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Pharmacognostical, physicochemical investigations and phytochemical of seeds of *Meyna laxiflora Robyns*

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ABSTRACT

The ***Meyna laxiflora*** **Robyns** belongs to the family **Rubiaceae.** In the present work, pharmacognostic studies of this medicinal plant was attempted which included physicochemical, phytochemical, macroscopic, microscopic studies and organoleptic evaluation. The plant powder characteristics were also elucidated. The physicochemical analyses were done by using WHO recommended parameters such as loss on drying, ash values (total ash, water soluble ash, acid insoluble ash, sulphated ash, carbonated ash, and nitrated ash), extractive values and Foaming index. The seeds were vertically oblong and elliptical in outline. The surface is reddish brown, smooth and membranous. Microscopical study depicts a elliptical in outline and shows thin dark seed coat enclosing massive perisperm and cellular endosperm with a central embryo. The powder of the seed consists of abundant seed coat sclereids and parenchyma cells of cotyledons. Foaming index value of powdered seeds of *M.laxiflora* Robyns was less than 100. Qualitative Phytochemical Analysis of ethanolic and aqueous extracts of powdered seeds of *M.laxiflora* Robyns showed the presence of Alkaloids, Tannins and Phenolic compounds, Flavanoids, Seroids, Proteins and amino acids. The pharmacognostic characters enlisted in this study will help in identification of the crude drug; the standardization parameters laid down will ensure the efficacy of drug and also distinguish the drug from its adulterants. The distinguishing characters will also be helpful for the preparation of monograph of this plant.

Keywords: ***Meyna laxiflora*** **Robyns**, macroscopic, microscopic, cotyledonous, Phytochemical.

INTRODUCTION

Recently, there has been a renewed interest in medicine which has always been thought to be safe for the treatment of infections originated from microbial and non-microbial [1,2]. Pharmacognostic investigation implicates standardization and authentication of crude drugs and generally this study was conducted in order to identify controversial species of plants [3]. The Pharmacognostic evaluation which includes macroscopic/organoleptic, microscopic, physicochemical and phytochemical analyses assesses the quality and purity of crude drugs in order to establish the standard pieces of information that will form the monographs to be used for their correct identification [3,4].

Meyna laxiflora Robyns also known as Vangueria spinosa, belongs to the family of **Rubiaceae.** In tamil its known as Manakkarai. It is a genus of about 6500 species tree, shrubs plant natives of Northern Bengal to Burma from the thick rain forest region. The fruit and the leaves are edible. The leaves are used as fodder, but are of an inferior quality. The dry fruits of *M.laxiflora* Robyns believed to be narcotic and are reported to be used for boils and dysentery. The powdered leaves are used by ethno pharmacists for curing diphtheria. *M.laxiflora* Robyns mainly is used in Ayurveda; Pinditaka. Madana or Mainphala are misleading synonyms. It is equated with Randia dumetorum Poir [5]. The present paper deals with the standardization of seeds on the basis of various Pharmacognostic, physico chemical and phytochemical parameters. The determination of these characters will aid future investigators in their Pharmacological analysis of this species.

MATERIALS AND METHODS

Plant collection and athentification

The plant specimens for the proposed study were collected from the trees carefully because they have thorns.

The plant parts of *M.laxiflora* Robyns was collected from the dense forest in the Bhimashankar range (The three lakes -Tansa, Vaitarna and Bhatsa) in Maharastra and it was identified by Dr. P.Jayaraman, Plant Anatomy Research Centre (PARC), Sakthi Nagar, West Tambaram, Chennai.

Care was taken to select healthy plants and normal organs. The required samples of different organs were cut and removed from the plant and fixed in FAA (Farmalin-5ml + Acetic acid-5ml + 70% Ethyl alcohol-90ml). After 24 hrs of fixing, the specimens were dehydrated with graded series of tertiary – butyl alcohol as per the schedule given by [6]. Infiltration of the specimen was carried by gradual addition of paraffin wax (melting point 58-60 C) until TBA solution attained super saturation. The specimens were cast into paraffin blocks [7-9].

Sectioning

The paraffin embedded specimens were sectioned with help of Rotary Microtome. The thickness of the sections was 10-12 µm. Dewaxing of the sections was by customary procedure. The sections were stained with toluidine blue as per the method published by [10] since toluidine blue is a polychromatic stain, the staining results were remarkably good: and some cytochemical reactions were also obtained. The dye rendered pink colour to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage, blue to the protein bodies etc. Wherever necessary sections were also stained with safranin and Fast-green and IKI(for starch).

Photomicrographs

Microscopic descriptions of tissues are supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon Lab Photo 2 microscopic Unit. For normal observation bright field was used. For the study of crystals, starch grains and lignified cells, polarized light was employed. Since these structures have birefringent property, under polarized light they appear bright against dark back ground. Magnifications of the figures are indicated by the scale-bars. Descriptive terms of the anatomical features are as given in the standard books [11].

Pharmacognostic study
Macroscopic study

The macroscopic studies were carried out using organoleptic evaluation method. The arrangement, size, shape, base, texture, margin, apex, veination, colour, odour, taste of leaves and stem were observed [12]. Macroscopic and microscopic characters were studied as described in quality control method [12]. Photographs at different magnifications were taken by using digital camera.

Microscopic study

Microscopic study was carried out by preparing thin sections of stem and leaf. The thin sections were further washed with water, stained with safranin, fast green and mounted in glycerine for observation and confirm its lignifications (10x, 40x) [13].

Powder Microscopy

The powder microscopy of the whole plant powder was studied using standard procedure by capturing the images of different fragments of tissues and diagnostic characteristic features were recorded [13].

Physicochemical parameters

The moisture content (loss on drying), ash values and extractive values of the dried aerial parts were determined using standard procedures [14-16].

Physico – chemical properties
Determination of Total Ash Value

Accurately weighed about 3 g of air dried powdered drug was taken in a tarred silica crucible and incinerated by gradually increasing the temperature to make it dull red hot until free from carbon. Cooled and weighed, repeated for constant value. Then the percentage of total ash was calculated with reference to the air-dried drug. (**Table No 1**).

Determination of Acid insoluble Ash Value

The ash obtained as directed under total ash was boiled with 25 ml of 2N HCl for 5 minutes. The insoluble matter was collected on an ash less filter paper, washed with hot water, dried the filter paper, ignited and weighed. Then, the percentage of acid insoluble ash with reference to the air-dried drug was computed.

Determination of Water - soluble Ash Value

The total ash obtained was boiled with 25 ml of water for 5 minutes. The insoluble matter was collected on an ash less filter paper, washed with hot water and ignited for 15 minutes at a temperature not exceeding 450^0C. The weight of insoluble matter was subtracted from the weight of total ash. The difference in weight represents the water – soluble ash. The percentage of water-soluble ash level was calculated with reference to the air-dried drug.

Determination of Sulphated Ash value

About 3 g of accurately weighed air dried powdered drug was taken in a tared silica crucible, which was previously ignited and weighed. The contents were ignited gently at first and subsequently the drug was thoroughly charred. The crucible was cooled and residue was moistened with 1 ml of concentrated sulphuric acid, heated gently until the white fumes were no longer evolved and ignited at 800 ^{0}C ± 25 ^{0}C until all the black particles has disappeared. The crucible was allowed to cool, few drops of sulphuric acid was added and again heated. The ignition was carried out as before, allowed cooling and weighed to get a constant weight (difference not more than 0.5 g between two consecutive readings). The percentage of sulphated ash was calculated with reference to the air-dried drug. All the ash values were calculated and recorded.

Determination of Alcohol Soluble Extractive Value

Accurately about 5g of the air-dried coarse powder of the seeds of *Meyna laxiflora* Robyns was macerated with 100 ml of ethanol in a closed flask for 24 hours, shaken frequently during the first 6 hours and allowed to stand for 18 hours. Thereafter, it was filtered rapidly taking precautions against loss of the solvent. Out of that filtrate, 25 ml of the filtrate was evaporated to dryness in a tarred flat-bottomed shallow dish, dried at 105^0C and weighed. The percentage of ethanol soluble extractive value was calculated with reference to the air-dried drug and the results were recorded.

Determination of Water Soluble Extractive Value

Accurately weigh about 5 g of coarsely powdered drug and macerate it with 100 ml of distilled water in a closed flask for 24 hours, shaking frequently during the first 6 hours and allowing to stand for 18 hours. Thereafter, it was filtered rapidly taking precautions against loss of the solvent. Then, 25 ml of the filtrate was evaporated to dryness in a tarred flat-bottomed shallow dish, dried at 105^0C and weighed. The percentage of water-soluble extractive was calculated with reference to the air dried drug and the results were recorded

Loss on drying

Loss on drying is the loss in weight in % W/W determined by means of the procedure given below. It determines the amount of volatile matter of any kind (including water) that can be driven off under the condition specified (desiccators or hot air oven).

Determination of foaming index

Weighed accurately about 1 g of coarsely powdered drug and transferred to 500 ml conical flask containing 100 ml of boiling water. The system was maintained at moderate boiling at 80-90^0C for about 30 minutes. Cooled and filtered into a volumetric flask and sufficient water was added through the filter to make up the volume to 100 ml (V_1). Ten cleaned and stoppered test tubes of uniform dimensions were taken and marked from 1 to 10. Subsequently, from the made up solution in the volumetric flask measured and transferred the successive portions of 1, 2, 3 ml up to 10ml solution and adjusted the volume of the liquid in each tube with water to 10 ml. Stoppered the tubes and shaken them in a lengthwise motion for 15 seconds uniformly and allowed to stand for 15 minutes and measure the height. If the height of the foam in every tube is less than 1 cm, the foaming index is less than 100 (not significant). Here, the foam was more than 1cm height after the dilution of plant material in the fourth tube. The corresponding number of the test tube was the index sought, if the height of the foam in every tube is more than 1cm, the foaming index is more than 1000. In this case, 10 ml of the first decoction of the plant material is measured and transferred to a 100 ml volumetric flask (V_2) and volume is made to 100 ml and followed the same procedure.

$$\text{Foaming index} = \frac{1000}{a} (V_1)$$

$$\text{Foaming index} = \frac{1000}{a} (V_2)$$

Where, a = volume (ml) of decoction used for preparing the dilution in the tube where exactly 1 cm or more foam was observed.

Phytochemical screening

The powdered aerial parts were subjected to preliminary chemical tests for the presence of various primary and secondary metabolites including carbohydrates, tannins, flavonoids, alkaloids, cardiac glycosides, saponins, anthraquinones, sterols and triterpenes using standard procedures [16-18].

RESULT AND DISCUSSION

Macroscopical studies of Meyna laxiflora Robyns seeds

Fig No: 1. Exomorphic Flowers of *Meyna laxiflora* Robyns

Appearance: A Ripe fruit has up to 7 seed sand the seeds are kidney shaped, Smell: Faintly pungent smell, Taste: Seeds are bitter. Colour: Reddish Brown, Season : During the near rainy season (June- August). (Fig 1-3)

Fig No: 2. Exomorphic Almost Ripe Fruits of *Meyna laxiflora* Robyns

Fig No:3. Exomorphic Features of the dry seeds of *Meyna laxiflora* Robyns

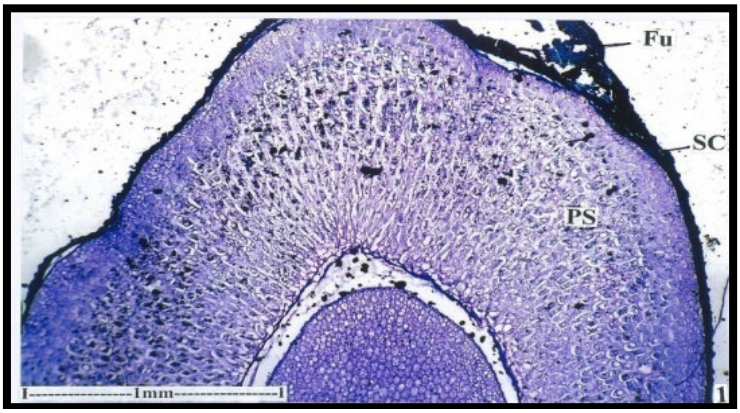
Microscopical characters

Microscopical studies

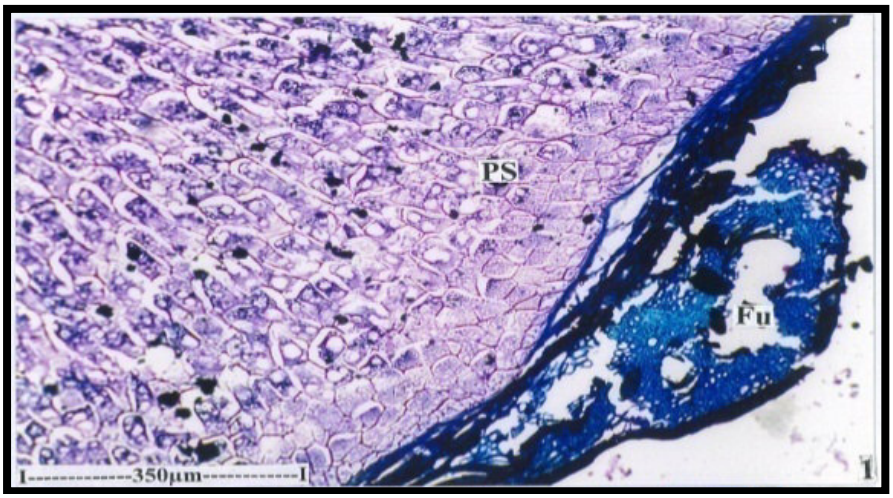
The seed is vertically oblong and elliptical in outline. The surface is reddish brown, smooth and membranous. It 3 mm thick in the median part and 5 mm wide in horizontal plane.

Microscopic features

In transverse –vertical section, the seed is elliptical in outline and shows thin dark seed coat enclosing massive perisperm and cellular endosperm with a central embryo (Fig:10; 11). These are a small dark tenant of the funicule at one end of the seed (Fig:4; 5). The funicle consists of sclerenchymatous tissue (Fig:5).

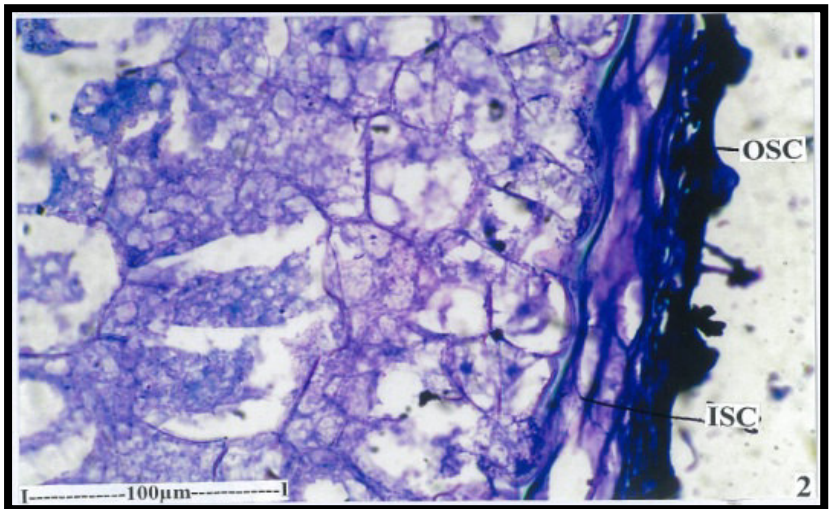


(Fu-Funicle; SC- Seed Coat; PS-Perisperm.)
Fig:4. T.S of seed upper portion



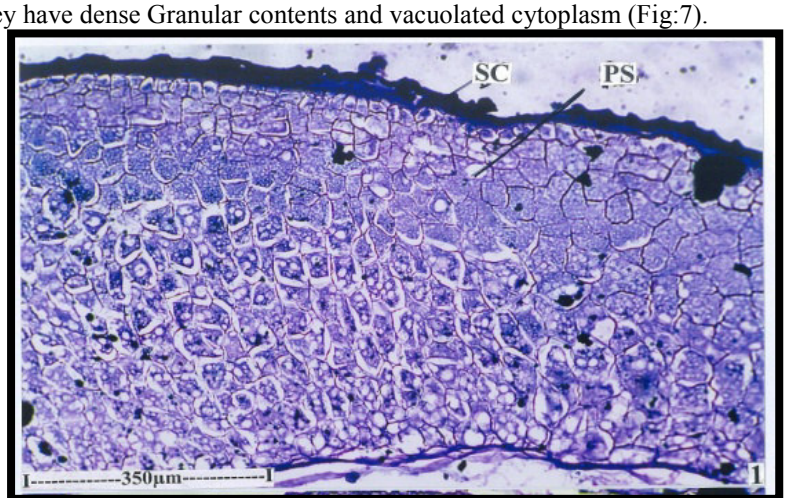
(Fu-Funicle; PS-Perisperm.)
Fig: 5. T.S of the seed through funicle with Perisperm

The seed coat in uniformly in thickness all around the seed . The surface is undulate due to the presence of shallow pits and wide ridges. The seed is narrow dark line measuring 40 µm in thickness. It has outer sclerotic test and inner two or three layers of elongated spindle shaped parenchymatous cells (Fig:6).



(ISC-Inner seed coat; OSC- Outer seed coat.)
Fig:6. T.S of the seed – seed coat and perisperm magnified

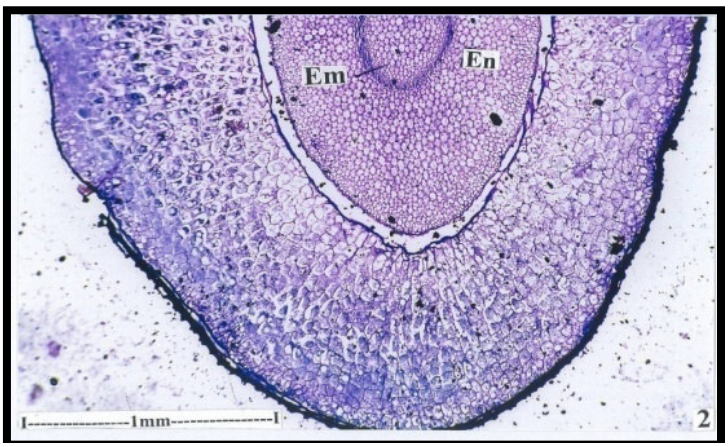
Perisperm is wide and parenchymatous firmly attached to the seed coat. It is 500 µm wide. The cells are angular in outline, compact and thin walled; they have dense Granular contents and vacuolated cytoplasm (Fig:7).



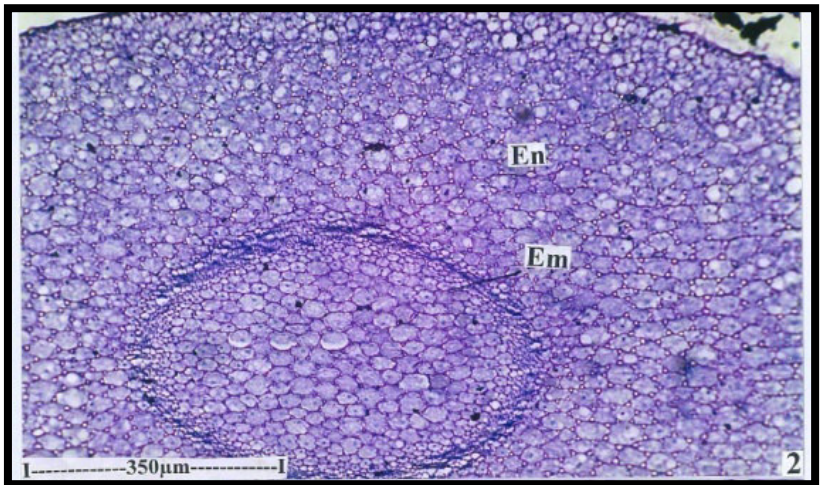
(SC- Seed Coat; PS-Perisperm.)
Fig:7. T.S of seed – seed coat and perisprem

Endosperm

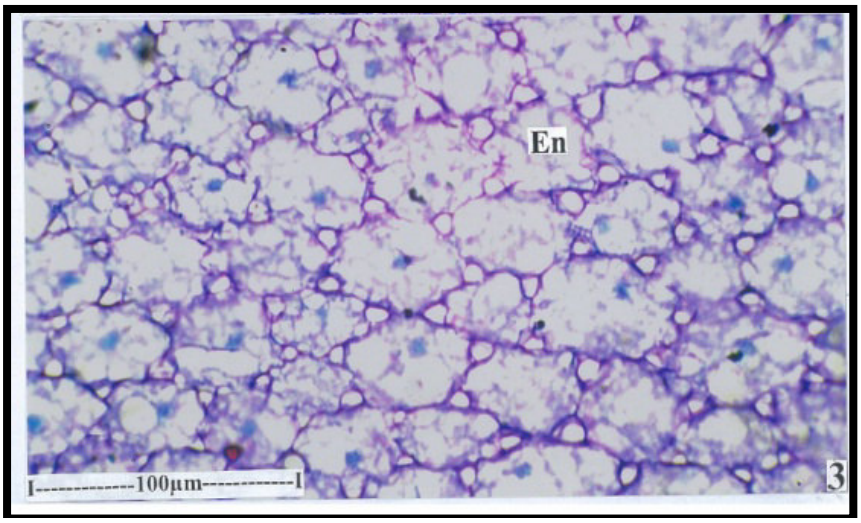
The endosperm consists of thin walled criclular cells; these are triangular inter cellular space in between the cells which form characteristic pattern. The cells are highly evacuated with central nuclei. (Fig:8; 9; 10)



(Em- Embryo; En-Endosperm)
Fig:8. T.S of seed lower portion



(Em- Embryo; En-Endosperm)
Fig:9. T.S of seed – Embryo and endosperm



(En-Entosperm; Em-Embryo; PS- Perisperm; SC – Seed coat.)
Fig:10. Endosperm cells magnified

Powder microscopy

The powder of the seed consists of abundant seed coat sclereids and parenchyma cells of cotyledons. Seed coat sclereids are abundant in the powder they differ in size and shape (Fig:11). The tracheids are mostly elongated and some of that resemble the fibers some of the trachides are elongated with tapering ends (Fig:12); some of the trachides have spindle shaped with one and tapering (Fig:12) and the other end is rounded (Fig:13). Some of the sclereids are slightly covered with short lateral lobes (Fig:13). These also long fibre- like sclereids which are known as fibre-sclereids (Fig:14). They are narrow, long and pointed the tip. The sclereids are thick walled with narrow lumes and canal like pits. They have lignified walls and hence they appear bright under the polarized Light microscope (Fig:14).The sclereids range in length from 80-280 µm and 15-20 µm in thickness.

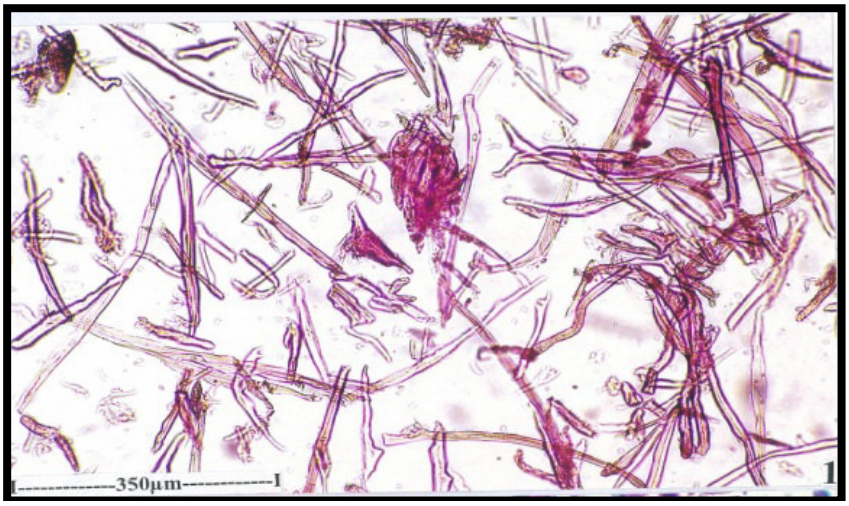
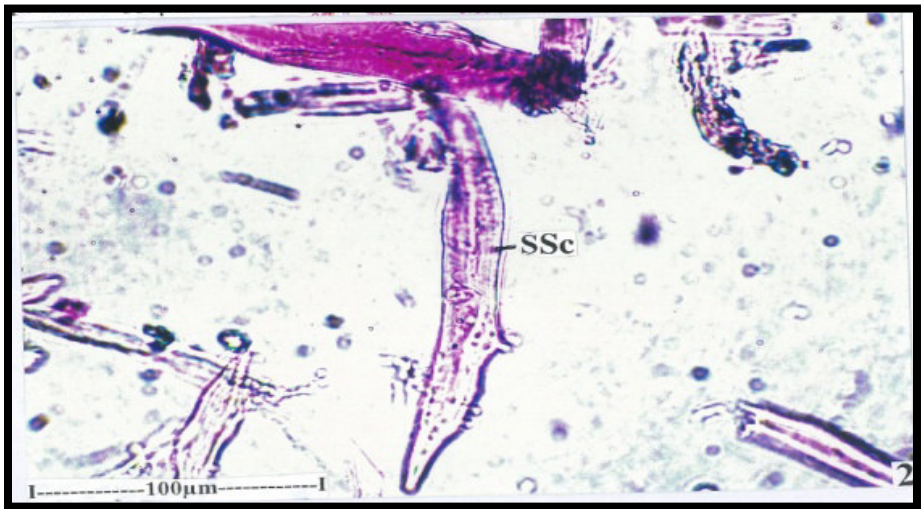
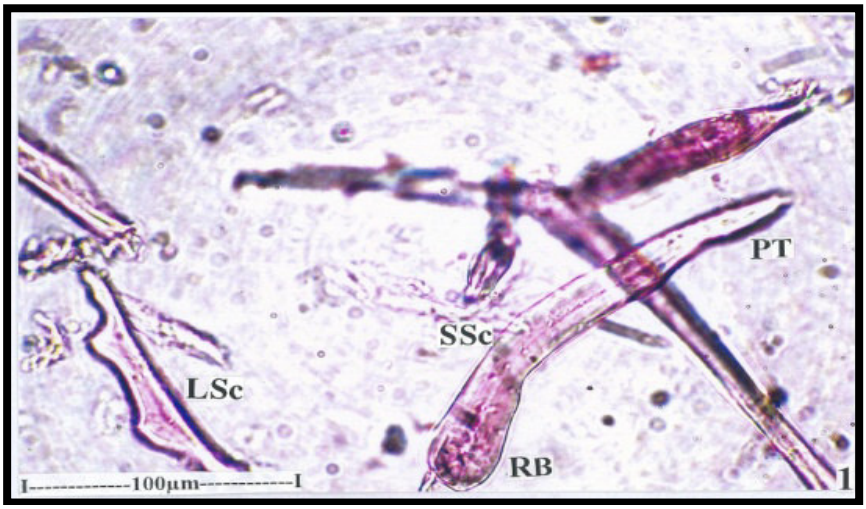


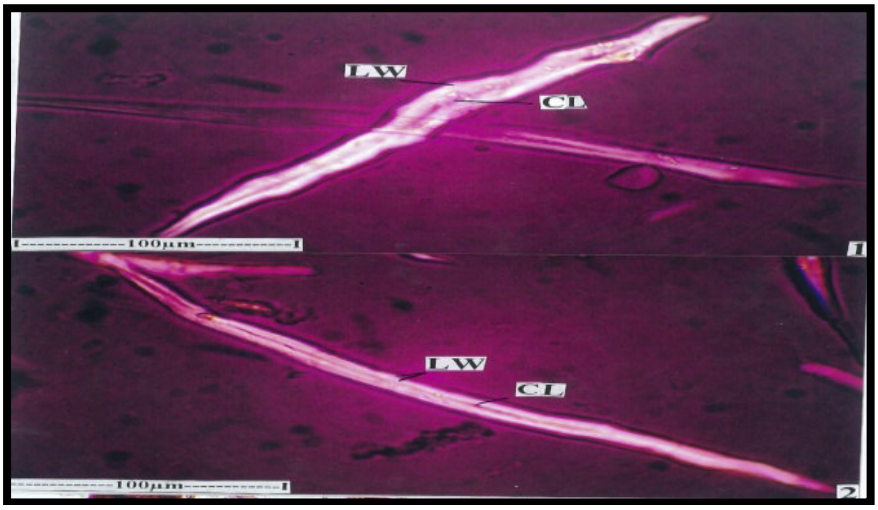
Fig:11. Scattered seed coat sclereid of different morphological types



(SSc- Short Sclereid.)
Fig:12. Short, Wide sclereid



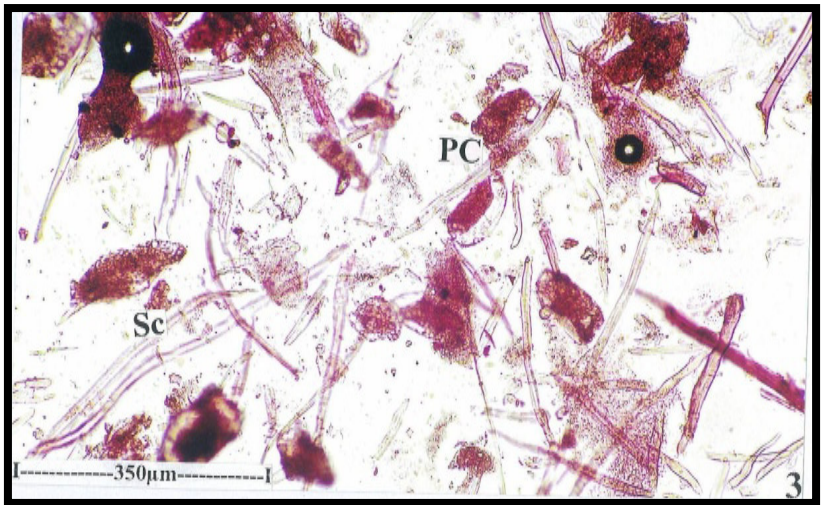
(LSc-Lobeel Sclereid; PT-Pointed Tip; RB-Rounded Base; SSc-Shhort sclereid.)
Fig:13. Lobed, Short sclereid and thick short sclereid



(CL-Cell Lumen; LW-Lumen Wall.)
Fig: 14. Fibre sclereid showing lignified cell walls and narrow lumen as seen under polarized light microscope

Parenchyma cells

There are numerous parenchyma cells of the cotyledonous (embryo) scattered in the powder (Fig:15).These cells are rectangular squarish or polygonal in out line. They contain dense granular bodies which are starch grains. The cells 50 µm wide and 100 µm long.



(PC-Parenchyma Cell; SC-Sclereids.)
Fig: 15. Parenchyma cells of the embryo with dense granular contents.

Physico – chemical parameters

The Total Ash value, Acid -insoluble Ash, Water Soluble Ash, Sulphated Ash was s found to be 5.56, 1.21, 2.14, 1.82 respectively. **(Table:1).**

Table 1: Physico – chemical parameters for the powdered seeds of *Meyna laxiflora* Robyns

S.No	Parameters	%W/W
	Ash Values	
1.	(a) Total Ash	5.56
	(b) Acid -insoluble Ash	1.21
	(c) Water Soluble Ash	2.14
	(d) Sulphated Ash	1.82

2.	Extractive Values	
	a) Alcohol Soluble Extractive	0.82
	b) Water Soluble Extractive	11.24
3.	Loss on Drying	12.31

Foaming index

Foaming index is mainly performed to determine the saponin content in an aqueous decoction of plant material. Thus, the foaming index value of powdered seeds of *Meyna laxiflora* Robyns was less than 100. **(Table:2).**

Table No. 2: Foaming index of the powdered seeds of *Meyna laxiflora* Robyns

S.No	Test volumetric flask no. (10ml)	Height of foam (cm)
1.	1	0.1
2.	2	0.3
3.	3	0.6
4.	4	0.7
5.	5	0.7
6.	6	0.8
7.	7	0.8
8.	8	0.8
9.	9	0.9
10.	10	0.9

Phytochemical screening

Phytochemical Analysis of ethanolic and aqueous extracts of powdered seeds of *Meyna laxiflora* Robyns showed the presence of Alkaloids, Tannins and Phenolic compounds, Flavanoids, Seroids, Proteins and amino acids. The pharmacognostic characters enlisted in this study will help in identification of the crude drug; the standardization parameters laid down will ensure the efficacy of drug and also distinguish the drug from its adulterants. The distinguishing characters will also be helpful for the preparation of monograph of this plant. **(Table:3)**

Table 3: Data for Qualitative Phytochemical Analysis of ethanolic and aqueous extracts of powdered seeds of *Meyna laxiflora* Robyns

Phytoconstituents	Aqueous extract	Ethanolic extract
Alkaloids	+	+
Saponins	–	–
Glycosides	–	–
Carbohydrates	–	–
Tannins and Phenolic compounds	+	+
Flavonoids	+	+
Steroids	+	+
Proteins and Amino acids	+	+
Terpenoids	–	–
Fats	+	+
Gums and Mucilage	-	-
Lignins	-	-

(+) : Present, (-) : Absent

CONCLUSION

The present work focused on pharmacognostical, physicochemical and preliminary phytochemical examinations of the seeds of *Meyna laxiflora* Robyns. The outcomes of this study exhibited relevant pieces of information that could help to check the adulterations and could be used for the standardization and pharmacopoeial parameters development of *M.laxiflora* Robyns.

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