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ANALYTICAL STUDY OF VACHA (ACORUS CALAMUS LINN.)

Dr. Srishti Kharkwal[1]*, Dr. D. C. Singh[2], Dr. Hemant Kharkwal[3], Dr. Seema Joshi[4]

[1*]Ph.D. Scholar, Rishikul Campus, P.G. Department of Dravyaguna, Haridwar, Uttarakhand.

[2]Prof. and H.O.D., Campus Director, P.G. Department of Dravyaguna, Rishikul Campus, Haridwar, Uttarakhand.

[3]Ph.D. Scholar, Department of Public Health, Jigyasa University, Dehradun, Uttarakhand.

[4]Prof. and H.O.D., Department of Kriyasharir, Rishikul Campus, Haridwar, Uttarakhand.

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***Corresponding Author**
Dr. Srishti Kharkwal
Ph.D. Scholar, Rishikul Campus, P.G. Department of Dravyaguna, Haridwar, Uttarakhand.

ABSTRACT

Background: Vacha (*Acorus calamus* Linn.) is used for various conditions of ailments in traditional system of medicine since ancient times. Vacha is mentioned as one of the Rasayana dravya and its regular intake is said to make one endowed with sharp intellect and sweet voice. Acharya Charaka has mentioned Vacha in Lekhaniya, Arshoghna, Triptighna, Asthapnopaga, Shirovirechana, Sanjnasthapana, Sitaprashamana Mahakashaya which indicates its therapeutic importance in Ayurveda. **Objective:** This study is designed to establish the various pharmacognostical, physiochemical and phytochemical standards of *Acorus calamus* Linn. for its correct identification and authentication. **Material and Methods:** Vacha (*Acorus calamus* 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 *Acorus calamus* 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 values were found in the sample of Vacha (*Acorus calamus* Linn.):**Rf Value-254nm**-0.02, 0.85, **366nm**-0.02, 0.19, 0.43, 0.62, 0.81, 0.85, **After Derivatized 366nm-**

0.02, 0.19, 0.24, 0.28, 0.39, 0.43, 0.71, 0.81, 0.85, 0.97 **Conclusion:** Pharmacognostical, physiochemical and phytochemical profiling will be helpful in identification and authentication of *Acorus calamus Linn*. and the parameters which are established from this study may be helpful in standardization of Vacha (*Acorus calamus*) Linn.

KEYWORDS: Vacha, *Acorus calamus* Linn., Physiochemical, phytochemical, microscopical etc.

INTRODUCTION

Traditional herbal medicine and their preparations have been widely used for the thousands of years in developing and developed countries owing to its natural origin and lesser side effects or dissatisfaction with the results of synthetic drugs. In olden days vaidyas used to treat patients on individual basis and prepare drug according to the requirement of the patient but now the scene has changed, herbal medicines are being manufactured on large scale where manufacturers come across many problems such as availability of good quality raw material, authentication of raw material, availability of standards, proper standardization methodology of single drugs and formulation, quality control parameters etc; hence the concept of quality from very first step is paramount factor must get good attention. The chemistry of plants involves the presence of therapeutically important constituents usually associated with many inert substances (coloring agents, cellulose, lignin etc). The active principles are extracted from the plants and purified for therapeutic utility for their selective pharmacological activity. So quality control of herbal crude drugs and their constituents is of great importance in modern system of medicine. Lack of proper standard parameters for the standardization of herbal preparation and several instances of substandard herbs, adulterated herbs come into existence. To meet new thrust of inquisitiveness, standardization of herbals is mandatory (Chaudhry, 1999; Kokate, 2005; Raina, 2003; Raven, 1999; Yan, 1999).

Hence every single herb needs to be quality checked to ascertain that it confirms to quality requirement and delivers the properties consistently. Standardization assures that products are reliable in terms of quality, efficacy, performance and safety.

Vacha (*Acorus calamus* L.) is exceedingly influential and renowned drug in Ayurveda well known for medhya karma (that which improve memory & intellect). The rhizome of this plant has been indicated as brain tonic in weak memory (API). In Sanskrit the word Vacha means that which improves speech or enhances the power of speech. It is commonly known

as Sweet flag, a tall perennial wetland monocot plant from the Acoraceae family. It is exceedingly common in Manipur and Naga Hills and on the edges of lakes & streams. It is found throughout India under cultivation as well as in the wild state, in plains, lower elevations and in Himalayan upto altitude of 1800m. Vacha (*Acorus calamus* Linn.), an indigenous drug of India belongs to family Acoraceae. It is delineated under various therapeutical groups like Lekhaniya, Triptighna, Arshoghna dashemani etc., by Acharya Charaka, Pippalyadi, Vachadi etc., ganas by Acharya Sushruta and Mustadi, Vatsakadietc., gana by Vagbhata. The pharmacognostical characters of Vacha are described through various synonyms like Shadgrantha (Having six nodes), Ugragandha (Having strong aroma), Lomasha (Having small hairs), Golomi (Having small hairs like cow) etc. It has important pharmacological properties like Deepana (Appetizer), Pachana (Digestive), Vamaka (Emetic), Lekhaniya, Medhya (brain tonic), Kanthya (Good for throat), Sanjnasthapana (Restores lost conciousness), Vedanasthapana (Anodyne) etc., and hence used extensively in therapeutics.[1-11]

So, In this study, Vacha (*Acorus calamus* Linn.) was selected for their authentication and standardization because of its highly valuable therapeutic properties.

MATERIAL AND METHODS[12]

1. Material

Plant Material

The plant material which was taken for study is *Acorus calamus* Linn.

Acorus calamus Linn. is taken as a source of Vacha.

Plant Collection and authentication

- The genuine sample was collected after identifying the source of plant as per standard description.
- The genuine sample of *Acorus calamus* Linn. rhizomes (Vacha) 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,

2. METHODS

2.1 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, Vacha 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 (Hgcl2) 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. (Fig. No.3, 4).

Table No. 1: Genuine Sample of Vacha (*Acorus calamus* Linn.) with Herbarium accession number.

S.No	Name of Plant	Date of Collection	Place of Collection	Accession No.
1.	*Acorus calamus* L.	27/08/2022	Haridwar, Uttarakhand	14407

3. ORGANOLEPTIC / MACROSCOPIC STUDY

3.1 Procurement of raw materials

Procurement of herb, Vacha (Rhizome of *Acorus calamus* Linn.) was followed as described in collection of materials.

3.2 Method

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

4. MICROSCOPIC STUDY

4.1 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.

4.2 Materials

Fresh rhizome of botanically identified plant of Vacha (*Acorus calamus* Linn.) was collected from Haridwar, Uttarakhand. Rhizomes were washed, cut into pieces and preserved in Formalo-acetyl-alcohol (FAA) and labeled for pharmacognostical study.

4.3 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.

4.4 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.

5. 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.

5.1 Materials

5.1.1 Plant material: Procurement of herb, Vacha (Rhizome of *Acorus calamus* Linn.) was followed as described in collection of materials.

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

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

5.2 Methods

5.2.1 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 separated foreign matter was weighed and percentage was calculated.

Calculation

$$\text{Foreign matter} = 100 \times \text{Weight of foreign matter} / \text{Weight of sample}$$

5.2.2 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 weighings is less than one milligram. Record the lowest mass obtained.

Calculation

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

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

5.2.3 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

$$\text{Total ash (on dry basis), \% by mass} = 100 \times (M_1-M_2) / M_1 - M$$

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

5.2.4 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 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

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

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

5.2.5 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

$$\text{Water soluble ash, \% by mass} = 100 \times (M3-M) / M2 - M$$

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

5.2.6. 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

5.2.7. 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

5.2.8 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.

5.2.9 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 AND RESULTS

VACHA (ACORUS CALAMUS LINN.)

Pharmacognostical Study

Morphological Features

Table No. 2: Morphological Features of Vacha *(Acorus calamus Linn).*

S.NO	FEATURES	*Acorus calamus* Linn.
1.	Habit	perennial semiaquatic herb of marshy places with woody rhizome.
2.	Habitat	Wild or cultivated throughout the country ascending upto 1800m in the Himalayas.
3.	Stem	Stout and creeping
4.	Leaves	Two ranked, distichous, sword shaped (ensiform), erect, sharp pointed, 15-75cm long, closely sheathing each other, midrib is stout, striate parallel venation, glabrous, weakly differentiated into petiole and blade.
5.	Inflorescence	Spadix, scapes 3 angled, spathe is leaf like long, grren and narrow, flower densly covered with spadix, compactly arranged, greenish yellow, bracts and bracteoles are absent.
6.	Rhizome	Sub-cylindrical or compressed, upto 20cm long, 1-1.5cm broad, light brown in colour, upper surface shows triangular leaf scars, lower surface bear small, raised circular root scars.

ORGANOLEPTIC STUDY

Table No. 3: Organoleptic Study of Genuine Sample of *Vacha* Rhizome.

S.No	Rhizomes	Genuine Sample of Vacha
1.	Shape	Sub-cylindrical to slightly flattened rhizome, somewhat tortuous or rarely straight.
2.	Size	Cut pieces of 1-9 cm long, and 0.5-1.5cm broad
3.	Colour	Light brown with reddish to pinkish tinge externally, buff colour internally
4.	Surface	Upper side marked with alternately arranged, large, broadly, transverse leaf scars which almost encircle the rhizome, at nodes leaf sheath mostly having an appearance present, lower side shows elevated tubercular spots of root scars
5.	Odour	Aromatic
6.	Taste	Pungent and bitter
7.	Fracture	Short

MICROSCOPIC STUDY

Table No. 4: T. S of Genuine Sample of *Vacha* Rhizomes.

S.NO	Parameters	T.S of Genuine Sample of *Vacha* (*Acorus calamus* Linn.)
1.	Epidermis	Single layer of epidermis
2.	Cortex	Cortex composed of spherical to oblong thin walled cells of various sizes, cells towards periphery, smaller, somewhat collenchymatous, more or less closely arranged cells towards inner side, rounded and form a network of chains of single row of cells, enclosing large air spaces.
3.	Endodermis	Distinct
4.	Vascular bundles	Several concentric vascular bundles arranged in ring towards endodermis, a few vascular bundles scattered in ground tissue.
5.	Starch	Starch grains, simple and spherical, present in cortex region and ground tissue.

Powder Microscopy of *Acorus calamus* Linn.

Buff coloured; shows fibres, reticulate, annular vessels and simple spherical starch grains. Shows transversely cut fragments of cortical aerenchymatous tissue embedded with oleo-resin cells; plenty of simple and compound, small sized starch grains scattered as such or embedded in the parenchymatous cells; transversely cut fragments of outer cortex showing a layer of epidermis and underlined cells of collenchyma; fragments of epidermal cells of scaly leaves embedded with stomata, overlapping with underlined collenchymatous tissue; spiral and reticulately thickened vessels, longitudinally cut thin-walled pericyclic fibres.

Physiochemical Analysis of *Acorus calamus* Linn.

FOREIGN MATTER

Table No. 5.1: Genuine Sample of Vacha.

S.No	Sample	Foreign Matter	Standard as per API
1.	***Vacha***	0.50%	Not more than 1%

MOISTURE CONTENT

Table No. 5.2: Genuine Sample of *Vacha.*

S.No	Sample	Moisture Content
1.	*Vacha*	11%

pH VALUE

Table No. 5.3: Genuine Sample of *Vacha.*

S.No	Sample	pH Value
1.	***Vacha***	5.9

AQUEOUS EXTRACTIVE VALUE

Table No. 5.4: Genuine Sample of *Vacha.*

S.No	Sample	Aqueous Extractive Value	Standard as per API
1.	***Vacha***	18%	Not less than 16%

ALCOHOL EXTRACTIVE VALUE

Table No. 5.5 Genuine Sample of *Vacha.*

S.No	Sample	Alcohol Extractive Value	Standard as per API
1.	***Vacha***	9%	Not less than 9%

TOTAL ASH VALUE

Table No. 5.6: Genuine Sample of *Vacha.*

S.No	Sample	Total Ash Value	Standard as per API
1.	***Vacha***	6.8%	Not more than 7%

ACID INSOLUBLE ASH VALUE

Table No. 5.7 Genuine Sample of *Vacha.*

S.No	Sample	Acid Insoluble Ash Value	Standard as per API
1.	***Vacha***	1%	Not more than 1%

WATER SOLUBLE ASH VALUE

Table No. 5.8: Genuine Sample of *Vacha.*

S.No	Sample	Water Soluble Ash Value
1.	***Vacha***	4.9%

CHROMATOGRAPHIC STUDY

THIN LAYER CHROMATOGRAPHY (TLC)

Table No. 6: TLC of *Vacha.*

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

Test	Genuine Sample of Vacha (*Acorus calamus* Linn.)
R_f Value	**254nm**-0.02, 0.85 **366nm**-0.02, 0.19, 0.43, 0.62, 0.81, 0.85 **After Derivatized 366nm**- 0.02, 0.19, 0.24, 0.28, 0.39, 0.43, 0.71, 0.81, 0.85, 0.97

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 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.

VACHA (*ACORUS CALAMUS* LINN.)

MORPHOLOGY

The source of the drug under present study as *Vacha* is rhizomes of *Acorus calamus Linn.* Vacha (*Acorus calamus* Linn.) is a tall, perennial wetland monocot, 1-4 feet tall of the Araceae or Acoraceae family. It is an aromatic marsh herb with creeping root stock. The seemingly numerous plants seen above ground in a population probably arise from a single plant connected by an extensive underground rhizome. (Fig. No.1, 2, Table No. 2)

ORGANOLEPTIC STUDY

Organoleptic study was reported in TABLE No. 3

Rhizomes of Vacha (*Acorus calamus* Linn.) were Sub-cylindrical to slightly flattened, somewhat tortuous or rarely straight, nearly about cut pieces of 1-9 cm long, and 0.5-1.5cm broad. Light brown in colour with reddish to pinkish tinge externally and buff colour internally. Upper side surface marked with alternately arranged, large, broadly, transverse leaf scars which almost encircle the rhizome, at nodes leaf sheath mostly having an appearance present, lower side surface shows elevated tubercular spots of root scars. Fracture was short exposing buff colour. Rhizome was found aromatic in odour and Bitter & pungent in taste. (Fig. No.2)

MICROSCOPIC STUDY

Microscopic study was reported in TABLE No. 4, Fig. No. 5

In the T.S of Vacha (*Acorus calamus*Linn.), single layer of epidermis was present, Cortex was composed of spherical to oblong thin walled cells of various sizes, cells towards periphery were smaller and somewhat collenchymatous, more or less closely arranged, cells towards inner side was rounded and form a network of chains of single row of cells, enclosing large air spaces. Endodermis was distinct. Several concentric vascular bundles arranged in ring towards endodermis and a few vascular bundles scattered in ground tissue. Starch grains are simple and spherical, present in cortex region and ground tissue.

Powder Microscopy

Buff coloured; shows fibres, reticulate, annular vessels, lignified parenchyma and lignified polygonal cells and simple spherical starch grains. (Fig. No.6)

PHYSIOCHEMICAL STUDY

Physiochemical study was reported in TABLE No. 5.1-5.8

All physiochemical parameters including moisture content, ash content, acid insoluble ash content, water-soluble extract content, ethanol-soluble 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 *Acorus calamus* Linn. Was 11% 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 Vacha exhibited total ash content below the permissible limits of 7% as per the required API specification. Total ash value for *Acorus calamus* Linn. was found 6.8%.

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. Herein, all the tested herbal powders exhibited moderate-to-high water soluble extractive value, thereby, indicating their purity. Water soluble extractive value was found for *Acorus calamus* Linn. was 18%. Alcohol soluble extractive value for *Acorus calamus* Linn. was found 9%. Furthermore, the pH value of *Acorus calamus* was found 5.9, thereby, indicating the sample of drug to be safe for human usage.

CHROMATOGRAPHIC STUDY

Chromatography study was reported in TABLE No. 6

Thin Layer Chromatography (TLC)

Thin Layer Chromatography of genuine sample of *Acoruscalamus* Linn. wasdeveloped using mobile Phase-Toluene : Ethyl Acetate : Formic Acid- 6:4:0.2. After developing fingerprints, the plates were dried and visualised under Anisaldehydesulphuric 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 Vacha(Acoruscalamus Linn.):

Rf Value

254nm-0.02, 0.85

366nm-0.02, 0.19, 0.43, 0.62, 0.81, 0.85

After Derivatized 366nm- 0.02, 0.19, 0.24, 0.28, 0.39, 0.43, 0.71, 0.81, 0.85, 0.97 (Fig. No.7)

LEAVES **INFLORESCENCE**

FIG. NO. 1: Vacha (*Acorus calamus* Linn.)

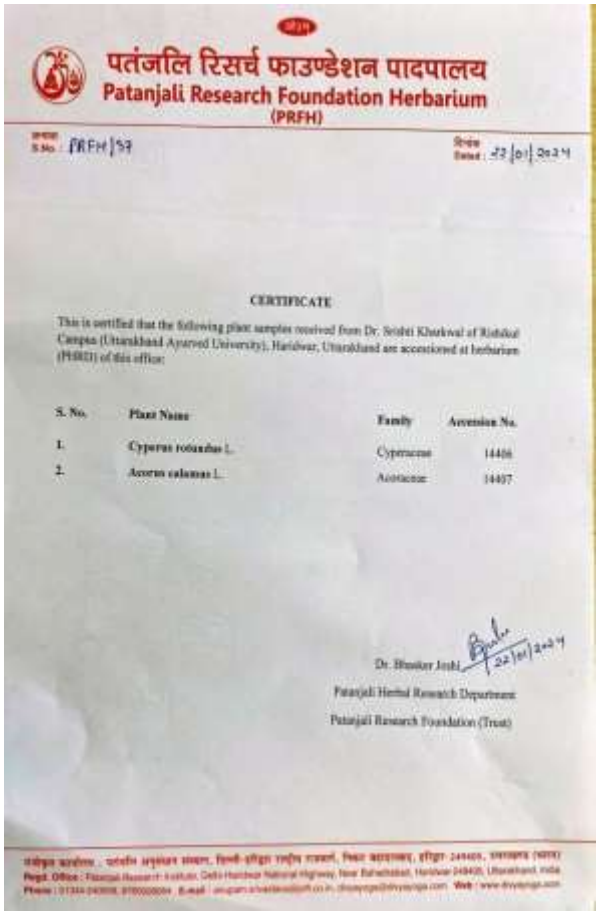
All the images presented were taken by author

RHIZOME

FIG. NO. 2: Vacha (*Acorus calamus* Linn).

All the images presented were taken by author

Fig.No.3 Herbarium Specimen of Vacha.



पतंजलि रिसर्च फाउण्डेशन पादपालय
Patanjali Research Foundation Herbarium
(PRFH)

CERTIFICATE

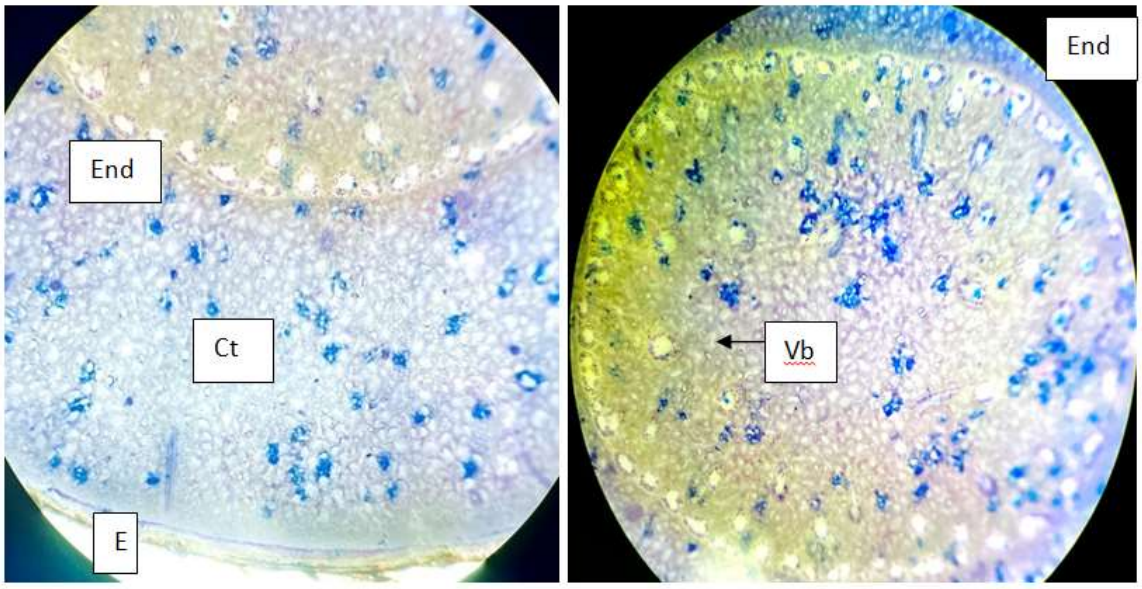
S. No.	Plant Name	Family	Accession No.
1.	Cyperus rotundus L.	Cyperaceae	14406
2.	Acorus calamus L.	Acoraceae	14407

Dr. Bhaskar Joshi
Patanjali Herbal Research Department
Patanjali Research Foundation (Trust)

Fig. No. 4 Herbarium Authentication Certificate.

All the images presented were taken by author

TRANSVERSE SECTION



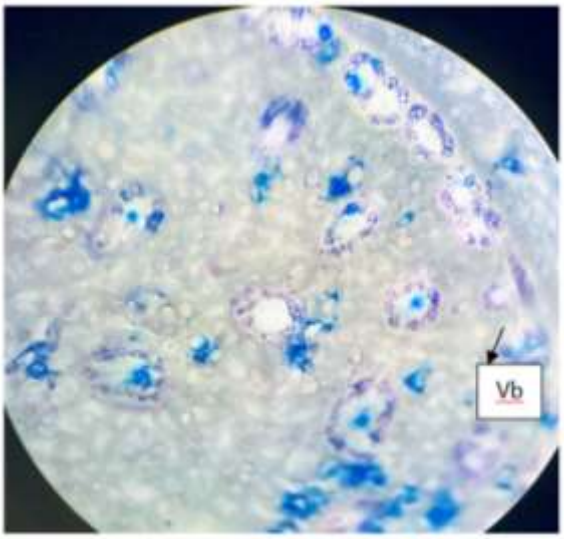
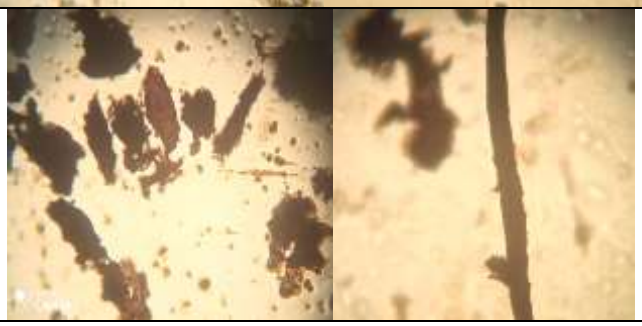


FIG. No. 5: T.S. of Rhizome of Vacha (*Acorus calamus* Linn.)

E : Epidermis, End : Endodermis, Ct : Cortex, Vb : Vascular bundles

All the images presented were taken by author

S. no.	Staining dye	Image	Character
1.	Safranine		stained lignified parenchyma and Lignified polygonal cells
2.	Methylene blue		Parenchyma cluster and stained pith cells
3.	Ferric chloride		Stained Polyphenolic cells, Mucilage cell cluster

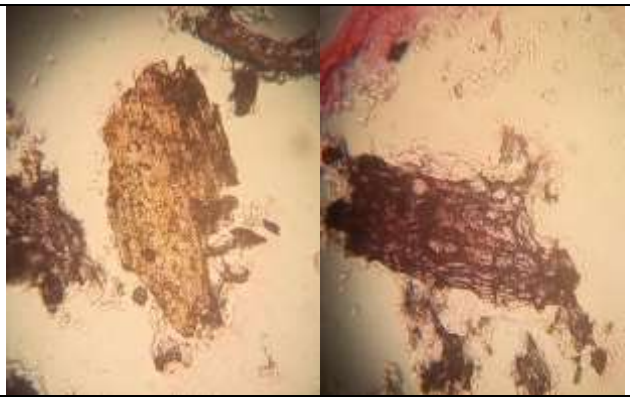
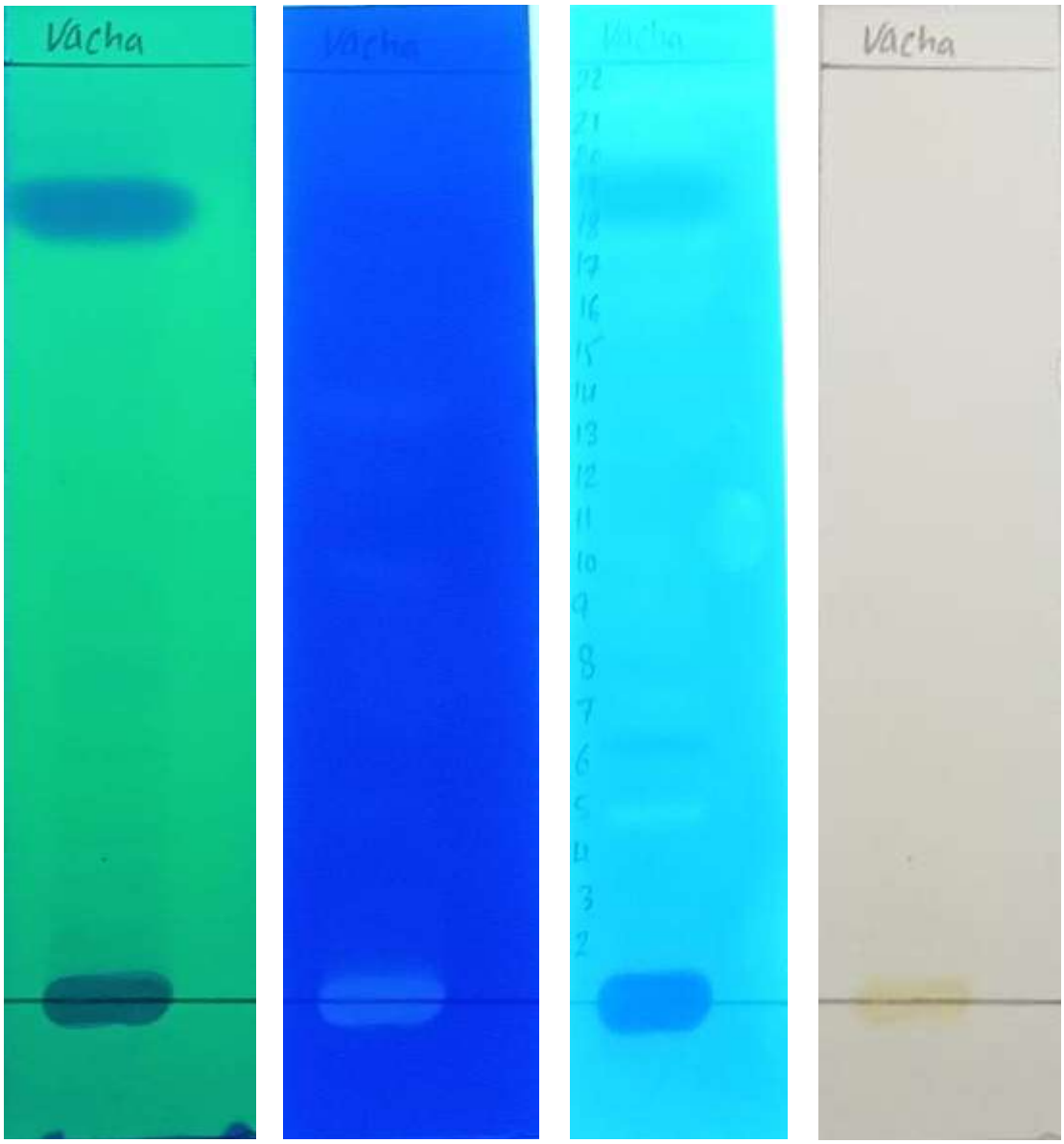
4.	Eosine		parenchymal cells and pink stained Lignified parenchyma

Fig.No. 6 Powder Microscopy of Vacha (*Acorus calamus* Linn.).



254nm **366nm** **After derivatized** **White light**

Fig. No. 7: TLC Analysis of *Acorus calamus* Linn.

S.R. LABS & RESEARCH CENTRE

OFFICE & WORK: F-14, VIDYA VIHAR, NEAR R.T.O. OFFICE, NEAR CBI PHATAK, JAGATPURA, JAIPUR, RAJASTHAN-302017, INDIA

Website: www.srlabsindia.com, e-mail: srlabjpr@gmail.com, bd@srlabsindia.com | mob: +91-7340031402-03

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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

Fig.No.8 Certificate of Analytical Study of Vacha (*Acorus calamus* Linn).

CONCLUSION

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

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