

Antioxidant activity of methanol extract of *Diplazium esculentum* (Retz.) Sw. leaves collected from Aceh

Halimatussakdiah*, U Amna, V Mardina

Chemistry Department, Faculty of Engineering, Universitas Samudra, Langsa, Aceh, Indonesia

*halimatussakdiah@unsam.ac.id

Abstract. Cell damage mediated by free radicals is one of the main causes of many dangerous diseases such as cancer, autoimmune disorders, rheumatism, cataracts, aging, cardiovascular disease, diabetes, arthritis, Parkinson, Alzheimer and neurodegenerative diseases. The prevention of dangerous diseases caused by free radicals can be done by developing raw materials for natural antioxidant drugs that can reduce free radicals by giving one of their electrons to produce neutral molecules that are not harmful to the human body, such as fern (*Diplazium esculentum* (Retz.) Sw.). *D. esculentum* collected from Aceh was extracted in methanol. The antioxidant activity of this extract was determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity assay. The result showed very good activity of the DPPH antiradical efficiency of methanol extract of *D. esculentum* with IC₅₀ values of 123.958 ppm. Based on phytochemical screening, *D. esculentum* contained polyphenol compounds that have good activity as antioxidant. This result indicated that *D. esculentum* has potential as antioxidant and can be applied in the development of new medicines.

1. Introduction

Every organism needs oxygen to survive. Oxygen can be a very reactive molecule that can damage organisms by producing a free radical, namely Reactive Oxygen Species (ROS) from the reduction and oxidation reaction of cell. ROS is a free radical which is responsible for several diseases such as cancer, autoimmune, rheumatic, cataract, aging, cardiovascular disease, diabetes, arthritis, Parkinson's disease, Alzheimer's and neurodegenerative diseases [1, 2, 3]. In general, free radicals are produced by endogenous sources, such as mitochondrial leakage, respiratory outbursts, enzyme reactions, autoimmune reactions, and environmental sources such as smoking, pollutants, ultraviolet and ionization radiations, and xenobiotic [1]. In addition, oxidative processes are one of the most important routes for producing free radicals in food, drugs, and even in living systems. Today there has been increased interest in the potential for therapeutic medicinal plants as antioxidants in reducing oxidative stress tissue injury [4]. Cell damage mediated by ROS is one of the main causes of many diseases and hence the development of natural antioxidants continues to be carried out because it is very beneficial for human health [5].

Antioxidants are chemical compounds that can contribute one or more electrons to free radicals to scavenge free radicals activity. The human body does not have an excessive amount of antioxidant reserves. Therefore, human body needs exogenous antioxidants which include beta carotene, vitamin C, vitamin E, zinc (Zn), and selenium (Se) in the case of exposure to excess radicals. Antioxidants are divided into two types, namely synthetic antioxidants and natural antioxidants. Synthetic antioxidants are often avoided because of their dangerous side effects. Natural antioxidant is an indispensable alternative because they

can protect the human body from damage caused by ROS, inhibit degenerative diseases and also inhibit lipid peroxide in food. Antioxidants prevent damage caused by free radicals by scavenging them so they can protect the body from oxidative stress [6]. The search for the potential of medicinal plants as an alternative source of new medicines has received great attention because of its low price and few side effects [7].

Plants are the main source of food, animal feed, fuel, and are also categorized as a source of medicines or called medicinal plants [8]. Medicinal plants play an important role in the human health sector since the beginning of civilization. Different parts of plants used for medical healing purposes have been developed since ancient times [9]. One of plants that had antioxidant activity is edible fern (*Diplazium esculentum* (Retz.) Sw.) [1, 2, 4, 5, 6, 10, 11]. Fern plants belonging to the Athyriaceae family are one of the lowland plants that are widespread in several regions in Indonesia, including the Aceh Province. In general, Acehnese community consume ferns as one of the vegetables and a small group of people use their leaves as insect repellents.

Apart from being an antioxidant, several studies have reported that ferns have some other bioactivities such as antibacterial [12, 13]), antimicrobials [11], analgesics [14], hepatoprotective [15], and anti-inflammatory [15]. Those activities are caused by the content of secondary metabolites in ferns. Based on phytochemical screening, ferns contain compounds such as alkaloids, terpenoids, steroid saponins, tannins, phenols and flavonoids [2, 10, 16, 17]. Generally, polyphenol compounds such as phenols and flavonoids are the most dominant contained in ferns.

Among many natural antioxidants; Ascorbic acid, carotenoids and phenolic compounds are more effective in inhibiting lipid peroxidation by deactivating lipoxygenase, binding to free radicals and ROS by spreading the reaction cycle and for chelating heavy metal ions [4]. Flavonoids and phenolic compounds have been shown to eliminate or deactivate free radicals, in addition to being able to protect lipids and vitamin C from destruction in oxidative processes [3].

2. Materials and Methods

2.1. Plant Material

The leaves of *D. esculentum* (Retz.) Sw. were collected from Blangkejeren, Aceh (Indonesia) in February 2018.

2.2. Instrument

Rotary evaporator (Buchi R-100) and spectrophotometry Ultraviolet (AE-S60-2UP UV).

2.3. Preparation of Extract

The air-dried leaves (1 kg) of *D. esculentum* (Retz.) Sw. were ground and extracted with methanol by maceration method for 3 x 24 h, the maceration process was repeated until got clear filtrate. The extract solution was filtered and evaporated by rotary evaporator to give methanol extract [17].

2.4. Phytochemical Screening

2.4.1. Alkaloid.

About 2 g of samples were crushed then added 1 mL of ammonia. Furthermore, 10 mL of chloroform was added, then crushed and filtered. The filtrate was added 10 mL of 2 N H₂SO₄, shaken vigorously, left for a minute until the sulfuric acid solution and chloroform

separated. The sulfuric acid layer is taken and divided into three test tubes and each test tube is tested by Meyer, Dragendorff, and Wagner reagents to determine the presence of alkaloids. The addition of Meyer reagent established white precipitate, Dragendorff reagent caused reddish precipitate, and Wagner reagent raised yellow precipitate. Those results indicate the presence of alkaloids [17].

2.4.2. Terpenoid, Steroid, and Saponin.

Two grams of methanol extract was partitioned with hexane. The soluble extract in hexane was tested with the Liberman-Bourchard reagent. The blue or green color exhibits the presence of steroids and red color indicates terpenoids. The insoluble residue in hexane is added water and shaken vigorously. The presence of the stable foam for 30 minutes indicates the existence of saponins, if it shows positive for saponins, the solution was hydrolyzed with HCl then tested with the Liberman-Bourchard reagent. The green or blue color indicates the presence of steroidal saponins and the purple or red color shows the existence of terpenoid saponins [17].

2.4.3. *Flavonoid*. The concentrated methanol extract was partitioned with hexane then the residue was extracted with 10 mL of 80% ethanol, subsequently added 0.5 mg of magnesium and 0.5 M HCl. The pink or purple color shows the presence of flavonoids [17].

2.4.4. *Phenol*. Methanol extract tested by Ferric Chloride. Add 3 – 4 drops of FeCl₃ solution into extract, the formation of bluish black color exhibits the phenol compound [17].

2.4.5. *Tannin*. About 0.5 g of methanol extract was boiled in 10 ml of water in the test tube and then filtered. Add a few drops of 0.1% FeCl₃. Forming of a brownish green or bluish black color indicates tannins [17].

2.5. DPPH Radical Scavenging Activity Assay

The free radical 2,2-Diphenyl-1-picryl-hydrazyl (DPPH) was used to determine the free radical scavenging activity of the methanol extract of *D. esculentum* at concentration of 25, 50, and 100 ppm using the spectrophotometric method. As a comparison, the antioxidant activity of vitamin C was tested with concentration of 3, 6, 9, 12 and 15 ppm. Different concentrations of methanol extract and vitamin C (4 mL) were put in the test tube and then 1 mL of a methanolic solution of 0.4 mM DPPH was added. The reaction mixtures were homogenized using a vortex mixer and incubated at 37°C in the dark for 30 minutes. The absorbances of test mixtures were read at 517 nm against a blank. Scavenging activity was calculated using the following formula.

$$\text{DPPH Scavenging (\%)} = \frac{(A_0 - A_s)}{A_0} \times 100$$

Where, A₀ is the absorbance of the blank (DPPH) and A_s is the absorbance of the sample. The inhibitory concentration of the extract that caused 50% inhibition (IC₅₀) was calculated by equation Y = a + bX which obtained from the intersection of lines between % inhibition and the concentration [18].

3. Results and Discussion

3.1. Phytochemical Screening

Phytochemical screening is a preliminary test to determine the content of secondary metabolites in plants. It is one of approach in the medicinal plants research to detect plant secondary metabolites based on its classes. Secondary metabolites are compounds produced by plants as protectors, insect attracting hormones, and others. Phytochemical screening of

methanol extract of *D. esculentum* leaves has reported previously [17] which is shown in Table 1.

Table 1. Phytochemical screening test of *D. esculentum* (Retz.) Sw

Secondary Metabolites	Methanol Extract
Alkaloid	-
Terpenoid	-
Steroid	+
Saponin	+
Flavonoid	-
Phenol	+
Tannin	+

3.2. Antioxidant Activity

Methanol extract was investigated for antioxidant activity according to the method carried out by Ginting et al. [18]. The antioxidant activity was measured using the UV-Vis Spectrophotometer and absorbance was measured at 517 nm. DPPH will be reduced by the process of donating hydrogen or electrons that caused the colour change from violet to yellow. A change in colour intensity is proportional to the number of electron donations which followed by a decrease in DPPH absorbance. The greater the decrease in DPPH absorbance indicates the stronger the antioxidant activity.

Table 2. Antioxidant activity of methanol extract of *D. esculentum* (Retz.) Sw

Concentration (ppm)	Absorbance	% Inhibition	IC ₅₀ (ppm)
25	0.451	45.00	123.958
50	0.440	46.34	
100	0.420	48.78	

The intersection between % inhibition and the concentration in Table 2 produces a curve with the regression equation $Y = a + bX$ which is used to determine the IC₅₀ value of methanol extract and vitamin C antioxidant activities.

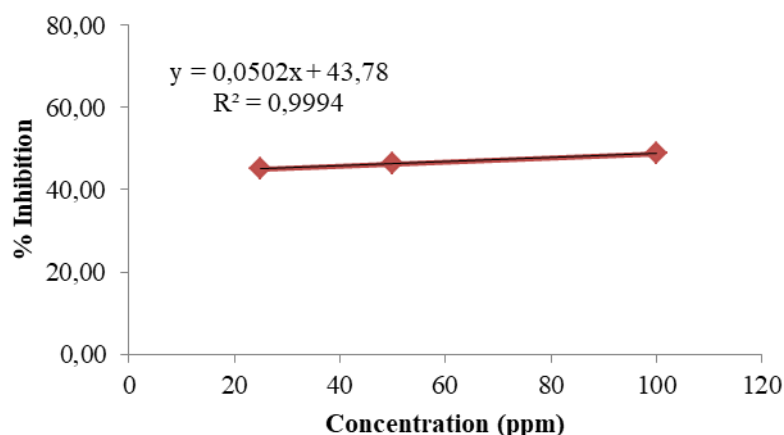


Figure 1.Antioxidant activity curve of methanol extract

Table 2 and Figure 1 showed that the antioxidant activity of methanol extract was 123.96 ppm. The IC₅₀ values of positive control (vitamin C) were obtained at 6.07 ppm. The result of this study can be categorized very strongly based on the range of antioxidant activity reported by Mangkasa et al. [19]. An antioxidant would be very strong if the IC₅₀ value ranged >150 ppm, the strong activity had IC₅₀ values of 150 – 300 ppm, moderate activity had IC₅₀ values of 300 – 400 ppm, and weak activity had IC₅₀ values of 400 – 500 ppm. In this case, this study showed that antioxidant activity of methanol extract was very good compared to the ethanolic extract of fern leaves reported by Junejo et al. [6] with IC₅₀ value of 138.8 ppm. The high antioxidant activity of fern (*D. esculentum*) is very closely related to the content of secondary metabolites, especially phenolic compounds in it. Phenolic compounds can donate hydrogen atoms to DPPH free radicals to form stable reduced DPPH (DPPH-H). The higher the phenolic content, the more DPPH radicals react and caused the concentration decreases [20].

4. Conclusion

Methanol extract of *D. esculentum* leaves contains steroid, saponins, phenols and tannins compounds. These compounds provided strong antioxidant activity in methanol extract with IC₅₀ values of 123.96 ppm and the IC₅₀ value of vitamin C was obtained at 6.07 ppm. The results of this study indicate that fern is very potential as antioxidants, so it can be used as a reference for further research in developing new natural antioxidants.

5. References

- [1] Roy S, Hazra B, Mandal N, and Chaudhuri T K 2013 assessment of the antioxidant and free radical scavenging activities of methanolic extract of *Diplazium esculentum*. *International Journal of Food Properties*, **16** 1351–70
- [2] Choudhury J, Majumdar S, Roy S, and Chakraborty U 2017 antioxidant activity and phytochemical screening of two edible wetland pteridophytes *Diplazium esculentum* (Retz) Sw and *Marsilea minuta* L. a comparative study *World Journal of Pharmaceutical and Medical Research*, **3**(9) 195-203
- [3] Hernández Zarate M S, Abraham Juárez M D R, Cerón García A, Ozuna López C, Gutiérrez Chávez A J, Segoviano Garfías J D J N, and Avila Ramos F 2018. flavonoids,

- phenolic content, and antioxidant activity of propolis from various areas of guanajuato, Mexico *Food Science and Technology* **38**(2) 210-15
- [4] Nair A G, Nikhila G S, Sangeetha G and Swapna T S 2015 free radical scavenging activity of methanolic extract of *Diplazium esculentum* – a wild fern of western ghats *International Journal of Current Research*, **7**(06) 17209-13
 - [5] Sarkar P and Dalal A 2016 evaluation of antioxidant activity of *Diplazium esculentum* and *Enhydra fluctuans* of west bengal *IOSR Journal of Pharmacy and Biological Sciences*, **11**(6) Ver. I 45-50
 - [6] Junejo J, A, Gogoi G, Islam J, Rudrapal M, Mondal P, Hazarika H, and Zaman K 2017 exploration of antioxidant, antidiabetic and hepatoprotective activity of *Diplazium esculentum* - a wild edible plant from north eastern India *Future Journal of Pharmaceutical Sciences* 1-9
 - [7] Hossain M S, Akter S, Begum Y, and Bulbul I J 2016 analgesic and anti-inflammatory activities of ethanolic leaf extract of *Phyllanthus acidus* L. on swiss albino mice *European Journal of Medicinal Plants* **13**(1) 1-10
 - [8] Patel D K 2015 diversity of underground medicinal and aromatic plants and their regeneration for further *ex situ* conservation in herbal garden *Journal of Biodiversity & Endangered Species* **3**(1) 1-3
 - [9] Kalita G J, Rout S, Mishra R K, and Sarma P 2015 traditionally used medicinal plants of bajali sub-division, barpeta district, assam *Journal of Medicinal Plants Studies* **3**(2) 8-17
 - [10] Das B, Paul T, Apte K G, Chauhan R, and Saxena R C 2013 evaluation of antioxidant potential & quantification of polyphenols of *Diplazium esculentum* Retz. with emphasis on its HPTLC chromatography *Journal of Pharmacy Research* **6** 93-100
 - [11] Akter S, Hossain M M, Ara I, and Akhtar P 2014 investigation of in vitro antioxidant, antimicrobial and cytotoxic activity of *Diplazium esculentum* (Retz) Sw. *International Journal of Advances In Pharmacy, Biology and Chemistry* **3**(3) 723-733
 - [12] Amit S, Sunil K, Bhatt S P, and Arvind N 2011 antibacterial activity of *Diplazium esculentum* (Retz.) Sw *Pharmacognosy Journal* **3**(21) 77
 - [13] Ullah M O, Haque M, Urmi K F, Zulfiker A H M, Anita E S, Begum M, and Hamid K 2013 anti-bacterial activity and brine shrimp lethality bioassay of methanolic extracts of fourteen different edible vegetables from bangladesh *Asian Pacific Journal of Tropical Biomedicine* **3**(1) 1-7.
 - [14] Chawla S, Chawla S, Ram V, Semwal A and Singh R 2015 analgesic activity of medicinally important leaf of *Diplazium esculentum* *African Journal of Pharmacy and Pharmacology* **9**(25) 628-32
 - [15] Nair A G, Nikhila G S, Sangeetha G and Swapna T S 2015 in vitro hepatoprotective and anti-inflammatory activities of *Diplazium esculentum* (Retz) Sw. – a wild fern from western ghats *International Journal of Institutional Pharmacy and Life Sciences* **5**(2) 341-48
 - [16] Tongco J V V, Villaber R A P, Aguda R M, and Razal R A 2014 nutritional and phytochemical screening, and total phenolic and flavonoid content of *Diplazium esculentum* (Retz.) Sw. from philippines *Journal of Chemical and Pharmaceutical Research* **6**(8) 238-42
 - [17] Halimatussakdiah, Amna U, and Wahyuningsih P 2018 preliminary phytochemical analysis and larvicidal activity of edible fern (*Diplazium esculentum* (Retz.) Sw.) extract against *Culex*. *Jurnal Natural* **18**(3) 141-47

- [18] Ginting B, Mustanir, Helwati H, Desiyana L S, Eralisa, and Mujahid R 2017 antioxidant activity of n-hexane extract of nutmeg plants from South Aceh province *Jurnal Natural* **17**(1) 39-44
- [19] Mangkasa M Y, Rorong J A, and Wuntu A D 2018 uji fitokimia dan aktivitas antioksidan dari ekstrak daun bawang kucai (*Allium tuberosum* Rottl. Ex Spreng) Menggunakan Spektrofotometer Uv-Vis *PHARMACON Jurnal Ilmiah Farmasi – Unsrat* **7**(4) 12 - 22
- [20] Adawiah, Sukandar D, and Muawanah A 2015 aktivitas antioksidan dan kandungan komponen bioaktif sari buah namnam. *Jurnal Kimia VALENSI: Jurnal Penelitian dan Pengembangan Ilmu Kimia* **1**(2) 130-136