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Research Article

# PHYTOCHEMICAL ANALYSIS AND COMPARATIVE ANTIPARKINSON ACTIVITY OF FOUR SPECIES OF *MUCUNA*

**KARUNANITHI M[1]*, DAVID RAJ C[1], BRINDHA P[1], JEGADEESAN M[2], KAVIMANI S[3]**

**[1]Centre for Advanced Research in Indian System of Medicine, Shanmugha Arts, Science, Technology and Research Academy Deemed University, Thanjavur, Tamil Nadu, India. [2]Department of Environmental Sciences and Medicinal Botany, Tamil University, Thanjavur, Tamil Nadu, India. [3]Department of Pharmacology, College of Pharmacy, Mother Theresa Post Graduate and Research Institute of Health Sciences, Puducherry, India. Email: karuna@carism.sastra.edu**

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**ABSTRACT**

**Objective:** The aim was to study the antiparkinson activity in the seed extracts of four species of *Mucuna*.

**Methods:** The hydroalcoholic extracts of seeds of four species of *Mucuna* were evaluated for antiparkinson activity of after a preliminary phytochemical study. The activity was measured in rats by indirectly measuring the decrease in malondialdehyde level, decrease in tongue protrusion frequency, and reduction in vacuous chewing movement after administering reserpine at the dose of 1 mg/kg. The dose levels of four species of *Mucuna* seed extract were kept at 100, 200, and 300 mg/Kg.

**Results:** Extracts exhibited potent antiparkinson activity and achieved statistically significant p values compared with control group. The study corroborates and compares all four species of *Mucuna.*

**Conclusion:** Among the extracts, the highest percentage of antiparkinson activity was recorded for *Mucuna pruriens*.

**Keywords:** *Mucuna pruriens,* Cochinchinesis, Utilis, Deeringiana, Antiparkinson activity.

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## INTRODUCTION

Parkinson's disease belongs to a group of conditions called movement disorders. It is characterized by muscle rigidity, tremor, a slowing of physical movement (bradykinesia) and in extreme cases, a loss of physical movement (akinesia) [1]. It is a clinical syndrome characterized by lesions in the basal ganglia, predominantly in the substantia nigra [2]. As a rule, PD begins between the ages of 40 and 70 years, with peak age onset in the seventh decade. The prevalence of PD is approximately 160 cases per 100,000 populations, and the incidence is about 20 cases per 100,000 populations. The pathological changes of PD may appear as early as three decades before the appearance of clinical signs [3]. The cause of PD is probably multifactorial, with contributions from hereditary predisposition, environmental toxins, and aging [4]. The most widely used form of treatment is L-DOPA in various forms. L-DOPA is transferred into dopamine in the dopaminergic neurons by L-aromatic amino acid decarboxylase.

*Mucuna pruriens*(L.) DC., the velvet bean, is one of the important herbal drugs in the Indian Systems of Medicine. Seeds of this plant contain a high amount of L-DOPA [5]. The seeds are used in the treatment of many common ailments and also as a food. The seeds have been sold in the herbal drug market in many parts of India in the name of "atmagupta" or "poonaikali" [6]. In our survey, we found that seeds of many other species other than *M. pruriens* are sold as "poonaikali" in Tamil Nadu and "atmagupta" or "kawanch" in other states of India. It is frightening to note that the crude drug traders and traditional physicians and pharmaceutical manufacturers, who use this seed for preparation of medicine, are unaware of its identity and its adulterants. As "poonaikali" is a common drug and big demand in India and abroad, it is essential to standardize the scientific lines for identifying the authentic drug and to detect the adulterants. Although many pharmacological works on seeds of *M. pruriens* have been carried out, comparative scientific work of *M. pruriens* and its adulterants is not available.

The seeds of *M. pruriens* are reported to have antiparkinson activity along with other activities. Commonly *M. pruriens* is adulterated with other species of the genus such as *Mucuna deeringiana, Mucuna utilis, and Mucuna cochinchinesis*. This department has worked on seeds of *Mucuna* and its adulterants on the basic phytochemical and pharmacological works [7]. In this work, we would study comparative and detailed phytochemistry and pharmacology of the seeds of *M. pruriens* and its adulterants.

## MATERIALS AND METHODS

### Collection of seed samples

Seeds of "poonaikali" (minimum 2 kg) were procured from different herbal drug stores in Madurai, Thanjavur, and Chennai. Some of the seed samples were collected from the Herbal Garden of Tamil University.

### Preparation of extract

The collected seed samples were dried in the open sunlight for 2 days. Then, the dried seeds were cleaned. Foreign matter, broken seeds, and immature seeds were removed from the sample. The seeds were stored in a suitable plastic container and kept at room temperature. Then, the seeds were powdered mechanically to 60 mesh size. The seed powder was soaked in 70% ethanol for 72 h with occasional shaking. The solvent was decanted and filtered. The marc was subjected to further extraction by repeating the procedure thrice. The solvent was removed by distillation under vacuum. Previous work on the seeds of *Mucuna* species [7] had revealed the presence of most of the active principles in alcoholic and water extracts. Hence, hydroalcoholic extract was chosen for the analytical and pharmacological study.

### Powder analysis

Behavior of seed powder with different chemical reagents was carried out as mentioned by Kay L [8] Johnson [9] and Birch and Doughty [10].

**Qualitative phytochemical studies**

Qualitative phytochemical analysis was done using the procedure of Kokate (1994). Alkaloids, carbohydrates, tannins and phenols, fixed oils and fats, saponins and gums, and mucilages were qualitatively analyzed.

**Quantitative phytochemical studies**

The estimation of total alkaloid was performed by gravimetric method [11] total protein [12], total tannins [13], and total phenolic content [14].

**Thin layer chromatography (TLC) studies**

Extracts mixed with 0.1N HCl were spotted on silica gel G coated plates. N-Butanol, glacial acetic acid, and water in the ratio of 2:1:1 was used as mobile phase. Freshly prepared mixture of an equal proportion of 10% ferric chloride and 5% potassium ferricyanide was used as detecting agent.

One study was carried out using the above procedure with *M. pruriens* powder and a marketed pharmaceutical product containing *Mucuna*.

Another study was carried out with four types of extract as four spots and using L-DOPA standard as the fifth spot.

**Animal studies**

*Antiparkinson activity [15,16]*

Animals

Recently, a promising animal model of tardive dyskinesia has been proposed: The increase in tongue-protrusion frequency induced by repeated administration of reserpine (RE) in rats [17]. In this regard, although RE is not classified as a neuroleptic, it was used as an antipsychotic agent and has been associated with the development of tardive dyskinesia [18]. This RE-induced oral dyskinesia in rats has several other features that are consistent with this movement disorder, including persistence following interrupted administration, and a reported dose-dependent blockade induced by a D2 selective antagonist. As with tardive dyskinesia, RE-induced oral dyskinesia is exacerbated by dopamine agonists like amphetamine [19].

Furthermore, despite the fact that RE-induced tongue-protrusion dyskinesia in rats develops very rapidly (few days) after high doses [20]; this fact offers an outstanding methodological advantage over other proposed rodent models of tardive dyskinesia.

An important hypothesis recently receiving considerable interest is the proposal that the symptoms of Parkinsonism are due to neurotoxic effects of free radical byproducts from dopamine (DA) metabolism. The increase in DA turnover is produced from blockage of dopamine receptors by neuroleptics [21]. Dopamine undergoes monoamine oxidase-catalyzed oxidative deamination to 3,4-dihydroxyphenyl-acetaldehyde (DOPAL), which is metabolized primarily to 3,4-dihydroxyphenylacetic acid. DOPAL is a reactive radical and toxic to dopaminergic cells [22]. DOPAL injection into the substantia nigra of rats resulted in DA neuron loss [23].

There were 84 male albino mice weighing 20–40 g housed under conditions of controlled temperature (20±2°C) and lighting (12 h light/12 h dark, lights on at 7:00 am). Food and water were available *ad libitum* throughout the experiment. The mice were brought to the experimental laboratory 7 days before the beginning of the experiment and immediately housed at random in groups of 6 animals per cage. All procedures involving laboratory animal use were in accordance to the Institute Animal Ethics Committee regulations approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Drugs

The drugs used in these experiments were obtained from Sigma (St. Louis, MO). RE was dissolved in 50 µl of glacial acetic acid and then diluted to the correct concentration with distilled water, and injected at the dose of 1 mg/kg. Vehicle consisted of the same amount of acetic acid and water used in the RE solution. Hydroalcoholic extract of *M. pruriens* was dissolved in 1% tween 80.

**Experimental procedures**

Mice were randomly divided into 14 groups of six animals each, which received a hydroalcoholic extract of *Mucuna* species at the dose of 100, 200, and 300 mg/kg orally for the period of 16 days. On days 14 and 16, 1 mg/kg i.p. RE was injected 1 h after the drug and vehicle treatment. After 24 h of the last RE injection, all the animals were subjected to behavioral observation as follows:

Animal behavior was initially observed in a glass cage. Mirrors were placed under the floor and behind the back wall of the cage to permit observation of oral movements when the animal faced away from the observer. In this experimental stage, oral movement and tremor were observed. The frequency of tongue protrusion and vacuous chewing movements (VCM) was determined using hand-operated counters. In the present study, tongue protrusion was operationally defined as a visible extension of the tongue outside of the mouth and not directed at anything. Individual tongue protrusions during a short time of oral dyskinesia were each preceded by visible retraction of the tongue [19]. If tongue protrusions occurred during a period of grooming, they were not taken into account. The incidence of tongue protrusions was measured continuously for 10 min. VCMs were scored during 5 min observation period, according to a five-point scale (0=absent, 1=minimal, 2=mild, 3=moderate, and 4=severe). A VCM consisted of a rapid movement of the jaw which resembles chewing but did not appear to be directed at any particular stimulus [24].

Stopwatches were used to quantify the duration of generalized body tremor, expressed in seconds. These parameters were measured continuously for 15 min.

The animal's forepaws were then placed on a horizontal glass bar (2 mm diameter) elevated 4.5 cm above the observation floor, and the time elapsing before the animal removed its forepaws from the bar and placed them on the floor was measured. This test was repeated 3 times during a session, with the longest elapsed time representing the catalepsy score, expressed in seconds [25].

**Statistical analysis**

Statistical analysis was performed with GraphPad Prism 5 Software (GraphPad Prism Software Inc. San Diego, California, USA). VCMs and tongue protrusions are considered to be nonparametric. Thus, the data were analyzed by Kruskal–Wallis analysis of variance followed by the Dunn's Multiple Comparisons test. Tremor and catalepsy are considered to be parametric, and the data were analyzed by ANOVA followed by the Tukey test. A probability of $p<0.05$ was considered to show significant differences for all comparisons made.

**RESULTS**

**Morphology of seeds**

Seeds of different samples of *Mucuna spp*. were purchased from different places of Tamil Nadu such as Madurai, Thanjavur, and Chennai. Botanical identity of the samples was established based on the morphology of the seeds (plates 1–4).

Morphological description and dimensions of seeds of different samples are given in Table 1 (plates 1–4). Color of the seeds, weight/100 seeds, dimensions of seeds, thickness of seed coat, dimensions of raphae, thickness of cotyledon, and loss on drying were noted (Fig. 1).

The seeds of *M. pruriens* and its adulterants could be distinguished by their size and shape. Thus seeds of *M. pruriens, M. cochinchinensis,* and *M. deeringiana* are oval in shape and smooth and glossy. Seeds of *M. utilis* are angular in shape and smooth and glossy. On the basis of the dimensions, the seeds of *mucuna* species could be grouped as medium

and small. *M. deeringinana* and *M. utilis* are medium in size followed by *M. cochinchinensis*. Among all the seed samples, *M. pruriens* seeds are the smallest.

Weight of 100 seeds of the four samples has correlation to their size class. Thus, among the medium-sized class seeds (*M. deeringiana* and *M. utilis*), *M. deeringiana* has the highest weight. Even though *M. cochinchinensis* is smaller in size the weight of 100 seeds is slightly higher in proportion. *M. pruriens* seeds have lowest weight/100 seeds, among all the samples (Table 1).

**Analytical values**

Analytical values such as total ash, acid insoluble ash, acid soluble ash, and solubility percentage in water for all the four samples were analyzed, and their values are tabulated in Table 2. Total ash value is more or less same in all seed samples. *M. utilis* has slightly higher ash value. Acid-insoluble ash value is the highest (0.1222) in *M. utilis* and is lowest in *M. deeringiana* (0.0588%), medium values in *M. pruriens,* and *M. cochinchinensis* were noted. Value of solubility percentage in water was higher for *M. pruriens* seeds.

**Qualitative phytochemical studies**

Qualitative phytochemical analysis for alkaloids, carbohydrates tannins, phenols, gums and mucilage, fixed oils and fats, saponins and steroids was screened in four types of seed samples and was recorded in Table 4. Extractive values in solvents yielded distinct values for the four seed samples. *M. deeringiana* and *M. utilis* did not have any extractive value in pet ether. All the seed samples had higher extractive values in water followed by an alcohol. Lower values were observed for all the samples in chloroform. The order of extractive values in alcohol is MD>MU>MC>MP; and in water MU>MC>MP>MD; and in benzene MD>MU>MC>MP (Table 3).

**Alkaloids**

Presence of alkaloids was noted in all the seed samples in benzene, chloroform, alcohol, and aqueous extracts. Out of the four extracts, aqueous extracts of the samples gave more amounts of alkaloids followed by benzene extracts.

**Preparation of hydroalcoholic extract**

The collected seed samples were thoroughly shade dried for 2 days. Then, the dried seeds were cleaned and any foreign matter, broken seeds and immature seeds were removed. The seeds were stored in a plastic container at room temperature. Then, the seeds were powdered separately in a mechanical way to 60 mesh size. The seed powder was soaked in 70% ethanol for 72 h with occasional shaking. The solvent was decanted and filtered. The marc was subjected to further extraction by repeating the procedure thrice. The solvent was removed from the extract by vacuum distillation.

**Quantitative phytochemical studies**

*Estimation of protein, carbohydrate, and alkaloids*

Total protein, carbohydrate, and alkaloids in seeds of *M. pruriens* and its adulterants were estimated. Highest protein content (32%) was obtained in dried samples of *M. pruriens* followed by *M. utilis* (28%). Lowest protein content (25%) was observed in *M. cochinchinensis* seeds.

Maximum carbohydrates content (53%) was observed in *M. deeringiana* followed by *M. pruriens* (41%). In *M. utilis* lowest carbohydrate (40%) was observed.

Total alkaloids of seed samples reveal highest content (0.82%) in *M. utilis*. Alkaloid content in seeds is in the following order: MU>MP>MD>MC.

Phenols and tannin are present in seeds of *M. pruriens,* and its adulterants were estimated (Table 5). Highest phenol content (6.63%) was observed in *M. utilis*. *M. cochinchinensis* showed lowest (2.13%) phenol content and *M. pruriens* seeds had 2.15%.

Estimation of tannins in seeds showed the highest value (2.1%) in *M. deeringiana*. *M. pruriens* had 0.3% of tannins.

**Table 1: Macroscopic details of seeds of *M. pruriens* and its adulterants**

| S. No | Parameters | *M. pruriens* | *M. cochinchinensis* | *M. deeringinana* | *M. utilis* |
|---|---|---|---|---|---|
| 1 | Color | Black | Dull white | Black | Grey with black spots |
| 2 | Weight/100 seeds (gm) | 32.72 | 90.64 | 162.14 | 122.36 |
| 3 | Dimensions of seed L×B × T (mm) | 12×9 × 6 | 14×10×7 | 16×11×8 | 15×11×8 |
| 4 | Thickness of seed coat (mm) | 0.20 | 0.25 | 0.17 | 0.12 |
| 5 | Dimensions of raphae LxB | 5×2 | 7×2 | 6×2 | 7×3 |
| 6 | Thickness of cotyledon (mm) | 5.32 | 6.08 | 7.61 | 7.64 |
| 7 | LOD (%) | 5.71 | 7.13 | 3.54 | 10.28 |

*M. pruriens: Mucuna pruriens, M. cochinchinensis: Mucuna cochinchinensis, M. deeringinana: Mucuna deeringinana, M. utilis: Mucuna utilis*

**Table 2: Analytical values (in percentage) of seed powders**

| S. No | Parameters | *M. pruriens* | *M. cochinchinensis* | *M. deeringinana* | *M. utilis* |
|---|---|---|---|---|---|
| 1 | Total ash value | 3.2732 | 3.0728 | 3.0553 | 3.2908 |
| 2 | Acid-insoluble ash value | 0.0854 | 0.1231 | 0.0588 | 0.1222 |
| 3 | Acid soluble ash value | 3.1878 | 2.9497 | 2.9965 | 3.1686 |
| 4 | Solubility percentage in alcohol | 3.0000 | 2.9600 | 6.9200 | 5.7600 |
| 5 | Solubility percentage in water | 25.5300 | 21.2900 | 19.4700 | 18.500 |

*M. pruriens: Mucuna pruriens, M. cochinchinensis: Mucuna cochinchinensis, M. deeringinana: Mucuna deeringinana, M. utilis: Mucuna utilis*

**Table 3: Successive extractive values of seeds in percentage**

| S. No. | Name of the samples | Benzene | Pet ether | Chloroform | Alcohol | Water |
|---|---|---|---|---|---|---|
| 1 | *M. pruriens* | 1.932 | 0.014 | 0.106 | 0.857 | 10.059 |
| 2 | *M. cochinchinesis* | 2.634 | 0.354 | 0.226 | 1.697 | 11.021 |
| 3 | *M. deeringiana* | 5.086 | - | 0.569 | 17.354 | 7.499 |
| 4 | *M. utilis* | 4.269 | - | 1.011 | 5.856 | 12.555 |

*M. pruriens: Mucuna pruriens, M. cochinchinensis: Mucuna cochinchinensis, M. deeringinana: Mucuna deeringinana, M. utilis: Mucuna utilis*

**Table 4: Qualitative phytochemical screening of hydroalcoholic extracts of seeds**

| Alkaloids | Reagent/test | *M. pruriens* | *M. cochinchinensis* | *M. deeringinana* | *M. utilis* |
|---|---|---|---|---|---|
| | Mayer's reagent | + | + | + | + |
| | Dragendorff's reagent | + | + | + | + |
| | Hager's reagent | + | – | – | – |
| | Wagner's reagent | + | – | + | – |
| | Mayer's reagent | + | + | + | + |
| | Dragendorff's reagent | + | + | + | + |
| | Hager's reagent | + | + | + | + |
| | Wagner's reagent | + | + | + | + |
| Carbohydrate | Molisch's reagent | + | + | + | + |
| | Fehling's reagent | – | – | – | – |
| | Benedict's reagent | + | + | + | + |
| Tannins and phenols | Ferric chloride | + | + | + | + |
| | Gelatin Solution | + | - | + | + |
| | Lead acetate | + | + | + | + |
| Gums and mucilage | Precipitation test | + | + | + | + |
| Fixed oils and fats | Paper pressing | + | + | + | + |
| Saponins | Foam Test | + | + | + | + |
| Steroids | Lieberman's test | – | – | – | – |

+: Presence, –: Absence. *M. pruriens: Mucuna pruriens, M. cochinchinensis: Mucuna cochinchinensis, M. deeringinana: Mucuna deeringinana, M. utilis: Mucuna utilis*

**Table 5: Quantitative estimation of protein, carbohydrate, alkaloids, phenols, and tannins**

| S. No | Name of the samples | Protein (%) | Carbohydrate (%) | Alkaloids (%) | Phenols (%) | Tannin (%) |
|---|---|---|---|---|---|---|
| 1 | *M. pruriens* | 32 | 41 | 0.48 | 2.15 | 0.3 |
| 2 | *M. cochinchinesis* | 25 | 41 | 0.13 | 2.13 | 0.6 |
| 3 | *M. deeringiana* | 27 | 53 | 0.45 | 5.31 | 2.1 |
| 4 | *M. utilis* | 28 | 40 | 0.82 | 6.63 | 1.3 |

*M. pruriens: Mucuna pruriens, M. cochinchinensis: Mucuna cochinchinensis, M. deeringinana: Mucuna deeringinana, M. utilis: Mucuna utilis*


**Fig. 1: Morphology of seeds. (a) Seeds of *Mucuna pruriens*, (b) seeds of *Mucuna cochinchinensis*, (c) seeds of *Mucuna deeringiana*, and (d) seeds of *Mucuna utilis***

**TLC**

*Coenzyme Q10 by TLC*

Hydroalcoholic extracts of all the four species were spotted and compared with the coenzyme $Q_{10}$ as the fifth spot in two solvent systems dioxane: water (50:50) and chloroform: methanol (55:45). Spots in the four extracts and the standard confirmed the presence of coenzyme $Q_{10}$ at 254 nm 366 nm and after color development. The hydroalcoholic extract was dissolved in chloroform, and it is used for TLC analysis. The solvent systems used were dioxane: water (50:50) and chloroform: methanol (55:45). The spraying reagent used was 5% phosphomolybdic acid in ethanol. The developed blue colored spots were visualized after spraying 5% phosphomolybdic acid in ethanol.

**Antiparkinson activity**

Antiparkinson activity is measured indirectly by the following activity:

1. Decrease in malondialdehyde (MDA) level
2. Decrease in tongue protrusion frequency (TPF) and
3. Decrease in VCM.

The effect of hydroalcoholic extracts of MC, MD, MP, and MU was observed in the above activity and data recorded (Figs. 2-4).

Free oxygen radicals can induce lipid peroxidation in cells; MDA is formed during oxidative degeneration and accepted as an indicator of lipid peroxidation. RE increased the level of lipid peroxide, MDA, from 1.2±0.1125 to 2.5±0.175 (µmol/g tissue). MP and MD at the dose of 300 mg/kg prevented the RE-induced elevation in MDA levels and significantly ($p<0.01$) decreased its elevated levels to 1.4±0.1025 and 1.5±0.0875 respectively (Fig. 2), when compared with disease control group. MU treated group does not produce a significant change in MDA levels when compared with RE alone treated group.

Extracts of MC, MD, MP, and MU were administered at three different dosages (100, 200, and 300 mg/Kg) and TPF was observed (Fig. 3). It was noted that TPF was increased three-fold in the control group. There was a dose-dependent response for all the four extracts in decreasing the TPF. TPF was significantly reduced by an extract of MC, MD,and MU and more significantly reduced by MP at the dose of 300 mg/Kg.

Effect of hydroalcoholic extract of MC, MD, MP, and MU on VCM frequency is shown in Fig. 4. Animals that received RE exhibited an

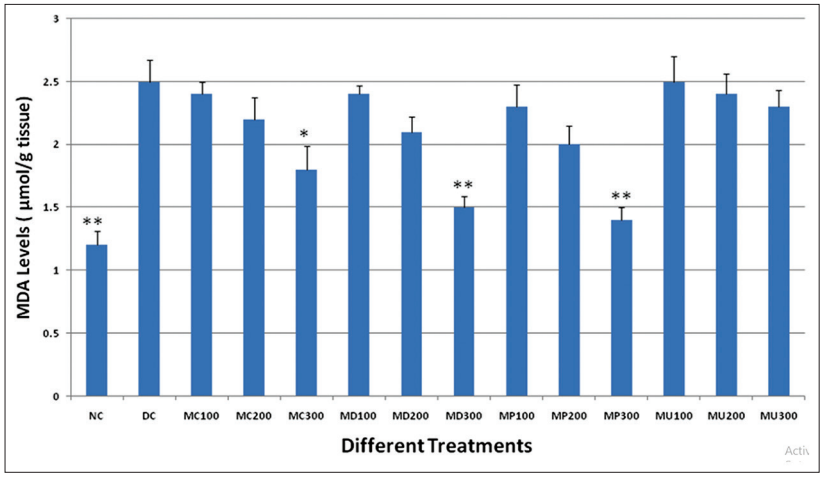


**Fig. 2: Effect of extracts of MC, MD, MP, and MU on malondialdehyde. The values are in mean±standard error of the mean, n=6, *p<0.05 and **p<0.01 compared with disease control group**

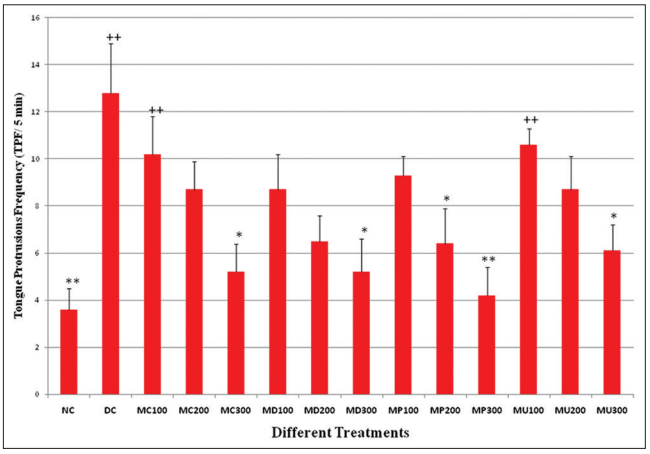


**Fig. 3: Effect of extracts of MC, MD, MP, and MU on tongue protrusion frequency. The values are in mean±standard error of the mean, n=6, *p<0.05 and **p<0.01 compared with disease control group. ++p<0.01 compared with normal control group**

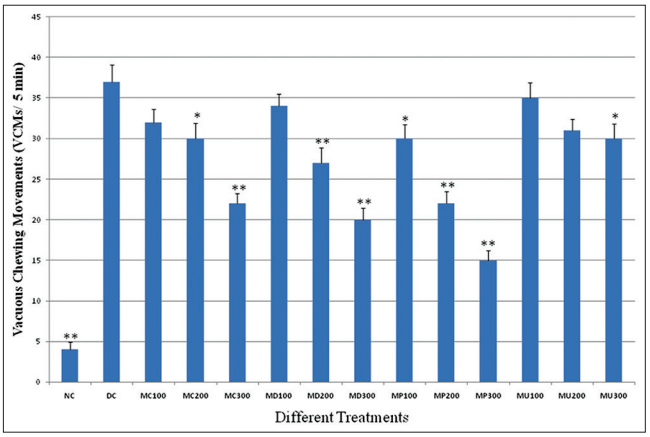


**Fig. 4: Effect of extracts of MC, MD, MP, and MU on vacuous chewing movement. The values are in mean±standard error of the mean, n=6, *p<0.05 and **p<0.01 compared with disease control group**

increase in VCMs compared to vehicle-treated animals (p<0.01). All the *Mucuna* species except MU produced dose-dependent effect. MC, MD, and MP at the dose of 200 mg/kg showed less significant (p<0.01) decrease in VCM, when compared with diseased control, whereas MU at the dose of 300 mg/kg showed significant (p<0.05) reduction in VCM, when compared with RE alone treated group.

## DISCUSSION

The macroscopic study of the *M. pruriens* and the adulterants shows the difference in size, color, and weight. The phytochemical study reveals the presence of alkaloids, carbohydrate, tannins, Phenols, gums, mucilages, fixed oils, and saponins. The high-performance TLC was carried out after dissolving extracts in 0.1 m hydrochloric acid in methanol. The four species were kept as 1–4 tracks and the 5$^{th}$ track was standard L-DOPA.

The last track is of the reference standard L-DOPA showing a single peak at the $R_f$ value of same 0.43. This confirms the presence of L-DOPA in all varieties and the only difference in the quantification.

RE interferes with the storage of dopamine (and also of noradrenaline and 5-hydroxytryptamine) in synaptic vesicles, leading to depletion of dopamine in nerve terminals. Its central action produces sedation, hypokinesia, rigidity (catalepsy), and often tremor [26,27]. The effect of RE on spontaneous locomotor activity is frequently used as a model of the motor disturbance of Parkinson's disease [28-30] and several clinically used antiparkinson drugs (e.g., dopamine receptor agonists, L-DOPA + benserazide, amantadine, and trihexyphenidyl) have been shown to improve this motor impairment [30]. This suggests that the RE model showed good face and predictive validity [31]. *M. pruriens* increased brain dopamine level [32]. Fachinetto and colleagues [33] have reported that animals with VCMs have significantly higher lipid peroxide expressed as thiobarbituric acid reactive substances in the striatum, suggesting increased lipid peroxidation, and free radical production in these animals.

Another source of neuronal oxidative damage is related to calcium overload. It was hypothesized that prolonged stimulation of N-methyl-D-aspartate (NMDA) and glutamate receptors can induce massive cell death in the brain (excitotoxicity), by causing calcium overload in post-synaptic neurons [34]. Hernández-Fonseca *et al*. [35] have reported that calcium influx through NMDA receptors is involved in reactive oxygen species (ROS) production and neuronal damage. Lipid peroxidation is considered as a major mechanism of oxygen radical toxicity, thereby altering membrane permeability. Persistent activation of NMDA and non-NMDA glutamate ionotropic receptors mediates calcium entry and ROS production which is well-recognized perpetrators of neuronal oxidative damage [36]. Calcium channel blockers also prevent calcium-overload in ischemic rat brains through their effect on the L-type calcium channel leading to suppression of formation of oxygen-derived free radicals and lipid peroxidation [37]. Our results show that *Mucuna* species markedly reduced the elevated lipid peroxide levels, MDA, that

were augmented after RE treatment. The elevated MDA level is better reduced by *M. pruriens* extract when the second highest activity goes to *M. deeringiana. M. utilis* is getting third place, and *M. cochinchinensis* is in the last. This amelioration in oxidative stress parameters was accompanied by a moderate reduction in behavioral abnormalities such as VCM and TPF.

RE induced tongue protrusions to seem to be a better model of tardive dyskinesia. Indeed, acute dystonia frequently develops after the first dose of neuroleptic, whereas RE produces a decrease in tongue protrusion in animals observed 6 h after the first injection (Neisewander *et al.*, 1994, 1996). The increase in tongue protrusion, however, is not observed until 24 h after the second RE injection or 72 h after a single injection [37]. In addition, Sussman *et al.* demonstrated that RE-induced spontaneous oral dyskinesia (i.e., spontaneous increase in TPF) persisted above control level for at least 84 days post-treatment, despite depletion of dopamine in the caudate putamen. These findings suggest that tongue protrusion is not an acute RE-elicited effect, but rather spontaneous oral dyskinesia that develops as a result of a persistent neuropathological change. In our study, the TPF is much lowered by *M. pruriens* extract at the dose of 300 mg/kg. *M. deeringiana* and *M. cochinchinensis* are having nearly equal effect in reducing TPF. *M. utilis* is comparatively less effective in the minimization of TPF. The reduction of VCM is highest in *M. pruriens* extract, and *M. deeringiana* and *M. utilis* are also having remarkable effect. *M. cochinchinensis* is showing poor performance in bringing down VCM. The content of L-DOPA and neuroprotective antioxidants is more in *M. pruriens* and *M. utilis*. The level of significance of reduction of VCM is higher in these two samples. This confirms the L-DOPA content plays a major role in the treatment of Parkinsonism and the antioxidants complement the activity.

## CONCLUSION

From the present investigation, it is concluded that out of four samples of "*Poonaikali*," *M. pruriens* is the authentic and effective drug. Although *M. cochinchinensis*, *M. deeringiana,* and *M. utilis* are adulterants, they could be used as substitutes for *M. pruriens*. *M. pruriens* is the authentic and effective drug in the treatment of Parkinson's disease. The L-DOPA content is more in *M. pruriens.* Further clinical trial is needed to support this conclusion. As on offshoot of this work, an activity guided fraction of *M. pruriens* seeds, isolations, purification, and characterization of compounds in the extracts could be carried out for future work.

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