Medicinal Values Of The Leaf And Husk Aqueous Extract Of Velvet Beans (*Mucuna flagellipes*) HOOK. F.

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Abstract

The aim of this study was to investigate the phytochemical (qualitative and quantitative) constituents of the leaves and husks of Mucuna flagellipes for the treatment of different ailments. Standard procedures were used to assess the phytochemical screening of Mucuna flagellipes leaves and husks. The presence of nine qualitative phytochemical substances was found in the leaves and husk of the plant under investigation, albeit in varying amounts. The leaf screening results show that alkaloid and flavonoid concentrations were high (+), tannin, terpenoid, and saponin concentrations were low (+), and steroids and resin were not found (-). The husk of the plant under study was also subjected to qualitative screening. The discovery identified nine plant compounds, but in varying concentrations. Terpenoid and resin were absent, but flavonoid, steroid, and cardiac glycoside were (+), and alkaloid, tannin, and saponin were plentiful (++). The quantitative analysis of the plant's leaves reveals that cyanogenic glycoside is the most abundant chemical, accounting for 9.13 percent, followed by flavonoid at 6.48 percent. Quantitative screening of the plant's husk reveals that haemaglutinin is the most abundant chemical, accounting for 40.47 percent of the total, followed by cyanogenic glycoside (9.13 percent), and phytate and oxyalate, which account for 0.02 percent and 0.17 percent, respectively. Finally, the findings demonstrate that the plant under investigation has a variety of chemical components that have an impact on both modern and traditional medical practices.

Key Word: Aqueous extract-phytoconstituents, cyto-toxicity, Mucuna flagellipes.

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I. Introduction

The use of plants in the management and treatment of diseases started with life. In more recent years, with considerable research, it has been found that many plants do indeed have medicinal values ^{1, 2}. *Mucuna flagellipse* is one of these medicinal plants. Mucuna is a genus of roughly 100 recognized species of climbing vines and shrubs belonging to the fabaceae family that can be found globally in tropical forests ². *Mucuna flagellipes*, also known as "agbara" in Igbo areas of eastern Nigeria, is a powerful forest climber. Its seeds are used as thickeners in soups. *Mucuna flagellipes* leaves are used to treat a variety of ailments, including diabetes, cancer, arthritis, dysentery, cardiovascular disease, and more. Squeezing the leaves of *Mucuna flagellipes* in water and taking them orally to increase blood levels is a typical practice among rural residents in Eastern Nigeria.

Therapeutic plants are important species of plants that are used for medicinal purposes to alleviate ailments and improve human health, according to traditional medicinal practices and modern scientific

investigations. These plants are thought to be rich in chemicals that can be exploited in medication synthesis and manufacture¹. Phytoconstituents are a group of chemical compounds found in plants³. Phytoconstituents help plants by performing secondary activities such as assisting plant development, protecting plants by activities defense mechanisms, and providing color, taste, and flavor⁴. Natural products and their derivatives have fewer adverse effects and are more effective than synthetic alternatives ⁵. Flavonoids, terpenoids, alkaloids, oxalate, and other plant-derived components are found in a variety of plant parts, including the root, stem, leaf, shoot, and bark, and perform biological functions that increase therapeutic effects such as anti-carcinogenic, antimutagenic, anti-inflammatory, and anti-oxidant qualities. ⁵. The scientific process of evaluating, studying, extracting, experimenting, and therefore identifying different classes of phytoconstituents present in various portions of the plant for the discovery of medications, with the active components being taken for further investigation and research. The method was qualitative, and it was referred to as phytochemical screening. The findings of the study could lead to the development of effective medications to treat a variety of ailments. A huge number of researchers from all around the world have looked into the impact of plant extracts on microorganisms. For 80 percent of the world's population, their use in traditional health cures is the most popular. Multiple drug resistance has emerged in many bacteria in recent years, prompting the quest for novel antibiotic sources. Plant secondary metabolites in the form of phytochemicals, vitamins, and minerals are thought to be the dietary ingredients contributing to these plant materials' protective effects.

Saponins, tannins, flavonoids, alkanoids, terpenoids, steroids, and other biologically active chemical components found in medicinal and aromatic plants have restorative qualities ⁵. These plants are also said to contain additional compounds that help to reduce the effects of the medicinal substances ⁴. This diet, which is high in fruits and vegetables, protects against a number of ailments, especially cardiovascular disorders ⁶ In Nigeria, there are many uncommon and useful plants from which essential pharmaceuticals could be derived, as well as agents that could be used as starting materials for the prospective synthesis of certain useful drugs ².

This study was therefore designed to investigate the phytochemical (qualitative and quantitative) constituents of the leaves and husks of *Mucuna flagellipes* for the treatment of different ailments.

II. Materials And Methods

The samples were collected from a forest in Izzi, Ebonyi Local Government Area, Ebonyi State, Nigeria in July 2018. The plant was identified and authenticated as *Mucuna flagellipes* Hook. F. at Nnamdi Azikiwe University, Botany Department in Awka with the authentication number of N.A.U.H/No 15. The plant was dried in the oven, ground into a fine powder with a mortar and pestle, and then sieved through a 20mm mesh sieve. After that, the samples were placed in polyethylene bags and maintained in desiccators until they were analyzed.

Qualitative Analysis

The leaves and husks of *Mucuna flagellipes* were analyzed phytochemically using standard methods. The methods used are:

Test for alkaloids: Meyer's test ⁷: 1 mL of Meyer's reagent was added to 2 mL of extract. Alkaloids were detected by the presence of a pale yellow precipitate.

Test for flavonoids ⁷: The Shinoda test: a small magnesium chunk was warmed in 4 mL of extract solution, 1.5 mL of 50% methanol solution was mixed, and 5-6 drops of concentrated HCl were added, resulting in a red coloration of the flavonoids.

Test for steroids⁷: A drop of conc. H2 SO4 was added to 1 g of plant extract diluted in a few drops of acetic acid. The presence of steroids was detected by the presence of a green color.

Test for tannin⁷: 3-4 drops of 10% FeCl3 were added to the diluted extract, resulting in a blue color for gallic tannins and a green color for catechol tannins.

Test for Protein⁸: In a test tube, 2 drops of million reagents were added to a small amount of the filtrate. A white precipitate indicated the presence of protein.

Test for Resins ⁷: About 0.2g of powdered components was extracted with 15ml of 90% ethanol. The alcoholic extract was mixed with 20ml of distilled water in a beaker. After the formation of a precipitate, the presence of resins was not detected.

Test for saponins⁹: In 20 mL of distilled water, 2 g of powdered sample was cooked. 10 mL of filtrate and 5 mL of distilled water were vigorously quivered. Saponins were detected by the development of foaming.

Test for cardiac glycosides.⁷: 5 mL of plant extract was treated with 2 mL of glacial acetic acid and one drop of FeCl3 solution in a total volume of 5 mL. A violet ring or a greenish ring may form around it, indicating the presence of cardiac glycosides.

Test for terpenoids. ⁹. 2 mL of chloroform and 3 mL of conc. H2 SO4 were added to 0.2 g of each sample. The presence of terpenoids was indicated by the reddish-brown coloring.

Quantitative Phytochemical Analysis

Determination of Oxalate (by Titration Method)¹⁰.

There are three primary processes for this test: digestion, oxalate precipitation, and permanganate titration. **Digestion**

(i) In a 250 ml volumetric flask, 2 g of sample was suspended in 190 ml of distilled water.

(ii) 10 mL of 6 M HCl was added, and the suspension was digested for 1 hour at 1000 °C.

It was allowed to cool, and then was created to a volume of up to 250 ml before filtering.

Precipitation of oxalate

Four drops of methyl red indicator were applied to duplicate portions of 125ml of the filtrate in beakers. Following that, drop by drop, NH4OH solution was added until the test solution changed from salmon pink to a pale yellow color (pH4-4.5). Each portion was then heated to 900 °C, cooled, and filtered to remove the ferrous ion precipitate. The filtrate was heated to 900 °C again, and 10 mL of a 5% CaCl2 solution was added, stirring frequently. After heating, it was cooled and kept at 250°C overnight. The fluid was then centrifuged for 5 minutes at 2500rpm. After decanting the supernatant, the precipitate was completely dissolved in 10 mL of 20% (v/v) H2S04 solution.

Titration of Permanganate

The total filtration resulting from the digestion of 2g of flour reaches 300ml at this point. For a light pink color that lasts 30 seconds, 125ml aliquots of the filtrate were heated to near boiling, and then titrated against a 0.05M standardized KMNO4 solution to produce a light pink color.

Five grams (5g) of the sample were weighed into a 250ml beaker, and then 200ml of 20% acetic acid in ethanol was added, covered, and left to stand at 250 °C for four hours. This was filtered using filter paper, and the filtrate was concentrated to one quarter of its original volume using a water bath (Memmert). Drop by drop, concentrated ammonium hydroxide was added to the extract until it precipitated completely. The precipitate was collected and rinsed with dilute NH4OH once the entire solution had settled (1 percent ammonia solution). Then filtered with filter paper that has been pre-weighed. The alkaloid residue on the filter paper was dried in an oven (precision electro-thermal model BNP 9052 England) at 800 degrees. The alkaloid content of the sample was calculated and expressed as a percentage of the total weight.

Determination of flavonoid; 10g of plant material was extracted several times with 100ml of 80% aqueous methanol at room temperature. Whatmann filter paper No. 42 was used to filter the entire solution (125 mm). The filtrate was then transferred to a crucible and dried over a water bath before being weighed at a consistent weight of 10 .

Determination of saponin; ¹⁰ · 5g of the material was immersed in 20 percent acetic acid in ethanol for 24 hours in a 500 °C water bath. This was filtered, and the extract was concentrated to one-quarter of its original volume using a water bath. Drops of concentrated NH4OH were added to the extract until it was completely precipitated. The entire solution was allowed to settle before the precipitate was collected and weighed using filtration. The saponin content was weighed and a percentage was calculated.

Determination of Cardiac Glycoside: 1 mL of 3,5-DNS (Dinitro Salicylic acid) solution in methanol and 1 mL of 5% aqueous NaOH were added to 1 ml of extract. The boiled sample was filtered after boiling for 2 minutes (until a brick-red precipitate was visible). Prior to filtering, the weight of the filter paper was measured. The filter paper with the absorbed residue was dried in an oven at 500 degrees Fahrenheit until it was completely dry, and the weight of the filter paper with residue was recorded.

Determination of Tanins¹⁰: 100ml of petroleum ether was poured into a conical flask with 20g of crushed material and covered for 24 hours. After that, the sample was filtered and set aside for 15 minutes to allow the petroleum ether to evaporate. It was then re-extracted by soaking it for 4 hours in 100ml of 10% acetic acid in ethanol. After that, the sample was filtered, and the filtrates were collected.

Determination of alkaloids ¹⁰; 25ml of NH4OH was added to the filtrate. The alkaloids were cooked using an electric hot plate to eliminate any remaining NH4OH. The remaining volume was found to be 33 milliliters. We took 5ml of this and mixed it with 20ml of ethanol. It was titrated with 0.1 M NaOH until a pink end point was obtained, using phenolphthalein as an indicator.

Determination of Phytate: Three 250ml conical flasks were weighed with 0.2g of each of the different processed Mucuna. Each sample was soaked in 100ml of 2 percent concentrated HCL for 3 hours prior to filtering. Each sample was laced with 50ml of each filtrate in a 250ml beaker and 100ml of distilled water. Ten milliliters of 0.3 percent ammonium thiocynate solution were used as an indicator, and the solution was titrated with standard iron (III) chloride solution (0.00195g iron per 1 ml).

A dry sample of 2 g was boiled with 50 ml of ether for the extraction of the phenolic compound for 15 minutes. Then 5 ml of the extract was pipette into a 50-ml flask, and 10 ml of distilled water was added. For colour development, 2 ml of ammonium hydroxide solution and 5 ml of concentrated amyl alcohol were also added to react for 30 minutes. Also, 2 ml of the samples were added to a test tube, and 1 ml of ferric chloride was added as well. The development of a greenish-brown precipitate indicated the presence of phenols.

Determination of Heamaglutinin was determined by adding 20 ml of 0.9 percent NaCL to two grams of each sample and rapidly agitating the suspension for one minute. After allowing the supernatant to settle for 1 hour, the material was centrifuged at 2000 rpm for 10 minutes before being filtered. Each of the supernatants was collected and utilized as a crude agglutination extract. At 420 nm, the absorbance was measured.

Determination of Steroid ¹⁰: The alkaline dry ash technique was used to identify steroids by placing 0.5g of each sample into nickel crucibles. 1ml of a solution containing 0.5M sodium hydroxide and 0.1M potassium nitrate was added to the samples, stirred, and dried. Following that, the containers were wrapped in aluminum foil and placed in a muffle furnace. The samples were heated to 2500°C for 15 minutes, then to 4800°C for another 15 minutes, before being raised to 5800°C. They were kept at this temperature for 3 hours before being allowed to cool down to room temperature. The resulting ash was then removed with 2ml of 1.0ml sodium hydroxide, made up of double distilled water. The solution was collected for 20 minutes at 2500g in polypropylene centrifuge tubes, and the supernatant solution was collected for iodine analysis. The biological matrix was damaged by the heat. Potassium nitrate was employed to accelerate the oxidation of the organic waste, while sodium hydroxide was utilized to maintain the steroid in a nonvolatile form. Then, at 350°C, 1 mL of sample solution and 1 mL of arsenic reagent were added to the cuvette. The addition of 1 mL of ceric reagent kicked off their action. The change in absorbance at 420 nm was used to compute the initial reaction rate.

Determination of Cynogenic Glycoside¹⁰:

(i) In an 800ml Kjeldahl flask, 100ml of H2O was added, and 10–20 powdered samples were allowed to pass through the N0.20 sieve.

(ii) And was allowed to macerate for 2 hours at room temperature.

(iii) The distillate was collected in 20 mL of 0.02N AgNO3 acidified with 1 mL of HNO3 after steam distilling 100 mL of H2O.Before distillation; the tips of the condenser were dipped below the surface of the liquid in the receiver.

(iv) After passing 150ml through the gooch wash receiver and gooch with little H2O, the distillate was filtered.(v) Using the Fe alum indicator, excess AgNO3 was titrated with filtrate and washed with 0.02N KCN. 1.58mg HCN in 1ml of 0.02N AgNO3.

Parameters	Presence (concentration/amount)
Alkaloid	++
Flavonoid	++
Tannin	+
Protein	+
Terpenoid	+
Saponin	+
Steroid	
Resin	
Cardiac glycoside	+

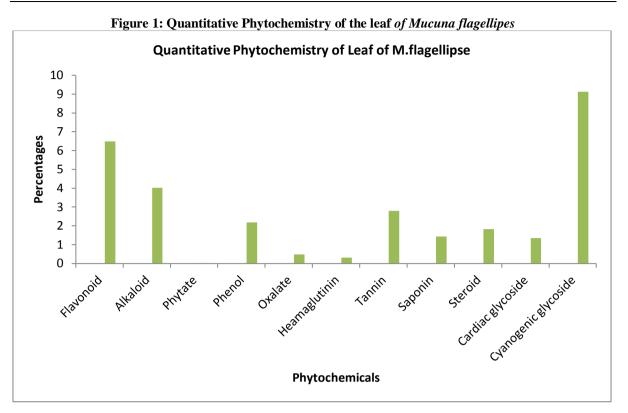
III. Results And Discussion Table 1: Qualitative phytochemistry of the leaf of *Mucuna flagellipes*

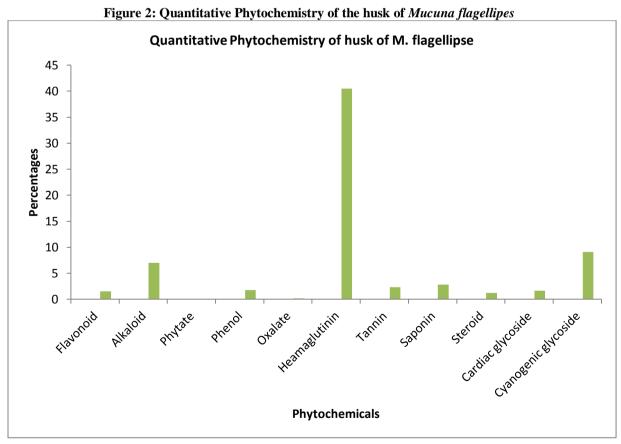
Keys: ++ = present in high amount, + = present in trace amount, - = absent

Table 2: Qualitative	Phytochemistry	of the husk	of Mucuna flagellip	es
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Parameters	Presence(concentration/amount)	
Alkaloid	++	
Flavonoid	+	
Tannin	++	
Protein	+	
Terpenoid	-	
Saponin	++	
Steroid	+	
Resin	-	
Cardiac glycoside	+	

Keys: ++ = present in high amount, + = present in trace amount, - = absent





IV. Discussion

Using established procedures, the leaves and husk of *Mucuna flagellipes* were evaluated qualitatively and quantitatively for phytochemicals.

The qualitative phytochemical examination of *M. flagellipes* leaf is shown in Table 1. The qualitative screening revealed the presence of nine essential plant chemicals: alkaloids, flavonoids, tannins, resins, saponins, steroids, terpenoids, cardiac glycosides, and proteins. Alkaloid and flavonoid concentrations were high +; tannin, protein, terpenoid, saponin, and cardiac glycoside concentrations were low +; and steroids and resin were absent -. Unlike the leaves of other therapeutic plants, the leaves of *M. flagellipes* have a considerably high concentration of alkaloids and flavonoids. This characteristic endows the plant with powerful medical properties, such as lowering the risk of cardiovascular disease and cancer ¹¹. The presence of phytochemical classes like flavonoids, alkaloids, and tannin had a cytotoxic effect ¹². Because of their color and fragrance, flavonoids were thought to have anti-cancer properties. Saponin is also known for its cholesterol-lowering benefits, as well as its cytotoxic, antibacterial, and antiviral characteristics ¹³. Tannin has an anticancer property that can be seen in its growth inhibitory action. ^{14, 15}. Plants with high flavonoid content could be effective as antibacterial agents, even in their most basic forms. According to ¹⁶, the presence of a significant amount of alkaloid provides the plant with a unique preventive feature against animals, particularly herbivores and bacterial attacks; that is, the presence of alkaloid provides a significant defense mechanism against animal attacks. The phytochemical contents of *M. flagellipes* husk were studied gualitatively. Phytochemical compound screening on the husk, as opposed to the leaf, reveals nine key plant phytochemicals: alkaloid, flavonoid, tannanin, resin, saponin, steroid, cardiac glycoside, terpernoid, and protein. Alkaloid, tannin, and saponin are plentiful ++, while flavonoid, protein, steroid, and cardiac glycoside are present in trace amounts +, and terpenoid and resin are absent -. The presence of a significant concentration of flavonoids in both cases shows that the plant could be employed to treat oxidative cell damage ¹⁷. The findings are consistent with the past study of ¹⁶. Who discovered that the strong alkaloid present in the plant husk made it extensively beneficial as a medication for many centuries. Mucuna flagellipes husk contains the flavonoid, terpenoid, saponin, which is an antioxidant, anti-inflammatory, and antibacterial agent ¹⁸. They also aid in the reduction of blood pressure and the prevention of heart disease.

The leaves of *M. flagellipes* contained eleven phytochemicals, of which cyanogenic glycosides were determined to have the greatest percentage composition or concentration of these compounds at 9.13%. This was found to be identical to previous works. Furthermore, this discovery shows that this plant may be a source of natural bioactive chemicals that have previously been used in both modern and traditional medical treatments. According to previous reports, Mucuna flagellipes contains an alkaloid that has been used as a medicine by humans for many years. Saponin has also been reported to have therapeutic effects ¹⁹. The plant has a high percentage of cyanogenic glycosides, which gives it therapeutic properties. Flavonoids were discovered to be second to cyanogenic glycosides in terms of proportion, at 6.48%. Unlike cyanogenic glycosides, flavonoids have a strong anti-inflammatory, anti-oxidant, and anti-microbial effect on the plant. It also provides the plant with a robust defense against herbivores and viruses. Phytate, on the other hand, had the lowest percent at 0.023%, while heamaglutinin came in second with 0.31%. The rest is summarized in figure 1. Because of its ability to mix with extracellular and soluble proteins, as well as the bacteria cell wall, the presence of steroids, alkaloids, and cardiac glycosides gives the plant powerful therapeutic effects on the biological system against bacterial and a wide range of other microbial infections¹⁹. On the husk of Mucuna flagellipes, quantitative phytochemical screening was performed. Unlike the leaf, the plant's husk contains compounds as well. As shown in Figure 2, the presence of phytate, haemaglutinin, alkaloid, cardiac glycoside, oxalate, saponin, tannins, and cyanogenic glycosides were discovered. However, heamaglutinin was discovered to have the largest percentage, 40.477% by composition, followed by cyanogenic glycosides at 9.132%, with phytate having the lowest proportion of 0.023% and oxylate following at 0.178%. The significant concentration of heamaglutinin and cyanogenic glycoside in the husk is consistent with ²⁰ findings; the presence of heamaglutinin in the plant makes it an excellent cure for congestive heart failure.

V. Conclusions

In the last few years, with considerable research, it has been found that many plants are a rich source of phytochemicals that can be used to treat a variety of diseases. According to the findings, *Mucuna flagellipse* contains a considerable amount of tannin, terpenoid, flavonoid, steroid, alkaloid, cardiac glycoside, saponin, and other compounds, according to qualitative screening. Quantitative screening also revealed the presence of phytate, haemaglutinin, alkaloid, cardiac glycoside, oxalate, saponin, tanins, and cyanogenic glycoside. The identification of numerous phytoconstituents found in plant extracts was aided by phytochemical screening. The aqueous extract's phytochemicals had a minor inhibitory effect on the growth. This research aided in determining the cytotoxic effect of phytoconstituents found in plant extracts on live cells. The findings,

therefore, laid a solid foundation for further research into the isolation and characterization of phytoconstituents from *Mucuna flagellipes* and other plants for drug development.

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

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