minutes as high as 1-10 cm.

Alkaloid test

2 mL of extract sample was added 8 mL of heated water for ± 10 minutes, filtered and put into three test tubes. Added 6 drops of Meyer Reagent to the first test tube, 6 drops of Dragendrof Reagent on the second test tube, and 6 drops of Bouchardat Reagent on the third test tube. Positive Results: there is white sediment on Alkaloids with Meyer Reagent, there is orange sediment on Alkaloids with Dragendrof Reagents, and there are brown deposits on Alkaloids with Bouchardat Reagent.

Thin Layer Chromatography Test

2 g of extract sample was added to 10 mL of Ethanol P.A, then filtered and put into a test tube. The sample is sprayed in a stationary phase (silica gel 60F254) and then eluted using each mobile phase according to the compound identification. TLC results are checked under the TLC Scanner so that the Rf (retention factor) is identified.

Identification of Flavonoids

The mobile phase used in the identification of flavonoids is acetyl acetate: formic acid: aquadest (85:10:15). A positive reaction is indicated by the formation of a graph with a certain Rf value. Flavonoids of Quercetin Rf type 0.85-0.90, Hyperoside Rf 0.45-0.50, Quercitrin Rf 0.60-0.65, Routine 0.25-0.30.

Identification of Tanin

The mobile phase of n-butanol: acetic acid: aquadest (4: 1: 5). A positive reaction is shown by the Rf value which ranges from 0.70 to 0.80

Identification of Polyphenols

Mobile phase Toluent: Etyl acetate (93: 7). A positive reaction is indicated by the formation of Rf 0.25 - 0.35

Identification of Terpenoids and Saponins

N-Hexane: Etyl acetate (4: 1) mobile phase. A positive reaction is indicated by the formation of Rf 0.20-0.25.

Identification of Alkaloids

The mobile phase of Methanol: NH4OH (200: 3). Positive reactions are indicated by the Rf value of around 0.55 - 0.75

3. RESULTS

Based on the results of phytochemical screening showed that P. *vittata* L. in Baluran National Park with P. *vittata* L. at Malang State University there were differences in secondary metabolite content. P. *vittata* L.. at Malang State University contains secondary metabolites of flavonoids, terpenoids, polyphenols, and alkaloids, whereas P. *vittata* L.. in Baluran National Park does not contain flavonoids. The absence of flavonoids in P. *vittata* L.. in the National Park is due to both abiotic and biotic factors that affect the formation of secondary metabolites. Factors that can affect the production of secondary metabolites are the composition of culture media, temperature, light, humidity, genetic factors and environmental stress (Rao, 2002).

4. DISCUSSION

P. *vittata* L.. in general, can grow at alkaline pHs such as areas contaminated with arsenic (Ma et al. 2001) and copper (Zheng and Xu, 2008). The productivity of P. *vittata* L. secondary metabolites is influenced by soil texture and nutrient content. Soil texture in

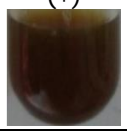
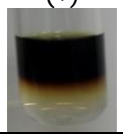
Baluran National Park has a clay sandy soil texture (Figure 1) while in the area of Malang State University has sandy clay soil (Figure 2). Different soil textures are likely to affect the element's nutrient content. The pH of the soil in Baluran National Park and in Malang in this study is almost the same, which is 6

Figure 1. Baluran soil texture

Figure 2. Malang Land Texture

Soil nutrients give affect the formation of secondary metabolites. It was found that the results of phytochemical screening of P. *vittata* L. in National Park Baluran had not detected flavonoid and P. *vittata* L. candles at the State University of Malang contained flavonoids. The differences in these compounds were influenced by the precursors of their secondary metabolite biosynthesis and soil texture in the place where P. *vittata* L.. was grown. The presence of flavonoid compounds in P. *vittata* L. extract at Malang State University made it possible for high soil nutrients to contain Ca. P. *vittata* L.., which lives in nutrients that are poor in Calcium, will also produce a small number of secondary metabolites, especially flavonoids. This is because the function as an enzyme activator, where there are three important enzymes involved in the biosynthesis pathway of phenolic compounds including flavonoids, and acts as a protective enzyme against various environmental stresses, namely POD (peroxidase), PPO (polyphenol oxidase) and PAL (phenylalanine amonialyase) (Ningsih, 2014).

Table 1. Results of phytochemical screening *Pteris vittata* extract in Baluran National Park and Malang State University environment.

Test		Extract of *Pteris vittata*	
		Baluran	**Malang**
Flavonoid		Negative (-) 	Positive (+) 
Terpenoid		Positive (+) 	Positive (+) 
Polyphenol		Positive (+) 	Positive (+) 
Alkaloid	Meyer	Positive (+) 	Negative (-) 
	Dragendorf	Positive (+) 	Positive (+) 

5. CONCLUSION

The environment in which *P. vittata* L.. is grown (nutrient content and soil texture) affects the formation of secondary metabolites

6. SUGGESTION

Further research is needed regarding the chemical content of soil and soil nutrients contained in the soil of both places

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