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Extraction of Siamih (Ageratum conyzoides L) Leaves as a source of natural antioxidants using The DPPH Method

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ABSTRACT: The use of antioxidants has been developed in various fields, such as in food and for health. In this study, phytochemical testing of siamih (Ageratum conyzoides L) leaf extract was carried out to determine the secondary metabolites in Siamih leaf and to determine the antioxidant activity of the leaves. The research conducted was the preparation of siamih leaf extract, phytochemical test of Siamih leaf, antioxidant activity test, and the use of the DPPH procedure. The use of a microplate reader with a maximum measurement wavelength of 520 nm. namely inhibition concentration. Based on this research, the illustration of the methanol of siamih leaf extract has a greater value than the sample of Siamih leaf extract of n-hexane.

Keywords: antioxidants, Siamih, DPPH method, extraction

I. INTRODUCTION

Free radical is a compound or molecule that has one or more unpaired electrons, this causes the compound to be very reactive to look for a partner, by binding to the molecular electrons that are located around it. Free radicals can come from within the body as an important part of the activity of metabolic processes. On the other hand, free radicals originating from nature or outside the body can be caused by environmental aspects, including smoking habits, use of pesticides in food, pollution and radiation [1]. Antioxidant compounds continue to be popularly used in the food or health sector. In the food sector, antioxidants can function as preservatives. Meanwhile, in the health sector, the content of antioxidant compounds can function as a prevention for various chronic diseases. Antioxidant compounds have been scientifically proven to reduce the effects of chronic diseases, such as cancer, and chronic heart disease [2].

Antioxidants can be used to prevent chronic diseases such as cancer and chronic heart disease, which are antioxidants that come from chemicals. As we all know, the chemicals used will definitely have a negative impact on health [3]. Recently, researchers have been interested in the use of natural ingredients as antioxidants, because the method of adding chemicals can have a negative impact on health and are not environmentally friendly. Likewise, the addition of antioxidants that contain these chemicals, of course, will be more expensive because the materials used are not economical [4]. Therefore, the use of natural materials is becoming better and more attractive. Like the use of Siamih (Ageratum conyzoides L) leaf extract as an antioxidant, it can be said that it has not been found as a natural antioxidant for health in humans.

Antioxidants have the ability to neutralize free radicals [5]. When antioxidants bind free radicals by accepting or donating electrons, the antioxidants will not turn into free radicals and will remain stable. Antioxidants are found in many vegetables, fruits and medicinal plants [6].

The use of natural ingredients as traditional medicine is not feasible only because of the experience that has been passed down or given by the community from generation to generation, but the medicinal plants to be used need scientific proof. Medicinal plants are known to have active compounds that can be effective for the treatment of diseases. Various studies have been tried to see the pharmacological activity and chemical content of natural ingredients. Natural ingredients that have been reported to have some pharmacological activity are Siamih (Ageratum conyzoides L). The ability of medicinal plants describes a very large source of biological energy to be raised as raw materials sourced from herbal medicinal plants as medicinal plants where there are thousands of plant species that have been used as an alternative for medicinal raw materials.

The benefits of medicinal plants are the cause of the presence of secondary metabolites with various structures molecules and levels of biological activity so that they can reduce and cure various diseases. Therefore, the use of a medicinal

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plant against a disease is the reason for the presence of secondary metabolite compounds, for example, Ageratum conyzoides L [7-8].

Siamih (Ageratum conyzoides L) leaf have many benefits, one of which is as a wound healing medicine and as an antibacterial, this is because siamih leaves contain secondary metabolite compounds such as terpenes, sterols, flavonoids, alkaloids, benzofuran, chromen, chromon, coumarin, oil. volatiles and tannins [9]. The development of research on the reasons for the use of siamih leaf as a medicine for wounds and indigestion can be related to its antibacterial activity [10].

This research was conducted with the use of Siamih leaf extract (Ageratum conyzoides L) as an antioxidant in the human body, using phytochemical testing to identify secondary metabolite compounds contained in Siamih leaf extract, and to ascertain the amount of antioxidant activity that has been generated from existing secondary metabolites. in siamih leaves using the DPPH method.

II. EXPERIMENTAL

Preparation of Siamih (Ageratum conyzoides L) leaf extract

The fresh leaf of Siamih are dried and then mashed. After that it was weighed 400 grams then extracted using 2000 mL of methanol for 3 days with 3 replications. The extract was filtered and the solvent evaporated using a rotary evaporator. The concentrated extract was obtained about 5 g, then placed in a 100 mL beaker to make an inhibitor solution with different concentrations of 1 g/L, 2 g/L, 4 g/L, 6 g/L, and 8 g/L.

Phytochemical test of the leaves of Siamih

The procedure for checking the content of flavonoid, triterpenoid, steroid, phenolic compounds is: 1 g of fresh sample is put in a test tube, then macerated using methanol that has been heated (over a water bath) using 15 minutes. After that it is filtered and put into another test tube and let all the methanol evaporate to dry. Then add chloroform and water with a ratio of 1:1 each as much as 5 mL, shake well, then put it in a test tube, leave for a moment until 2 chloroform-water arrangements are formed. The basic chloroform arrangement was used for checking triterpenoid compounds and steroids. On the other hand, the water structure was used for checking phenolic compounds and flavonoids.

Flavonoid Check

Part of the water arrangement is taken and transferred using a pipette into the response tube, after that add concentrated hydrochloric acid and some magnesium powder, the occurrence of an orange to red color shows the presence of flavonoids

Phenolic Check

A part of the water arrangement is taken and transferred by pipette into a small response tube, after which add $FeCl_3$ to create a blue color indicating the presence of phenolic compounds.

Checking Triterpenoids and Steroids

The chloroform layer that is present is taken a little and put into 3 holes of the drop plate and let it dry, into one hole the drop plate is added a drop of acetic anhydride and a drop of concentrated H_2SO_4. The occurrence of a green or blue patch indicates the presence of steroids. Conversely, the occurrence of mera indicates the presence of triterpenoids.

Alkaloid Check

Samples of 2-4 g were cut into small pieces, then mashed in a mortar with a small accumulation of sand and 10 mL of chloroform-ammonia 0.05N, after which it was stirred or crushed for a long time. The solution is filtered with a small funnel, in which a cotton swab must be placed as a filter and the results of the filter are put into a test tube, then add 10 drops of H_2SO_4 2N and shake for a long time. Let it sit for a while until the separation of the acid and chloroform composition was created. The acid sequence was taken by pipette push and transferred into a tube. After that add Meyer reagent, a positive reaction was indicated by the presence of a white precipitate.

Antioxidant Activity Test

The antioxidant activity test was tried using a two fold delution microplate reader with the DPPH (1,1-diphenyl-2– picryl hydrazyl) method at a wavelength of 520 nm. The sample is 2 mg in 2 mL MeOH in this case illustration concentration of 1000 mg/mL. Line A was subjected to inserting a sample of 100 µL (the plate consisted of rows A-H each with 12 holes). A total of 50 µL of MeOH was inserted into each hole in the B-F row. Line A was pipette 50 µL and inserted into line B, line B is pipette 50 µL inserted into line C and tried to line F, line F was pipette 50 µL then

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discarded, so that a concentration of 1000 mg/mL, 500 mg/mL, 250 mg/mL was obtained 125 mg/mL, 62.5 mg/mL, and 31.25 mg/mL. On the other hand, the G-H line was filled with 50 µL MeOH, specifically on the H row it was filled with only holes 1- 6.

The A-G line was added with DPPH as much as 80 µL with a concentration of 40 mg/mL, then incubated for 30 minutes. The activity of radical scaling was measured as the absorbance shrinkage of DPPH with a microplate reader and information processing. The positive control used as a comparison was vitamin C with a concentration of 50 mg/mL. The positive control used as a comparison was vitamin C with a concentration of 50 mg/mL. The % inhibition value is calculated by the following formula:

$$\% \text{ Resistance} = \frac{(A_{control} - A_{sample})}{A_{control}} \times 100$$

Information: $A_{control}$ = The absorbance does not contain a sample

A_{sample} = Absorbance of the sample

III. RESULT AND DISCUSSION

Phytochemical Test of Siamih (Ageratum conyzoides L) Leaf Extract

Table 1. Phytochemical Test Results

Chemical Compounds	Test results
Alkaloids	+
Flavonoids	+
phenolic	+
Triterpenoids	+
Steroids	-

Description: + states the presence of these chemical compounds in siamih leaf extract

Phytochemical test was carried out to determine the presence or absence of these compounds in Siamih leaf extract. Table 1 shows that siamih leaf extract contains alkaloids, flavonoids, phenolics, and triterpenoids. This was evidenced by the presence of a sign (+) in Table 1.

Antioxidant activity test of Siamih leaf extract

The antioxidant activity of the siamih leaf extract was tested using the DPPH procedure with UV-Vis spectrophotometry with a maximum wavelength of 517 nm. The amount of antioxidant activity was indicated by the value of IC_{50}, which is the concentration of the illustrative solution needed to limit 50% free radical DPPH [11]. The IC_{50} value for each extract with n-hexane and methanol was determined using a linear regression equation from the illustrated concentration bond curve to percent inhibition with the equation Y = ax + b, illustration concentration (ppm) as the axis (X) and the percentage value inhibition as the (Y) axis [12]. Based on the linear regression equation from Figure 1 and Figure 2, the bond between the concentration of the extract and the inhibition percentage, the IC_{50} values were 1133.8964 ppm, 708.8541 ppm, respectively.

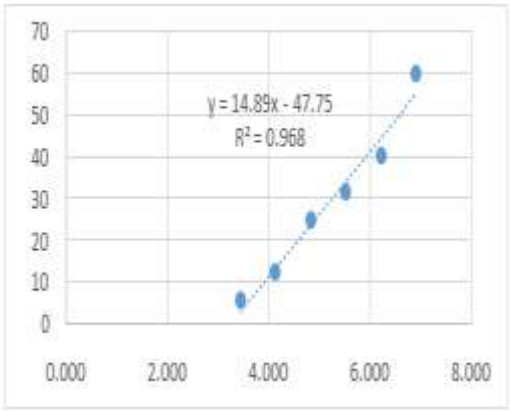

Figure 1. n-hexane

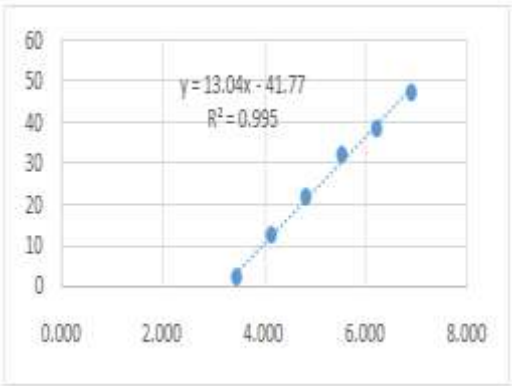

Figure 2. Methanol

Comparison of the type of solvent that has been used in the extraction process affects the antioxidant activity obtained. Based on the antioxidant activity test, it shows that the use of methanol solvent gives the smallest IC_{50} value (the highest activity) compared to the use of n-hexane solvent [11,13]. This is predictable because in the illustration of siamih leaf there are many bioactive compounds with polar characteristics when compared to nonpolar and semipolar bioactive compounds which cause polar solvents (methanol) to bind more bioactive compounds found in siamih

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leaf [11]. Antioxidants are very strong if they have an IC_{50} value of less than 50 ppm, a strong antioxidant has an IC_{50} value located in the range of 50 ppm to 100 ppm, antioxidants again have an IC_{50} value between 100 ppm to 150 ppm, weak antioxidants have a range of 150 ppm to 200 ppm and a value IC_{50} greater than 200 ppm is a very weak antioxidant [13-15]. In particular, a compound is said to have very strong antioxidant activity when IC_{50}; 50 ppm, solid if the IC_{50} is 50-100 ppm, again if the IC_{50} is 101-250, and weak if the IC_{50} is 251-500 ppm [12,16].

The results have shown that all extracts have better antioxidant activity than polar or non-polar solvents. The activity comparison obtained by each of these extracts may be due to the presence of a comparison of the content and the amount of active compounds contained in the extract, so that the antioxidant activity obtained is also different. [17] There are more active compounds from some of the antioxidant compounds that have polar characteristics than those with non-polar ones in siamih leaf [11].

IV. CONCLUSION

Based on the research results obtained siamih leaf extract (Ageratum conyzoides L) contains alkaloids, flavonoids, phenolics, and triterpenoids. The results of the extract on the percentage of inhibition, the IC_{50} values were 1133.8964 ppm, 708.8541 ppm, respectively. The results of the methanol of siamih leaf extract had a greater value than the extract of the n-hexane of siamih leaf extract. This shows that siamih leaf extract has better antioxidant activity in polar than non-polar solvents.

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