Analysis of Bioactive Compounds and DPPH Radical Inhibition Activity of Hydroethanolic Extracts from *Mucuna pruriens* Seeds

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Abstract. *Mucuna pruriens* is a medicinal plant known to contain various bioactive compounds, including L-DOPA, alkaloids, phenolics, and tannins, with potential as a natural antioxidant. This study aimed to evaluate the characteristics of *Mucuna pruriens* seed simplicia and its hydroethanolic extract, identify its secondary metabolites, and determine its antioxidant activity. Simplicia were characterized through moisture and ash content analysis, and extraction was carried out using maceration with hydroethanol (1:1) solvent. The results showed a moisture content of 2% and an ash content of 2.1%, meeting the standards of the Indonesian Herbal Pharmacopoeia. The extract yield was 17.6%, indicating good extraction efficiency. Phytochemical screening revealed the presence of alkaloids, phenolics, tannins, and triterpenoids. Antioxidant activity was assessed using the DPPH method, yielding an IC_{50} value of 2.18 ppm, indicating a powerful antioxidant category. These findings suggest that the hydroethanolic extract of *Mucuna pruriens* seeds has high potential as a natural antioxidant source for health and cosmetic applications.

keywords: Antioxidant activity, bioactive compounds, DPPH assay, hydroethanolic extract, *Mucuna pruriens*.

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1. Introduction

In recent years, plant-based medicines have attracted global attention due to their natural origin, affordability, and ease of use [1,2]. In fact, approximately 80% of the population in developing countries tends to use traditional herbs according to the World Health Organization (WHO) [3]. Numerous studies have been conducted to demonstrate the presence of various medicinal compounds, including tannins, flavonoids, and saponins, in various plant tissues, such as leaves, seeds, and fruits. It has been reported that these natural products are used to develop approximately 44% of new primary drug discoveries. Concerns about the side effects of synthetic compounds also drive the increasing interest in using medicines from natural sources. For example, synthetic antioxidants common in the pharmaceutical industry, namely butyl hydroxyanisole (BHA), butyl hydroxytoluene (BHT), and tert-butylhydroquinone (TBHQ), have been reported to cause DNA damage, carcino genicity, and oxidative stress when misused. This condition highlights the need to explore potential natural sources as safer and more effective alternatives in pharmaceutical practice. *Mucuna pruriens*, a member of the leguminosae family, genus Mucuna, is commonly known as the velvet bean [4]. Physically, this annual herbaceous plant has long, pea-like fruits covered with golden-brown hairs. The seeds are black or shiny brown, oval-shaped, and about 12 mm long [4]. This plant has been reported to possess various biological activities, including anti-Parkinson's disease, antioxidant, antitoxin, antiglycemic, antimicrobial, and aphrodisiac effects [5]. Specifically, *Mucuna pruriens* seeds themself are also known to contain various secondary metabolites, such as alkaloids, phenolics, tannins, and triterpenoids, which result in their ability as antioxidant, anti-inflammatory, and neuroprotective agents [6]. In Indonesia, the productivity of this plant can reach 3–4 tons/ha, surpassing soybean productivity, which is typically around 1–2 tons/ha. However, despite its high productivity, its potential for medical or therapeutic applications remains largely unexplored.

When preparing medicinal herbs, the extraction process serves as a fundamental step [2]. One commonly used extraction method is maceration, which involves soaking powdered plants in a specific solvent at room temperature. In this technique, the selection of a suitable solvent is an essential point as the type of solvent determines its ability to isolate a wide array of active plant material or secondary metabolites from the plant [3]. Specifically, the maceration technique, which utilizes hydroethanolic solvents (a mixture of water and ethanol), is widely employed due to its ability to extract polar and semi-polar compounds [7] effectively. This solvent combination maximizes the recovery of bioactive compounds with high extract yields, while maintaining the stability of heat-sensitive compounds [8].

According to the author's knowledge, the thorough examination of the medicinal potential of *Mucuna pruriens* seed extracts using hydroethanolic solvents is limited, particularly regarding the relationship between the phytochemicals contained and their biological activity. Furthermore, there are still a few studies that systematically link the physicochemical characteristics of the medicinal plant (such as water and ash content) to the quality of the extract and the resulting antioxidant activity. Therefore, this research will focus on providing a comprehensive analysis of the hydroethanolic extract of *Mucuna pruriens* seeds, starting from the characterization of the medicinal plant, extraction efficiency, qualitative secondary metabolite content, and evaluation of antioxidant activity. This finding is expected to provide a scientific contribution in supporting the potential use of *Mucuna pruriens* as a source of natural antioxidants for therapeutic applications in the health sector.

2. Material and Methods

2.1. Material

Mucuna pruriens seeds were obtained from a local farmer in Jember Regency, Indonesia. In addition, this research used analytical-grade reagents from Merck, such as 96% ethanol, Mayer's, Wagner's, Dragendorff's, Boucharde's, Salkowski's, Lieberman-Burchard's reagents, concentrated HCl, magnesium powder, and 10% sodium hydroxide (NaOH); chloroform, 10% ferric chloride ($FeCl_3$), and 2,2-diphenyl-1-picrylhydrazyl (DPPH).

2.2. Sample Preparation

The *Mucuna pruriens* seeds were initially sorted and washed with deionized water to remove dirt and contaminants. Then, the seeds were dried at 60°C for 48 hours using an oven. The dried seeds were ground using a blender and sieved through a 40-mesh sieve to obtain a homogenous powder for subsequent analysis [1].

2.3.Physicochemical Analysis

2.3.1. Moisturise content analysis

The moisture content of 1 gram of seed powder was determined using a moisture analyzer to ensure it was below the standard limit of ≤ 10%.

2.3.2. Total ash content analysis

Around 1 g of powder (W_1) was weighed and placed into a pre-weighed silicate crucible (W_0). The mixture was slowly heated in a furnace at 600 ± 25°C until a white or greyish ash was formed. Then, the crucible was cooled in the desiccator and weighed again (W_2). The total ash content was calculated as:

$$\% \text{ Total Ash} = \frac{W2-W0}{W1} \text{ x } 100\% \quad (1)$$

2.4.Extraction of Mucuna prurienta seeds

The seed powder was extracted by maceration using 96% ethanol. A 100 g of the powdered seeds was macerated with 96% ethanol at a sample-to-solvent ratio of 1:10 (w/v). The extraction was performed in two stages: the first stage involved using 700 mL of solvent, followed by a second maceration with an additional 300 mL of solvent. The combined filtrates were concentrated using a rotary evaporator at 40 rpm and 60–70°C to yield a paste. The percentage yield of the extract was calculated using the following equation:

$$\text{Yield (\%)} = \frac{weight\ of\ extract\ (g)}{weight\ of\ simplisia\ (g)} \text{ x } 100 \quad (2)$$

2.5. Phytochemical Screening of Hydroethanolic Extract of Mucuna prurient seeds

The ethanolic extract was used for qualitative analysis to detect the presence of major secondary metabolites, following the methods described by previous research [15]:

- **Alkaloids:** The presence of alkaloids was evaluated using four reagents: Mayer's, Wagner's, Dragendorff's, and Bouchardat's reagents. For each test, twenty drops of liquid extract were treated with ten drops of the respective reagent. A positive result was indicated by the formation of a white precipitate (Mayer's test), a reddish-brown precipitate (Wagner's test), an orange or yellow precipitate (Dragendorff's test), or a brownish precipitate (Bouchardat's test).
- **Flavonoids:** Flavonoids were detected using two tests: the Shinoda and Sodium Hydroxide Test. In the Shinoda test, the formation of a pink to red color upon adding twenty drops of the sample with three drops of concentrated HCl and magnesium powder indicated the presence of flavonoids. For the Sodium Hydroxide test, the development of an intense yellow or reddish color after adding twenty drops of the sample to a 10% NaOH solution indicated a positive result.
- **Saponins:** Twenty drops of liquid extract and twenty drops of hot distilled water were mixed and gently shaken continuously for 15 minutes. A positive saponin result is indicated by the formation of a stable foam layer approximately 1 cm high.
- **Steroids and Triterpenoids:** These compounds were screened using the Salkowski and Lieberman-Burchard tests. In the Salkowski test, twenty drops of the sample were mixed with twenty drops of chloroform and 10 drops of Salkowski reagent and then vortexed. The appearance of a brown ring at the interface between the extract/chloroform layer, and with Salkowski's reagent, indicated the presence of steroids. In the Lieberman-Burchard test, twenty drops of the sample were mixed with ten drops of the Lieberman-Burchard reagent. The mixture was incubated for 5 minutes. A blue-green color indicated sterols, while a pink to purple-red color indicated the presence of triterpenoids.
- **Tannins (Polyphenols):** Tannins were detected using Braymer's test. The development of a greenish-black coloration upon adding ten drops of a 10% ferric chloride ($FeCl_3$) solution to the extract, followed by twenty drops of the extract, indicated a positive result.

2.6. Determination of Antioxidant Activity

The antioxidant activity of the Mucuna pruriens seed extract was determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay. A stock solution of the extract (100 µg/mL) was prepared by dissolving 10 mg of the extract in 100 mL of ethanol. A stock was diluted to obtain a range of concentrations, including 12.5, 25, 50, 100, and 200 ppm solutions. After that, 1 mL of each extract concentration was mixed with 3 mL of 0.1 mM DPPH solution. The mixtures were incubated in the dark at room temperature for 30 minutes. Then, the absorbance of each solution was measured at 517 nm using a UV-Vis spectrophotometer. Quercetin (10, 20, 30, and 50 ppm) was used as the positive control. The percentage of DPPH radical inhibition was calculated using the following equation:

$$\% \text{ inhibition} = \frac{(AControl\ absorbtion - ASampel\ absorbtion)}{AControl\ absorbtion}\ x\ 100 \quad (3)$$

Where Acontrol is the absorbance of the DPPH solution without the extract, and Asample is the absorbance of the sample mixture.

3. Result and Discussion

3.1. Physicochemical properties of *Mucuna pruriens* seed powder

The evaluation of physicochemical properties, such as water and ash content, serves as a crucial quality parameter to ensure the therapeutic effectiveness of the final herbal product. The results of the water and ash content tests are reported in Table 1.

Table 1. The characteristics of *Mucuna pruriens* seeds simplicia.

Parameters (%)	Result (%)	Standard (%)
Water content	2	≤ 10
Ash content	2,1	≤ 6

Determination of water content is related to the purity of the extract. Excessive moisture promotes damage and degrades the stability and effectiveness of the active compounds in herbal medicine, whereas low water content is crucial for extending their shelf life by inhibiting microbial growth. The standard water content for herbal medicines should not exceed 10% according to the requirements of the Indonesian Herbal Pharmacopoeia. Our results showed that the water content of the *Mucuna pruriens* used was 2%. This finding indicates that the sample has good stability and low microbial contamination.

Another important characteristic of herbal medicines is ash content, as this parameter reflects the mineral content of the herbal plant. A low ash content suggested that the plant material has high purity and is free from inorganic contamination. Furthermore, a low ash content may indicate that the drying process is effective enough to prevent significant loss of mineral content and maintain its quality. The requirement for total ash content according to the Farmakope Herbal Indonesia is no more than 6%. Our research results showed that the ash content of the *Mucuna pruriens* powder was 2.1%. This finding provides additional evidence that our tested sample is of high quality.

3.2. Phytochemical screening of Hydroethanolic Extract *of Mucuna pruriens* seeds.

The selectivity, solubility, and safety of the solvent are important considerations when extracting phytochemicals from natural sources using maceration techniques, as they significantly impact their effectiveness [2]. This research used a 1:1 ethanol: aquadest mixture for the extraction of *Mucuna pruriens* seeds, as this mixture is highly effective in extracting a wide array of bioactive compounds. Aquadest, a strongly polar solvent, can break down cell walls and dissolve polar components, while ethanol is efficient in extracting semi-polar compounds [8]. In addition, particle size is also known to enhance solvent penetration and solute diffusion [2]. Hence, *Mucuna pruriens* seeds were initially ground and sieved using a 40-mesh screen to ensure homogeneity. This combination was expected to enhance extraction efficiency and increase phytochemical content, thereby increasing its antioxidant activity. The yield from the extraction of *Mucuna pruriens* seeds with a 1:1 Hydroethanolic solvent was 17,6%.

Then, the preliminary phytochemical screening was conducted on the hydroethanolic extract of *Mucuna pruriens* seeds to identify the secondary metabolites in the herb. The qualitative analysis was performed using standard colorimetric tests, and the results are summarized in Table 2. Generally, the tests were positive for alkaloids, phenols, tannins, and triterpenoids, indicating the presence of these compounds in the extract. However, the

negative analysis also found flavonoids, saponins, and steroids, suggesting the absence of these secondary metabolites.

Table 2. Preliminary phytochemical screening of Hydroethanolic Extract of *Mucuna pruriens* seeds

Phytoconstituents	Test	Result	Remarks
Alkaloid	Mayer's Reagent	+	Alkaloids are present
	Wagner's Reagent	+	
	Dragendroff's Reagent	-	
	Bucardat	+	
Fenolik	Ferric Chloride Test	+	Phenols are present
Tanin	Ferric Chloride Test	+	Tanin are present
Triterpenoid	Salkowoski	+	Triterpenoids are present
Flavonoid	Alkali solution test	-	Flavonoids are absent
Saponin	Emulsion Test	-	Saponins are absent
Steroid-terpenoid		-	Steroid-terpenoids are absent

The presence of alkaloids in the *Mucuna pruriens* seed extract aligns with its known role as a primary source of L-DOPA [27,28], given that L-DOPA is a fundamental compound in the biosynthetic pathway of various alkaloids. This compound serves as a direct precursor to crucial neurotransmitters, including dopamine, norepinephrine, and epinephrine, and is used as a medication for the treatment of Parkinson's disease [10]. The identification of triterpenoids also adds to their therapeutic value, as this compound is known to possess prospective neuroprotective effects against Alzheimer's, Parkinson's, and Cerebral ischemia disease via anti-inflammatory, antioxidant, and anti-apoptotic effects.

Furthermore, the positive results obtained from the presence of phenols and tannins are also an important finding. These secondary metabolite compounds are widely recognized for their antioxidant, anti-inflammatory, cardioprotective effects. They are associated with a low risk of chronic diseases, such as cardiovascular disease, cancer, and diabetes [11]. Overall, the phytochemical profile of the *M. pruriens* seed extract provides a strong potential pharmacological activity.

3.3.Antioxidant effect of Hydroethanolic Extract *of Mucuna pruriens* seeds

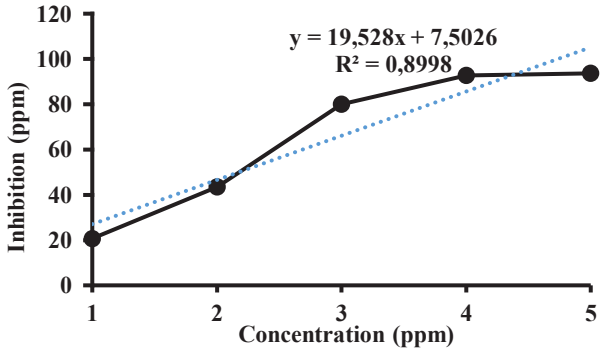

Fig. 1. Standard curve from *the hydroethanolic extract of Mucuna pruriens seeds*

The antioxidant effect of *Mucuna pruriens* seed hydroethanolic extract was examined using the DPPH free radical scavenging assay, with quercetin as the positive control. The DPPH method is one of the most widely used techniques for evaluating antioxidant activity due to its ease, rapidity, and sensitivity, requiring only a small sample [12]. The inhibition activity of the extract toward the DPPH radical was measured by its IC_{50} value. The IC_{50} value implies the concentration of a compound at which 50% of radical is inhibited, serving as a parameter to assess the effectiveness of potential antioxidant compounds.

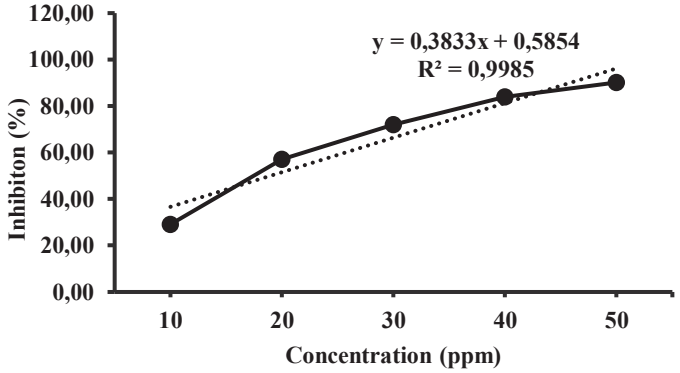

Fig. 2. Standard curve from quercetin

The IC_{50} measurement of *Mucuna pruriens* seed extract and the quercetin standard solution, using the spectrophotometric method, was derived from a linear regression curve. The extract sample had a standard curve equation of $y = 19.529x + 7.5015$; $R^2 = 0.8998$. Meanwhile, the standard curve for quercetin produced the equation $y = 0.3833x + 0.5854$, with $R^2 = 0.9985$. Subsequently, these two linear equations were used to calculate the % inhibition in the extract sample and standard solution, respectively, at the concentration range used.

Table 3. Antioxidant test results from Hydroethanolic Extract *Mucuna pruriens* seeds and quercetin as a control

Sample	Concentration (ppm)	Absorbance	% Inhibition	IC_{50} (ppm)
Hydroethanolic Extract *of Mucuna pruriens* seeds	12.5	0.887	20.692	2.18
	25	0.633	43.411	
	50	0.225	79.905	
	100	0.081	92.785	
	200	0.071	93.649	
Quercetin	50	0.097	90.17	12.891
	40	0.160	83.83	
	30	0.278	71.91	
	20	0.425	57.09	
	10	0.702	29.10	

The hydroethanolic extract of *M. pruriens* seeds has an IC_{50} value of 2.18 µg/mL (Table 3). Under the same experimental conditions, the standard antioxidant quercetin exhibited an IC_{50} value of 12.891 µg/mL. The IC_{50} value of the extract was significantly lower than that of quercetin, indicating a superior radical scavenging capacity and excellent antioxidant activity. In addition, according to the established classification system for antioxidant activity (Table 4), a substance with an IC_{50} value below 50 µg/mL is categorized as a "very potent" antioxidant. The IC_{50} results of the extract were significantly below the standard, indicating that the extract exhibited substantial antioxidant activity.

Table 4. Classification of antioxidant activity based on the IC_{50} Value

IC_{50} (µg/mL)	**Antioxidant Activity**
< 50	Very strong
50 – 100	Strong
100 – 150	Moderate
150 – 200	Weak
> 200	Very weak

This high antioxidant activity can be originated from the presence of bioactive compounds present in the extract, as confirmed by the phytochemical screening. It is noteworthy that M. pruriens contains a high concentration of L-DOPA, an alkaloid compound well-known for its strong antioxidant activity, as well as its therapeutic effects for Parkinson's disease. Previous research has shown that L-DOPA is identified as a primary contributor to the significant antioxidant activity and protection of cells from oxidative stress [14-15]. In addition to the alkaloids, the radical scavenging effect of the extract can be attributed to the presence of other phytochemicals, including triterpenoids, phenols, and tannins, as described before [11]. This result highlighted that the hydroethanolic extract of Mucuna pruriens seeds has excellent potential as a source of natural antioxidants and can be applied in products related to oxidative stress-related damage.

4. Conclusion

In conclusion, the characterization of *Mucuna pruriens* seed simplicia meets the established requirements for both water and ash content of 2% and 2.1%, respectively. Immersing this sample in a hydroethanolic solvent through maceration methods yielded 17.6%, leading to the identification of several bioactive compounds, including alkaloids, phenols, tannins, and triterpenoids, which suggests the efficiency of the extraction process. Furthermore, the antioxidant testing using the DPPH method demonstrated significant antioxidant activity with an IC_{50} value of 2.18 ppm. Overall, the hydroethanolic *Mucuna pruriens* seed extract can be concluded to be a potent antioxidant, highlighting its potential in the medical and therapeutic fields.

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