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Evaluation of L-dopa, proximate composition with *in vitro* anti-inflammatory and antioxidant activity of *Mucuna macrocarpa* beans: A future drug for Parkinson treatment

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ABSTRACT

Objective: To investigate *L*-3, 4-dihydroxyphenylalanine (L-dopa, anti-Parkinson drug), anti-inflammatory activity, proximate nutritional composition and antioxidant potential of *Mucuna macrocarpa* (*M. macrocarpa*) beans.

Methods: L-dopa content was determined and quantified by high performance thin layer chromatography and reversed phase high-performance liquid chromatography (RP-HPLC) methods. Anti-inflammatory activity was performed by *in vitro* protein denaturation inhibition and human red blood cell membrane stabilisation activity. Proximate composition and elemental analysis were also investigated. The antioxidant potential (2,2-diphenyl-1-picrylhydrazyl, N-N-dimethyl-phenylenediamine and ferric-reducing antioxidant power) of *M. macrocarpa* beans were evaluated by using different extraction solvents. The RP-HPLC analysis also quantified significant phenolics such as gallic acid, tannic acid, *p*-hydroxybenzoic acid and *p*-coumaric acid.

Results: RP-HPLC quantification revealed that *M. macrocarpa* beans contain a high level of L-dopa [(115.41 ± 0.985) mg/g] which was the highest among the *Mucuna* species from Indian sub-continent. Water extract of seed powder showed strong anti-inflammatory and antioxidant potential. Proximate composition of *M. macrocarpa* beans revealed numerous nutritional and anti-nutritional components. RP-HPLC analysis of major phenolics such as tannic acid (43.795 mg/g), gallic acid (0.864 mg/g), *p*-coumaric acid (0.364 mg/g) and *p*-hydroxybenzoic acid (0.036 mg/g) quantified successfully from *M. macrocarpa* beans respectively.

Conclusions: This study suggests that *M. macrocarpa* is a potential source of L-dopa with promising anti-inflammatory, antioxidant and nutritional benefits.

1. Introduction

Parkinson's disease is the second most neurological disorder occurs after the Alzheimer's disease [1]. Degeneration of dopaminergic neurons and Lewy body's formation in the substantia nigra pars compacta region of the brain are the main hallmark of Parkinson's disease [2]. Some factors have a critical role in the development of Parkinson's disease like age, genetics, exposure to an excess of iron, pesticides, fungicides and toxins [3]. Mechanism of dopaminergic cell degeneration involves microglial activation, excitotoxicity, oxidative stress, neuroinflammation and mitochondrial dysfunction which ultimately leads to apoptosis [4].

L-3, 4-dihydroxyphenylalanine (L-dopa) is the principal drug used for the management of Parkinson's disease at earlier stages of disease progression. There are several sources of L-dopa (synthetic and biological). Plants are the most popularly used source to treat Parkinson's disease due to their advantage of

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comprising several other metabolites including phenolics, flavonoids and alkaloids which assist treatment through various pathways [5]. *Mucuna pruriens* (*M. pruriens*), a well-known species from genus *Mucuna* is commercialised for L-dopa extraction and has been successfully exploited for the Parkinson's disease management [6]. Genus *Mucuna* comprises nine species and four varieties throughout India [7]. Unfortunately, most of the species have been remained neglected for their investigation regarding their valuable properties. As Parkinson's disease occurrence is continuously rising, the demand of L-dopa is also increasing concurrently. Hence, the finding of novel potential L-dopa sources is of great importance.

Inflammation is a crucial function of the immune system to resist microbial infections and to provoke anti-inflammatory molecules. Several chronic disorders such as Alzheimer disease and Parkinson's disease happen due to long-term neuroinflammation [8]. For the treatment of inflammatory disorders, various drugs are available in the market. However, long-term use of these drugs may lead to immunodeficiency and gastrointestinal disorder [9]. In this direction, investigation of new anti-inflammatory molecules with potential for the alleviation of inflammation is an essential task. Plants are the excellent source of polyphenols, terpenoids, anthocyanidins, carotenoids, flavonoids, glucosinolates, isoflavonoids, and phytoestrogens. These molecules have health beneficial effects through antioxidant and anti-inflammatory mechanism [10].

Free radicals produced in cellular metabolism lead to the pathogenesis of Parkinson's disease by producing reactive oxygen and nitrogen species [11]. Brain cells are highly vulnerable to oxidative stress because of polyunsaturated fatty acids in the cell membrane, poor catalase, superoxide dismutase and glutathione peroxidase activity and higher content of iron generating free radicals [12]. Researches on animal models of Parkinson's disease, cell culture, postmortem brain tissue and the investigation of human genetics demonstrates the contributions of oxidative stress and mitochondrial dysfunction in Parkinson's disease development [13]. Free radicals generate oxidative stress which leads to cell impairment and destruction [14]. To reduce the oxidative stress, supplementation of external antioxidant is required. Several secondary metabolites such as phenolics, flavonoids, alkaloids derived from plants show superfluous antioxidant potential [15]. In Indian system of Ayurveda, various medicinal plants with surplus antioxidant activity have been reported and extensively used for the management of neurodegenerative diseases [16].

Mucuna macrocarpa (*M. macrocarpa*) is a species predominately found in Taiwan and Southeastern Asia including India and China [17]. It is a large and woody climber, up to 70 m long stem, 20–25 cm in diameter, trifoliate leaves and 5–25 cm long inflorescences. Pods are woody, linear straight or slightly curved (40–42 cm long) having 9–13 seeds per pod. Seeds are flattened round and brown with hard seed coat and an average weight of 5–7 g [17]. Recently, anti-leukaemic, antioxidant and antimicrobial properties of *M. macrocarpa* bark and root extract have been reported [18]. However, *M. macrocarpa* seed has remained uninvestigated for its enormous medicinal and nutritional properties. Hence, the present study attempted to evaluate L-dopa content, proximate nutritional composition, anti-inflammatory and antioxidant potential of *M. macrocarpa* beans.

2. Materials and methods

2.1. Standard chemicals and reagents

The chemicals, standard and reagents used in the present work were of analytical and high performance liquid chromatography grade. 2, 2-diphenyl-1-picrylhydrazyl (DPPH), 2, 4, 6-tripyridyl-s-triazine, N-N-dimethyl-phenylenediamine (DMPD), ammonium acetate, and acetonitrile were purchased from Sigma (St. Louis, MO, USA). Ferric chloride and other chemicals were procured from S.D. Fine Chemicals Ltd. (Mumbai, India) and Himedia (Mumbai, India). The reference standards for phenolics *viz*., gallic acid, tannic acid, 3, 4-dihydroxybenzoic acid, *p*-coumaric acid, vanillic acid and ferulic acid were purchased from Himedia (Mumbai, India).

2.2. Collection of plant material

The *M. macrocarpa* seeds were collected from Darjeeling, West Bengal, India. Herbarium of plant material maintained at the Botany Department, Shivaji University, Kolhapur (Voucher No.-Swaroopsingh 030 SUK). The *M. macrocarpa* seeds were grinded (after removing seed coat) in a mechanical grinder to obtain fine powder and stored at −20 °C refrigerator for further analysis.

2.3. Qualitative and quantitative analysis of L-dopa

Qualitative and quantitative investigation of L-dopa was conducted according to the method described by Rathod *et al.* [19]. Shortly, 50 mg of *M. macrocarpa* powder was mixed with 15 mL 0.1 N aqueous HCl and mixture then sonicated for 20 min. Further, the volume was adjusted to 50 mL with 0.1 N HCl. The sample was then filtered through 0.45 mm nylon filter (Axiva filters). The filtered sample was used for the qualitative analysis by using high-performance thin layer chromatography (HPTLC) (E. Merck, Darmstadt, Germany). The sample was spotted on the silica-coated aluminium sheet (10 cm × 10 cm with 0.2 mm thickness) using a Camag Linomat V automatic sample spotter (Muttenz, Switzerland). *n*-butanol, acetic acid and water were used as mobile phase at 4:1:1 proportion [20]. Thin layer plates scanning was done at 280 nm with the help of Camag thin layer scanner III.

Reverse phase high-performance liquid chromatography (RP-HPLC) analysis was performed by Shimadzu prominence unit with degasser DGU-20A 5R, low-pressure quaternary pump LC 20 AD and SPD-M20A as a photo diode array detector. Water, methanol and acetonitrile (5:3:2) containing 0.2% triethylamine used as mobile phase having pH 3.3 [19]. A total of 20 µL volume was used for the injection. Nova-Pak C18 column (4 µm, 4.6 mm × 250 mm) from water (Milford, USA) was used for the chromatographic separation.

2.4. In vitro anti-inflammatory activity

Sample preparation was carried out by adding 100 mg *M. macrocarpa* seed powder in 10 mL distilled water, extracted in mortar petal and centrifuged at 10 000 rpm for 10 min. The supernatant was collected and calculated the effective yield. Further, the supernatant was kept in the refrigerator until further analysis.

2.4.1. Bovine serum albumin (BSA) anti-denaturation assay

BSA protein anti-denaturation assay was performed according to the method described by Grant *et al.* [21] with minor changes. In brief, 1 mL of extracted samples of *M. macrocarpa* bean with different concentrations was allowed to react with 1 mL of BSA solution (1% BSA in 50 mM Tris buffer, pH 6.5). Diclofenac was used as a standard anti-denaturating drug with various concentrations. The reaction mixture was kept at room temperature for 20 min for the incubation and then kept to 64 °C in water bath for 5–10 m till mixture got turbid. After cooling, the absorbance of the reaction mixture was recorded at 660 nm by spectrophotometer to measure the turbidity. Distilled water was used as a control. The experiment was conducted in triplicate number for statistical analysis. The percentage of protein denaturation inhibition was calculated as follows:

$$\%\ \text{denaturation inhibition} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

where, $A_{Control}$ stands for absorbance of the control and A_{Sample} stands for absorbance of the sample.

2.4.2. Human red blood cell membrane stabilisation test

Freshly collected 10 mL of human blood sample was centrifuged at 3 000 rpm for 10 min to obtain packed red blood cells. The collected red blood cells were then washed for three times with equal volume of normal saline by centrifugation at 3 000 rpm for 10 min. The collected human red blood cell membranes were measured and re-constituted with normal saline to obtain 10% v/v suspension [22].

The hypotonicity induced haemolysis of human red blood cell membrane was performed according to Bhurat *et al.* [23]. In short, the reaction solution consists of 2 mL hypo saline, 1 mL 0.15 M phosphate buffer (pH 7.4), 1 mL *M. macrocarpa* extract (200 µg–600 µg/mL) in an isosaline and 0.5 mL 10% reconstituted human red blood cell. Distilled water was used to prepare control instead of hypo saline. The mixture then was kept at room temperature for 30 min and centrifugation was carried out at 3 000 rpm for 20 min. The supernatant was collected and used to measure the haemoglobin content at 560 nm by a spectrophotometer. Aspirin was used as a standard drug.

$$\text{Percentage of stabilization} = 100 - \frac{A_{test}}{A_{control}} \times 100$$

where, A_{test} stands for absorbance of test and $A_{control}$ stands for absorbance of control.

2.5. Biochemical analysis

2.5.1. Sample preparation

M. macrocarpa seed powder (200 mg) was added with 10 mL D/W and kept on rotator shaker for 3 h at room temperature and extracted using mortar and pestle. Then centrifugation was carried out at 8 000 rpm for 15 min, the supernatant was collected and sediment re-extracted. After the 2–3 times centrifugation, supernatant collected altogether and kept in the refrigerator for further biochemical analysis.

2.5.2. Nutritional and anti-nutritional parameters analysis

The prepared sample was used for the analysis of various nutritional and anti-nutritional parameters. Total ash content of *M. macrocarpa* seed powder was analysed by the Association of Official Agricultural Chemists method [24]. Total solid was determined as described by James [25]. Crude fibre content of the beans was calculated according to Association of Official Agricultural Chemists method [26]. Protein and carbohydrate content was estimated by using Lowry's method [27] and Anthrone method [28]. The fat content of the sample was estimated by extracting sample in diethyl and petroleum ether solution and results were expressed in percentage of fat according to the method described by James [25]. The energy content of the sample was determined according to the formula described by Carpenter *et al.* [29] with considering proximate analysis data. Starch content of the seed flour was estimated by McCready *et al.* method [30]; in short, the powder was treated with 80% ethanol and perchloric acid was added to extract the starch. The proanthocyanidin content was estimated according to a method of Sun *et al.* [31] and results were expressed as catechin equivalents per gram (mg CAE/g) of dry weight.

Tannin and phytic acid content of the seed was quantified by the Folin-Denis colorimetric [32] and Gao *et al.* method [33]. Total saponin was determined according to a method of Harborne *et al.* [34].

2.5.3. Mineral determination

Mineral quantification of seed powder was carried out by preparing ash of seed flour using Bunsen flame for 20 min. The prepared ash was kept in cleaned porcelain crucibles at 550 °C in a muffle furnace to analyse the metal content of the sample by using atomic absorption spectrophotometer (Perkin–Elmer 8650). The phosphorus content of the digested sample was estimated according to by Nahapetian and Bassiri method [35].

2.6. Total phenolic content, total flavonoids content and antioxidant potential

M. macrocarpa seed powder was extracted in four different extracting solvents (water, methanol, ethanol and acetone) at the concentration of 1 mg/mL. Samples were prepared using shaking at (120 ± 2) rpm for 2 h and sonicated for 10 min in a sonication bath. The extracted sample then was centrifuged at 10 000 rpm for 15 min and the supernatant was collected. The centrifugation procedure was repeated twice. The supernatant was pulled together, and effective yield of respective sample was calculated and diluted to known concentration for further study.

2.6.1. Total phenolics content and flavonoids content

The total phenolics content of *M. macrocarpa* seed powder was quantified spectrophotometrically according to a method of Singleton and Rossi [36]. Gallic acid was used as standard and results were expressed per gram (mg GAE/g) of dry weight. The flavonoids content was determined by the method of Chang *et al.* [37]. Total flavonoids content was calculated by comparing quercetin as a standard and results were expressed as milligram of quercetin equivalent to per gram of dry powder.

2.6.2. Antioxidant activity study

Radical scavenging activity of different solvent extracted *M. macrocarpa* seed powder samples was estimated by DPPH [38], DMPD [39] and ferric-reducing antioxidant power (FRAP) [40] assay. DPPH and DMPD activity was expressed in terms of percent radical scavenging activity, whereas, FRAP activity in terms of optical density at 593 nm.

2.7. RP-HPLC-DAD determination of phenolic acids

Sample preparation for the quantification of phenolics was done by continuous shaking with constant stirring for (110 ± 2) rpm at room temperature [(25 ± 2) °C]; methanol was used as a solvent for the extraction. The extracted sample was then passed through 0.45 mm nylon filter (Axiva filters), the final volume was adjusted with methanol and stored in the amber vial at 4 °C until RP-HPLC analysis. A solvent system consisting of water, acetonitrile and glacial acetic acid in the 90:5:5 proportion [41] and 20 µL was used as injection volume. The flow rate of mobile phase was 1 mL/min with 15 min run time and 280 nm was used as absorbance channel.

2.8. Statistical analysis

All experimental analysis was performed in triplicates and results were calculated as a mean ± SD value. The data were statistically analyzed by GraphPad Prism 5 software using Dunnett multiple range test to check the significant differences among the mean values.

3. Results

3.1. Qualitative and quantitative analysis of L-dopa

The qualitative (HPTLC) (Figure 1) and quantitative (HPLC) analysis of L-dopa from *M. macrocarpa* beans were carried out. The qualitative HPTLC evaluation confirmed L-dopa content in the seed sample. RP-HPLC quantification revealed that *M. macrocarpa* beans contain a high level of L-dopa [(115.410 ± 0.985) mg/g] (Figure 2).

3.2. In vitro anti-inflammatory potential

In the present research, *M. macrocarpa* seed powder showed strong protein anti-denaturation activity. Albumin denaturation inhibition activity of *M. macrocarpa* sample at various concentrations has been shown in Table 1. *M. macrocarpa* water extract showed strong inhibition of protein denaturation (90.13% ± 6.25%) at 500 µg/mL concentration, whereas, standard drug diclofenac showed (98.35% ± 5.12%) inhibition at 500 µg concentration. *M. macrocarpa* seed powder was also examined on the human red blood cell membrane stabilisation activity and showed excellent anti-inflammatory activity in a concentration-dependent manner (Table 1). The *M. macrocarpa* bean showed maximum membrane stabilisation activity (59.53% ± 4.23%) at 500 µg/mL concentration. However, aspirin was used as a standard drug and showed (62.35% ± 1.35%) activity for the same concentration.

3.3. Biochemical analysis

3.3.1. Nutritional and anti-nutritional factors analysis

Proximate composition of *M. macrocarpa* beans revealed numerous nutritional and anti-nutritional components. The composition of seed showed ash (23.0 ± 4.5) mg/g, total solid (957.5 ± 11.6) mg/g and fibre (74.8 ± 2.8) mg/g. The total protein content of *M. macrocarpa* seed was (90.7 ± 2.8) mg/g. It was observed that *M. macrocarpa* seed contains a moderate level of total carbohydrate (123.0 ± 8.2) mg/g. The total calorific value of seed was 95.56 kcal. The crude fat and starch contents were (11.20 ± 1.27) and (29.00 ± 2.61) mg/g respectively. The *M. macrocarpa* seed showed (36.0 ± 4.7) mg/g of proanthocyanidin. *M. macrocarpa* bean also revealed major anti-nutritional factors. Seed powder showed (97.2 ± 6.4) mg/g of phytic acid, (136.60 ± 27.12) mg/g of tannin and (47.2 ± 5.8) mg/g of saponin level. The mineral analysis of *M. macrocarpa*

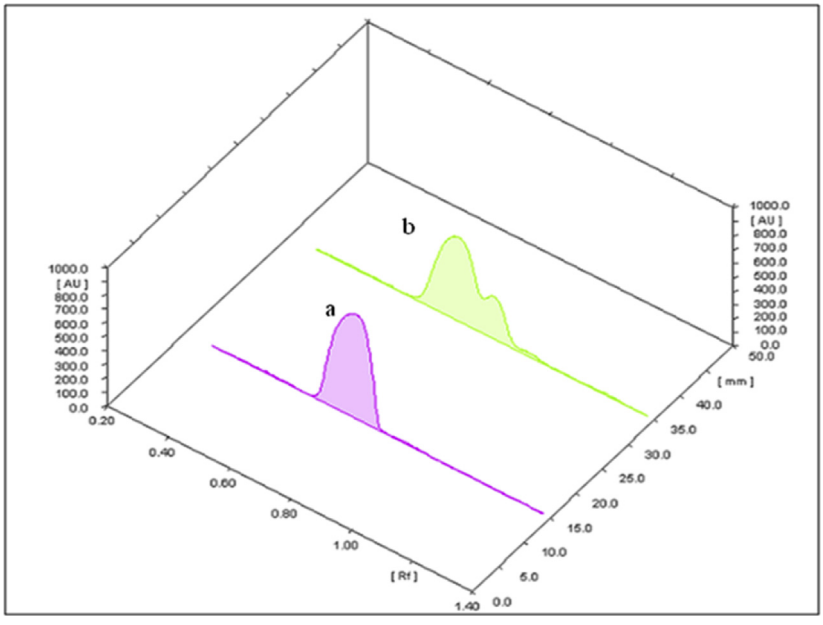

Figure 1. HPTLC analysis of *M. macrocarpa* seeds for L-dopa.
HPTLC chromatograms. (a) Std L-DOPA; (b) *M. macrocarpa* seed sample (n = 3).

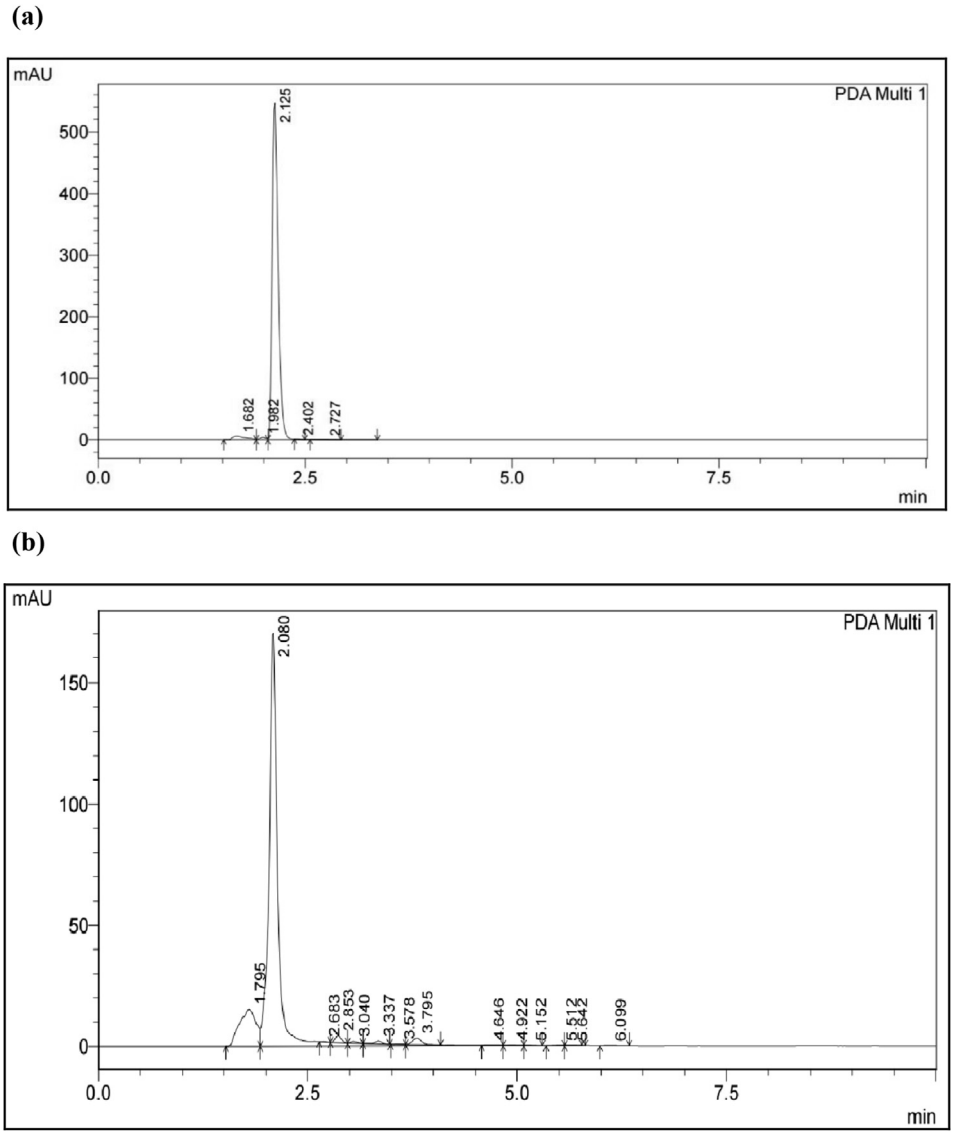

Figure 2. HPLC analysis of *M. macrocarpa* seeds for L-dopa.
(a) HPLC profile of standard (L-dopa); (b) HPLC profile of *M. macrocarpa* seeds (n = 3).

Table 1
Human red blood cell membrane stabilisation activity and heat induced BSA anti-denaturation activity of *M. macrocarpa* seed (mean ± SD) (n = 3).

Concentration (µg)	Aspirin[a]	*M. macrocarpa*[a]	DCF[b]	*M. macrocarpa*[b]
100	42.62 ± 3.60	32.33 ± 3.85	66.11 ± 5.53	61.23 ± 4.23
200	49.56 ± 1.34	44.25 ± 4.76	77.77 ± 4.56	72.68 ± 5.21
300	54.25 ± 4.23	48.04 ± 3.76	88.47 ± 6.21	81.87 ± 5.89
400	58.21 ± 2.78	52.76 ± 2.49	95.82 ± 6.45	85.40 ± 4.84
500	62.35 ± 1.35	59.53 ± 4.23	98.35 ± 5.12	90.13 ± 6.25

[a] % Human red blood cell membrane stabilisation activity. [b] % inhibition of protein denaturation.

beans explored high calcium and sodium level. The content of nitrogen, phosphorus, potassium, sulphur, and magnesium was in moderate level. The trace elements iron, boron, copper, manganese, zinc, iron, molybdenum were also recorded at a lesser concentration (Table 2).

3.4. Total phenolics and total flavonoids content

In the present research work, total phenolics content and total flavonoids content were evaluated from *M. macrocarpa* beans by using four different solvents (Table 3). The *M. macrocarpa* water extract showed higher phenolic and flavonoids content *viz*., (103.030 ± 1.122) mg GAE/g and (129.166 7 ± 0.956 0) mg CHE/g, while *M. macrocarpa* methanol extract showed (87.060 ± 0.695) mg GAE/g and (139.166 7 ± 1.238 0) mg CHE/g respectively.

3.5. Antioxidant potential

3.5.1. DPPH free radical scavenging activity

The DPPH radical scavenging activity of differentially extracted *M. macrocarpa* seed was carried out in comparison

Table 2
Minerals content of *M. macrocarpa* seed.

Parameters	Analysis
Nitrogen %	0.22
Phosphorous %	0.19
Potassium %	0.17
Calcium %	3.50
Magnesium %	0.14
Sulphur %	0.18
Sodium %	8.75
Zinc PPM	131.89
Ferrous PPM	≤1.00
Copper PPM	91.00
Manganese PPM	209.97
Molybdenum PPM	1 133.70
Boron PPM	4.87

Table 3
Total phenolics (mg GAE/g) and total flavonoids (mg CHE/g) content of *M. macrocarpa* seed (mean ± SD) (n = 3).

Solvents	Phenolics (mg GAE/g)	Flavonoids (mg CHE/g)
MMWE	103.03 ± 1.12	129.16 ± 0.95
MMME	87.06 ± 0.69	139.16 ± 1.23
MMEE	66.96 ± 0.95	91.66 ± 1.02
MMAE	14.84 ± 0.68	14.66 ± 0.48

MMWE: *M. macrocarpa* seed water extract; MMME: *M. macrocarpa* seed methanol extract; MMEE: *M. macrocarpa* seed ethanol extract; MMAE: *M. macrocarpa* seed acetone extract.

with ascorbic acid as an internal standard. Increase in antioxidant activity with a simultaneous increase in sample concentration was observed. *M. macrocarpa* water extract showed (18.92% ± 1.86%) activity at 10 µg concentration and (89.46% ± 1.76%) at 50 µg (Table 4). DPPH radical scavenging activity of *M. macrocarpa* methanol extract was ranged from (16.03% ± 1.86%) to (86.04% ± 3.61%) respectively for the lower and higher concentration of sample under study. *M. macrocarpa* ethanol extract showed (14.11% ± 0.93%) to (78.04% ± 3.95%) while *M. macrocarpa* acetone extract contains low DPPH radical scavenging activity (8.69% ± 0.90%) to (45.46% ± 2.84%). The ascorbic acid showed (23.13% ± 1.97%) and (97.65% ± 3.52%) activity at the 20 µM and 100 µM concentration respectively. The order of DPPH radical scavenging activity for the different solvents was water > methanol > ethanol > acetone.

3.5.2. DMPD radical scavenging activity

The DMPD radical scavenging potential of *M. macrocarpa* seed powder using different solvents has been depicted in Table 6. *M. macrocarpa* water extract showed (8.98% ± 1.05%) activity at 20 µg concentration while (44.84% ± 3.10%) at 100 µg. *M. macrocarpa* methanol extract showed (8.30% ± 0.32%) activity at 20 µg and (40.23% ± 2.80%) at 250 µg concentration. *M. macrocarpa* ethanol extract and *M. macrocarpa* acetone extract showed negligible DMPD radical scavenging activity. The ascorbic acid showed (10.02 ± 1.02)% and (51.34 ± 2.81)% activity at 50 µM and 250 µM concentration respectively. The trend of DMPD radical scavenging activity for the different solvents was water > methanol > ethanol > acetone (Table 5).

3.5.3. FRAP activity

Table 6 represents ferric reducing antioxidant potential of *M. macrocarpa* beans in form of absorbance values. In the present study, *M. macrocarpa* methanol extract showed good

Table 4
DPPH radical scavenging activity of *M. macrocarpa* in percentage (%) (mean ± SD, n = 3).

Concentration (µg)	Std. Ascorbic acid	Water	Methanol	Ethanol	Acetone
10	23.13 ± 1.97	18.92 ± 1.86	16.03 ± 1.86	14.11 ± 0.93	8.69 ± 0.90
20	41.42 ± 1.84	34.12 ± 1.16	30.29 ± 1.93	25.96 ± 2.13	14.56 ± 0.73
30	64.88 ± 1.90	55.01 ± 1.93	52.63 ± 2.05	45.92 ± 2.92	26.45 ± 2.10
40	86.85 ± 2.52	68.56 ± 1.61	65.01 ± 3.14	60.88 ± 2.88	33.47 ± 2.31
50	97.65 ± 3.52	89.46 ± 1.76	86.04 ± 3.61	78.04 ± 3.95	45.46 ± 2.84

Table 5
DMPD radical scavenging activity of *M. macrocarpa* in percentage (%) (mean ± SD, n = 3).

Concentration (µg)	Std. Ascorbic acid	Water	Methanol	Ethanol	Acetone
20	10.02 ± 1.02	8.98 ± 1.05	8.30 ± 0.32	4.74 ± 0.55	2.67 ± 0.19
40	17.47 ± 1.64	13.03 ± 1.86	12.04 ± 1.15	7.090 ± 1.161	4.29 ± 1.03
60	29.49 ± 2.10	24.43 ± 2.10	21.00 ± 1.64	11.28 ± 1.88	7.02 ± 0.68
80	42.38 ± 2.40	36.14 ± 2.53	28.48 ± 2.11	12.81 ± 1.30	10.74 ± 1.25
100	51.34 ± 2.81	44.84 ± 3.12	40.23 ± 2.80	15.45 ± 1.71	13.24 ± 1.00

Table 6
FRAP assay of *M. macrocarpa* expressed in OD at 593 nm (mean ± SD, n = 3).

Concentration (µg)	Std. Ascorbic acid	Water	Methanol	Ethanol	Acetone
10	0.70 ± 0.11	0.49 ± 0.05	0.51 ± 0.06	0.40 ± 0.02	0.18 ± 0.02
20	1.06 ± 0.08	0.91 ± 0.05	0.90 ± 0.08	0.80 ± 0.09	0.24 ± 0.09
30	1.60 ± 0.07	1.43 ± 0.07	1.30 ± 0.04	1.22 ± 0.07	0.42 ± 0.08
40	1.90 ± 0.11	1.61 ± 0.10	1.71 ± 0.09	1.51 ± 0.08	0.72 ± 0.07
50	2.37 ± 0.09	1.67 ± 0.14	2.05 ± 0.08	1.60 ± 0.07	1.07 ± 0.10

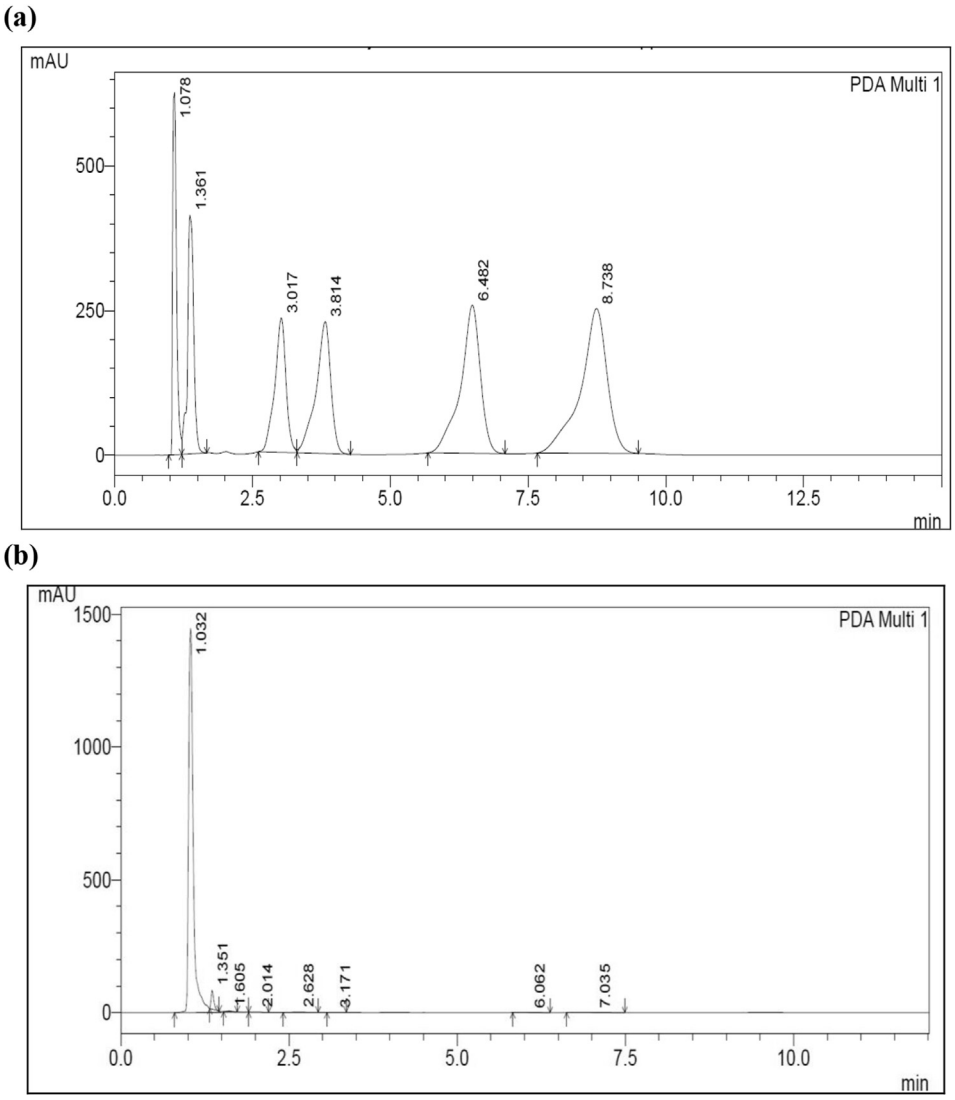

Figure 3. HPLC chromatogram for analysis of phenolics in *M. macrocarpa* seed powder.
(a) HPLC profile of standard phenolics compounds P-1.062 (tannic acid), P-1.36 (gallic acid), P-3.245 (*p*-hydroxybenzoic acid), P-4.083 (vanillic acid), P-7.18 (*p*-coumaric acid), P-9.601 (ferulic acid), (b) HPLC profile of *M. macrocarpa* seed powder extract showing tannic acid, gallic acid, *p*-hydroxybenzoic acid and *p*-coumaric acid (n = 3).

ferric reducing capacity *viz*., (0.51 ± 0.06) OD at 10 µg concentration while (2.05 ± 0.08) OD at 50 µg. The ascorbic acid showed (0.70 ± 0.11) OD and (2.37 ± 0.09) activity at the 10 µg and 50 µg concentration respectively. The order of FRAP activity for the different solvents was methanol > water > ethanol > acetone.

3.6. Quantitative RP-HPLC analysis of major phenolic compounds

Quantitative analysis of major phenolics from *M. macrocarpa* beans was carried out with a comparison of the standard RP-HPLC chromatographic profile. RP-HPLC revealed that *M. macrocarpa* bean contains tannic acid (43.795 mg/g), gallic acid (0.864 mg/g), *p*-coumaric acid (0.364 mg/g) and *p*-hydroxybenzoic acid (0.036 mg/g) (Figure 3).

4. Discussion

L-dopa is important molecule used for the synthesis of the neurotransmitter dopamine and acts as a prime drug for the symptomatic management of Parkinson's disease. Synthetic L-dopa causes several secondary complications after prolonged use for the treatment [42]. Hence, research is being focused on the natural sources of L-dopa with special emphasis on herbal medicines. The plants have the advantage that, despite specific drug content, it also shows additional properties like antioxidant activity, anti-inflammatory property which may reduce the adverse effects of the synthetic counterpart. RP-HPLC quantification proved that *M. macrocarpa* beans contain (115.410 ± 0.985) mg/g of L-dopa which is the highest among the *Mucuna* species from Indian sub-continent. The well-known species of *Mucuna*, *M. pruriens* contain 12.5–91.6 mg/g of L-dopa [6] whereas, species of *Mucuna* like *M. pruriens* var. *utilis* and *Mucuna sanjappae* contains 12.5–91.6 mg/g [6] and 73 mg/g respectively [7]. The RP-HPLC revealed the higher content of L-dopa in *M. macrocarpa* beans which accelerate their exploitation for the management of Parkinson's disease.

Neurodegenerative disease such as Alzheimer disease and Parkinson's disease are caused due to prolong neuroinflammation [8]. In the animals, most of the inflammatory reaction happens due to secretion of lysosomal enzymes and denaturation of protein

which causes a variety of impairments in surrounding tissues [22]. Several synthetic drugs show dose-dependent activity for inhibition of protein denaturation [43]. However, long-term use of these drugs may lead to several secondary complications [9]. Herbal drugs usually contain a variety of bioactive compounds which show strong anti-inflammatory and antioxidant potential. Some of these biomolecules can penetrate the blood–brain barrier and can be deployed for the use of central nervous system related neuroinflammatory disorders [44]. The findings of this study indicate that *M. macrocarpa* beans have excellent anti-inflammatory activity. *M. macrocarpa* beans show strong inhibition of protein denaturation with maximum membrane stabilisation activity.

Proximate composition of *M. macrocarpa* beans revealed numerous nutritional and anti-nutritional factors. It was observed that *M. macrocarpa* seed contains a moderate level of total carbohydrate and lower level of protein and energy as compared to *M. pruriens* var. *utilis* and *Mucuna sanjappae* [7]. *M. macrocarpa* beans also showed the excellent content of proanthocyanidin which is well-known for antimicrobial, anti-allergic, antihypertensive and antioxidant activity [45]. Anti-nutritional factors are the components found in the leguminous seeds having a negative role in the digestion of protein, carbohydrates and minerals [7]. Therefore, determination of anti-nutritional factors is an essential feature for the consideration of leguminous seeds for food or medicinal purpose. *M. macrocarpa* seed has a moderate level of phytic acid and saponin, whereas, high level of tannin was observed. According to some previous reports, anti-nutritional factors also exert beneficiary effects on the treatment of different diseases through diverse molecular pathways. For instance, iron-induced oxidative stress has a critical role in Parkinson's disease generation [12] and phytic acid acts as natural iron chelators reducing the adverse effect of iron accumulation in the brain [46]. Saponins are well known anti-cancer agents whereas tannin is used as an anti-inflammatory molecule for the inhibition of tissue swelling [47].

Phenolic and flavonoid compounds are the major secondary metabolites having strong therapeutic perspective [48]. Plant phenolics and flavonoids prevent heart disease, reduce inflammation, lower the incidence of cancers and diabetes, reduce rates of mutagenesis in human cells and prevents neurodegenerative disease like Alzheimer and Parkinson's disease [49]. Measurement of these secondary metabolites using single extraction solvent may not give a precise conclusion. Hence, in the investigation, we evaluated several phytocomponents using four different solvents. The *M. macrocarpa* water extract and *M. macrocarpa* methanol extract showed higher phenolic and flavonoids, whereas, *M. macrocarpa* ethanol extract and *M. macrocarpa* acetone extract showed a low level of phenolics and flavonoids extraction capacity. It was explored that, total phenolics content and total flavonoids content were significantly affected by the extracting solvents. The order of total phenolics content extraction for the different solvents was water > methanol > ethanol > acetone. The finding in work revealed that *M. macrocarpa* seed powder has significant levels of phenolics and flavonoids as compared to *M. pruriens* var. *utilis* [50] which may react with free radicals to recover oxidative stress-induced cell damage. The present work also has shown that *M. macrocarpa* seed powder has enormous levels of phenolics and flavonoids as compared to *M. pruriens* var. *utilis* [50], which may react with free radicals to release oxidative stress.

Oxidative stress has been evidenced for the pathogenesis of several disorders [51]. The imbalance between the bodily antioxidant system and generated oxidants creates oxidative stress ultimately resulting in different disorder including neurological disorders [52]. To surmount such cases, we have to rely on external sources such as herbal drugs and food-based sources of antioxidants [53]. *M. macrocarpa* seed powder extract showed excellent *in vitro* antioxidant potential by DPPH free radical scavenging activity, DMPD radical scavenging activity and FRAP activity. *M. macrocarpa* water extract and *M. macrocarpa* methanol extract showed significant reduction of DPPH radicals into DPPH-H molecule by hydrogen-donating antioxidant potential. The order of DPPH radical scavenging activity for the different solvents was water > methanol > ethanol > acetone respectively. In DMPD radical scavenging activity, the hydrogen ions are transferred from the antioxidant molecules to stabilise the free radical and DMPD solution is decolorised [7]. The DMPD radical scavenging potential of *M. macrocarpa* seed powder was explored with different solvents. Water and methanol extract showed excellent radical scavenging activity as compared to ethanol and acetone. The order of DMPD radical scavenging activity for the different solvents was water > methanol > ethanol > acetone. FRAP results revealed that the *M. macrocarpa* methanol extract has good potential of reducing ferric radicals than other solvent extracts. The order of FRAP activity for the different solvents was methanol > water > ethanol > acetone. The excellent antioxidant potential of *M. macrocarpa* beans suggests its possible use to cure oxidative stress-related disorders including Parkinson's disease.

Plants contain numerous phytocomponents such as polyphenols, flavonoids and terpenoids which have the antioxidant and anti-inflammatory potential [10]. RP-HPLC analysis showed the presence of major phenolics such as tannic acid, gallic acid, *p*-coumaric acid and *p*-hydroxybenzoic acid in *M. macrocarpa* beans. Tannic acid has an enormous role in biological activities and used for the treatment of cancer, neuroinflammation, and several microbial and oxidative stress-related disorders [54]. Gallic acid plays a significant role in anti-Parkinson's disease and anti-amyloid fibril formation activity [55]. *p*-hydroxybenzoic acid is mostly used as preservatives in food and beverage industries [56], whereas, *p*-coumaric acid has application in aromatic and cosmetic products [57].

As compared to commonly used *M. pruriens*, *M. macrocarpa* possesses a higher level of anti-Parkinson drug L-dopa with essential secondary metabolites. As Parkinson's disease cases are increasing continually, there are tremendous scopes for exploring *M. macrocarpa* for effective management of Parkinson and other oxidative stress-related disorders. It will also ease the burden created by commonly used *Mucuna* species.

In conclusion, in recent years, genus *Mucuna* has gained more attentions for the research because of its strong medicinal and food potential. There is an increasing demand of L-dopa for the Parkinson's disease treatment which is creating a burden on the commonly used *Mucuna* species (*M. pruriens*). Hence, exploitation of underutilised *Mucuna* species like *M. macrocarpa* is a need of time. This study proves *M. macrocarpa* as a potential source of L-dopa with promising anti-inflammatory and antioxidant activity. The present study also motivates its conservation and improvement by advanced

biotechnological tools, as it is endemic and threatened by increasing urbanisation. Further research is needed to explore this plant for anti-Parkinson's disease and other oxidative stress-related disease combating properties.

Conflict of interest statement

The authors declare no conflict of interest.

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