



ISSN (E): 2277- 7695  
 ISSN (P): 2349-8242  
 NAAS Rating: 5.03  
 TPI 2018; 7(11): 273-278  
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 www.thepharmajournal.com  
 Received: 16-09-2018  
 Accepted: 17-10-2018

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## Nutraceutical and phytochemical investigation of *Mucuna pruriens* seed

**G Shanmugavel and G Krishnamoorthy**

**Abstract**

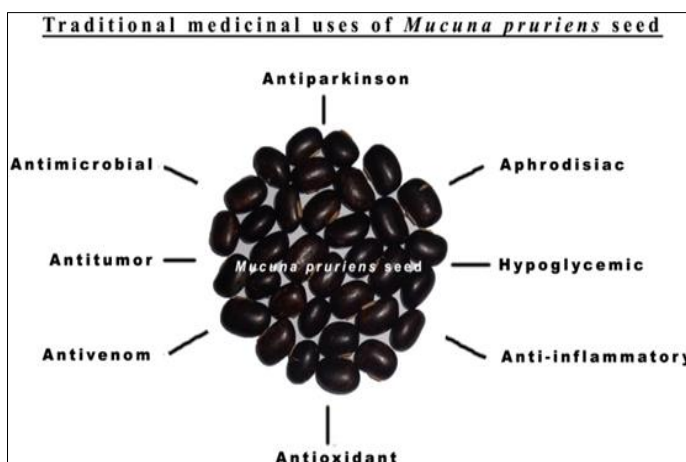
The aim of the present study was to scrutinize the presence of nutritional and phytochemicals contents of the *Mucuna pruriens* (MP) seed. MP flour was evaluated for proximate analysis. The phytochemical validation was performed by biochemical test, Fourier transform-infrared spectroscopy (FT-IR) and Gas Chromatograph-Mass Spectroscopy (GC-MS) in seed extract. The proximate analysis of MP flour showed relatively high carbohydrate value with good energy value. The qualitative phytocontent analysis indicated presence of alkaloids, flavonoids, glycosides, saponins, steroids, tannin and terpenoids. The infrared spectral data revealed the presence of characteristic functional groups of amines, amides, phosphine, fluorides, bromides, iodide, aliphatic and aromatic nitro compounds. The GCMS analysis showed the presence of alkaloid, carboxylic acid, terpenoid, polyphenol and other secondary metabolites. The present study was revealed that *Mucuna pruriens* seed as rich source of nutrient and phytochemicals, which could be utilized as good therapeutic agents.

**Keywords:** *Mucuna pruriens*, nutraceutical, phytochemical, FTIR spectroscopy, GCMS analysis

**1. Introduction**

Nature has been the best source for drug and its precursors. Natural products and their derivatives discovered years ago are still considered as useful therapeutics even today. The majority of drugs available today were discovered either from chance observations or from the screening of synthetic or natural product libraries. Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity. However, this approach is labor and time-intensive and researchers in the pharmaceutical industry are constantly developing methods with a view to increasing the efficiency of the drug discovery process <sup>[1]</sup>.

The continuing malnutrition with population growth in developing countries has promoted scientists to seek more esoteric plant species. In spite of an urgent need to meet the nutritional requirements of the ever increasing populations, the availability of cheap protein resources has remained relatively unexplored <sup>[2]</sup>. Knowledge of the chemical constituents of plants is desirable, not only for the discovery of therapeutic agents. It also valued the chemical substances in the medicinal plant that produce a definite therapeutic action on the human body. In addition, the knowledge of the chemical constituents of plants would further be helpful to find out the actual value of folkloric remedies.

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*Mucuna pruriens* Linn. (Fabaceae), commonly known as velvet bean, is a climbing annual legume, endemic in India and in other part of the tropical countries. About 15 species of *Mucuna* found in the forests and plains in India. All the parts of *Mucuna* contain valuable medicinal properties and there is a demand of *Mucuna* in Indian drug market. *M. pruriens* is the authentic and effective drug in the treatment of Parkinson's disease [3]. Seed posses antiparkinson, aphrodisiac, hypoglycemic, antivenom, antioxidant, antimicrobial, anti-inflammatory and antitumor activities [4]. Hence, there is great need to evaluate the safety of the phytochemicals present in the herbal plants. The present study deals with a qualitative analyze over the phytochemical constituents present in the methanolic extract of *M. pruriens* seed.

## 2. Materials and methods

### 2.1 Plant sample collection

The seeds of *M. pruriens* were collected from the local Ayurvedic shop in Pondicherry and the identity was confirmed by traditional and expects of plant biologist. Shade dried seeds were pulverized into fine powder using electric blender and stored in air-tight container for further use.

### 2.2 Preparation of Seed extract

50 g of the dried seed powder was soaked in 200 ml methanol (Analytical grade) in a conical flask and wrapped with aluminum foil for 72 hours with occasional shaking. After 72 hours, the extract was filtered using Whatman filter paper No: 1. then the solvent was removed from the extract by vacuum distillation. The concentrated seed extract (120 mg) was dried and stored at 4°C for phytochemical validation by biochemical test, Fourier transform-infrared spectroscopy (FT-IR) and Gas Chromatograph-Mass Spectroscopy (GC-MS).

### 2.3 Proximate analysis of *M. pruriens* flour

The proximate analysis of *M. pruriens* flour was performed by triplicate. Moisture analysis was performed by drying in oven at 105°C, until a constant weight was obtained. Ash was determined by incineration in muffle furnace at 600°C for about 2 hrs. The protein percentage was obtained by using Kjeldhal method [5]. The Soxhlet extraction methodology was used for the extraction and determination of lipid [6]. The crude fibre was determined by gravimetric method [7].

Carbohydrate percentage was calculated based on formula described by Müller and Tobin [8].

Carbohydrates (%) = 100 – [crude protein (%) + crude lipid (%) + crude fiber (%) + ash (%)].

Energy value was calculated using the formula of Osborne and Voogt [9].

Energy value (kcal/100g) = [crude protein (%) × 4] + [crude lipid (%) × 9] + (crude carbohydrates (%) × 4).

### 2.4 Phytochemical analysis

Phytochemical analysis of *M. pruriens* seed extract for qualitative detection of Alkaloids, flavonoids, glycosides, saponins, steroids, tannin and terpenoids was performed by following standard methods of Parekh and Chanda [10].

#### 2.4.1 Test for Alkaloids

The small portion extract were stored separately with a few drops of dilute hydrochloric acid and filtered. The filtrate was tested with various alkaloidal agents, such as Mayer's reagent

(cream precipitate) dragendorff's reagent (orange brown precipitate).

#### 2.4.2 Test for Flavonoids

5 ml of dilute ammonia solution was added to a portion of aqueous filtrate of plant extract followed by addition of concentrated H<sub>2</sub>SO<sub>4</sub>. A yellow coloration absorbed in extract indicated presence of flavonoids.

#### 2.4.3 Test for Glycosides

Small quantity of extract was hydrolysed with dilute hydrochloric acid for few hours in water bath and was subjected to Liebermann-Burchard's test to detect absence of different glycosides (pink to red colour indicates presence of glycosides).

#### 2.4.4 Test for Saponin

1 ml extract and 1 ml alcohol diluted with 20 ml distilled water and shake well about 15 minutes. 1 cm layer of foam indicated presence of saponin.

#### 2.4.5 Test of Steroids

2 ml acetic anhydride was added to 0.5 g methanolic extract with 2 ml H<sub>2</sub>SO<sub>4</sub>. The colour changed from violet to blue which indicates presence of steroid.

#### 2.4.6 Test for Tannin

Extract is treated with vanillin hydrochloric acid reagent pinkish red colour is formed it indicate the formation of phloroglucinol.

#### 2.4.7 Test for Terpenoids (Salkowski test)

5 ml extract was mixed in 2 ml of chloroform and 3 ml conc. H<sub>2</sub>SO<sub>4</sub> was added carefully to form a layer. A reddish brown coloration of the interface was formed indicated presence of terpenoids.

### 2.5 Fourier transform-infrared spectroscopy (FT-IR) analysis

The qualitative analysis of the active principles of *Mucuna pruriens* seed extract was done by FTIR method, described by Kemp [11]. Potassium bromide discs were prepared by grinding the seed extract (5 mg) with dry potassium bromide (KBr). The mixture was thoroughly mixed in a mortar and pressed at pressure of 6 bars within 2 min to form a KBr thin disc. Then the disc was placed in a sample cup of a diffuse reflectance accessory. The frequency of the spectra set to analysis was between 4000 - 400 cm<sup>-1</sup> wave number and the vibration spectrum was recorded as graphical chart. The IR spectrum was obtained using FTIR analysis (Shimadzu, Japan).

### 2.6 Gas Chromatograph-Mass Spectroscopy (GC-MS)

GC-MS analysis of methanolic extract of *M. pruriens* seed was performed using a Perkin-Elmer Clarus 680 Gas Chromatograph equipped and coupled to a Mass spectrometry - Perkin Elmer Clarus 600 (Software - Turbomass version 5.4.2) spectrometer with an Elite - 5 (5% diphenyl 95% dimethyl polysiloxane), 30.0 mm X 0.25 mm X 250µm of capillary column. The instrument was set at an initial temperature of 60 °C, and maintained this temperature for 2 min. At the end of this period the oven temperature was rise up to 300 °C, at the rate of an increase of 10 °C/min, and maintained for 6 min. Injection port temperature was ensured

at 250 °C and Helium flow rate at 1 ml/min. The ionization voltage was 70 eV. The samples were injected in split mode as 10:1. Mass spectral scan range was set at 50-600 ( $m/z$ ). The ion source temperature was maintained at 240 °C and Interface temperature was at 240 °C. The solvent delay time was of 2 min. using computer searches on a NIST-2008 MS data library and comparing the unknown component mass spectrum obtained through GC-MS, compounds present in the samples were identified.

### 3. Results and discussion

Nutraceutical analysis indicates the nutrients present in the seed of which carbohydrate content is relatively high with 54.1%, moisture with 9.8%, protein with 12.1%, lipid with 6.9%. The total energy value was confined to be 327 Kcal/100g (Table. 1). Nwaoguipe *et al.* [12] analyzed MP flour and found 60% carbohydrates, 28.2% proteins and 2.7% lipids. Tavares *et al.* [13] reported that MP flour has high protein, carbohydrate and significant ash contents. Variations in proximate composition with present study may be justified by different varieties and cultivation conditions such as soil characteristics, temperature, humidity and rainfall volume [14].

**Table 1:** Proximate analysis of *M. pruriens* flour

Parameters	Results
Moisture	9.8 ± 0.04
Ash	3.8 ± 0.02
Protein	12.1 ± 0.09
Lipid	6.9 ± 0.13
Crude fibre	13.3 ± 0.18
Carbohydrate	54.1 ± 0.25
Energy Value (kcal)	327 ± 5.36

Values are mean ± standard error of triplicate

The phytochemical investigation of the medicinal plant will be helpful for evaluation of medicinal value and preparation of modern drugs and medicines. The prediction of medicinal plants relies on the type of solvent used for extraction. Traditionally, methanol is used as a primary solvent for medicinal plants. Hence in the present study we used methanol as a solvent for extraction of seed powder. Phytochemical analysis helps to reveal the chemical nature of

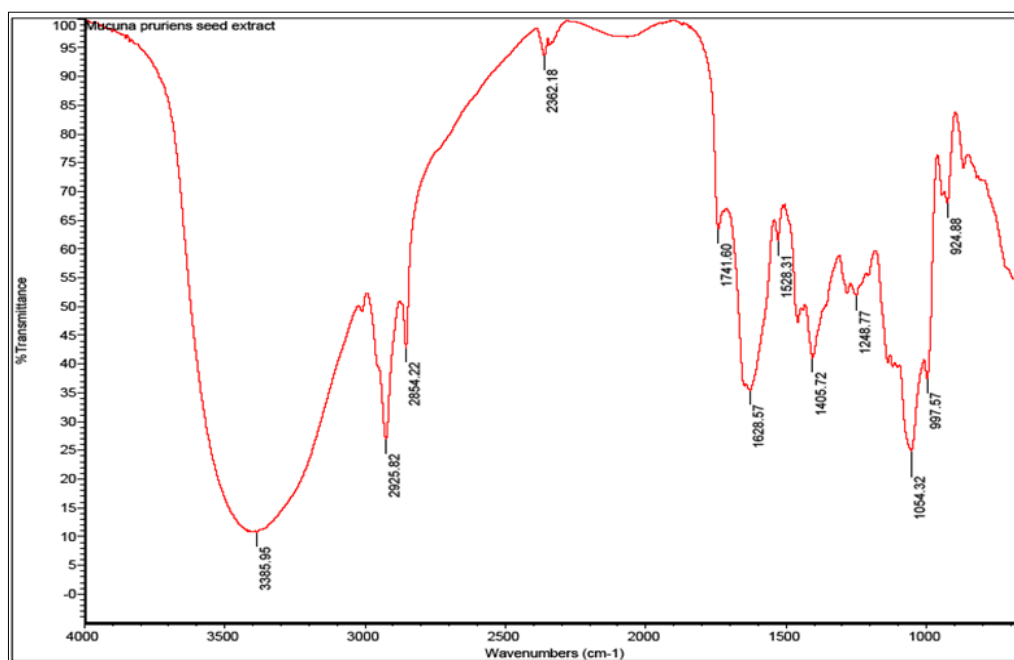
the constituents of *Mucuna pruriens* seed extract. The phytochemical constituents of *M. pruriens* seed extract as given in the Table 2. Qualitative analysis showed the presence of the alkaloids, flavonoids, glycosides, saponins, steroids, tannin and terpenoids in the seed extract. Kavitha [15] also reported that *M. pruriens* seed extract contains alkaloids, phenols, flavonoids, terpenoids, coumarin, quinones, steroids, saponins and tannins.

**Table 2:** Phytochemical constituents of *Mucuna pruriens* seeds extract

S. No	Phytochemical constituents	<i>Mucuna pruriens</i> seeds extract
1	Alkaloids	Presence
2	Flavonoids	Presence
3	Glycosides	Presence
4	Saponins	Presence
5	Steroids	Presence
6	Tannin	Presence
7	Terpenoids	Presence

Alkaloids are naturally occurring chemical compounds containing basic nitrogen atoms. They often have pharmacological effects and are used as medications and recreational drugs [16]. Flavonoids from the medicinal plant extract are water soluble antioxidants and free radical scavengers which are capable of preventing oxidative cell damage and have strong anticancer activity [17]. Flavonoids enhance the effects of Vitamin C and function as antioxidants. 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 [18]. The glycosides are beneficial in lowering blood pressure [19]. The sterols possess antibacterial and antibiotic activity and have been shown to act as inhibitors of tumor promotion *in vivo* [20]. The presence of these sterols has been reported to account for the exertion of antimicrobial activity by plants containing them [21]. Tannin is an astringent, plant polyphenolic compound that binds to and precipitates proteins and various other organic compounds including amino acids and alkaloids [22]. The

earlier study on antibacterial action of phytochemicals from *M. pruriens* seed extract [23], also support to present findings. The FTIR spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation. The Infra-red spectroscopic (IR) analysis of the *M. pruriens* seed extract in a band width ranging from 400 to 4000  $\text{cm}^{-1}$ , revealed the presence of different functional groups (Fig. 1). The peaks showed that the *M. pruriens* seed extract have the compounds like amines, amides, phosphine, fluorides, bromides, iodide, aliphatic and aromatic nitro compounds. Further, it indicates the possible chemical bond and compound type as follows, O-H, N-H stretching for alcohol (3385.95 $\text{cm}^{-1}$ ), N-H stretching and for amine salt (2925.82, 2854.22, 2362.18  $\text{cm}^{-1}$ ), C=O stretching for amide (1741.60, 1628.57  $\text{cm}^{-1}$ ), Asymmetric stretching for aliphatic and aromatic nitro compounds (1528.31 $\text{cm}^{-1}$ ), P-CH<sub>3</sub> bending for phosphine (1405.72  $\text{cm}^{-1}$ ), C-F, C-Br stretching for fluoride and aryl bromide (1248.77  $\text{cm}^{-1}$ ), C-Br, C-I stretching for bromide and iodide (1054.32) and P-H stretching for phosphine (997.57, 924.88  $\text{cm}^{-1}$ ) was observed.

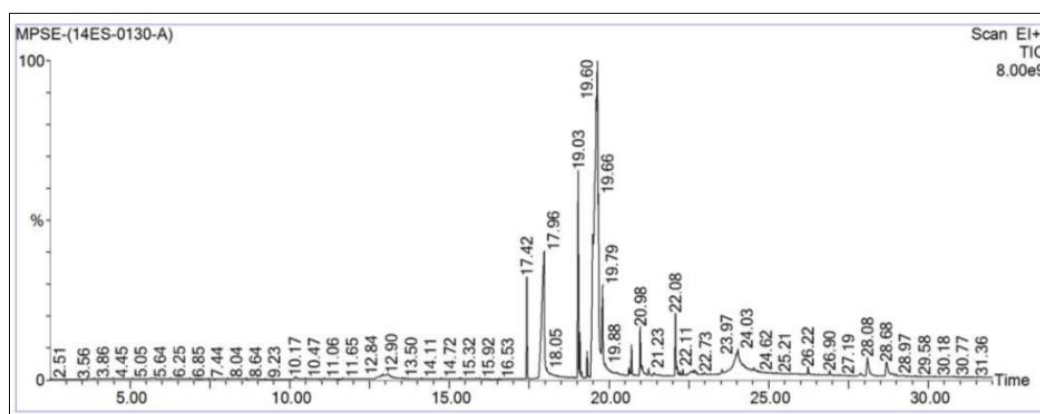


**Fig 1:** FT-IR Spectroscopic analysis of *Mucuna pruriens* seed extract

FTIR is one of the most widely used methods to identify the chemical constituents elucidate the compounds structures. It has been used as requisite method to identify medicines in pharmacopoeia of many countries [24]. Among the functional groups observed in the extracts, OH group was found in the seed of *M. pruriens*. As OH group has got the ability of forming hydrogen bonding capacity, presence of OH group indicates the higher potential towards inhibitory activity against microorganisms. The antibacterial activity of the *M. pruriens* seed extracts may be also due to by the presence of these functional compounds such as aromatic acids, carboxylic acid and bromides.

The GC-MS Chromatogram of the methanolic seed extract of *M. pruriens* (Fig. 2) showed seventeen peaks indicating the presence of eleven significant compounds. The qualitative analysis identified the various phytochemicals compounds. Interpretation of mass spectrum GC-MS was conducted using

the database of NIST data Library. Ethyl 9, 12-Hexadecadienoate (53.36%) was found as the major components followed by N Hexadecanoic Acid (17.72%), Methyl 11,14-Octadecadienoate (7.30%), (IS,15S)-Bicyclo [13, 10] Hexadecan-2-one (7.12%), Methyl 11-Methyl-Dedecanoate (3.42%), Pentadecanoic acid, 15-Bromo (2.89%), Cyclobutane carboxylic acid, 2-Dimethyl amino ethylether (2.02%), Stigmasterol (1.75%), cyclobutanone, 2-Tetradecyl (1.62%), Gamma-Sitosterol (1.31%) and I-Propyl 9,12-Octadecadienoate (1.17%). The analysis showed the presence of alkaloid, carboxylic acid, terpenoid, polyphenol and other secondary metabolites most of them are of pharmacological importance in terms of antioxidant and antimicrobial. Many of these polyphenolic compounds from plant origin were seemed to have potential antioxidant properties. Polyphenol's affects bacterial growth through cytotoxic mechanisms.



**Fig 2:** GC-MS chromatogram of *M. pruriens* seed extract

*Mucuna pruriens* methanolic extract had significant *in vitro* lipid peroxidation and antimicrobial activity [25]. *M. pruriens* seed was effective antimicrobial agent to treat bacterial infection, apart from their roles as food additives and supplements. It can also be utilized as effective and cheap source of antimicrobial agent [26]. The methanol extract of *M.*

*pruriens* effectively inhibits the pathogens compared to acetone and ethanol extracts [27]. The methanolic extract of *M. pruriens* seed exhibited the significant antibacterial effect against *V. harveyi* and *V. cholerae* [23].

The extensive antibiotics use making disease causing bacteria more resistant to the drugs. The active compounds in these

plants should be traced out which could find place in the treatment of various bacterial infections where they can be used as alternatives to conventional antibacterial synthetic drugs. Application of phytochemicals in aquaculture to overcome the drawbacks of chemical therapeutics usage to control bacterial infection. This is relatively new venture and the potential of the herbals with multifunctional active principles are promising [28]. Shanmugavel and Krishnamoorthy [23] suggest that *M. pruriens* seed extract have a potent antibacterial phytochemicals, which may be used as medicated feed for shrimps to manage the vibriosis.

#### 4. Conclusion

The present study was revealed that *Mucuna pruriens* seed as rich source of nutrients and phytochemicals. The proximate analysis showed *M. pruriens* flour has high carbohydrate content with good energy value. The phytochemical screening of seed extract indicates the presence of alkaloids, flavonoids, glycosides, saponins, steroids, tannin and terpenoids. The FTIR analysis of seed extract revealed the presence of characteristic functional groups of alcohol, amines, amides, phosphine, fluorides, bromides, iodide, aliphatic and aromatic nitro compounds. The GCMS analysis showed the presence of alkaloid, carboxylic acid, terpenoid, polyphenol and other secondary metabolites are responsible for various medicinal properties of seed. This seeds could be utilized as good therapeutic agents and an alternative source of useful drugs. Further investigation is needed to isolate the bioactive compounds of this seed for novel drug formulation, which can use as one of the new approaches to prevent and control of infectious diseases.

#### 5. Acknowledgment

We are very grateful to the Director and Head, Department of Zoology, K.M. Centre for PG Studies, Puducherry, India, for providing us the facilities to carry out this research work successfully.

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