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Antioxidant Activity Test of Tetanus Leaf (*Leea aequata* L.) Ethanol Extract with DPPH Method

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Abstract

Free radicals play an important role in oxidative stress, which can lead to a number of degenerative diseases, including heart disease, cancer and premature aging. Natural and artificial ingredients can be used to make antioxidants. The use of synthetic antioxidants is starting to be limited because excessive use can cause negative side effects, such as the accumulation of toxins in the body. This study aims to evaluate the antioxidant activity of tetanus (*Leea aequata* L.) leaf extract using DPPH method. Free radicals generated during metabolism can cause cell damage and contribute to various degenerative diseases. Therefore, it is important to find effective sources of antioxidants. In this study, Tetanus leaves were extracted using 96% ethanol solvent through maceration method. Antioxidant activity was evaluated by measuring the IC_{50} value against DPPH radical. The results showed that tetanus leaf extract had an IC_{50} value of 39.484 ppm, which indicated significant antioxidant activity. These results indicate that tetanus leaf extract can function as a free radical scavenging agent, which could potentially serve as a raw material in the development of herbal medicine to prevent oxidative stress-related diseases.

Keywords: Antioxidant, Tetanus leaf (Leea aequata L.), DPPH, free radicals, IC_{50}

Abstrak

Radikal bebas berperan penting dalam stres oksidatif, yang dapat menyebabkan sejumlah penyakit degeneratif, termasuk penyakit jantung, kanker dan penuaan dini. Bahan alami dan buatan dapat digunakan untuk membuat antioksidan. Penggunaan antioksidan sintetik mulai dibatasi karena penggunaan berlebihan dapat menimbulkan efek samping negatif, seperti penumpukan racun dalam tubuh. Penelitian ini bertujuan untuk mengevaluasi aktivitas antioksidan dari ekstrak daun titanus (Leea aequata L.*) dengan menggunakan metode DPPH. Radikal bebas yang dihasilkan selama metabolisme dapat menyebabkan kerusakan sel dan berkontribusi terhadap berbagai penyakit degeneratif. Karena itu, penting untuk menemukan sumber antioksidan yang efektif. Dalam penelitian ini, daun titanus diekstraksi menggunakan pelarut etanol 96% melalui metode maserasi. Aktivitas antioksidan dievaluasi dengan mengukur nilai* IC_{50} *terhadap radikal DPPH. Hasil penelitian menunjukkan bahwa ekstrak daun titanus (Leea aequata* L.*) memiliki nilai* IC_{50} *sebesar 39,484 ppm, yang menunjukkan aktivitas antioksidan yang signifikan. Hasil ini mengindikasikan bahwa ekstrak daun titanus (Leea aequata* L.*) dapat berfungsi sebagai agen penangkal radikal bebas, yang dapat berpotensi sebagai bahan baku dalam pengembangan obat herbal untuk mencegah penyakit terkait stres oksidatif.*

Kata Kunci: *Antioksidan, Ekstrak Titanus (Leea aequata* L.*), DPPH, Radikal Bebas,* IC_{50}

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INTRODUCTION

Reactive oxygen species (ROS) and free radicals are naturally produced by human cells as metabolic byproducts. Oxidative stress results from an imbalance between free radicals and antioxidants, which occurs when the body's defenses cannot keep up with the amount of free radicals. In addition to regular metabolism, exposure to cigarette smoke, car exhaust and air pollution can also generate free radicals[1]. If left unchecked, these unstable and reactive free radicals can cause degenerative diseases including cancer, heart disease and premature aging by causing damage to cell structures through chain reactions. Due to their ability to neutralize free radical molecules, antioxidants are very important substances for maintaining health. ability to stop the body's oxidative process, which is the root cause of many diseases[2].

Natural and artificial ingredients can be used to make antioxidants. The use of synthetic antioxidants is starting to be restricted as overuse can have negative side

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effects, such as toxic buildup in the body[3]. Herbal medicine has long used traditional medicinal plants as raw materials. Tetanus leaf (*Leea aequata* L.) is one type of herbal plant.

This plant is used to treat a number of diseases. In the Tanah Karo region of North Sumatra Province, this plant is used as a traditional wound and antitetanus medicine. The leaves of tetanus are proven to contain antioxidant chemicals. Natural ingredients that have antioxidant properties include plants that contain bioactive substances such as alkaloids, flavonoids, tannins, steroids, and glycosides[4]. The aim of the research was to see the antioxidant activity of tetanus leaf extract.

METHODOLOGY

Materials and Tools

The samples used in this study were tetanus leaves (*Leea aequata* L.), obtained from the village of Deli Tua Barat, Kec. Deli Tua, kab. Deli Serdang, North Sumatra, *2,2-diphenyl-1-picrylhydrazyl* (DPPH), 96% ethanol, quercetin, vitamin c, methanol p.a. The tools used in this research are laboratory glassware, analytical balance, rotary evaporator, waterbath, cuvette, UV-Vis-spectrophotometer.

Sample Preparation

After the tetanus (*Leea aequata* L.) leaf samples were wet sorted, separated from the stalk, and then washed with water. After being separated from the stalk, the leaves were weighed, and dried. The dried tetanus leaves were weighed, and pulverized with a blender to produce dry simplisia, then put into a tight container [5,6].

Extraction

Simplicia of tetanus leaves (*Leea aequata* L.) was extracted by maceration method of 200 g of simplicia in 96% ethanol solvent for 1×24 hours. After three days, the extract was concentrated using a rotary evaporator and waterbath. By weighing the extract, the yield was determined by calculating the weight percentage (w/b) between the yield and the weight of the simplicia powder used[7,8].

Antioxidant Testing

This test was conducted to measure quantitative antioxidant activity using the DPPH test. Samples of tetanus leaf extract, vitamin c, and quercetin with concentration variations of 4, 8, 16, 32, and 64 ppm each as much as 2 ml, added with 2 ml of 100 ppm DPPH solution and placed in a dark room for incubation time. After that, the absorbance was measured at the maximum wavelength. The percentage of DPPH inhibition was calculated and the result was expressed as IC_{50} value (ppm)[9,10].

RESULTS AND DISCUSSION

After the tetanus leaf (*Leea aequata* L.) samples were collected, they were wet sorted, washed with clean running water, and shredded. Then put in a simplicia drying cabinet with a temperature of 40 ° C. Then the simplicia is pulverized with a blender [5].

A thick 96% ethanol extract of 130 grams was made from 200 mg of simplicia, resulting in a yield value of 26% for the thick extract. Ethanol 96% is suitable for use as a solvent because it is a universal

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solvent that can attract polar, semi-polar, and nonpolar compounds [11].

Antioxidant Testing

The DPPH method is a quantitative method to measure the antioxidant activity contained in a sample [12]. Measurement of antioxidant activity with the DPPH method has several advantages compared to other methods, namely simple, fast, and does not require a lot of chemical reagents [13]. The principle of quantitative measurement of activity using DPPH is the change in intensity of the purple color of DPPH which is proportional to the concentration of the DPPH solution. DPPH free radicals that have unpaired electrons will give a purple color[14]. The color changes to yellow when the electrons are paired. Purple discoloration of DPPH occurs due to the silencing of free radicals due to the reaction between DPPH molecules and hydrogen atoms released by compounds in the sample. The compound *2,2-Diphenyl-1-Picrylhydrazine*, which changes the color of DPPH from purple to yellow. Determination of the value of free radical suppression activity is expressed by the IC_{50} value, the UV-Vis spectrophotometer will detect a change in color, which will result in a change in absorbance at the maximum wavelength of DPPH [15,16].

Table 1. Antioxidant Test Results

Sample	IC_{50} (ppm)	Category
Tetanus Leaf Extract (*Leaa aequata* L)	39.484 ppm	Very Strong
Quercetin	10.086 ppm	Very Strong
Vitamin C	5.848 ppm	Very Strong

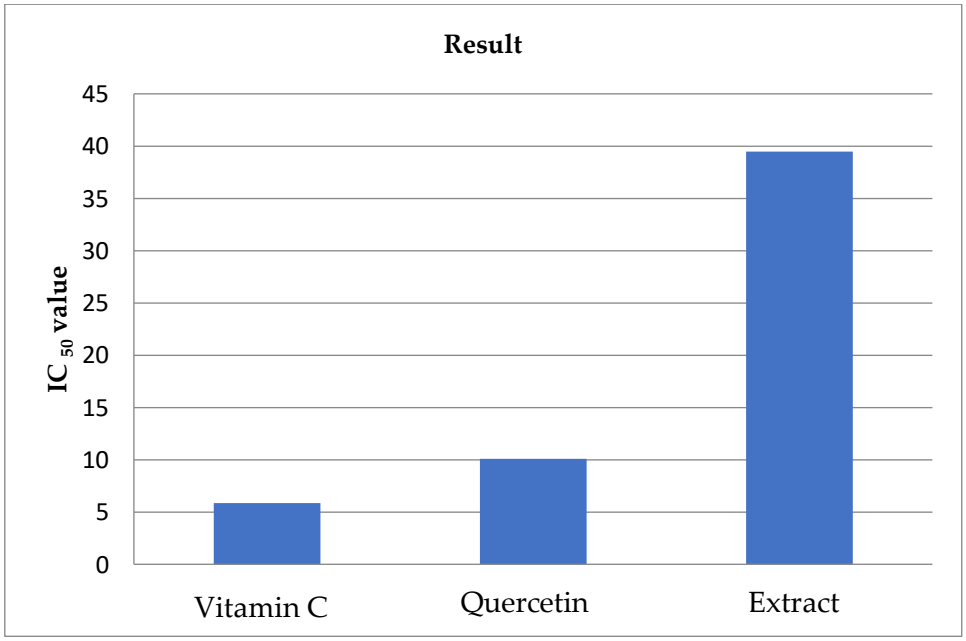

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Figure 1. IC_{50} values (antioxidant activity) of the comparator and tetanus (*Leea aequata* L.) leaf extract**.**

The final result of the antioxidant activity test is the IC_{50} value, which is a parameter used to assess the strength of compounds that have potential as antioxidants. This study found the IC_{50} value of vitamin c was 5.848 ppm, quercetin 10.086 ppm, and extract 39.484. These results are in line with previous research which showed the IC_{50} value of the extract was 6.09 ppm, indicating that the sample showed very strong antioxidant activity[17,18]

The tetanus leaf extract (*Leea aequata* L.) has the ability as an antidote to DPPH free radicals. Compared to positive controls such as vitamin c and quercetin showed promising results, although the IC_{50} value was higher than vitamin c (5.848 ppm) and quercetin (10.086 ppm), this shows that the stronger the antioxidant activity, the lower the IC_{50} value. So it can be concluded that tetanus leaf extract has a very strong antioxidant activity value because the IC_{50} value obtained is below 50 ppm. [19,20]

CONCLUSIONS

From the results of this study it can be concluded that antioxidant activity testing using the DPPH method shows tetanus leaf extract (*Leea aequata* L.) has a good ability to neutralize free radicals. The test results showed the IC_{50} value of tetanus leaf extract of 39.484 ppm.

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