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Pharmacognostic characterization of Mung Beans (Vigna radiata (L. R. Wilczek) Seeds for identification and evaluation

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Abstract
Seeds of Mung beans (Vigna radiata L.R. Wilczek) belongs to family Fabaceae, an important food source and traditional medicinal cereals in the Indian system of medicine. In recent years, they have also gained popularity due to their dynamic therapeutic potential. However, before conducting research, it is essential to authenticate them. Botanical microscopy is the most convenient technique for authenticating plant material. So, this study examines detailed pharmacognostic features of seeds of Mung beans using a bright-field microscope and polarized light microscope. In addition to microscopic studies, various physicochemical parameter of seeds of mung beans were also evaluated like total ash value, water-soluble ash, acid-soluble ash, swelling index, foaming index and extractive values of different extracts. Morphologically, seeds were olive-green in colour, elliptical in shape, smooth and shiny with long white raphe. The embryo has located in the middle part of the seeds, surrounded by endospores. The endosperm cells are large elliptical in shape, thin-walled and dense accumulating starch grains as well as narrow straight micropylar canal starting from the outer surface of the embryonal chamber that is some of the diagnostic features observed from the anatomical study. In powder microscopy, palisade sclereids or columnar sclereids and starch grains were found to be abundant. The results of the pharmacognostic study of seeds of Mung beans can serve as a valuable source of data and provide suitable significant standards for the taxonomic identification and evaluation of quality. Researchers may also use supportive data while preparing monographs. It may serve as a supporting reference for future investigations and several applications.

Keywords: seeds of Mung bean, botanical microscopy, phytochemical constituents, extractive values

1. Introduction

Seeds of Mung beans (V. radiata (L.) R. Wilczek) is usually identified as a 'Green pearl' of Asia. It has been extensively used as a traditional food in the whole World [1]. The Mung beans is commonly named as mash, golden gram, green gram, and Mung beans. The species compasses from small herbs to large tropical canopy trees. This plant species are found in the humid tropics, temperate zones, high land, arid zones and in low lands [2]. This is the best source of protein, minerals, and vitamins. The previous names of Mung beans were Phaseolus aureus or Phaseolus radiata. Mung beans are found to be moved from the genus Phaseolus to genus Vigna in the 1970s. According to the International Legume Database and Information Service reports several synonyms were found i.e., Vigna radiata var. glabra (Roxb) Verdc, Vigna radiata var. grandiflora, Vigna radiata var. glabra, Vigna radiata var. setulose (Dalzell), Vigna radiata var. sublobata (Roxb) have been cited in the literature. It is native to Bangladesh, India, Lesser Sunda Is, Myanmar, Laos, New South Wales, Pakistan, Yemen Queensland, Sri Lanka, Northern Territory, Taiwan, Thailand, Vietnam, West Himalaya and Western Australia. In India farmers usually have been cultivating seeds of Mung beans since 3500 years ago. These cultivated Mung beans spread rapidly from India to China and various regions of Southeast Asia. Mung beans plant contains plenteous health benefits to prevent human diseases [3].

Traditionally, seeds of Mung beans is used against alcoholism. This information was mentioned in the classical book (CAO-GAND-MU) i.e., the composition of the book of Materia Medica [4]. Generally, it is used as an antipyretic, antiscorbutic, diuretic, antidote, antihypertensive and anticancer properties. An oriental herbalist recommended it for aches, heat, high blood pressure and inflammation. Mung beans seeds used either raw or cooked at matured poultice just because of its curative potential for polyneuritis galinarum. However, in India, the mung bean seeds are used for cough, paralysis, fever, rheumatism. They are used as a hot and tonic, and considered for piles liver diseases. Mung beans root are known to be narcotic and are given as a healing property in bone aches [4, 5].

It is found that Mung bean seeds has a lot of pharmacological activity such as anticancer [6], antihyperlipidemic [7, 8], antihypertensive [9], antidiabetic [10], antioxidant [11, 12], anti-viral [13], antifungal and antibacterial [14]. Mung beans can also to reduce high-fat diet-induced obesity and disorder [15] as well as usefull in liver diseases [16]. It also increases muscular strength and effective in rheumatism [17], and anti-alzheimer [18]. However, Literature revealed that no detailed pharmacognostic study has not been carried out on the seeds of Mung seeds (V. radiata (L.) R. Wilczek) for its proper authentication, hence the present investigation was undertaken. The aim of present study to evaluate various pharmacognostical parameters such as macroscopic, microscopy, powder microscopy physicochemical, and phytochemical studies of Mung bean seeds.

2. Materials and Methods for Anatomical Studies

2.1. Collection of specimen

Mung beans were planted in late May to early June in Gurugramm, India. Mung beans had beared flower within 50 to 60 days after planting and had continued flowering for about a month. Seeds of Mung beans were collected in month of August. The plant was identified by the National Institute of Science Communication and Information Resources (NISCAIR), located in New Delhi, India, and voucher specimen of the plant (No. NISCAIR/RHMD/Consult/2018/3245-46) is kept for further reference. After confirmation and identification, the sample was subjected to further Pharmacognostical studies. Care was taken to select healthy seeds. Seeds of Mung beans were fixed in an FAA (Formalin – 5ml + Acetic acid –5ml + 70% ethyl alcohol – 90ml). After 24 hours of fixing, the specimens were dehydrated with a graded series of tertiary - butyl alcohol. Infiltration of the specimens was carried by the gradual addition of paraffin wax (melting point 58 - 60 ^{0}C) until the tertiary butyl alcohol solution attained supersaturation. The specimens were cast into paraffin blocks [19]

a. Sectioning

The paraffin-embedded specimens were sectioned with the help of a Rotary microtome. The thickness of the section was measured from 10 to 12µm. Dewaxing of the sections were done by the customary procedure [19]. The sections were stained with Toluidine blue. Glycerin mounted temporary preparations were made for macerated materials. Powder material of different parts was cleared with NaOH and mounted in glycerin medium after staining. Different cell components were studied and measured [20, 21].

b. Photomicrographs

Microscopic descriptions of tissues are supplemented with micrographs wherever necessary. Photographs of different magnification were taken with the Nikon labphoto 2 microscopic unit. For normal observation bright field was used. For the study of crystals, starch grain, and lignified cells, polarized light was employed. Since these structures have birefringent property, under polarized light they appear bright against a dark background. Magnifications of the figures are indicated by the scale- bars. Descriptive terms of the anatomical features are as given in the standard anatomy books [22].

c. Physicochemical and phytochemical analysis

Physicochemical values such as the percentage of ash values and extractive values were determined according to the well-established official methods and procedures [23, 24]. Preliminary Phytochemical of screening of different were carried out using the standard procedures [25].

3. Result

3.1. Macroscopic characteristics

The leaves, seeds, flowers, stems, pods, roots of the Mung beans plants have major constitutes. Plant leaves are found to be trifoliate, entire leaflet, ovate to rhombic, acuminate 5-15 cm in length. Leaves are greenish to yellow, odorless, bitter. Stems are green, odorless and slightly bitter. Pods can be identified by 5.5-10 cm in length and thin cylindrical with short pubescences. Generally, Stems are long, thin, round, cylindrical, hairy with a twinning to its upper branches, and 50-160 cm long. Flowers are yellow to yellowish-green clusters of 10-25 flowers on long pedicels. Seeds are more or less globular, often green in color and having a fine surface, wavy ridges sometimes may be almost invisible, characteristics of odor, sweet, mucilaginous after soaking in water. Roots are small rootlets with parent root. Roots are light brown/ dark yellowish, odorless, bitter. Generally, flat hilum is covered by white rough layer

3.2. Anatomy of seeds

The seeds are thick, short and cylindrical. In the cross-section view, the seeds appear broadly oblong with a short wide ridge on one side in the Figure of the seed Fig. (1). Since, Toluidine blue is a polychromatic stain. The staining results were remarkably good, and some cytochemical reactions were also obtained. The dye rendered pink color to the cellulose wall, blue to the lignified cell, dark green to violet to the mucilage, blue to the protein bodies, etc, wherever necessary sections were also stained with safranin and fast green and Iodine for starch, and, the same consists of thin seed coats and large portion start containing endosperm. The embryo is located in the middle part of the seeds, surrounded by endosperm Fig. (1) also, there is a narrow straight micropylar canal starting from the outer surface of the embryonal chamber Fig. (2) however, the endosperm cells are large elliptical in shape, thin-walled and posses dense accumulating starch grains.

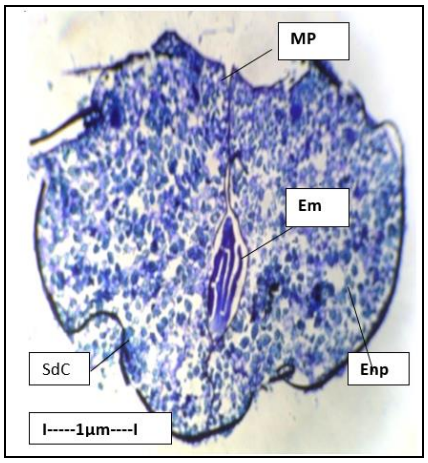

Fig 1: T.S of seed showing the seed coat, endosperm of starch grains and two embryos. (4x) (Enp: Endosperm; Em: embryos; Mp: micropylar canal; Sdc: seed Coat)

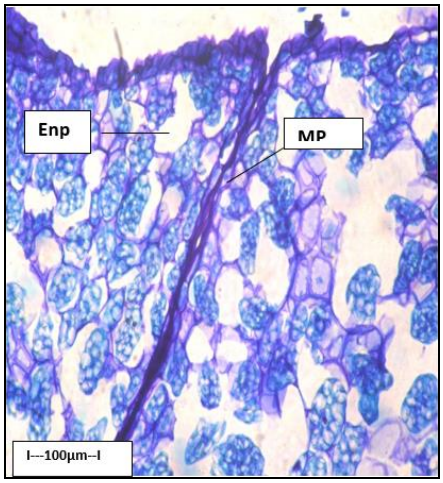

Fig 2: T.S. of seed showing micropylar canal with thick-walled cells. (20x) (Enp: Endosperm; Em: embryos; Mp: micropylar canal; Sdc: seed Coat)

The epidermal layer epicarp of the seed consists of thick-walled cells with conical outgrowth and outer tangential walls. Epidermis layer is wide, rectangular, thin-walled parenchyma cell as described in Fig. (3). the endosperm cells are in the central part of the seed are wide, angular, compact, and thick-walled as described in Fig. (4).

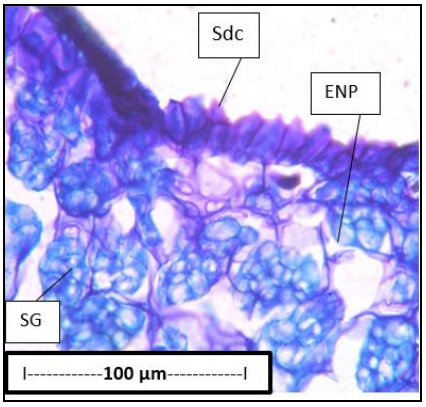

Fig 3: T.S of a portion of the seed coat. (40x). (Enp: Endosperm; Sdc: Seed coat; SG: Starch Grains)

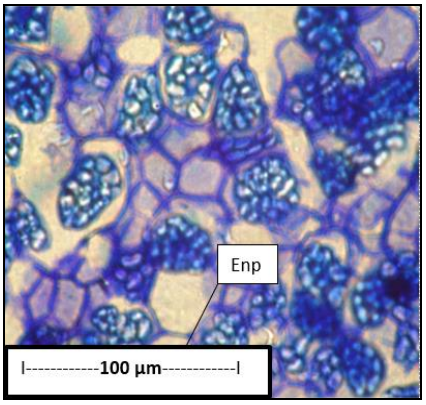

Fig 4: Cells in the endosperm, processing starch grains within the cells. (40x) (Enp: Endosperm)

The seeds contain two embryos situated along the long axis of the embryo sac. The embryo consists of two long flat cotyledons and thick cylindrical radicals. The cotyledonous of the embryo are intricate with each other. They consist of dark-stained cells as shown in (Fig. 5)

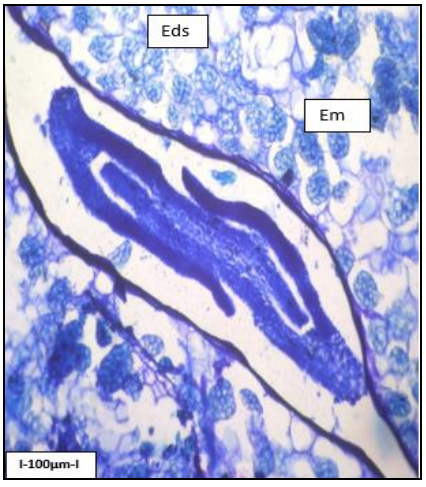

Fig 5: Sectioned view of the seed showing two embryoes (10x) (Em; Embryo; Eds: Endosperm)

3.3. Powder microscopy of the seed

Powder preparations of the seeds show the following characteristics Palisade sclereids or columnar sclereids are common in the powder (Fig. 5, 6, 7). These sclereids from the sclerotic of the seed coat. The vertically elongated thick-walled with the central part being hollow and the terminal part being dilated (Fig. 6). These cells are 40 µm long and 12µm thick. Starch grains are abundant in the powder when viewed under the polarized light, Starch grains appear bright white (Fig. 6 and 7). When stained with KI, the grains appear dark (Fig. 6). The starch grains are cylindrical, elliptical (Fig. 6 and 7). Starch grains bearing cells: Apart from isolated starch grain, these are cells in which, the starch grains are densely packed (Fig.8). These cells are wide, angular, and thin-walled. Starch grains were also present within the cells. Small fragments of the outer seed coat are seen on the surface with outer ends of the columnar in shape. Sclereides are visible as compact, highly thick-walled cells. The intercellular spaces of the cells are thick and vary in outline. (Fig. 9). The peeling of the seed coats is seen in the outer and inner surface. The inner surface of the seed coat is less distinct while the outer surface is well defined with angular cells. These cells have a circular and a compact pit (Fig.10).

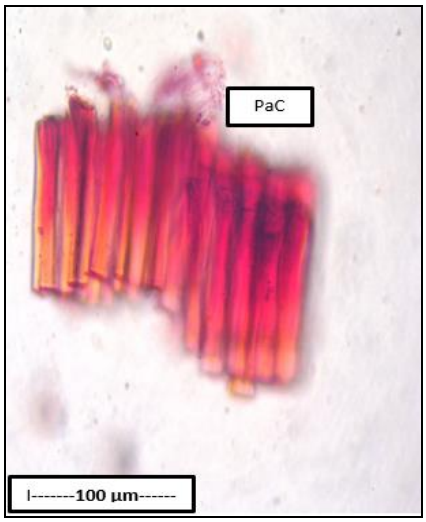

Fig 6: Columnar sclerids forming palisade layer in the outer seed coat (PaC: Palisade cell)

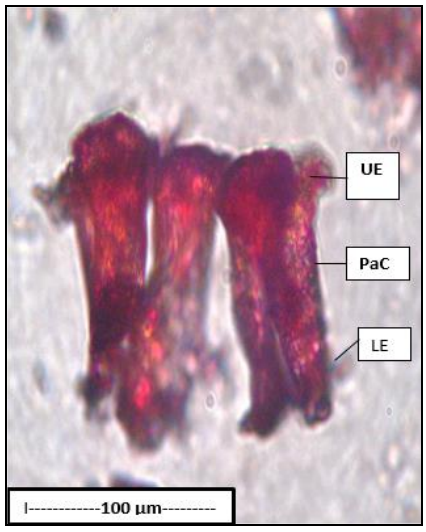

Fig 7: Vigna powder microscopy, Palisade cells-(80X) Columnarsclerids forming palisade layer in the outer seed coat

d. Phytochemical analysis of different extracts of Mung bean seeds.

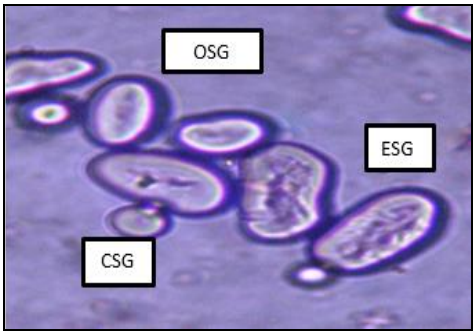

Fig 8: Starch grains as seen under bright fields. Starch grain- (40X) CSG: Circular starch grains; OSG: Ovate starch grains, SG: Starch grains

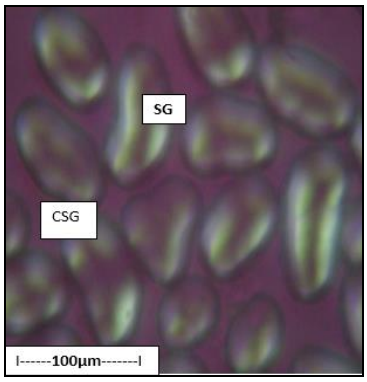

Fig 9: Mungbean Seeds (Vigna radiata (L.) R. Wilczek), Starch grains are seen under polarized light – (40X), SG: Starch grain

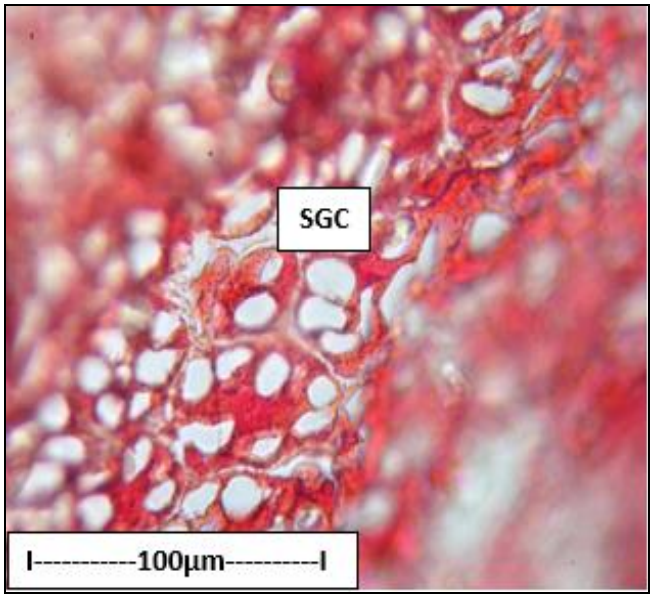

Fig 10: Starch containing endosperm cells (40x) (SGC: Starch grains counter)

The four extracts made from seeds of V. radiata were subjected to initial phytochemical investigation using standard methods and results revealed that ethanol extract showed the presence of phenol, saponin, flavonoids, terpenoids, and tannins. Water extract showed the presence of phenol, carbohydrates saponin, flavonoids, terpenoids, tannins and proteins. Chloroform extract indicated the presence of lipid and terpenoids. Petroleum ether extract showed the presence of lipid. No phytochemical was found in chloroform extract. Results are reported in the Table – 1.

Table 1: Phytochemical analysis of different extracts of Mung bean seeds.

Phytochemical	Chemical test	Pet.ether extract	Chloroform extract	Ethanol extract	Water extract
Alkaloids	Dragendorffs Test	-	-	-	-
	Mayer Test	-	-	-	-
Phenol	Ferric chloride test			++	+++
Saponin	Foam test	-	-	+	++
Carbohydrates	Molisch test	-	-	-	++
	Fehling's test	-	-	-	++
Flavanoids	Shinoda test	-	-	++	++
	Lead acetate test	-	-	++	++
Terpenoids	Salkowski test	-	++	++	+
Tannin	Acetic acid test	-	-	+	++
Protein	Biuret test	-	-	-	+++
Lipids	Sudan IV test	+++	-	-	-
	Copper acetate test	+++	++	-	-

(Positive sign indicate the presence of various phytoconstituents with respective to concentration like + (good), ++ (better), +++ (best) and negative sign indicate the absence of phytoconstituents).

e. Physicochemical parameter

Physicochemical analysis of seeds of Mung beans (V. radiata) powder were also evaluated for foreign organic matter, moisture content, swelling index, ash value and extractive values. Total ash value, water-soluble ash, acid-soluble ash, swelling index and moisture content were found to be 3.9, 0.9%, 0.5%, 8.7% and 8.6%. The foaming index was found to be less than 100. Different quantitative parameters were established. The extractive values of ethanol, and water were found to be 0.62%, and 7% as shown in Table-2

Table 2: Physiochemical analysis of seeds of Mung beans

Sr.No.	Physicochemical Constant	Seeds of Mung beans % w/w
1.	Total ash	3.9 %
2.	Water-soluble ash	0.9 %
3.	Acid insoluble ash	0.5 %
4.	Foaming index	<100
5.	Swelling index	8.7 %
6.	Moisture content	8.6%
7.	Ethanol	0.7%
8.	Water	8 %

Discussion

In human nutrition, seeds of Mung beans have been focused on great importance to solve malnutrition problems. It has extensively used as a traditional food in the whole world. It is the best source of protein, minerals, and vitamins, so there is urgent need to develop a pharmacognostical standardization method for taxonomical identification and quality evaluation.

The standardization of a crude drug is an integral part of establishing its correct identity and descriptions. Pharmacognostic standards must be established before including any crude drug in a herbal pharmacopeia. The macroscopical characters of the seeds V. radiata can serve as diagnostic parameters. Microscopic studies are one of the best, simplest, and cheapest methods to start with for establishing the correct identity of the source of herbal materials.

The pharmacognostic standards for seeds carried out for the first time in this study. Microscopical studies clearly indicated the presence of a micropylar canal, which starting from the outer surface of the seed and ending in the embryonal chamber. The endosperm cells are large, and elliptical in shapes. Epicarp and endocarp are two layers of seeds and endosperm situated at the center. Two embryos embedded in the embryo sac, and seeds have covered by a seed coat.

Cells of endosperm were possess starch grains within the cells as well as two embryos with thin cotyledons, and thick radical. In powder characteristics of seeds found that columnar sclereids were formed palisade layers into the outer seed coat, and contain palisade cells. Starch grains found to be in a different shape i.e, Circular starch grains, Elliptical starch grains, ovate starch grains. Starch grains were containing endosperm cells, and seed coats contained palisade sclereids.

The inner surface of the seed coat and outer surface seed coat contains seed cells.

Ash values and extractive values may be used as a reliable aid for detecting adulteration. Ash values of crude drug provide a clear idea of the mineral composition, and other numerous impurities present together with the crude drug. Extractive values studies are primarily useful for the dedication of exhausted and adulterated drugs. Extractive values also help to evaluate all chemical parts present within the crude drug and also helps inside the estimation of particular ingredients soluble in particularly solvents.

Various other physciochemical parameters of Total ash, water-soluble ash, acid insoluble ash, foaming index, swelling index and moisture content evaluation have been performed. Extractive value of water extract of Mung bean seeds was found to be significantly higher than alcohol, chloroform and petroleum ether extractive values.

Conclusion

The present study on the seeds of Mung beans (V. radiata) may satisfy the basic needs of various Pharmacognostics parameter and provide suitable significant standards for the taxonomic identification and evaluation of quality. Researchers may also use supportive data while preparing monographs. It may serve as a supporting reference for future investigations and several applications.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

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