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# DETERMINATION OF TOTAL FLAVONOID LEVELS AND ANTIOXIDANTACTIVITY FROM ETHANOL EXTRACT INSULIN LEAVES *(SMALLANTHUS SONCHIFOLIUS)* WITH DPPH METHOD (2,2-DIPHENYL-1-PICRYLHYDRAZYL)

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## ABSTRACT

The insulin or yacon plant (*Smallanthus sonchifolius*) is a plant that comes from the Andes Mountains, Peru. Insulin leaves contain protein, lipids, fiber, saccharides, catechones, terpenes, and flavonoids. Flavonoids are one of the phenol group compounds known to have free radical scavenger properties, inhibit hydrolysis, oxidative enzymes, and work as anti-inflammatory. Insulin leaves are very suitable for people with diabetes mellitus.This study aims to determine the total levels of flavonoids in the ethanol extract of insulin leaves and to test the antioxidant activity using DPPH(2,2-diphenyl-1-pikrilhidrazil) and ascorbic acid as a comparison. The determination was made at the Jatinangor Herbarium, Plant Taxonomy Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Padjadjaran University, Bandung. Extraction was carried out by maceration method using ethanol 96% with a yield of 10.90%. Determination of total flavonoid levels of ethanol leaf insulin extract was determined based on the absorbance value measured at a wavelength of 437 nm using a quercetin comparison. The results of the determination of total flavonoid levels were 24,905 mg QE / gr. The antioxidant activity test used a UV-Vis spectrophotometer with the DPPH method by ethanol extract of insulin leaves at a wavelength of 517 nm. The results of the antioxidant activity test of the ethanol extract of insulin leaves showed an $IC_{50}$ value of 4,583 µg/mL, indicating very strong antioxidant activity with the DPPH method.

**KEYWORDS:** Insulin leaves, total flavonoids, antioxidants, DPPH.

## INTRODUCTION

Insulin plant or yacon (Smallanthus sonchifolius) is a plant originating from the Andes Mountains, Peru. Is a sunflower family that grows in warm places with an altitude of up to 3200 meters. Yacon plant leaves are known to contain phenol components, such as chlorogenic, caffeic, and feluric (Pahlawan et al., 2016). The part of the plant used for treatment is the leaf. Insulin leaves can be consumed by boiling. Insulin leaves are very suitable for people with diabetes mellitus. Insulin leaves can be boiled or cooked with tea and taken two to three times a day to lower and control blood sugar levels (Putri, 2016). Flavonoids are one of the largest natural phenol group compounds found in all green plants (Markham, K.R 1988). According to Pourmorad (2006) one class of polyphenolic compounds is known to have properties as a free radical scavenger, an inhibitor of hydrolysis enzymes, oxidative, and also works as an anti-inflammatory. Flavonoids are a group of compounds that are not resistant to heating and are easily oxidized at high temperatures.

Based on this description, it is necessary to conduct more intensive research on testing the total flavonoid content of the ethanol extract of insulin leaves.

Antioxidants are substances that protect the body from the effects of free radicals that damage body cells and trigger degenerative diseases such as diabetes, heart disease and cancer.

A compound is said to have antioxidant properties if it is able to donate one or more electrons, then convert the oxidant compound into a more stable compound (Pietta, 2000).

In connection with the above background, in order to increase the utilization of insulin leaves as a source of medicine, this study was conducted to determine total flavonoid levels and test antioxidant activity by the DPPH method.

## Experiment

### Materials

Erlenmeyer, analytical balance, brown bottle, knife, spatel, watch glass, porcelain cup,volumetric flask, funnel, beaker, measuring cup, drop pipette, volume pipette, oven, evaporator, bath water, test tube, moisture balance, UV –Vis spectrophotometer Ethanol 96%, $AlCl_3$, quercetin, HCL, acetic acid, DPPH, Ascorbic acid, aluminum foil, filter paper, distilled water, $H_2SO_4$, Dragendroff's reagent, Mayer's reagent, Mg, NaCl 10%, $FeCl_3$.

### Preparation of sample

The collection of insulin leaves (Smallanthus sonchifolius) was obtained from Mataburanga Village, Konawe Islands Regency, Southeast Sulawesi.

The insulin leaves are cleaned under running water, drained and then dried in the sun. The dried insulin leaves are powdered using a blender. A total of 500 grams of insulin leaves simplicia which has become powder, sieved using a mesh sieve number 60, then weighed again and obtained 300 grams of powder.

### Extraction

150grams of insulin leaves powder was extracted using 96% ethanol solvent by maceration method for 3 x 24 hours protected from light while stirring occasionally, then filtered using filter paper to obtain the filtrate. Then evaporated using a Rotary Vacuum Evaporator at a temperature of 40$^{o}$C, obtained a thick extract and then evaporated using a water bath at a temperature of < 50$^{o}$C to obtain a concentrated extract of insulin leaves as much as 16,36 g with a yield of 10.90%.

### Phytochemical Screening

Phytochemical screening is a preliminary stage that can provide an overview of the content of certain compounds in natural materials to be studied (Fajriyah dkk, 2018). Screening for alkaloids, flavonoids, tannins and saponins was carried out.

### Total Flavonoid Content (TFC)

Total flavonoid content with quercetin as a comparison, 15 mg of extract, dissolved in 10 Ml of ethanol, in order to obtain a concentration of 1500 ppm. From this solution, 1 mL of pipette was added, 1 mL of 2% $AlCl_3$ solution and 1 mL of 120 mM potassium acetate were added (Irvan Ipand et al., 2016) Samples were incubated for one hour at room temperature.

The absorbance was determined using UV-Vis spectrophotometry at a maximum wavelength of 437 nm. (Stankovic, 2011). The total flavonoid content was figured as g quercetin equivalent per 100 g extract.

### DPPH scavenging activity

Preparation of DPPH solution by mixing 4 mg of DPPH with 96% ethanol in a 100 mL volumetric flask to obtain a concentration of 100 ppm. Then the absorption was measured at a wavelength of 400-600 nm using UV-Vis spectrophotometry. 20 mg of ethanol extract of insulin leaves was dissolved with ethanol in a 10 mL volumetric flask and then centrifuged. Various concentrations of 10 ppm, 20 ppm, 30 ppm, 40 ppm and 50 ppm were made, homogenized and incubated for 30 minutes in a dark place at 37°C. The absorbance was measured at a wavelength of 517 nm using UV-Vis spectrophotometry. The $IC_{50}$ value is calculated using the linear regression equation formula.

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**LEMBAR IDENTIFIKASI TUMBUHAN**
No.18/HB/07/2020

Herbarium Jatinangor, Laboratorium Taksonomi Tumbuhan, Departeman Biologi FMIPA UNPAD, dengan ini menerangkan bahwa :

| | |
|---|---|
| Nama | : Nurlinda Sapitri |
| NPM | : D1A191849 |
| Instansi | : AL-GHIFARI |

Telah melakukan identifikasi tumbuhan, dengan No. Koleksi: -

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|---|---|
| Tanggal Koleksi | : 25 Juli 2020 |
| Lokasi | : Jabar |

Hasil Identifikasi,

| | |
|---|---|
| Nama Ilmiah | : ***Smallanthus sonchifolius*** **( Poepp. ) H. Rob.** |
| Sinonim | : *Polymnia sonchifolia* Poepp. |
| Nama Lokal | : Daun Insulin |
| Suku/Famili | : Asteraceae |

Klasifikasi (Hirarki Taksonomi)

| | |
|---|---|
| Kingdom | Plantae |
| Divisi | Magnoliophyta |
| Class | Magnoliopsida |
| Ordo | Asterales |
| Famili | Asteraceae |
| Genus | *Smallanthus* |
| Species | *Smallanthus sonchifolius* ( Poepp. ) H. Rob. |

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**Figure 1: Result of determination.**

**Figure 2: Insulin Leaves.**

**Table 1: Chemical screening of extract.**

| Secondary metabolites | Solvent | Extract |
|---|---|---|
| Alkaloid | Dragendroff | + |
| | Mayer | + |
| Flavonoid | Magnesium | + |
| Tannin | FeCl3 | + |
| Saponin | HCl | + |

(+) = detected

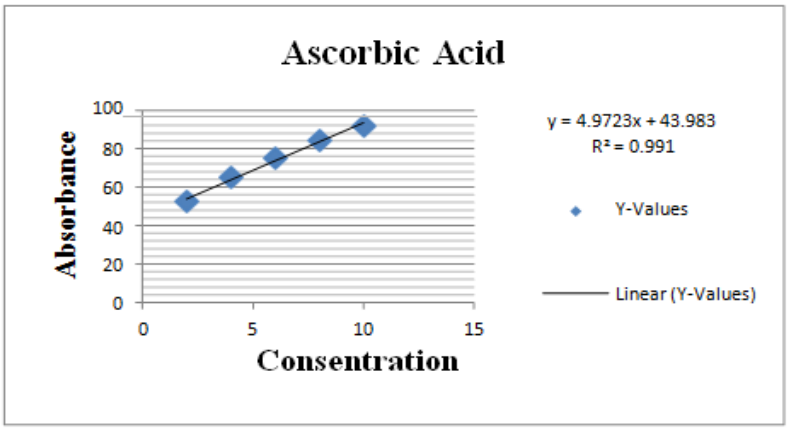


**Figure 3: Standard Curve of Linear Regression % inhibition of Antioxidant Activity of Ascorbic Acid with DPPH.**

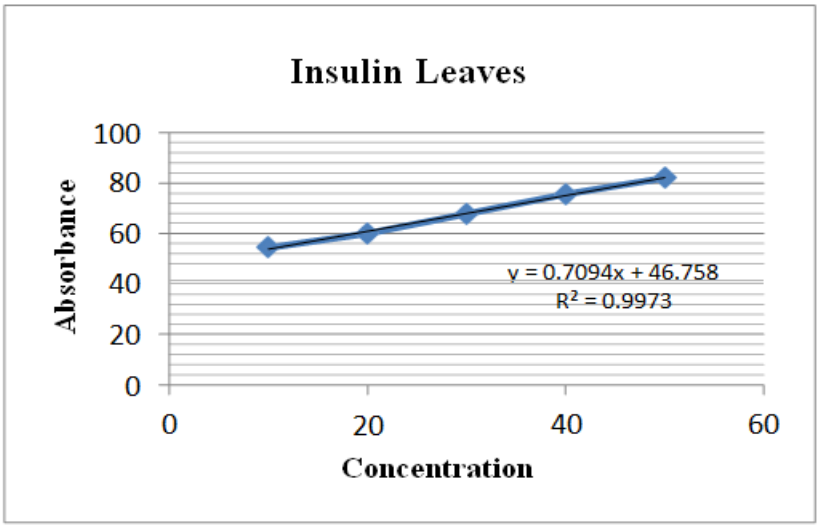


**Figure 4: Standard Curve of Linear Regression % Antioxidant Activity Ethanol Extract Insulin Leaves with DPPH.**

## RESULTS AND DISCUSSION

Plant determination was done in Plant Taxonomy Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences (FMIPA) Universitas Padjadjaran Jatinangor and stated that the plants used were insulin leaves (Smallanthus sonchifolius) (Figure 1) Characterization of simplicia exhibited that water content 2,4% (Wiendarlina et al, 2018) and loss drying 13,37%. Results insulin leave extraction 16,36 g with a yield of 10,90%.

The chemical screening was performed in extracts to find out the presence of secondary metabolites such as alkaloids, flavonoids, tannins, saponins in extracts. The results of the phytochemical screening can be seen in Table 1

The total flavonoid test results were calculated in mg QE/100g and the total flavonoid content of the ethanol extract of insulin leaves was 24,905 mg QE/100g Measurement of antioxidants by the DPPH method was characterized by a purple to yellow color change after being incubated for 30 minutes. The DPPH method was chosen because it is simple, easy, fast and sensitive and requires a small sample to determine the antioxidant activity of natural compounds (Hanani et al., 2005).

Ascorbic acid was used as a comparison because it has very strong antioxidant properties (Haeria, 2016). The $IC_{50}$ calculation results for Ascorbic acid is 1,210 µg/mL exhibit very strong antioxidant activity and ethanol extract of insulin leaves is 4,583 µg/mL, exhibit very strong antioxidant activity.

The standard linear regression curve of inhibition of antioxidant activity of Ascorbic acid and ethanol extract of insulin leaves by DPPH can be seen in Figures 3 and 4.

## CONCLUSION

The total flavonoid content of the ethanol extract insulin leaves (Smallanthus sonchifolius) was 24,905 mg QE/100g and $IC_{50}$ antioxidant activity 4,583 µg/mL, indicating a very strong antioxidant activity by the DPPH method.

## ACKNOWLEDGEMENT

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