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Phytochemical Composition of *Selaginella* spp. from Java Island Indonesia

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Abstract

For a long time, people in West Java, Indonesia have used *Selaginella* as a traditional cure for several ailments including fever, minor wounds, broken bones, women's health disorders or postnatal bleeding. However, information on the active compounds of the plant from Java Island has not been fully expounded. The objective of the research was to reveal the diversity of bioactive compounds and amentoflavone content of *Selaginella* from Java Island in order to optimize the use of this plant as a medicinal plant. *Selaginella* plants collected from 29 locations in Java were extracted and subjected to colorimetric and thin layer chromatography test to qualitatively analyze the bioactive compounds of alkaloid, phenol, and steroid. In addition, high performance liquid chromatography was performed to analyze the amentofalvone content of *Selaginella* extract. The research result showed that all the examined *Selaginella* species contained alkaloid, flavonoid, saponin, tannin, and steroid, but they did not contain hydroquinone. Seven of them contained the biflavonoid type of amentoflavone. The highest amentoflavone concentration, 6.87 ppm, was found in *S. subalpina* that originated from Gedung Songo, Central Java. Those results indicated that more than one *Selaginella* species originating from Java Island had marked potential for use as medicinal plants.

Abstrak

Komposisi Fitokimia *Selaginella* spp. Pulau Jawa Indonesia. Masyarakat di Jawa Barat, Indonesia sudah lama menggunakan *Selaginella* untuk menyembuhkan secara tradisional beberapa penyakit seperti panas, luka ringan, patah tulang, masalah kewanitaan, dan pendarahan setelah melahirkan. Namun, informasi tentang senyawa aktif dari *Selaginella* yang berasal dari Pulau Jawa belum sepenuhnya diketahui. Tujuan penelitian ini adalah untuk mengungkapkan keanekaragaman senyawa bioaktif dan kandungan amentoflavon dari *Selaginella* yang berasal dari Pulau Jawa dengan maksud untuk memaksimalkan pemanfaatan tumbuhan ini sebagai tumbuhan obat. Bahan *Selaginella* yang dikoleksi dari 29 lokasi di Pulau Jawa diekstraksi dan digunakan sebagai bahan dalam uji *colorimetric* dan *thin layer chromatography* untuk menganalisis secara kualitatif senyawa bioaktif alkaloid, phenol, dan steroid. Selain itu, *high performance liquid chromatography* dilakukan untuk menganalisis kandungan amentofalvone dari ekstrak *Selaginella*. Hasil penelitian menunjukkan bahwa semua spesies *Selaginella* yang diuji mengandung alkaloid, flavonoid, saponin, tannin, and steroid, tetapi tidak mengandung hydroquinon. Tujuh spesies *Selaginella* berisi biflavonoid tipe amentoflavon. Kandungan amentoflavon tertinggi yaitu konsentrasi 6.87 ppm dihasilkan oleh *S. subalpina* yang berasal dari Gedung Songo, Jawa Tengah. Hasil ini mengindikasikan bahwa lebih dari satu spesies *Selaginella* yang berasal dari Pulau Jawa sangat potensial untuk digunakan sebagai tumbuhan obat.

Keywords: amentoflavone, biflavonoid, diversity, Selaginella

1. Introduction

In recent years, the need for medicine has risen in proportion to the increase of socially transmissible diseases. The use of alternative medicine from plants in traditional remedies has been used in many places around the world. Herbal products from medicinal plants are preferred because of their higher safety, efficiency, and cultural acceptability over drugs. Besides these reasons they also have less side effects and testing time. Khanna *et al.* [1] showed that chemical compounds of herbs is a part of the plant's physiological and ecological functions, therefore it is believed to have better compatibility with the human body.

Selaginella, a perennial herbaceous genus belonging to the *Selaginellaceae* (Pteridophytes) family, is distributed almost worldwide. It has simple and very small leaves, usually much less than 10 mm long without lateral veins arranged in four ranks with two lateral rows of larger leaves and two rows of smaller median leaves. All sporangia fall at the end of the branches [2]. Previous studies showed that *Selaginella* is rich with an active compound called biflavonoid, a secondary metabolite that is formed from the dimer of flavone and flavanon structures with 5,7-4'- oxigenated pattern. For centuries, people, especially in China, have used *Selaginella* as a traditional medicine, for curing various diseases including hepatitits and cancer or as an antioxidant [3]. Pan *et al.*, Gayatri *et al.*, Sah *et al.* [4-6] have evaluated the active compound and its biochemical and pharmaceutical functions in different *Selaginella* species. The extract of *S. Doederleinii* contained alkaloid, phytosterol and saponin compounds [4]. The extract of *S. involvens* with the concentration of 2 mg/mL could inhibit lipid peroxidation at almost 50% [5]. The extract of *S. Bryopteris* increased cell growth and protected against dead cells induced by oxidative stress [6], while the extract of *S. Tamariscina* contained a strong antioxidant that was able to reduce blood sugar levels and function as a lipid peroxide serum and increase insulin serum [7].

Indonesia is one of the world's countries that has mega plant diversity, including diversity of *Selaginella*. It has been reported by Alston [8] that there have been 23 *Selaginella* species identified in Java Island. For centuries people from West Java have used this genus as a remedy for curing many ailments such as fevers, wounds, broken bones, female disorders and postnatal bleeding. In spite of its benefits, *Selaginella* that originated from Java Island has not been expounded, scientifically examined, and exposed as a traditional medicinal plant. Therefore, scientific investigation, in particular elucidation of its active compound content is necessary in order to optimalise the use of this genus as a source of natural medicine. In the future, we hope that the active compounds of *Selaginella* can be extracted and massively produced. This paper revealed the content of active compounds as well as the flavonoid amentoflavone in 9 species of *Selaginella* originating from Java, Indonesia.

2. Methods

Sample collection, preparation and identification. The sample was collected from 29 locations in Java Island and was prepared for plant extraction. Plant identification was performed by using identification keys for ferns [8-10] and comparing the materials to herbarium specimen collections belonging to the Research Center of Botany, LIPI, Cibinong, Bogor.

Bioactive compound analyses. The bioactive compounds were extracted and analyzed using procedures developed by Gayathri *et al.* [5] and de Oliveira *et al.* [11]. All plant parts were dried and ground to powder, and then extraction was performed using ethanol and *n*-hexane. For the ethanol extract, dry powder of *Selaginella* was extracted using ethanol (5 g plant powder/100 ml ethanol) by constantly mixing the suspension for 4 hours, then it was filtered using Whatman filter paper. The filtrat was then dried using a rotary evaporator at 40 °C for 4-8 hours until all the solvent evaporated. Hexane extraction was carried out using the same procedure as ethanol extraction with 2 g plant powder per 100 ml *n*-hexane.

Qualitative analyses of the bioactive compounds, i.e. alkaloid, phenol, and steroid, were carried out using the colorimetric method based on the standard phytochemical analysis for plants as described by Harborne [12]. The intensity of the color represents the relative content of the bioactive compounds. The bioactive compounds were then verified using thin layer chromatography (TLC) methods. In order to determine the biflavonoid content of *Selaginella*, a part of the plant extract was fractionated using sephadex column chromatrography with a combination of ethanol and *n*-hexane as eluen. All fractions were then quantitatively analyzed using high performance liquid chromathography (HPLC) with amentoflavone as an internal standard.

3. Results and Discussion

The qualitative phytochemical compositions of *Selaginella* is shown in Table 1. All tested *Selaginella* species contained alkaloid, flavonoid, saponin, and steroid compounds. Tannins were only found in some species. This research showed that *Selaginella* was not only rich in flavonoid compounds, but also contained all the most important bioactive compounds of plants, such as alkaloid, tannin, flavonoid, saponin, and phenolic compounds [13]. All compounds have a good effect on human health when they are used properly. Saponins form strong insoluble complexes with cholesterol therefore, it is believed to be useful in the human diet for controlling cholesterol. Beside that it has antifungal and antibacterial properties that are important as components of cosmetic products [14].

Apart from the antioxidant activity, flavonoid also possesses anti-inflammatory, anti-allergic, hepatoprotective, anti-thrombic, antiviral, and anti-carcinogenic activities. Alkaloids have diuretic, antipasmodic, anti-inflammatory, and analgesic effects [15]. Different classes of steroids have different functions. Anabolic steroids increase muscle mass. Anti-inflammatory steroids can reduce swelling, pain, and other manifestations of inflammation [14]. Tannins

Table 1. The Result of Phytochemical Test of *Selaginella* from Java Island

Plant Species	Alkaloid	Phenolic				Steroid
		Flavonoid	Tannin	Saponin	Hydroquinone	
S. ornata	+	+	+	+	-	+
S. plana	+	+	-	+	-	++
S. opaca	+	+	-	+	-	+++
S. remotifolia	+	++	+++	+	-	++
S. willdenovii	+	+	-	++	-	+
S. subalpina	+	+	-	+	-	++
S. aristata	+	+	++	+	-	+
S. involvens	+	+	+++	+	-	++
S. intermedia	++	+	+	+	-	+++

Note: Relative bioactive content: - = absent, + = low, ++ = medium, +++ = high

may be used medicinally as antidiarheal, haemostatic, and antihemorhoidal compounds. Tannins can also be effective in protecting the kidneys [16]. Tannins have also shown to have potential antibacterial and antiparasitic effects [17-19]. It is believed that tannins isolated from the stem bark of *Myracrodruon urundeuva* may have neuroprotective functions capable of reversing hydroxydopamine-induced toxicity. The plant is also potential as a therapeutic agent, which may be beneficial to patients with neurological disease [20]. Sung *et al.* [21] showed that tannins extracted from green tea waste product could be a natural source for antioxidants as well as antibiotics. All those properties possessed by the bioactive compounds of plants may explain the use of *Selaginella* species as a medicinal plant in West Java communities.

Table 1 also showed that all the tested *Selaginella* extracts did not contain hydroquinone. The absence of this compound makes them safe to be consumed because it is extremely dangerous and may lead to the development of cancer. Hydroquinone is an aromatic organic compound that is a type of phenol, having the chemical formula $C_6H_4(OH)_2$. This compound has been used for decades as a skin lightening agent. However, the use of hydroquinone in cosmetics has been banned since January 2001 because of the mid-term effects like leukomelanoderma en confetti and exogenous ochronosis [22].

The verification of phytochemical components using TLC analysis showed that the type of eluen affected the result. The TLC analysis for alkaloids showed that the eluen *n*-hexane was able to extract alkaloid from all tested *Selaginella* species, but analysis using ethanol eluen was only able to extract alkaloid from two species (Table 2). Those results indicated that the eluen *n*-hexane was better than that of ethanol for extracting *Selaginella* alkaloids. Flavonoid was found in all tested *Selaginella* species, and the highest level of flavonoid was in *S. remotifolia* (Table 1). The TLC analysis showed that ethanol eluen was better for extracting flavonoid

Table 2. The Result of TLC Analysis of Alkaloid in *Selaginella* Species

Species	*n*-hexane eluen		Ethanol eluen	
	Alkaloid	Rf	Alkaloid	Rf
S. ornata	+	0.65	-	-
S. plana	+	0.65 &0.80	-	-
S. opaca	+	0.65	-	-
S. remotifolia	+	0.65 &0.80	-	-
S. willdenovii	+	0.65 &0.80	-	-
S. subalpina	+	0.8	-	-
S. aristata	-	-	-	-
S. involvens	+	0.8	-	-
S. intermedia	-	-	+	0.80

Note: + = present, - =absent

than that of *n*-hexane (Table 3). Tannins were found in 5 species, namely; *S. ornata, S. remotifolia, S. aristata, S. Involvens* and *S. Intermedia* (Table 1). Meanwhile saponins were identified in all the tested species, and the highest saponin content was found in *S. willdenovii.* Steroids were also found in all species (Table 1 and 4). In general, the TLC analysis showed that the *n*-hexane eluen was better than that of the ethanol eluen. The identification of active compounds of *Selaginella* extraction indicated that *Selaginella* has good potential as a source of active compounds (secondary metabolites), especially flavonoid, which has great potential as a source of natural medicine.

The active compounds identified from 9 *Selaginella* species were described in Table 5. Extract contents were varied among species ranging from 6.01 to 14.22% of plant dry weight. The content of active compound was highest in *S. willdenovii*, while the lowest content of active compound was found in *S. intermedia.* Both these species originated from Cangkuang, Sukabumi (West Java).

Table 3. The Result of TLC Analysis of Flavonoid on *Selaginella* Species

Species	Hexane eluen		Ethanol eluen	
	Flavonoid	Rf	Flavonoid	Rf
S. ornata	+	0.73	+	0.09 & 0.26
S. plana	+	0.73 & 0.91	+	0.09 & 0.26
S. opaca	+	0.73 & 0.91	+	0.09 & 0.26
S. remotifolia	+	0.73 & 0.91	+	0.26
S. willdenovii	+	0.91	+	0.09 & 0.26
S. subalpina	-	-	+	0.09 & 0.26
S. aristata	-	-	+	0.09 & 0.26
S. involvens	+	0.73	+	0.09
S. intermedia	+	0.73 & 0.91	+	0.09

Note: + = present, - = absent

Table 4. The Result of TLC Analysis of Steroid in *Selaginella* Species

Species	Hexane eluen		Ethanol eluen	
	Steroid	Rf	Steroid	Rf
S. ornata	+	0.95	-	-
S. plana	+	0.95	-	-
S. opaca	+	0.95	-	-
S. remotifolia	+	0.95	-	-
S. willdenovii	+	0.95	-	-
S. subalpina	+	0.95	-	-
S. aristata	+	0.95	-	-
S. involvens	+	0.95	-	-
S. intermedia	+	0.95	-	-

Note: + = present, - = absent

Table 5. Species Names, the Origin of Sample, Simplisia Weight, Extract Percentage and Amentoflavone Concentration in *Selaginella* Species

Species	The origin of Samples	Simplisia weight (g)	Extract weight (g)	Extract % (w/w)	Amentoflavon concentration (ppm)
S. ornata	Cibodas	16.79	1.43	8.51	ud
S. plana	Gunung Lawu	18.37	1.37	7.47	ud
S. opaca	Dieng	16.31	1.42	8.69	1.44
S. remotifolia	Dieng	21.21	1.90	8.96	2.28
S. willdenovii	Cangkuang	19.40	2.76	14.22	2.46
S. subalpina	Gedung Songo	8.22	0.77	9.41	6.87
S. aristata	Batuseribu	1.48	0.16	10.83	2.81
S. involvens	Temanggung	2353	2.06	8.75	2.15
S. intermedia	Cangkuang	20.33	1.22	6.01	6.03

*ud= undetectable

Flavonoid amentoflavone was identified in seven *Selaginella* species, but it was not detected in two other species. The concentration of amentoflavon varied among seven species ranging from 1.44 to 6.87 ppm with the highest content found in *S. subalpina* that originated from Gedung Songo (Central Java). Even though amentoflavone was not detected in *S. plana*, and *S. ornata*, it does not mean that the species have no flavonoid because we know from Table 1 that all species have flavonoid, indicating that those two species have different types of flavonoid. There are several types of flavonoids known in *Selaginella*, such as gingketin and robusflavon [23]. It is also possible that one *Selaginella* species has more than one phenolic compound. For example, the extract of *S. Doederleinii* has 11 phenolic compounds, which are 5 lignans: (-)-lirioresitol A, lirioresitol B, (+)-wikstromol, (-)-nortracheloside and matairesinol; 2 phenylpropanon: 3-hydroxy-1(3-methoxy-4-hydroxy-phenyl)–propan-1-one and 3-hydroxy-1-(3,5-dimethoxy-4-hydroxyphenil)-propan-1-one; and 4 biflavonoids: amentoflavone, 7,7"–di-0-methylamento-flavone, 7, 4', 7", 4"'-tetra-0-methylamentoflavone and heveaflavone [24]. The results of this research suggested that more than one species of *Selaginella* originating from Java Island have great potential for medicinal plants.

4. Conclusions

All the examined *Selaginella* species contained alkaloid, flavonoid, saponin, and steroid compounds, but only some *Selagnella* species contained tannins. Hydroquinone was not found in all the examined *Selaginella* species. The content of each compound varied among species. The extract concentration ranged from 6.01-14.22% of the dry matter with the highest concentration found in *S. willdenovii* that originated from Cangkuang, Sukabumi (West Java).

Seven species have flavonoid amentoflavone with various concentrations ranging from 1.44 to 6,87 ppm. The highest concentration of amentoflavone was found in *S. subalpina* originating from Gedung Songo (Central Java). More than one *Selaginella* species has the potential as medicinal plants.

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