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ORIGINAL RESEARCH ARTICLE

# Analytical Study of *Mustak* (Cyperus rotundus Linn.)

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## ABSTRACT

**Background:** Mustak (*Cyperus rotundus* Linn.) is an aquatic or semi-aquatic herb and found throughout India. The therapeutic utility of Mustak is wide ranging and is mainly based on its chief action as sangrahaka, dipana, pachana according to pharmacological consideration in India medicine. In the Charak Samhita, the plant *Mustak* has been described under *Lekhaniya, Triptighna, Kandughna, Stanayashodhana and Trishnanigrahana mahakashyaya* which indicates its therapeutic importance in Ayurveda. In Astang Hridya, *Mustak* has been described under *Kaphagna Gana, Mustadi Gana, Vachadi Gana* and *Tikata gana*. It is widely used for intestinal problems, indigestion, diarrhoea, dysentery, vomiting, fever and also used as a hypocholesterolaemic drug. **Objective:** This study is designed to establish the various pharmacognostical, physiochemical and phytochemical standards of *Cyperus rotundus* Linn. for its correct identification and authentication. **Material and Methods:** Mustak (*Cyperus rotundus* Linn.) was collected from their natural habitat and botanically identified. Oraganoleptic study, transverse sections and powder microscopy of the rhizomes of the drug were carried out. The other investigations included determination of various standardization parameters such as physiochemical, phytochemical analysis of the drug. **Results:** Morphological features, organoleptic characters and microscopic features of the *Cyperus rotundus* Linn. were analysed. All physiochemical parameters of the plant including foreign matter, moisture content, total ash value, acid insoluble ash value, water soluble ash value, water-soluble extract content, alcohol-soluble extract content and pH were assessed and found to be within permissible limits. The following $R_f$ value were found in the sample of Mustak (*Cyperus rotundus* Linn.): **$R_f$ Value- 254nm**-0.02, 0.12, 0.22, 0.26, 0.35, 0.38, 0.50, 0.65, 0.72, 0.80, 0.87, **366nm**-0.02, 0.06, 0.08, 0.16, 0.22, 0.80, **After Derivatized 366nm**- 0.02, 0.06, 0.12, 0.22, 0.31, 0.40, 0.50, 0.76, 0.80, 0.83, 0.92, 0.96 **Conclusion:** Pharmacognostical, physiochemical and phytochemical profiling will be helpful in identification and authentication of *Cyperus rotundus Linn*. and the parameters which are established from this study may be helpful in standardization of Mustak (*Cyperus rotundus* Linn.).

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## INTRODUCTION

*Cyperus rotundus* Linn., (Family-Cyperaceae), also known as purple nutsedge or nutgrass, is a common perennial weed with slender, scaly creeping rhizomes, bulbous at the base and arising singly from the tubers which are about 1-3 cm long. Rhizome emitting long, slender, wiry stolons ending in a fleshy, blackish tuber. The source plant *Cyperus rotundus* Linn. of drug

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Mustak occurs throughout India particularly in marshy and moist areas ascending to 6,000 ft. elevation in different regions where the localities with water courses or any other similar watery or aquatic situations along which the plants find their suitable habitat. It is a common annual weed of the pasture lands, road sides and other moist places in the plains and also in the hilly region.

Plant flowers and fruits during rainy to spring season. Mustak is an important drug of Ayurveda, described under *Lekhaniya, Triptighna, Kandughna, Stanayashodhana and Trishnanigrahana mahakashyaya* of Charak Samhita and the drug is used in the various classical formulations and some of the reputed classical formulations viz. Karpuradyarka, Kanṭakaryavaleha, Chyavanaprash, Chaturbhadra kvatha-churṇa, Punarnavadi kvatha-churṇa, Kumkumadi taila, Punarnavadi mandura, Karpura rasa and Candanadi louha etc. Besides classical formulations, the drug Mustak is commercially exploited for using in various medicinal products in pharmaceutical field.

The therapeutic utility of Mustak is wide-ranging and is mainly based on its chief action as Sangrahaka, dipana, pachana (and amapachana) according to pharmaco-clinical consideration in Indian medicine. It is very useful in Sthoulya (Obesity) because of its properties like lekhaniya, dipaniya, pachaniya etc. The drug Mustak is prescribed in vomiting, dyspepsia, anorexia, flatulence, diarrhoea, chronic dysentry, colitis, excess thirst, worms and allied ailments of digestive system. Along with other therapeutic applications, The Ayurvedic Pharmacopoeia of India indicated the use of the rhizome in rheumatism, inflammations, dysuria, puerperal diseases and obesity.

The tubers of *Cyperus rotundus* Linn. are rich in Cu, Fe, Mg and Ni. Beta-sitosterol, isolated from the, tubers, exhibits significant anti-inflammatory activity against carrageenan- and cotton pellet induced oedema in rats; the activity is comparable to hydrocortisone and phenyl-butazone when administered intraperitoneally.

The alcoholic and aqueous extracts of the tubers possess lipolytic action and reduce obesity by releasing enhanced concentrations of biogenic amines from nerve terminals of the brain which suppress the appetite centre. Presence of eudalne group of sesquiterpenic compounds of sesquiterpene alcohol, isocyperol is said to play an important role in lipid metabolism[1-14].

So, Mustak is very important and useful medicinal herb which has many pharamacological actions.

## MATERIALS AND METHODS[15-18]

### MATERIAL

**Plant Material:**

The plant material which was taken for study is *Cyperus rotundus* Linn.

*Cyperus rotundus* Linn. is taken as a source of Mustak.

**Plant Collection and authentication**

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- The genuine sample was collected after identifying the source of plant as per standard description.
- The genuine sample of *Cyperus rotundus* Linn. rhizomes (Mustak) was collected from Haridwar District, State- Uttarakhand, India.

Collected sample was authenticated by the researcher and supervisor with specimen deposited in the repository of Patanjali Research Institute, Haridwar,

## METHODS

### Herbarium and authentication of collected genuine plant material

A herbarium is a collection of dried, pressed plant specimens mounted on specified herbarium sheets bearing detailed data label and stored in a herbarium cabinet in a climate controlled room. A herbarium can be thought of as a dried plant library, the pages of the books are the sheets of plants.

For the preparation of Herbarium sheets and other study purposes, plant specimens were collected from their native habitat during the time of flowering. For Herbarium, Mustak was collected from Bahadrabad, Haridwar, Uttarakhand.

During field visit the complete plant materials (including root and inflorescence) were taken. These plant specimens were dried with the help of bloating paper. These bloating papers were regularly changed to check fungal infection. When the plant specimens were entirely dried then it was poisoned with 9% (w/v) mercuric chloride ($HgCl_2$) in alcohol and were kept in bloating sheet to absorb excessive alcohol. Poisoned plant materials were placed in mounting sheet and mount. Now with the help of expert taxonomist and authentic literature the plants were identified.

Collected roots (rhizome) were washed with running water and kept for drying under the shade for further study. The procured dried rhizomes part were labeled, packed and subjected for organoleptic and other analytical studies. The authentication of plant material collected for study was done at Herbarium section of Patanjali research Institute governed under Patanjali Research Foundation Trust, Haridwar, Uttarakhand. Patanjali Research Foundation Herbarium (PRFH) verified with the original Herbarium sheets for the correct identity of plant, also one specimen of plant deposited in PRFH and gave the accession no. for the plant. (As seen in Table 1, Figure 3 & 4)

**Table 1** Genuine Sample of Mustak (*Cyperus rotundus* Linn.) with Herbarium accession number

| S.No | Name of Plant | Date of Collection | Place of Collection | Accession No. |
|---|---|---|---|---|
| 1. | *Cyperus rotundus* L. | 28/09/2022 | Haridwar, Uttarakhand | 14406 |

## ORGANOLEPTIC/ MACROSCOPIC STUDY

### Procurement of raw materials:

Procurement of herb, Mustak (Rhizome of *Cyperus rotundus* Linn.) was followed as described in collection of materials.

### Method:

All the collected genuine samples were dried and studied organoleptically with naked eye,

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magnifying lens and measuring tape with the help of Pharmacognostical parameters i.e. appearance shape, size, surface, color, odour, taste, fracture and findings were recorded.

**MICROSCOPIC STUDY**

**Microscopy:**

Microscopic study of crude drug is another aid of Pharmacognosy which can be helpful in the process of standardization of medicinal plants. This study can be helpful in identifying genuine drug by their known histological characters through Transverse section (T.S.) and powder microscopy which can help in evaluation of different constituents by using different staining reagents.

**Materials:**

Fresh rhizomes of botanically identified plant of Mustak (*Cyperus rotundus* Linn.) were collected from Haridwar, Uttarakhand. Rhizomes were washed, cut into pieces and preserved in Formalo-acetyl-alcohol (FAA) and labeled for pharmacognostical study.

**Methods:**

Specimens were soaked in water or Formalo-acetyl-alcohol (FAA) depending upon the hardness of the sample and transverse sections were taken using sharp razor blades. Few microscopic sections were cut by Microtome sectioning. Numerous temporary and permanent mounts of the microscopical sections of the specimen were made and examined microscopically. The section has been passed by double staining methods. Different staining reagents were applied on transverse sections so as to differentiate between different cell wall components.

**Powder microscopy:**

Powder characteristics, Preliminary examination and behaviour of the powder with different chemical reagents were carried out and microscopical examination was carried out as per reported methods.

**PHYSIOCHEMICAL STUDY**

Characterization of the powdered herbal material was conducted by following the standardized guidelines for the determination of foreign matter, moisture content, total ash content, acid insoluble ash content, water soluble ash content, water-soluble extract content, alcohol soluble extract content and pH value.

**Materials:**

Plant material: Procurement of herb, Mustak (Rhizome of *Cyperus rotundus* Linn.) was followed as described in collection of materials.

**Instruments:** Petri dish, desiccators, oven, crucible, muffle furnace, Whatman filter paper No. 42 etc.

**Chemical:** 5N Hydrochloric acid, alcohol, distilled water etc.

**Methods:**

**Foreign matter:**

50 gm of the drug sample was taken and weighed and spread out in a thin layer. Then any foreign matter like moulds, insects, animal fecal matter, other contaminations such as earth, stones and extraneous material or any other drug found adulterated was detected by inspection with the unaided eye or by the use of a lens. Then this

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separated foreign matter was weighed and percentage was calculated.

**Calculation:**

**Foreign matter = 100 × Weight of foreign matter / Weight of sample**

**Determination of moisture content:**

Weigh accurately 5 gm of the plant material in a Petri dish previously dried to a constant weight in an electric oven. Place the dish in an electric oven, maintained at 105°±1°C for five hours. Cool the dish in a desiccator and weigh with the lid on. Repeat the process of heating, cooling and weighing at half-hour intervals until the loss in weight between two successive weightings is less than one milligram. Record the lowest mass obtained.

**Calculation:**

Moisture content, % by mass (m) = 100 X ($M_1$ - $M_{2)}$ / $M_1$ - M

Where,

$M_1$ gm = wt of the dish with sample before drying

$M_2$ gm = wt of the dish with sample after drying

M gm = wt of the empty dish

m = moisture content, % by mass

**Determination of total ash:**

The total ash method is designed to measure the total amount of material remaining after ignition. Silica Crucible was cleaned, dried well and labelled with glass pencils and then weighed to constant weight. Transfer the dried material obtained for Moisture analysis, in crucible. Heat the crucible carefully for one hour over a hot plate to char the material. Then ignite the charred material in the Muffle Furnace at 550 to 600°C untill the grey ash is obtained (by confirming no specks of carbon are visible). Then, crucible was taken out and allowed to cool in a desiccator and weighed. Repeat the process of igniting, cooling and weighing at half-hour intervals until the difference in mass between two successive weighing is less than one milligram. Record the lowest mass. Retain the total ash for determining the acid-insoluble ash.

**Calculation:**

Total ash (on dry basis), % by mass = 100 × ($M_1$-$M_2$) / $M_1$ - M

Where,

$M_1$ gm = Wt of crucible+ sample (dried before ash)

$M_2$ gm = Wt of crucible+ ash (after ignition)

Mgm = Wt of empty crucible

**Determination of acid insoluble ash:**

The crucible containing total ash was taken; dropwise 25 ml of dilute 5N Hydrochloric acid was added. Covered with a watch-glass and heated on a water bath for 10 minutes. Allowed to cool and filter the contents of the dish through a Whatman filter paper No. 42 or its equivalent ash less filter paper. Wash the filter paper with hot water until the filtrate was neutral (free from the acid). The filter paper containing the insoluble matter was transferred to the original crucible. It was dried on a hot plate for few minutes, and then transferred to hot air oven where it was kept for 3 hrs at 135 plus/minus 2 degree Celscius. Ignite in a muffle furnace at 550 plus/minus 600 degree Celscius for one hour. The residue was allowed to cool in a suitable

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desiccator for 30 minutes and weighed without delay. Repeat the process for igniting in the muffle furnace for 30 minutes, cooling and weighing until the difference between two successive weighing is less than one milligram. Record the lowest mass.

**Calculation:**

Acid insoluble ash, % by mass = 100 X ($M_3$ - M) / $M_2$- M

Where,

M gm = Wt of empty crucible

$M_2$ gm = Wt of crucible+ sample (after ash)

$M_3$ gm = Wt of crucible i.e., acid insoluble ash

**Water soluble ash:**

Ash in a crucible was taken and 25ml distilled water was added and kept on hot plate for 10 minutes. Then cool and filter with Whattman no. 42 and wash with hot water until and unless the pH is neutral. After neutralization process took a filter paper and kept on the same crucible and ignited the material in muffle furnace at 450-600°C for 1 hour. The weight of the insoluble matter was subtracted from the weight of ash. The percentage of water-soluble ash with reference to the air-dried drug was calculated.

**Calculation:**

Water soluble ash, % by mass = 100 × (M3-M) / M2 - M

Where,

M gm = Wt of empty crucible

$M_2$ gm = Wt of crucible + sample (after ash)

$M_3$ gm = Wt of crucible i.e., acid insoluble ash

**Determination of alcohol soluble extractive:**

5 gm of the air dried drug was taken, coarsely powdered and macerated with 100 ml of alcohol of specified strength in a closed flask for twenty-four hours, shaking frequently during six hours and allowed standing for eighteen hours. It was rapidly filtered; taking precautions against loss of solvent, 25 ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish, and dried at 105 degree, to constant weight and weighed. The percentage of alcohol-soluble extractive with reference to the air-dried drug was calculated.

**Calculation:**

Weight of sample taken = W1gm

Weight of empty petridish = W2 gm

Weight of dish + extractive residue = W3 gm

Alcohol soluble extractive %= W3 - W2 / W1 X 25 X 100 X 100

**Determination of water soluble extractive: Same procedure was followed as for determination of alcohol-soluble extractive, using distilled water instead of ethanol**

**Calculation:**

Weight of sample taken = WI gm

Weight of empty petridish = W2 gm

Weight of dish + extractive residue = W3gm

Water Soluble extractive % = W3-W2/ W1 x 25 x 100 x 100

**Determination of pH value:**

5 gm of the powdered drug was taken in a beaker with 50 ml fresh distilled water. It was stirred well with the help of a glass rod and allowed to stand for 2 hours. It was then filtered and pH was determined using pH meter at 25 degree celsius.

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## CHROMATOGRAPHIC STUDY

### Procedures for TLC fingerprint profile

### TLC PROFILE:

### Conditions

Stationary phase: Pre-coated silica gel 60 $F_{254}$ aluminum plates

Mobile phase: Toluene: Ethyl Acetate : Formic Acid- 6:4:0.2)

Chamber Saturation Time: 20 minute.

Test Solution: 1 gm of formulation dissolved in methanol and then filter the liquid extract. Make the volume up to 10 ml with methanol.

Visualization & Detection: 254 nm, 366 nm

### Procedure

Take previously washed with methanol and dried TLC plate and fix dimension at X position and mark from base with help of pencil at 10 mm and 90 mm. and also left 15 mm from both sides of plate. Apply the test sample solution for each 20 µl in the form of bands. Allow the solvent to be evaporated and place the plate in the saturated tank, possibly vertical and so that spots or bands are above the level of mobile phase. Close the tank and allow standing at room temperature until mobile phase ascended to the marked line. Remove the plate and dry and visualize as in UV-Vis light at 254 nm and 366 nm.

## OBSERVATION & RESULTS

### MUSTAK (CYPERUS ROTUNDUS LINN.)

### Pharmacognostical Study

### Morphological Features

**Table 2** Morphological Features of *Mustak* (*Cyperus rotundus* Linn.)

| S.NO. | FEATURES | *Cyperus rotundus* Linn. |
|---|---|---|
| **1.** | Habit | Erect and perennial glabrous herb with woody subterranean stoloniferous rhizome. |
| **2.** | Habitat | Weed of marshy and moist areas in plains and hilly region, found throughout India and mostly in North-east part. |
| **3.** | Stem | Stems nodose at base, trigonous and 10-60 cm. high. |
| **4.** | Leaves | Leaves basal, shorter or longer than the stem, narrowly linear, finely acuminate at apex and 10-18 cm. long & 0.3-0.5 cm broad. |
| **5.** | Inflorescence | Inflorescence, umbel of more or less condensed spikes, flowers reddish-brown, Bracts 3, variable in length, the longest reaching 15cm long, but sometimes abbreviated and much shorter than the head. |
| **6.** | Rhizome | Oval to spindle shaped, generally range from 1.5-3.5cm. in length, 0.5-2.5cm. in diameter. |

## ORGANOLEPTIC STUDY

**Table 3** Organoleptic Study of Genuine Sample of *Mustak* Rhizome

| S.No | Rhizomes | Genuine Sample of *Mustak* (*Cyperus rotundus* Linn.) |
|---|---|---|
| **1.** | Shape | Ovoid, bluntly conical and spindle shape, rhizome and stolon having number of wiry roots |
| **2.** | Size | Vary in size and thickness but approximately 1-2.5 cm in length and 0.5-1cm. in thickness. |
| **3.** | Colour | brownish black externally and starchy white internally |
| **4.** | Surface | Rough with striations and covered with flexuous hairs. |
| **5.** | Odour | Pleasant |
| **6.** | Taste | Bitter, pungent & astringent in taste |
| **7.** | Fracture | Not easily breakable due to smaller size, Short exposing white interior with light brown dots |

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## MICROSCOPIC STUDY

**Table 4** T. S of Mustak (*Cyperus rotundus* Linn.) Rhizomes

| S.No | Parameters | T.S of Genuine Sample of *Mustak* ( *Cyperus rotundus* Linn.) |
|---|---|---|
| 1. | Epidermis | Single layered epidermis |
| 2. | Cortex | Cortex, wide and composed of uniformly round, thin walled, parenchymatous cells, ground tissue of cortex consists of circular to oval , thin walled, parenchymatous cell with small intercellular spaces |
| 3. | Endodermis | Distinct and Single layered |
| 4. | Vascular bundles | Numerous, collateral vascular bundles present inner to the pericycle region |
| 5. | Starch | Cells of pith and cortex region contain starch |

### Powder Microscopy of Mustak (*Cyperus rotundus* Linn.)

Powder - Creamish-brown; shows reddish-browm cells, reticulate and simple pitted vessels; fibre-like, closely packed sclerified cells, narrow vessels with scalariform thickness and oblique pore from the remnants of leaves simple, round to oval, starch grains.

## PHYSIOCHEMICAL ANALYSIS OF (CYPERUS ROTUNDUS LINN.)

### FOREIGN MATTER

**Table 5** Genuine Sample of *Mustak*

| S.No | Sample | Foreign Matter | Standard as per API |
|---|---|---|---|
| 1. | *Mustak* | 0.55% | Not more than 2% |

### MOISTURE CONTENT

**Table 6** Genuine Sample of *Mustak*

| S.No | Sample | Moisture Content |
|---|---|---|
| 1. | *Mustak* | 8.67 % |

### pH VALUE

**Table 7** Genuine Sample of *Mustak*

| S.No | Sample | pH Value |
|---|---|---|
| 1. | *Mustak* | 5.2 |

### AQUEOUS EXTRACTIVE VALUE

**Table 8** Genuine Sample of *Mustak*

| S.No | Sample | Aqueous Extractive Value | Standard as per API |
|---|---|---|---|
| 1. | *Mustak* | 15% | Not less than 11% |

### ALCOHOL EXTRACTIVE VALUE

**Table 9** Genuine Sample of *Mustak*

| S.No | Sample | Alcohol Extractive Value | Standard as per API |
|---|---|---|---|
| 1. | *Mustak* | 5% | Not less than 5% |

### TOTAL ASH VALUE

**Table 10** Genuine Sample Of *Mustak*

| S.No | Sample | Total Ash Value | Standard as per API |
|---|---|---|---|
| 1. | *Mustak* | 4.15% | Not more than 8% |

### ACID INSOLUBLE ASH VALUE

**Table 11** Genuine Sample of *Mustak*

| S.No | Sample | Acid Insoluble Ash Value | Standard as per API |
|---|---|---|---|
| 1. | *Mustaka* | 0.94% | Not more than 4% |

### WATER SOLUBLE ASH VALUE

**Table 12** Genuine Sample of *Mustak*

| S.No | Sample | Water Soluble Ash Value |
|---|---|---|
| 1. | ***Mustak*** | 2.02% |

## CHROMATOGRAPHIC STUDY

**Table 13** THIN LAYER CHROMATOGRAPHY (TLC)

(Mobile Phase-Toluene: Ethyl Acetate: Formic Acid- 6:4:0.2)

| Test | Genuine Sample of *Mustak* (*Cyperus rotundus* Linn.) |
|---|---|
| $R_f$ Value | **254nm**-0.02, 0.12, 0.22, 0.26, 0.35, 0.38, 0.50, 0.65, 0.72, 0.80, 0.87<br>**366nm**-0.02, 0.06, 0.08, 0.16, 0.22, 0.80<br>**After Derivatized 366nm**- 0.02, 0.06, 0.12, 0.22, 0.31, 0.40, 0.50, 0.76, 0.80, 0.83, 0.92, 0.96 |

## DISCUSSION

This section of research work will provide a brief overview of the present study to the reader. In this section, significance of the findings have been described in light of what was already known about this subject and new understandings or fresh insights about the research work which

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have been undertaken for the present study. The discussions and scholastic deliberation will enlighten the ayurvedic fraternity to know both the theoretical and practical aspects of the present work.

**Morphology of Mustak ( *Cyperus rotundus* Linn. )**

Mustak is a weed of marshy lands and other moist places in the plains and hilly region and found throughout India and mostly in North-east part. *Cyperus rotundus* Linn. is a pestiferous perennial weed with the dark green glabrous culms, 0.5-2ft high, arising from a system of underground tubers. It is found throughout India up to an elevation of 6000ft. The plant has an elaborate underground system consisting of tubers, rhizome and roots. The tubers are white and succulent when young and hard and black when mature. It thrives on all kind of soils under varying climate conditions. Once established the plant spreads rapidly. Regeneration is mainly through underground rhizomes. The tubers of the plant have an aromatic odour. The dry tubers are sold in markets and used in medicine, perfumery and for the preparation of fragrant sticks called agarbatties. The tubers are said to be diaphoretic and astringent and in indigenous medicine they are given for disorders of the stomach and irritation of the bowels. (As per Table 2, Figure 1-3)

The genuine sample of Mustak i.e. rhizomes of *Cyperus rotundus* Linn. was collected from Haridwar, Uttarakahand. Collected sample was botanically authenticated by the researcher and supervisor with specimen deposited in the repository of Patanjali Research Institute, Haridwar. Collected genuine sample was dried and preserved for further organoleptic and other analytical studies. (As per Figure 4-5)

**ORAGANOLEPTIC STUDY**

Rhizomes of Mustak were ovoid, blunt and spindle in shape, vary in size and nearly about 1-3.5 cm in length and 0.5- 2 cm. in thickness. They were not easily breakable due to smaller size and hardened nature and the fracture was short exposing white interior with light brown dots. Odour of rhizome was pleasant and rhizomes were found Bitter, pungent, astringent in taste. (As per Table 3, Figure 6-8)

**MICROSCOPIC STUDY**

In the T.S of Mustak (*Cyperus rotundus* Linn.), single layer of epidermis was present, cortex was wide uniformly round and thin walled, endodermis was distinct, single layered and made up of rounded or barrel like cells, the ground tissue inner to the pericycle contains a large number of small rounded vascular bundle, Cells of pith and cortex region contain starch. (As per Table 4, Figure 9)

**Powder Microscopy**

Tannin, Cellulose, Mucilage, Cutin were present in the genuine sample of Mustak. (As per Figure 10)

**PHYSIOCHEMICAL STUDY**

All physiochemical parameters including foreign matter, moisture content, ash content, acid insoluble ash content, water soluble ash content, water-soluble extract content, alcohol soluble

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extract content and pH were assessed and found to be within permissible limits.

Moisture content is a critical indicator of the quality of the material. Excessive or deficient moisture content of a substance can adversely impact the physical properties of a material. An excess of water in herbal materials will encourage microbial growth, where as a dearth of moisture content will lead to deterioration of the bioactivity and quality of the plant material. Fundamentally, the moisture content should never be more than 50% of the total soluble content of the plant material. Moisture content was found for *Cyperus rotundus* Linn. is 8.67%

Secondly, the ash content is a measure of the concentration of minerals and other inorganic matter present in the plant material. However, total ash content is not sufficient alone in determining the quality of plant materials. Hence, acid insoluble ash and water soluble ash content are also used as indices of the quality of herbals. Sample of Mustak exhibited total ash content below the permissible limits of 8% as per the required API specification. Total ash value for *Cyperus rotundus* Linn. was found 4.15%.

Moreover, water-soluble extractive value plays an important role in the evaluation of herbal samples, whereas, a lesser extractive value is indicative of adulteration or incorrect processing of herbals. Here in, all the tested herbal powders exhibited moderate-to-high water soluble extractive value, thereby, indicating their purity. Water soluble extractive value for *Cyperus rotundus* was found 15%. Alcohol soluble extractive value for *Cyperus rotundus* was found 5%. Furthermore, the pH value of *Cyperus rotundus* was found 5.2, thereby, indicating the sample of drug to be safe for human usage. (As seen in Table 5-12)

**CHROMATOGRAPHIC STUDY**

**Thin Layer Chromatography (TLC)**

Thin Layer Chromatography of genuine sample of *Cyperus rotundus* Linn. was developed using mobile Phase-Toluene : Ethyl Acetate : Formic Acid- 6:4:0.2. After developing fingerprints, the plates were dried and visualised under Anisaldehyde sulphuric acid reagent. Sample has found lot of unknown chemical constituents, were separate in mobile solution Toluene: Ethyl Acetate : Formic Acid- 6:4:0.2. The following $R_f$ value were found in the sample of Mustak (*Cyperus rotundus* Linn.):

**$R_f$ Value-**

**254nm**-0.02, 0.12, 0.22, 0.26, 0.35, 0.38, 0.50, 0.65, 0.72, 0.80, 0.87

**366nm**-0.02, 0.06, 0.08, 0.16, 0.22, 0.80

**After Derivatized 366nm**- 0.02, 0.06, 0.12, 0.22, 0.31, 0.40, 0.50, 0.76, 0.80, 0.83, 0.92, 0.96 (As seen in Table 13), (As per Figure 11)

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**Figure 1** Leaves of *Mustak*

**Figure 2** Stem of *Mustak*


**Figure 3** Inflorescence of *Mustak*

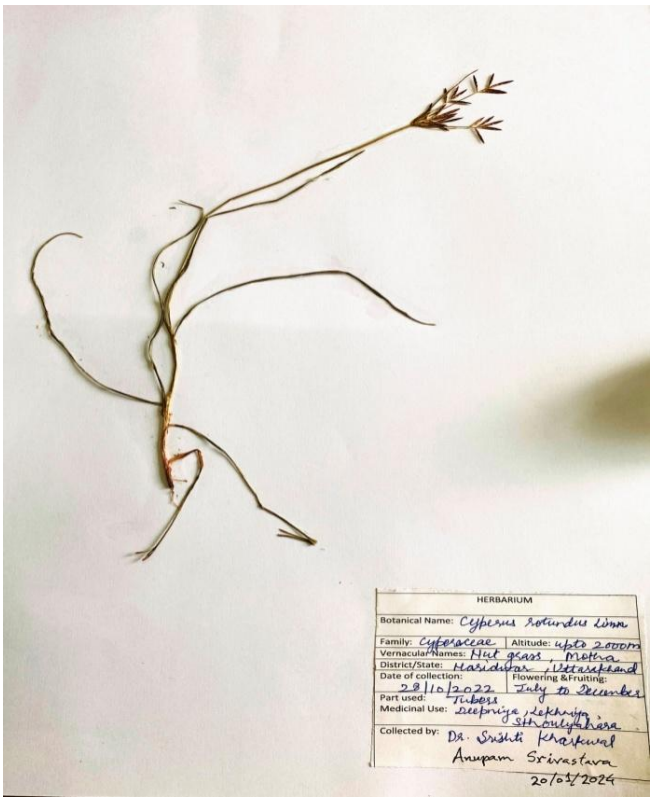


**Figure 4** Specimen of Mustak

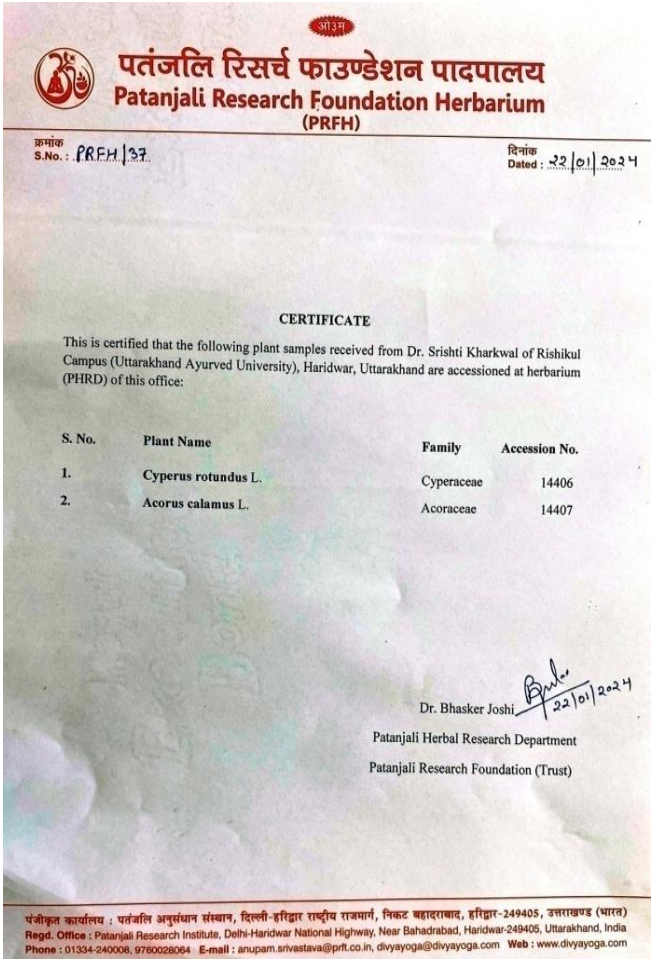


**Figure 5** Herbarium Authentication Certificate

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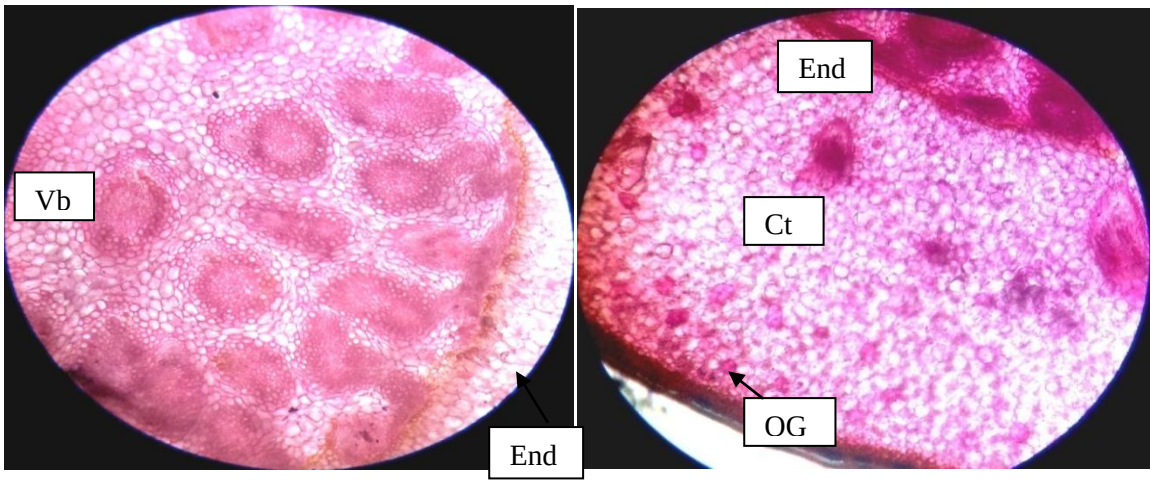
**Figure 6** Rhizomes of *Mustak* (*Cyperus rotundus* Linn.)


**Figure 7** Fresh tuber of *Mustak*
**Figure 8** Dried tubers of *Mustak*

T.S. of Rhizome of *Mustak* (*Cyperus rotundus* Linn.)




E: Epidermis, End : Endodermis, Ct : Cortex, Vb : Vascular bundles, Pi : Pith, OG : oil globules All the images presented were ta by author

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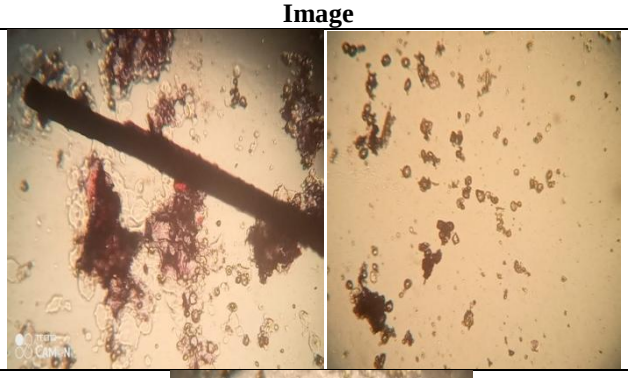
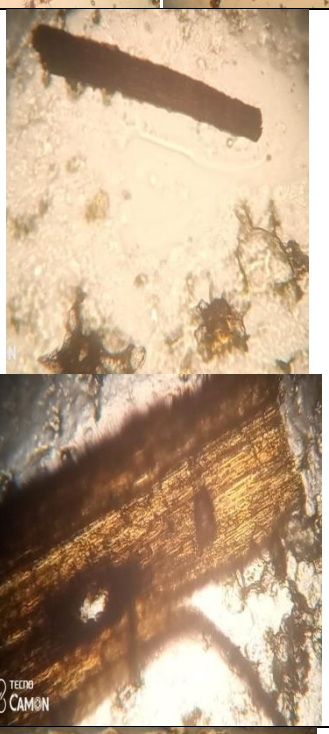
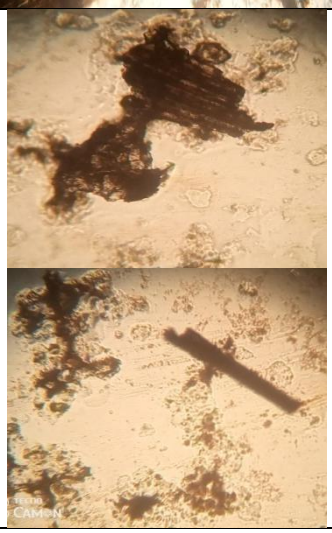
**Figure 10** Powder Microscopy of Mustak (*Cyperus rotundus* Linn.)

| S. no. | Staining dye | Image | Character |
|---|---|---|---|
| 1. | Safranine |  | pink stained lignified parenchyma and Lignified cells |
| 2. | Methylene blue |  | Parenchyma cluster and stained cells |
| 3. | Ferric chloride |  | Parenchymatous cells, Mucilage cell cluster |

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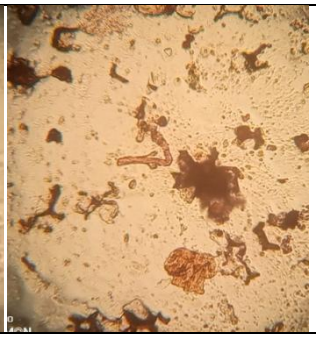
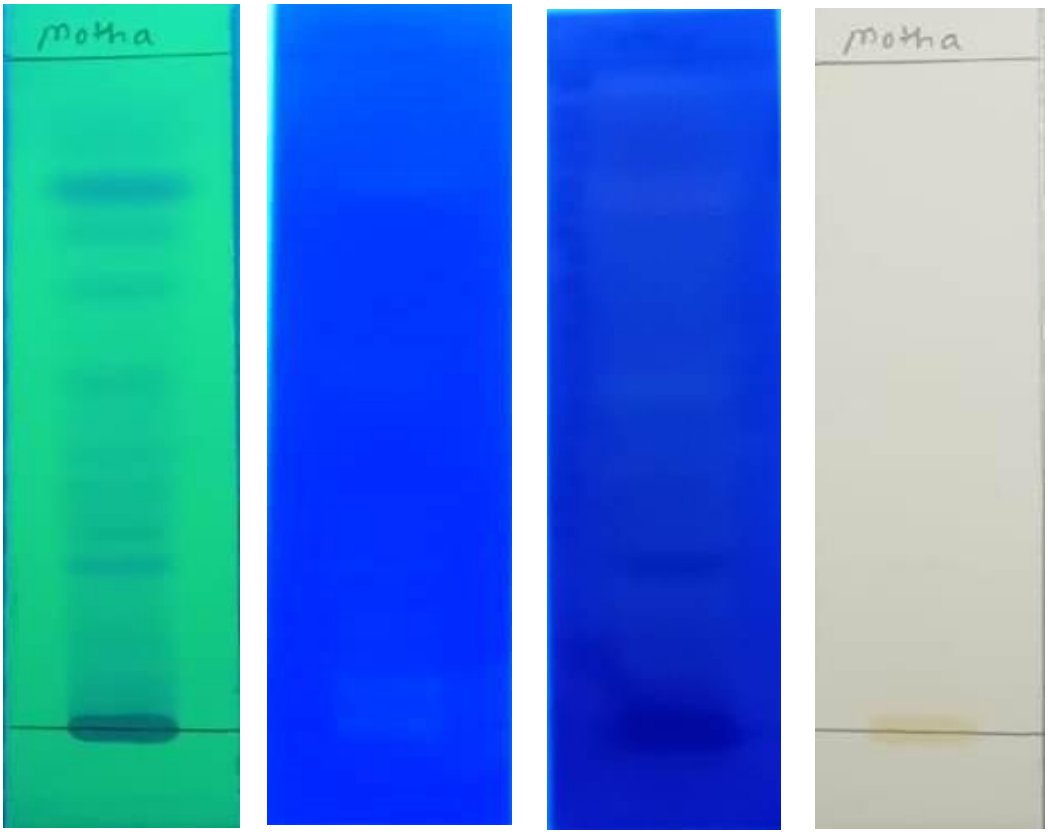
| | | | |
|---|---|---|---|
| 4. | Eosine |   | Oil containing parenchymal cells and Lignified parenchyma |

**Figure 11** TLC Analysis of *Cyperus rotundus* Linn.




254nm 366nm 366nm After derivatized White light

**Figure 12** Certificate of Analytical Study of Mustak (*Cyperus rotundus*Linn.)

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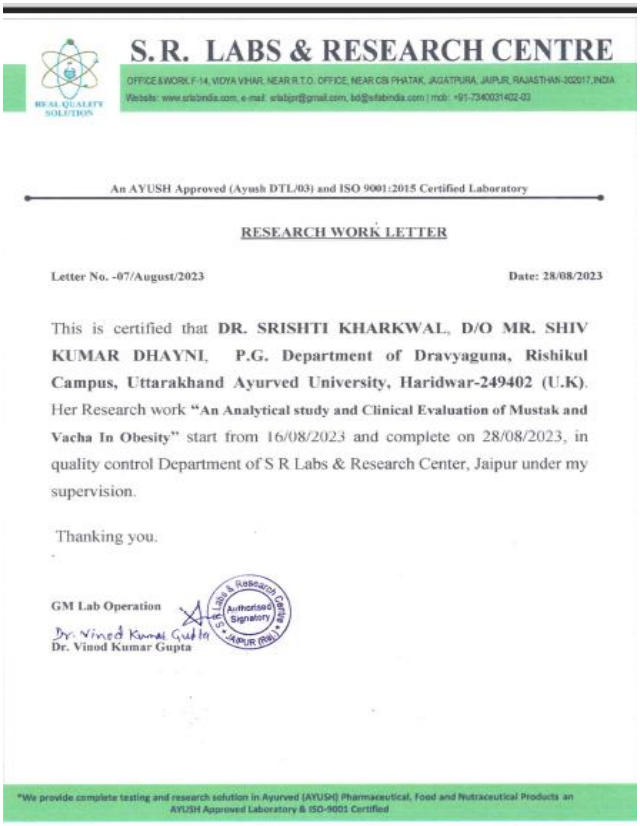

S.R. LABS & RESEARCH CENTRE

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RESEARCH WORK LETTER

Letter No. -07/August/2023 Date: 28/08/2023

This is certified that **DR. SRISHTI KHARKWAL, D/O MR. SHIV KUMAR DHAYNI, P.G. Department of Dravyaguna, Rishikul Campus, Uttarakhand Ayurved University, Haridwar-249402 (U.K).** Her Research work **"An Analytical study and Clinical Evaluation of Mustak and Vacha In Obesity"** start from 16/08/2023 and complete on 28/08/2023, in quality control Department of S R Labs & Research Center, Jaipur under my supervision.

Thanking you.

GM Lab Operation
Dr. Vinod Kumar Gupta

Authorised Signatory

*We provide complete testing and research solution in Ayurved (AYUSH) Pharmaceutical, Food and Nutraceutical Products an AYUSH Approved Laboratory & ISO-9001 Certified

## CONCLUSION

In this study, different quality control parameters (Pharmacognostical, Physiochemical and Phytochemical) have been established for *Cyperus rotundus* Linn. These parameters would be useful as an analytical tool for the standardization of the rhizome of *Cyperus rotundus* Linn. since these features are distinctive for the identification of *Cyperus rotundus* Linn. This study would be helpful in authentication of Mustak (*Cyperus rotundus* Linn.).

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