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Determination of Total Saponin Content and Antioxidant Activity Using DPPH (2,2-diphenyl-1-picrylhydrazyl) Method of 70% Ethanol Extract of Sidaguri Leaves (Sida rhombifolia L.)

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Abstract

Health is the most important and primary thing in human life. Industry and human lifestyle development have both positive and negative impacts. One of the adverse consequences to be aware of is the proliferation of a variety of degenerative diseases. Sidaguri leaves (Sida rhombifolia L.) grow naturally on roadsides, grasslands, forests, fields, areas exposed to bright sunlight, and slightly sheltered places. It is a plant that is widespread in tropical areas ranging from lowlands to 1,450 m above sea level throughout the world. The plant's chemical content includes alkaloids, hypoparin, flavonoids, triterpenoids, sterols, sugars, vaccissinol, vaccissinone, betaine, and phenylalanineSidaguri leaves (Sida rhombifolia L.) are medicinal plants from the Malvaceae family. Almost all parts of this plant can be used for medicinal purposes, particularly in alternative medicine. This study aimed to determine the total saponin content in a 70% ethanol extract of Sida rhombifolia L. Sidaguri leaves, extracted using the maceration method and a 70% ethanol solvent. Using vanillin, H_2SO_4, and diosgenin as standards, we used UV-Vis spectrophotometry to find out how much total saponin was present. We measured antioxidant activity using the DPPH free radical scavenging test. The test showed that the 70% Sidaguri ethanol extract had a total saponin content of 196.28 ± 1.60 mg DE/g. It also had an IC_{50} value of 208.63 ± 7.73 mg/mL against DPPH in the antioxidant activity test. This shows the potential of Sidaguri leaf extract as a natural antioxidant.

Keywords: *Antioxidants, Sidaguri Leaves, Diosgenin, DPPH, UV-Vis Spectrophotometry.*

1. Introduction

Indonesia potentially encompasses some of the most environmentally varied environments globally. The World Health Organisation (WHO) claimed in 2008 that 68% of the world population still subscribed to traditional medicine. Over 80% of people worldwide use traditional medicine to keep themselves healthy, with the majority of these treatments relying on the usage of plants to treat illnesses. One of the plants used for medicine is the sidaguri leaf (Sida rhombifolia L). Health is the most critical and fundamental aspect of human existence [1]. The development of human existence and industry has both positive and negative effects. One of the adverse consequences to be aware of is the proliferation of a variety of degenerative diseases. Sidaguri leaves (Sida rhombifolia L.) grow naturally on roadsides, grasslands, forests, fields, areas exposed to bright sunlight, and slightly sheltered places. It is a plant that is widespread in tropical areas ranging from lowlands to 1,450 m above sea level throughout the world [2]. The plant's chemical content includes alkaloids, hypoparin, flavonoids, triterpenoids, sterols, sugars, vaccissinol, vaccissinone, betaine, and phenylalanine. Sidaguri also contains ephedrine, calcium oxalate, tannins, saponins, phenols, and essential oils. People commonly use Sidaguri leaves for various diseases such as influenza, fever, tonsillitis, diphtheria, tuberculosis (scrotal dermatitis), colitis, red intestine, jaundice, malaria, urinary tract stones, abdominal pain, bleeding hemorrhoids, haematemesis, and delayed menstruation parasites [3].

Antioxidants, in the chemical sense, are compounds that donate electrons. Antioxidants function by donating one electron to an oxidant compound, thereby diminishing its activity. By compensating for a deficiency of electrons and inhibiting the chain reaction of free radical formation, antioxidants stabilize free radicals [4]. Saponins are secondary terpenoid metabolite compounds that are present in the roots, epidermis, leaves, seeds, and fruits of numerous plants. They serve as a defense mechanism. The ability to form compounds with cholesterol, the appearance of a bitter flavor, and the production of stable foam in liquid solutions are all signs that saponins are present [5]. In general, in the same plant, immature plants have a higher saponin content than mature plants. According to reports, saponins have the potential to act as antioxidants, reducing superoxide through the formation of hydroperoxide intermediates, thereby preventing biomolecular damage by free radicals [6] [7].

There are several methods for making extracts, including maceration [8]. We use the maceration method to extract samples that are relatively heat resistant; we do this by immersing the sample in a solvent for a specific duration, typically 24 hours, without the use of a heater [9]. The maceration method is advantageous in that it does not require complicated equipment and is relatively inexpensive. The 1,1-diphenyl-2-picrylhydrazyl (DPPH) method is one method used to measure antioxidant activity [10]. Antioxidants will interact with DPPH to neutralize and lessen its free radical nature, whether by electron transfer or hydrogen radicals. The color of the solution changes from dark purple to vibrant yellow when the DPPH free radical couples all of its electrons, and the absorbance at 517 nm disappears [11]. The objective of this work is to calculate the overall saponin content and assess the antioxidant activity of the 70% ethanol extract of sidaguri leaf using the DPPH method.

2. Research Method

The study used the following instruments: a blender, test tube, glass jar, filter paper, stirring rod, water bath, oven, refrigerator, beaker glass, measuring cup, measuring flask, vacuum rotary evaporator (Eyela), analytical balance (Ohaus), UV-Vis spectrophotometer (Shimadzu), spatula, micropipette, quartz cuvette, aluminum foil, and weighing bottle. Fresh sidaguri leaves were used as the test materials at the Spice and Drug Research Centre (BALITRO), Bogor. It was 70% ethanol, diosgenin powder, DPPH, aquades, methanol p.a., 2N HCl, H_2SO4, $FeCl_3$, Mg powder, gelatin, NaCl, chloroform, and anhydrous acetic acid that were used. We prepared a calibration curve using the diosgenin standard series [12]. We expressed the total saponin content as the equivalent weight of diosgenin per gram of powdered simplicia. We calculated the total content determination and antioxidant activity using the linear regression equation: y = b x + a.

We used the maceration method to extract sidaguri leaves. We put 500 g of sidaguri leaf powder into a container and added 5 L of 70% ethanol. We periodically stirred the soaking for the first 6 hours and then left it for 18 hours. After that, the container was placed in a place protected from direct sunlight [13]. Next, we filtered the macerate to remove the dregs, and then repeated the process with a 70% ethanol solvent, applying the same method. We concentrated the resulting macerate in a vacuum rotary evaporator set to 50 °C, and then we evaporated it in a water bath till we obtained a thick extract. The yield was then measured, recorded, and calculated [6]. Following a mixture of 1 milliliter of 2N HCL and 9 milliliters of water, the extract was heated to a boil, cooled, and filtered before being split into three containers. The first tube produced a white or yellow precipitate when Mayer's reagent was added. After adding Bouchardat's reagent to the second tube, a brown to black precipitate was produced [14].

We added Dragendorf's reagent to the third tube, resulting in a brick-red precipitate. We added 2-3 mL of ethanol to the extract, heated it, cooled it down, and filtered it. We added 10 drops of HCl(p) and 100 mg of Mg powder to the filtrate until a red-orange to red-purple color formed [2]. The extract should first be mixed with 10 mL of hot distilled water, let it settle, shaken rapidly for 10 seconds, and then mixed with 2N HCl [15]. Foam will form if it is visible for at least ten minutes and grows to a height of one to ten centimeters (Hanani, 2015). Heat, cool, and filter the extract after adding 10 milliliters of distilled water to it. Hanani (2015) adds 1% gelatin and 10% NaCl to the filtrate to produce a white precipitate. Warm the extract, pour in 2 milliliters of ethanol, let it settle, and then strain it. To produce a reddish-purple color indicative of triterpenoids and a greenish-blue tint indicative of steroids, add three drops of ether, three drops of anhydrous acetic acid, and one or two drops of H2SO4(p) to the filtrate [15]. After adding 10 mL of distilled water to the extract, it should be heated, cooled, and filtered. A green to blackish blue color is produced by adding FeCl3 to the filtrate [16].

Weigh out ten milligrams of diosgenin and dissolve it in twenty milliliters of 70% ethanol to make the diosgenin stock solution. A stock standard of 500 ppm will result from this. Subsequently, we dilute the 130-ppm concentration by pipetting 2.6 ml into a 10 ml measuring vial and adding 70% ethanol until the limit is reached [17]. Create five series of solutions using the Lambert-Beer formula to produce the diosgenin standard curve. Develop the diosgenin standard solution by employing five standard series, each of which has concentration variations of 40, 70, 100, 130, and 160 ppms. Take 0.25 ml of each concentration and pour it into a 5 ml vial. After that, stir the mixture thoroughly and add 2.5 ml of 72% sulfuric acid reagent and 0.25 ml of vanillin reagent. Warm the mixture for 5 minutes in a water bath at 60 °C. Allow the item to cool. After that, allow it to sit for a period of 10 minutes. In order to ascertain the saponin concentrations, we weighed 100 mg of viscous sidaguri leaf extract and dissolved it in a 100-ml measuring flask containing 70% ethanol. We added 70% ethanol solvent to a 10 ml vial containing 1 ml of the extracted solution in order to reach the limit. Next, we added 2.5 ml of 72% sulfuric acid reagent and 0.25 ml of vanillin reagent to the 0.25 ml of liquid that we had pipetted into a 5-ml vial. We then shook the mixture until it was completely smooth. After cooling the combination and heating it for five minutes at 60 °C in a water bath, we allowed it to stand for the five-to-ten-minute extract operating period. Three replications of the extract solution's absorbance were measured at a wavelength of 535.70 nm.

We calculated the total saponin levels using the following formula:

Formula Level = …… (1)

$$\frac{X \times Fp \times V}{g\ sample}$$

X = concentration obtained from the linear regression equation
Fp = dilution factor
V = sample volume
g = sample weight

Test For Antioxidant Activity - To make 0.1 mM DPPH Solution, 3.9432 mg of DPPH powder (BM 394.32 g/mol) had to be mixed with methanol in a 100-ml measuring flask until the mark appeared. Preparing a Blank Solution Using a pipette, we transferred 3 ml of the 0.1 mM DPPH solution into a test tube. We added 1 ml of methanol p.a. to the mixture and stirred until it was homogeneous. To find DPPH's maximum wavelength at 0.1 mM, we pipetted 3 ml of the DPPH solution, added 1 ml of methanol p.a., and shook it. It was then left in a dimly lit area for thirty minutes. The absorption was measured with a UV-Vis spectrophotometer, and a wavelength in the range of 400 to 800 nm was chosen.

3. Result And Discussions

Ethanol Extract of Sidaguri Leaves, by using 70% ethanol solvent, we macerated 500 g of sidaguri leaf powder. The dry simplicia yield obtained was 2 kg from 7 kg of fresh simplicia. We used 500 g of simplicia powder for maceration, ensuring the extraction results were not excessive for this research process. A 70% ethanol extract of sidaguri leaves yielded 35.5%. This yield is quite large, and in accordance with the Indonesian herbal pharmacopoeia, it should not be less than 7.4%. The maceration process using 70% ethanol solvent, which is polar, allows polar compounds to dissolve in polar solvents [6]. We conduct phytochemical screening tests to identify the compounds present in the extract. This screening test for phytochemicals contains assays for steroids, alkaloids, flavonoids, phenols, triterpenoids, saponins, and tannins. The phytochemical screening's results are shown in Table 1.

Table 1. Results of Sidaguri Leaf Extract Phytochemical Screening

No.	Screening Test	Results
1	Phenolics	+
2	Flavonoids	+
3	Alkaloids	+
4	Saponins	+
5	Tannins	+
6	Steroids	+
7	Triterpenoids	+

According to previous researchers, sidaguri leaves are rich in alkaloids, flavonoids, phenolics, saponins, and tannins. After adding $FeCl_3$, the phenol test produces a blackish green color change. The phenolic test employs 3–4 drops of iron (III) chloride. The addition of iron (III) chloride salt solution causes the phenolic compounds to exhibit a green to black blue coloration. The flavonoid test yields positive results, indicating the formation of an orange color through the use of magnesium as a reducing agent. In this test, we carry out the reduction process in an acidic atmosphere with the addition of hydrochloric acid. If you mix an extract with concentrated magnesium hydrochloric acid, the samples that contain flavonoids turn yellow [18]. When distilled water and 2N HCL are added, a stable foam is formed, which is how the saponin test is identified. The presence of alkaloids in Sidaguri leaf extract is demonstrated by the precipitates that form when Mayer, Dragendorf, and Bouchardat reagents are added: a white precipitate, an orange precipitation, and a brown precipitate, respectively. Meanwhile, it was discovered that a 70% ethanol extract of sidaguri leaves tested positive for triterpenoids and secondary metabolites of steroids. The triterpenoid test is detected by the addition of chloroform, anhydrous acetic acid, and H2SO4, while the steroid test is characterized by the production of a green tint.

We determined the saponin levels using Pasaribu et al. method. Diosgenin, a group of steroids and triterpenoids easily obtained, served as the standard in this study. This method uses vanillin reagents and 72% sulfuric acid; the reaction between vanillin and sulfuric acid produces vanillin sulfate acid, which has a color change from the standard and sample. A UV-Vis spectrophotometer can spectrophotometrically determine the levels of a colored compound. We investigated the extract solution's absorbance at a wavelength of 449 nm through three replications [19]. The maximum wavelength of diosgenin was observed at 449 nm, with an operating time of 10 minutes. We created the diosgenin standard curve by calculating the minimum limit concentration (c-min) and maximum limit concentration (c-max), resulting in five series of concentrations. The c-min and c-max calculations yielded five concentrations, namely 40, 70, 100, 130, and 160 ppm. We used an incubation time of 10 minutes to make the standard curve, which we then read at a wavelength of 449.00 nm. Furthermore, we conducted the absorbance measurement at each concentration of the prepared solution [20].

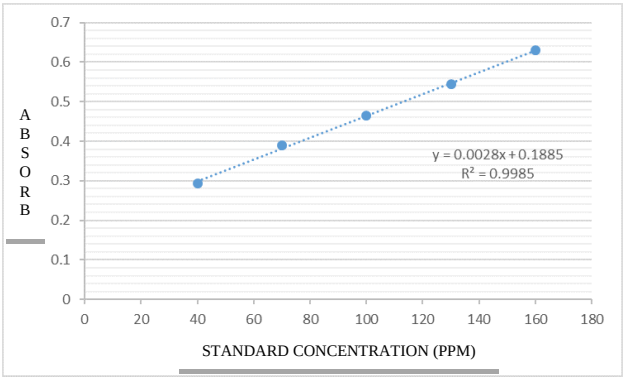

Fig 1. Calibration Curve

We created a linear regression equation, y = 0.0028x + 0.1885, using the standard diosgenin concentration and its absorption on the UV-vis spectrophotometer. This allowed us to achieve an R2 value of 0.9985 (Figure 1). The diosgenin calibration curve serves as a comparison to determine the concentration of saponin compounds in a 70% ethanol extract of sidaguri leaves.

The subsequent step is to ascertain the content of the sample in order to ascertain the levels of the analyzed compounds once the calibration curve of the standard solution has been obtained. The extract of sidaguri leaves in 70% ethanol contains a total saponin content of 196.28 ± 1.60 mg DE/g. There have been no reports yet on the total saponin content of sidaguri leaves. However, Singh and Mendhulkar (2015) found 296 mg DE/g of total saponin in a sample of Abutilon indicum (Linn.), a species of Malvaceae collected in Mumbai, India. The saponin content in A [21]. indica appears to be higher than that found in 70% sidaguri leaf extract. Numerous factors, such as plant species, growth environment, and the kind of extraction solvent utilized in the study, could be to blame for this variation. Antioxidants are substances with the power to counteract free radicals, shielding the body's biological system from the harmful consequences of reactions or processes that result in excessive oxidation. We use spectrophotometric antioxidant activity measurement with the DPPH technique. The absorption has decreased, indicating that DPPH radicals have been captured. Plant saponins are capable of absorbing free radicals.
The antioxidants in the sidaguri leaf extract suppress the solution's concentration, causing DPPH to change from purple to yellow. Color fading will result in a drop in the UV-vis spectrophotometer's visible light absorption value. The objective of determining the maximal wavelength is to ascertain the wavelength at which the DPPH solution exhibits the highest level of absorption. Finding the longest wavelength involves using a control solution, which is DPPH dissolved in methanol with no sample added. This is done to make sure that other compounds in the sample don't change the longest wavelength of DPPH. Previous researcher conducted DPPH wavelength testing at 400-800 nm. The study's results indicate that the DPPH solution's maximum wavelength is 513 nm, with an absorbance value of 0.411. The percentage value of DPPH radical inhibition (%) can be determined using the acquired absorbance value. The sidaguri leaf extract's IC_{50} value in the DPPH-based antioxidant experiment was 208.63 ± 7.73 mg/mL. An evaluation of the antioxidant activity of 70% ethanol extract of sidaguri leaves revealed that the extract had a moderate level of activity, with a IC_{50} value that ranged from 100 to 250 µg/mL. This means that the ethanol extract sample of this plant could be used as a source of antioxidants.

4. Conclusion

The study looked at the total saponin levels and antioxidant tests of a 70% ethanol extract of sidaguri leaves (Sida rhombifolia L) using the DPPH method. The sidaguri leaf saponin test showed that it was positive, making foam, and had a diosgenin content of 196.28 ± 1.60 mg DE/g. In the antioxidant test of sidaguri leaves using the DPPH method, there was an IC_{50} value of 208.63 ± 7.73 µg/mmL. The sidaguri leaf plant has the potential to act as a natural antioxidant.

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