Do Altitude Influence the Characterization of *Dryopteris hirtipes* (Bl.) Kuntze Biomedicinal Profile? A Study of Fascinating Fern from Baluran National Park and Raden Soerjo Grand Forest Park

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**Abstract.** Secondary metabolites of fern are increasingly recognized as medicine ingredient. The total of secondary metabolites in plants may form maximally related to the altitude, environment, nutrient, biotic and abiotic stresses. *Dryopteris hirtipes* (Bl.) Kuntze is easily found in Baluran National Park and Raden Soerjo Grand Forest Park. This study aimed to investigate the differences between secondary metabolites of *Dryopteris hirtipes* (Bl.) Kuntze from Baluran National Park and Raden Soerjo Grand Forest Park. The samples were taken from Baluran National Park which has a height of 0-1000 masl, with a rainfall intensity about 2000-4000 mm per year, and Raden Soerjo Forest Park which has an altitude of 1000-3000 masl, with rainfall intensity 1488 mm per year. The research was conducted by Thin Layer Chromatography (TLC) method. Samples of *Dryopteris hirtipes*’ rhizome and fronds were made as powder then extracted using methanol (1:10). Methanol leaf extract subsequently tested by TLC test. Both of *Dryopteris hirtipes* (Bl.) Kuntze from Baluran National Park and Raden Soerjo Grand Forest Park have similarity chemical content of secondary metabolites such as alkaloid, terpenoid-saponin, tannin, but polyphenols only found from Baluran National Park, and flavonoids only found from Raden Soerjo Grand Forest Park.

**Keywords:** Baluran National Park, *Dryopteris hirtipes*, Raden Soerjo Grand Forest Park Secondary Metabolites, TLC method,

1. **Introduction**

Ferns are the nonflowering vascular plant, in the plant kingdom classified as Pteridophyte. The life cycle of pteridophytes shows alternation of generations as the gametophyte phase and sporophyte phase, but it is dominated by the sporophyte phase [1]. Gametophyte phase is a macroscopic thallus, simple in structure, photosynthetic free living, and short-lived. This thallus contains sperm-producing antheridium and egg-producing archegonium [2]. The sporophyte phase consists of a complex plant body composed of highly specialized roots, stems, and leaves (known as fronds). The frond arises from the stem which is a rhizome growing on or just beneath the soil surface, even though the fronds
of tree ferns emerge from an apical region atop a trunk comprised of pith parenchyma, fibers, and adventitious roots. The dominant sporophyte produces spores through meiosis.

About 10,560 species from fern have been recorded globally [3,4]. The recent modern and comprehensive classification utilizing a community-based approach treats an estimated 11,916 species in 337 genera, 51 families, 14 orders, and two classes [5]. These ferns are widely distributed in various habitats and also as epiphytes, aquatic, terrestrial, or lithophytes [6]. Mostly fern is found in upper-mid to higher altitude, moist, and shady places [7].

Fern is one of the oldest plants on earth; thus, the medical uses of fern is transmitted through centuries [8]. However, the fern is remaining undere xplored in medical aspects in comparison to other vascular plants. Several researchers have studied 48 families of fern, most of them, specifically 31 of them are medicinal pteridophytes [9]. Ferns produce various secondary metabolites with different bioactivities that increasingly recognized as medicine ingredient and could potentially be useful in for the remedy and cure of certain diseases [10]. Studies on the pharmacology and phytochemistry of fern may report novel therapeutic agents by developed active principles [11].

Latest pharmacology and phytochemistry studies showed that ferns contain flavonoid [5], polyphenol [12], alkaloid [13], isoquercetin [14], terpenoid [15], steroid [16], tannin [17]. Flavonoid from Cheilanthes tenuifolia possesses as potent of antibacterial, antioxidant, and anticancerous activities [18]. Adiantum capillus-veneris Linn performs as antidiabetic, analgesic, antibacterial, antifungal, wound healing, anti-inflammatory, antioxidant, antiurolithiatic and detoxifying effects in modern medicine [19]. Cyrtomium fortunei (J.) Smith consist of 23 compounds exhibited moderate antiproliferative activities that could inhibit the growth of human carcinoma cells [20]. More than 20 species of Genus Dryopteris are used as folk medicine. A recent study in modern pharmacological shows that the plants in genus Dryopteris have antiviral, antitumor, antimicrobial, anti-inflammatory, and antioxidative activities [21].

The total production of secondary metabolites in plants may form maximally related to the environment, biotic, abiotic stresses, and nutrient. Secondary metabolites content such as berberine and total phenolics and flavonoid of Thalictrum foliolosum is varied by altitudinal variation. Thalictrum foliolosum in low elevation has more berberine, while the total phenolic and flavonoid increased at higher elevation [22]. Biotic and abiotic stresses in plants tend to increase the production of secondary metabolites [23]. Secondary metabolite accumulation is strongly dependent on environmental factors such as temperature, light, soil fertility soil water, and salinity. Nevertheless, the actual synthesis and accumulation of various secondary metabolites were generally modulated by several environmental factors simultaneously [24].

Baluran National Park and Raden Soerjo Grand Forest Park are Indonesian conservation areas located in East Java having different topographies. Ferns are easily found of them because of their altitude and moisture affect fern habitat preferences. Raden Soerjo Forest Park which has an altitude of 1000-3000 masl higher than Baluran National Park which has a height of 0-1000 masl. One type of fern that grows mostly in Baluran National Park and Raden Soerjo Grand Forest Park and potentially used as a medicine is Dryopteris hirtipes (Bl.) Kuntze. D. hirtipes has morphological characteristics including stems in the form of erect rhizoma, longitudinal scales, and pale leaves with tapered tips and flat edges, attractively shaped leaves on stems and strands, fronds have pinna and lobes, dry compilation indusium, spores in kidney form and flat-convex [25].

There are less recent studies showing about the secondary metabolites of Dryopteris hirtipes. The isolation, identification, and characterization of compounds from fern secondary metabolites could advance to biotechnological and medical benefits. Throughout the different topography between Baluran National Park and Raden Soerjo Grand Forest Park, the author will examine whether it affects the presence of secondary metabolites from Dryopteris hirtipes (Bl.) Kuntze. Thus, the purpose of this study is to investigate the differences between secondary metabolites of Dryopteris hirtipes (Bl.) Kuntze from Baluran National Park and Raden Soerjo Grand Forest Park.
2. Methods
The research used qualitative methods. The samples are rhizome and frond of *Dryopteris hirtipes* (Bl.) Kuntze and was obtained in April - July 2018. Samples collected from Baluran National Park which have a height of 0-1000 masl, representing low altitude site, with rainfall intensity around 2000-4000 mm per year, temperatures around 25-26 °C, and Raden Soerjo Great Forest Park which has a height 1000-3000 masl, representing high altitude site, with rainfall intensity around 1488 mm of rain per year, and high humidity. The research was conducted by Thin Layer Chromatography (TLC) method.

This study started from the exploration stage which includes the identification and characterization of plants; then the sample (rhizome and frond of *Dryopteris hirtipes* (Bl.) Kuntze) is dried and made powder then extracted using methanol (1:10). TLC test began with taking 2 g of extract sample, then added eluent. Then filtrate filtration was carried out and put into a test tube; then it was sprayed on 60F254 silica gel plate.

The Eluent used to identify alkaloid is methanol: concentrated NH$_4$OH with ratio 200: 3. Alkaloids are identified with Rf 0.55-0.75. The eluent used to identify flavonoid is Ethyl Acetate: Formic Acid: Aquades with ratio 85: 10: 15. Flavonoid is identified with Rf 0.85-0.90 (Quercetin), Rf: 0.60-0.65 (Quercitrin), Rf: 0.45-0.50 (Hyperoxide), Rf:0.25-0.30 (Rutin). The Eluent used to identify polyphenol is Toluene: Ethyl Acetate with ratio 93: 7. Polyphenol is identified with Rf 0.25-0.35. The Eluent used to identify terpenoids-saponin is n-Hexane: Ethyl Acetate with ratio 4:1. Terpenoids-saponins is identified with Rf 0.20-0.25. If the results of the Terpenoid TLC analysis (+), then the results of the Saponin TLC analysis are also (+). This is because Saponins are a form of glycosides with sugar molecules that are bound to Triterpenoid or Steroid aglycones. Triterpenes and Steroids are two of several types of Terpenoids. The Eluent used to identify tannin is n-Butanol: Acetate Acid: Aquades with ratio 4: 1: 5. Tannin is identified with Rf 0.70-0.80.

3. Results and Discussion
The results of Thin Layer Chromatography (TLC) of *Dryopteris hirtipes* (Bl.) Kuntze from Baluran National Park test were presented in Table 1, while the other samples of *Dryopteris hirtipes* (Bl.) Kuntze from Raden Soerjo Grand Forest Park were presented in Table 2. TLC results showed that *Dryopteris hirtipes* (Bl.) Kuntze from Baluran National Park and Raden Soerjo Grand Forest Park positively containing alkaloids, terpenoid-saponin, and tannins which are characterized by Rf values around 0.35-0.76 included in the alkaloid identification range, Rf values around 0.10-0.61 included in the terpenoid-saponin identification range, and the Rf value around 0.50-0.77 included in the tannin identification range. *Dryopteris hirtipes* (Bl.) Kuntze from the Baluran National Park contains no flavonoids and positively contains polyphenols with Rf values around 0.26-0.58 in the range of identification of polyphenols. *Dryopteris hirtipes* (Bl.) Kuntze from the Raden Soerjo Grand Forest Park is negative for polyphenols and is positively containing flavonoids with Rf values around 0.10-0.94 in the range of identification of flavonoids.

Different altitude of their habitat influences the secondary metabolites of *Dryopteris hirtipes*. Polyphenols were found in lower altitude and flavonoids were found in higher altitude. This result is in line with the study of secondary metabolites characterization in *Pluce indica* from three different kinds of altitude. *Pluce indica* has higher total phenolic content in lower altitude and higher flavonoid content in higher altitude [26].

The content of secondary metabolites of *Dryopteris hirtipes* (Bl.) Kuntze from Baluran National Park and the Raden Soerjo Grand Forest Park tested showed differences. The composition of the methanol extract of secondary metabolites *Dryopteris hirtipes* (Bl.) Kuntze from Baluran National Park are alkaloids, flavonoids, terpenoids -saponin, and tannin, whereas methanol extract of secondary metabolites *Dryopteris hirtipes* (Bl.) Kuntze from Raden Soerjo Grand Forest Park are alkaloids, polyphenols, terpenoids-saponins, and tannins. Various ecological factors greatly influence the synthesis of secondary metabolites such as flavonoid and polyphenols during the growth and development of plants in addition to the influence of genetic factors. The height of the area affects the temperature and humidity of the region. Low temperatures can affect the content of secondary metabolites.
metabolites. Environmental conditions also play a role in the biosynthesis of secondary metabolites pathways [24].

**Table 1.** Thin Layer Chromatography (TLC) *Dryopteris hirtipes* (Bl.) Kuntze from Baluran National Park

| Plant Section | RF (Retention factor) Value of Chemical Content |
|---------------|-----------------------------------------------|
|               | Alkaloids | Flavonoids | Polyphenols | Terpenoid - Saponin | Tannin |
| Rhizome       | 0.35      | -          | 0.26        | 0.20                | 0.62   |
| *Dryopteris hirtipes* (Bl.) Kuntze | 0.75      | 0.56       | 0.28        | 0.71                |
| Frond         | 0.35      | -          | 0.31        | 0.18                | 0.65   |
| *Dryopteris hirtipes* (Bl.) Kuntze | 0.76      | 0.58       | 0.27        | 0.77                |

**Table 2.** The Results of Thin Layer Chromatography (TLC) *Dryopteris hirtipes* (Bl.) Kuntze from Raden Soerjo Forest Park

| Plant Section | RF (Retention factor) Value of Chemical Content |
|---------------|-----------------------------------------------|
|               | Alkaloids | Flavonoids | Polyphenols | Terpenoid - Saponin | Tannin |
| Rhizome       | 0.73      | 0.10       | -           | 0.10                | 0.59   |
| *Dryopteris hirtipes* (Bl.) Kuntze | 0.76      | 0.26       | 0.33        | 0.17                | 0.64   |
|               | 0.62      | 0.73       | 0.82        | 0.75                | 0.94   |
| Frond         | 0.74      | 0.09       | -           | 0.11                | 0.50   |
| *Dryopteris hirtipes* (Bl.) Kuntze | 0.76      | 0.33       | 0.44        | 0.17                | 0.55   |
|               | 0.60      | 0.75       | 0.82        | 0.61                | 0.77   |

Based on the primary data in Table 1, there is no flavonoid derived from *Dryopteris hirtipes* (Bl.) Kuntze from Baluran National Park. Based on its altitude, Baluran Nation Park is lower than Raden Soerjo Grand Forest Park. This view was supported by the study that flavonoid derived from *Hypericum perforatum* has more amount in higher altitude. The amount of rutin, one of the flavonoid types, in plants grown at higher altitude was 4-fold higher than in the plants grown at lower altitude. This strong correlation between rutin content and the altitude of the grown site was particularly to the discrepancy of solar radiation levels [27]. The higher solar radiation at higher altitudes affected the secondary metabolite profiles, the more increased light irradiation, the more increased contents of flavanols [28].

Interestingly, elevating temperature, considering higher temperature happen in lower elevation, resulted in a decrease in the amounts of total flavonoids [29]. However, the temperature has a less dominant effect on the production of flavanol. One of the types of flavonoids, quercetin will increase when the temperature is lower. The prominent factor is total irradiation because there is no clear evidence that the period of reduced FR/Ratio at dawn and the end of a day, may influence the accumulation of flavonoids [28]. The biosynthetic of flavonoid is in the cytosol. Flavonoids transported to cell walls or vacuoles after biosynthesis [30]. Lately, some studies have reported at biosynthesis of flavonoid from the perspective of molecular. Flavonoid biosynthesis would involve the
interaction of WD40 proteins, DNA binding R2R3 MYB transcription factors, and MYC-like basic helix-loop-helix (bHLH) [31]. DNA binding R2R3 MYB transcription factors are influenced by specific light wavelengths when regulating the biosynthesis of distinct flavonoids [32].

From Table 2 Dryopteris hirtipes (Bl.) Kuntze from Raden Soerjo Grand Forest Park has no polyphenols. Based on its altitude, Raden Soerjo Grand Forest Park is higher than Baluran National Park. Regardless, it has been reported that there was a strong correlation at regions with different ecological conditions, the higher the altitude, the higher the content of total polyphenol [22,33]. Baluran National Park and Raden Soerjo Grand Forest Park are in tropical conditions; thus, temperature decreases with the increase in altitude, but solar UV radiation increases with an increase in altitude. Therefore, the more UV radiation at higher altitudes, the more polyphenols produced [34]

However, this result is in line with the recent study that suggests that the lower the altitude, the more polyphenols, are produced by the plant [35]. Based on the obtained results, plants may adapt their polyphenol content in response to changing environmental conditions. It may be caused by gene and environmental interaction that alter the biosynthesis pathways of polyphenols [36]. Additionally, the total phenolic content is directly proportional with longer day length, but temperature also plays a role in the contents of some polyphenols [12]. Temperature, altitude, ultraviolet radiation as environmental parameters may cause the difference in an actual level of phenolics [17]. The actual synthesis and accumulation of various secondary metabolites were modulated by more than one factor. Many environmental factors simultaneously influence the secondary metabolites compound [7].

In other words, secondary metabolites of a species that live in a different habitat with different environmental stress would have a different presence and level of secondary metabolites compounds. Another vascular plant also showed the similarity, the higher the Fallopia japonica and Larix kaempferi altitude, the higher their flavonoid contents [37]. Study about phytochemistry and pharmacology of secondary metabolites may consider the environmental factor of plant species to report novel therapeutic agents.

4. Conclusion
Both of Dryopteris hirtipes (Bl.) Kuntze from Baluran National Park and Raden Soerjo Grand Forest Park positively contained alkaloids, terpenoid-saponin, and tannins. Nonetheless, Dryopteris hirtipes (Bl.) Kuntze from the Baluran National Park contained no flavonoids and positively contained polyphenols, whereas Dryopteris hirtipes (Bl.) Kuntze from the Raden Soerjo Grand Forest Park contains no polyphenols and positively contains flavonoids.

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