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Antioxidant and Antimicrobial Activity: The Potency of *Selaginella intermedia* Leaves Against Oral Pathogen

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ABSTRACT
Selaginella intermedia is one of the traditional medicine in East Kalimantan, which use as some traditional medicinal uses. *S. intermedia* is usually found in the forest floor, especially for this study we obtain from Kebun Raya Unmul Samarinda (KRUS) in East Kalimantan. The aim of this study is to inhibit the radical scavenging agent (DPPH) and antimicrobial assay against *Streptococcus sobrinnus* and *Porphyromonas gingivalis.* The control positive of this study is chlorhexidine. The sample extracted with ethanol and obtain 4.33 % extract from extract yield 2.23 g. *S. intermedia* has a good activity to inhibit radical scavenging agent (DPPH) with a value of 65 % at concentration 100 ppm. *S. intermedia* also has antimicrobial activity with the minimum inhibitory concentration (MIC) value of 4 mg/ml and minimum bactericidal concentration (MBC) value of 4 mg/ml against *S. sobrinus*. In a case against *P. gingavalis* antimicrobial activity with the MIC value of 4 mg/ml and MBC value of 4 mg/ml. The ability of *S. intermedia* is thought to be due to the presence of secondary metabolites such as alkaloids, flavonoids, and terpene groups. Further research is ongoing to find out deeply about *S. intermedia* influence on dental caries.

Keywords: *Selaginella intermedia, Antioxidant, Antimicrobial, East Kalimantan*

1. INTRODUCTION

Medicinal plants have been considered the primary source of medicine since ancient times, and 70% of the worldwide population still relies on plants for traditional medicine [1]. Meanwhile, Indonesia's rainforest has a thousand plants that can be utilized for biological activities [2. Indonesia comprises 110 million hectares and serves about 80% of the world`s medicinal and other plants. It is estimated that 28,000 plant species exist in the Indonesian forest. Among them are 7,000 medicinal plants, which account for 90% of medicinal plants in Asia. So far, 1000 species have been identified and utilized for traditional medicines [3].

Medicinal plants are used as a source of potent and powerful drugs. Hence medicinal plant extracts are used as drugs that possess various medicinal properties [4]. Moreover, medicinal plants' effects have been found in the experiences through trial and error for hundreds of centuries searching for disease treatment [5,6]. According to the World Health Organization (WHO), 80% of people still rely on plant-based traditional medicines for primary health care [7]. On the other side, Fabricant and Farnsworth reported 98 medicinal plants were related to the intended use of native folk ethnopharmacological [8]. Nevertheless, medicinal plants are an essential source of new chemical substances with potential therapeutic effects [9].

The medicinal plants are distributed in the rainforest of East Kalimantan. Some indigenous people utilize the rainforest for almost every aspect of their lives because the rainforest provides their food, shelter, tools, clothing, and medicinal purposes. Moreover, they utilize many parts of the plants, including roots, leaves, bark, stems, and fruits [11,12]. Unfortunately, the utilization of medicinal plants by the local people has not yet been well documented. But there are few studies of traditional medicines used by the indigenous tribal people in Borneo (Kalimantan). Nevertheless, botanists, pharmacologists, pharmacognosists, anthropologists, and phytochemists are still researching to discover something new [10].

Selaginella intermedia (cakar ayam) is the medicinal plant used by the traditional tribe with traditional and modern. Besides used as a medicine, the community also uses it as ornamental plants and vegetables. S. intermedia are distributed in the regions of Java, Malaysia, Borneo, Papuasia, and Philippines. *S. intermedia* contains many secondary metabolites such as alkaloids, phenols (flavonoids, tannins, saponins), and terpenoids (triterpene and steroids) [14]. the content is possible to have natural ingredients that can be produced for antimicrobials and antioxidants agent. Although there have been many studies related to the potential of S. intermedia as a medicinal plant, there is still little information on the oral pathogen. Here, the present data on antioxidant and antimicrobial activities against the oral pathogen.

2. MATERIAL AND METHODS

2.1. Plant Materials and Chemicals

Leaves and roots medicinal plants were collected from KRUS (Samarinda Educational Forest), East Kalimantan, Indonesia. The plant was Sellagenila intermedia. The plant was identified by a taxonomist, Raharjo M.P from Mulawarman University, and confirmed by references. The plant materials were dried for three days and grounded with a blender to small pieces. DPPH (1,1-diphenyl-2-picrylhydrazyl) was purchased from Tokyo Kasei Kogyo (Tokyo, Japan). DMSO (dimethyl sulfoxide) was purchased from Merck (Darmstadt, Germany). Ascorbic acid was obtained from Sigma (St. Louis, MO, USA). Nutrient Broth was obtained from Difco (Detroit, MI, USA). Other chemicals were of HPLC grade or the highest purity commercially available.

2.2. Extraction

Ground plant samples were extracted successively with methanol at room temperature with continuous shaking on a shaker (7400 Tubingen; Edmun Buchler, Germany) for 48 h. This process was repeated following filtration of the suspension through Whatman filter paper. The crude methanol extracts were evaporated at 400C and put in a vacuum oven to near dryness.

2.3. Antioxidant Assay

The sample was first dissolved in DMSO and used at a 3 times dilution for the actual experiment. The DPPH radical scavenging method was performed as previously described by Arung et al. [13]. UV absorption was measured on a Shimadzu UV-VIS 1240 spectrophotometer (Shimadzu Corp., Kyoto, Japan).

2.4. Antimicrobial Assay

The microdilution technique using 96-well microplates was used to obtain the extract's MIC values against microorganisms throughout this study. The serially diluted extracts were added to the 96-well and incubated with *S. sobrinus* for 24 h or *P. gingivalis* for 72 h at 37^{o}C under an anaerobic condition inoculumns, 106 cell/ml for *S. sobrinus*, and 108cell/ml for *P. gingivalis*. The final concentration of extract ranged from 4mg/ml to 0.015 mg/ml. The chlorhexidine was used as a positive control. Microbial growth was indicated by adding 50 µl of 0.2 mg/ml iodonitrotetrazolium chloride (INT) to microplate wells and incubated at 37^{o}C for three hours. The MIC was defined as the lowest concentration that inhibited the color change of INT. For sequential MBC determination, 10 µl from wells that showed no change in color into 100 µl fresh medium then incubated 24h for *S. sobrinus* or 72 h for *P. gingivalis* under the anaerobic condition at 37^{o}C. The MBC was determined by adding 50 µl of 0.2 mg/ml INT to microplate wells and incubated at 37^{o}C for three hours.

3. RESULT AND DISCUSSION

The potential for medicinal plants as a source for new drugs has been mostly unexplored as of yet. Among the estimated 250,000-500,000 plant species, only a small percentage has been phytochemically investigated, and few fractions have been examined in the biological or pharmacological screening tests [15]. Thus a very narrow sampling regarding the effects of medicinal plants has been elucidated so far. Hence, random screening is most productive in antibiotics in discovering new biologically active molecules [16,17] as it is known that the plants had been used traditionally for centuries which was then revealed by modern scientific studies to contain antibiotic properties.

3.2. Antimicrobial Activity

The traditional tribe usually used s. intermedia as medicate of wounds, menstruation fitness, heart disease, and anti-inflammatory. Percentage ethanol extract of S. intermedia with a value of 4.33 % with extract yield was 2.23 g. Recent development of microbial resistance high response to the new antibiotics led from the new antimicrobial agent. Our research for antimicrobial bioactivity use S. intermedia revealed belongs antimicrobial activity. The result of the antimicrobial assay of the plant extract is shown the capability of *S. intermedia* inhibition with MIC (minimum inhibitory concentration) value of lower than 4000 ppm, and the indicator was the yellow color of well shown the bacterias weren't growing and MBC (minimum bactericidal concentration) value of 4000 ppm. The ethanolic extract of *S. intermedia* showed a good effect

against *Streotococcus sobrinus* and *Porphyromonas gingavalis* relative to chlorhexidine as a standard drug. *S. sobrinus*, known as a gram-negative and *P. gingavalis* as a gram-positive, the both of acquainted as an anaerobic bacterium, in a normal site human oral flora and is involved in dental caries and inflammatory gum disease, such as periodontal disease.

Figure 1 *Selaginella intermedia*

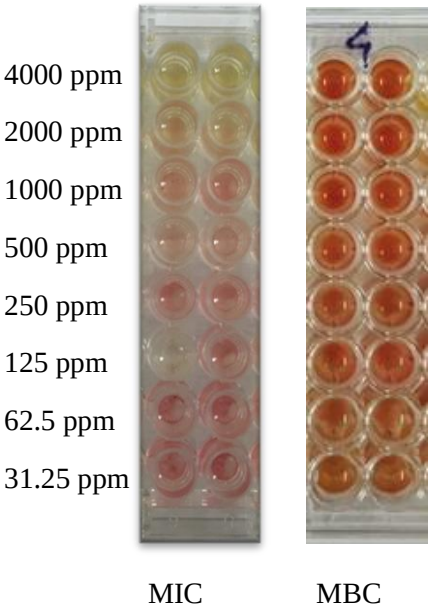

Figure 2 MIC and MBC of *Selaginella intermedia*

3.1. Antimicrobial Activity

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Table 1. Antimicrobial activity of *Selaginella intermedia* against *Streptococcus sobrinus* and *Porphyromonas gingavalis*

Sample	*Streptococcus sobrinus*		*Porphyromonas gingavalis*	
	MIC	MBC	MIC	MBC
Selaginella intermedia	< 4000 ppm	>4000 ppm	< 4000 ppm	>4000 ppm
Positive Control	< 31,25 ppm	31,25 ppm	< 31,25 ppm	31,25 ppm

S. sobrinus is one of the most cariogenic bacteria of mutants streptococci [18]. Although the human flora is quite diverse and complex, one species of mutants streptococci, *S. sobrinus*, has been implicated as a primary etiology agent of dental caries [19]. One of the most important virulence factors of this species is its ability to produce glucosyltransferase (GTase) [23]. GTase is the key enzyme that catalyzes the introduction of a glucose moiety from sucrose to the adhesive glucans and contributes significantly to dental caries formation [24]. Consequently, the plant extracts' potent activity toward antimicrobial activity for the treatment of dental caries suggested by inhibition of glucosyltransferase activity and depress the growth of *S. sobrinus*.

P. gingivalis is a gram-negative oral anaerobe, a pathogenic bacteria species conducting initiation and causation periodontitis, an inflammatory disease that destroys the tissues supporting the tooth, which eventually may lead to tooth loss [20,21], rheumatoid arthritis, atherosclerosis, and orodigestive cancers [22]. Moreover, orodigestive cancers represent a large proportion of the total malignancies worldwide and include cancers of the oral cavity, gastrointestinal tract, and pancreas [22]. Therefore, the result suggested that the extracts may be used to inhibit and kill bacteria growth and etiological agent.

3.2. Antioxidant Activity

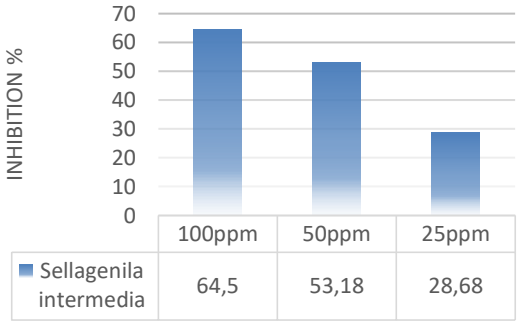

Figure 3 Antioxidant activity of *Selaginella intermedia* against DPPH

The antioxidant activity of the plant extracts was evaluated by DPPH radical scavenging mechanism. The chemical compound DPPH is a stable free radical with scavenging abilities and is often used to assess the antioxidant activity of various types of samples [25]. Therefore, the selective choice of a potential plant from the natural resource capable of radical scavenging abilities of samples. The antioxidant activities of the leaves and roots of the collected medicinal plants were given in Figure 3. The results are shown as the relative activities against the standard ascorbic acid.

The result showed the effective antioxidant activity was displayed by S. intermedia at 100 ppm with a value of 64.5 % DPPH inhibition and concentration 50 ppm and 25 ppm with a value of 53.18 % and 28.68 %, respectively. The screening and characterization of antioxidants derived from natural sources have gained much attention, and efforts have been put into identifying compounds as suitable antioxidants to replace synthetic ones [26]. Moreover, through investigation of phytochemical was able to evaluate the compound group and perform isolation. However, to reveal and search the remedies against free radical-mediated disease significant to analyze potent antioxidant properties to prevent an oxidative reaction in food, protect against DNA damage, carcinogenesis, and possible substances with a wide range of pharmacological and anti-fungal properties. Further investigation on isolation and characterization of bioactive compounds derived from natural extracts in progress.

4. CONCLUSION

The present work has proved that the extracts of the hole part of *S. intermedia* from East Kalimantan, Indonesia. It possessed strong antimicrobial and antioxidant properties. The extract of S. intermedia was shown the potential to inhibit *S. sobrinus* and *P. gingavalis*. The extracts of S. intermedia exhibited potential as inhibitory of GTase. Moreover, the extracts of S. intermedia exhibited DPPH radical-scavenging activity. This study aims to further develop some of these Indonesian species into medicinal plants as a scientific basis.

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