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PHENOLIC TOTAL CONTENT AND ANTIOXIDANT ACTIVTY OF ETHANOL EXTRACT OF MARKISA KONYAL (*Passiflora ligularis*) SEEDS

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D e t a i l A r t i k e l

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K a t a K u n c i

Konyal passion fruit seeds (Passiflora ligularis F. lobata)
Extract
Antioxidant Activity
DPPH

P e n u l i s K o r e s p o n d e n s i

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A B S T R A C T

Konyal passion fruit (*Passiflora ligularis* f. *lobata*) has been widely used as fruit juice by the community, it is necessary to conduct research to explore the potential of konyal passion fruit as a natural medicine. This study aims to determine the total phenolic compound content and antioxidant activity of ethanol extract konyal passion seeds using UV-Vis Spectrophotometry method. The sample was macerated with 70% and 96% ethanol solvent, repeated three times and the filtrate was concentrated at 40°C. The extract obtained was determined by the concentration of phenolic compounds using the Follin - Ciocalteau method and the antioxidant activity test using the DPPH method with gallic acid as a comparison compound. The determination of the total phenolic compound content was obtained at 77,73 µg/mL at a wavelength of 747 nm. The antioxidant activity test of gallic acid obtained IC_{50}: 2,04 µg/mL, and the antioxidant activity test of the sample obtained IC_{50}: 21,89 µg/mL at a wavelength of 515 nm. Based on the IC_{50} value, it can be concluded that the antioxidant activity of the ethanolic extract of passion fruit seeds is in the very strong category, namely in the range <50 µg/mL. The equivalence of antioxidant activity of ethanolic extract of konyal passion seeds with gallic acid is 1:10,73 mg, meaning that 1 mg gram of gallic acid is equivalent to 10,73 mg of ethanolic extract of konyal passion fruit seeds.

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INTRODUCTION

The study of free radicals and antioxidants is an emerging topic in current research trends. Free radicals are chemical compounds that in excessive amounts can damage health. Free radicals come from within the body produced by complex chemical processes and also from the external environment such as pollutants, chemical radiation, fast food, and toxins (Ionita, 2021). Therefore, to protect the body from free radical attacks, a material that functions as an antioxidant is needed (Ramadhan, 2020).

One of the plants that produce natural antioxidants is the Passiflora genus, also known as passion fruit. Some species of this genus include Passiflora edulis, Passiflora flevicarpa, Passiflora ligularis and Passiflora quadrangularis (Karmila, 2013).However, for konyal passion fruit, there is not much information on its bioactivity studies

Passion fruit can reduce muscle tension, lower anxiety, headaches, muscle spasms, and lower blood pressure. Meanwhile, the leaves are for insomnia. In addition, passion fruit also has anti-bacterial power and can also be used to treat malaria (Hana Shovyana & Karim Zulkarnain, 2013). In previous research conducted by (Tisa, 2021) Click or tap here to enter text.It was reported that passion fruit peel extract has antioxidant activity, namely with an IC50 value of 53.34 µg/mL (strong category) tested using the DPPH (1,1-Dipheny1-2-Piery Ihydrazy1) method.

Based on this information, the researcher continued to explore the total phenolic and antioxidant activity of passion fruit seeds konyal. Determination of total phenolic content using Follin-Ciocalten method because it is a simple, sensitive and thorough method. Antioxidant activity testing in this study used the DPPH method with gallic acid as the standard comparison compound.

RESEARCH METHODOLOGY

Tool and Materials

The tools and materials used are in accordance with previous research by (Tisa, 2021).

Sampel Preparation

The samples taken were fresh ripe passion fruit seeds (Passiflora ligularis {. lobata) taken in the Alahan Panjang area, Lembah Gumanti District, Solok, West Sumatra Province. The seeds and membranes of passion fruit are separated using a coconut milk sieve to make the separation of seeds and membranes easier and faster. After that, the passion fruit seeds were washed thoroughly with water and air dried for approximately 7 days. Passion fruit seed extract was extracted using maceration method using 70% and 90% solvents. Combine all the macerates obtained, then concentrated with a rotary evaporator at 40° until a thick extract is obtained. (Corey & Su, 2017). Furthermore, the maximum absorption wavelength and calibration curve of gallic acid-folin ciocalteu were measured according to previous research by (Tisa, 2021).

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a. Determination of Total Phenolic Compound Level

To measure the absorbance, 0.5 mL of sample solution was mixed with 5 mL of Follin-Ciocalteu recombinant (diluted 1:10) with distilled water. Then, leave at room temperature for 15 minutes and measure the absorbance with a UV-VIS spectrophotometer at the maximum wavelength three times. The linear regression equation formula obtained from the calibration curve of the standard solution was used to calculate the total phenolic compound content.

b. Determination of DPPH Maximum Absorption Wavelength

Pipetted into a 4 mL vial of freshly made 35 g/mL D PH solution, add 2 mL of methanol, and let stan for 30 minutes in a dark place. Test the absorbance using UV-VIS spectrophotometer with a length of 400-800 nm.

c. Determination of Antioxidant Activity Of Markisa Konyal Seed Extract

A sample of 25 mg was dissolved with methanol in a 25 mL volumetric flask to the limit, obtaining a standard solution of 1000 g/mL concentration. ug/mL. From the standard solution of 1000 µg/mL standard solution was pipetted 2.5 ml, then dissolved into a 25 ml volumetric flask so as to obtain a solution with a concentration of 100 µg/mL. from the 100 µg/mL solution was pipetted (0.5; 1; 1.5; 2; 2.5) mL. Then add methanol in a 10 mL volumetric flask until the limit mark. So as to obtain samples with a concentration of (5; 10; 15; 20; 25). µg/mL. Pipette each concentration as much as 2 mL of sample solution using a micro pipette and enter into the vial, then add 4 mL of DPPH 35 µg/mL. The mixture was homogenised and left for 30 minutes in a dark place, measure the absorbance using UV-VIS Spectrophotometer at the wavelength of maximum absorption. Determine the antioxidant activity by calculating the % inhibition. And regression equation of concentration value and percent inhibition. From the regression equation, find the IC valueso by entering the value of 50 into the regression equation.

RESULT AND DISCUSSION

Result

The results of determining the maximum wavelength of gallic acid standard solution by Folin-Ciocalteau method measured by Visible spectrophotometer obtained maximum absorption at a wavelength of 747 nm with an absorption of 0.496.

In the absorption measurements for the determination of gallic acid calibration curve, the regression equation y = 0,043 + 0,00569x was obtained with correlation coefficient (r) = 0,9974. As shown in Figure 1.

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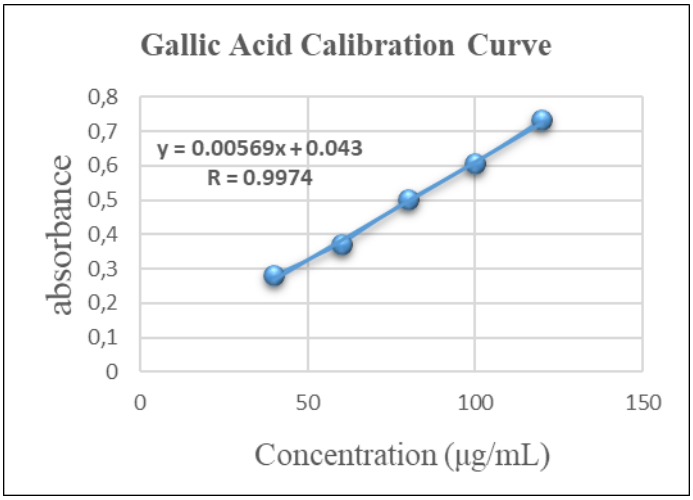

Figure 1. Calibration Curve of Gallic Acid + Follin-Ciocalteau Concentration

Based on the calculation, the phenolic compound content of the markisa konyal sample solution is 7,77%.

Table 1. Calculation Data of Phenolic Compound from Sample Solution

Repetition	Absorbance	Phenolic Compound Concentration (%)
1	0,480	7,68
2	0,485	7,76
3	0,491	7,87
Avarage		7,77

Determination of the maximum wavelenght of DPPH solution measured by Visible spectrophotometer obtained maximum absorption at waveleght 515 nm with absorption of 0,570. Result calculation of IC_{50} of gallic acid standard solution is 2,04 µg/mL.

Tabel 2. IC_{50} of Gallic acid standards

Gallic Acid Concentration (µg/mL)	DPPH absorbance	Absorbance of Gallic Acid + DPPH	% Inbition	IC_{50} (µg/mL)
0,5	0,513	0,454	11,50	
1	0,513	0,385	24,95	
1,5	0,513	0,320	37,62	**2,04**
2	0,513	0,262	48,92	
2,5	0,513	0,201	60,81	

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Tabel 3. IC50 of Sampel Solution + DPPH

Concentration of Markisa konyal seeds extract (µg/mL)	DPPH absorbance	Absorbance of Sampel + DPPH	% Inhibition	IC50 (µg/mL)
5	0,608	0,499	17,92	
10	0,608	0,442	27,30	
15	0,608	0,386	36,51	**21,89**
20	0,608	0,326	46,38	
25	0,608	0,267	56,08	

DISCUSSION

This study used samples of passion fruit seeds konyal from Alahan Panjang area in Gumanti Valley District Solok, West Sumatra Province. Parts of the roots, stems, fruits, flowers, and leaves were taken to the Herbarium ANDA in the Department of Biology, Faculty of Mathematics and Natural Sciences (FMIPA), Andalas University to be identified. The purpose of identification is to ensure that the plants used in the study are the real ones. The identification results show that the plant parts used in this study are the seeds of passion fruit konyal (Passiflora ligularis f. lobata), with a letter provided by the Herbarium of Andalas University Padang.

The extraction process is carried out by maceration method. The solvent used is ethanol, because it is cheap, relatively non-toxic and universal (able to attract polar and non-polar compounds) making it easier to attract phenolic compounds present in simplisia.

The first extraction process was carried out for 48 hours using 70% ethanol because the sample contains a high enough water content so that it can develop and open the pores of the sample which was previously dry (Verawati et al., 2017). The second extraction used 96% ethanol by soaking the sample for 48 hours. This maceration process was repeated three times to optimise the withdrawal of secondary metabolite compounds present in the sample. During maceration, there is a concentration equilibrium between the solvent used and the simplisia, therefore it is necessary to stir and change the solvent repeatedly (Asra et al., 2019). The results showed that phenolic compounds affect the antioxidant activity of ethanol extract of konyal passion fruit seeds.

Determination of total phenolic content of ethanol extract of markisa konyal seeds was carried out using *Follin-Ciocalteu* method. The principle of *Follin-Ciocalteu* method is the oxidation reaction of phenol compound in an alkaline atmosphere by Folin-Ciocalteu reagent which will produce a blue coloured complex that provides strong (Blainski et al., 2013).

The maximum wavelength of gallic acid at concentration 500 g/mL was 747 nm with an absorbance of 0,496. Furthermore, the absorbance of gallic acid at concentration 40, 60, 80,

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100, dan 120 µg/mL was measured. The goal was to find the calibration curve of gallic acid with Folin-Ciocalteu reagent, so a linear regression equation (y) = 0,043+0,00569x with r=0,0074 was obtained.

Table.1 shows the total phenolic content of 7,77% from the ethanol extract of markisa konyal seeds.

The DPPH (1,1-Diphenyl-2-picrylhydrazyl) method is a method used in measuring antioxidant activity because it is simple and fast, the sample used is very small to test antioxidant activity (Leaves, 2014). The maximum absorption area of DPPH is measured by UV-Vis spectrophotometer in the range of 400-800 mm using ethanol solvent as a control solution (Kandi & Charles, 2019). The results obtained the maximum absorption wavelength of DPPH was 515 am at a concentration of 35µg/ml with an absorbance of 0.570. The results showed that ethanol extract of konyal passion fruit seeds had an IC5O value of 21.8983 µg/ml for antioxidant activity, while the IC50 value of gallic acid as the opponent was 2.0398 µg/ml. A compound has very strong antioxidant activity if the Ic50 value is less than 50 µg/mL, strong if the IC50 value ranges from 50-100 µg/mL, moderate if the IC50 value ranges from 101-250 µg/mL, weak if 250-500 µg/mL, and inactive if >500 µg/mL ((Kedare & Singh, 2011). This limitation indicates that thanol extract of passion fruit seeds has a strong level of antioxidant activity.

CONCLUSION

From the result of the research that has been carried out, conclusion can be drawn:

1. The total phenolic content of markisa konyal seed was 7,77 %.
2. The IC50 value of ethanol extract of markisa konyal seeds of 21,8983 g/mL showed strong antioxidant activity through DPPH method.

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