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Proximate constituents and L-3,4-dihydroxyphenylalanine levels in *Mucuna pruriens* seed powder and baked biscuits therefrom

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Seeds of *Mucuna pruriens* (*Mp*), a leguminous plant, are known for their high content of L-3,4-dihydroxyphenylalanine (L-DOPA) and are used in the management of Parkinson's disease (PD) in Indian traditional medicine. These seeds also contain valuable nutrients and phytochemicals with potential health benefits. This study aims to evaluate the physicochemical, phytochemical, nutritional, and certain neuroactive constituents of *Mp* seed powder and to assess its application in neuro-nutraceutical development. Dried *Mp* seed powder and baked biscuits prepared using the dough made of this seed powder are chemically analysed for contents of moisture, ash, water- or alcohol-soluble extract, phytochemical constituents (phenols and tannins), and nutritional value compounds (protein, fat, fibre, carbohydrate, sources of bioenergy). L-DOPA contents in both *Mp* powder and *Mp* biscuits were quantified using a high-performance liquid chromatography-electrochemical detection method. *Mp* powder is rich in proteins, fibres, and phytochemicals, and contains a significant amount of L-DOPA, the precursor of the neurotransmitter dopamine. Upon baking, the biscuits retained L-DOPA, albeit with a 55% reduction during thermal processing. The presence of appropriate bioactive/pharmaceutical compounds, such as phenols and tannins, was confirmed by phytochemical screening. *Mp* seed powder is a promising functional ingredient for the development of nutraceutical foods. The successful retention of L-DOPA in baked biscuits suggests its potential application as a medicinal food in the management of Parkinsonian syndromes in PD patients. Further clinical validation is needed to confirm L-DOPA bioavailability for therapeutic relevance, which is in progress.

Keywords: Ayurveda, Biscuit formulation, L-3,4-Dihydroxyphenylalanine, *Mucuna pruriens*, Neuronutraceuticals, Parkinson's disease, Seed-powder

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Introduction

Ayurveda is the ancient system of medicine practised in India. The approach to health is holistic, mainly through the integration of herbal remedies, dietary interventions, and lifestyle modifications. Ayurvedic medicines cure rather than provide symptomatic relief by improving basic metabolism, strengthening immunity, reducing stress, aiding detoxification, and, in short, aiding daily living by boosting cellular metabolism. Many major chronic and neurodegenerative diseases are cured with the Ayurveda system of therapeutics, including pre-preparation and penance, making it an invaluable treatment mode in this modern world. Ayurveda takes a unique approach to the treatment of these diseases with the help of procedures and medicinal treatment using herbal and herbo-mineral drugs. Classical Ayurveda texts describe *Kapikachu* (in Ayurveda) or *Mucuna pruriens* (*Mp*) as having rejuvenating, nootropic and aphrodisiac properties[1]. Traditionally, it is used for treating symptoms of Parkinson's disease (PD), including tremors, stiffness, and motor decline, and reflects Ayurvedic treatment insight into neurological dysfunctions[2].

Mp is a climbing annual legume belonging to the family Fabaceae, commonly known as 'velvet bean'. L-3,4-dihydroxyphenylalanine (L-DOPA) is a major bioactive phytochemical constituent present in *Mp* seeds, which is the gold-standard treatment for controlling the motor symptoms of PD. Earlier studies reported that whole *Mp* seed powder provided effective relief from PD symptoms with fewer side effects than synthetic L-DOPA; although issues with palatability and bulk dosing are noted as concerns[3,4]. There are also studies reporting that *Mp* seed powder had a faster

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onset and longer duration of action than standard L-DOPA/carbidopa, without increasing dyskinesia, indicating its potential as a safer alternative medicine for PD patients, sensitive to synthetic drugs[5]. The *Mp* formulation, 'HP-200' was studied by the global 'Parkinson's Study Group' and reported that a 12-week-long study using HP-200 significantly improved PD symptoms based on UPDRS scores, with only mild gastrointestinal side effects[6]. A clinical study using an Ayurvedic formulation containing *Mp*, *Withania somnifera*, *Sida cordifolia*, and *Hyoscyamus niger*, administered in milk, reported to improve UPDRS scores in PD patients[7]. Studies by Damodaran & Ramaswamy[8] and Kasture *et al*.[9] have consistently isolated L-DOPA from the seeds of *Mp*. It contains high levels of starch, protein, and fibre, enabling it to be utilised as a nutraceutical and functional medicinal ingredient in the formulation of various food products[10].

In Ayurveda therapeutics, drug preparations are of prime importance. *Bhaishajya Kalpana* is a well-developed discipline concerned with the formulation of ayurvedic medicines and dosage forms. In this branch of Ayurveda, several processing methods for drugs are mentioned, which can convert drugs into many forms or preparations to obtain the optimum medicinal benefits of the drugs, and also help prevent the development of disagreeability to a particular form of drug and improve the palatability of drugs. Due to the bulkiness of herbal medicines in their original form (in ground powder or suspension forms), and associated non-palatability, and difficulty in administering orally, it has become difficult to routinely consume these herbal medicines regularly for prolonged periods. Efforts to develop food-based nutritional supplements using herbs or herbal materials can serve as an alternative in this context. It is absolutely necessary that these formulations are viable and acceptable to patients.

Biscuits are the oldest and most popular bakery product, consumed by people of all ages worldwide. Some of the reasons for the acceptance are comparatively low cost, tastes can be created in acceptable flavours, and they will have a longer shelf life. Two of the major disadvantages of biscuits are typically high fat and refined sugar content, which are contraindicated for normal health. Nutritional and medicinal qualities of biscuits can be enhanced by the enrichment with ingredients such as medicinal plant extract or powder. With the positive, rapid growth of the biscuit industry, there is a huge scope for research into diversifying baked products into medicated products, such as biscuits. The use of herbal powders will not only enhance nutritional value but also help overcome the unpalatability of these ground forms and powders[11,12]. A unique study reported by Ezegbe *et al.* detailed the physicochemical parameters and sensory analysis of biscuits incorporated with up to 25% *M. pruriens* seed powder, providing direct methodological precedent for our 30% baked biscuit formulation, the study mainly focusing on L-DOPA contents in the raw seed powder, and its retention in the product, showcasing its therapeutic benefit[13].

Therefore, the primary objective of the present study is to assess the feasibility of making edible food products from *Mp* seed powder, while preserving its active constituents. The formulation is evaluated for its nutritional composition, sensory attributes, and retention of L-DOPA post-processing. Furthermore, our ongoing research aims to extend this work into preclinical and clinical studies to assess the safety, bioavailability, and therapeutic impact of this formulation. By combining Ayurvedic wisdom with contemporary food science, this research would contribute to the development of safe, acceptable, and effective dietary strategies for the management of neurodegenerative diseases.

Materials and Methods

Plant collection and identification

Seeds of *M. pruriens* were procured from Ambuja Institute of Ayurveda Research and Documentation (AIARD), Udayamperoor, Ernakulam, Kerala, India, and the purity and identity of the samples were authenticated by an Ayurveda *Dravya guna* (Materia Medica and Pharmacology) specialist, Dr. P. P. Dileep Kumar, of the Institute. (AIARD, Ernakulam, 23 Aug 2019).

Processing of seeds

Seed samples were washed and dried in the shade before powdering, employing an electric mixer. The seed powder was stored in air-tight glass containers at 30°C.

Preparation of *Mp*-seed powder-based food formulation

Biscuits were prepared by incorporating *the Mp* seed powder along with wheat flour. Butter and sugar (2.25 g each) were creamed well in a glass bowl. To this butter-sugar mixture, the powder blend (3.50 g wheat flour and 1.50 g *Mp* seed powder) along with

6.25 mg baking soda was added, and the mixture was mixed thoroughly in a blender. Water was added to make the dough, which was then rolled into sheets of uniform thickness. Circular uniform discs were cut using a tubular punching device into round pieces and baked in a microwave oven on a baking tray at 180°C. Each of these was baked for 12 minutes for uniform consistency. These baked cookies were allowed to cool to room temperature for 20 minutes and packed in air-tight containers, batch wise.

Proximate composition

An analytical study of the Mp seed powder and the biscuit formulation was conducted in the Quality Control Lab of the Cashew Export Promotion Council of India, Kollam, Kerala. The Proximate analysis included moisture content, total ash, acid-insoluble ash, water-soluble ash, alcohol-soluble extractives, and water-soluble extractives.

Determination of moisture content[14]

Weighed exactly (2.0 g) (w_1) powdered sample in a wide petri dish, transferred the petri dish into a hot air oven, and kept it for drying at 105± 5°C for 3 to 4 hours. The petri dish was removed and transferred to a desiccator. It was allowed to cool to room temperature. The weight (w_2) was noted then the heating, cooling, and weighing process was repeated until a constant weight (w_3) was obtained.

Moisture content[14] was calculated as $\{(w_2–w_3)/ w_1\}$x 100.

Determination of total ash

Accurately weighed (2.0 g) (w_1) powdered sample in a silica dish, and incinerated it at a temperature not exceeding 450°C until free from carbon, cooled and weighed. Since a carbon-free ash could not be obtained, the ash was washed with water, and the residue was collected on Whatman No. 1 ash-free filter paper; the filter paper containing the residue of the drug was dried and then ignited in a previously weighed silica crucible (w_2) at a temperature not exceeding 450°C till a constant weight was obtained (w_3). The percentage of total ash[14] with respect to *Mp* seed powder was calculated.

Percentage of Total ash = $\{(w_3-w_2)/ w_1\}$x 100.

Determination of acid-insoluble ash

Ash from an accurately weighed (2.0 g) (w_1) powdered sample was obtained by the same procedure given above, and the weight was determined. The ash obtained was boiled for five minutes with 25 mL 2.0 N HCl. The insoluble matter was collected on a Whatman No. 1 ash-free filter paper, washed with hot distilled water, dried and ignited in a previously weighed silica crucible (w_2) at a temperature not exceeding 450°C to get a consistent weight (w_3). The percentage of acid-insoluble ash[14] in the sample was calculated with reference to the dried drug.

Percentage of acid insoluble ash = $\{(w_3-w_2)/ w_1\}$x 100.

Determination of water-soluble ash

An accurately weighed (2.0 g) (w_1) powdered sample was incinerated to obtain the ash, which was then weighed. The insoluble matter was collected on ashless filter paper, washed with 25 mL of double-distilled water, dried, and ignited in a previously weighed silica crucible (w_2) at a temperature not exceeding 450°C to obtain a constant weight[14] (w_3). The percentage of acid-insoluble ash in the sample was calculated with reference to the dried drug, i.e.,
Percentage of water-soluble ash = $\{(w_3-w_2)/ w_1\}$x 100.

Determination of extractive values

Alcohol soluble extractives

Mainly represent the percentage of organic plant constituents, such as alkaloids, phenols, flavonoids, sugars, volatile oils, resins, steroids, and glycosides, present in the prepared sample.

Procedure

Exactly (5.0 g) of accurately weighed, air-dried (w_1), and powdered sample was macerated with 100 mL of alcohol (99%) in a closed flask for 24 hours. The contents were occasionally shaken for the first 6 h, which was then allowed to stand for the next 18 hours. After 24 hours, this was rapidly filtered separately, with precautions taken to prevent solvent loss. The filtrate was evaporated to dryness in two separate pre-weighed beakers and dried at 105°C. It was weighed again after heating, and weighing continued until a constant weight (w_2) was obtained. The percentage of cold alcohol-soluble extractive was calculated with respect to air-dried drug[14].

Percentage of extractive value = (w_2/w_1) x 100

Water-soluble extractives

The powdered sample (5.0 g) (w_1) was transferred into a round-bottom flask. About 100 mL of chloroform-water (100 mL distilled water + 2.5 mL

chloroform) was added, and the contents were shaken occasionally for 24 hours. It was then filtered rapidly, evaporated, and dried at 105°C to constant weight, and weighed (w_2). The percentage of water-soluble extractive was calculated with reference to air-dried drug[14].

Percentage of extractive value = (w_2/w_1) x 100

Phytochemical screening

Quantitative estimation of phenols and tannins was evaluated under phytochemical analysis[15].

Determination of total tannins

The tannin content of the sample was determined by the Folin Denis method. For preparing the extract, 1.0 g of the sample was mixed with distilled water at a 1:10 (w/v) ratio, agitated for 30 minutes at room temperature, and filtered. Then known amount of sample extracts was mixed with 5.0 mL of the Folin Denis reagent and Na_2CO_3 solution and made up to 100 mL. Incubated for 90 minutes at room temperature. Their absorbance was measured at 760 nm. The tannin content was expressed as mg tannic acid equivalents/100 mg of sample.

Determination of total phenols

Total phenols were determined by the Folin-Ciocalteu spectrophotometric method. For estimating total phenol content, a sample (200 mg) was extracted with 10 mL of methanol. The mixture was shaken for 30 minutes at room temperature. The mixture was centrifuged at 500 rpm for 15 minutes, and the supernatant (extract) was used for the analysis. One mL of the extract was treated with 0.5 mL of Folin-Ciocalteu reagent. After incubation for 5 minutes, 1 mL of 5% Na_2CO_3 solution was added, mixed well and kept in the dark for 1 hour. The absorbance was measured at 725 nm.

Nutritional profiling of the seeds

The nutritional profiling of the seed powder included the determination of various nutritional factors in the drug samples and biscuit products. Crude fibre, energy value, total protein, carbohydrates and total fat were analysed[16].

Estimation of crude fibre

For the determination of crude fibre, 1.0 g of the sample (w_1) was taken in a beaker and diluted with 50 mL of dilute sulphuric acid (2.5%). The mixture was heated to boiling. Boiling was continued for exactly 30 minutes. After boiling, the contents were filtered through fine linen held in a funnel and then washed with boiling water. The residue was then boiled in 50 mL of 2.5% sodium hydroxide solution for 30 minutes. After boiling, the flask was removed from the heating element and the contents were filtered. The residue was thoroughly washed with 20 mL of 99.8% ethanol, twice with 20 mL of diethyl ether and thrice with 20 mL of acetone. The insoluble residue obtained after these washings was dried at 105°C, weighed (w_2) and incinerated in a muffle furnace for 3 hours, and weighed (w_3).

Percentage of crude fibre = $\{(w_2-w_3)/w_1\}$x 100.

Estimation of total proteins

To determine crude protein content, a sample was initially digested using 1.0 g of the powdered sample in a Kjeldahl flask. To this, copper sulphate (0.45 g), potassium sulphate (15.0 g), and concentrated sulphuric acid (40 mL) with 2-3 small glass beads were added. For digestion of the sample, the flask was placed in an inclined position on the stand in the digestion chamber at 420°C for 30 minutes. After cooling the digested sample, 200 mL water was added slowly. The digested sample was placed in the distillation unit. A conical flask containing 25 mL of boric acid (containing indicator) was placed under the condenser outlet. The alkali (25 mL of 40% NaOH) was dispensed and distilled for 4 minutes. The ammonium borate solution formed was titrated with 0.1 M sulphuric acid to a purplish-grey endpoint. Using 0.1 M sulphuric acid for titration, % Nitrogen content = (0.28 X volume (mL) of 0.1 M sulphuric acid used in the titration) divided by weight of sample, 1.0 g.

% Protein = 6.25 x % nitrogen.

Estimation of carbohydrates

Total carbohydrates were calculated as follows, after determining the percentage of moisture (A), total protein (B), fat (C) and total ash (D).

Total Carbohydrates in % = 100 – (A + B + C + D)

Estimation of fat

Five grams of the sample (w_1) were accurately weighed in a thimble and placed in the Soxhlet apparatus dried at 100±2°C and weighed (w_2).

Extraction with petroleum ether for 16 hours in the Soxhlet apparatus was performed, and the solvent was evaporated from the flask on a water bath using a rotary evaporator. The traces of residual solvent were

removed by heating the flask in a hot-air oven for 30 minutes. The flask was cooled and weighed (w_3) to obtain total fat.

Crude fat, percent by mass =$\{(w_3-w_2)/ w_1\}$x 100.

Estimation of energy value

After determining the percentage of total protein, fat and carbohydrate, the energy Value was calculated as follows:

Energy value = (9 x fat%) + (4 x protein%) + (4 x carbohydrate%).

HPLC-coupled with an electrochemical detector for the analysis of L-DOPA

High-performance liquid chromatography for the analysis of L-DOPA was performed at the Centre for Drug Discovery Lab, Inter University Centre for Biomedical Research, and Super Speciality Hospital. Chemicals used: Pure L-DOPA (PHR 1271 Sigma-Aldrich), acetonitrile (catalogue no: 61830025001730, E-Merck), heptane sulphonic acid (catalogue no: 94373 SRL), triethylamine (catalogue no: T0886 Sigma-Aldrich), ethylenediaminetetraacetic acid (catalogue no: E5134, Sigma-Aldrich), orthophosphoric acid (Catalogue no: 5.43828.0250, E-Merck), n-Hexane (catalogue no: 1.04368.2521, E-Merck).

Extraction of M. pruriens seeds using 0.1 N HCl

Mp seed powder (1.5 g) was taken in a conical flask, and the powder was soaked in 50 mL of n-hexane for 48 hours, with the supernatant decanted and stored, and fresh solvent added every 24 hours. The entire supernatant was collected as an n-hexane extract. The residue was then kept under vacuum for 12 hours. The resulting residue was extracted in 0.1 N HCl by refluxing in a water bath at 80°C for 30 minutes[17]. The extract was decanted and stored at 4°C. For analysis of the contents, the extract was deproteinised with 0.4 M $HClO_4$ containing 0.01% EDTA, centrifuged, and the supernatant was diluted using 0.2 M $HClO_4$ containing 0.01% EDTA and assayed using HPLC coupled with an electrochemical detector[7].

Extraction of medicated formulation

Biscuits were weighed and powdered. Defatting was achieved by extracting the sample twice with n-hexane (25 mL). After n-hexane extraction, the residue was kept under vacuum (0.1 MPa) for 12 h. Further extraction was carried out twice with 50 mL of 0.1 N HCl by refluxing in a water bath at 80°C for 30 minutes. Extracts were pooled together, filtered, centrifuged, and made up to 100 mL with 0.1 N HCl. For L-DOPA analysis, the extract of medicated biscuit prepared was filtered through a 0.22 µm syringe filter and diluted 2000 times by adding 0.2 M $HClO_4$/0.01% EDTA. Ten microlitres of this diluted extract was injected into an HPLC HiCHROM Ultrasphere 5 IP column through a Rheodyne injector, connected to an electrochemical detection system, where the oxidation potential was kept at +740 mV.

HPLC analysis of L-DOPA content

Preparation of different concentrations of standard L-DOPA

A stock solution of L-DOPA was prepared at a concentration of 10 mM. L-DOPA concentrations (100, 200, 400, 600, 800 nM) were prepared in 0.1 M perchloric acid containing 0.01% EDTA, and 10 µL of each sample was injected into the HPLC system, equipped with an electrochemical detector.

HPLC conditions were: flow rate 1.2 mL/min, applied potential +0.74 V, and the sensitivity of detection set at 2 nA. Mobile phase composition was 8.65 mM heptane sulphonic acid, 0.27 mM ethylene-diaminetetraacetic acid, 0.4-0.45% triethylamine, 0.32-0.35% orthophosphoric acid, and 13% acetonitrile. pH was maintained at 3. The analytical column used was HiCHROM Ultrasphere 5 IP (250 x 4.6 mm, 5 µm particle size). Injections of the samples and the standards were manually carried out using a 25 µL air-tight Hamilton syringe through a Rheodyne injector[18].

Results and Discussion

Biscuits were formulated by blending *Mp* seed powder with other essential ingredients. Control biscuits were prepared using wheat flour alone (Fig. 1). From the seed powder, brown biscuits of homogenous texture were prepared. The seed powder contributed a darker colour than that of the conventional wheat-based biscuits, which can be attributed to inherent pigments or Maillard browning

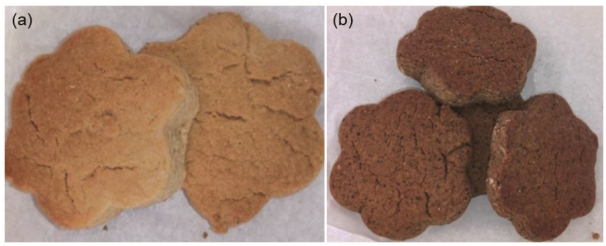

Fig. 1 — (a) Control biscuits, with a light colour of yellowish brown or mauve colour, and (b) Biscuits incorporating *Mp* seed powder along with wheat flour had a darker, Maillard chocolatey-brownish appearance.

during baking. The final appearance was satisfactory; the shape was well retained, and the surface colour was even, indicating good mixing and uniform heat and moisture transfer during baking. The surface had a slight graininess, probably contributed by the fibre component of the seed powder, but it did not detract from its appearance. Previous studies have also reported the inclusion of herbal ingredients at 30% while maintaining sensory acceptability[19,20].

Proximate analysis

The proximate analysis of the seed powder and the biscuits formulated using this powder is presented in Table 1. The parameters analysed included moisture, total ash, acid-insoluble ash, water-soluble ash, alcohol-soluble extractive, and water-soluble extractive.

The moisture content decreased from 9.7% in the powder to 3.7% in the biscuits, evidently due to dehydration during baking. The lesser moisture in biscuits could provide a better shelf life and significantly lower microbial spoilage chances. Moisture should be regulated to prevent decomposition, which may result in chemical changes and/or microbial infestation, which can affect the drug quality.

The ash value, which represents the total mineral mass, decreased slightly in the biscuits (2.6%) compared with the powder (3.4%), but was higher than in commercial biscuits (0.9%). The reduction could be caused by leaching out during mixing or slight degeneration at high temperatures. Still, the ash content indicated the retention of substantial mineral content, supporting the nutritional value of the product. Acid-insoluble ash represents the silica (especially as sand, siliceous earth) present in the sample. Water-soluble ash is the water-soluble portion of the total ash[21].

The extractive values provide the approximate measure of the chemical constituents present. Alcohol soluble and water-soluble extract values (2.5 and 13.4% respectively) indicate that the presence of water-soluble extractives is almost six times more than the alcohol-soluble extractives in the seed powder. The increase in water-soluble extractive values from seed powder to biscuits suggests that thermal processing during baking may have enhanced the release or solubility of bioactive compounds, including sugars, amino acids, and certain phenolic compounds. High water-soluble extractive value indicates the presence of acids, sugars and inorganic compounds, and high alcohol-soluble extractive value indicates the presence of medium polar constituents such as steroids, phenolics, flavonoids and glycosides. This enhancement in extractive values is beneficial, as it is suggestive of improved functional and nutritional quality of the final product[22]. Physicochemical viability and sensory acceptance at 25% incorporation of *Mp* seed powder were established by Ezegbe *et al.*[13]. The present study validates 30% incorporation and highlights the therapeutic value of biscuits containing *Mp* seed powder by assessing L-DOPA levels after baking. No study reports L-DOPA retention in *M. pruriens* seed powder or biscuit formulations post-baking. The present study demonstrates the nutraceutical properties of our *Mp*-based neuro-nutraceutical, a potential safe treatment option for PD patients.

Phytochemical screening

The qualitative and quantitative phytochemical analyses of seed powder and the biscuits developed from it revealed the presence of several bioactive compounds. Table 2 summarises the presence and concentrations of key phytochemicals, including phenols and tannins.

The *Mp* seed powder demonstrated a rich phytochemical profile, with the presence of phenols and tannins. Phenolic compounds are known for their strong antioxidant activities, which play a crucial role in preventing oxidative stress-related tissue damage, health issues and some common diseases. Previous studies have reported *Mp* seeds to have the potential as a natural antioxidant owing to the presence of higher phenolic content[23,24]. The reduction in its levels after baking (*i.e.*, from 2.9 to 0.08% for phenolics) is expected, as thermal processing can degrade heat-sensitive bioactive compounds[25].

Table 1 — Proximate composition of seed powder and formulated biscuits

S. No.	Parameters	*Mp* seed powder (in % value)	*Mp* biscuit (in % value)
1	Moisture content	9.7	3.7
2	Total ash	3.4	2.6
3	Acid-insoluble ash	0.5	Nil
4	Water-soluble ash	2.0	0.4
5	Alcohol-soluble extractive	2.5	31.1
6	Water-soluble extractive	13.4	40.3

Table 2 — Phytochemical contents of seed powder and formulated biscuit

S. No.	Parameters	*Mp* seed powder (%)	*Mp* biscuit (%)
1	Total phenolic content	2.9	0.080
2	Tannin	0.2	0.090

Tannins, while present in lower concentrations (0.2%), also contribute to the antioxidant effect. Previous studies have reported free radical scavenging activity and antioxidant effects of *Mp*, which were attributed specifically to tannins and flavonoids[15]. Their reduction during baking likely results from heat sensitivity and interactions with other components such as proteins and carbohydrates, which may affect the bioavailability of these valuable biomolecules in a patient treatment scenario.

Overall, the phytochemical profile confirms that the *Mp* seed powder is a rich source of natural bioactive and neuroactive molecules, and despite some losses during baking, the biscuits retain significant levels of these beneficial compounds. This supports their potential use as a neuro-nutraceutical aimed at delivering health benefits beyond basic nutritive addition.

Nutritional profile

The nutritional composition of *Mp* seed powder and the corresponding biscuits were evaluated based on key macronutrients: protein, fat, carbohydrate, dietary fibre, total sugars, and energy content (kcal). The values for each macromolecule from *the Mp* seed powder and biscuits are given in Table 3.

The seed powder exhibited high protein content (47%), indicating its potential as a valuable ingredient for protein-enriched food products. Upon baking into biscuits, the protein content decreased to 9.7%, which can be attributed to the possible denaturation during thermal processing. Nevertheless, the protein level in the biscuits remained slightly higher than in conventional wheat-based biscuits, which typically range from 7-8%[12].

Crude fat content was higher in the biscuits (17.4%) than in the seed powder (2.6%), likely due to the addition of butter during baking. Fat contributes to flavour and texture, enhancing the sensory qualities of the final product. Fibre content was significantly reduced from 6.5% in seed powder to 2.2% in biscuits. Heat treatment can cause the breakdown of insoluble fibres, and dilution with other ingredients may further reduce the value. Nevertheless, the fibre content remained high relative to standard biscuits (1.1%), which supports the *Mp* seed powder's role in gastrointestinal health[21].

Carbohydrate content increased in biscuits (47.6%) compared to seed powder (39.8%), largely due to the contribution of refined powder and sugar used in biscuit preparation. This increase also led to a higher energy value, rising from 361 kcal/100 g in the powder to 386 kcal/100 g in the biscuits. The carbohydrate content of the Mp biscuit (47.6%) was found to be comparably lower than that of standard commercial biscuit products (50.2%)[21]. In summary, the nutritional profile of the biscuits formulated from *Mp* seed powder demonstrates a functional balance of macronutrients. While baking and formulation lead to some nutrient losses (notably protein and fibre), the final product remained a nutrient-dense, high-energy snack.

Mp seed powder demonstrated nutritional composition (proteins & fibre) with a superiority with respect to the protein content than that of non-germinated *Mp* nutritional composition, and with regard to common legume pulses (soybean, cowpea), *Mp* exhibited higher nutritional value. These superior seed nutritional characteristics confirm the practicability for 30% biscuit fortification, positioning *Mp* as a suitable PD patients' nutraceutical with added therapeutic benefits[26-28].

The study reported by Sánchez-Velázquez *et al.*[29], revealed the impact of physical and chemical processing on the *in vitro* protein quality, levels of bioactive compounds, and antioxidant potential of selected pulses. They have concluded that baking-induced protein reductions accompany significantly enhanced digestibility of the remaining proteins and improved phytochemical liberation. These points validate our observed decrease in *Mp* biscuit protein, indicating optimal processing for enhanced bioavailability and increased extractive values. Siddhuraju and Becker[30] also reported a similar thermal processing effect of *Mp* seeds.

Table 3 — Nutritional composition of seed powder and formulated biscuit

S. No.	Parameters	*Mp* seed powder (%)	*Mp* biscuit (%)
1	Total protein	47	9.7
2	Total fat	2.6	17.4
3	Carbohydrates	39.8	47.6
4	Crude fibre	6.5	2.2
5	Energy	361 Kcal	386 Kcal

Determination of L-DOPA content in *Mp* seed powder and biscuit formulation employing HPLC

L-DOPA standard curve is plotted for a sensitivity of 1 to 8 pmol/10 µL as shown in Fig. 2. With the help of a standard curve prepared for L-DOPA concentration *vs*. peak area under the curve for different concentrations of L-DOPA for the set HPLC conditions, the quantification of L-DOPA in the

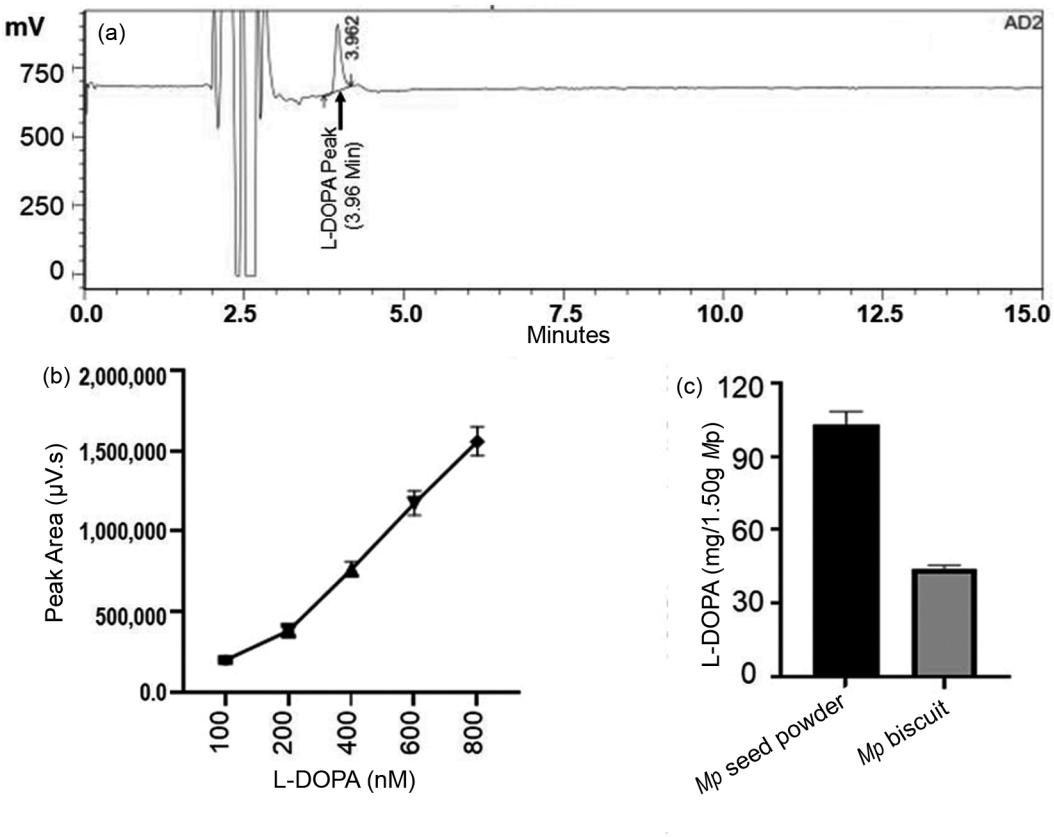

Fig. 2 — (a) Representative HPLC chromatogram of L-DOPA standard showing the peak at a retention time of 3.8 minutes, (b) HPLC-Electrochemical detection made for standard concentrations (1.0-8.0 pmol/10.0 µL), and (c) Quantitative analysis of *Mp* seed powder and the medicated formulation; L-DOPA content was determined employing an HPLC-electrochemical detection method.

samples were achieved. A representative chromatogram of a L-DOPA standard of known concentration is shown in Fig. 2a.

The extract of *Mp* seed powder in 0.1 N HCl was golden yellow in colour. When analysed using HPLC-electrochemistry, it showed the L-DOPA content to be in the range 92.2 - 116.5 mg for 1.5 g of *Mp* seed powder, which corresponds to 6.15-7.7%. Many previous studies have evaluated L-DOPA content in *Mp* seed extracts employing varied, yet sensitive methods. An earlier study reported the L-DOPA content using HPTLC in 0.1 N HCl extract of different varieties of *Mp* seed powder, and the level ranged from 2.23-5.36%[17]. In the study by Katzenschlager *et al.*, L-DOPA content of *Mp* preparation employing chromatographic method was reported to be 4.86%[5]. In another study, aqueous extract of powdered *Mp* seeds showed 6.5% of L-DOPA content[31]. Quantitative analysis of *Mp* extract using HPTLC densitometry scanning and digital imaging method detected 5.6% L-DOPA[32]. Mugendi *et al.*[33] determined L-DOPA in the range of 6.7-7% when the HPLC method was employed. These studies also reported other compounds such as phenols (7%), phytates (0.9%) and trypsin-inhibitor unit activity (5.1 TIU).

The study by Nagashayana *et al.*[7] reported L-DOPA content in *Mp* to be 2.86-3.1% in an Ayurveda formulation in milk, which was prepared for patient consumption. Our present study reports comparatively higher L-DOPA content in *Mp* seed powder. Concentration of L-DOPA in the range of 100 nM to 800 nM showed a sensitivity of 1 to 8 pmol/10 µL. Regression analysis showed a highly significant correlation coefficient (R^2 = 0.9993592), as seen in Fig. 2b. HPLC-electrochemical detection analysis of *Mp*-medicated biscuits (prepared using 1.5 g of seed powder) showed a concentration of L-DOPA in the range of 41.50-51.50 mg, as seen in Fig. 2c.

The biscuit formulation containing 1.5 g *Mp* seed powder was found to contain 2.77-3.43% L-DOPA, which is 55% lower than the initial L-DOPA content in the seed powder. It clearly indicated that L-DOPA is reasonably resistant to the thermal processing conditions encountered in the present processes;

however, high temperature during the baking process may cause a reduction in the level of L-DOPA[34].

Three to six grams of *Mp* seed powder could be consumed daily in accordance with Ayurveda principles[2]. Given that each biscuit contains 1.5 g of *Mp* seed powder, consuming four biscuits at a time could add about 6 g of the herbal powder. Such a dosage strategy guarantees an effective normal prescribed dose of L-DOPA medication, in accordance with the recommended therapeutic dosage, since 45% of the active molecule is retained after baking processes.

A previous study developed *Mp*-based effervescent powder and suspension, as new drug delivery platforms and analysed their physical, chemical, and microbiological stability. They have reported L-DOPA stability more in powders than in suspensions. Nanogel-jelly formulation developed from *Mp* extract provided efficient oral L-DOPA delivery, demonstrating physical/chemical stability and neuroprotective efficacy in PD animal models[34,35].

Vaidya *et al.*[3] also conducted one of the first clinical investigations of whole *Mp* seed powder (40–60 g/day) in PD patients. The treatment achieved similar motor symptom control with fewer side effects, including reduced motor complications. The preparation was well tolerated overall, although several participants reported an unpleasant taste and complained of the bulkiness of the dose. The study emphasised *Mp* as a potentially more natural and safer source of L-DOPA than synthetic levodopa. However, the palatability and dosing practicality (bulk and taste) were obvious limitations, emphasising the necessity of more palatable delivery formats, such as standardised extracts or food-based preparations[3]. Muralidharan and Warrier[4] reported a case of Parkinsonism in which an Ayurvedic management protocol comprising combined external treatment and an oral dose of *Mp* seed powder (10 g/day) over a 10-month period provided significantly moderate relief of symptoms in an 8-year chronic case study[4]. Emerging clinical evidence suggests that higher doses of *Mp* are well tolerated in humans. HP-200, an Ayurveda formulation, derived from *Mp* endocarp, at a dose of 7.5 g thrice daily was well-tolerated by PD patients and the treatment showed improvement in the disease symptoms[6]. For instance, a study by Katzenschlager *et al.* demonstrated that a single 30 g dose of *Mp* powder resulted in a faster onset of action and longer duration of motor response compared to standard L-DOPA/carbidopa therapy, without a significant increase in dyskinesias or adverse effects[5]. These clinical findings indicate that increasing the *Mp* dosage beyond traditional limits may be feasible and safe, particularly when aiming to compensate for L-DOPA losses during food processing. However, it is essential to monitor patients for potential side effects, such as dyskinesias, and required to adjust dosages accordingly.

Conclusion

Findings from the present study highlight the potential of *Mp* seed powder as a valuable neuro-nutraceutical, particularly for PD patients. The *Mp* seed powder contained a rich profile of proteins, dietary fibres and phytochemicals, including significant levels of L-DOPA, a natural precursor to dopamine and a key therapeutic drug moiety prescribed in modern Allopathic medicine, for PD management. HPLC analysis confirmed that L-DOPA remained present even after lengthy procedures and baking, suggesting that nutraceuticals such as *Mp*-seed powder-enriched biscuits could serve as a diet-cum-medicine source of this neuroactive compound for PD patients. Incorporation of *Mp* powder into biscuits enhanced the nutritional and functional profile of the final product. The inherent neuroprotective phytochemicals, such as phenols, tannins, natural proteins, and L-DOPA, significantly enhance its therapeutic value. The present study strongly supports the feasibility of developing *Mp*-based edible neuro-nutraceuticals for treating PD patients, providing additional natural dietary supplements that may complement conventional pharmacotherapy.

Conflict of interest

No conflict of interest to state.

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