JURNAL BIOMEDIKA
Volume 13 No. 02 September 2020
P-ISSN: 1979 - 035X (printed) & E-ISSN: 2302 – 1306 (online)
DOI: https://doi.org/10.31001/biomedika.v13i2.900

The Measurement of Antioxidant Activity of Velvet Beans (*Mucuna pruriens*) and Velvet Beans (*Mucuna pruriens*) in Coffee Preparations

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ARTICLE INFO

Article History:
Received: July, 2020
Revise: December, 2020
Accepted: February, 2021

ABSTRACT

Antioxidant activity tests of methanol extracts of velvet beans (*Mucuna pruriens*) and velvet beans (*Mucuna pruriens*) in coffee preparations have been previously carried out by several researchers. This present study aims to examine the phytochemical and antioxidant activities of methanol extracts of *Mucuna pruriens* and *Mucuna pruriens* in coffee preparations. The methanol extract was tested for the phytochemical and antioxidant activities by applying DPPH (1,1-Dhiphenyl-2-picrylhydrazil) method. The results of the phytochemical test showed that the methanol extracts of the *Mucuna pruriens* and *Mucuna pruriens* in coffee preparations contained secondary metabolites of flavonoids, alkaloids, saponins, terpenoids, and tannins. The outcomes of the antioxidant activity test revealed that the methanol extracts of the *Mucuna pruriens* and *Mucuna pruriens* in coffee preparations had antioxidant activities, as indicated by the IC_{50} values of 42.09 ppm and 37.23 ppm. It was also revealed that the antioxidant content of *Mucuna pruriens* in coffee preparations was stronger than the content in the velvet beans alone.

Keywords:
Antioxidant; DPPH method; *Mucuna pruriens* and *Mucuna pruriens* in coffee preparations; phytochemicals.

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INTRODUCTION

Diabetes mellitus (DM) is currently the fourth leading cause of death in the world (Akinchietal., 2008). The prevalence of DM cases worldwide is estimated to continue to reach 366 million people in 2030 and 80-90% of the patients are estimated to suffer from type 2 DM (Chen et al., 2012; Szkudelski, 2012). This type of Diabetes mellitus is characterized by hyperglycemia due to a decrease in insulin secretion triggered by insulin resistance (American Diabetes Association, 2015). Hyperglycemic conditions can be caused by an increase in the production of free radicals or Reactive Oxygen Species (ROS). Increased ROS activity in the body can harm various tissues, one of which is tissue damage in the form of microvascular abnormalities in kidney tissue (Tiwari et al., 2013).

Velvet beans (*Mucuna pruriens*) contain phenolic compounds, which in general can act as antioxidants. The research conducted by Kottai Muthu et al. (2010) reported that the methanol extract of velvet beans contains phenolic and tannin antioxidant compounds. Antioxidants are electron donors that can inhibit oxidative reactions by binding to free radicals and highly reactive molecules. Antioxidants play a role in fighting against free radicals in the body to prevent oxidative damage without becoming free radicals themselves (Widyawati, 2016).

The research on in vitro antioxidant activity concluded that methanol extract of velvet beans (*Mucuna pruriens*) has antioxidant activity and thus can capture 90.16% free radicals of 1,1-diphenyl-2-picrylhydrazyl (DPPH) with a 100 μg/mL concentration. The qualitative test results depicted the presence of phenolic compounds with the total phenolic levels of 33.04 mg/g using the Folin-Ciocalteau test (Rajeshwar, et al., 2005).

In this present study, velvet beans are processed into coffee powder because coffee is one of the favorite drinks of most people in Indonesia so that is easily accepted. Moreover, velvet bean coffee has a higher digestibility value because it contains nutrients that are easily digested in the digestive system. Velvet beans in coffee preparation have been consumed by some people in Sumberlawang, Sragen, Central Java, suffering from Diabetes mellitus to reduce blood glucose levels. There have been studies on the antioxidant levels in velvet seeds; however, studies on the antioxidant levels of velvet seeds in coffee preparations have not been carried out.

Based on the above explanation, this study is conducted to investigate the secondary metabolite compounds contained in velvet beans and velvet beans in coffee preparations, as well as evaluate the antioxidant activities of velvet beans and velvet beans in coffee preparations to investigate the extract that has the stronger antioxidant activity than the other.

MATERIALS AND METHODS

Materials and Equipment

The materials used in this research were velvet beans, distilled water, 96% ethanol, concentrated sulfuric acid, 10% NaOH, concentrated HCl, Mayer reagent, Wagner reagent, Dragendroff reagent, glacial acetic acid, $FeCl_3$, and 2,2-Diphenyl-1-picrylhydrazyl (DPPH).

Research Procedure

Preparation and Extraction of Velvet Beans and Velvet Bean Coffee

Velvet beans were collected and dried using an oven at 40°C. After that, the beans were ground and made into powder. For making coffee

preparation, velvet beans were roasted under low heat until brown and separated from the husk using a blender. 150 grams of velvet bean simplicia and velvet bean coffee were macerated with 1500 ml of 96% methanol and stored in a closed room for five days. On the fifth day, the materials were filtered and the obtained filtrates were concentrated using a rotary vacuum evaporator at a temperature of 45°C until thick extracts were yielded. The thick extracts were then stored in the refrigerator at a temperature of 10°C (Harborne, 1987)

Rendement Calculation

Extract samples were dried in an oven at a temperature of 100°C in an hour, weighed, and calculated for the rendement by using the following formula.

$$= \frac{\text{Weight after extraction}}{\text{Weight before extraction}} \text{ X } 100\%$$

Phytochemical Test

Flavonoid Test

A total of 0.5 grams of the extract was added to 100 ml of hot water and then stirred for five minutes. After that, the extract was sieved to obtain filtrate, which was later used as the solution. The filtrate was put into two tubes. The first tube was added with concentrated H_2SO_4, while the second tube was added with 2N NaOH. The tubes were shaken strongly and if an orange color was formed, it indicated the presence of flavonoid compound (Harborne, 1987).

Alkaloid Test

A total of 0.5 grams of the extract was added with 1 ml of 2N hydrochloric acid and 9 ml of distilled water, heated over a water bath for two minutes, cooled down, and then filtered. The filtrates obtained were used for the alkaloid test. The filtrates were then put into three test tubes, each of which contained 0.5 ml of filtrate. Each test tube was added with a different reagent. Test tube 1 was added with 2 drops of Mayer's reagent and a positive result is shown if a white precipitate is formed. Test tube 2 was added with 2 drops of Wagner's reagent and a positive result is shown if a brown precipitate is formed. Test tube 3 was added with 2 drops of Dragendroff's reagent and a positive result is shown if orange sediment is formed. Alkaloids are positive if there are deposits obtained from at least two of the three experiments above (Harborne, 1987).

Saponin Test

A total of 0.05 grams of the extract was put in a test tube, added with hot water, cooled down, and then shaken vigorously for 10 seconds. The reaction is positive if the steady foam is formed for not less than 10 minutes, 1 cm to 10 cm in height. With the addition of 1 drop of 2 N hydrochloric acid, the foam does not disappear (Harborne, 1987).

Terpenoid Test

A total of 0.5 grams of the extract was put in a dry test tube, added with 10 drops of anhydrous acetic acid and 3 drops of concentrated sulfuric acid. A positive reaction is indicated by the formation of a red solution that turns blue and green for the first time (Harborne, 1987).

Tannin Test

A total of 0.05 grams of the extract was added with 50 ml of hot water, boiled for 5 minutes, and then filtered. Half of the filtrate obtained was added with 10% FeCl3 solution. Positive results are indicated by the formation of a greenish color (Harborne, 1987).

Antioxidant Test

1 mL of ethanol extract of velvet beans and velvet bean coffee were taken, added 1 mL of DPPH each, vortexed, and incubated for 30 minutes at room temperature. The adsorption was measured at a wavelength of 515 nm. DPPH absorption measurement was carried out as a blank at the same wavelength. After the absorbance value was attained, the percentage of damping was calculated using an equation (Valentao, P. et al., 2001). The following is the calculation for the percentage of inhibition.

$$= \frac{\text{Blank abs} - \text{Sample abs}}{\text{Blank abs}} \text{X100\%}$$

From the obtained percentage of damping, the IC_{50} value was determined, the concentration that can inhibit 50% of free radicals.

RESULT AND DISCUSSION

The percentage of rendement was used to determine the weight of the extract produced compared to the weight of simplicia. The rendements of velvet beans and velvet bean coffee are presented in Table 1. Phytochemical screening was carried out on the extracts of velvet beans and velvet bean coffee to determine the chemical content of the secondary metabolites contained in the samples. Based on the phytochemical screening of 96% ethanol extracts of velvet beans and velvet beans coffee, positive results were obtained for all the secondary metabolites tested. The types and results of the phytochemical screening are demonstrated in Table 2.

Table 1. The Results of Rendement Analysis of Velvet Beans and Velvet Bean Coffee

Dry Weight (g)	Ethanol Extract	Weight (g)	Rendement (%)
150	Velvet beans	20.01	13.34
	Velvet beans in coffee preparation	18.18	12.12

Table 2. The Results of the Phytochemical Screening of Ethanol Extracts of Velvet Beans and Velvet Bean Coffee

Type Compound	Ethanol Extract	Result (+/-)
Flavonoid Test	Velvet beans	+
	Velvet bean coffee	+
Alkaloid Test	Velvet beans	+
	Velvet bean coffee	+
Saponin Test	Velvet beans	+
	Velvet bean coffee	+
Terpenoid Test	Velvet beans	+
	Velvet bean coffee	+
Tanin Test	Velvet beans	+
	Velvet bean coffee	+

Description:
+ : contains secondary metabolite compounds
- : does not contain secondary metabolite compounds

The results of the simplicia phytochemical screening in this study show that the samples contained flavonoids, alkaloids, saponins, terpenoids, and tannins. This is in line with the findings of the study by Murugan (2005) that velvet beans contain alkaloids, tannins, saponins, flavonoids, and triterpenoids.

Flavonoids are simple phenolic compounds with broad-spectrum in biochemistry including anti-oxidant, anti-mutagenic, and anti-cancer activities (Beta et al., 2005). The methanol extract of velvet beans donates hydrogen to the radical 1,1-diphenyl-2-picrylhydrazyl (DPPH). The research conducted by Kottai Muthu et al (2010) reported that the ethanol extract of velvet beans contains a number of phenolics. Further, in vivo research showed that the ethanol extract of velvet beans can act as natural antioxidants, which may be useful in preventing various oxidative stresses.

The results of the examination of antioxidant levels of velvet beans and velvet bean coffee ethanol extracts using the DPPH method are shown in Table 3.

Table 3. The IC_{50} Value of DPPH Antioxidant Activities

Sample	IC_{50} ppm
Ethanol extract of velvet beans	42.09
Ethanol extract of velvet beans in coffee preparation	37.23

According to Phogpaichit et al. (2007), a compound is said to be a free antiradical that is

very strong if the IC_{50} value is <10 μg/mL, strong if the IC_{50} value is between 10 and 50 μg/mL, moderate if the IC_{50} value ranges from 50-100 μg/mL, weak if the IC_{50} value ranges from 100 - 250 μg/mL and inactive when the IC_{50} value is above 250 μg/mL. When viewed from the IC_{50} value, velvet beans have an IC_{50} value of 42.09 ppm and velvet beans in coffee preparations have an IC_{50} value of 37.23 ppm, signifying that both are included in a "strong" category.

In general, compounds with bioactivity as polyphenol antioxidants belong to compounds that have a hydroxyl group. Polyphenol compounds inhibit free radicals by donating protons and forming stable radicals. The formation of stable radicals happens since the free electrons contained in these radicals are stabilized by electron delocalization due to the resonance in the aromatic ring. Moreover, compounds are considered to have antioxidant activity, such as polyphenol, flavonoids, and quinones.

The estimation of antioxidant activity with DPPH radical aims to determine the ability of the extracts of velvet beans and velvet beans in coffee preparations in capturing radical compounds or the ability to be antioxidant compounds. The estimation of antioxidants was performed using the DPPH method.

DPPH is a free radical that is stable and is beneficial to evaluate the reduction of free radicals in natural materials. The reaction principle of this method is that DPPH will be reduced by the hydrogen or electron donation process so that the color will change from violet to yellow with a change in color intensity that is proportional to the number of electron donations followed by a decrease in DPPH absorbance (Dris and Jain, 2004).

Compounds contributing to this condition can be considered antioxidants or radical scavengers. The greater the decrease in the absorbance of DPPH is, the stronger the antioxidant activity will be. The color-changing process of the DPPH solution due to the reaction with antioxidants can be seen in Figure 1.

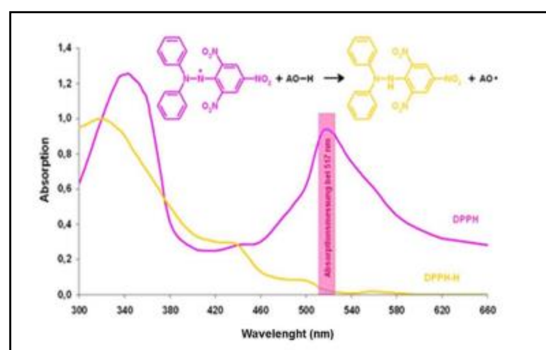

Figure 1. The DPPH Solution Color Change due to Reaction with Antioxidants (Morales-Gonzalez, 2013)

CONCLUSION

The phytochemical contents of the extracts of velvet beans and velvet beans in coffee preparations are similar, which are flavonoids, alkaloids, saponins, terpenoids, and tannins. The antioxidant activities of both ethanol extracts are very strong, although the antioxidant activity of velvet beans in coffee preparation (37.23 ppm) is higher than that of velvet beans (42.09 ppm).

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