



## Comparative Phytochemical Profiling and Antioxidant Activities of *Mucuna pruriens* Accessions: Insights into Bioactive Compounds and Therapeutic Potential

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### Abstract

This study investigates the comparative phytochemical profiling and antioxidant activities of different accessions of *Mucuna pruriens*, a leguminous plant renowned for its medicinal properties. The phytochemical composition of each accession was analyzed to identify bioactive compounds such as alkaloids, flavonoids, tannins, saponins, and phenolic compounds. Antioxidant activities were evaluated using two distinct assays: the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay, which measures the ability to neutralize free radicals, and the nitric oxide (NO) scavenging assay, which assesses the capacity to inhibit nitric oxide production, a mediator of oxidative stress. The results demonstrated significant variations in both phytochemical composition and antioxidant activity across the different accessions. Some accessions exhibited higher concentrations of flavonoids and phenolics, which correlated with stronger antioxidant potential. These findings suggest that the genetic diversity among accessions of *Mucuna pruriens* plays a crucial role in determining their phytochemical richness and antioxidant efficacy.

**Keywords:** *Mucuna pruriens*, Phytochemicals, Minimum Inhibitory Concentration (MIC), Antioxidant.

### Introduction

*Mucuna pruriens* commonly known as velvet bean, has garnered significant attention due to its pharmacological and therapeutic properties. This leguminous plant is traditionally used for managing a variety of ailments such as Parkinson's disease, male infertility, and neurological disorders, mainly due to its rich content of bioactive compounds [1].

The therapeutic potential of *Mucuna pruriens* is largely attributed to its diverse phytochemical profile, which includes alkaloids, phenolics, flavonoids, and saponins [2]. The variation in phytochemical composition among different accessions of *Mucuna pruriens*, particularly from various geographical regions, can result in differences in their biological activity [3].

Therefore, phytochemical profiling is essential to determine the specific compounds responsible for their medicinal properties. In recent years, advanced techniques have been used to assess the antioxidant potential of plant extracts, which play a pivotal role in protecting against oxidative stress-induced diseases [4]. The DPPH (2,2-diphenyl-1-picrylhydrazyl) and nitric oxide (NO) assays are among the most reliable methods used to evaluate the free radical scavenging capacity of plant extracts. These assays measure the plant's ability to neutralize reactive oxygen species, thus providing insights into their potential to combat oxidative stress [5]. By comparing the antioxidant activity of different accessions of *Mucuna pruriens* through these assays, the study aims to identify which accession has the highest therapeutic potential.

*Mucuna pruriens* (L.) is a plant belonging to the Fabaceae family, commonly known as velvet bean, “Karara” in Hausa, “Ukpo” in Igbo and “Werepe” in Yoruba, typically found in tropical regions and used for various purposes in traditional medicine in several countries. In India, it is used as a uterine stimulant and aphrodisiac [6] and also used against snake bites [7]. Traditionally, the seeds of *M. pruriens* are used as a tonic and aphrodisiac for male virility. Seeds contain L-DOPA (3,4-dihydroxyphenylalanine) content, which is a precursor of the neurotransmitter has been found to be used in the treatment of Parkinson’s disease and mental disorder [8]. Several studies have shown that L-3,4-dihydroxyphenylalanine (L-DOPA), lectin, isoflavones, and some alkaloids contained in

*M. pruriens* seeds are responsible for the enormous bioactivity of its crude extracts [9].

Recently, several therapeutic approaches have been adopted to make healthy living affordable to all regardless of their status in the society, since synthetic products are not only expensive but also comes with adverse effect on the body [10]. The challenges posed by the cost and safety of synthetic drugs have raised the need to explore underutilized plant species reputed for high medicinal significance. From previous reports, different parts of *Mucuna* have proven to be good for diverse therapeutic purposes [11]. Despite a large literature on *Mucuna*, its biological activities have not been adequately exploited for their therapeutic potential. Besides multi-drug resistance to synthetic drugs found in some communities, which is a major setback in the fight against malaria and other chronic diseases, the long-term usage of synthetic drugs could be detrimental. Countering the predisposing factors to these phenomena is a major concern for pharmaceutical industries. Some of these drugs are not readily available, not affordable, or could be imbued with myriads of adverse effects upon usage, thereby causing an ethical and medical dilemma [12]. Therefore, this study is aimed at comparative phytochemical profiling and antioxidant activities of *Mucuna pruriens* accessions: insights into bioactive compounds and therapeutic potential.

## Materials and Methods

### Sample Collection

The plant samples were collected from different accessions in Ibadan, Oyo state. The samples were properly cleaned and pulverized separately using clean mortar and pestle. The pulverizing step will increase the surface area of the *Mucuna pruriens*, making it easier for the phytochemicals to be released during the extraction process. The pulverized samples were poured into clean containers (separately) and taken for extraction.

### Extraction

The extraction was carried out using the method described by [13] of which n-hexane was used here instead, with Soxhlet extraction in the ratio 1:3 (w/v). 100 g of pulverized sample was measured into a porous bag (thimble) made of clean cloth and sealed, after which it was placed in the sample chamber of the Soxhlet apparatus. Subsequently, it was extracted with 300 ml of n-hexane continuously for duration 3hrs at a temperature of 60 °C. Following this, the solvent was then recovered, and the resulting extracts of each accession (A and B) was poured into a clean beaker and placed on a water bath to remove the solvent completely and to obtain a pure form of *Mucana pruriens* extracts.

### Phytochemical Screening

Phytochemical examinations were conducted on the extract using standard procedures to ascertain its constituents [13]. The screenings were thus conducted as follows;

### Test for Saponins (Frothing test)

The extract was dissolved in a test tube with 1 ml of water, and 10 ml of distilled water was added. The mixture was shaken continuously for 30 seconds. The solution was allowed to stand for 15 minutes. The formation of persistent froth indicates the presence of saponins.

### Test for Flavonoids (Sodium hydroxide test)

To a small portion of extract dissolved in 1 ml of water to which two drops of 10% Sodium hydroxide was added. A yellow colouration indicated the presence of flavonoids.

### Test for Alkaloids (Mayer's Test)

To 2 ml of the acidic solution of the extract in a test tube, 3 drops of Mayer's reagent was added. A cream precipitate indicated the presence of alkaloids.

### Test for Tannins (Lead acetate test)

To a small portion of extract dissolved in 1 ml of distilled water, 4 drops of lead acetate solution were added. The formation of a cream-colored precipitate indicates the presence of tannins.

### Test for Terpenoids

To a small portion of the sample dissolved in chloroform and equal amount of sulphuric acid (H<sub>2</sub>SO<sub>4</sub>) was added and the formation of a reddish-brown ring at the interface of the mixture indicates the presence of terpenoids.

### Test for Phenol

To a small portion of extract dissolved in distilled water, a drop of 10% ferric chloride (FeCl<sub>3</sub>) was

added, the formation of a dark green colouration indicates the presence of phenol.

#### **Test for Anthraquinone**

To a small portion of extract dissolved in chloroform, aqueous ammonium hydroxide (NH<sub>4</sub>OH) and a faint pinkish colouration in the aqueous upper layer indicates the presence of anthraquinone.

#### **Test for Cardiac glycoside**

To a small portion of extract in a test tube 1mL of concentrated acetic acid (CH<sub>3</sub>COOH) containing traces of ferric chloride (FeCl<sub>3</sub>) was added followed by the addition of equal volume of concentrated sulphuric acid (H<sub>2</sub>SO<sub>4</sub>) along the side of the test tube the formation of a reddish-brown ring at the interface of the mixtures indicates the presence of cardiac glycoside.

#### **Quantitative phytochemical screening**

##### ***Sample preparation***

Each of the extracts (50 g; A and B) were weighted and dissolved in 2.5 ml of 50% methanol to give a concentration of 20 mg/ml for each sample. The quantitative phytochemical screening of the Phenolic acid, flavonoids, tannins, alkaloids, saponins and the Nitric oxide assay were done using standard methods as described by [14].

#### **Antioxidant Assay**

##### **DPPH Scavenging Activity**

The DPPH antioxidant assay was carried out on the *mucana pruriens* extracts according to [15] with slight modifications.

#### **Nitric oxide assay**

The Nitric oxide assay was carried out on *mucuna pruriens* extracts according to [1] with slight modifications as well.

#### **Characterization**

Characterization of the phytochemical to analyze and compare the chemical composition and presence of bioactive compounds across accessions were carried out using Fourier Transform Infrared Spectroscopy (FT-IR) at the R&D Laboratory, Defense Industries Corporation of Nigeria (DICON), Kaduna state, Nigeria. Following the method of spectral analyses reported by.

#### **Results and Discussion**

##### **Qualitative Phytochemical Screening**

Phytochemical screening of seed extracts *Mucuna pruriens* from both accessions was performed to determine the presence and concentration of secondary metabolites like alkaloids, terpenoids/steroids, flavonoids, phenolic compounds, anthraquinones, saponins, tannins, and cardiac glycosides. Extracts from both accessions were tested to have all of the above secondary metabolites present although not in the same concentration as listed in the Table 1

**Table 1: Qualitative Phytochemical Screening**

| Tests                      | Extract A | Extract B |
|----------------------------|-----------|-----------|
| <b>Alkaloids</b>           | ++        | +         |
| <b>Steroids/Terpenoids</b> | +         | +         |
| <b>Flavonoids</b>          | +         | +         |
| <b>Phenolics</b>           | +         | +         |
| <b>Anthraquinones</b>      | +         | +         |
| <b>Saponins</b>            | ++        | +         |
| <b>Tannins</b>             | +         | ++        |
| <b>Glycosides</b>          | +         | +         |

Key: + present, ++ high

The result obtained here is in agreement with that obtained by [16] of which soluble carbohydrates and deoxy-sugers were also present in their research. Similarly, the phytochemicals detected were present in the result obtained by [13], with the exception of alkaloids and phenolics.

#### Quantitative Phytochemical Analysis

Absorbance for phenolic, flavonoids, tannins, alkaloids, saponins were read at a wavelength of 765 nm, 510 nm, 725 nm, 470 nm and 544 nm respectively, using the micro plate spectrophotometer. The concentrations obtained are shown in table 2.

**Table 2: Quantitative Phytochemical Analysis**

| Phytochemicals    | Concentration (g) |           | Total Content (µg/ml) |           |
|-------------------|-------------------|-----------|-----------------------|-----------|
|                   | Extract A         | Extract B | Extract A             | Extract B |
| <b>Phenolic</b>   | 0.371             | 0.378     | 18.55                 | 18.90     |
| <b>Flavonoids</b> | 5876              | 6069.33   | 293,800               | 303,466   |
| <b>Tannins</b>    | 141.125           | 153.875   | 7056.25               | 7693.75   |
| <b>Alkaloids</b>  | 1.007             | 0.631     | 50                    | 31.535    |
| <b>Saponins</b>   | 120.4             | 81.6      | 120,400               | 81,600    |

The results obtained for the quantitative phytochemical analysis carried out, showed that there are significant amount of the tested phytochemicals present in the extract. These results

differ slightly with the results obtained by [17] where the total phenol content, flavonoids and proanthocyanidins contents obtained from the spectral result were 3730.1 mgGAE/g, 63.03 mg

QE/g and 18.92 mg CE/g respectively. This differences could be as a result of geographical location of the *Mucuna pruriens*.

### Antioxidant Assay

#### DPPH Scavenging Activity

Absorbance was read at a wavelength of 520 nm from the micro plate spectrophotometer for DPPH and the following readings were obtained as shown below;

**Table 3: Results for DPPH Scavenging Activity**

| Extracts/Blank | First Readings | Second Readings | Average reading | %Inhibition |
|----------------|----------------|-----------------|-----------------|-------------|
| Blank          | 1.698          | 1.552           | 1.1415          |             |
| A              | 1.036          | 1.034           | 1.1035          | 0.269       |
| B              | 1.053          | 1.118           | 1.086           | 0.233       |

#### Nitric Oxide Assay

Absorbance was read at a wavelength of 542nm from the micro plate spectrophotometer for DPPH

and the following readings were obtained as shown below;

**Table 4: Results for Nitric Oxide Assay**

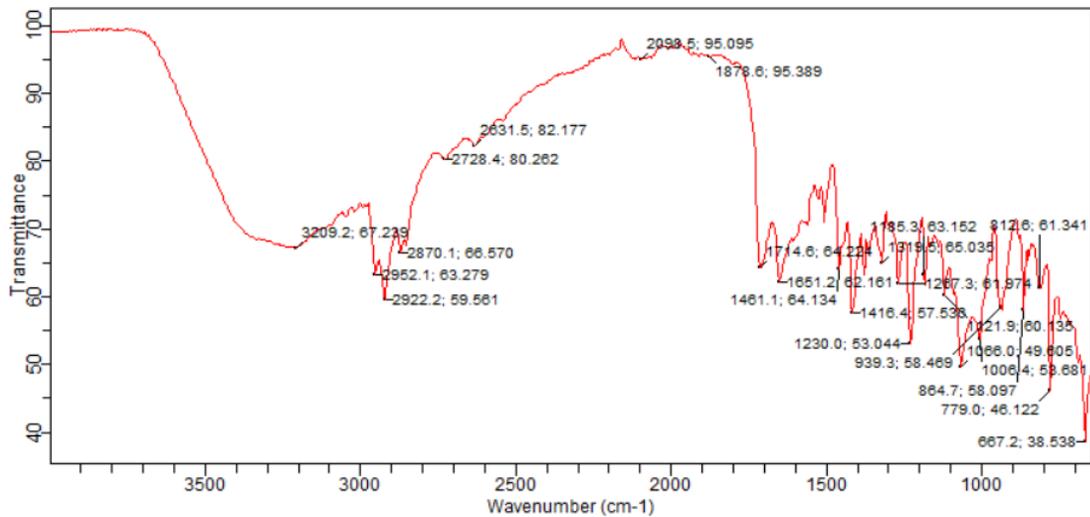
| Extracts/Standard        | First Readings | Second Readings | Average reading | %Inhibition |
|--------------------------|----------------|-----------------|-----------------|-------------|
| Standard (ascorbic acid) | 1.615          | 1.627           | 1.621           |             |
| A                        | 1.481          | 1.389           | 1.435           | 0.115       |
| B                        | 1.571          | 1.460           | 1.516           | 0.065       |

The DPPH scavenging activity showed very little % inhibition compared to [17] of which the % inhibition were mostly above 20% with

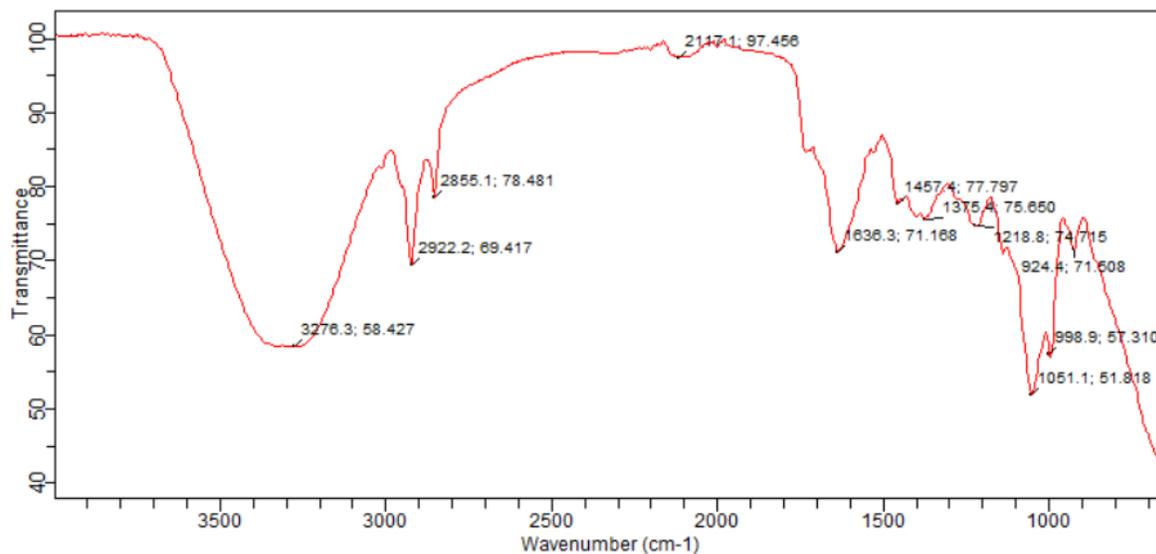
concentrations of 0.0025, 0.005, 0.01, 0.02 and 0.04 mg/ml of the crude extract.

## FTIR Analysis

### Extract A



### Extract B



The peaks as seen in the FT-IR spectra of extract A and B indicates that *Mucuna pruriens* extract

contains compounds like amine, phosphine, amides, aliphatic and aromatic nitro compounds.

The FTIR spectra of Extract A and Extract B provide valuable insights into the chemical composition of *Mucuna pruriens* extract. FTIR spectroscopy is a widely used analytical technique that identifies functional groups in a sample by measuring the absorption of infrared radiation at different wavelengths. Each peak in the spectra corresponds to a specific vibrational mode of a chemical bond, allowing researchers to determine the types of compounds present.

In both extracts, a broad absorption band observed between 3200–3400  $\text{cm}^{-1}$  suggests the presence of amine ( $-\text{NH}_2$ ) functional groups. These groups are commonly found in proteins, alkaloids, and other nitrogen-containing compounds, which are known to contribute to the plant's medicinal properties. Additionally, peaks appearing in the 2200–2300  $\text{cm}^{-1}$  range indicate the presence of phosphine ( $-\text{P}-\text{H}$ ,  $\text{P}=\text{O}$ ) stretching vibrations, suggesting that phosphorus-based metabolites are present in the extract.

The spectra also reveal significant peaks between 1650–1700  $\text{cm}^{-1}$ , which are characteristic of amide ( $\text{C}=\text{O}$ ) stretching. Amides are often associated with proteins and peptides, indicating that the extract may contain bioactive compounds derived from these sources. Another set of peaks in the 2800–3000  $\text{cm}^{-1}$  range corresponds to C-H stretching in aliphatic hydrocarbons, confirming the presence of organic molecules with long-chain carbon structures.

A key observation in both spectra is the presence of peaks around 1350–1550  $\text{cm}^{-1}$ , which are indicative of aromatic nitro ( $\text{NO}_2$ ) compounds. These functional groups are commonly found in various secondary metabolites and may contribute to the extracts' biological activity, such as its antioxidant and anti-inflammatory effects.

While Extract A and Extract B share similar functional groups, slight differences in peak intensities and positions suggest variations in concentration or extraction conditions. These differences may arise due to factors such as solvent polarity, extraction temperature, or purification techniques.

### Conclusion

In conclusion the results of this study indicate significant differences in the phytochemical composition and antioxidant activity among various accessions of *Mucuna pruriens*. Accessions from extract A shows higher antioxidant activities than that of B, as measured by DPPH and nitric oxide scavenging assays. These findings suggest that genetic variation plays a critical role in determining the medicinal potential of *Mucuna pruriens*, highlighting the importance of selecting specific accessions for therapeutic applications.

Further isolation and structural characterization of individual phytochemicals responsible for the antioxidant activity should be pursued. Techniques such as HPLC and NMR could be employed to identify.

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