

Seed coat study and preliminary phytochemical analysis of *Hibiscus manihot* Linn./*Abelmoschus manihot*(L.) Medik. (Raan Bhindi)) seeds of family Malvaceae.

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Abstract: India being a rich repository of medicinal plants in world market. The seeds being a complex organ of multiple origin. Metabolically seed most active that secretes and synthesizes efficient compounds that can be exploited for drug formulations. The present study investigated seed morphology, anatomy and preliminary phytochemical analysis of *Hibiscus manihot* Linn. The micromorphological characters of spermoderm, have been found to be consistent variations specifying the seed taxa. Scanning electron microscopy (SEM) of seed surface shows tufts of papillate hairs, trichomes with ornamented cellular variations. Seed anatomy also helps to study the internal cellular structure of the seedcoat. Different cellular variations present in them. Presence of epidermis, palisade layer, tannin cells and endosperm layer in cellular variations. The preliminary phytochemical analysis in seed detected various phytochemicals present in them which is very helpful for drug preparations. Flavonoids, phenols, fixed oil and fats were detected in aqueous, methanol, ethyl acetate extract. In methanol large number of phytochemicals were detected as compared to aqueous and ethyl acetate. Medicinally seeds are very important. The study helps for identification of seeds, solving taxonomic problems and for therapeutic efficacy also.

Keywords: Seed morphology, Scanning electron microscopy (SEM), Seed anatomy, Phytochemical analysis, Malvaceae.

1. INTRODUCTION:

The *Hibiscus manihot* Linn. or *Abelmoschus manihot* (L.) Medik. plant belongs to family Malvaceae. It is also known as aibika, raan bhindi or jangli bhindi, sunset muskmallow, sunset hibiscus. It is a flowering plant, tropical subshrub or shrub native to parts of Asia and Australia. Traditionally used for many purposes. Inflorescence is solitary cymes or axillary cymes. Flower is complete, bisexual, large and showy. It is also known as edible hibiscus. (Fig-01, Fig-02). *Hibiscus manihot* Linn. seeds are very important for study purposes, it contain morphological, anatomical surface and cellular variations and high medicinal value. Seeds are evolved as a unique structural and functional entity to face the challenges imposed by changing environmental conditions. seed is a fertilized or mature ovule (Green, *et al.* 1984).

To study the micromorphological characters of seeds, the Scanning Electron Microscopic studies on the seed coat surface (spermoderm) is an advanced field. Seed coats surface show structural marking of great diversity. Anatomical features of seed coat also help to determine the internal cellular variations. Phytochemical are naturally occurring compounds present in plants. It is also known as phytonutrients. It gives various health benefits. It indicates the various bioactive compounds. It includes carbohydrates, glycosides, flavonoids, tannins, steroids, proteins and others. India is considered to be leading exporter of the medicinal plants in the world market. Major importer of medicinal plants from India are U.S.A., Japan, Germany, U.K., France and Switzerland.



Fig-01



Fig-02

Fig-01- Habit of *Hibiscus manihot* Linn. Whole plant body with flowering and fruiting. **Fig-02-** Inflorescence Solitary cymes or axillary cymes with pale yellow color flowers.

2. Materials and methods: -

Sample collection: - Seeds of family Malvaceae like *Hibiscus manihot* or *Abelmoschus manihot* (L.) Medik. were collected from local places of Amravati district. For seed coat study, all the seeds parameters were studied using dissecting and binocular microscope. Digital weighing balance was used for weighing the seeds in mg. The morphological observations of seeds were done followed by their photography, using 1 cm. scale.

Seed coat morphology (SEM): - To study the seed coat morphology scanning electron microscopy is most important. For this purpose, the individual seeds were dipped in alcohol for 5-10 min. to remove the dust from them. The seed mounted on pin type stubs using double sided adhesive tape or conductive silver paint to prevent charging of the surface during scanning and then coated with a very thin layer of gold in a polaron sputter coating unit. For spermoderm study of seed photomicrograph were taken in the scanning electron microscope (SEM) (LEO 430) at Birbal Sahani Institute of Paleobotany, Lucknow.

Seed coat anatomy: - For the anatomical observation of seed coat study take the transverse sections of seed coat. Using permanent slide preparation method or double staining method place the section on various alcohol grades like 30%, 50%, 70%, 90% absolute alcohol, xylene, DPX etc. The staining like safranin and light green stain used for staining.

Preliminary phytochemical tests: - The preliminary phytochemical analysis is most important for detection of various chemical constituents. Trease and Evans (1989) test were done. Qualitative phytochemical analysis of the crude powder of the seeds of the plant for the identification of phytochemicals like alkaloids, flavonoids, glycosides, phenol, tannins, steroids, protein, saponin, carbohydrates, terpenoids, amino acids, fixed oil and fats. Preliminary phytochemical test was done using different extract.

3. Observations: -

Seed morphology: - For the identification of seed there are various parameters which are helpful in distinguishing the taxa at suprageneric level. These parameters include morphology, anatomy, information of various types of seeds life size, shape, colour, surface, symmetry, medicinal value of seeds, value of seed in trade and marketing systems. Externally seed 0.45 cm. - 0.20cm, spheroidal, kidney shape, greenish, 18.03mg, radial, median, linear, surface hairy, seed show black color with brownish hairy coating on it, cellular network surface contain finger print like structure, hilar region shows tufts of hairy deposition. (Fig-03, Fig-04)



Fig-03

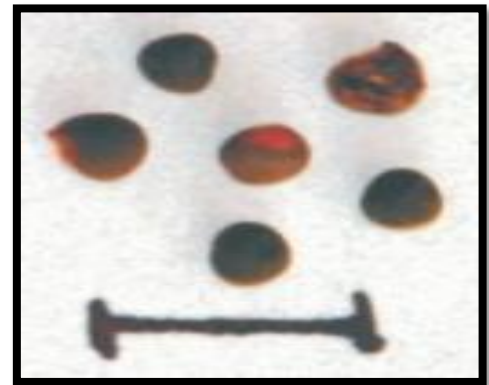


Fig-04

Fig-03- Dehiscence of fruit (Black color seeds inside the fruit) of *Hibiscus manihot* Linn. **Fig-04 -** Dry seeds bilobed, kidney shape.

Scanning electron microscopy: - The SEM investigation i.e. Scanning electron microscopy play a very important role in differentiating and identification of micromorphological characters of seeds. The scanning electron microscopy (SEM) shows whole view of seed with hairy deposition on surface. Semicircular linings of hairs on surface of seed. Papillate, epidermal hairs in bunches on the surface with penta to hexagonal interconnected warts. The seed surface show cellular reticulation. Near hilar region trichomatous tuft of hairy deposition present. Elongated, thick, sporadic hairs on hilar region. (**Fig-05, Fig-06**)

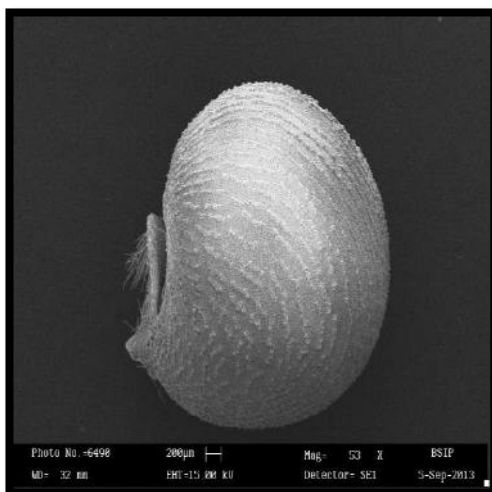


Fig-05

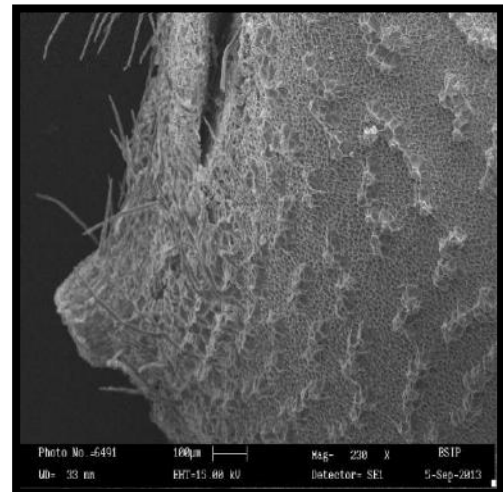


Fig-06

Fig-05- 53X- Scanning electron microscopic view of *Hibiscus manihot* Linn. Whole seed. **Fig-06-** 230X- Magnified view (SEM) of Seed surface of *Hibiscus manihot* Linn., tufts of hairs near hilar region, Papillate hairy deposition in bunches.

Seed anatomy: - Seed coats attributes complexity to certain types of seeds; like very hard seeds, seeds with “ornamented” coats and seed with fleshy coats. Internally the transverse section of seed shows the epidermal layer thin and wavy. Compactly arranged palisade cells are present. The inner layer developed tannin cells as pigmented zone. The palisade cell measures 81.41µm in length and 23.26µm in breadth. Below its endosperm layer developed. The pigmented cell measures 46.52µm in length and 23.26µm in breadth (**Fig-07, Fig-08, Fig-09, Fig-10**)



Fig-07



Fig-08

Fig-07-X160- T.S. of seed coat of *Hibiscus manihot* Linn. With epidermis, palisade, tannin cells. **Fig-08- X640** -T.S. of seed coat of *Hibiscus manihot* Linn. with higher magnification shows wavy epidermis, parenchymatous cells, elongated palisade cells, endosperm region with tannin deposition.

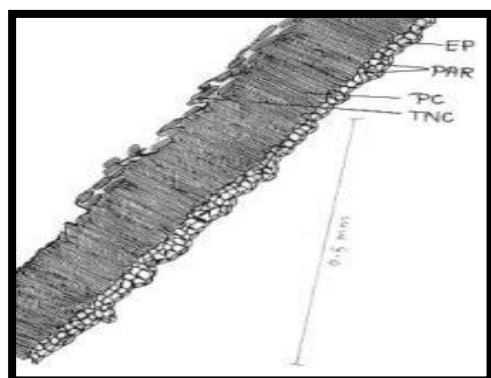


Fig-09

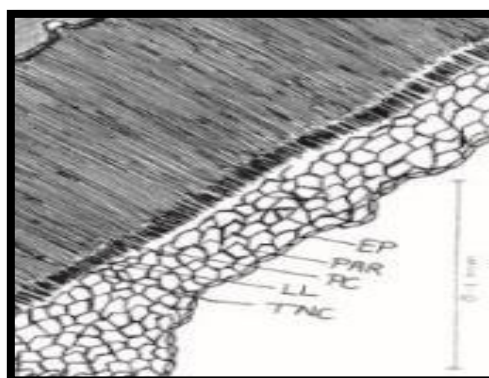


Fig-10

Fig-09-X100- T.S. of seed coat of *Hibiscus manihot* Linn. **Fig-10-X400-** T.S. of seed coat of *Hibiscus manihot* Linn. with higher magnification. shows cellular variations with parenchymatous cells.

Medicinal uses: - *Abelmoschus manihot* L. (Medik.) seeds medicinally important, diuretic pass, used to treat the adverse effects of defecation, edema, urinary calculi etc., it is rich in fat oil (DUAN Qiong-hui *et al.* 2018). Seeds used to make perfume. (<https://tropical.theferns.info/viewtropical.php?id=Abelmoschus+manihot>). *Hibiscus manihot* L. seed oil is high-value edible vegetable oil that also has a variety of important health functions, and concerns about its pressing method have great significance for commercial and consumer health. (Xinlei Yan *et al.* 2023) Medicinally whole seed or seed coat used for preparation of various drugs. Various chemical compositions present inside the seed are effective for drug preparation. Phytochemicals from medicinal plants are receiving greater attention in scientific literature, in medicine and in the world economy in general (Bruni 2003).

Observation table: - 01- Preliminary phytochemical observations of *Hibiscus manihot* Linn. plant part (Seed) with various extracts.

Sr no.	Phytochemical	Plant part (Seed powder)		
		Aqueous	Methanol	Ethyl acetate
01	Alkaloids	+	-	-
02	Flavonoids	+	+	+
03	Glycosides	+	+	-

04	Phenols	+	+	+
05	Tannin	+	+	-
06	Steroids	-	+	+
07	Protein	-	+	-
08	Saponin	-	+	+
09	Carbohydrate	+	-	+
10	Terpenoids	-	+	-
11	Amino acids	+	+	-
12	Fixed oil and fats	+	+	+

Present (+), Absent (-)

From the above observations it is seen that the various phytochemicals present in the *Hibiscus manihot* Linn. seeds which were detected by preliminary phytochemical test. The *Hibiscus manihot* Linn. seeds detected flavonoids, phenols, fixed oil and fats in aqueous, methanol, ethyl acetate extract while alkaloid detected in aqueous extract, protein and terpenoid in methanol extract. Glycosides, tannin and amino acids detected in aqueous and methanol extract. Steroid, saponin in methanol and ethyl acetate extract. Carbohydrate detected in aqueous and ethyl acetate extract. Methanol extract detected the highest number of phytochemicals. (Table- 01)

4. Discussion:

In Malvaceae, seed coats divided into two layers. Outer thick and impervious to water, inner thin. Symmetrical variation with hairy seedcoat which is impervious to water. According to Chuang and Heckard (1972) the seed coat pattern is diversified among species and furnish an important feature for classification. *Hibiscus manihot* Linn. shows epidermis wavy, presence of palisade and tannin cells. There are numerous surface variations observed in seed. Scanning electron microscopy with higher range of magnification and it provide an important tool for more precisely characterizing seed surface (Johnson, *et al.* 2004).

The seeds or seed coat are known to have capacity to store various alkaloids or chemicals. The phytochemical present in the *Hibiscus manihot* Linn. seeds gives anti-inflammatory, antimicrobial and antioxidant properties. All the phytochemical present in the seeds are very important for health benefits. Seeds have high medicinal properties. Herbal medicine is the oldest form of medicine. The use of herbs as potential agent to treat various ailments and its use in new drug formulation in gaining wide acceptance through the world (Gupta and Hore 2003). These phytochemical gives potential therapeutic applications. Identification of seeds by means of morphological and anatomical characters is very important for testing seed samples in agri and horticulture and in Paleobotany and archeology. (Boesewinkel and Bouman 1984). Spermoderm variation through SEM is one of the most important modern identification technique. The micro morphological characters of seeds were investigated through this special technique. Seed recognition is an important diagnostic feature and have great applied value in various scientific disciplines. Above study shows that the morphological, anatomical and phytochemical analysis of seeds of *Hibiscus manihot* Linn. is important for identification of seeds, for increasing the taxonomical knowledge. Preliminary test is helpful for detection of various phytochemical which helps for drug preparation and useful on various diseases.

Acknowledgement: - The authors are thankful to the Director, Birbal Sahni Institute of Paleobotany, Lucknow for extending SEM facilities and Central Instrumentation Center (CIC) Sant Gadge Baba Amravati University, Amravati for slide photography.

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