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Flavonoid Characterization in Ferns from Baluran National Park

Eko Sri Sulasmi*, Zauhara Faiqohtun Wuriana, Ratna Suryaningtya Sari, Uun Rohmawati

Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Malang, Jl. Semarang 5 Malang 65145, Indonesia

*Corresponding author: eko.sri.fmipa@um.ac.id

Abstract: Flavonoids belong to the largest group of a phenolic compound found in plants. The presence of these flavonoids is significant for the plants themselves such as protecting plants from UV-B, insect pests, biotic, and abiotic stresses. More than 10,000 varieties of flavonoids have been already identified. Several plants can synthesize flavonoids, one of which is ferns. Cosmopolitan ferns are found in tropical and subtropical regions like Baluran National Park. The ecosystem in Baluran National Park has components that can show different sensitivity level to the factors influencing the environment, either in terms of biotic and abiotic factors. Same species of plants in different environments can be different significantly in their secondary metabolite content. The primary purpose of this research was to determine the types of flavonoids ferns in Baluran National Park. This study was conducted in April-June 2018. The extraction of samples was conducted using 96% methanol solvent and the analysis using the thin layer chromatography. The results showed the fourth type of flavonoid rutin, hyperoside, quercitrin, and quercertin contained in *Pseudocyclosorrus ochthodes* (Kunze) Holttum, *Phymatodes scolopendria* (Burm.) Ching. *Stenochlaena palustris* (Burm.), except routine not found in *Dryopteris hirtipes* (Bl.) Kuntze Beddome. *Pteris vittata* L. does not contain all four types. Types of flavonoids from ferns in Baluran National Park are routine, hyperoxide, quercitrin and quercetin.

Keywords. Flavonoids, Fern, Thin Layer Chromatography, Baluran National Park

1. Introduction

Flavonoids are the largest group of a phenolic compound found in plants. The presence of these flavonoids is essential for the plants themselves such as protecting plants from UV-B [1], insect pests, biotic and abiotic stresses [2], microbial defensive compounds [3]. Over 10,000 varieties of flavonoids have been identified such as isoflavones, flavanols (quercetin, routine, kaempferol, myricetin, and fisetin), flavanones (hesperitin, naringin, hesperidin), flavones (tangeretin, apigenin, and luteolin), chalcones (arbutin, phloretin), flavanols, anthocyanins, dihydroflavonols, etc [4–7]. Various types of flavonoids have the benefits and functions as antibacterial [8] and antimutagenic activity such as apigenin, flavones, flavanones, isoflavone, quercetin [9]. Types of flavonoids that function as anti-inflammatory and analgesic effects are luteolin, hesperidin, quercetin and apigenin [10]. Flavonol glycosides have been potent antioxidant [11]. The various types of flavonoids are not all found in one plant. Several plants only synthesize flavonol namely ferns [12]. Types flavonoids flavones and biflavones contained in primitive herbaceous ferns [13].

Over 2000 years, Pteridophytes have known to humans to have a medicinal value compared to Angiosperms, but very little application of these plants in modern world medicines [14]. Ferns can be used as a medicinal ingredient for a component in a variety cosmetic, pharmaceutical, and medicine including anticancer [15], anti-inflammatory, antitumor, antineoplastic [10] because it contains flavonoids such as flavonol, biflavones, and flavones. *Pteris vittata* from Guangzhou China has four types of flavonoids such as kaempferol, quercetin, kaempferol3-o-d-glucopyranoside and routine [25]. *Cheilanthes tenuifolia* has two types of flavonoids such as rutin and quercetin [26]. *Dryopteris erythosora* has ten types of flavonoids such as Dihydromyricetin, 7-hydroxyflavone, biochanin A, formononetin, apigenin, kaempferide, myricetin, daidzein, catechin and daidzin [27]. Flavonoids secondary metabolites contained in ferns was influenced by biotic and abiotic factors like environmental factors including temperature, light intensity, water availability, type and composition of soil which influence the productivity and quality of these medicinal plants [16]. Ferns is a cosmopolitan was found in tropical and sub-tropical regions like Baluran National Park, East Java Indonesia [17].

Based on the Review Report of Flora Potential of Baluran National Park in 2013, there are 13 types of ferns in Baluran National Park. Some species that have been found *Pseudocyclosorrus ochthodes* (Kunze) Holttum, *Dryopteris hirtipes* (Bl.) Kuntze, *Phymatodes scolopendria* (Burm.) Ching., *Pteris vittata* L. and *Stenochlaena palustris* (Burm.) Beddome. Baluran National Park has a dry climate type with temperatures ranging between 27.2 – 30.9 °C with an average humidity at 77%. The ecosystem in Baluran National Park has varied greatly which causes vulnerability to be biotic and abiotic factors [18]. Extreme weather conditions in Baluran National Park will affect the production of secondary metabolites of plants there. Secondary metabolites can be different even though the plants is a species but different in their environment [12]. Although some previous studies have been done to explore the fern flavonoids profile in various environments, there is still lack information about the flavonoid's characteristics in ferns particularly origin from Baluran National Park with their specific extreme.

2. Methods

This study was conducted in April- June 2018. The fern samples collected originated from Baluran National Park, Situbondo, East Java and analyzed the sample in Materia Medica, Batu-Malang, East Java Province. The samples used were identified in the Herbarium Malangensis of Universitas Negeri Malang. Accordingly, the alignment data showed that the species for this samples were *Pseudocyclosorrus ochthodes* (Kunze) Holttum, *Dryopteris hirtipes* (Bl.) Kuntze, *Phymatodesscolopendria* (Burm.) Ching., *Pteris vittata* L. and *Stenochlaenapalustris* (Burm.) Beddome. The samples were dried and then mashed by blending or grinding then extracted with 96% methanol solvent. Extraction of samples used 96% methanol solvent until 24 hours. The methanol extract then was filtered, using filter paper and then evaporated used a rotary vacuum evaporator until obtained a thick extract. In the final step, the extract was stored in a refrigerator with a temperature at ± 4 °C.

The identification of flavonoids was made by thin layer chromatography method. About 2 grams of the samples (extract) was added with 10 mL of ethanol P.A. and filtered into a test tube. The samples were plotted in a silica plate 60F254 at a distance of 1 cm from the bottom line and 1 cm from the edge. Next, this samples were eluted by using ethyl acetate: format acid: sterile distilled water with composition (85: 10: 15) respectively. The Rf value was calculated by Camag TLC Scanner 3, and the final data were analyzed qualitatively.

3. Results and Discussion

The morphology feature of ferns of our samples from Baluran National Park is presented in Figure 1.

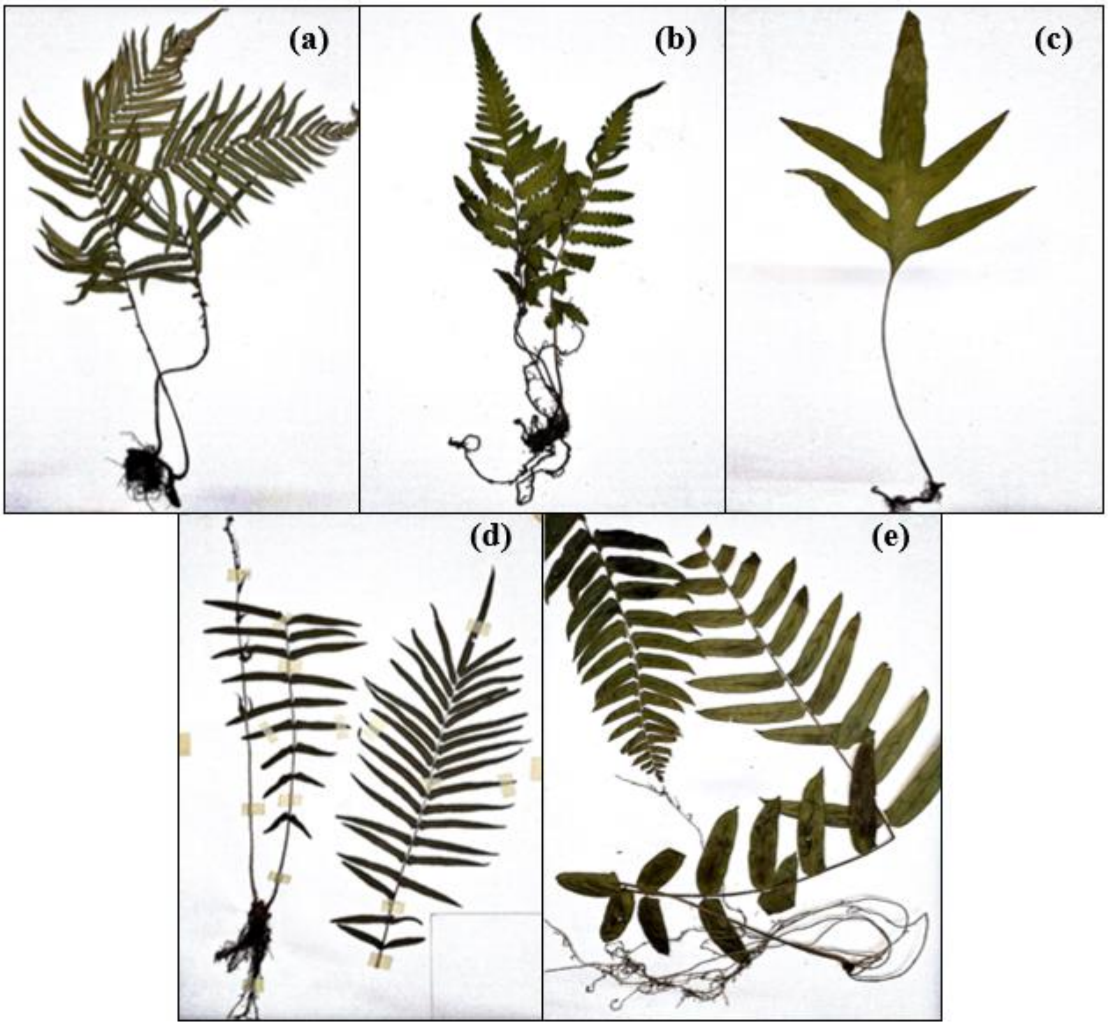

Figure 1. The morphology of ferns from Baluran National Park samples. (a) *Pseudocyclosorus ochthodes* (Kunze) Holttum, (b) *Dryopteris hirtipes* (BI.) Kuntze, (c) *Phymatodes scolopendria* (Burm.) Ching, (d) *Pteris vittata* L., (e) *Stenochlaena palustris* (Burm.) Beddome.

Based on Figure 1. the fern morphology from Baluran National Park including *Pseudocyclosorus ochthodes* (Kunze) Holttum has frond long, sori always distinctly sepramedial with a few short hairs and sometimes glands [19]. The morphology of *Dryopteris hirtipes* (BI.) Kuntze has rhizome short and brown color, stipes stramineous densely scaly at base more sparsely upwards, rachis grooved on upper surface densely scaly throughout with narrow, sori in two or three indistinct rows near costa. *Phymatodes scolopendria* (Burm.) Ching has rhizome widely, rhizome scales dark brown, fronds widely spaced, sori in 1 until 2 regular to irregular lines on either side of the costae, slightly sunken into lamina [28]. *Pteris vittata* L. has short scaly rhizomes and light brown scales, stipes solid scaly at the bottom and the lamina imparipinnate, the lower simple pinnae gradually be smaller down for only the middle or upper auricles that are linear, terminal pinnae usually much longer, rachis notched on the upper surface, smooth scaly, branched veins, sori marginal continuous along margin of pinnae. *Stenochlaena palustris* (Burm.) Beddome has rhizome long climbing on tree trunks, frond long, pinnae up to 15 pairs, opposite, simple or branched veins form a series of narrow coastal areola/pinnae with sporangia throughout the lower surface. The results of flavonoids analysis by thin layer chromatography from 5 fern species in the Baluran National Park test was presented in Table 1.

Table 1. Flavonoids type by thin layer chromatography method from fern species in Baluran National Park

Samples	**Flavonoids**	
	Rf	**Type**
***Pseudocyclosorus ochthodes* (Kunze) Holttum**	0. 14- 0.31	Rutin
	0.33- 0.48	Hiperoside
	0.60- 0.81	Quercitrin
	0.81- 0.89	Quercetin
***Dryopteris hirtipes* (BI.) Kuntze**	0.32 – 0.45	Hiperoside
	0.58 – 0.71	Quercitrin
	0.88 – 0.96	Quercetin
***Phymatodes scolopendria* (Burm.) Ching**	0.17 – 0.28	Rutin
	0.32 – 0.47	Hiperoside
	0.59 – 0.72	Quercitrin
	0.87 – 0.94	Quercetin
***Pteris vittata* L.**	-	-
***Stenochlaena palustris* (Burm.) Beddome.**	0.19 – 0.28	Rutin
	0.40 – 0.48	Hiperoside
	0.50 – 0.62	Quercitrin
	0.79 – 0.88	Quercetin

Based on Table 1, *Pseudocyclosorus ochthodes* had 4 positive spots from flavonoid compounds. The spot was identified around Rf 0.14- 0.31, 0.33- 0.48, 0.60- 0.81, and 0.81- 0.89. *Dryopteris hirtipes* (BI.) Kuntze had 3 positive spots of flavonoid compounds. The spot was identified was around Rf 0.32 - 0.45, 0.58 - 0.71 and 0.88 - 0.96. *Phymatodesscolopendria* (Burm.) Ching had 4 positive spots from flavonoid compounds. The spot was identified around Rf 0.17 – 0.28, 0.32 – 0.47, 0.59 – 0.72, and 0.87 – 0.94. *Pteris vittata* L. does not contain flavonoid compounds which prove that the value of Rf is not detected in the test. *Stenochlaenapalustris* (Burm.) had 4 positive spots from flavonoid compounds. The spot was identified around Rf 0.19 – 0.28, 0.40 – 0.48, 0.50 – 0.62, and 0.79 – 0.88. The flavonoid types in ferns from Baluran National Park was proven in the chromatogram spectrum in Figure 2.

Based on Figure 2, *Pseudocyclosorusochthodes* had 4 groups of flavonoid compounds with different polarity, as evidenced by the presence of 4 chromatogram peaks, namely routine, hyperoside, quercitrine, and quercertin. *Dryopteris hirtipes* (BI.) Kuntze as evidenced by the presence of 3 chromatogram peaks, namely the type of hyperoside, quercitrine, and quercertin. *Phymatodesscolopendria* (Burm.) Ching, evidenced by the presence of 4 chromatogram peaks, namely routine, hyperoside, quercitrine, and quercertin. *Stenochlaena palustris* (Burm.) as evidenced by the presence of 4 chromatogram peaks, namely routine, hyperoside, quercitrine, and quercertin.

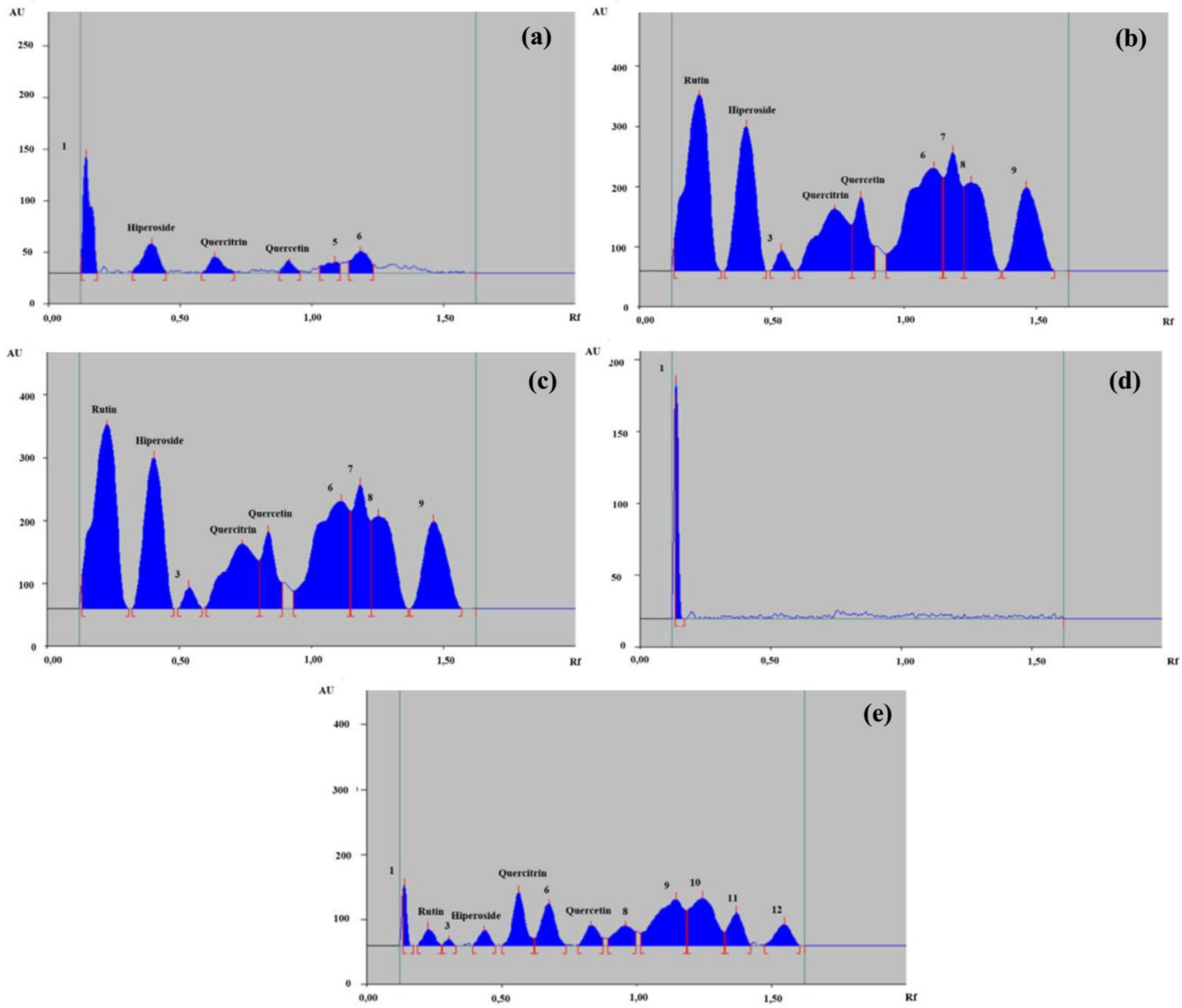

Figure 2. The chromatogram spectrum of ferns from Baluran National Park samples. (a) *Pseudocyclosorus ochthodes* (Kunze) Holttum, (b) *Dryopteris hirtipes* (BI.) Kuntze, (c) *Phymatodes scolopendria* (Burm.) Ching, (d) *Pteris vittata* L., (e) *Stenochlaena palustris* (Burm.) Beddome.

Each plant species has a different type of flavonoids [19]. Each type of flavonoids in a variety of plant species has its respective roles towards abiotic pressure and interactions with other organisms are herbivores and pathogenic microbia [20]. Environmental factors can affect the type and content of active substances. In this study, *Pseudocyclosorus ochthodes*, *Phymatodes scolopendria,* and *Stenochlaena palustris* have the same four types of flavonoids, *Dryopteris hirtipes* only has three types of flavonoids, while Pteris vittata L. does not contain flavonoids. This difference can be influenced by several environmental factors, such as temperature, light and, soil water content that affect the biosynthesis of flavonoids. Climatic factors often have a very large effect on biosynthesis and the quality of secondary metabolites in plants [21]. Plants cannot be separated from environmental influences such as light, temperature, and drought [22], temperature stress can cause to some biochemical, molecular, and physiological changes in plants metabolism such as protein denaturation or impaired membrane integrity.

Some of these changes can be effect in changes in the concentration of secondary metabolites in plant tissues which are often used as indicators of a stress injury in plants. Ferns which lives in nutrients that are poor in calcium will also produce a small number of secondary metabolites, especially flavonoids. According to research Elhaak [23] states that treatment with the addition of

$CaCl_2$ and NaCl generally increased total phenolic compounds. This is because the function as an enzyme activator, where there are three important enzymes involved in the biosynthesis pathway of the phenolic compound including flavonoids, and acts as a protective enzyme against various environmental stresses, namely POD (Peroxidase), PPO (Polyphenol Oxidase) and PAL (Phenylalanine Ammonia Lyase) [24]. Similar research looked at the *Asplenium ceterach* L fern in Serbia. Serbia is a country with changes in the minimum and maximum temperature extreme [20]. *Asplenium ceterarch* L. in Serbaria contain flavonoid such as rutin, catechin, gallocatechin, epigallocatechin, epicatechin [21].

4. Conclusion

The types of flavonoids from 4 species of ferns in Baluran National Park are routine, hyperoxide, quercitrin, and quercetin and *Pteris vittata* L. do not contain all four types.

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