

Full Length Research Paper

## Nutritional composition, phytochemicals and microbiological quality of the legume, *Mucuna pruriens*

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The aim of this study was to evaluate the nutritional and phytochemical compositions and microbiological quality of seeds of the legume *Mucuna pruriens* (MP) grown in northeastern Brazil. MP flour and extract were produced and evaluated for proximate, mineral, and phytochemical compositions, fatty acid profile, and microbiological quality. MP flour and seed extract showed 43.12 and 43.4% of protein, 7 and 7.6% lipid matter, 37.19 and 33.33% starch, and 5.64 and 2.36% fiber ( $p < 0.05$ ), respectively. Abundant minerals found were in both the extract as flour, such as potassium (635 and 679 mg/g), iron (79 and 158 mg/g), and phosphorus (83 and 93 mg/g). Flavonoids, steroids, and saponins were detected. The main fatty acids found were myristic, palmitic, oleic and linoleic acids. Microbiological evaluation did not indicate the presence of pathogenic or spoilage microorganisms. MP produced in Northeastern Brazil is an alternative source of carbohydrate, fiber, protein, essential fatty acids, minerals, saponins and flavonoids, which may encourage its potential consumption and marketing.

**Key words:** Mineral composition, microbiological quality, phytochemicals, legume.

### INTRODUCTION

*Mucuna pruriens* (MP) is a member of the *Fabaceae* family, composed of approximately 650 genera and 2,000 species. This leguminous plant grows pods about 12 cm long that contain about 7 seeds of varied coloration from beige to brown and black, and also striped ones. This legume is also known as “velvet bean, lion bean, nescafe, and cowage”, among others. It is a leguminous plant originating in India and cultivated in Sri Lanka,

Malaysia, southeastern Asia, and in tropical regions of Central and South America (Hammerton, 2003). MP is largely grown in northeastern Brazil, where it is used as “green manure” due to its capacity to fix nitrogen for the soil or as a supplement for animal feed and in a minor volume used as food source or in the traditional medicine (Raina et al., 2012), being an important source of income for many farmers and consequently for the economy.

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Several studies have highlighted some medicinal properties of this plant and its use has been proposed especially in the treatment of Parkinson's disease (Tharakan et al., 2007). Studies have shown hypoglycemic (Majekodunmi et al., 2011), anti-inflammatory and diuretic (Bala et al., 2011), hypolipidemic (Eze et al., 2012), and aphrodisiac properties (Suresh et al., 2009), and other studies have shown its androgenic (Muthu and Krishnamoorthy, 2011; Ahmad et al., 2012) and estrogenic activities (Shahaji and Parnu, 2011).

Studies analyzing the composition of MP grown in India have shown high percentages of protein (28.23%) and carbohydrate (60.03%) and a low content of lipids (2.69%), and a mineral composition of good nutritional quality (Siddhuraju and Becker, 2003). Regarding the phytochemical composition, Manalisha and Chandra (2012) detected the presence of proteins, carbohydrates, resins, flavonoids, alkaloids, sterols, phenols, and glycosides in MP seeds after washing and drying in the shade. antinutritional components such as tannins, phytic acid, enzyme inhibitors, trypsin, saponins, lectins, as well as hemagglutinin activity and high levels of L-dopa (Vadivel and Pugalenthi, 2010; Nwaoguikpe et al., 2011; Ahmad et al., 2012), which require treatments such as peeling, cooking, irradiation, and others to be inactivated (Betancur-Ancona et al., 2008). Studies on the nutritional composition of MP have characterized this leguminous plant grown mainly in India; however, it is known that the nutritional composition of foods is influenced by climatic variations, soil composition, temperature, surrounding vegetation, and rainfall volume (Luizão, 2007). Accordingly, the aim of this study was to evaluate the nutritional and phytochemical composition and microbiological quality of *M. pruriens* grown in northeastern Brazil, with the purpose of encouraging the human consumption of this legume.

## MATERIALS AND METHODS

### Preparation of *M. pruriens* flour and seed extract

MP seeds (4 kg) were obtained in the local market of the city of João Pessoa - PB. To prepare the flour, seeds were washed twice in tap water, subsequently dried with absorbent paper at room temperature (26°C), crushed in grinder, and sieved to obtain a fine powder, which was kept in a drying oven at 50°C for 100 h. MP extract was obtained by hydroalcoholic extraction by adding 200 mL distilled water and 200 mL alcohol in 100 g of dry flour. This mixture was homogenized for 72 h and sieved to remove solid residues, being then submitted to water bath at 45°C for 24 h to form a creamy material of beige-brown coloration. The samples were frozen and then lyophilized at -48°C with pressure of 130 mmHg in lyophilizer model Liotop, L101 (São Carlos, São Paulo, Brazil) for 24 h (Muthu and Krishnamoorthy, 2011; Suresh et al., 2009).

### Nutritional composition of *M. pruriens* flour and extract

#### Proximate composition

All chemical composition analyses were performed in triplicate.

Moisture analysis was performed by drying in oven at 105°C, and ash by incineration in muffle furnace at 550°C; the protein percentage was obtained using the Kjeldhal method, the Soxhlet methodology was used for the extraction and determination of total lipids and the amount of carbohydrate was obtained by determining the starch and fiber contents (AOAC, 2002).

#### Fatty acid profile

The lipid extract was initially obtained by the method of Folch et al. (1957) and this extract was used to obtain methyl esters by esterification (Hartman and Lago, 1973). The identification and quantification of methyl esters were performed on gas chromatograph model GC-Master (Ciola & Gregori Ltda, São Paulo, Brazil) with flame ionization detector. The chromatographic conditions were: fused silica polyethylene glycol column (Carbowax 20 M) with 30 m in length, 0.53 mm in diameter and 0.25 µm of film thickness in the stationary phase. The temperatures used were: vaporizer: 150°C, detector 200°C and oven programming: 80°C for 3 min, 10°C/min up to 120°C, remaining at 200°C for 6 min and later decreasing 3°C/min up to 180°C. The mobile phase was hydrogen at flow rate of 5 mL/min. The injected volume was 1 µL, with a split ratio of 1:25. The characterization of fatty acids was performed by comparison of mass spectra obtained with standards that were also injected into the chromatograph.

#### Mineral composition

The analysis of minerals present in MP was performed by energy-dispersive X-ray fluorescence spectrometry (Teixeira et al., 2012). Initially, the samples were dried at 75°C for 1 h in watch glasses. Then, the samples were placed in appropriate sample holders of the Energy Dispersive X-ray Spectrometer device (model EDX-720, Japan), being sealed on both ends with thin polypropylene film and at one end a hole was opened to prevent extrusion of samples when activating the vacuum to be then analyzed by EDX.

#### Phytochemical screening

Phytochemical screening of MP flour and extract to detect the presence of flavonoids, saponins, tannins, sterols, and alkaloids was performed according to methodology proposed by Matos (2009). For the detection of flavonoids, chloroform was added for the separation of layers. Then, methanol was added and evaporation was conducted in rotary evaporator. The dissolved material was distributed into two test tubes, where 10% HCl solution and magnesium tape were added, leaving to react until the tape disappeared and observing the appearance of pink color (positive test). In the second tube, acetone, oxalic acid and boric acid were added. The mixture was dried in water bath, ethyl ether was added and the fluorescence was observed under UV spectrophotometer at wavelength of 510 nm (Biospectro model SP-220/Brazil).

Qualitative analysis of saponin was performed using the foam test, which consisted of dissolving the sample in water in a test tube, stirring for 1 min and leaving to rest for 10 min. After the rest, the foam disappeared, which characterizes negative test for this phytochemical. The test for tannins was carried out with evaporation of samples to complete drying, and filtering with cotton funnel. The filtrate was distributed in six test tubes, the first three were tested with 0.5% gelatin and the others with 2% iron chloride at different concentrations (0.5, 1.0 and 2.0 mL), observing the formation of precipitates, which indicates the presence of tannins. For the analysis of steroids, samples were submitted to evaporation until complete drying, adding chloroform for dissolution. Then, the samples were divided into three test tubes with 0.12, 0.25 and 0.5

**Table 1.** Proximate composition of *Mucuna pruriens* flour and seed extract.

Proximate composition	Flour	Extract
Protein (%)	43.12±0.19	43.40±0.07
Starch (%)	37.19±0.4	33.33±1.6
Crude fiber (%)	5.64±0.3	2.36±0.06
Lipids (%)	7.00±0.5	7.60±1.4
Moisture (%)	8.20±0.04	9.90±0.02
Ash (%)	3.10±0.04	2.90±0.02
Total energy (Kcal)	384.24±2.46	375.32±7.06

Means ± standard error with different letters in the same row differ by the Student t test ( $p < 0.05$ ).

mL. Chloroform, acetic anhydride and concentrated sulfuric acid were added to each tube. The results were observed according to the color standard, where the color blue indicated the presence of steroids.

Qualitative analysis of alkaloids was performed by evaporation of the alcoholic extract to complete drying, and alkalinizing the medium with 1% sodium hydroxide. Distilled water with chloroform was added; the system was filtered with cotton filter and the extract was separated from the chloroform layer. Then, 1% hydrochloric acid was added to the chloroform layer, stirred and allowed to settle until it becomes clear. Subsequently, it was distributed into four test tubes of 1 mL, followed by testing with the following reagents: Boucharadat, Mayer, Dragendorff and silicotungstic acid, observing the formation of precipitate if positive.

#### Microbiological evaluation

The prior preparation of MP extract and flour samples for microbiological analysis consisted of homogenizing the products with 0.1% of buffered peptone water (BPW), where  $10^{-1}$ ,  $10^{-2}$  and  $10^{-3}$  dilutions were prepared. The microbiological quality of MP flour and extract was assessed by counts of coliforms at 35°C, standard plate count of mesophilic aerobic bacteria, yeasts and molds, *Staphylococcus aureus* and *Salmonella* spp. (Vanderzant and Splittstoesser, 1992). All analyses were performed in triplicate.

#### Statistical analysis

The results regarding the proximate composition were submitted to the Student's t-test at 5% significance level ( $p < 0.05$ ) using the GraphPad InStat software version 3.0.1 (GraphPad InStat, San Diego, CA, USA).

## RESULTS AND DISCUSSION

### Proximate composition

Difference ( $p < 0.05$ ) in the percentages of moisture, ash, starch and fiber was observed between MP flour and extract, which is justified by the different processing techniques that the samples were submitted (Table 1). Both MP flour and extract have high protein content (from 43.12 to 43.40%) and carbohydrates as starch (from 37.19 to 33.33%) and significant ash content (from 2.90 to 3.10%). By comparing with results obtained by

Josephine and Janardhanan (1992), the present study obtained higher protein percentage (34.4%), similar lipid percentage (7.7%) and lower carbohydrate percentage (45.9%). In relation to results obtained by Siddhuraju et al. (1996), results obtained in this research were higher for lipids and protein (6.7 and 31.5%, respectively) and lower for carbohydrates (52.5%). Nwaoguie et al. (2011) analyzed raw MP flour and found 28.2% proteins, 2.7% lipids and 60% carbohydrates and when raw MP flour was submitted to water logging followed by cooking, only reduction in the protein content was observed. The fiber results of MP flour and extract are lower than the fiber percentage of cowpea ( $19.4 \pm 1.07$ ) determined by Frota et al. (2008); however, MP flour can be considered as a source of fibers, since it exceeded the minimum 3 g fibers/100 g of food (Brasil, 1998). The grinding and sifting processes that the MP grain was submitted to may be responsible for this decrease, since some amount of bark was removed during processing. Moreover, the extraction process that the flour was submitted to may have removed additional amounts of bark, so there was difference between fiber values obtained between flour and extract. Variations in proximate composition reported in literature and compared with the present study may be justified by different varieties and cultivation conditions such as soil characteristics, temperature, humidity and rainfall volume (Freire Filho, 2011).

### Fatty acids profile

The main fatty acids detected in MP flour were myristic, palmitic and linoleic acids. MP extract showed higher percentages of myristic, palmitic, oleic and linoleic acids (Table 2). The present study detected higher concentrations of saturated fatty acids in MP flour and similar proportion of saturated and polyunsaturated fatty acids in the MP extract; similar results were found by Frota et al. (2008), who analyzed cowpea and found higher concentrations of linoleic acid followed by palmitic and stearic acids, so that the highest percentage obtained was of saturated fatty acids. Among the fatty acids of higher

**Table 2.** Fatty acids profile of *Mucuna pruriens* flour and seed extract.

Fatty acids	Flour	Extract
Myristic acid - C 14:0	52.05 ± 3.3	23.8 ± 3.3
Palmitic acid - C 16:0	14.15 ± 1.0	12.61 ± 0.4
Stearic acid - C18:0	4.82 ± 0.53	4.94 ± 0.2
Saturated fatty acids (%)	71.02	41.35
Oleic acid - C18:1	8.02 ± 0.6	17.07 ± 0.88
Monounsaturated fatty acids (%)	8.02	17.07
Linoleic acid - C 18:2	18.51 ± 1.5	37.46 ± 1.71
Linolenic acid - C 18:3	2.44 ± 0.17	4.11 ± 0.13
Polyunsaturated fatty acids (%)	20.95	41.57

Means ± standard error with different letters in the same row differ by the Student t test ( $p < 0.05$ ).

**Table 3.** Mineral composition of *Mucuna pruriens* flour and seed extract.

Mineral	Flour (mg/g)	Extract (mg/g)	*DRI (adults)
Potassium	635	679	4.700 mg
Iron	158	79	14 mg
Phosphorus	83	93	700 mg
Calcium	70	68	1.000 mg
Sulfur	38	48	N/A
Zinc	6	34	7 mg
Copper	3	34	900 µg
Magnesium	**nd	20	260 mg

\*Daily recommended intake (Brazil, 2005); \*\* nd = not detected; N/A = no recommended daily intake.

concentration in MP flour and extract, saturated fatty acids myristic and palmitic acids can raise total cholesterol and all its fractions, participating in the development of atherosclerosis in humans. Monounsaturated and polyunsaturated oleic and linoleic acids in MP extract have antiatherogenic action, since they reduce total and LDL-C cholesterol without reducing HDL-C. Furthermore, they increase vasodilation and reduce platelet aggregation (Nicod et al., 2015). Polyunsaturated fatty acids present in higher concentration in the MP extract are considered essential in humans because there is no endogenous biosynthesis of these nutrients. They are important for maintenance of cell membranes, brain function and nerve impulse transmission, and take part in the transfer of atmospheric oxygen to the blood plasma, hemoglobin synthesis and cell division (Martin et al., 2006). The American Heart Association (Stone et al., 2013) limits the intake of saturated fatty acids to 7% the total calories ingested per day, 20% for monounsaturated fatty acids and 10% for polyunsaturated fatty acids. Both samples have a high content of saturated fatty acids;

however, the MP extract shows fatty acids profile more beneficial to health than MP flour, since it presents greater proportion of monounsaturated and polyunsaturated fatty acids.

### Mineral composition

Mineral composition of MP showed amounts of potassium and iron in the flour, and potassium and phosphorus in the extract, and magnesium was only detected in the extract. The presence of toxic minerals under the Brazilian law was not detected (Brasil, 2005) (Table 3). By comparing the concentration of minerals present in MP flour or extract with values proposed by the DRI for adults (FAO/WHO/UNU, 2001), it was observed that MP flour or extract have concentrations higher than recommendation for the following minerals: iron, copper and zinc. Both samples had values greater than 10% the DRI values for minerals potassium and phosphorus. Magnesium was found only in the MP extract, representing

7.7% of the recommended daily intake. Only calcium showed concentration lower than 10% the DRI values for flour (7%) and extract (6.8%). Vegetables are natural sources of magnesium, iron, zinc, and copper (Mesquita et al., 2007). Vadivel and Janardhanan (2005) found higher potassium concentrations (83.51%), followed by calcium (30.45%) and magnesium (20.88%) and Bhat et al. (2008) found 24.5% of phosphorus, 6.65% of calcium and 1.94% of selenium in *M. pruriens*. Other vegetables such as soybeans, widely consumed by the Brazilian population, presents varying concentration of minerals such as calcium (275 mg/g), phosphorus (674 mg/g) and iron (53 mg/g) (Penha et al., 2007). Bean, a major Brazilian food source, shows 0.72 g potassium/100 g, 1.51 g calcium/100 g, 0.45 g phosphorus/100 g and 126.9 mg iron/kg (Mesquita et al., 2007).

Minerals play important and specific roles in the body. Potassium is important for cardiac patients who wish to reduce blood pressure through diet (Terker et al., 2015). Iron stands out for participating in oxidation and reduction reactions, respiratory and blood transport of oxygen and carbon dioxide and enzyme activation (Moura and Canniatti-Brazaca, 2006). Phosphorus participates in essential functions of the organism, since DNA and RNA are based on phosphate, a source of energy in the form of ATP, cell membrane component, formation of hydroxyapatite (a component of teeth and bones) and others (Rostami et al., 2014). The main function of calcium is to gain and maintain bone mass and density, transmembrane transport, nerve transmission and muscle contraction (Pedrosa and Castro, 2005).

### Phytochemical screening

There was no (-) alkaloids and tannins in MP, but a weak presence (+) of steroids and saponins and moderate presence (++) of flavonoids in MP. MP is widely used as aphrodisiac and stimulant of testosterone biosynthesis (Suresh et al., 2009; Muthu and Krishnamoorthy, 2011). It may be due to the high concentration of this steroid in the grain, since steroids stimulate the production of anabolic androgenic hormones; however, in this study, it was observed that steroids are weakly present in MP grown in northeastern Brazil. Still, Manalisha and Chandra (2012) evaluated the phytochemical composition of MP and observed absence of saponins, weak presence of resins, tannins, flavonoids, alkaloids, steroids, phenols and glycosides. Phytochemical analysis showed moderate presence of flavonoids in the MP extract. Flavonoids comprise a large group of polyphenolic compounds responsible for a variety of pharmacological activities related to its antioxidant activity, inducing protective enzyme systems in humans against infectious, degenerative, cardiovascular and age-related diseases. These phytochemicals are widely distributed in foods of plant origin and cannot be synthesized by humans or animals;

therefore, foods that are sources of these nutrients should be consumed (Kumar and Pandey, 2013). There is a great demand for the consumption of food or food supplements rich in flavonoids; however, it is difficult to identify an average dietary intake of this phytochemical, since there is a wide distribution in plant food sources (Izzi et al., 2012). Accordingly, there has been an increasing interest in the production of foods with therapeutic potential attributed to the presence of flavonoids (Kumar and Pandey, 2012), which can boost MP consumption after the conduction of *in vivo* studies evaluating the antioxidant capacity of the grain.

Saponins also play an important role due to their activities beneficial to health such as significant anticholesterolemic, antidiabetic and anticancer activity, as well as the ability to reduce plasma cholesterol concentration (Park et al., 2001). Despite many beneficial effects attributed to saponins, these substances are also considered anti-nutritional factors. These compounds are very common in foods of plant origin and are considered anti-nutritional substances because they reduce the nutritional value of foods, decreasing the digestibility and absorption of nutrients. When in excess, saponins can be toxic and lead to undesirable side effects (Santos, 2006). Antinutritional factors have led the scientific community to find ways to reduce their concentration or inactivate them, especially through the application of methods such as cooking, roasting or drying, considering that the majority of anti-nutritional factors are sensitive to high temperatures. In addition to these techniques, fermentation, alkaline solubilization, or isoelectric precipitation can also be used to reduce antinutritional factors that are insensitive to high temperatures (Betancur-Ancona et al., 2008).

### Microbiological evaluation

The tested samples showed no contamination by coliforms at 35°C and no *S. aureus* or *Salmonella* spp. were isolated. The MP extract showed mesophilic aerobic bacteria count of  $1.0 \times 10^3$  CFU/g, and this value is within standards established by the National Health Surveillance Agency (ANVISA) from  $1.0 \times 10^3$  to  $1.0 \times 10^5$  CFU/g (Brasil, 2001). Yeast and mold counts were  $2.34 \times 10^6$  CFU/g in the extract and  $3 \times 10^2$  CFU/g in the flour. The MP extract and flour obtained showed no contamination by pathogenic and spoilage microorganisms and can be considered suitable for human consumption.

### Conclusions

*M. pruriens* flour and extract are sources of carbohydrates, fiber and protein and exhibit considerable amounts of iron, potassium and phosphorus for humans.

Moreover, they present essential fatty acids, and the presence of flavonoids in this vegetable represents possible antioxidant activity. The microbiological evaluation indicated satisfactory sanitary quality of the product. These results may encourage the consumption and marketing of this vegetable in the domestic and international market as food source or ingredient in industrial formulations.

## Conflict of interests

The authors did not declare any conflict of interest.

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