Extraction and stability analysis of antioxidant activity from *Stenochlaena palustris*

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Abstract:

Stenochlaena palustris is an edible fern from the *Blechnaceae* family and is a known natural antioxidant sources. Antioxidants are compounds with a molecular structure that is able to donate its electrons to free radical molecules and terminate the chain reaction. Antioxidants have a function to prevent various diseases caused by oxidative stress. The human body will be healthier if vegetables and fruits with high antioxidant content are sufficiently consumed. The aims of this research was to determine the proper maceration solvent to obtain an *S. palustris* extract with the highest antioxidant activity and to analyze the antioxidant stability through different temperatures, pH, light condition, and time of the extract *S. palustris* from the selected solvent. The solvents used were ethanol 70% and distilled water with three different extraction times, 12, 24, and 48 hours at room temperature. It is revealed that maceration using distilled water for 48 hours had the highest antioxidant activity compared to other extraction samples using DPPH assay. The extract with highest antioxidant activity was observed through stability test with different temperatures (5^0C, 30^0C, 50^0C, 70^0C, 90^0C), pH (4, 5, 6, 7), and light conditions (dark and bright). The stability test revealed that the antioxidant activity, total phenolic and flavonoids content of the selected extract on pH 4 and 5 stored at 70^0C was more stable than the other conditions.

Keywords: *S. palustris*, antioxidant activity, extraction, stability.

Introduction

Free radicals trigger cell damage by pairing the unpaired electron with other molecule, which this unification will lead to oxidative stress process in cells and molecules. Furthermore, oxidative stress resulted from free radicals will contribute to several chronic diseases, for instance, diabetes mellitus, heart disease, cancer, stroke, and Alzheimer [1]. Therefore, in order to neutralize free radicals, a human body definitely needs antioxidant. Antioxidants are abundant in some plants are present in seeds, vegetables, and fruits [2]. One of the sources of natural antioxidant is from *Kalakai* plant or *Stenochlaena palustris* that is arises in tropical district especially Central Kalimantan [3]. As supported by Ho *et al* (2010), bioactive components of ferns mainly belong to phenolic, flavonoids, alkaloid, and terpenoid families. Dai & Mumper (2010) added that phenolic compounds and flavonoids have been demonstrated to be potent antioxidant. According to the experiment done by Chai *et al* (2012), *S. palustris* had total of 51.69 mg/g dry matter polyphenol content and 58.05 mg/g dry matter flavonoids. In previous experiments, the antioxidant extract of *S. palustris* plant was obtained through maceration. Fidrianny *et al* (2013) reported that antioxidant activity is influenced by the polarity of solvent and length of extraction time. Thus, extraction of *S. palustris* in different choices of solvent and length of times needs to be done in order to determine which condition will provide maximum extraction based on antioxidant activity.

Furthermore, the utilization of antioxidant compounds in food industries is getting better along with the growing of free radical awareness [8]. Some of studies have showed the relationship between antioxidant-rich foods with prevention of human diseases [9]. Antioxidant as one of functional characteristic that is had by *S. palustris* is the potential for its utilization as food ingredient. Additionally, *S. palustris* could be harvested in the wild and sold in food markets [6]. However, *S. palustris* plant has not been utilized on food products industrially. Means, the proper condition to maintain its antioxidant activity during food processing is still unknown. In this research, analysis of *S. palustris* plant as a source of antioxidant in foods and beverages and its stability condition will be performed.

Materials and methods

The materials which were used in this research are the *Kalakai* plant or *S. palustris*, which were obtained from Central Kalimantan, Indonesia. The used chemical substances were ethanol 70%, distilled water, DPPH (1,1-diphenyl-2-picrylhydrazyl), methanol, Folin-Ciocalteu reagent, galic acid, deionized water, quercetin, aluminum chloride dehydrate, ethanol 70%, ethanol 96%, sodium carbonate, sodium nitrite, sodium hydroxide, pH buffer solution 4-7.

DPPH radical scavenging activity for antioxidant activity analysis, total phenolic content, and total flavonoids content of *S. palustris* extract was determined using the method which previously modified by Chai *et.al* (2012).

Sample preparation

S. palustris plant from Kalimantan was used as the raw material. The plant was cleaned from dirt, washed, drained, and weighed as wet weight. Then, the plant was oven dried for 24 hours at 40°C to totally remove the moisture content and weighed again as a dry weight. Next, the dried sample was blended into powder, sieved and put to plastic bag in order to avoid humidity and filth

Extraction

After the powder of *S. palustris* plant was obtained from prepared sample, the powder and solvent solutions (ethanol and water) were combined with ratio 1:20 [6]. Using water bath shaker, the extraction of antioxidant from *S. palustris* was started at two different types of solvent which are ethanol and water and three different times which are 12, 24, 48 (hours). Further, the maceration process was stopped and the samples were filtered with filter paper and vacuum filtration. Then, the samples were centrifuged at 6000 rpm at room temperature for 20 minutes. The most proper maceration method was next determined after the data analysis was taken from UV-Visible Spectrophotometer on purpose of obtaining antioxidant activity, total phenolic content, and total flavonoid content of the sample extract.

Antioxidant activity assay

DPPH radical scavenging activity of *S. palustris* extract was determined using the method which previously modified by [6]. In preparing the DPPH assay, 39.4 mg of DPPH were diluted with 1 L of analytical grade methanol to make 0.10 mM DPPH solution. In undertaking the analysis, 1 ml of *S. palustris* extract was mixed with 1 ml of 0.10 mM DPPH solution. Next, the mixture was left for 30 minutes in dark condition. Afterwards, the absorbance of the mixture was measured at 517 nm using UV-Vis spectrophotometer. Likewise, the blank sample was prepared by replacing DPPH solution with methanol. DPPH scavenging ability (%) was estimated as follows:

$$\text{DPPH radical scavenging ability (\%)} = (A\_control\text{-}A\_sample)/A\_control \times 100 \quad (1)$$

The results are showed as IC50 number, which represents the capability of the concentrations of sample extract to inhibit 50% of DPPH radicals [6].

Total phenolic content analysis

The concentration of total phenolics in *S. palustris* was completed using a Folin-Ciocalteu colorimetric assay [6]. Gallic acid was used as a standard. Zero to 100 mg/l gallic acid were prepared to make a standard curve. In conducting the analysis, 0.2 ml of *S. palustris* extract was added with 0.8 ml of deionized water and 0.1 of Folin-Ciocalteu reagent. Then, the mixture was incubated at room temperature for 3 minutes. Afterwards, the previous mixture was added with 0.3 ml of sodium carbonate (Na_2CO_3) (20% w/v). Next, the mixture was incubated again at room temperature but for 120 minutes. After that, the absorbance of the mixture was measured at 765 nm using UV-Vis spectrophotometer. Total phenolic content was represented in mg gallic acid equivalents (GAE)/g dry matter.

Total flavonoid content analysis

The concentration of total flavonoids in *S. palustris* extract was accomplished using a method adapted from [6]. Zero to 500 µg/ml quercetin were dissolved in 80% analytical grade ethanol and next used as a standard calibration curve. In undertaking the analysis, 0.2 ml of *S.palustris* plant extract was added to 0.15 ml of $NaNO_2$ (5% w/v). Then, the mixture was incubated for 6 min at room temperature. Next, 0.15 of $AlCl_3.6H_2O$ (10% w/v) was added to the mixture and abandoned again at room temperature for 6 minutes. Afterwards, the mixture was mixed together with 0.8 ml of sodium hydroxide (10% w/v). After that, the mixture was left again for 15 minutes at room temperature. The mixture was then read at 510 nm using UV-Vis spectrophotometer. For the blank, the sample of *S.palustris* extract was replaced with water. Total flavonoid content was expressed in mg quercetin equivalents/g dry matter.

Stability Test

The stability test during storage was done by firstly conducting an extraction of antioxidant by using the obtained solvent and time for *S. palustris* dried plant. Afterwards, the extract will be then divided into four different food pH levels; 4, 5, 6, 7 with the addition of pH buffer solution 4-7. The stability during storage were observed at 5^{o}C, 30^{o}C, 70^{o}C, and 90^{0}C. Next, the samples also will be tested through light exposures; in dark and transparent vials. The stability test resulted the data analysis of antioxidant activity (DPPH), total phenolic content, and total flavonoid content.

Results and discussion

Stenochlaena palustris was extracted through various treatments in purpose of determining the most proper condition of extraction. The treatments included two factors. The factors are three different extraction times (12, 24, 48 hours), and 2 different solvents (ethanol and water). Extraction with water as the solvent and 48 hours as the extraction time was found to be the most proper condition to obtain high antioxidant activity, total phenolic and flavonoids content of *S. palustris*.

Table 1. Antioxidant activity, Total phenolic content and Total flavonoid content of *S. palustris* extract extracted by water for 48h.

Response	**Content**
Antioxidant Activity (IC_{50}), µg/ml	4.20 ± 0.01
Total Phenolic Content, mg GAE/g	4.85 ± 0.07
Total Flavonoids Content, mg QE/g	4.24 ± 0.04

The stability test was completed with four different pH levels, which were pH 4, 5, 6, and 7 storage for 6 days showed in Figure 1. It is presented that pH 4 had lowest decreasing value of percent antioxidant activity during 6 days storage followed by pH 5. This result was supported by previous research that is stated that plant aqueous extracts were able to inhibit H_2O_2 as free radicals more effectively at acidic pH [11]. Additionally, *S. palustris* extract was proven to have high average amount of antioxidant activity compare with vitamin C [12]. Vitamin C is an electron donor and it is also a powerful water-soluble antioxidant in humans [13]. Moreover, Vitamin C is more potent as antioxidant at lower pH [14].

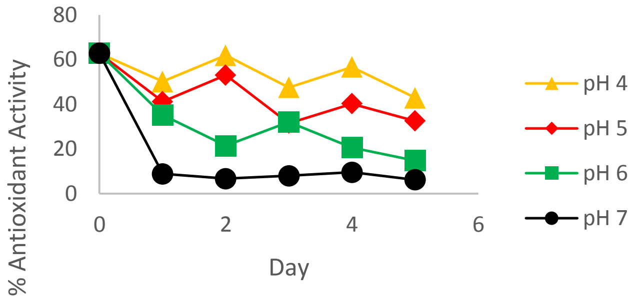

Figure 1. Antioxidant activity at pH 4-7 during stability

During storage, total phenolic content of *S. palustris* extract was also observed in pH 4, 5, 6, and 7. As the result, during storage phenolic content in pH 5 showed higher value than other pH condition (Figure 2). Some naturally occurring polyphenolic compounds are damaged when exposed to higher pH [15]. High amount of hydroxycinnamic acids and their proportion in total phenolic content was found to increase in the extract of *S. palustris* [6]. Total flavonoids content of *S. palustris* extract was also observed in pH 4, 5, 6, and 7 during storage. It is presented that total flavonoid in pH 5 had higher value compared to other pH treatment. Anthocyanins were likely the key of flavonoids compounds responsible for radical scavenging activity in the extract of young fronds of *S. palustris* [6]. Further, anthocyanins are considered as flavylium cation, which is stable in acidic pH.

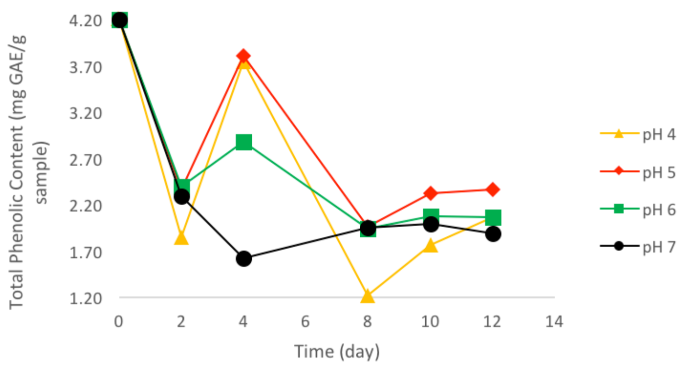

Figure 2. Total phenolic content at pH 4-7 during stability

The stability test on different temperatures was observed to find out the effect of thermal coverage on antioxidant from *S. palustris*. The stability test was completed with five different temperatures, which were 5^{o}C, 30^{o}C, 50^{o}C, 70^{o}C, and 90^{o}C. pH 4 and 5 were not too influenced by heat treatment since the data indicates a tendency of higher antioxidant activity, phenolic and flavonoids content. In contrary, pH 6 and 7 were affected by heat treatment where there was a decrease in antioxidant activity, phenolic and flavonoids as well. Many phenolic compounds are easily hydrolyzed and oxidized when temperature is increased [5] and some naturally occurring polyphenolic compounds are damaged when exposed to high pH [15]. The temperature of 70^{o}C was found to be the suitable temperature storage condition for antioxidant activity and total flavonoids content, while 50^{o}C is the suitable temperature storage condition for total phenolic content.

Conclusions

Extraction with distilled water and for 48 hours were found to be the suitable condition for antioxidant properties in *S. palustris* extract. During stability, pH 4 showed higher antioxidant activity and pH 5 showed both higher phenolic and flavonoids content. 70^{o}C was found to be suitable for antioxidant activity and flavonoids content, while 50^{o}C was suitable for phenolic content.

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