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Variations in the L-DOPA Content, Phytochemical Constituents and Antioxidant Activity of Different Germlines of *Mucuna pruriens* (L.) DC.

B. Rakesh[1], K. Hima Bindu[2] and N. Praveen[1,*]

[1]Department of Life Sciences, CHRIST (Deemed To Be University), Hosur Road, Bengaluru-560 029, India
[2]Principal Scientist, Flower and Medicinal Crops, ICAR-Indian Institute of Horticultural Research, Bengaluru-560089, India

*Corresponding author: E-mail: praveen.n@christuniversity.in

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In this study a 'wonder plant' *Mucuna pruriens* (L.) DC., which is commercially important medicinal plant of the Fabaceae family known for its treatment in Central Nervous System disorders like Dementia, Parkinson's, Alzheimer's, *etc*. have been selected. Different germplasms have been collected to analyze the phytochemical variations between them and quantify the L-DOPA in root, stem, leaves and seeds of all the five germlines using HPLC. Along with the biochemical assays, antioxidant activity by DPPH, phosphomolybdneum method, the metal chelating and reductive potential activity of all the germplasms were studied. All parts of the plant have shown the presence of L-DOPA but, seeds have the highest quantity followed by the roots, stem and leaves. Arka Shubra seeds showed high L-DOPA content (51.9 mg/g) while the other germplasms showed L-DOPA ranging from 43-45 mg/g. Highest content of carbohydrates (258.8 mg/g) and phenolics (157.0 mg/g) was seen in the seeds of Arka Aswini. While the seeds and leaves of Arka Charaka showed high protein (332.2 mg/g) and flavonoid (10.2 mg/g) content, respectively. High proline (1.74 mg/g) was observed in the seeds of Arka Shubra. Antioxidant studies revealed that Arka Charaka and Arka Daksha to be having high reductive power and free radical scavenging activity by phosphomolybdate method while high metal chelating activity was observed in Arka Aswini (88.7%) and high antioxidant activity by DPPH method was seen in Arka Shubra (86.5%).

Keywords: Antioxidant activity, Phytochemicals, Germplasms, L-DOPA, *Mucuna pruriens*.

INTRODUCTION

Mucuna pruriens (L.) DC. commonly known as 'velvet bean' has been used in Ayurvedic traditional medical system from time immemorial and also mentioned in various kinds of ancient literatures of India [1]. It has been reported in many studies that they are used to cure many medical ailments since 1500 BC [2]. In Sanskrit it is referred to as 'Kapikachhu' or 'Atmagupta', in Hindi, it is known as 'Kiwach', 'Nasaguni' in Kannada and 'Poonaikali' in Tamil [3]. *Mucuna pruriens* is an annual, herbaceous, climber grown in damp, deciduous plains of Indian, American and African countries [4]. The leaves are opposite, trifoliate, lanceolate with broad ovate leaflets which are unequal at base. Flowers are white to purplish borne in hanging clusters in pendulous racemes. Fruits are in characteristic pods [5]. *Mucuna*, a genus of Fabaceae family (subfamily Papilionaceae) consisting of 150 species around the globe of which 15 species are reported in India and a particular species called *Mucuna pruriens* is a medicinally valuable crop [6]. Two varieties of *Mucuna pruriens* is found in India. One being *M. pruriens* var. *pruriens* which is a wild variety found with a black seed coat. These possess silky, velvet-like reddish-brown irritating trichomes over the pod which causes severe itching if comes in contact. The itchy irritation is due to the secretion of monoamine neurotransmitter 5-hydroxy tryptamine (serotonin) from the trichomes. Due to which they are used in haptic sensation [7]. Also, human contact results in itchy dermatitis due to mucunain production [8]. The other variety being *Mucuna pruriens* var. *utilis* which is a cultivated variety with ellipsoid, oblong seeds with different shapes and seed coat. These pods contain non-irritant trichomes. Due to the presence of these dense silky trichomes which give a velvety appearance, these are commonly known as velvet bean [9].

It is mainly cultivated for pods and leaves, which are used as fodder, famine food, *etc*. [10]. It is a tropical legume used as green manure and cover crop due to its high nitrogen-fixing

capacity and aggressive growth [4]. Some tribal groups in the Benin Republic and Vietnam use this crop as a biocontrol agent against problematic grasses like *Imperata* [11]. Seeds are roasted, grounded and consumed as a substitute for coffee in tribal communities of Mexico and Guatemala. While in some parts of Africa, seeds are used as soup thickeners [12]. All parts of this plant are used in ancient medicines [13]. *Mucuna pruriens* increases testosterone levels when consumed hence the seeds are used as a spermatogenic agent or aphrodisiac for male virility [14], diabetes, leukoderma [15]. Vigorex-SF capsule containing *Mucuna* seed powder is administered to treat male sexual dysfunction, improve libido. The seed extracts neutralize the venom from *Echis carinatus*, *Najas putatrix* and King Cobra. *Rhinax,* a herbal formulation of this wild pulse used for its anti-hepatotoxicity activity [16]. Metabolites like tryptamine, 5-hydroxytryptamine, mucunine, mucunadine, prurienine, pruieninine [17] and catecholamines like isoquinolines are reported from *Mucuna* seeds [18]. The hair on the pods is used as vermifuge by the tribal group Garos of Meghalaya and in Nigeria, they are consumed with honey to expel intestinal worms. Roots are used for the treatment of cholera, dropsy, constipation, ulcer and fever. Leaves are used for cephalalgia, debility and to relieve inflammation [19]. Apart from these, all parts are known for their antivenom, anthelmintic, hypoglycemic, hypocholesterolemic properties. They are also involved in learning and memory enhancement along with treating irregular menstruation, pain and numbness in joints [20].

M. pruriens is noted for its principal metabolite L-DOPA (3,4-dihydroxy-L-phenylalanine) or levodopa, which was first chemically synthesized by a Polish biochemist Casmir Funk in 1911, later in 1913, Marcus Guggenheim isolated L-DOPA for the first time from *Vicia faba* [21]. L-DOPA is used as a potent drug for the treatment of various neurological disorders like Parkinson's disease, dopamine-responsive dystonia, *etc.* [22]. Parkinson's is caused due to the death of dopaminergic neurons in the substantia nigra of brain, which results in the depletion of dopamine secretion in the striatum [23]. L-DOPA, a non-protein, catecholic amino acid synthesized by the oxidation of tyrosine, is produced by shikimic acid pathway in plants [24]. L-DOPA crosses the blood-brain barrier in humans and restores the dopamine levels by decarboxylation reaction. Thus, L-DOPA is a valuable precursor for numerous neurotransmitters and catecholamines like dopamine, epinephrine (adrenaline) and nor-epinephrine (nor-adrenaline) [9].

The estimated global demand for L-DOPA is 250 tons per year [25]. It is extremely difficult to meet the global demand due to the complexity of extraction procedures, low germination and growth rate. Many research institutes develop different germplasms with superior characters and high metabolite content across the globe. Hence, it is important to screen and select the germplasm before proceeding with biotechnological works like *in vitro* and molecular approaches. Therefore, in the study, we have attempted to screen the different germlines available in Bangalore city, India by various biochemical experiments, antioxidant assays and quantifying the L-DOPA content in roots, stem, leaves and seeds to select the elite variety for further studies.

EXPERIMENTAL

Sample collection: Five different germplasms of *Mucuna pruriens* (L.) DC. namely, Arka Aswini (As), Arka Charaka (Ch), Arka Daksha (Da), Arka Dhanvantri (Dh) and Arka Shubra (Sh) were collected from ICAR-Indian Institute of Horticulture Research, Bengaluru, India. The seed morphology of all the collected germplasm is shown in Fig. 1. All the five germplasm varieties were sown separately in uniformly sized pots filled with an equal quantity of soil-mixed with humus. From each variety, triplicates were maintained and their growth was monitored in the Greenhouse, CHRIST (Deemed to be University), Bangalore, India. After 40-45 days fresh sample of roots, stem and leaves were collected for some experiments and the rest was shade dried at room temperature along with seeds and then finely powdered and stored at 4 ºC for various biochemical analysis.

Quantification of L-DOPA using HPLC: L-DOPA of different parts of all the germplasm was quantified following the method of Chinapolaiah *et al.* [26]. Dry weight (2.0 g) of root, stem, leaf and seed of all the five germplasm was weighed separately, finely powdered and taken in a conical flask. To this, 20 mL of freshly prepared 0.1M orthophosphoric acid was added and covered using aluminum foil. Conical flasks were kept in an orbital shaker at 150 rpm for 30 min at room temperature, filtered using Whatman filter paper and then the filtrate was centrifuged at 10,000 rpm for 10 min. The clear supernatant was collected in an Eppendorf tube and preserved for the HPLC analysis. The sample was freshly prepared and stored at -20 ºC. It was analyzed within 24-48 h as L-DOPA gets readily oxidized. The samples were filtered through a 0.45 µM nylon membrane and subjected to HPLC analysis. The L-DOPA was quantified using RP-HPLC system (Shimadzu) equipped with C-18 column (Sharpsil-U, 250 (L) × 4.6 mm ID), SCL-40 system controller, SIL-40C XS auto-injector, coupled with a UV detector (SPD-40). The mobile phase was phosphate buffer (pH 2.5) and acetonitrile (80:20, v/v) in an isocratic elution manner; a flow rate of 0.5 mL/min and the column temperature of 40 ºC was maintained. The detection wavelength was set at 280 nm. The injection volume was 5 µL. The chromatography system was equilibrated by the mobile phase. The L-DOPA standard (HPLC purity- 99.3%) was procured from Natural Remedies, Bengaluru, India.

Preparation of methanolic extracts: Dry weight (0.2 g) of root, stem, leaf and seed of five germlines was added to 5 mL methanol and covered with aluminum foil and left for 48 h for the complete exudation of phytochemicals. They were filtered using Whatman filter paper and the filtrate was collected and evaporated completely. To the residue obtained after evaporation, methanol was added and re-dissolved to obtain a stock concentration of 10 mg/mL. This methanolic extract of plant was stored at 4 ºC and used for various biochemical assays.

Phytochemical screening

Estimation of total chlorophyll by Arnon's method: Chlorophyll content in the plant sample was estimated according to reported method [27]. Fresh leaf sample (0.5 g) was was added in a 10 mL of pre-chilled 80% acetone and ground

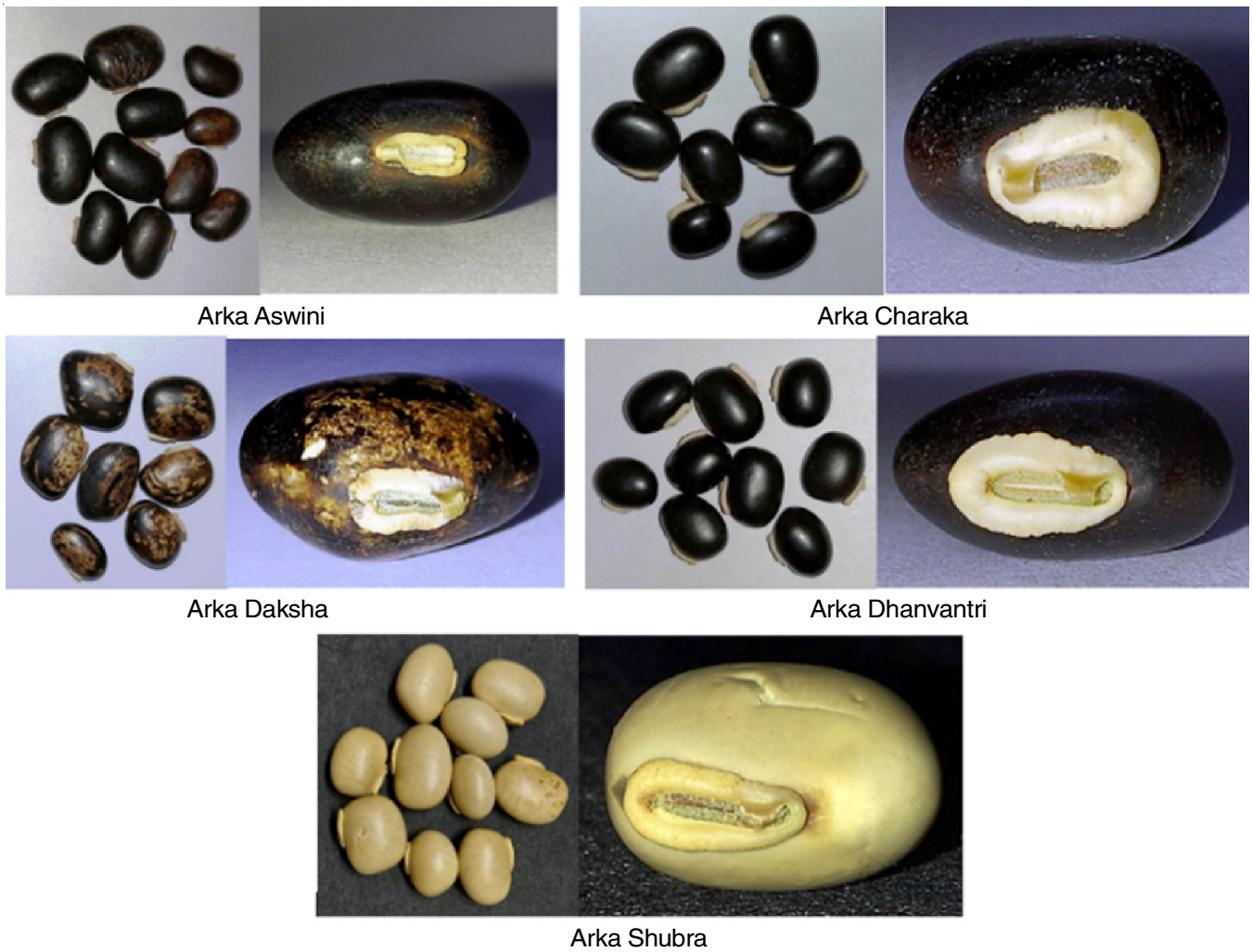

Fig. 1. Seed morphology of five different germplasms of *Mucuna pruriens* (L.) DC. collected from IIHR, Bengaluru

well. The extraction was repeated 2-3 times until decolouration of tissue was observed. The contents along with the decolourized sample were added and covered with aluminum foil and left overnight in dark for complete exudation of chlorophyll. After 24 h, the solution was centrifuged at 10,000 rpm for 10 min, supernatant collected and stored. The extract was diluted and the absorbance was measured at 645 and 663 nm with acetone as blank using a microplate reader (BIO-RAD, iMARK™, Japan).

Chlorophyll 'a', chlorophyll 'b' and total chlorophyll were calculated using the formula:

$$\text{Chlorophyll 'a'}\left(\frac{\text{mg}}{\text{g}}\text{ of FW}\right)=\left(12.7(A_{663})-2.69(A_{645})\times\frac{V}{1000\times W}\right)$$

$$\text{Chlorophyll 'b'}\left(\frac{\text{mg}}{\text{g}}\text{ of FW}\right)=\left(22.9(A_{645})-4.68(A_{663})\times\frac{V}{1000\times W}\right)$$

$$\text{Total chlorophyll}\left(\frac{\text{mg}}{\text{g}}\text{ of FW}\right)=\left(20.2(A_{645})-8.02(A_{663})\times\frac{V}{1000\times W}\right)$$

where V = final volume of chlorophyll extract; W = fresh weight.

Estimation of total carbohydrates by phenol-sulphuric acid method: The total carbohydrates in root, stem, leaf and seeds of all the five germplasm were quantified according to Karibasappa *et al.* [28], which was modified accordingly. Fresh sample (0.1 g) was taken in a pestle and mortar to which 5 mL of 2.5 N HCl was added and grinded well. The contents along with the sample were transferred to a test tube, covered with aluminium foil and incubated in a boiling water bath for 3 h. Later this was allowed to cool down and sodium carbonate was added to neutralize it until the effervescence ceased. This was made up to 25 mL by adding distilled water and centrifuged at 5000 rpm for 5 min. Extract (supernatant, 10 µL) and distilled water (90 µL) was added and mixed well. To this, 100 µL of 5% phenol was added followed by 500 µL of 96% sulphuric acid and shaken well. This was incubated at room temperature for 30 min. Later the absorbance of golden-red colour was read at 490 nm using a microplate reader (BIO-RAD, iMARK™, Japan) against blank. To 100 µL of distilled water, all the reagents were added except plant extract and served as blank. Glucose was taken as a standard to calculate the carbohydrate content in the given samples.

Estimation of total proteins by Lowry's method: Total proteins in the sample were estimated by modified Lowry's method [29]. Fresh sample (0.2 g) was taken and 10 mL of phosphate buffer (pH 7.0) was added and grinded well using

pestle and mortar. This was centrifuged at 5000 rpm for 5 min and the supernatant was collected. Extract (20 µL) was taken and 80 µL of distilled water was added followed by the addition of 500 µL of reagent C and left for 10 min. Later, 50 µL of Folin-Ciocalteau (FC) reagent (diluted with distilled water in the ratio 1:1 v/v) was added and incubated in dark for 30 min. The absorbance of the bluish colour was read at 660 nm using a microplate reader (BIO-RAD, iMARK™, Japan). For 100 µL of distilled water, all the reagents were added except plant extract and considered as blank. Bovine serum albumin (BSA) was taken as standard and the graph was plotted using which the unknown was calculated. Reagent C was prepared by adding 50 mL of reagent 'A' (2% Na_2CO_3 in 0.1 N NaOH) and 1 mL of reagent 'B' (0.5% $CuSO_4$ in 1% potassium sodium tartrate).

Estimation of total phenolics by Folin-Ciocalteau's method: Total phenolics in the sample were estimated by modified Folin-Ciocalteu's (FC) method [30]. To a 50 µL of methanolic extracts, 450 µL of distilled water was added to dilute the extract and 150 µL of FC reagent (diluted with distilled water in the ratio 1:1 v/v) was added and vortexed. To this 500 µL of 20% (w/v) Na_2CO_3 was added and incubated in dark for 1 h. The absorbance of greenish-blue colour developed was read at 650 nm using a microplate reader (BIO-RAD, iMARK™, Japan). For 500 µL of methanol, all the reagents were added except plant extract and considered as blank. Gallic acid was taken as a standard to determine the phenolic content of the samples.

Estimation of proline: Proline in the different parts of all the germplasm was estimated by the acid-ninhydrin method [31]. Fresh sample (0.2 g) was homogenized using 10 mL of 3% sulpho-salicylic acid. The extract was centrifuged at 5000 rpm for 5 min. Then 200 µL of extract (supernatant), 200 µL glacial acetic acid and 200 µL acid ninhydrin were mixed and incubated in a boiling water bath for 1 h. After which they are immediately placed in an ice-bath to arrest the reaction and 400 µL toluene was added, vortexed well and left for 10 min to settle down. The upper pinkish colour layer was separated and absorbance was read at 520 nm using a micro-plate reader (BIO-RAD, iMARK™, Japan). Toluene was taken as blank. Proline was taken as standard and a graph was plotted from which the proline content in the samples was estimated.

Acid ninhydrin solution was prepared by adding 1.25 g of ninhydrin to 30 mL of warm glacial acetic acid and 20 mL of 6 M phosphoric acid with continuous stirring until dissolved. This was freshly prepared and used within 24 h.

Estimation of total flavonoids: Total flavonoids in the plant sample were estimated by the aluminum chloride method [32]. To 100 µL methanolic extract, 400 µL methanol was added to dilute its concentration. To this 100 µL of 10% aluminum chloride solution was added and mixed well. Later 100 µL of 1M sodium acetate was added and incubated in dark at room temperature for 45 min. The absorbance of the developed golden-yellow colour was read at 415 nm (BIO-RAD, iMARK™, Japan). Methanol (500 µL) and all the reagents were added except plant extract and taken as blank. Quercetin was taken as a standard to calculate the concentration of flavonoids in the plant sample.

Antioxidant studies

DPPH method: The antioxidant activity of plant samples was estimated using modified DPPH (2,2′-diphenyl-1-picryl-hydrazyl method [33]. A sample (25 µL) was taken and made up to 600 µL by adding methanol. To this 200 µL of 0.004% DPPH solution (4 mg of DPPH dissolved in 100 mL of methanol) was added, mixed well and incubated in dark for 30 min. Later the absorbance was read at 517 nm using UV-VIS spectrophotometer (Shimadzu, UV-1900). Methanol was taken as blank. The experiment was carried under dark condition as DPPH is light sensitive. Butylated hydroxyanisole (BHA) was taken as standard. Percentage of antioxidant activity was calculated using the formula:

$$\text{Radical scavenging activity (\%)} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

where, control was 600 µL methanol + 200 µL DPPH solution Sample was plant extract made up to 600 µL using methanol + 200 µL DPPH solution.

Phosphomolybdate method: Antioxidant activity of plant samples was determined by the phosphomolybdate method as reported by Praveen *et al.* [34]. A methanolic plant extract (100 µL) was taken in a sterile Eppendorf tube to which 1 mL of phosphomolybdate reagent was added and incubated in the water bath at 90 ºC for 90 min. Later, it was allowed to cool down to room temperature and the absorbance for the greenish colour developed was measured at 695 nm using UV-VIS spectrophotometer (Shimadzu, UV-1900). To 100 µL of methanol, 1 mL of phosphomolybdate reagent was added and considered as blank. Ascorbic acid was taken as standard.

Phosphomolybdate reagent was prepared by adding equal volumes of 28 mM sodium phosphate, 0.6 M sulphuric acid and 4 mM ammonium molybdate.

Estimation of metal chelating activity: Metal chelating activity of different parts of all the germlines was quantified following the method of Pavithra & Vadivukkarasi [35]. The methanolic extract (50 µL) was taken and made up to 1 mL by adding methanol. To this 50 µL of 2 mM $FeCl_2$ was added and mixed well. Later 200 µL of 5 mM ferrozine was added and incubated under dark at room temperature for 10 min. The absorbance of the pinkish colour developed was measured at 562 nm using UV-VIS spectrophotometer (Shimadzu, UV-1900). Methanol was taken as blank. Butylated hydroxyanisole (BHA) was used as standard. Percentage of metal chelating activity was calculated using the formula:

$$\text{Metal chelating activity (\%)} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

where, control is 1 mL methanol + 50 µL $FeCl_2$ + 200 µL ferrozine. Sample is plant extract made up to 1 mL using methanol + 50 µL $FeCl_2$ + 200 µL ferrozine.

Estimation of reductive potential: The reducing potential of the sample was estimated by according to Jung *et al.* [36] method. The methanolic extract (5 µL) was taken to which 95 µL of methanol was added. To this 250 µL of phosphate buffer (0.2 M, 6.6 pH) was added and mixed well. Then 250 µL of

1% potassium ferrocyanide was added and incubated in the water bath at 50 ºC for 20 min. Later to this 250 µL of 10% trichloroacetic acid was added and shaken well using a vortex. From this 250 µL of the solution was taken into new Eppendorf tubes and 250 µL of distilled water was added. Now 100 µL of 0.1% $FeCl_3$ was added and vortexed. The absorbance of the blue-coloured product was measured at 700 nm using UV-VIS spectrophotometer (Shimadzu, UV-1900). Methanol as blank, while ascorbic acid and BHA were used as standards.

Statistical analysis: All the experiments were carried out in triplicates. The data were analyzed statistically by One-way ANOVA using IBM SPSS Statistics software version 21.0. The significance of differences among means was carried out using Duncan's Multiple-range test at $p \leq 0.05$. The results were expressed as means of triplicates ± SE.

RESULTS AND DISCUSSION

L-DOPA quantification by HPLC method: L-DOPA was quantified using an RP-HPLC and the result is represented in Fig. 2 with the chromatograms of few samples and standard L-DOPA in Fig. 3. It was observed that all parts of the plant contain L-DOPA but it is present in higher quantities in seeds, followed by the roots. The least amount of L-DOPA was reported to be in stem (Da, Dh) and leaves of few germplasms (As, Ch, Sh). Among the seeds, Sh showed high L-DOPA content (51.99 mg/g DW) followed by Ch (45.37 mg/g). The seeds of other germplasms show no drastic variations and contain L-DOPA in the range of 43-44 mg/g. However, the least was observed in As. When considering the roots, Da shows a high quantity (13.2 mg/g) of the metabolite followed by Ch (11.88 mg/g) and As (9.54 mg/g). The least was observed in the roots of Dh (8.14 mg/g). Among the stem, Ch (10.83 mg/g) and Da (2.03 mg/g) showed the highest and least L-DOPA content, respectively. While in the leaves, Dh (7.38 mg/g) and Sh (2.91 mg/g) showed the highest and least L-DOPA content, respectively.

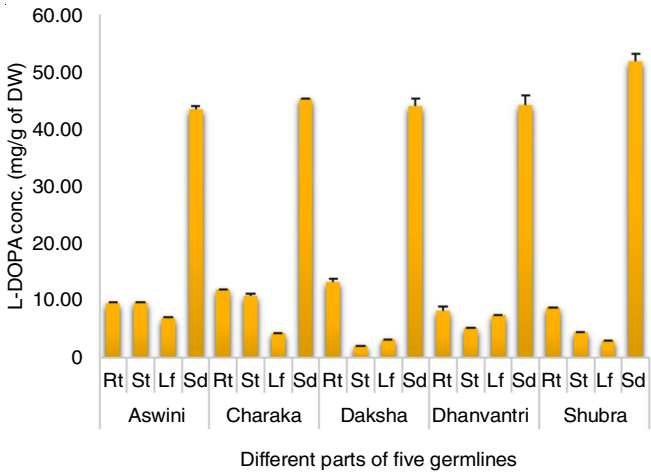

Fig. 2. Graphical representation of L-DOPA concentration in different partsof five germplasms. Each value represents the mean of triplicates ± SE

Overall, the Sh germplasm can be considered as the best germplasm with regard to its L-DOPA content in the seeds. Patil *et al.* [37] reported that some species like *M. macrocarpa* and *M. atropurpurea* showed the highest L-DOPA content, which was 116.0 mg/g and 108.3 mg/g, respectively. While a few accessions of *M. pruriens var utilis* including Dh germplasm showed L-DOPA content ranging from 50.1-76.1 mg/g. The least was observed in *M. gigentea*. Patil *et al.* [38] also reported the L-DOPA content in a new species of *Mucuna* which was found to be 73 mg/g. Chinapolaiah *et al.* [26] reported in their study that As and Dh to be having an L-DOPA content of 39.7 and 43.1 mg/g which is in concordance with the present study. They also reported the L-DOPA content of many other germplasms to be ranging from 29.4-69.9 mg/g.

Chlorophyll estimation: The total chlorophyll in mg/g of fresh weight (FW) for all the germplasms has been tabulated in Table-1. Out of all the five germplasms, the leaves of Sh

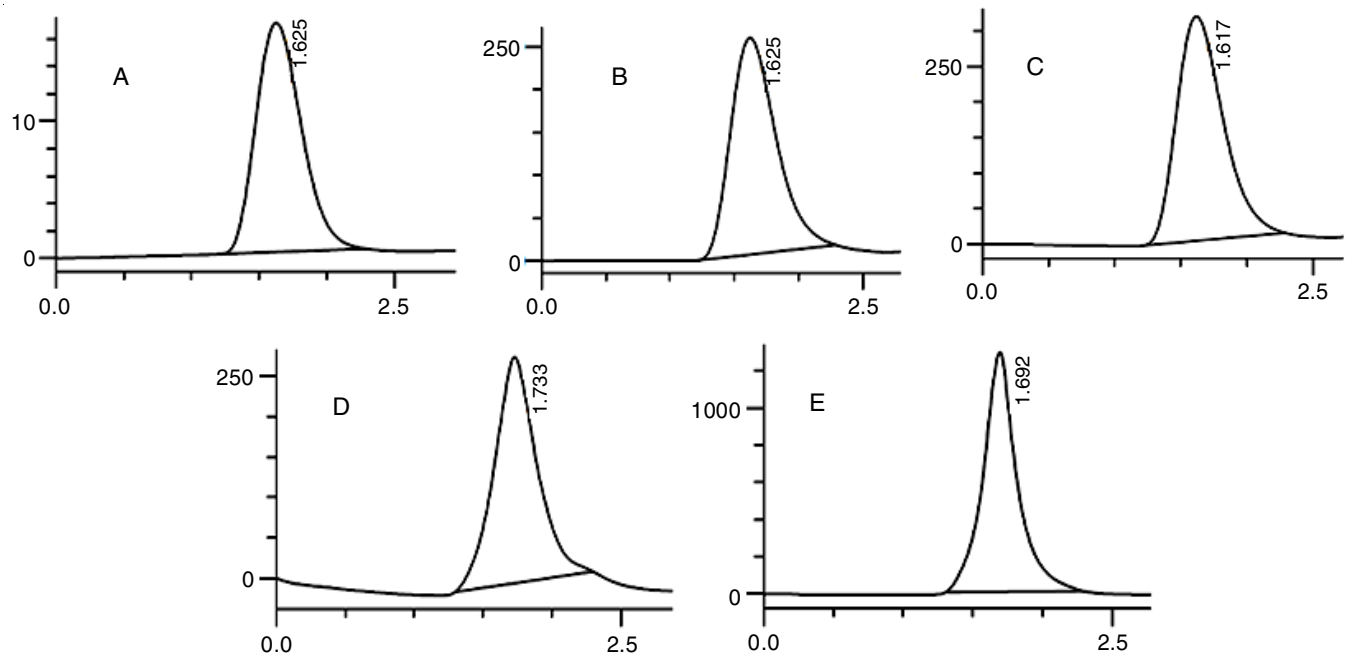

Fig. 3. HPLC chromatogram for A- standard L-DOPA, B-root, C-stem, D-leaf and E-seed (B-E different parts of Arka Aswini)

TABLE-1
TOTAL CHLOROPHYLL CONTENT (mg/g of FW)
IN FIVE GERMPLASMS OF *M. pruriens*

Variety	Total chlorophyll (mg/g) of fresh tissue
Arka Aswini	8.90 ± 0.01b
Arka Charaka	10.19 ± 0.01b
Arka Daksha	5.43 ± 0.01c
Arka Dhanvantri	9.87 ± 0.07b
Arka Shubra	14.96 ± 0.02a

Values are means of triplicates ± SE. Means followed by the same letter within columns are not significantly different ($p \leq 0.05$) using Duncan's multiple range test.

show the highest chlorophyll content followed by Ch and Dh. There are no much significant variations observed within Ch, Dh and As germplasms. The least amount of chlorophyll is seen in Da, which has 2.7-fold lesser amount when compared to Sh. Chlorophyll content in different germlines of *Mucuna pruriens* was reported to be ranging from 41.17 to 49.03 Soil Plant Analysis Development (SPAD) units according to Hadapad *et al.* [39]. The total chlorophyll reported in the present study is in concordance with the studies of Pugalenthi & Vadivel [40].

Carbohydrate estimation: The total carbohydrates present in root, stem, leaves and seeds of all the five germplasms were estimated using the phenol-sulphuric acid method, quantified spectrophotometrically and tabulated in Table-2. The seeds are generally showing higher carbohydrate content than the other parts while the roots are showing the least carbohydrate content. The carbohydrate content in the seeds of the present study is reported to be in the range of 112.2-258.8 mg/g of FW. Out of all the five germplasms, the seeds of As shows the highest carbohydrate content, followed by the seeds of Da and Dh. While considering the leaves, Da shows the highest carbohydrate content followed by the leaves of Ch. Similarly, the stem of Da also shows the highest carbohydrate content followed by the stem of Dh and Ch. Root and stem of As, root of Dh and leaves of Sh shows minimum carbohydrate content with no much notable variations between them. Overall, the highest carbohydrate content in seeds is seen in As, the highest carbohydrate content in leaves, stem and roots is observed in Da. Hence, Da seems to be a better germline when considering carbohydrates. Oko *et al.* [41] reported that the leaves of *Mucuna poggei* to have 115.5 mg/g of total carbohydrates. This is similar to the leaves of the five-germplasm taken up in this study where the carbohydrate content is between 73.8-183.2 mg/g FW. In a study of Renata *et al.* [42] that the carbohydrate content like starch in seeds was found to be as 333.3-371.9 mg/g, which was higher than the seeds.

Protein estimation: Total proteins in all the parts of the five germplasm were estimated by Lowry's method using acid ninhydrin. The protein content expressed in mg/g of FW is given in Table-2. Proteins were found to be higher in seeds of all the germplasms when compared with other parts. The seeds of Ch showed the highest protein content followed by the seeds of Sh and Dh with no much variation. Following the seeds, a high amount of proteins was observed in the roots where As showed the highest protein content among the roots and the least was seen in Sh. The protein content in stem and leaves were almost near in all the germplasms. The highest quantity of protein in leaves and stem was observed in Ch and Sh, respectively. Overall, there is no much significant variation seen within the germplasms, but Ch can be considered as germplasm with a good quantity of proteins. Total proteins in three different germplasms of *M. pruriens* were quantified by Josephine & Janardhanan [43] and found to be 307.0, 260.0 and 279.0 mg/g in Begur, Silent Valley, Lucknow germplasms,

TABLE-2
TOTAL CARBOHYDRATE, PROTEINS AND PROLINE CONTENT IN ROOT,
STEM, LEAVES AND SEEDS OF ALL THE FIVE GERMPLASMS

Variety	Part	Code	Total carbohydrates (mg/g) of fresh weight	Proteins (mg/g) of fresh weight	Proline (µg/g) of fresh weight
Arka Aswini	Root	AS-R	67.2 ± 8.5 g	82.7 ± 19.7 d	714.5 ± 21.1 b
	Stem	AS-st	64.8 ± 8.7 g	45.6 ± 1.3 de	713.9 ± 65.3 b
	Leaves	AS-lf	119.8 ± 45.6 defg	46.8 ± 4.6 de	893.1 ± 24.6 ab
	Seeds	AS-sd	258.8 ± 50.7 a	266.6 ± 17.3 c	1113.5 ± 36.6 ab
Arka Charaka	Root	Ch-R	84.2 ± 12.1 efg	64.3 ± 10.7 de	874.8 ± 60 ab
	Stem	Ch-st	113.5 ± 4.1 defg	46.2 ± 3.8 de	1315.7 ± 69.4 ab
	Leaves	Ch-lf	163.5 ± 37.7 bcde	58.1 ± 7.9 de	526.5 ± 50 b
	Seeds	Ch-sd	112.2 ± 39.3 defg	332.2 ± 12.9 a	1286.8 ± 32.7 ab
Arka Daksha	Root	Da-R	147.8 ± 3.2 cdefg	75.2 ± 17.3 de	694.5 ± 12.4 b
	Stem	Da-st	159.5 ± 16.7 bcdef	48.1 ± 7.0 de	996.2 ± 39.2 ab
	Leaves	Da-lf	183.2 ± 4.7 abcd	48.5 ± 4.6 de	713.3 ± 58.7 b
	Seeds	Da-sd	229.5 ± 20.6 ab	281.6 ± 3.9 bc	915.5 ± 68.4 ab
Arka Dhanvantri	Root	Dh-R	71.8 ± 3.8 g	72.2 ± 17.6 de	1038.1 ± 28.4 ab
	Stem	Dh-st	139.5 ± 37.4 cdefg	41.2 ± 6.3 e	721.0 ± 25.6 b
	Leaves	Dh-lf	79.5 ± 13.6 efg	44.3 ± 9.4 de	976.8 ± 31.3 ab
	Seeds	Dh-sd	202.8 ± 32.1 abcd	305.2 ± 19.7 ab	1386.4 ± 30.9 ab
Arka Shubra	Root	Sh-R	76.2 ± 0.6 fg	51.2 ± 6.3 de	1712.9 ± 45.7 a
	Stem	Sh-st	99.5 ± 6.6 defg	52.9 ± 2.3 de	867.2 ± 72.7 ab
	Leaves	Sh-lf	73.8 ± 28.4 g	54.7 ± 2.1 de	943.8 ± 0.00 ab
	Seeds	Sh-sd	137.2 ± 21.2 cdefg	310.83 ± 21.7 ab	1741.2 ± 0.00 a

Values are means of triplicates ± SE. Means followed by the same letter within columns are not significantly different ($P \leq 0.05$) using Duncan's multiple range test.

respectively. The seeds of all the five germplasm also showed similar results, where the proteins were ranging from 112.0-258.0 mg/g. Renata *et al.* [42] reported that the seeds are rich in protein content (431.2 mg/g) and is comparatively higher than the protein content reported in this study. Chinapolaiah *et al.* [26] studied 58 genotypes collected across India and reported the protein content in As and Dh germplasm to be 258.7 and 358.7 mg/g which is in concordance with the present study.

Proline estimation: Proline content was estimated in all the parts of the germplasm and the results are given in Table-2. High proline content was observed in the seeds of germplasms like Sh, Dh, Da and As. While in Ch, the stem shows high proline content. Even the roots of Sh, Dh, Da shows high proline content than the stem and leaves. The highest proline content was observed in the roots, seeds of Sh followed by seeds of Dh and stem of Ch. The leaves of Dh and Sh showed high proline content followed by As and Ch with lesser variations. The least amount of proline was found to be in leaves of Ch and root of Da. Overall, Sh represents the germplasm with high proline content followed by Dh. Josephine & Janardhanan [43] reported the proline content in three varieties of *M. pruriens* seeds to be as 9.36, 7.34 and 9.51 g/100 g protein in Begur, Silent Valley and Lucknow varieties, respectively. Adebowale *et al.* [44] reported proline content in *M. pruriens* to be 125 mg/g crude protein. Mahesh *et al.* [45] studied the effect of salinity in different germplasms of *M. pruriens* and noted the proline content varied from 3.0 mg/g to 9.0 mg/g with effect to the intensity of the salt stress. In present study, the proline content in different germplasms was in the range of 0.5-1.71 mg/g of FW.

Phenolic estimation: Phenolic estimation in all the germlines was carried out using FC reagent. It was observed that in few germlines (As, Ch, Da) seeds showed high phenolic content while in others (Dh and Sh) roots showed high phenolic content. Among the seeds, Dh showed high phenolic content followed by As and Da. The least was observed in Sh. Among the roots, Da showed high phenolic content followed by Sh and As. Leaves and stem of Ch showed more phenolic content and the least among the leaves was observed in Dh while least in the stem was seen in As. Overall, Ch is found to have higher phenolic content when compared with other germlines (Table-3). Ethyl acetate and methanolic extracts of *M. pruriens* showed the whole plant is rich in phenolics [46]. Marimuthu *et al.* [47] reported the total phenolics to be 40.16 and 42.81 mg/g in the seeds of white and black varieties, respectively. The phenolics in current germplasms were found to be between 57.1-162.6 mg/g, which is higher than the previous studies.

Flavonoid estimation: Total flavonoids were estimated by the aluminum chloride method which revealed that leaves showed high flavonoid content followed by seeds, stem and least in roots. Among the leaves of Ch and Da showed high flavonoids followed by As and Sh. The least was observed in Dh. While in the case of seeds, Ch showed the highest amount followed by As. The least was observed in Dh. Also, it was noted that there is minimal variation among the seeds. The stem of Sh and Da shows high flavonoids followed by Ch and the least was observed in As. Roots of Da showed a high amount followed by As and Sh and the least was observed in Dh and Ch with no much variations. Therefore, it can be concluded that out of all the five germlines Ch and Da showed high flavonoid content. Total flavonoids in two germplasm were studied by Marimuthu *et al.* [47] and reported to be 3.43 and 3.81 mg/g in seeds of white and black variety, respectively. In another study by Nwaoguikpe *et al.* [48], where they reported the flavonoid content in seeds to be 3.1-4.2 mg/g. Krishnaveni & Hariharan [49] reported the flavonoid content in *Mucuna* seeds to be 4.7 mg/g. Similar results have been obtained in present work where the flavonoid content was in the range between 2.8-10.3 mg/g.

TABLE-3
TOTAL PHENOLIC (EXPRESSED AS GALLIC ACID EQUIVALENTS) AND FLAVONOID (EXPRESSED AS QUERCETIN EQUIVALENTS) CONTENT IN ROOT, STEM, LEAVES AND SEEDS OF ALL THE FIVE GERMPLASMS

Variety	Part	Code	Total phenolic content (mg/g GAE)	Total flavonoid content (mg/g equivalent of quercetin)
Arka Aswini	Root	AS-R	129±3.3 bcde	3.9±0.2 ef
	Stem	AS-st	57.1±2.9 i	3.3±0.6 f
	Leaves	AS-lf	88.3±1.8 fgh	9.2±0.3 a
	Seeds	AS-sd	157.0±1.0 ab	6.4±0.2 bc
Arka Charaka	Root	Ch-R	119.1±2.2 cdef	2.8±0.2 f
	Stem	Ch-st	110.0±1.6 def	4.1±0.1 ef
	Leaves	Ch-lf	117.8±3.1 cdef	10.2±0.3 a
	Seeds	Ch-sd	139.5±3.8 abcd	6.6±0.1 bc
Arka Daksha	Root	Da-R	145.7±1.2 abcd	4.1±0.1 ef
	Stem	Da-st	77.1±1.2 fghi	4.8±0.4 de
	Leaves	Da-lf	105.1±4.2 efg	10.3±0.2 a
	Seeds	Da-sd	146.8±1.4 abc	6.1±0.1 bcd
Arka Dhanvantri	Root	Dh-R	118.0±2.4 cdef	2.9±0.1 f
	Stem	Dh-st	93.1±0.5 fgh	3.8±0.1 ef
	Leaves	Dh-lf	66.8±1.8 hi	7.2±0.2 b
	Seeds	Dh-sd	162.6±17.9 a	5.6±0.1 cd
Arka Shubra	Root	Sh-R	129.5±3.2 bcde	3.5±0.09 ef
	Stem	Sh-st	100.8±2.7 efg	4.8±0.1 de
	Leaves	Sh-lf	102.8±4.1 efg	9.1±0.3 a
	Seeds	Sh-sd	113.6±3.1 def	6.3±0.1 bc

Values are means of triplicates ± SE. Means followed by the same letter within columns are not significantly different ($p \leq 0.05$) using Duncan's multiple range test.

Antioxidant activity by DPPH method: DPPH is a stable, free radical which gives a purple-coloured solution and soluble in methanol. The antioxidant molecules present in the extracts donate an electron and reduces the DPPH thus decreasing the absorbance as the solution changes from purple to colourless. Therefore, higher the antioxidants present in the sample lower will be the absorbance [50]. The antioxidant activity of different parts of all the five germplasm was estimated by the DPPH method and the results are tabulated in Table-4. The seeds generally showed a high percentage of antioxidant activity followed by roots and then stem with no much variations. Comparatively, the leaves showed the least antioxidant activity. The highest antioxidant or radical scavenging activity was found to be in the seeds of Sh (86.5%) followed by the seeds of Da (84.4%) and the least among the seeds was found in As. Concerning the roots, the highest antioxidant power was found

to be in the root extracts of Sh (82.6%) followed by Da (80.7%) and the least among the roots was found to be in Ch. A similar trend was also observed in the stem extracts. The leaf extracts of Sh showed high antioxidant activity (60.9%) followed by Dh (57%) when compared with the leaves of other germplasm. The least activity was found in the leaves of As (28.2%). The percentage of antioxidant activity for the standard BHA was 93.3%. The Sh variety can be considered as a superior germplasm with respect to the antioxidant activity determined by the DPPH method as the seeds showed higher activity. Some compounds like phenolics, flavonoids, tannins are responsible for such antioxidant activity in plant extracts [51]. Antioxidant activity by DPPH method for ethanolic and aqueous extracts of *M. pruriens* was carried out by Agbafor *et al.* [52] where they reported that ethanolic extracts (41.40%) showed higher antioxidant activity than the aqueous extracts (32.6%), which is almost similar to the present study, where the methanolic extracts of the leaves showed the antioxidant activity in the range between 28-53%. Rajeshwar *et al.* [53] reported the methanolic extracts of seeds to be having an antioxidant activity of 90.16% by the DPPH method, which confirms the results obtained in this study where the seeds of five germplasm show 79.9-86.5% antioxidant activity.

TABLE-4
PERCENTAGE OF ANTIOXIDANT ACTIVITY BY DPPH METHOD AND METAL CHELATING ACTIVITY OF METHANOLIC EXTRACTS FROM DIFFERENT PARTS OF ALL THE FIVE GERMPLASMS OF *M. pruriens*

Variety	Part	Code	Percentage of antioxidant activity by DPPH method	Percentage of metal chelating activity
Arka Aswini	Root	AS-R	78.3±0.9 efg	46.5±6.9 de
	Stem	AS-st	76.4±0.2 fgh	88.7±0.8 g
	Leaves	AS-lf	28.2±1.3 m	55.1±2.0 e
	Seeds	AS-sd	79.9±0.5 cdef	52.7±2.5 e
Arka Charaka	Root	Ch-R	77.5±01.3 efg	12.3±2.1 a
	Stem	Ch-st	75.8±0.7 gh	58.1±1.9 e
	Leaves	Ch-lf	48.2±1.1 l	30.0±1.9 bc
	Seeds	Ch-sd	82.8±0.9 abcd	21.8±1.5 ab
Arka Daksha	Root	Da-R	80.7±1.1 bcde	9.9±1.4 a
	Stem	Da-st	77.7±0.5 efg	80.9±0.3 fg
	Leaves	Da-lf	53.3±2.3 k	37.4±2.3 cd
	Seeds	Da-sd	84.4±3.0 ab	29.3±0.2 bc
Arka Dhanvantri	Root	Dh-R	79.1±0.6 defg	15.8±0.2 a
	Stem	Dh-st	73.4±0.7 h	75.8±3.7 f
	Leaves	Dh-lf	57.0±0.9 j	47.0±0.3 de
	Seeds	Dh-sd	83.6±0.7 abc	21.6±2.1 ab
Arka Shubra	Root	Sh-R	82.6±1.1 bcd	9.6±0.3 a
	Stem	Sh-st	77.0±1.1 efgh	86.9±1.9 fg
	Leaves	Sh-lf	60.9±1.2 i	82.7±1.2 fg
	Seeds	Sh-sd	86.5±0.6 a	58.3±1.3 e
Butylated hydroxy anisole			93.3±0.87 a	5.1±0.28 h

Values are means of triplicates ± SE. Means followed by the same letter within columns are not significantly different ($p \leq 0.05$) using Duncan's multiple range test.

Metal-chelating activity: The results of metal chelating activity in different parts of five germplasms are given in Table-4. The stem shows a high percentage of metal-chelating activity followed by leaves and seeds. The root extract shows the least activity. The stem of As (88.7%) has high metal chelating activity followed by the stem of Sh (86.9 %) and the least among the stem is seen in Ch (58.1%). The highest and the least among the leaves as well as in seeds are found to be in Sh (82.7%) and As (55.1%). The highest activity among the root extracts was found to be in As (46.5%) followed by Dh (15.8%) and the least was observed in roots of Sh (9.6%). All the parts of the five germplasm show a high percentage of metal-chelating activity when compared with the activity of standard BHA (5.1%). Jimoh *et al.* [54] reported in their study that *M. pruriens* shows a high phyto-chelating effect, which coincides with the present study. Dhanasekaran *et al.* [55] also reported that the *Mucuna* seeds possess 15% of the metal chelating activity. But the seeds in the present study showed metal chelating activity between 21-58%, which is higher than the previous studies.

Antioxidant activity by phosphomolybdneum method: The results of the antioxidant activity in different parts of the germplasms are shown in Fig. 4. The roots are generally showing high antioxidant power when compared with the other parts. Following the roots are seed, leaf and stem extracts with no much variations among them. The highest activity was found in the root extracts of Ch (714.7 µg/mg) followed by Da (687 µg/mg) and the least is found to be in As (558 µg/mg). The high and least antioxidant activity in the leaf is found to be in Da (611 µg/mg) and Dh (494 µg/mg). Among the seeds, high activity is seen in Da (544 µg/mg) followed by Dh and Ch (541 and 541.3 µg/mg, respectively). Out of all the stem extracts of the five germplasm, the high activity was found in Sh (575.7 µg/mg). The least antioxidant activity in this method was reported to be in the stem extracts of As (452 µg/mg). All parts exhibited high free radical scavenging activity thus *M. pruriens* can be used as a natural antioxidant [56].

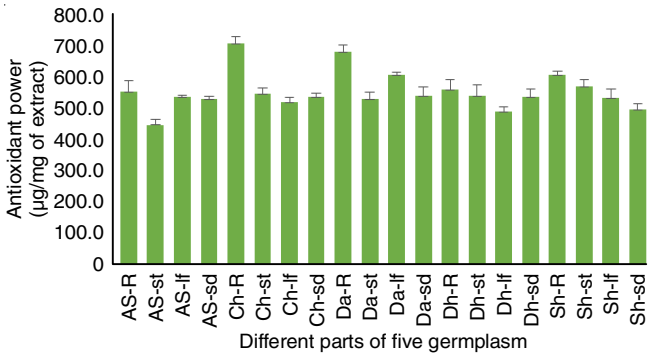

Fig. 4. Graphical representation of antioxidant power (equivalent to ascorbic acid µg/mg) by phosphomolybdneum method in different parts of five germplasms. Each value represents the mean of triplicates ± SE

Assay of reductive potential or reducing power: The reductive potential in different parts of the germplasms are shown in Fig. 5. Reducing power was generally found to be high in Ch and Da germplasms. Out of all the parts leaf showed high reductive potential followed by stem and root in a few germplasms (As, Ch and Da) while in others (Sh and Dh), it was followed by seeds and stem. The highest reducing power (0.47) was found in Ch stem and Da leaf. This was followed

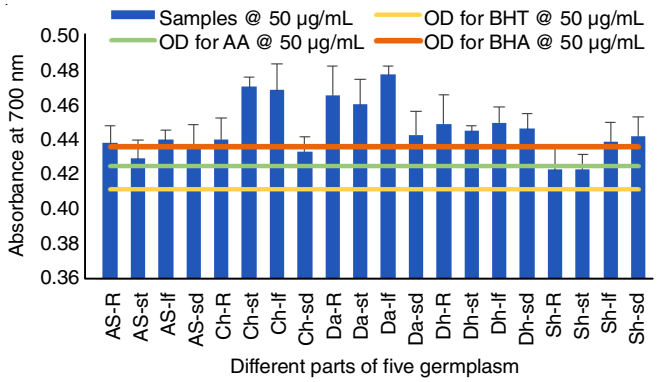

Fig. 5. Graphical representation of reducing power/reductive potential in methanolic extracts of different parts of methanolic extracts of five germplasms. Each value represents the mean of triplicates ± SE

by Ch leaf, Da root and stem (0.46). The least reducing power (0.42) was found in the methanolic extracts of As stem, Sh root and stem. The reducing power of standards like butylated hydroxyanisole (BHA), ascorbic acid (AA) and butylated hydroxytoluene (BHT) were 0.44, 0.43 and 0.41, respectively. Many parts of the germplasm showed almost the same or better reducing power than the standards taken at the same concentration. The results of present study were similar to the study by Jimoh *et al.* [54], where they found out that aqueous extract of *M. pruriens* showed almost the same reducing power as the standard drug (rutin) and it was also noted that the higher the concentration higher was the reductive potential [54]. Rajeshwar *et al.* [53] reported the absorbance for the reductive potential to be around 0.3-0.6 for the concentration between 50-200 µg/mL which is similar to present study where the absorbance is between 0.42-0.48. Siddhuraju & Becker [57] also reported the similar results.

Conclusion

From the present study, it can be concluded that different germplasms show a unique variation among the biochemical parameters studied. Moreover, all parts of the plant contain L-DOPA out of which seeds are rich in L-DOPA content followed by the roots and leaves. Since, L-DOPA being a principal metabolite, considering this metabolite alone it can be concluded that Arka Shubra as an elite germplasm out of the five germlines studied as their seeds contain the highest metabolite than the others. The biochemical assays revealed Arka Aswini to be having high carbohydrate and phenolics, Arka Charaka having high protein and flavonoid while Arka Shubra to be having a high proline content. Antioxidant activities proved that Arka Charaka and Daksha to be a better germplasm considering their reductive power. While Arka Aswini shows better free radical scavenging activity by DPPH method and high metal chelating activity when compared with the other germplasms. With these preliminary studies, the authors are further working on the biotechnological approaches for the production of L-DOPA.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. S. Chattopadhyay, S.K. Datta and S.B. Mahato, *Plant Cell Rep.*, **15**, 271 (1995); https://doi.org/10.1007/BF00193734
2. R. Katzenschlager, A. Evans, A. Manson, P.N. Patsalos, N. Ratnaraj, H. Watt, L. Timmermann, R van der Giessen and A.J. Lees, *J. Neural Neurosurg. Psychiatry*, 75, 1672 (2004); https://doi.org/10.1136/jnnp.2003.028761
3. S.N. Rai, V.K. Chaturvedi, P. Singh, B.K. Singh and M.P. Singh, *3 Biotech.*, **10**, 522 (2020); https://doi.org/10.1007/s13205-020-02532-7
4. K. S. Vishwakarma, S. I. Mohammed, A. R. Chaudhari, N. S. Salunkhe and V. L. Maheshwari, *Indian J. Nat. Prod. Res.*, **8**, (2017).
5. L. Sathiyanarayanan and S. Arulmozhi, *Pharmacogn. Rev.*, **1**, 1 (2007).
6. K. Lahiri, M.J. Mukhopadhyay, Y. Desjardins and S. Mukhopadhyay, *Nucleus (India)*, **55**, 1 (2012); https://doi.org/10.1007/s13237-012-0051-7
7. V. Rudra, J. Shankaraswamy, K. Nagaraju, B.S.K. Nikhi and G. Sathish, *Vigyan Varta*, **1(7)**, 10 (2020).
8. L.R. Lampariello, A. Cortelazzo, R. Guerranti, C. Sticozzi and G. Valacchi, *J. Tradit. Complement. Med.*, **2**, 331 (2012); https://doi.org/10.1016/S2225-4110(16)30119-5
9. K. Lahiri, M.J. Mukhopadhyay and S. Mukhopadhyay, *Plant Tissue Cult. Biotechnol.*, **21**, 115 (2011); https://doi.org/10.3329/ptcb.v21i2.10224
10. L. Misra and H. Wagner, *Phytochemistry*, **65**, 2565 (2004); https://doi.org/10.1016/j.phytochem.2004.08.045
11. O.N. Avoseh, I.A. Ogunwande, G.O. Ojenike and F.M. Mtunzi, *Nat. Prod. Commun.*, **15**, 1934578X2093232 (2020); https://doi.org/10.1177/1934578X20932326
12. B. Rakesh and N. Praveen, Biotechnological Approaches for the Production of L-DOPA: A Novel and Potent Anti-Parkinson's Drug from Mucuna Pruriens (L.) DC, In: Research Trends in Medicinal Plant Sciences, AkiNik Publications, vol. 6, pp. 179-204 (2020).
13. N. Alam, M. Anis, S.B. Javed and A.A. Alatar, *Plant Cell Tissue Organ Cult.*, **141**, 155 (2020); https://doi.org/10.1007/s11240-020-01776-8
14. B. Janarthanam and E. Sumathi, *Asian J. Pharm. Clin. Res.*, **8**, 4 (2015).
15. S. Biswas, A. Mukherjee, U.K. Mallick, G. Ghosh and B. De, *Indian Drugs*, **47**, 12 (2010).
16. M. Murugan and V.R. Mohan, *Sci. Res. Rep.*, **1**, 2 (2011).
17. B.G. Rathod and N.M. Patel, *Int. J. Pharm. Sci. Res.*, **5**, 08 (2014).
18. H.J. Wichers, J.F. Visser, H.J. Huizing and N. Pras, *Plant Cell Tissue Organ Cult.*, **33**, 259 (1993); https://doi.org/10.1007/BF02319010
19. M. Faisal, I. Siddique and M. Anis, *In Vitro Cell. Dev. Biol. Plant*, **42**, 59 (2006); https://doi.org/10.1079/IVP2005717
20. J.B. Vibha, K. Choudhary, M. Singh, M.S. Rathore and N.S. Shekhawat, *Plant Cell Tissue Organ Cult.*, **99**, 319 (2009); https://doi.org/10.1007/s11240-009-9607-x
21. A. Abbott, *Nature*, **466**, S6 (2010). https://doi.org/10.1038/466S6a
22. S. Raghavendra, C.K. Ramesh, V. Kumar and M.H.M. Khan, *Front. Life Sci.*, **5**, 127 (2011); https://doi.org/10.1080/21553769.2011.649188
23. R. Randhir, Y.I. Kwon and K. Shetty, *Bioresour. Technol.*, **100**, 4507 (2009); https://doi.org/10.1016/j.biortech.2009.01.078

24. C.A. Oviedo-Silva, M. Elso-Freudenberg and M. Aranda-Bustos, *Appl. Sci.*, **8**, 12 (2018); https://doi.org/10.3390/app8122431
25. G. Saranya, M.V. Jiby, K.S. Jayakumar, P. Padmesh Pillai and C. Jayabaskaran, *Phytochemistry*, **178**, 112467 (2020); https://doi.org/10.1016/j.phytochem.2020.112467
26. A. Chinapolaiah, H. B. K., M. G.N., V. Thondaiman, V. Keshava Rao, H. R. N. and S. K. S., *Ind. Crops Prod.*, **138**, 2019 (2019); https://doi.org/10.1016/j.indcrop.2019.06.010
27. K. Rajalakshmi and N. Banu, *Int. J. Sci. Res.*, **4**, 11 (2015); https://doi.org/10.21275/v4i11.nov151021
28. G. Karibasappa, N, V. Jain.M, A. Dodamani.S and G. Mali.V, *J. Educ. Health Promot.*, **6**, 90 (2017); https://doi.org/10.4103/jehp.jehp
29. O. Lowry, H, N. Rosebrough.J, L. Farr.A and R. Randall.J, *J. Biol. Chem.*, **193**, 265 (1951).
30. J.J. Chavan, N.B. Gaikwad, P.R. Kshirsagar and G.B. Dixit, *S. Afr. J. Bot.*, **88**, 273 (2013); https://doi.org/10.1016/j.sajb.2013.08.007
31. W. Claussen, *Plant Sci.*, **168**, 241 (2005); https://doi.org/10.1016/j.plantsci.2004.07.039
32. G.C. Bag, P. Grihanjali Devi and T. Bhaigyaba, *Int. J. Pharm. Sci. Rev. Res.*, **30**, 1 (2015).
33. S. Aryal, M.K. Baniya, K. Danekhu, P. Kunwar, R. Gurung and N. Koirala, *Plants*, **8**, 96 (2019); https://doi.org/10.3390/plants8040096
34. N. Praveen, M. Thiruvengadam, H.J. Kim, I.M. Chung and P. Kumar, *J. Med. Plants Res.*, **6**, 33 (2012); https://doi.org/10.5897/jmpr12.200
35. K. Pavithra and S. Vadivukkarasi, *Food Sci. Hum. Wellness*, **4**, 42 (2015); https://doi.org/10.1016/j.fshw.2015.02.001
36. W.S. Jung, I.M. Chung, S.H. Kim, A. Ahmad and N. Praveen, *J. Med. Plants Res.*, **5**, 32 (2011); https://doi.org/10.5897/jmpr11.1129
37. R. Patil, C. Aware, S. Gaikwad, M. Rajebhosale, V. Bapat, S. Yadav and J. Jadhav, *Proc. Natl. Acad. Sci., India*, **89B**, 1413 (2019); https://doi.org/10.1007/s40011-018-01071-9
38. R.R. Patil, A.R. Gholave, J.P. Jadhav, S.R. Yadav and V.A. Bapat, *Genet. Resour. Crop Evol.*, **62**, 155 (2014); https://doi.org/10.1007/s10722-014-0164-8
39. B. Hadapad, C.S. Ravi, M. Shivaprasad, H. Bindu, S. Nadukeri and Devaraju, *J. Pharmacog. Phytochem.*, **7(Suppl.3)**, 86 (2018).
40. M. Pugalenthi and V. Vadivel, *Genet. Resour. Crop Evol.*, **54**, 1117 (2007); https://doi.org/10.1007/s10722-006-9003-x
41. A.O. Oko, J.C. Ekigbo, J.N. Idenyi and L.U. Ehihia, *J. Biol. Life Sci.*, **3**, 1 (2012); https://doi.org/10.5296/jbls.v3i1.2218
42. L.T. Renata, S.S. Alexandre, R.N.C. Ana, R.P.S. Alexandre and S.A. Jailane, *Afr. J. Biotechnol.*, **14**, 676 (2015); https://doi.org/10.5897/AJB2014.14354
43. R.M. Josephine and K. Janardhanan, *Food Chem.*, **43**, 13 (1992); https://doi.org/10.1016/0308-8146(92)90235-T
44. Y.A. Adebowale, A. Adeyemi and A.A. Oshodi, *Food Chem.*, **89**, 37 (2005); https://doi.org/10.1016/j.foodchem.2004.01.084
45. S. Mahesh and N. Sathyanarayana, *J. Crop Sci. Biotechnol.*, **18**, 181 (2015); https://doi.org/10.1007/s12892-015-0019-7
46. C. Kavitha and C. Thangamani, *J. Med. Plants Res.*, **8**, 138 (2014); https://doi.org/10.5897/JMPR2013.5036
47. M. Marimuthu, U. Sundaram and P. Gurumoorthi, *Int. J. Pharm. Pharm. Sci.*, **5**, 2 (2013).
48. R.N. Nwaoguikpe, W. Braide, C.O. Ujowundu, *Pak. J. Nutr.*, **10**, 10 (2011); https://doi.org/10.3923/pjn.2011.947.951
49. M. Krishnaveni and D. Hariharan, *Int. J. Pharma Bio Sci.*, **7**, 2 (2017).
50. L.L. Mensor, F.S. Menezes, G.G. Leitao, A.S. Reis, T.C. dos Santos, C.S. Coube and S.G. Leitao, *Phytother. Res.*, **15**, 127 (2001); https://doi.org/10.1002/ptr.687
51. N.A. Khalaf, A.K. Shakya, A. Al-Othman, Z. El-Agbar and H. Farah, *Turk. J. Biol.*, **32**, 1 (2008).
52. K.N. Agbafor and N. Nwachukwu, *Biochem. Res. Int.*, **2011**, 1 (2011); https://doi.org/10.1155/2011/459839
53. Y. Rajeshwar, G. P. S. Kumar, M. Gupta and U. K. Mazumder, *Eur. Bull. Drug Res.*, **13**, (2005).
54. M.A. Jimoh, O.A. Idris and M.O. Jimoh, *Plants*, **9**, 1249 (2020); https://doi.org/10.3390/plants9091249
55. M. Dhanasekaran, B. Tharakan and B.V. Manyam, *Phytother. Res.*, **22**, 6 (2008); https://doi.org/10.1002/ptr.2109
56. B.J. Divya, M. Venkataswamy, K. Thyagaraju and K.T. Raju, *Indo Am. J. Pharm. Res.*, **7**, 1 (2017).
57. P. Siddhuraju and K. Becker, *J. Sci. Food Agric.*, **83**, 1517 (2003); https://doi.org/10.1002/jsfa.1587