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Phytochemical Profile and Pharmacological Activity of Local Velvet Bean (*Mucuna pruriens*) of Timor

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ABSTRACT
The processing of velvet beans (*Mucuna pruriens*) from Timor is traditionally done by boiling them repeatedly twelve times before consumption due to concerns about their antinutrient content. On the other hand, earlier research indicated that velvet beans possess bioactive compounds that may serve as antioxidant and antidiabetic agents. The purpose of this study was to identify the bioactive components in extracts of velvet beans grown by the Timorese community, assess the overall levels of secondary metabolite compounds, and evaluate their bioactivities through *in vitro* antioxidant and hyperglycemia tests. Velvet beans samples were extracted using maceration, soaking them in ethanol and n-hexane solvents, followed by qualitative phytochemical analysis. Additionally, quantitative determination of secondary metabolites was performed, along with pharmacological activity tests, which included assessing antioxidant activity and antidiabetic effects through the spectrophotometric method. The findings revealed that the local Timor velvet beans extract contained flavonoid compounds at a concentration of 12.77 ± 3.10 mg QE/g, while the levels of phenolic and tannin compounds in the extract could not be quantified due to an absorbance lower than the y-intercept. The DPPH test demonstrated that the antioxidant activity of the local Timor velvet beans extract was measured at 1,964.51 ppm, which is categorized as very weak in comparison to the standard ascorbic acid solution that registered a value of 5.39, classified as very strong. The antidiabetic assessment indicated that the extract could inhibit the activity of the α-glucosidase enzyme at a concentration of 1,000 ppm by 28.60 ± 1.10%, which is categorized as very weak relative to the results from acarbose. Further investigation is necessary to optimize the bioactive compounds and identify the content of anti-nutritional compounds, as well as to explore effective methods of processing local Timor velvet beans that can reduce the antinutritional compounds.

Keywords: *Mucuna pruriens*, Phytochemistry, Secondary metabolites, Antioxidant, Antihyperglycemic.

ABSTRAK
Pengolahan kacang beludru (*Mucuna pruriens*) lokal Timor secara tradisional dilakukan dengan cara perebusan berulang selama dua belas kali sebelum dikonsumsi karena diduga memiliki kandungan antinutrisi. Di sisi lain, penelitian terdahulu menunjukkan bahwa kacang beludru mengandung senyawa bioaktif yang dapat berperan sebagai antioksidan dan antidiabetes. Penelitian ini bertujuan mengidentifikasi komponen bioaktif dalam ekstrak kacang beludru yang dibudidaya oleh masyarakat Timor, penetapan kadar total senyawa metabolit sekunder, dan uji bioaktivitas melalui uji antioksidan dan uji hiperglikemia secara *in vitro*. Sampel kacang beludru diekstraksi secara maserasi atau perendaman menggunakan pelarut etanol dan n-heksana, setelah itu dilakukan analisis fitokimia secara kualitatif. Selanjutnya, dilakukan penentuan total senyawa metabolit sekunder secara kuantitatif dan uji aktivitas farmakologi yang meliputi uji aktivitas antioksidan dan antidiabetes menggunakan metode spektrofotometri. Hasil penelitian menunjukkan bahwa ekstrak kacang beludru lokal Timor mengandung senyawa flavonoid dengan konsentrasi 3.10 ± 0.39 mg QE/g, sedangkan senyawa fenol dan tanin dalam ekstrak kacang beludru lokal Timor tidak dapat ditentukan karena absorbansi yang dihasilkan lebih rendah dari y-*intercept*. Uji DPPH menunjukkan aktivitas antioksidan yang dihasilkan ekstrak kacang beludru lokal Timor yaitu sebesar 1.964,51 ppm dimana tergolong sangat lemah jika dibandingkan dengan nilai yang dihasilkan oleh larutan standar asam askorbat, yaitu sebesar 5.39 yang tergolong sangat kuat. Uji antidiabetes menunjukkan kemampuan penghambatan aktivitas enzim α-glukosidase pada konsentrasi 1,000 ppm sebesar 28,60 ± 1.10% yang tergolong sangat lemah jika dibandingkan dengan hasil yang ditunjukkan oleh akarbosa sebagai inhibitor standar. Perlu dilakukan penelitian lanjutan mengenai optimasi senyawa bioaktif dan identifikasi kandungan senyawa antinutrisi, serta pengujian metode pengolahan kacang beludru lokal Timor yang efektif yang dapat mereduksi senyawa antinutrisi.

Kata kunci: *Mucuna pruriens,* Fitokimia, Metabolit sekunder, Antioksidan, Antihiperglikemia.

INTRODUCTION

Velvet bean (*Mucuna pruriens*) is a variety of beans that has been historically utilized by the community as a food source, including in Timor Island. This bean is rich in protein and complex carbohydrates, making it a significant source of energy when eaten, thus serving as a staple food option (Boniface et al., 2024). The people of Timor process velvet beans by boiling them twelve times. This method of repeated boiling is applied to various types of beans that are believed to contain anti-nutritional compounds (Karangora et al., 2021). Research by Ezegbe et al. (2023) indicated that velvet beans possess antinutrients like trypsin inhibitors, phytates, and carbohydrates such as raffinose and stachyose, which cannot be digested properly.

Prior research indicated that velvet beans are rich in fiber and various vital minerals, including iron, calcium, phosphorus, sodium, magnesium, and vitamin A (Pathania et al., 2020). The investigation found that velvet beans have a crude carbohydrate content ranging from 42.79% to 64.88%, crude fiber between 5.3% and 11.5%, crude protein from 24% to 31.44%, and crude fat varying from 4.1% to 14.39% (Ezegbe et al., 2023). Research by Theansungnoen et al., (2022) indicated that velvet beans contain phenolic compounds that function as antioxidants. Additional studies demonstrate that the treatment associated with velvet beans consumption patterns is effective in lowering blood sugar levels (Yadav et al., 2024). It has been established that various types of antioxidant compounds are present in beans, including saponins and anthocyanins (Chávez-Santoscoy et al., 2016), flavonoids (Arinanti, 2018), as well as phenols and tannins (Yadav et al., 2017). It is known that antioxidants contribute to neutralizing detrimental free radicals in the body and may help prevent cancer, diabetes mellitus, and other inflammatory diseases.

Despite the concern of local communities about possible toxicity, velvet beans possess a variety of beneficial bioactive compounds that merit further research. Moreover, there are currently no studies that identify the bioactive or antinutrient compounds present in the local velvet beans from Timor, making it essential to conduct phytochemical analysis as a preliminary step to shed light on the bioactive compounds contained in these local velvet beans. Additionally, insights into the bioactivity of local velvet beans from Timor can be gathered through *in vitro* pharmacological testing, which includes evaluating antioxidant properties and antihyperglycemic activity.

RESEARCH METHODS

This study was performed between June and September 2024, involving the extraction procedures and qualitative analysis of phytochemical profiles conducted at the Laboratory of the Faculty of Agriculture, Science, and Health at Timor University. Following this, a comprehensive analysis of secondary metabolites and pharmacological activity assessments, which included tests for antioxidant activity and α-glucosidase enzyme inhibition, were carried out at the Indonesian Biotech Engineering Laboratory.

Local velvet beans from Timor were sourced from Sunsea Village, located in the Naibenu District of the North Central Timor Regency in NTT. The samples utilized were dried velvet beans that had been harvested and were ready for processing, with a two-month post-harvest shelf life. The velvet beans underwent sorting, then were ground and measured to a weight of 500 grams, followed by extraction with two different solvents, specifically ethanol and n-hexane. The samples were immersed for two days and then filtered, and the resulting liquid extracts were concentrated using a rotary vacuum evaporator to yield thick extracts.

Phytochemical screening of velvet beans extracts. The extracts were subjected to phytochemical tests, which involved a qualitative analysis of flavonoids, alkaloids, saponins, tannins, and triterpenoids.

- Alkaloids test employed two different types of reagents: Wagner and Mayer. A total of 0.1 grams of both ethanol and n-hexane extracts were taken, after which a 2% hydrogen chloride solution was introduced and tested with Wagner’s and Mayer’s reagents. The presence of alkaloids was indicated by a brown precipitate appeared in the samples treated with Wagner’s reagent and a white precipitate observed in the tube with Mayer’s reagent (Solichah et al., 2021).

- Flavonoids test was performed with Shibata's reagent. Extracts weighing 0.1 grams each were prepared for testing with Shibata's reagent. The presence of flavonoids was indicated by the development of a red or orange hue in the samples (Baud et al., 2014).
- Terpenoids test was conducted using the Liebermann-Burchard's reagent. For this, 0.1 grams of ethanol and n-hexane extracts were utilized and tested with the Liebermann-Burchard's reagent. The presence of terpenoids was indicated by the appearance of a reddish-brown or purple ring in the samples (Jafar et al., 2020).
- The phenol test was performed using a solution of ferric chloride. Extracts solution of 1 mL was mixed with 15 drops of a 1% ferric chloride solution in water. The presence of phenolic compounds was indicated by the emergence of a bluish-black color in the samples (Wilujeng & Anggarani, 2021).
- The tannins test was performed by weighing 0.1 grams of each of the extracts and then boiling them in 10 mL of water in test tubes, followed by filtering the mixtures. Subsequently, a few drops of 0.1% ferric chloride were added to verify the presence of tannins in the samples. The presence of tannins was indicated by a color change of each mixture to brownish green (Baud et al., 2014).

The analysis of total flavonoids was performed using a modified aluminum chloride method as described by Tang et al., (2020), with modifications. An extract volume of 80 µL was combined with 80 µL of a 2% aluminum chloride solution mixed with ethanol, followed by the addition of 120 µL of a 50 g/L sodium acetate solution, and then incubated for 150 minutes at 25°C. The absorbance of the resulting solution was recorded at a wavelength of 440 nm using a UV-Vis spectrophotometer. The total flavonoids content was expressed in mg equivalents of quercetin per gram of sample (mg QE/g) based on the quercetin standard curve ranging from 0 to 250 µg/mL.

The total phenolic content was analyzed using a spectrophotometric method with the Folin-Ciocalteu reagent (Tang et al., 2020). The test utilized a gallic acid standard solution with a concentration range of 0-250 µg/mL. A volume of 25 µL of the sample extract was combined with 25 µL of the Folin-Ciocalteu reagent, which had been diluted with water in a ratio of 1:3, followed by the addition of 200 µL of water, and the mixture was allowed to incubate at room temperature for 5 minutes. Subsequently, 25 µL of 10% sodium carbonate solution was incorporated into the mixture, which was then incubated in a dark environment for 60 minutes. After this period, the absorbance was assessed at a wavelength of 765 nm. The total phenolic content was represented in mg equivalents of gallic acid per gram of dry weight of the sample (mg GAE/g).

The analysis of total tannin compounds was determined by the vanillin and p-dimethylaminocinnamaldehyde methods (Samsonowicz et al., 2019), with modifications. An extract sample of 25 µL was combined with 150 µL of a 4% vanillin solution that had been diluted with methanol, followed by the addition of 25 µL of a 32% sulfuric acid solution, then incubated at 25°C for 15 minutes. The absorbance of the resulting solution was recorded at a wavelength of 500 nm. The total tannin compounds presented in the samples was quantified in mg equivalents of catechin per gram of sample (mg CE/g) based on a catechin standard curve ranging from 0 to 550 µg/mL.

Antioxidant activity test. The antioxidant activity was assessed using the DPPH method (Tang et al., 2020), with modifications. An aliquot of 40 µL of the ethanol extract was mixed with 40 µL of methanolic DPPH solution, then stirred and allowed to incubate at 25°C for 30 minutes. Absorbance measurements for a range of concentrations of the sample were taken at a wavelength of 517 nm. The antioxidant activity was reported in mg equivalents of ascorbic acid per gram of the sample (mg AAE/g), based on the standard curve of ascorbic acid (2-7 µg/mL).

Alpha-glucosidase inhibitory activity. The antidiabetic activity test utilizing the α-glucosidase inhibitory method was performed based on the procedure outlined by Etsassala et al. (2020), with modifications. Specifically, 50 µL of phosphate buffer (100 mM, pH = 6.8), 10 µL of α-glucosidase (1 U/mL), 20 µL of the sample, and a standard acarbose solution (ranging from 0.0008 to 0.1 µg/mL) were combined and incubated for 15 minutes at 37°C. After this incubation, 20 µL of a 5 mM 4-nitrophenyl β-D-glucopyranoside solution was introduced, followed by another 20-minute incubation at the same temperature. Upon completion of the incubation, 50 µL of a 0.1 M sodium carbonate solution was added. The absorbance of the resulting solution was measured at a wavelength of 405 nm. The percentage of inhibition was calculated using the standard curve of ascorbic acid (2-7 μg/mL).

The findings of the study regarding the total content of secondary metabolites and the pharmacological evaluations were presented as mean ± SEM. The significance of the results was assessed using the post-hoc LSD test. Data is regarded as significant if the p-value is below 0.05.

RESULTS

A qualitative analysis of phytochemicals was performed to determine the presence of secondary metabolite compounds in the extract obtained from a sample using particular reagents. In this research, phytochemical analysis was carried out on local Timor velvet beans that were extracted using two different solvents: ethanol and n-hexane, chosen according to the polarity of the solvents.

Table 1. The results of qualitative phytochemical evaluation of secondary metabolite compounds from ethanol and n-hexane extracts of local Timor velvet beans.

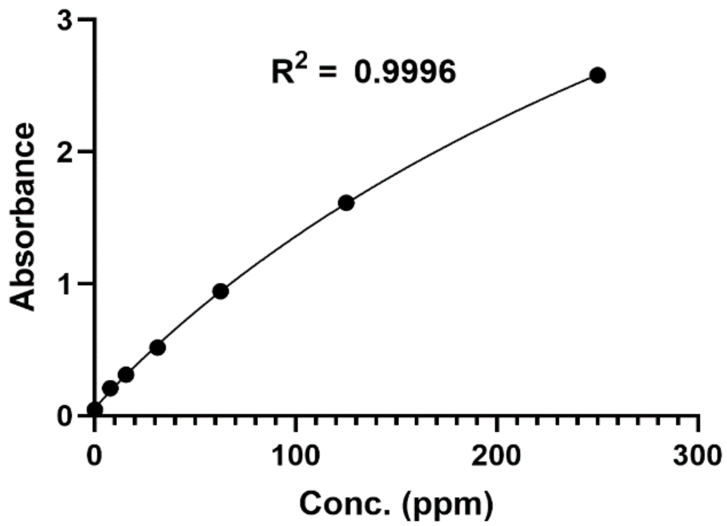
Secondary Metabolite	Reagents	Ethanol Solvent	n-Hexane Solvent
Alkaloids	Wagner	+	-
	Mayer	+	-
Flavonoids	Shibata	+	-
Terpenoids	Liebermann-Burchard	+	+
Phenolic compounds	ferric chloride	+	+
Tannins	ferric chloride	+	+

Table 1 shows that the ethanol extract of local Timor velvet beans contains alkaloids, terpenoids, flavonoids, phenolic compounds, and tannins, while the n-hexane extract comprises terpenoids, phenolic compounds, and tannins.

Table 2. Finding of the analysis of total flavonoid content.

Concentration (ppm)	Absorbance			Quercetin Equivalent/concentration
	1	2	3	
1,000.00	0.1161	0.1114	0.1029	3.10 ± 0.39

Based on the total flavonoids test results for local velvet beans of Timor presented in Table 2, the absorbance measurements for a 1,000 ppm concentration varied between 0.1029 and 0.1161. The total flavonoids content, represented in quercetin equivalents, yielded a result of 3.10 ± 0.39. The analysis utilized quercetin as the standard reference.



$$Y = \frac{7.353}{\left(\frac{487.5}{X}\right)^{0.9682} + 1} + 0.05566$$

Chart 1. The calibration curve of quercetin.

The fundamental concept of the $AlCl_3$ colorimetric method involves the creation of a stable acid compound complex with a ketone group present at the C-4 position, followed by the interaction of the hydroxyl group at C-3 or C-5 of flavones and flavonols with $AlCl_3$ (Fachriyah et al., 2020) as can be seen in Figure 1.

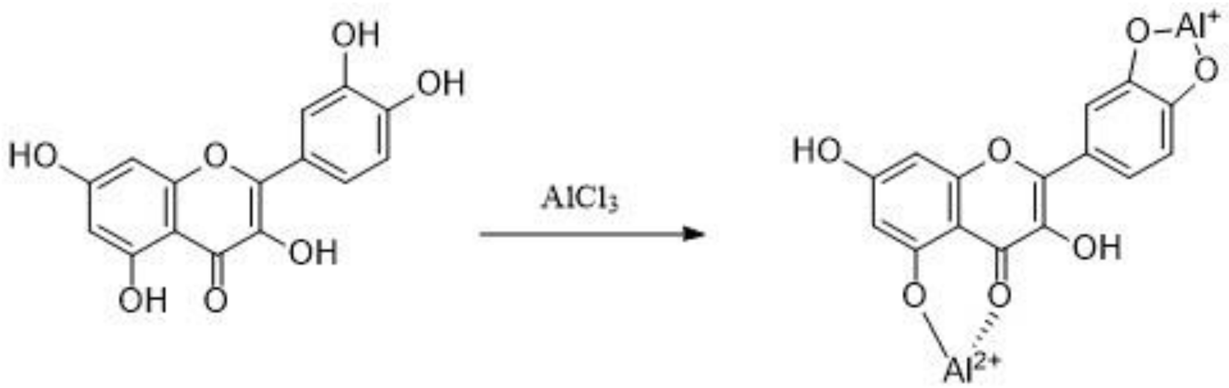


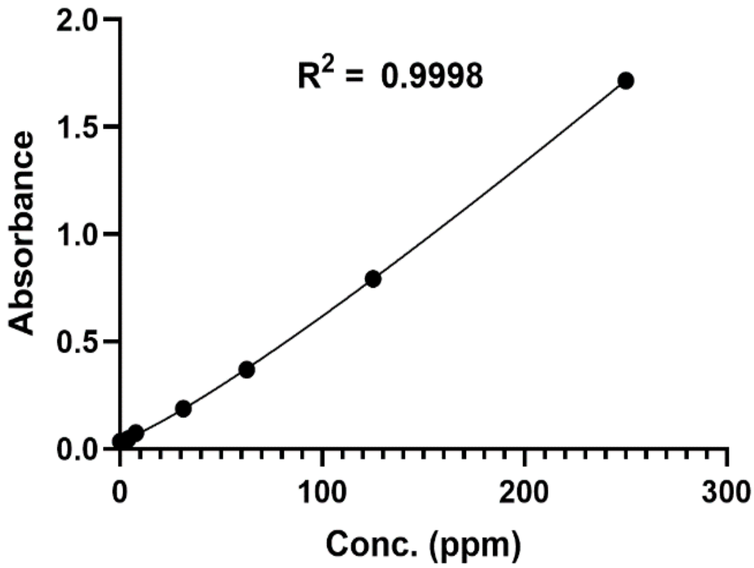
Figure 1. Reaction mechanism of flavonoid compounds with $AlCl_3$ (Fachriyah et al., 2020).

Table 3. Finding of the analysis of total phenolic content.

Concentration (ppm)	Absorbance			Gallic Acid Equivalent/concentration
	1	2	3	
1,000.00	0.0162	0.0174	0.0155	NA

Note: *Gallic acid equivalent is reported as NA (not available) due to absorbance lower than y-intercept*

The absorbance measurements for the total phenol test of the ethanol extract from local velvet beans of Timor presented in Table 3 reflect a concentration of 1,000 ppm. The recorded absorbance values were 0.0162, 0.0174, and 0.0155. However, information regarding the gallic acid equivalent (GAE) for this concentration is not available due to absorbance lower than the y-intercept. The analysis of total phenolic content was performed utilizing a gallic acid solution as a standard.



$$Y = \frac{67.68}{\left(\frac{5690}{X}\right)^{1.175} + 1} + 0.03688$$

Chart 2. The calibration curve of gallic acid.

Phenolic compounds are recognized for their antioxidant properties due to the presence of hydroxyl groups that can donate hydrogen atoms to free radicals, thereby preventing the oxidation process (Zhang et al., 2022).

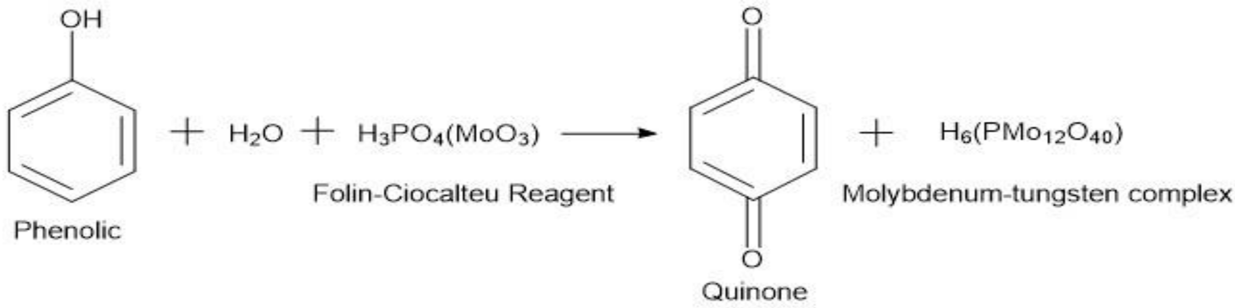


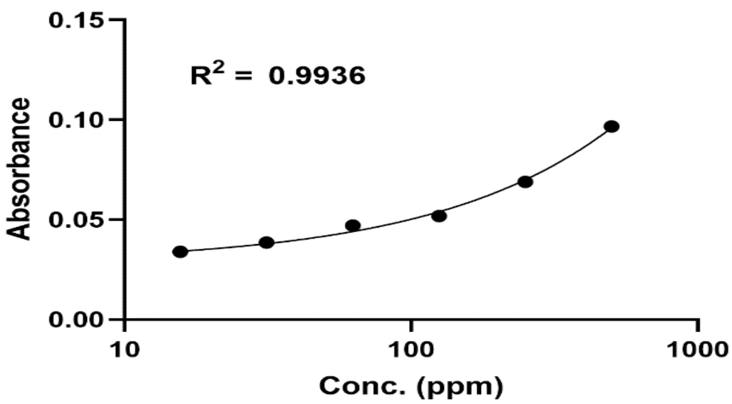
Figure 2. Specific reaction between phenolic and the Folin-Ciocalteu reagent (Mukhriani et al., 2019).

Table 4. Finding of the analysis of total tannins content.

Concentration (ppm)	Absorbance 1	Absorbance 2	Absorbance 3	Catechin Equivalent/concentration
1,000.00	0.0007	0.0001	-0.0007	NA

Note: *Catechin equivalent is reported as NA (not available) due to absorbance lower than y-intercept*

The result of the total tannin content analysis on the ethanol extract from local velvet beans of Timor is presented in Table 4. The concentration of the sample tested was 1,000 ppm, with recorded absorbance values of 0.0007; 0.0001; and -0.0007. The outcomes of this research indicate that there is no information available about catechin equivalent due to absorbance lower than the y-intercept. Catechin served as a reference standard, leading to the creation of a standard curve illustrated in the chart below.



$$Y = \frac{677.6}{\left(\frac{2.727e+08}{X}\right)^{0.6972} + 1} + 0.02816$$

Chart 3. The calibration curve of catechin.

Table 5. Finding of the antioxidant activity analysis.

Sample	Concentration (ppm)	% Inhibition	IC_{50}
Ascorbic acid	7.00	65,04 ± 1.45	5.39
	6.00	56.37 ± 1.71	
	5.00	48.11 ± 0.69	
	4.00	34.61 ± 0.88	
	3.00	26.07 ± 2.22	
	2.00	17.30 ± 2.53	
Velvet beans	2,500.00	59.59 ± 0.51	1.964,51
	1,250.00	37.48 ± 1.02	
	625.00	26.52 ± 0.15	
	312.50	24.35 ± 0.64	
	156.25	20.62 ± 0.74	
	78.13	19.96 ± 0.22	

Table 5 indicates that at the maximum concentration of 2.500 ppm, the extract displayed an inhibition level of 59.59 ± 0.51%. The percentage of inhibition declined with a reduction in extract concentration. At the minimum concentration evaluated (78.13 ppm), the inhibition percentage was recorded at 19.96 ± 0.22%. The findings from the antioxidant activity assessment of the ethanol extract from local velvet beans of Timor demonstrated that the extract possesses antioxidant activity, with an IC_{50} value of 1,964.51 ppm.

The antioxidant activity investigation of ethanol extract from velvet beans was conducted using the DPPH method by a UV-Vis spectrophotometer, which operates on the principle of light absorption at a designated wavelength. When DPPH is dissolved in ethanol, it exhibits a deep purple hue; however, when this solution interacts with other compounds capable of donating protons, it undergoes a color change to pale yellow (Fachriyah et al., 2020).

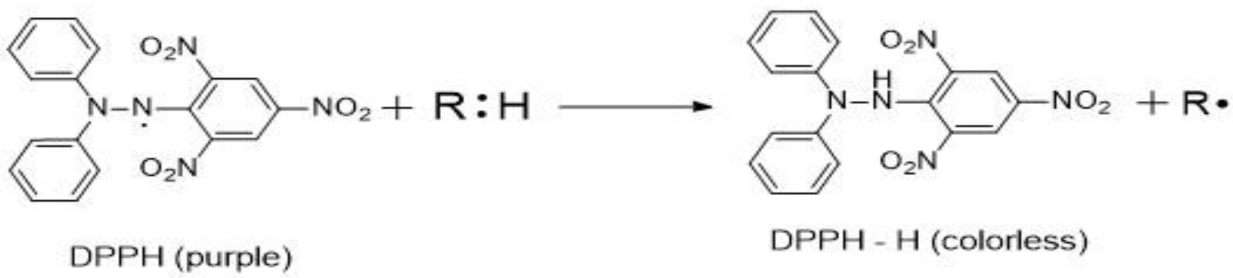
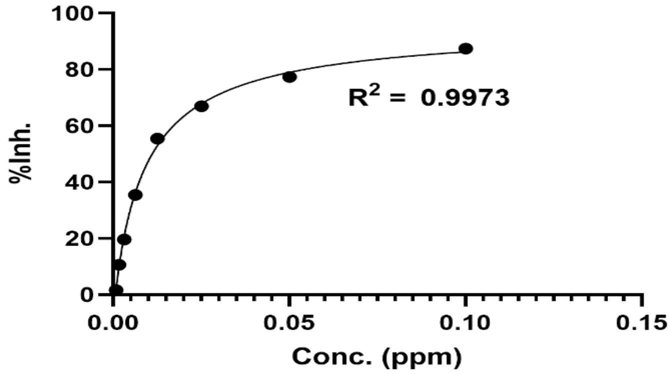


Figure 3. Mechanism of reaction between 2,2-diphenyl-1-picrylhydrazyl (DPPH) and antioxidants (Liang & Kitts, 2014). R:H = radical scavenger from the antioxidant; R• = antioxidant radical.

Table 6. Finding from the α-Glucosidase inhibition activity test.

Concentration (ppm)		Absorbance			% Inhibition
		1	2	3	
Acarbose	0.0008	1.8434	2.3537	0.7635	1.65 ± 0.81
	0.0016	10.0558	10.1032	11.7766	10.65 ± 0.98
	0.0031	20.0008	18.9090	19.8465	19.59 ± 0.59
	0.0063	34.2063	36.9358	35.3455	35.50 ± 1.37
	0.0125	55.7815	56.6359	54.0726	55.50 ± 1.31
	0.0250	67.2099	67.5897	66.1063	66.97 ± 0.77
	0.0500	77.7483	76.5260	77.8077	77.36 ± 0.72
	0.1000	87.6221	86.3760	88.2749	87.42 ± 0.96
Velvet beans	1,000.00	0.5964	0.5962	0.6123	28.60 ± 1.10

According to the information presented in Table 5, acarbose was used as a standard solution with various concentrations (0.0008 - 0.1 ppm). The percentage inhibition value increased with increasing concentration, from 1.65% at 0.0008 ppm to 87.42% at 0.1 ppm. The α-glucosidase inhibition activity test conducted on the ethanol extract of local velvet beans of Timor revealed that at a concentration of 1.000 ppm, the inhibition percentage recorded was 28.60 ± 1.10%.



$$Y = \frac{104.5}{\left(\frac{0.008486}{X}\right)^{0.93} + 1} - 8.646$$

Chart 4. The calibration curve of acarbose.

Acarbose is an example of an α-glucosidase inhibitor that functions by reducing glucose absorption in the intestine, assisting in the management of postprandial blood sugar levels. Consequently, the α-glucosidase enzyme is a key target for diabetes treatment (Altay, 2022). The reaction whereby the α-glucosidase enzyme is inhibited is outlined as follows.

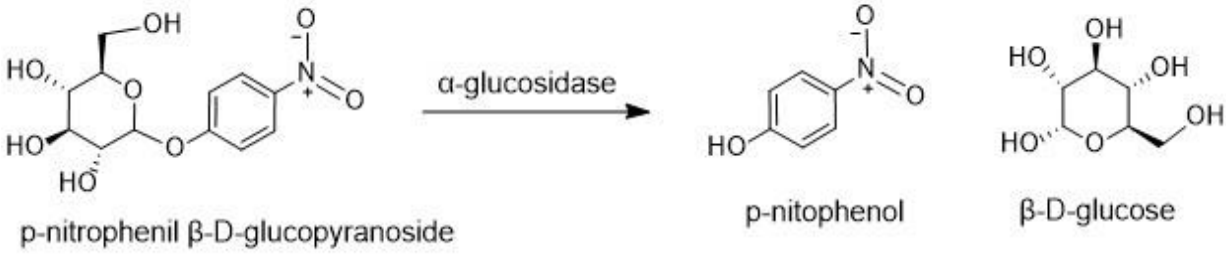


Figure 4. Enzymatic reaction equation for α-glucosidase and p-nitrophenyl α-D-Glucopyranoside (Sugiwati et al., 2010).

DISCUSSION

The phytochemical evaluation of local velvet bean (*Mucuna pruriens*) from Timor indicated the existence of alkaloids, terpenoids, flavonoids, phenolic compounds, and tannins, whereas the n-hexane extract contained terpenoids, phenolic compounds, and tannins. The identification of flavonoids and alkaloids corresponds with the research of Iamsaard et al., (2020), which verified the antioxidant properties of velvet beans. The presence of phenolic compounds in velvet beans has also been documented by Theansungnoen et al., (2022). These phytochemical results are in agreement with earlier studies and offer a detailed perspective on the pharmacological potential of velvet beans (Sowdhanya et al., 2024).

Flavonoids are substances characterized by functional groups attached to the carbon atoms of aromatic rings, enabling them to neutralize free radicals and serve as antioxidants (Wilujeng & Anggarani, 2021). The total flavonoids content of velvet beans extract was assessed through the $AlCl_3$ colorimetric method, with quercetin utilized as a standard reference. The result of this study indicated that the quercetin equivalent in the total flavonoids assay was 3.10 ± 0.39 mg QE/g, which is lower compared to the flavonoid levels reported in previous studies (Diniyah et al., 2023) (Theansungnoen et al., 2022). The flavonoids concentration in plants can differ due to factors such as genetic variation and environmental influences, compound stability, and the method of extraction employed (Prima et al., 2018).

Phenolic compounds are valuable metabolites found in plants that possess various biological activities, such as antioxidant, antimicrobial, antifungal, and anticancer effects (Alara et al., 2021). In this research, the assessment of total phenolic content in samples was conducted utilizing gallic acid (GAE) as a standard solution. Gallic acid was chosen as the standard solution due to its status as a natural and stable phenol, as well as its cost-effectiveness in comparison to other options. The findings indicated that the total phenolic content in the ethanol extract of velvet beans could not be quantified due to an extremely low absorbance value, while previous research presented different results where the total phenolic content in velvet beans was 233.80 mg GAE/100 g (Astawan et al., 2023), this could be caused by genetic, climatic, and geographical factors (Erizal et al., 2024). Besides that, the Folin-Ciocalteu method necessitates an alkaline setting for the optimal reduction of phosphomolybdate-phosphotungstate into molybdenum tungsten complex compounds. Failure to create these conditions may result in a diminished color intensity, influencing the absorbance value (Indriyah et al., 2023). Additionally, using standard solutions that are inappropriate or lack the same reactivity as the phenolic compounds present in the sample can also lead to lower absorbance measurements (Wilujeng & Anggarani, 2021).

Tannins are a group of secondary metabolites that exhibit anti-inflammatory, antibacterial, and natural antioxidant properties (Prasathkumar et al., 2021). The levels of tannin are assessed using the vanillin and p-dimethylaminocinnamaldehyde methods. Both methods rely on a colorimetric reaction involving tannins and specific reagents that generate colored complex compounds measurable via spectrophotometry (Karamac et al., 2007; Xia et al., 2023). Under acidic conditions, the interaction between vanillin and the flavonoids group found in condensed tannins leads to the formation of a red complex, the intensity of which can be quantified with a spectrophotometer at a specific wavelength (Herald et al., 2014). This method is highly sensitive and frequently employed for assessing the total tannins across various plant samples (Amarowicz & Pegg, 2024). The p-dimethylaminocinnamaldehyde (DMACA) method is commonly utilized due to its specificity for detecting certain types of condensed tannins (proanthocyanidins). When DMACA reacts under acidic conditions with proanthocyanidins, it produces a blue complex compound that can be spectrophotometrically measured to determine the concentration of tannin compounds present in a sample (Szymandera-Buszka et al., 2021).

The results of the research indicated that the level of tannin compounds in the ethanol extract of local Timor velvet beans could not be quantified due to an extremely low absorbance value recorded. This might be attributed to a very low concentration of tannins within the sample (Fajrina et al., 2016). The creation of complex compounds is suboptimal because the temperature, time, and pH levels of the reaction are not ideal, leading to low absorbance readings (Allo et al., 2022). This aligns with earlier research indicating that changes in temperature and extraction duration of velvet bean samples notably impact the total tannins (Diniyah et al., 2023).

IC_{50} (Inhibitory Concentration 50) refers to the concentration of antioxidants required to reduce 50% of DPPH free radical activity. A lower IC_{50} value indicates a more potent antioxidant activity of a compound. The typical classification of antioxidant activities according to IC_{50} values is as follows: very strong: $IC_{50} < 50$ ppm; strong: 50 ppm $\leq IC_{50} < 100$ ppm; moderate: 100 ppm $\leq IC_{50} < 150$ ppm; weak: 150 ppm $\leq IC_{50} < 200$ ppm; and very weak: $IC_{50} > 200$ ppm (Pratiwi et al., 2024; Zhang et al., 2006).

The IC_{50} value of the ethanol extract of velvet beans in this research was found to be 1,964.51 ppm, significantly higher than the threshold of 200 ppm; thus, it is classified as possessing very weak antioxidant activity. This outcome differs from the findings of Diniyah et al. (2023), which indicated that the antioxidant activity of the ethanol extract from velvet beans measured at 38.30 ppm, with the sample extraction performed at a temperature of 60°C for 360 minutes. Other studies reported significant antioxidant activity using various solvents (Kumbhare et al., 2023). This highlights that the choice of solvents and extraction parameters can influence the antioxidant activity in research samples (Sasadara & Wirawan, 2021; Prasetya et al., 2020). The variation in IC_{50} values can be attributed to several factors, including differences in the growth environments of the plants, the extraction methods employed, and variations in pH, temperature, and duration used (Amalia et al., 2023). Furthermore, our findings revealed that the ethanol extract from local Timor velvet beans possesses a relatively low total phenolic content and flavonoid compounds. These phenolic and flavonoid compounds are essential, as they can determine the level of antioxidant activity. A lower concentration of these bioactive compounds correlates with diminished antioxidant activity (Akhter et al., 2024). One approach to enhance the antioxidant activity in food ingredients is by combining them with other components through their processing into food or beverage products (Pandiansyah et al., 2024; Susilowati & Purwati, 2021).

The percentage inhibition value assessed in the antidiabetic evaluation through the α-glucosidase method indicates that the sample under investigation can decrease the activity of the α-glucosidase enzyme, achieving an inhibition value of 28.60 ± 1.10%. However, this percentage of inhibition is still comparatively low in contrast to the inhibition value exhibited by the standard acarbose solution. These findings suggest that while the ethanol extract of velvet beans shows potential as an α-glucosidase inhibitor, it is less effective than acarbose. The low inhibitory activity of the α-glucosidase enzyme could be caused by the low level of natural inhibitory compounds like flavonoids, tannins, and phenolic compounds present in the sample (Maryam et al., 2023). Moreover, crude extracts that comprise both active and inactive substances can diminish the inhibition effectiveness. The existence of non-active or potentially antagonistic compounds can disrupt the interaction between inhibitors and enzymes (Lu et al., 2023).

CONCLUSION

The qualitative phytochemical screening results revealed that the local Timor velvet bean extract contained alkaloids, terpenoids, flavonoids, phenolic compounds, and tannins. The total flavonoids content measured in the extract was 3.10 ± 0.39 mg QE/g, which is lower than in previous research, while the levels of phenolic compounds and tannins could not be quantified due to absorbance lower than the y-intercept. The presence of bioactive compounds in plants is affected by genetic, geographic, and climatic variables. The conditions under which extraction occurs, such as time and temperature, may also influence the results of the tests. The DPPH assay indicated that the antioxidant activity of the local Timor velvet bean extract was very weak in comparison to the standard solution, and the test for antidiabetic properties showed that the extract's ability to inhibit α-glucosidase enzyme activity was less effective compared to acarbose, which served as the standard solution. Further investigation is necessary to optimize the bioactive compounds and identify the content of antinutritional compounds, and also to explore the effective methods of processing local Timor velvet beans that can diminish antinutritional compounds to ensure their safety for consumption.

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REFERENCES

Akhter, M. J., Akhter, S., Islam, S., Sarker, M. S. H., & Hasan, S. M. K. (2024). Varietal influence on bioactive compounds and antioxidant activity in chilies during development stages. *Heliyon*, *10*(17), e37406. https://doi.org/10.1016/j.heliyon.2024.e37406

Alara, O. R., Abdurahman, N. H., & Ukaegbu, C. I. (2021). Extraction of phenolic compounds: A review. *Current Research in Food Science*, *4*, 200–214. https://doi.org/10.1016/j.crfs.2021.03.011

Allo, I. S., Suryanto, E., & Koleangan, H. S. J. (2022). Aktivitas Antioksidan Fenolik Bebas dan Terikat dari Tepung Cangkang Pala (Myristica fragrans Houtt). *Chemistry Progress*, *15*(2), 83–92. https://doi.org/10.35799/cp.15.2.2022.44496

Altay, M. (2022). Acarbose is again on the stage. *World Journal of Diabetes*, *13*(1), 1–4. https://doi.org/10.4239/wjd.v13.i1.1

Amalia, B. R., Muliasari, H., & Hidayati, A. R. (2023). Uji Aktivitas Antioksidan Kombinasi Ekstrak Kulit Pisang Kepok (Musa paradisiaca L.) dan Kulit Buah Naga Merah (Hylocereus polyrhizus (Weber) Britton & Rose) dengan Metode DPPH. *Jurnal Pharmascience*, *10*(1), 69-81. https://doi.org/10.20527/jps.v10i1.14863

Amarowicz, R., & Pegg, R. B. (2024). Condensed tannins-Their content in plant foods, changes during processing, antioxidant and biological activities. *Advances in food and nutrition research,* 110, 327-398. https://doi.org/https://doi.org/10.1016/bs.afnr.2024.03.001

Arinanti, M. (2018). Potensi senyawa antioksidan alami pada berbagai jenis kacang. *Ilmu Gizi Indonesia*, *1*(2), 134-143. https://doi.org/10.35842/ilgi.v1i2.7

Astawan, M., Faishal, M. A., Prayudani, A. P. G., Wresdiyati, T., & Sardjono, R. E. (2023). Effects of Seed Germination on Physicochemical and Bioactive Compounds Characteristics of Velvet Bean Tempe. *Current Research in Nutrition and Food Science*, *11*(2), 808–821. https://doi.org/10.12944/CRNFSJ.11.2.30

Baud, G. S., Sangi, M. S., & Koleangan, H. S. J. (2014). Analisis Senyawa Metabolit Sekunder dan Uji Toksisitas Ekstrak Etanol Batang Tanaman Patah Tulang (Euphorbia tirucalli L.) dengan Metode Brine Shrimp Lethality Test (BSLT). *Jurnal Ilmiah Sains*, *14*(2), 106-112. https://doi.org/10.35799/jis.14.2.2014.6065

Boniface, F., Washa, W. B., & Nnungu, S. (2024). Comparison of nutritional values of Mucuna pruriens L. (velvet bean) seeds with the most preferred legume pulses. *Food Production, Processing and Nutrition*, 6, 17. https://doi.org/10.1186/s43014-023-00187-4

Chávez-Santoscoy, R. A., Lazo-Vélez, M. A., Serna-Sáldivar, S. O., & Gutiérrez-Uribe, J. A. (2016). Delivery of flavonoids and saponins from black bean (Phaseolus vulgaris) seed coats incorporated into whole wheat bread. *International Journal of Molecular Sciences*, *17*(2), 222. https://doi.org/10.3390/ijms17020222

Diniyah, N., Bulgis, U. M., & Novi Marchianti, A. C. (2023). Antioxidant activity and phytochemical compositions of Mucuna pruriens L. in different conditions of time and temperature extraction. *IOP Conference Series: Earth and Environmental Science*, *1177*(1), 1–9. https://doi.org/10.1088/1755-1315/1177/1/012042

Erizal, E., Hanafiah, M., Mudatsir, M., Helmi, T. Z., & Yusuf, M. (2024). Determination of Total

Phenolic Content and Total Flavonoid Content of Nutmeg Flesh (Myristica Fragrans Houtt) Ethanol Extract from South Aceh Province. *IOP Conference Series: Earth and Environmental Science*, *1297*(1), 012053. https://doi.org/10.1088/1755-1315/1297/1/012053

Etsassala, N. G. E. R., Badmus, J. A., Marnewick, J. L., Iwuoha, E. I., Nchu, F., & Hussein, A. A. (2020). Alpha-glucosidase and alpha-amylase inhibitory activities, molecular docking, and antioxidant capacities of salvia aurita constituents. *Antioxidants*, *9*(11), 1–14. https://doi.org/10.3390/antiox9111149

Ezegbe, C. C., Nwosu, J. N., Owuamanam, C. I., Victor-Aduloju, T. A., & Nkhata, S. G. (2023). Proximate composition and anti-nutritional factors in Mucuna pruriens (velvet bean) seed flour as affected by several processing methods. *Heliyon*, *9*(8), e18728. https://doi.org/10.1016/j.heliyon.2023.e18728

Fachriyah, E., Kusrini, D., Haryanto, I. B., Wulandari, S. M. B., Lestari, W. I., & Sumariyah, S. (2020). Phytochemical Test, Determination of Total Phenol, Total Flavonoids and Antioxidant Activity of Ethanol Extract of Moringa Leaves (Moringa oleifera Lam). *Jurnal Kimia Sains dan Aplikasi*, *23*(8), 290–294. https://doi.org/10.14710/jksa.23.8.290-294

Fajrina, A., Jubahar, J., & Sabirin, S. (2016). Penetapan Kadar Tanin pada Teh Celup yang Beredar Dipasaran Secara Spektofotometri UV-VIS. *Jurnal Farmasi Higea*, *8*(2), 133–142.

Herald, T. J., Gadgil, P., Perumal, R., Bean, S. R., & Wilson, J. D. (2014). High-throughput micro-plate HCl-vanillin assay for screening tannin content in sorghum grain. *Journal of the Science of Food and Agriculture*, *94*(10), 2133–2136. https://doi.org/10.1002/jsfa.6538

Iamsaard, S., Arun, S., Burawat, J., Yannasithinon, S., Tongpan, S., Bunsueb, S., Lapyuneyong, N., Choowong-in, P., Tangsrisakda, N., Chaimontri, C., & Sukhorum, W. (2020). Evaluation of antioxidant capacity and reproductive toxicity of aqueous extract of Thai Mucuna pruriens seeds. *Journal of Integrative Medicine*, *18*(3), 265–273. https://doi.org/10.1016/j.joim.2020.03.003

Indriyah, S. N., Permatasari, D. A. I., & Pratama, K. J. (2023). Penetapan Kadar Fenolik serta Uji Aktivitas Antioksidan Ekstrak dan Fraksi Batang Bajakah Kalalawit (Uncaria gambir Roxb) Dengan Metode Frap. *USADA NUSANTARA: Jurnal Kesehatan Tradisional*, *1*(2), 147–158. https://doi.org/10.47861/usd.v1i2.347

Jafar, W., Masriany, & Sukmawaty, E. (2020). Uji Fitokimia Ekstrak etanol Bunga Pohon Hujan (Spathodea campanulata) secara In Vitro. *Biologi, Teknologi dan Kependidikan*, 8(1), 328–334. Retrieved from: https://jurnal.ar-raniry.ac.id/index.php/PBiotik/article/view/9658

Karamac, M., Kosinska, A., Rybarczyk, A., & Amarowicz, R. (2007). Extraction and chromatographic separation of tannin fractions from tannin-rich plant material. *Polish Journal of Food and Nutrition Sciences*, *57*(4), 471–474. Retrieved from: https://agro.icm.edu.pl/agro/element/bwmeta1.element.agro-article-e6e5bdf4-6eb8-4bf2-b624-7a6fc20b0283

Karangora, G. G. K., Lalel, H. J. D., Abidin, Z., & Rubak, Y. T. (2021). The Effect of Germination on Nutrition and Cyanide Acid Of Several Local Timor Lima Beans (Phaseolus lunatus L.). *Jurnal Agrisa*, *10*(2), 112–120. Retrieved from: https://ejurnal.undana.ac.id/index.php/agrisa/article/view/10361

Kumbhare, S. D., Ukey, S. S., & Gogle, D. P. (2023). Antioxidant activity of Flemingia praecox and Mucuna pruriens and their implications for male fertility improvement. *Scientific Reports*, *13*(1), 1–23. https://doi.org/10.1038/s41598-023-46705-9

Liang, N., & Kitts, D. D. (2014). Antioxidant Property of Coffee Components: Assessment of Methods that Define Mechanism of Action. *Molecules*, *19*(11), 19180–19208. https://doi.org/10.3390/molecules191119180

Lu, H., Xie, T., Wu, Q., Hu, Z., Luo, Y., & Luo, F. (2023). Alpha-Glucosidase Inhibitory Peptides: Sources, Preparations, Identifications, and Action Mechanisms. *Nutrients*, *15*(19), 4267. https://doi.org/10.3390/nu15194267

Maryam, S., Tahir, M., Azzahra, R., Farmasi, F., Makassar, K., & Selatan, S. (2023). Aktivitas Inhibisi Enzim Alfa-Glukosidase Dari Ekstrak Bunga Kersen (Muntingia calabura L.) Secara in Vitro. *Makassar Pharmaceutical Science Journal*, *1*(3), 2023–2150. https://journal.farmasi.umi.ac.id/index.php/mpsj

Mukhriani, M., Rusdi, M., Arsul, M. I., Sugiarna, R., & Farhan, N. (2019). Total Phenolic and

Flavonoid Content of Grapevine (Vitis vinifera L) Leaves Ethanol Extract. *Ad-Dawaa' Journal of Pharmaceutical Sciences*, *2*(2), 95-102. https://doi.org/10.24252/djps.v2i2.11503

Pandiansyah, A., Asiah, N., Ramadhan, K., & Ardiansyah, A. (2024). Aktivitas Antioksidan dan Profil Sensori Minuman Sari Kacang Merah (Phaseolus vulgaris L .) dengan Penambahan Ekstrak Jahe Merah Antioxidant Activity and Sensory Profile of Kidney Bean Drink (Phaseolus vulgaris L .) with Added Red Ginger Extract. *Jurnal Mutu Pangan*, *11*(2), 71–77. https://doi.org/10.29244/jmpi.2024.11.2.71

Pathania, R., Chawla, P., Khan, H., Kaushik, R., & Khan, M. A. (2020). An assessment of potential nutritive and medicinal properties of Mucuna pruriens: a natural food legume. *3 Biotech*, 10(6), 261. https://doi.org/10.1007/s13205-020-02253-x

Prasathkumar, M., Raja, K., Vasanth, K., Khusro, A., Sadhasivam, S., Sahibzada, M. U. K., Gawwad, M. R. A., Al Farraj, D. A., & Elshikh, M. S. (2021). Phytochemical screening and in vitro antibacterial, antioxidant, anti-inflammatory, anti-diabetic, and wound healing attributes of Senna auriculata (L.) Roxb. leaves. *Arabian Journal of Chemistry*, *14*(9), 103345. https://doi.org/10.1016/j.arabjc.2021.103345

Prasetya, I. W. G. A., Putra, G. G., & Wrasiati, L. P. (2020). Pengaruh Jenis Pelarut dan Waktu Maserasi terhadap Ekstrak Kulit Biji Kakao (Theobroma Cacao L..) sebagai Sumber Antioksidan. *Jurnal Rekayasa dan Manajemen Agroindustri*, 2503, 150-159 https://doi.org/10.24843/jrma.2020.v08.i01.p15

Pratiwi, I., Hasnaeni, H., & Handayani, S. (2024). Uji Aktivitas Antioksidan Ekstrak Etanol Sabut Buah Pinang (Areca catechu L.). *Jurnal Pharmascience*, *11*(1), 135- 143. https://doi.org/10.20527/jps.v11i1.16811

Prima, S. R., Munarsih, F. C., & Saadah, U. N. (2018). Perbandingan Jenis, Komposisi Dan Jumlah Pelarut Terhadap Uji Total Flavonoid Dari Daun Jawer Kotok (Plectranthus scutellarioides(L.) R.Br.). *Jurnal Farmasi Higea*, 10(2),154–162. Retrieved from: http://jurnalfarmasihigea.org/index.php/higea/article/view/192

Samsonowicz, M., Regulska, E., Karpowicz, D., & Leśniewska, B. (2019). Antioxidant Properties of Coffee Substitutes Rich in Polyphenols and Minerals. *Food Chemistry*, *278*, 101–109. https://doi.org/https://doi.org/10.1016/j.foodchem.2018.11.057

Sasadara, M. M. V., & Wirawan, I. G. P. (2021). Effect of extraction solvent on total phenolic content, total flavonoid content, and antioxidant activity of Bulung Sangu (Gracilaria sp.) Seaweed. *IOP Conference Series: Earth and Environmental Science*, *712*(1), 1–9. https://doi.org/10.1088/1755-1315/712/1/012005

Solichah, A. I., Anwar, K., Rohman, A., & Fakhrudin, N. (2021). Profil Fitokimia dan Aktivitas Antioksidan Beberapa Tumbuhan Genus Artocarpus di Indonesia. *Journal of Food and Pharmaceutical Sciences*, *9*(2), 443–460. https://doi.org/10.22146/jfps.2026

Sowdhanya, D., Singh, J., Rasane, P., Kaur, S., Kaur, J., Ercisli, S., & Verma, H. (2024). Nutritional significance of velvet bean (Mucuna pruriens) and opportunities for its processing into value-added products. *Journal of Agriculture and Food Research*, *15*, 100921. https://doi.org/10.1016/j.jafr.2023.100921

Sugiwati, S., Setiasih, S., & Afifah, E. (2010). Antihyperglycemic Activity of the Mahkota Dewa Leaf Extracts as an Alpha-Glucosidase Inhibitor. *Makara Journal of Health Research*, *13*(2), 74–78. https://doi.org/10.7454/msk.v13i2.364

Susilowati, I. T., & Purwati, P. (2021). The Measurement of Antioxidant Activity of Velvet Beans (Mucuna pruriens) and Velvet Beans (Mucuna pruriens) in Coffee Preparations. *Biomedika*, *13*(2), 117–122. https://doi.org/10.31001/biomedika.v13i2.900

Szymandera-Buszka, K., Gumienna, M., Jędrusek-Golińska, A., Waszkowiak, K., Hęś, M., Szwengiel, A., & Gramza-Michałowska, A. (2021). Innovative application of phytochemicals from fermented legumes and spices/herbs added in extruded snacks. *Nutrients*, *13*(12), 1–18. https://doi.org/10.3390/nu13124538

Tang, J., Dunshea, F. R., & Suleria, H. A. R. (2020). LC-ESI-QTOF/MS Characterization of Phenolic Compounds from Medicinal Plants (Hops and Juniper Berries) and Their Antioxidant Activity. *Foods*, *9*(1), 1–25. https://doi.org/10.3390/foods9010007

Theansungnoen, T., Nitthikan, N., Wilai, M., Chaiwut, P., Kiattisin, K., & Intharuksa, A. (2022). Phytochemical Analysis and Antioxidant, Antimicrobial, and Antiaging Activities of

Ethanolic Seed Extracts of Four Mucuna Species. *Cosmetics*, *9*(1), 14. https://doi.org/10.3390/cosmetics9010014

Wilujeng, D. T., & Anggarani, M. A. (2021). Determination of Total Phenolic, Total Flavonoids, and Antioxidant Activity of Single Bulb Garlic Extract (Allium sativum L.). *UNESA Journal of Chemistry*, *10*(3), 295–306.

Xia, M., Li, M., de Souza, T. S. P., Barrow, C., Dunshea, F. R., & Suleria, H. A. R. (2023). LC-ESI-QTOF-MS(2) Characterization of Phenolic Compounds in Different Lentil (Lens culinaris M.) Samples and Their Antioxidant Capacity. *Frontiers in Bioscience (Landmark Edition)*, *28*(3), 44. https://doi.org/10.31083/j.fbl2803044

Yadav, J. P., Pathak, P., Yadav, S., Singh, A., Palei, N. N., & Verma, A. (2024). In-Vitro evaluation of antidiabetic, antioxidant, and anti-inflammatory activities in Mucuna pruriens seed extract. *Clinical Phytoscience*, *10*(1), 21. https://doi.org/10.1186/s40816-024-00381-y

Yadav, M. K., Upadhyay, P., Purohit, S., Pandey, B. L., & Shah, H. (2017). Phytochemistry and pharmacological activity of Mucuna pruriens: A review. *International Journal of Green Pharmacy*, *11*(2), 69–73. https://doi.org/10.22377/ijgp.v11i02.916

Zhang, Q., Zhang, J., Shen, J., Silva, A., Dennis, D. A., & Barrow, C. J. (2006). A Simple 96-Well Microplate Method for Estimation of Total Polyphenol Content in Seaweeds. *Journal of Applied Phycology*, *18*(3), 445–450. https://doi.org/10.1007/s10811-006-9048-4

Zhang, Y., Cai, P., Cheng, G., & Zhang, Y. (2022). A Brief Review of Phenolic Compounds Identified from Plants: Their Extraction, Analysis, and Biological Activity. *Natural Product Communications*, *17*(1). https://doi.org/10.1177/1934578X211069721