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Ultrasound Assisted Ethanolic Extraction of *Ipomoea reptans* Poir Leaves Antioxidant Activities, Total Phenolic and Flavonoids

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Abstract

Ipomoea reptans Poir leaves contain phenolic and flavonoid compounds that can reduce free radicals and prevent diseases such as cardiovascular disease, coronary heart disease, and cancer. The drawback of conventional extraction is that it takes a long time, so a shorter extraction method is needed, one of which is the ultrasonic method. This study aims to test the antioxidant activity of ethanol extract of *Ipomoea reptans* Poir leaves extracted by ultrasonic method and determine the total phenolic and flavonoid content. *Ipomoea reptans* Poir leaves were extracted using the ultrasonic method at 50°C for 30 minutes with 70% ethanol solvent to obtain ethanol extract of *Ipomoea reptans* Poir leaves. Antioxidant activity test of ethanol extract of *Ipomoea reptans* Poir leaves using DPPH method and vitamin C as a comparison. Determination of total phenolic content using gallic acid with a maximum wavelength of 745.20 nm and operating time of 120 minutes. Determination of total flavonoid content using quercetin with a wavelength of 439.60 nm and operating time of 30 minutes. The results showed that the antioxidant activity of *Ipomoea reptans* Poir leaves obtained an IC_{50} value of 40.62 ppm (very strong). Total phenolic and flavonoids were 78.33 mgGAE/g extract and 1.03 mgQE/g extract.

Keywords : Antioxidant, Phenolics, Flavonoids, Ultrasound extraction, *Ipomoea reptans* Poirs

INTRODUCTION

Antioxidants are compounds that can reduce excessive free radicals in the body to decrease the occurrence of degenerative diseases such as coronary heart disease, cardiovascular disease, and cancer (Parwata, 2016). Synthetic antioxidants such as BHA, and BHT cause many side effects so there is still a need to find safer natural antioxidants (Marfel et al., 2017). One of the plants that can be utilized as a natural antioxidant is *Ipomoea reptans* Poir (Kurniawan et al., 2020).

The 70% ethanol extract of *Ipomoea reptans Poir* leaves contains tannin, flavonoids, steroids, alkaloids, and saponins (Veronika, 2020). The 96% ethanol extract of *Ipomoea reptans* Poir extracted by maceration method has an IC_{50} value of 178.3 µg/mL (weak activity) tested using the DPPH method (Hayati et al., 2015). Ultrasonic extraction method (sonication) is an effective and efficient non-conventional or non-thermal extraction method. This method, with the help of ultrasonic waves, can help to introduce solvents into plant cells to obtain more secondary metabolites (Hasriandi, 2022).

The sonication method has a much shorter time than the maceration method (Sayuti, 2017). *Rhodomyrtus tomentosa* (Aiton) Hassk leaves extracted using maceration, sonication, and reflux methods with 70% ethanol solvent obtained IC_{50} values of 15.33; 6.18; and 6.97 µg/mL using the DPPH method (Marwati et al., 2022). Based on this research, the sonication method produces extracts with the highest antioxidant activity when compared to maceration and reflux methods.

Sauropus androgynus (L.) Merr. leaves extracted using maceration, soxhlet, and sonication methods with 70% ethanol solvent obtained the largest total phenolic and flavonoid content of 12.05 mgQE/g extract and 42.96 mgGAE/g extract obtained from the ultrasonic method. The 70% ethanol extract of *Sauropus androgynus* (L.) Merr. leaves extracted by maceration, soxhlet, and sonication obtained the largest total flavonoid and phenolic content of 12.05 mgQE/g extract and 42.96 mgGAE/g extract obtained from the sonication method (Hikmawati et al., 2021).

Ipomoea aquatic Forsskal leaves contain flavonoid compounds such as quercetin (Wirasutisna et al., 2012). The search for antioxidant compounds from natural resources is necessary to fulfill the body's need for protection against free radicals. We can improve overall health and prevent degenerative diseases by relying on safe and effective natural sources. Therefore, it is important to continue exploring the potential of natural materials as valuable sources of antioxidants. Based on the above background, research was conducted to test the antioxidant activity of 70% ethanol extract of *Ipomoea reptans* Poir leaves from sonication extraction using the DPPH method. The extract was also determined for total phenolic and flavonoid content using gallic acid and quercetin.

MATERIALS AND METHODS

Equipment

Simplisia scales (Henher), sonication extraction apparatus (Grant), glassware (IWAKI pyrex), pollinator, electric balance (Ohaus), moisture balance (Ohaus), UV-Vis 1800 spectrophotometer (Shimadzu), rotary evaporator (Heidolph), drying cabinet (Navis), micropipette (Socorex).

Material

Ipomoea reptans Poir leaves, 70% ethanol (technical), vitamin C (Merck), DPPH (Merck), $AlCl_3$ (Merck), CH_3COOK (Merck), quercetin (Merck), ethanol (Merck), gallic acid (Merck), Na_2CO_3 Merck, Folin-Ciocalteu reagent (Merck), Mg powder (Merck), amyl alcohol (Merck), HCl (Merck), and $FeCl_3$ (Merck).

Methode

1. Plant determination

Determination of Ipomoea reptans Poirs plants was carried out at the Ecology and Biosystematics Laboratory of the Department of Biology, Faculty of Science and Mathematics, Diponegoro University. Pembuatan Serbuk Simplisia Umbi Wortel

2. Material gathering

Ipomoea reptans Poir leaves were harvested in 5 kg from Waru Village, Mranggen District, Demak Regency, Central Java Province. The Ipomoea reptans Poir leaves used were green and not yet yellowed.

3. Preparation of *Ipomoea reptans* Poir leaves powder

Ipomoea reptans Poir leaves totaling 5 kg were wet sorted to separate good and damaged leaves. The leaves were washed using running water to remove any dirt attached to the leaves. The leaves were dried using an oven at 50°C until dry. The leaves were pulverized using a pollinator. The powder that has been refined is checked for moisture content with a moisture balance. The requirement for water content is less than 10% (Depkes RI, 2017).

4. Preparation of 70% ethanol extract of Ipomoea reptans Poir leaves

Ipomoea reptans Poir leaves powder was weighed as much as 50 grams. The powder was put into a 500 mL Erlenmeyer and 500 mL of 70% ethanol was added. The ratio of material to solvent (1:10) b/v. Erlenmeyer was put into a sonicator (temperature 50℃ and time 30 minutes (Ningsih, 2023). The solution was filtered using flannel cloth. The filtrate obtained was concentrated using a rotary evaporator until it became a thick extract. The extract yield was calculated using the following formula:

$$\text{The extract yield} = \frac{viscous\ extract\ weight}{dry\ powder\ weight}\ x\ 100\%$$

5. Phyto-chemical screening (Shaikh and Patil, 2020)
   a. Phenolic compounds

The sample was weighed 50 mg, added 10 mL of 70% ethanol, and 2 drops of 10% $FeCl_3$ solution. The sample contains phenolics if the solution changes color to green, red, black, purple, or blue.

b. Flavonoid compounds

The sample was weighed 50 mg, added 10 mL of 70% ethanol, then filtered. The solution was divided into two equal test tubes, one of which was added with magnesium powder, 3 mL of amyl alcohol, and 1 mL of concentrated HCl through the tube wall. The sample contains flavonoids if an orange, red, or yellow color forms in the upper phase.

6. Determination of antioxidant activity (Chaves et al., 2020)

Antioxidant activity determination of the samples was conducted using the DPPH method with vitamin C as the comparator. Determination of the maximum wavelength was done by measuring the absorbance of DPPH 0.1 mM solution in the range of 450-550 nm. Determination of operating time was done by reacting vitamin C solution and DPPH and then measuring the absorbance every 5 minutes for 60 minutes. Vitamin C solution was made a concentration series of 1, 2, 3, 4, 5, and 6 ppm while the sample solution was made a concentration series of 10, 20, 30, 40, 50, and 60 ppm. The solution was reacted with DPPH during the operating time and then the absorbance was measured at the maximum wavelength. Data results in the form of absorbance values are used to calculate the % inhibition value.

$$\% \text{ inhibition value} = \frac{control\ absorbance - sample\ absorbance}{control\ absorbance}\ x\ 100\%$$

7. Determination of total phenolic and flavonoid content

a. Phenolic Content

Determination of total phenolic content was carried out using a standard curve of gallic acid. The gallic acid solution was made in concentration series of 50, 100, 150, 200, 250 and 300 ppm. Determination of the maximum wavelength was done by reacting the gallic acid solution concentration of 150 ppm 200 µL and 400 µL folin ciocalteu and 4 mL Na_2CO_3 7%. The solution was read for absorbance in the range of 600-800 nm. To determine the operating time, the same solution was read for absorbance every 5 minutes for 120 minutes at the maximum wavelength. A gallic acid standard curve was prepared by reacting a gallic acid solution of 50, 100, 150, 200, 250, and 300 ppm each 1 mL then added Folin-ciocalteu 0.4 mL, and Na_2CO_3 7% 4 mL. The solution was read for absorbance using the maximum wavelength and operating time. Determination of the total phenolic content of the sample was done by weighing 25 mg sample and dissolving it with 50 mL ethanol. The solution was taken as much as 200 µL in a 5 mL volumetric flask, added 400 µL of Folin-ciocalteu, and added 4 mL of 7% Na_2CO_3. The solution was allowed to stand for the operating time and then read the absorbance at the wavelength and operating time.

b. Flavonoid Content

Determination of total flavonoid content was carried out using the standard curve of quercetin. Quercetin solution was made at concentrations of 2, 4, 6, 8, 10, and 12 ppm. Determination of the maximum wavelength was done by reacting 1000 µL of quercetin solution of 6 ppm concentration and 200 µL of $AlCl_3$ 10% and 200 µL of CH_3COOK 1M. The solution was read for absorbance in the range of 400 - 500 nm. To determine the operating time, the same solution was read for absorbance every 5 minutes for 120 minutes at the maximum wavelength. The standard curve of quercetin was prepared by reacting quercetin solutions of 2, 4, 6, 8, 10, and 12 ppm with 1 mL each and adding 200 mL of 10% $AlCl_3$ and 200 mL of 1M CH_3COOK. The solution was allowed to stand for operating time and then the absorbance was read at the maximum wavelength. Determination of total flavonoid content of the sample was done by weighing 25 mg sample and dissolving it with 50 mL ethanol. The solution was taken 1 mL then added 200 µL $AlCl_3$ 10% and 200 µL CH_3COOK 1M. The solution was allowed to stand for operating time and then read the absorbance at the maximum wavelength.

c. Analysis Data

The standard curve equation was obtained from the concentration vs absorbance series graph. The absorbance value of the sample solution was included in the standard curve equation. Total phenolic and flavonoid levels were calculated with the following formula:

$$TPC/TFC = \frac{C\ x\ Fp\ x\ V}{g}$$

Information:
TPC = Total Phenolic Content
TFC = Total Phenolic Content
C = Sample concentration
Fp = Dilution factor
V = Extract volume (mL)
g = Sample weight used (gram)

RESULT AND DISCUSSION

Plant determination is carried out to accurately determine the identity of plants used in research so that there are no errors in the collection of materials used. The results of the determination of the plants used in this study show the following determination key: 1b-2b-3b-4b-6b-7b-9b-10b-11b-12b-12b-13b-14a 15a- (Gol 8. Scattered single leaf plants)-109a-110b-111b-112a-113a-114b-123b-Fam 107. Convolvulaceae-1b-Genus Ipomoea -1b- 3b-4b-5b-6a-Species: *Ipomoea reptans* Poir. It was clear that the plant used in this research is *Ipomoea reptans* Poir. The sorted fresh Ipomoea reptans Poir leaves used in this study were 2.325 kg. After drying, the resulting simplisia was 0.45 kg with a drying shrinkage of 19.35%. The water content of Ipomoea reptans Poir leaves simplicia obtained from drying is 5.0% which meets the water content requirement of < 10% (Herman et al., 2020).

The extraction method used in this study is ultrasonic wave-assisted extraction. This extraction uses ultrasonic wave radiation to generate cavitation energy to increase the solvent's ability to extract the compounds in the sample. When compared to reflux and maceration methods, the ultrasonic method can produce more antioxidant compounds in *Rhodomyrtus tomentosa* (Aiton) Hassk leaves (Marwanti et al., 2022). In this research, we used ethanol as a solvent because ethanol possesses both polar and nonpolar characteristics, which allows it to dissolve a wide range of compounds. Its polar -OH group behaves similarly to water, while its carbon chain provides nonpolar properties. This unique combination enables ethanol to effectively extract both hydrophilic and lipophilic substances from plant materials, making it versatile for extracting flavors, colors, and bioactive compounds (Fadhila et al., 2023).

Phytochemical screening was carried out to determine the class of chemical compounds contained in the sample. To determine the presence of flavonoids, magnesium powder, amyl alcohol, and concentrated HCl were added. Identification of flavonoids is positive if a yellow, or orange color is formed in the amyl alcohol layer. To determine the phenolic group is done with the addition of $FeCl_3$. Positive phenolic identification forms blue, red, black, purple, and green colors (Ramadhan et al., 2020). The results of phytochemical screening on ethanol extract of *Ipomoea reptans* Poir leaves are presented in Table 1. It can be seen that the ethanol extract of Ipomoea reptans Poir leaves contains phenolics and flavonoids.

Table 1. Phytochemical screening of ethanol extract of *Ipomoea reptans* Poir leaves

Active Compound	Method	Result
Flavonoid	Amyl alcohol test	+
Phenolic	Ferric chloride test	+

Antioxidant activity test of ethanol extract of Ipomoea reptans Poir leaves using DPPH method with vitamin C as the comparator. Determination of the maximum wavelength aims to make the absorbance of the sample at the maximum wavelength so that maximum results are obtained. The result of determining the maximum wavelength of 0.1 mM DPPH solution is 516.70 nm. Operating time is done to determine the reaction time required between the sample and the reagent. Determination of operating time is done by measuring the absorbance of DPPH and Vitamin C every 5 minutes for 60 minutes. Stable absorbance from the mixing of vitamin C and DPPH solution occurred at the 30th minute. This shows that at the 30th minute, the reaction between vitamin C and DPPH solution has been completed with an absorbance value of 0.529. The result of this research is strengthened by the research of Puspitasari (2019), namely that the maximum wavelength of DPPH and Vitamin C obtained was 517.4 nm and the operating time was 30 minutes. The results of absorbance measurements, percent inhibition, and IC_{50} values of ethanol extracts of *Ipomoea reptans* Poir leaves and vitamin C can be seen in Tables 2 and 3.

Table 2. IC_{50} values of ethanol extracts of *Ipomoea reptans* Poir leaves (sample)

Replication	Concentration	Absorbance Sample	% Inhibition	linear regression	IC_{50}
1	10	0.812	9.17	a = -3.564 b = 1.309 r^2 = 0.976	40.92
	20	0.737	17.56		
	30	0.523	41.50		
	40	0.436	51.23		
	50	0.342	61.74		
	60	0.247	72.37		
2	10	0.803	10.18	a = -1.260 b = 1.270 r^2 = 0.991	40.36
	20	0.694	22.37		
	30	0.537	39.93		
	40	0.431	51.79		
	50	0.339	62.08		
	60	0.242	72.93		
3	10	0.785	12.19	a = -1.439 b = 1.197 r^2 = 0.991	40.57
	20	0.663	25.84		
	30	0.568	36.47		
	40	0.417	53.36		
	50	0.354	60.40		
	60	0.251	71.92		

Absorbance DPPH = 0,894

Table 3. IC_{50} Values of Vitamin C

Replication	Concentration	Absorbance Vitamin C	% Inhibition	linear regression	IC_{50}
1	1	0.807	9.73	a = -5.063 b = 13.26 r^2 = 0.995	4.15
	2	0.726	18.79		
	3	0.573	35.91		
	4	0.461	48.43		
	5	0.358	59.96		
	6	0.220	75.39		
2	1	0.803	10.18	a = -4.064 b = 12.89 r^2 = 0.989	4.19
	2	0.726	18.79		
	3	0.570	36.24		
	4	0.465	47.99		
	5	0.361	59.62		
	6	0.236	73.60		
3	1	0.796	10.96	a = -2.632 b = 12.85 r^2 = 0.983	4.10
	2	0.696	22.15		
	3	0.564	36.91		
	4	0.468	47.65		
	5	0.349	60.96		
	6	0.219	75.50		

Absorbance DPPH = 0,894

The smaller the IC_{50} value, the higher the antioxidant activity. The 70% ethanol extract of *Ipomoea reptans* Poir leaves obtained an IC_{50} value of 40.62 ppm and an IC_{50} value of vitamin C of 4.15 ppm. The ethanol extract of *Ipomoea reptans* Poir leaves is categorized as a strong antioxidant while vitamin C is a very strong antioxidant. The antioxidant activity of *Ipomoea reptans* Poir leaves is influenced by the presence of flavonoids and phenolic compounds (Muflihah et al., 2021).

Determination of the total phenolic content of 70% ethanol extract of *Ipomoea reptans* Poir leaves using the folin ciocalteu method with gallic acid as a comparison. Results of determining the maximum wavelength of the gallic acid-folin ciocalteu complex 743 nm. The results of determining the operating time were carried out by measuring the absorbance of the gallic acid-folin ciocalteu complex every 5 minutes for 180 minutes. Stable absorbance from mixing gallic acid and folin ciocalteu occurred at 120 minutes. This shows that at the 120th minute the reaction to form the gallic acid-folin ciocalteu complex was complete with an absorbance value of 0.552. The result of this research is strengthened by the research of Affrelia (2023), namely that the maximum wavelength gallic acid-folin ciocalteu obtained was 739.6 nm and the operating time was 120 minutes. The standard curve graph for gallic acid can be seen in Figure 2.

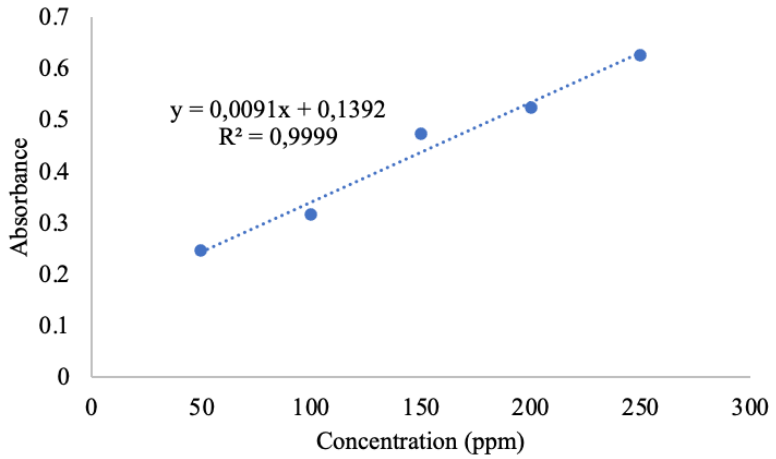

Figure 2. Gallic acid standard curves

The results of determining the total phenolic content of the 70% ethanol extract of Ipomoea reptans Poir leaves can be seen in Table 5. The total phenolic content of the 70% ethanol extract of Ipomoea reptans Poir leaves was 1.025 mgGAE/g extract. The presence of various phenolic acids in Ipomoea reptans Poir, including ferulic acid and hydroxybenzoic acid, has been confirmed, which contributes to antioxidant capacity (Fachriyah et al., 2024).

Table 4. The total Phenolic content of the 70% ethanol extract of Ipomoea reptans Poir leaves

Replication	Absorbance Value	Dilution	Phenolic Content (mgGAE/gram)	Average Phenolic Content (mgGAE/gram)
1	0.445		79.25	
2	0.436	5 kali	77.00	78.33
3	0.449		80.25	

Determination of total flavonoid content of 70% ethanol extract of Ipomoea reptans Poir leaves using Chang's method with quercetin as the comparator. The result of determining the maximum wavelength of the quercetin-$AlCl_3$ complex is 439.60 nm. The determination of operating time was done by measuring the absorbance of quercetin-$AlCl_3$ complex at every 5 minutes for 60 minutes. Stable absorbance of the results of mixing the quercetin solution and $AlCl_3$ occurred at the 30th minute. This shows that at the 30th minute, the reaction of quercetin-$AlCl_3$ complex formation has been completed with the absorbance value is 0.412. The result of this research is strengthened by the research of Amin (2019), in which the maximum wavelength quercetin-$AlCl_3$ complex obtained was 739.6 nm and the operating time was 120 minutes. The standard curve graph of quercetin can be seen in Figure 1.

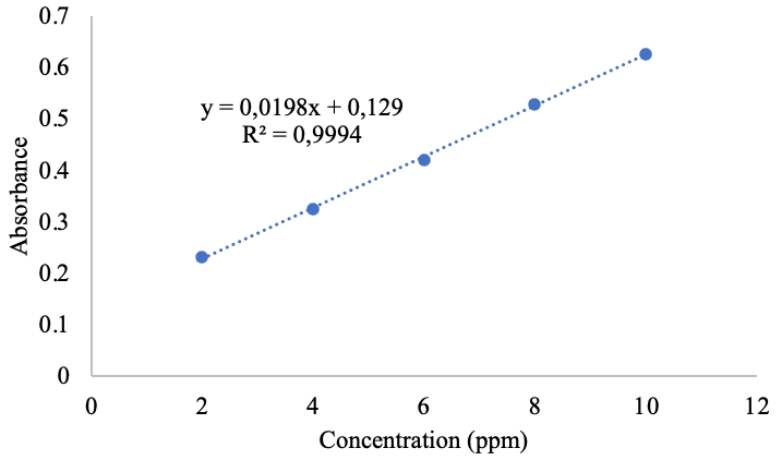

Figure 1. Quercetin standard curve

The correlation coefficient value in the linear regression equation obtained from the quercetin standard curve is close to 1, meaning there is a strong relationship between quercetin concentration and absorbance value (Shasia et al., 2021). The results of determining the total flavonoid content of the 70% ethanol extract of Ipomoea reptans Poir leaves can be seen in Table 5. The total flavonoid content of the 70% ethanol extract of Ipomoea reptans Poir leaves was 1.025 mgQE/g extract.

Table 5. The total flavonoid content of the 70% ethanol extract of *Ipomoea reptans* Poir leaves

Replication	Absorbance Value	Dilution	Flavonoid Content (mgQE/gram)	Average Flavonoid Content (mgQE/gram)
1	0.636		1.023	
2	0.635	None	1.021	1.03
3	0.638		1.030	

CONCLUSION

The IC_{50} value of 70% ethanol extract of Ipomoea reptans Poir leaves is 40.62 ppm. The total phenolic and flavonoid levels of the extract were 78.33 mgGAE/gram and 1.025 mgQE/gram. The ethanol extract of Ipomoea reptans Poir leaves can be considered as a natural antioxidant because it has very strong antioxidant activity based on the DPPH method.

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