Antioxidant Activity of Zodia (*Evodia suaveolens*) Protein Extract In Vitro

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Abstract. The zodia (*Evodia suaveolens)* plant is an Indonesian endemic that originated in Papua. Zodia leaves are empirically used as anti-mosquito and dysentery medications, and cooked bark is useful as a malaria fever reliever. Until present, no research has been conducted on the protein of the zodia plant extract that has demonstrated action. The goal of this study was to assess the antioxidant activity of the zodia plant protein extract as reflected by IC_{50} (inhibitory concentration). Various established procedures were used to determine antioxidant capacity, including radical cation, 2,2'-Azinobis 3-ethyl Benzothlazole-6-Sulfonic Acid ($ABTS^{\bullet+}$) assay, hydroxyl radical ($OH^{\bullet}$), and superoxide anion ($O_2^{\bullet-}$) scavenging. Data analysis was determined using ANOVA and continued with Duncan. The soluble protein in the stem has much stronger free radical scavenging ($ABTS^{\bullet+}$, $OH^{\bullet}$, and $O_2^{\bullet-}$) capabilities than the leaves and root. According to the research, soluble proteins in stems have a significant potential for usage as antioxidants.

1 Introduction

Free radicals are electrons that do not have a companion; therefore, they are unstable and have a high reactivity in an attempt to find a partner by binding electrons surrounding them [1]. Under normal conditions, the human body produces free radicals as a byproduct of cellular metabolism [1]. Free radicals can enter the body from outside sources such as pollution, radiation, medicines, and pesticides [2].

Free radicals are regularly created in biological systems by oxygen, nitrogen, and sulfur molecules. Reactive oxygen species (ROS), reactive nitrogen species (RNS), and reactive sulfur species (RSS) are the names given to this class of free radicals [3]. Hydroxyl radicals, superoxide anions, and hydrogen peroxide are examples of ROS. [1]. ROS are produced during cellular metabolism and physiological stimulation and play critical roles in gene expression, cell signalling, and ion transport [3]. However, significant amounts of free radicals can raise oxidative stress, which can damage biological molecules like DNA, carbohydrates, proteins, and lipids [2]. This damage can lead to diseases such as cancer, diabetes, liver damage, ageing, and rheumatoid arthritis [3].

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Antioxidants are substances that contribute positive electrons and can counteract the effects of free radicals [4]. Antioxidants are oxidation inhibitors that attach electrons to radicals, causing the free radicals to become stable. Antioxidants are known to reduce the risk of a variety of diseases, including cancer and heart disease [5].

Antioxidants can be found in naturally occurring compounds. The zodia plant (*Evodia suaveolens*) is an endemic plant in Indonesia that originated in Papua. This plant has been empirically cultivated by the community as an aesthetic plant and a mosquito-repellent plant [6]. There has never been any research done on the antioxidant capacity of zodia protein extract. Thus, the purpose of this study is to assess the antioxidant activity of protein extracts using methods such as the 2,2'-Azinobis 3-ethyl Benzothlazole-6-Sulfonic Acid ($ABTS^{\bullet+}$) assay, hydroxyl radical ($OH^{\bullet}$), and superoxide anion ($O_2^{\bullet-}$) scavenging.

2 Materials and methods

2.1 Extraction of protein

Extraction of protein from the zodia plant (leaves, stems, and roots) was accomplished by grounding fresh (1 gram) in Tris-HCl (50 mM, pH 7.6, 3 mL), followed by 15 minutes centrifugation at 4°C 10.000 rpm. The Bradford method [7] was then used to determine the amount of soluble protein in the pooled supernatant. A mixture of 45 µL of aquadest and 950 µL of Bradford solution was mixed with 5 µL of supernatant. At 595 nm, absorbance was measured using spectroscopy. To determine the protein concentration, the data were compared to the Bovine Serum Albumin (BSA) standard.

2.2 ($ABTS^{\bullet+}$) assay

The $ABTS^{\bullet+}$ radical cation assay was carried out using the method reported by Re et al. [8]. The ABTS solution was diluted with sodium phosphate buffer saline (200 mM, pH 7.4) to achieve absorbances ranging from 0.70 to 0.75 AU at 734 nm. At 734 nm, the absorbance was measured. Using the formula below, the antioxidant activity was calculated:

$$ABTS^{\bullet+} \text{ radical cation } (\%) = [(Ac\text{-}As)/Ac] \times 100\% \quad (1)$$

Ac = absorbance control and As = absorbance sample

2.3 $OH^{\bullet}$ scavenging

The Halliwell method was used to determine the hydroxyl radical scavenging activity [9]. The protein extract was treated for 1 hour at 37°C with 2-deoxy-D-ribose (28 mM, 10 µL) in KH_2PO_4 (20 mM, pH 7.4), EDTA (1 mM, 20 µL), $FeCl_3$ (10 mM, 2 µL), KH_2PO_4 (20 mM, pH 7.4, 145 µL), ascorbic acid (1 mM, 20 µL), and H_2O_2 (1 mM, 2 µL). After 30 minutes of incubation at 80°C, TBA (1%, 100 µL) and TCA (2.8%, 100 µL) were added to the solution. A spectrophotometer was used to detect the absorbance at 532 nm.

2.4 $O_2^{\bullet-}$ scavenging

Tang et al. methods were used to investigate superoxide anion radical scavenging [10]. The sample (200 µL) was immersed in Tris-HCl (10 mM, pH 8.2, 1.9 mL) for 10 minutes at room temperature. Pyrogallol (10 mM, 100 µL) was mixed with HCl (10 mM). A spectrophotometer was used to test the absorbance at 320 nm.

Analysis of variance (ANOVA) was used to assess all the experimental outcomes statistically. The significant difference was found by Duncan's test at $p<0.05$.

3 Results and discussion

3.1 Concentration of protein

The concentration of protein analysis using Bradford on zodia yielded varied results. Fig. 1a shows that the leaves have a high protein content of 5.30 μg/μl. This is due to the existence of the ribulose-1,5-bisphosphate carboxylase protein, which accounts for 28% of the total protein in the plant in some plants [11].

3.2 ($ABTS^{\bullet+}$) assay scavenging activity

Evaluating antioxidant activity on zodia plants is used to evaluate changes in antioxidant protein activity from different protein sources in zodia plants based on organs. Zodia antioxidant protein activity was assessed using the 2,2'-Azinobis 3-ethyl Benzothlazole-6-Sulfonic Acid (ABTS) assay. The benefit of utilizing this procedure is that it produces reasonably quick and cost-effective solutions. The ability of antioxidants to decrease ABTS can be evaluated using 734 nm spectrophotometry [8]. Fig. 1b and Table 1 show that, despite having the same concentration (5-20 μg/μl), zodia proteins based on plant tissue have distinct ABTS radical inhibition values. The antioxidant activity testing results utilizing the ABTS method with very low concentrations and IC_{50} values in leaves, stems, and roots were 9.67, 8.12, and 13.81 μg/μl, respectively. A low IC_{50} value indicated that protein zodia can be exploited as a source of antioxidant protein. This is due to the fact that antioxidants have a role in reducing cell damage induced by free radicals [12].

3.3 $OH^{\bullet}$ scavenging activity

Hydroxyl radicals are the most reactive radicals in the human body, and they can cause a variety of issues. Hydroxyl radicals are formed when hydrogen peroxide reacts with superoxide radicals, which is facilitated by transition metals. Fig. 1c and Table 1 depict how proteins from the zodia organ react with hydroxyl radicals. Furthermore, a protein isolated from stems has greater hydroxyl radical inhibitory action. Stem protein has a very low IC_{50} (12.87 μg/μl), whereas leaf and root IC_{50} values are 16.54 and 15.95 μg/μl, respectively. These findings indicate that proteins found in stems are more effective at combating hydroxyl radicals. Proteins can limit free radicals because the amino acids in them can contribute positive electrons to free radicals [13].

3.4 $O2^{\bullet-}$ scavenging activity

suppress superoxide radicals. When compared to leaf and root proteins, stem protein has the highest superoxide radical inhibition value. The IC_{50} value is lower in comparison to leaf and root proteins. The IC_{50} value of stem protein is 20.08 μg/μl, while the values for leaves and roots are 33.44 and 20.63 μg/μl, respectively. Proteins with high amounts of antioxidant amino acids have a stronger ability to ward against superoxide radicals. This is due to the fact that these amino acids contribute electrons to superoxide radicals [14].

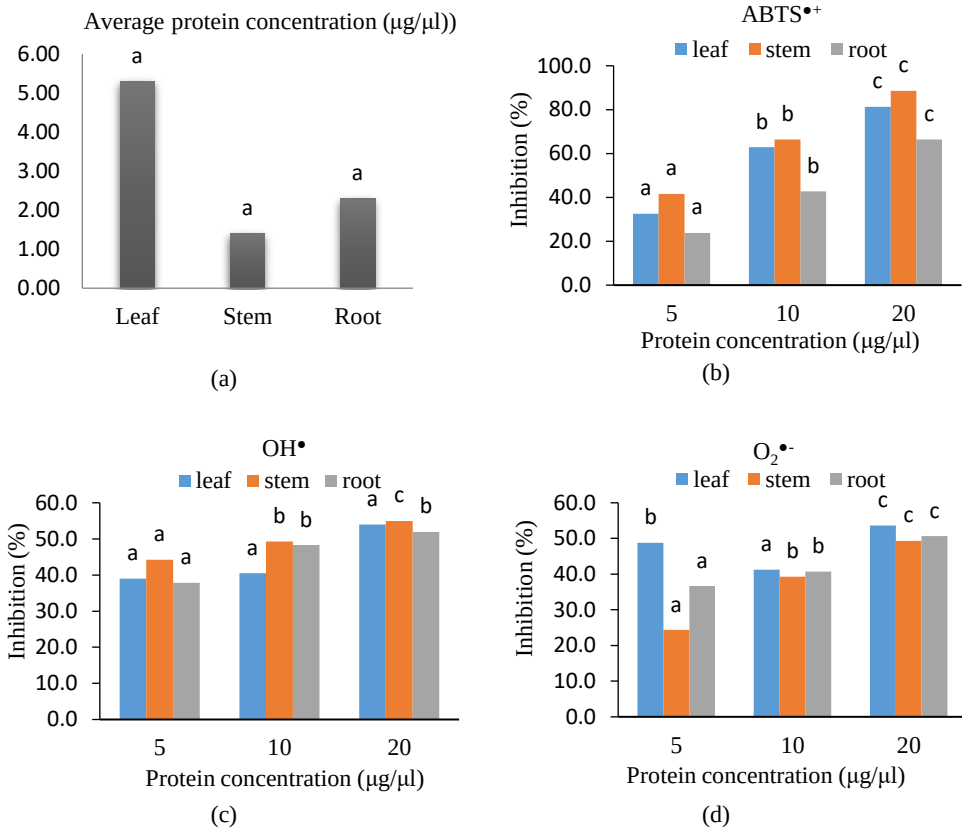

Fig. 1. Protein concentration and free radical scavenging activity of zodia

Table 1. IC_{50} value of the zodia protein's free radical inhibitory action

IC_{50} (µg/µl)			
	Leaf	**Stem**	**Root**
$ABTS^{\bullet-}$	9.67 ± 0.08^{a}	8.12 ± 0.81^{b}	13.81 ± 0.26^{a}
Hydroxyl Radical ($OH^{\bullet}$)	16.54 ± 0.05^{a}	12.87 ± 0.38^{b}	15.95 ± 0.45^{a}
Superoxide Radical ($O2^{\bullet-}$)	33.44 ± 0.05^{a}	20.08 ± 1.57^{a}	20.63 ± 0.87^{a}

4 Conclusion

$ABTS^{\bullet+}$, $OH^{\bullet}$, and $O_2^{\bullet-}$ scavenging analysis indicated that zodia protein extracts can suppress $ABTS^{\bullet+}$, $OH^{\bullet}$, and $O_2^{\bullet-}$ radicals. However, when paired with leaves and roots, protein extracted from stems provides the most effective resistance, as evidenced by the lower IC_{50} value. Collectively, zodia protein extract as a whole possesses antioxidant properties, especially in the stem protein.

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