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Antioxidant Activity of Methanol Extract of *Halodule uninervis* Seagrass from the Coastal of Lampung, Indonesia.

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ABSTRACT

The purpose of this study was to analyze phytochemistry contents and antioxidant activity of the methanol extractsfrom seagrass of *Halodule uninervis*. The samples were collected from the coastal of Lampung. Parameters of research included phytochemical content, DPPH scavenging activity and reducing power. The result showed content of phytochemical compounds of methanol extract seagrass are flavonoids, alkaloids, steriod and phenols. Antioxidant activity with DPPH method (IC50) of *H.uninervis* was 1.575 ppm. The highest of reducing power of *H.uninervis* was 1.381.

Keywords : Seagrass, *Halodule uninervis*, antioxidant.

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INTRODUCTION

Seagrasses are the marine flowering plants. Seagrasses such as *Amphibolis, Heterzostera, Phyllospadi, Posidonia, Pseudalthenia* and *Zostera* are mostly restricted to temperate seas and seven genera of seagrasses such as *Cymodocea, Enhalus, Halodule, Halophila, Syringodium, Thalasia* and *Thalassodendeon* are distributed in tropical seas [1]. Sea grass biomass is used as human food especially by coastal populations [2].

Numerous seagrasses have been shown to have antibacterial activities. *Halophila stipulacea, Cymodocea serrulata* and *Halodule pinifolia* [3], *Enhalus acoroides* [4] and *Enhalus acoroides, Thalassia hemprichii, Halodule pinifolia, Syringodium isoetifolium,* and *Cymodocea rotundata* [5] have been reported to exhibit antibacterial activity. Moreover, preliminary data suggest that seagrasesses could represent an interesting source of antilarvacidal [6] and antioxidant [7]. However, there is a little information regarding seagress from *Halodule uninervis* and their antioxidative activity.

MATERIALS AND METHOD

Preparation of Sample

Sea grass of *Halodule uninervis* was collected from Coastal of Lampung and immediately brought to the laboratory in sterile plastic bags containing water to prevent evaporation. Seagrasses were washed thoroughly with water to remove extraneous materials and shade-dried for 10 days at room temperature until constant weight obtained. The dried Seagrasses were powdered and stored in refrigerator for future use.

Preparation of Sea grass extract

Seagrass powder were soaked in 2 L with methanol (1:4 w/v), and kept for 10 days in a shaker. The extraction was repeated thrice and pooled. The dry aqueous extracts were stored in a refrigerator until further analysis.

Phytochemical Screening of *Halodule uninervis*

Flavonoids

Test of flavonoids were determined by Wadood et al method [8]. A sample was heated with 10 ml of ethylacetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1ml of dilute ammonia solution. A yellow colouration indicated the presence of flavonoid.

Alkaloids

Test of alkaloids were determined by Trease & Evan method [9].

Saponins

Test of saponin were determined by Wall et al method [10]. A sample was shaken with water in a test tube. Frothing which persists on warning confirmed the presence of saponins.

Steroids

Test of steroids were determined by Edeoga et al method [11]. 1 ml of acetic anhydride was added twice to 0.5 g of aqueous extract with 2 ml H_2SO_4. The colour changed from violet to blue or green in sample indicating the presence of steroids.

DPPH radical scavenging activity

DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity was determined by Hanani *et al*. method [12].

The antioxidant activity of each sample was expressed in percentage inhibition of free radicals which is calculated by the formula:

$$\% \text{ Inhibition} = \frac{\text{blanko absorbance} - \text{sample absorbance}}{\text{blanko absorbance}} \times 100\%$$

Concentration and barriers to samples the value of each plotted on the x axis and y. Obtained equation in the form [y = b(x) + a] is used to find the value of IC (Inhibitory concentration) with a stated value of y is 50 and the value of x as IC50. The data obtained analyzed descriptively.

Reducing power

Reducing power was determined by Oyaiza method [13]. The methanol extract of *Haloduleuninervis* was mixed with 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50^0C for 20 min. 2.5 ml of 10% trichoroacetic acid (TCA) was added to the mixture, followed by centrifugation at 3000 rpm for 10 min. The upper layer of solution (2.5 ml) was mixed with 2.5 ml of distilled water and 2.5 ml of 0.1% ferric chloride and the absorbance was read at 700 nm.

RESULT AND DISCUSSION

The phytochemical screening

As seen as Table 1 showed content of phytochemical compounds ofmethanolextract of seagrass were flavonoids, alkaloids, steriod, tannins and phenols.

Table 1. Phytochemical compound ofmethanol extract of *H.uninervis* Seagrass

	Flavonoid	Alkaloid	Saponin	Steroid
Extractof *Haloduleuninervis*	+	++	-	++
(+) indicates present while (-) indicates absent				

Flavonoid compounds have been found in methanol extract of *H.uninervis* seagrasses. Earlier study by Anwariyah [14] reported flavonoids have been found in seagrasses *C. rotundata*. Further screening of seagrass of *H.uninervis* showed the result of assayed of saponin compounds from seagrass of *H.uninervis* have not been found, due to the extracts not form foam. Saponin compounds also have been found in methanol extract of *H.uninervis* seagrasses. Saponin is a strong active compounds and cause foam when homogenized with water. Saponins are glycosides triterpena and sterols that have been detected in more than 90 tribes plants [15]. Steroids is typical of higher plants. We also detected a number of steroids in the methanol extract of *H.uninervis,* in a previous study of seagrass, steroid detected in extracts of *C. rotundata* with methanol, n-hexane and ethyl acetat [14]

DPPH radical scavenging activity

Antioxidant activities of extract of *Halodule uninervis* depicted in Figure 1. Increasing of concentration of *Halodule uninervis* indicates an increase in DPPH radical scavenging activity**.** The stable organic radical DPPH has been widely used in plant extracts [16], and foods[17], etc. The method is based on the reduction of alcoholic DPPH solutions at 517 nm in the presence of an hydrogen donating antioxidant (AH) due to the formation of the non-radical form DPPH-H.

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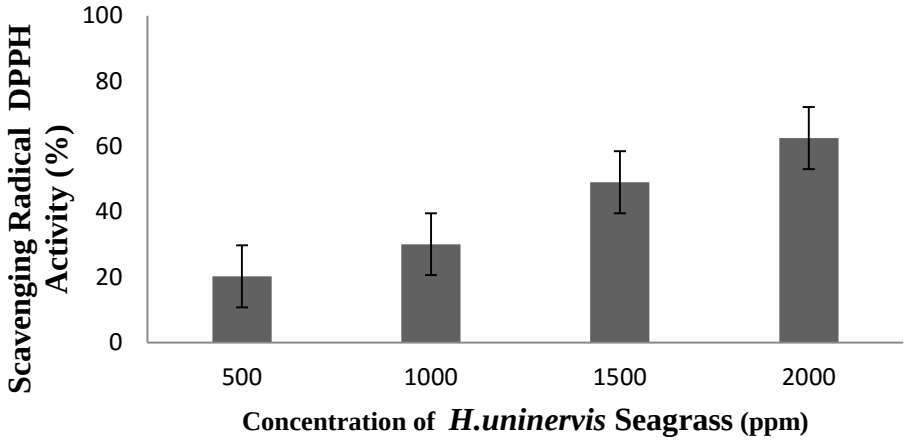

Figure 1. Scavenging radical DPPH activity ofmethanol extract of *H.uninervis* Seagrass

The IC50 of extract was 1.575 ppm. Antioxidants react with DPPH, reducing a number of DPPH molecules equal to the number of their available hydroxyl groups. Therefore, the absorption at 515 nm was proportional to the amount of residual DPPH [18]. Free radical scavenging activity of extract of *Halodule uninervis* could be due to their higher content of flavonoids components. Flavonoids have been interrupt the propagation of autoxidation by donating hydrogen atom of some hydroxyl (OH) bases that are attached outside the benzene rings, resulting in the formation of stable free radical [19]. These groups of polyphenolic compounds are very important in plant because they make their defense mechanisms [20].

Reducing power

Reducing power of extract of *Halodule uninervis* depicted in Figure 2. Increasing of concentration of *Haloduleuninervis*indicates an increase in reducing power

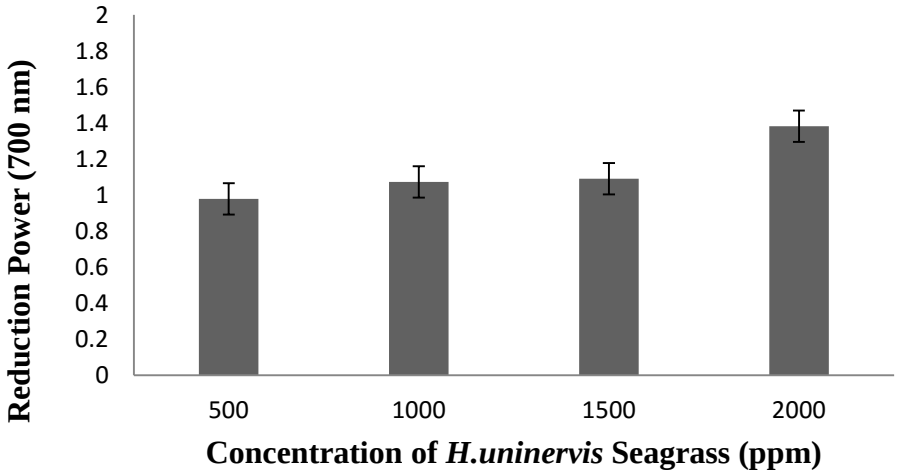

Figure 2. Reduction power of methanol extract of *H.uninervis* Seagrass

The reducing capacity of the extract is another significant indicator of antioxidant activity. In the reducing power assay, the presence of antioxidants in the sample would result in the reduction of Fe^{3+} to Fe^{2+} by donating an electron. Reducing power of a methanol extract of *H.uninervis* is probably due to the presence of group hydroxyl in the phenol compounds that can serve as an electron donor. Therefore, antioxidants are considered reducers and inactivating oxidants

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