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Comparative anatomical studies on seeds of *Mucuna* Adans. and *Canavalia* DC. species

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The corpus of anatomical knowledge has contributed tremendous share in systematic, interrelationship, physiology and pharmacognosy. Anatomical studies have become much relevant in the context of hundreds of new plants entering in to the crude phyto-drug markets to identify market samples which are sometimes adulterated with other species. The present study highlighted microscopic parameters of market samples of *Mucuna pruriens* (Linn.) DC., known as Atmagupta in Sanskrit and Konch in Hindi, to resolve the botanical identity of its seeds. Preliminary survey in Tamil Nadu also revealed that seeds of *Mucuna pruriens* and other six species, viz. *M. cochinchinensis* (Lour.) A. Chev.*, M. deeringiana* (Bort) Merr.*, M. utilis* Wight*, M. atropurpurea* (Roxb.) Wight & Arn.*, Canavalia ensiformis* (Linn.) DC. and *C. virosa* (Roxb.) Wight & Arn. are sold as *Poonaikali*. The features, viz., rim-aril, cuticle, palisade layer of osteo-sclereids or macrosclereids, hour glass cells, mesophylls and tracheid-bar are common to all the observed specimens. However, anatomical structures at hilar region are more important for diagnostic purpose. Shape and size of rim-aril also play major role in delimiting the taxa. The surface architecture of the seed at micropylar region also varies.

Keywords: Anatomical studies, Seeds, Atmagupta, Konch, *Mucuna, Canavalia,* Osteo-sclereids, Tracheid bar, Medicinal Plant.

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Introduction

Botanical identity of crude drugs in fragmentary form possesses a problem especially in the plant based pharmaceutical arena. Many crude drugs such as bark and root simulate the unidentified materials for adulteration of the drug with genuine ones. The organoleptic characters such as smell, taste, texture and some other parameters that could be retrieved with the help of the sensory organs have been one of the methods of diagnosis. However, this technique has its own limitations. The more dependable method for the identification of the phyto-drug is microscopic, anatomical and phytochemical parameters. The seeds of *Mucuna pruriens* (Linn.) DC. plant have been used as food, tonic and aphrodisiac by many tribal communities in India since many centuries[1]. Pharmacological studies have validated its various activities like anti-diabetic; aphrodisiac, anti-neoplastic, anti-epileptic and antimicrobial activities[2,3]. Its learning and memory enhancement, analgesic and anti-inflammatory, fertility (in men) and antivenom activities have also been reported scientifically[4-10]. The seeds of this plant are collected mostly in the wild. Various species of *Mucuna* are being sold in the market under the trade name Atmagupta. Preliminary survey in Tamil Nadu crude drug market revealed that seeds of *M. cochinchinensis* (Lour.) A. Chev.*, M. deeringiana* (Bort) Merr.*, M. utilis* Wight*, M. atropurpurea* (Roxb.) Wight & Arn.*, Canavalia ensiformis* (Linn.) DC. and *C. virosa* (Roxb.) Wight & Arn. are sold as 'Poonaikali' (Tamil vernacular name of *M. pruriens*)[11].

Comparative morphological and anatomical studies play an important role to test the purity of the crude drugs that is commercially available for the medicinal purposes. The present investigation was aimed to study the morphological and anatomical features of seed coat of all the market samples of 'Atmagupta' or 'Poonaikali' and made an effort to evolve diagnostic characters to distinguish the authentic one with their adulterants or substitutes. The seeds of *M. pruriens* and its adulterants could be distinguished by their size and shape. Seeds of *M. pruriens*, *M. cochinchinensis, M. deeringiana* and *C. ensiformis* are oval in shape

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and smooth and glossy. Therefore, seeds of these three species are commonly used as adulterants or substitutes. The seeds of *M. pruriens* are brown in colour with black flecks. Burnt seeds of *M. pruriens* are dark brown in colour along with ashes. Seeds of *M. cochinchinensis* are dull white in colour with grey striations. Seeds of *M. deeringiana* are dark brown in colour and that of *M. utilis* are grey with black spots. *C. ensiformis* seeds are ivory in colour and seeds of *C. virosa* are yellowish brown in colour. Seeds of *M. atropurpurea* are light to dark brown in colour (Plates 1). These morphological characters ensure the identification and authentication of the chosen medicinal plant product.

Plate 1— Seed morphology of *Mucuna* and *Canavalia* species: a. *M. pruriens* (Dried); b. *M. cochinchinensis*; c. *M. pruriens* (Burnt); d. *M. deeringiana*; e. *M. utilis*; f. *C. virosa*; g. *C. ensiformis*; h. *M. atropurpurea.*

Materials and Methods

Seeds of different samples of Poonaikali were purchased from Herbal drug stores from different places of Tamil Nadu like, Kanyakumari, Kalakad, Tirunelveli, Madurai, Thanjavur, Myladuthurai and Chennai. The samples were fixed in FAA. After 24 h of fixing, the specimens were dehydrated with graded series of tertiary-butyl alcohol as per the standard guidelines[12]. Infiltration of the specimens was carried out by gradual addition of paraffin wax (melting point 58-60°C) until tertiary-butyl alcohol solution attained super saturation. The specimens were cast into paraffin blocks by usual method[13]. Paraffin embedded specimens were sectioned with the help of Rotary Microtome. The thickness of the sections was 10 to 12 µm. Dewaxing of the sections was done by customary procedure[13]. The sections were stained with toludine blue (a polychromatic stain) as per the standard method[14]. The dye rendered pink colour to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage and blue to the protein bodies. Microphotographs of different magnifications were taken with Nikon Labshot 2 Microscopic Unit. Measurement of cells was made with micrometer. For each element, 15-20 measurements were taken and average is presented.

Results and Discussion

Transverse sections of seed coats at hilar region of the specimens reveal the characteristic features of legumes seeds, like, presence of large embryo, hard and dry testa. The seeds are exalbuminous. Various features of the seed coat of species are discussed below and key characters are summarized in Table 1.

M. pruriens

T.S. of seed coat at the hilar region reveals a dome shaped structure with a pair of wing shaped rim-aril (Plate 2a). The rim-aril is made up of loosely arranged hyphae-like thin walled parenchyma cells. Rim aril is the continuation of the funiculus layer. The palisade layers are narrow and radially elongated the palisade zone is 115 µ in height. The narrow elongated tracheid bar lies below the micropyle. The tracheid elements are linear and compact with scalariform thickening. The tracheid bar is surrounded by 2-5 layers of small, isodiametric and compactly arranged thin walled parenchymatous cells at the distal and basal ends. Adjacent to the hilar region below the palisade layer lays a single layer of hour glass cells. The ground tissue of this region consists of stellate parenchyma. Testa consists of palaside layer followed by a layer of hour glass cells (Plate 3a). The hour glass cells are dumb-bell shaped measuring 100 µ length with unequal heads.

M. cochinchinesis

The hilar region shows dome like structure at the micropylar end flanked by a pair of wing like rim-aril with tapering ends pointing towards the micropyle (Plate 2b). The palisade layer is thicker (length of the macrosclereid is 130 µ) than that in *M. pruriens*. Testa consists of a single layer of palisade cells followed by a single layer of hour glass cells (Plate 3b). The dimensions is 100 × 62 × 22 × 52 µ. The ground tissue consists of 10-12 layers of oval to hexagonal shaped loosely arranged parenchyma cells.

M. deeringiana

The seed coat reveals (Plate 2c) the following zones; funicular layer with a pair of clubbed globular shaped rim-aril, palisade layer, stellate parenchyma. Tracheid bar is oval shaped and uniformly surrounded by 3 layers of isodiametric parenchyma cells. The distinguishing feature is the presence of two layers of sclerenchyma lying below the palaside layer in the hilar region. The length of palisade cell is 110 µ. The hour glass cell is with unequal ends (Plate: 3c).

Table 1 — Dimension of isolated elements in seed coats of *M. pruriens* and its adulterants

Sample	Macro sclereids L × B (µ)	Hour glass cell L × B.E × Col × N.E (µ)	Stellate parenchyma L × B (µ)	Parenchyma L × B (µ)
Mucuna pruriens (dried)	115 × 9	100 × 60 × 25 × 40	80 × 20	12 × 70
M. cochinchinensis	130 × 11	100 × 62 × 22 × 52	102 × 30	80 × 50
M. deeringiana	110 ×11	75 × 62 × 20 × 30	74 × 24	60 × 40
M. utilis	100 × 30	100 × 60 ×30 × 40	120 × 20	95 × 80
Canavalia ensiformis	128 × 10	60 × 50 × 10× 50	112 × 22	134 × 86
C. virosa	190 × 20	80 × 30 × 20 × 30	80 × 20	80 × 60
Mucuna atropurpurea	430 × 7	140 × 76 × 25 × 35	140 × 32	120 × 88

(L – Length; B – Breadth; B.E. – Broad End; N.E. – Narrow End)

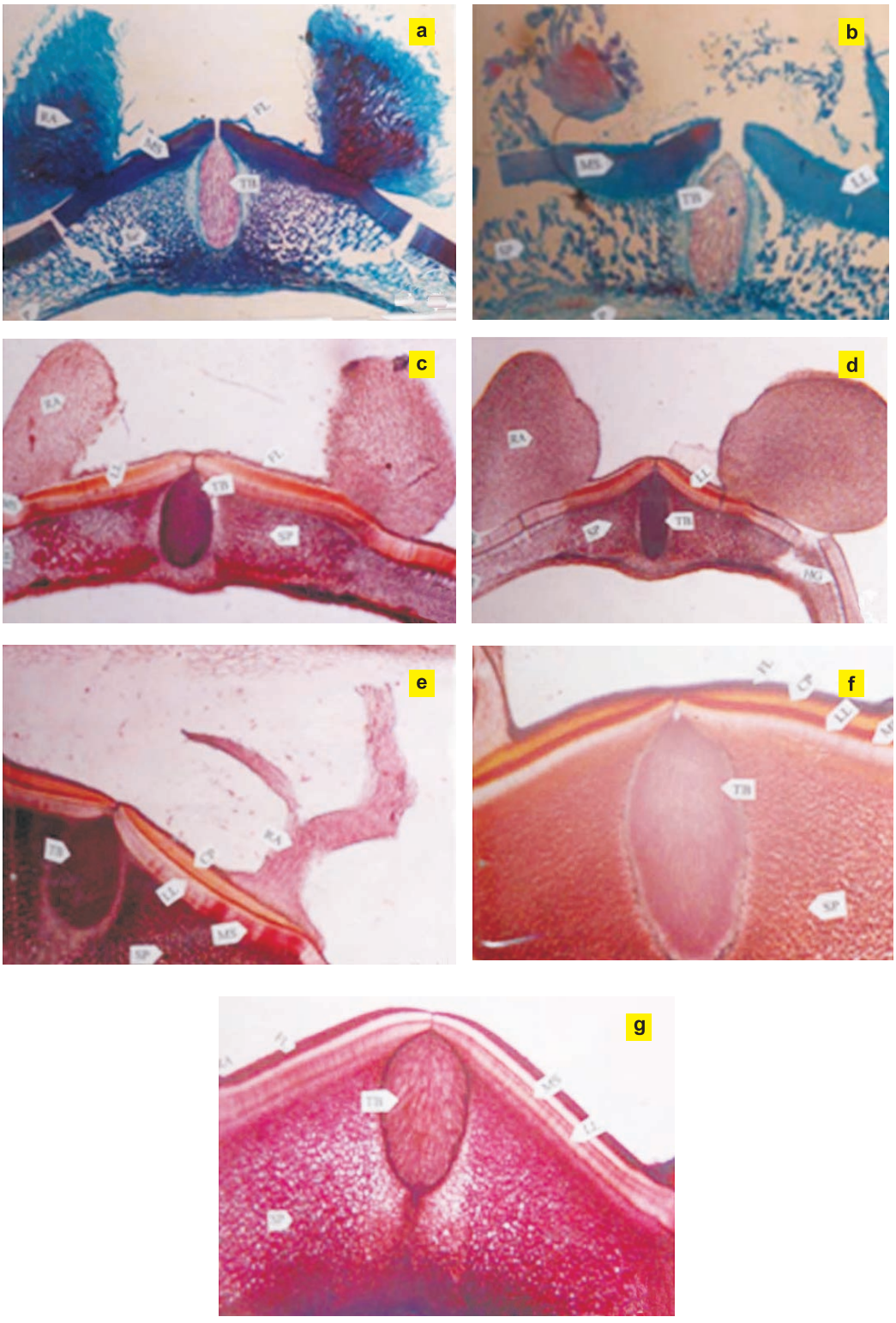

Plate 2— T. S. of seed coat through hilum region: a. *M. pruriens; b. M. cochinchinensis; c. M. deeringiana; d. M. utilis;* e. *C. ensiformis; f. C. virosa; g. M. atropurpurea* (CP- Confluent Palisade layer, HG- Hour Glass Cell, LL-Linear Ludidda, MS-Macrosclereids, P-Parenchyma, RA- Rim Aril, SP- Stellate Parenchyma, TB- Tracheid Bar, FL- Funiculus Layer).

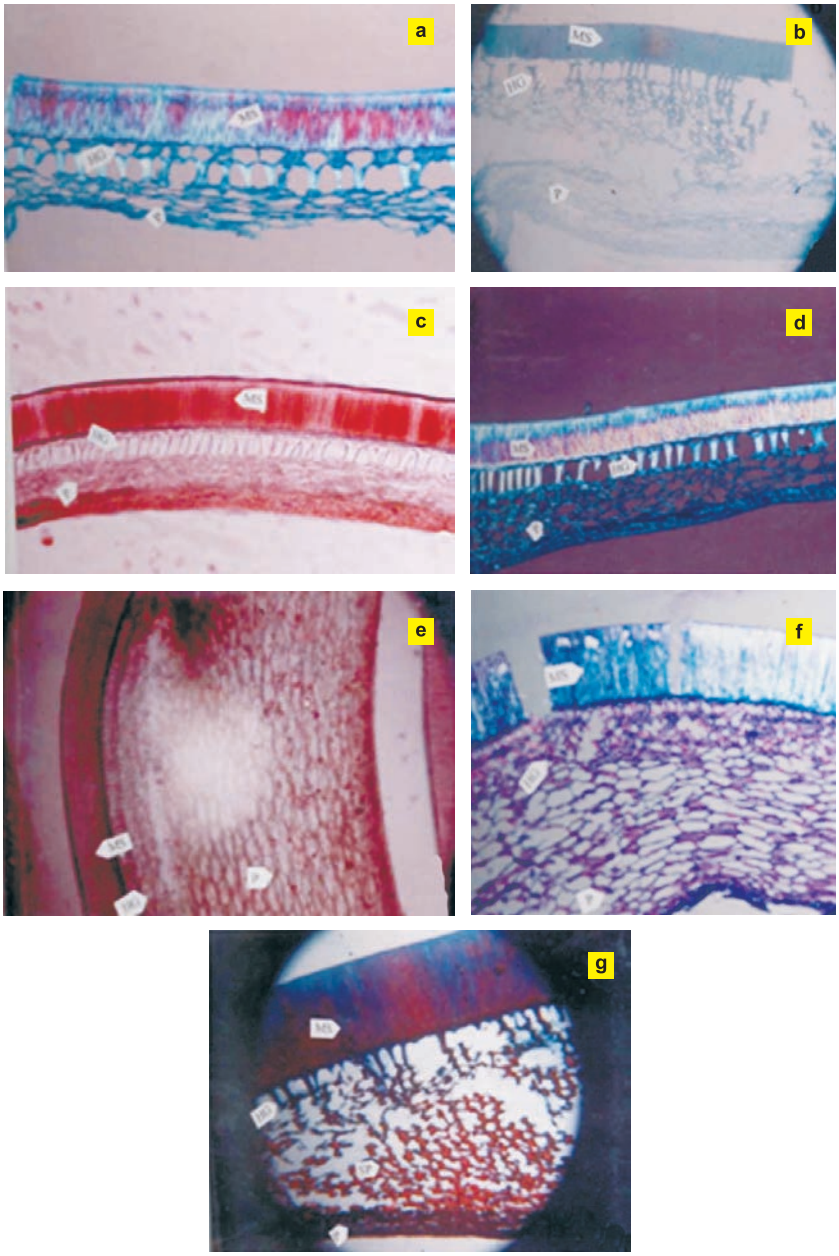

Plate 3— T. S. of seed coat through of non-hilum region: a. *M. pruriens; b. M. cochinchinensis; c. M. deeringiana; d. M. utilis;* e. *C. ensiformis; f. C. virosa; g. M. atropurpurea* (CP- Confluent Palisade layer, HG- Hour Glass Cell, LL-Linear Ludidda, MS-Macrosclereids, P-Parenchyma, RA- Rim Aril, SP- Stellate Parenchyma, TB- Tracheid Bar, FL- Funiculus Layer).

M. utilis

Seed coat at the hilar region (Plate 2d) has an elevated dome structure at the micropylar end and flanked by oval to globular shaped rim-aril. The seed coat (Plate 3d) is strikingly thinner (0.12 mm) compared to the seed coats of the other samples.

Canavalia ensiformis

The surface in the hilar region (Plate 2e) is flat with a long narrow, curved horn-like rim-aril which is incurved towards the micropyle. The funiculus layer adnate to the seed coat consists of vertical elongated cells. The size of the funiculus layer is almost equal to

the palisade layer of the seed coat. The funiculus layer has two zones: upper dark zone and inner light coloured zone. The palisade layer of the seed coat has a prominent linear lucida. The micropyle opens into an oval to globular tracheid bar, where the tracheids are arranged in a non-storied, irregular manner. The tracheid cells have pitted thickening in lateral wall. The tracheid bar is surrounded by layers of elongated parenchyma cells. In the (Plate 3e) non-hilar region also a single layer of palisade is present. But, next to this layer, 3 layers of hour-glass cells are present. The seed coat is thicker than that of all other samples analysed (Table 1). Below this many layers of parenchyma are present. The hour glass cells are typical dumb-bell shaped with equal sized ends on both ends. These cells are smaller in size (60 × 50 × 10 × 50μ).

C. virosa

The rim-aril is small loops like a beak of a bird (Plate 2f). The funiculus layer consists of two zones: the outer dark brown layer and inner light brown layer. The outer layer consists of tangentially elongated and crushed cells embedded in dark brown secretions. The macrosclereids has a clear linea lucida. The funiculus layer and palaside layer together gives the appearance that there are two super imposed layers of palisade cells. The tracheid bar is oval to cylindrical shaped with irregularly arranged tracheid cells. The tracheid cells are pitted in nature. The tracheid bar surrounded by two layers of thin walled elongated parenchyma cells. Below the tracheid bar, a few layers of thickened sclerenchyma cells and 3-4 layers of thin walled parenchyma cells are also present. Following the macrosclereids many layers of stellate parenchyma are present which are also smaller in size. The testa region adjacent to the hilum is having 5 layers of hour-glass cells but the number of layers decreased (up to 3 layers) towards the antiraphal end. The hour glass cells (Plate 3f) are small, regular, dump-bell shaped with equal ends (80 × 30 × 20 × 30 μ). Below the hour glass cells 14 layers of thin walled (80 × 60 μ) parenchyma are present in which the innermost layers are smaller in size.

Mucuna atropurpurea

The T.S. of seed coat resembles the other species of *Mucuna* but the size and quantity of all the cells are larger and thicker (Plate 2g). The tracheid bar is oval in shape unlike other species of *Mucuna*, there is no parenchyma cells surrounding the tracheid bar. Again the rim-aril is much reduced to a small papilla like projection. The non-hillar region of seed coat is filled up with thick walled stellate parenchyma (Plate 3g) but at the innermost region only a few layers of thin walled parenchyma cells are present. The palisade cells are very large with dimension, 430 × 70 μ (LxB). It is to be noted that length of palisade cells are almost 4 times greater than that in seeds of other species of *Mucuna*.

Seed coats of *M. pruriens* and its adulterations show variations in their anatomical features which aid in diagnosing. Anatomical structures at hilar region are more important for diagnostic purpose. Size and shape of rim-aril, surface at micropylar end, palisade layer, tracheid bar and its wall thickening and hour glass cells form distinguishing features. It was interesting to note that seed of all species of *Mucuna* observed in this study have unequal hour glass cells, i.e., cells with unequal ends. Whereas *Canavalia* sp. have hour glass cells with equal ends. Seeds of *C. ensiformis* and *C. virosa* have two zones of funiculus layer and have confluent palisade cells. These confluent palisade of funiculus layer is absent in all the chosen species of *Mucuna*. Shape and size of rim-aril also play major role in delimiting the taxa. Wing shaped rim-aril are found in *M. pruriens* and *M. cochinchinesis*; aril with tapering end in *M. cochinchinensis*; club shaped in *M. deeringiana*; oval to globular in *M. utilis*; horn-like and curved in *C. ensiformis*; small beak-like in *C. virosa* and small papillose projection in *M. atropurpurea*. Surface architecture of the seed at micropylar end also varies; dome shaped in *M. pruriens*; elevated dome shaped in *M. cochinchinensis* and *M. utilis*; concave in *M. deeringiana* and *M. atropurpurea*; and flat in *C. ensiformis*. The shape of tracheid bar and all thickening in all seed coats observed also show remarkable similarities and dissimilarities. In *C. ensiformis* and *C. virosa* tracheid in the tracheid bar have pitted thickening while those in *Mucuna* sp. have scalariform thickening. Largest palisade cells, 430 μ long, are found only in *M. atropurpurea*. Thickness of seed coat is also the greatest in *M. atropurpurea*. Thinnest seed coat was observed in *M. utilis*.

Different species of *Mucuna* and *Canavalia* could be identified based on key characters of seed coat

Table 2 — Key characters of seed coat of *Mucuna* and *Canavalia* species

	Species
Funiculus layer thick with confluent palisade cells. Tracheid cells in tracheid bar with pitted thickening. Hour glass cells with equal ends	
Rim-aril curved and horn like	*C. ensiformis*
Rim-aril small and beak like	*C. virosa*
Funiculus layer thin. Absence of confluent palisade cells. Tracheid cells in tracheid bar with scalariform thickening. Hour glass cells with unequal ends	
Shape of aril: Wing-shaped	
Length of palisade cells < 110 µ	*M. pruriens*
Length of palisade cells > 110 µ	*M. cochinchinesis*
Shape of aril oval to globular	
Concave micropylar surface	*M. deeringiana*
Elevated dome micropylar surface	*M. utilis*
Rim-aril small sized and papillose	*M. atropurpurea*

given in Table 2. Structure of seeds and seed coats of legumes have peculiar characters and are stable in wide geographical and climatic conditions and hence amenable for identification[15].

Conclusion

Thus in addition to chemical, morphological studies, anatomical studies could help in checking adulteration in crude drugs of similar appearance. This type of studies will help in detecting unscrupulous act practiced by traders to increase the supply of crude drug and their monetary profit. None the less, as the preparation of herbal drugs is increasing, there is an urgent need to have a quality control check on the various formulations to assure a supply of efficacious drugs. Further supporting researches are also very much needed for all the ISM drugs.

References

1 Vasudeva Rao MK and Shanpru R, Some plants in the life of the Garos of Meghalaya, *In*: Glimpses of Indian Ethnobotany, Jain S.K (Ed), Oxford and IBH Publishing co, New Delhi, 1981, 153-160.

2 Akhtar MS, Qureshi AO and Igbal J, Antidiabetic evaluation of *Mucuna pruriens* seed, *J Palc Med Assoc,* 1990, **40**(7), 174-50.

3 Sathiyanarayanan L and Arulmozhi S, *Mucuna pruriens* A Comprehensive Review. *Phamracog Rev*, 2007, **1**(1), 157-162.

4 Poornachandra MN, Khanam S, Shivananda BG, Shivananda, TN and Dris R, *Mucuna pruriens* (LDDC)-A novel drug for learning and memory retrieval, *J Food Agric Environ*, 2005, **3**(3&4), 13-15.

5 Rajendran V, Joseph T and David J, *Mucuna pruriens* decreases sexual activity in female rats, *Indian Drugs* 1997, **34**(3), 136-139.

6 Shukla KK, Mahdi AA and Ahmad MK, *Mucuna pruriens* reduces stress and improves the quality of semen in infertile men, *eCAM Advance Access,* DOl. 10.1093/ecam/nem171. (www.creativecommons.org), 2007.

7 Guerranti R, Aguiyi JC, Neri S, Leoncini R, Pagoni R and Marinello E, Proteins from *Mucuna pruriens* and enzymes from *Echis carinatus* Verom, *The J Biol Chem*, 2002, **277**(19), 17072-17078.

8 Fattepur SR and Gawade SP, Preliminary screening of herbal plant extracts for anti-venom activity against common sea snake (*Erhydrina schistosa*) poisoning, *Pharmac Mag,* 2008, **1**(3), 87- 92.

9 Hishika R, Shastry S, Shinde S and Gupta SS, Preliminary, phytochemical and anti-inflammatory activity of seeds of *Mucuna pruriens*, *Indian J Pharmacol*, 1981, **13**(1), 97-98.

10 Buckles D, Velvet bean: a new plant with a history, *Econ Bot*, 1995, **40**, 13-25.

11 Vijayambika C, Pharmacognostical studies on *Mucuna pruriens* (L.)DC. and its adulterants, Ph.D Thesis, Dept. of Siddha Medicine, Tamil University, Thanjavur, India, 2003.

12 Sass JE, Elements of Botanical Microtechnique. McGraw Hill Book Co INC New York and London, 1940, 222.

13 Johansen DA, Plant Microtechnique, McGraw–Hill Book Company Inc. New York 1940, 523.

14 O'Brein TP, Feder N and Mcull ME, Polychromatic staining of plant cell walls by toludine blue – O, *Protoplasma,* 1964, 59, 364-373.

15 Begchi GD and Tripathy T, Scanning electron microscopic studies on some medicinally important seeds of Papilionaceae, *Geophytology,* 1986, **16**(1), 122-128.