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PHARMACOGNOSTICAL AND PHYTOCHEMICAL EVALUATION OF VACHA RHIZOME (*ACORUS CALAMUS LINN.*) - A RESEARCH ARTICLE

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ABSTRACT

Vacha is the important medicinal plant used in *Ayurveda* traditional medicine to treat different ailments and maintain health condition. *Vacha* is a strongly aromatic gregarious perennial herb, with close set distichously arranged, erects narrowly ensiform, glossy green leaves, arising from a partially underground creeping and branching rhizome. The present study was carried out to investigate morphological, microscopical, physicochemical and phytochemical studies of *Vacha* rhizome. Morphological studies showed the presence of various diagnostic characters like brownish colour, cylindrical, branched shape, aromatic odour, pungent/bitter taste. In the microscopical studies, t.s. showed the presence of epidermis, cortex, fibrovascular bundle, endodermis contain parenchymatous cells and vascular bundle and powder of drug shows vessels with starch grain. Ash value, extractive value, foreign matter, moisture content and TLC were determined for quality standard of drugs. Phytochemical investigation shows the presence of alkaloids, carbohydrate, glycoside, phenolic compounds and tannins. The result of the study could be useful for identification and preparation of monograph of the plant.

Keywords: *Acorus Calamus*, Pharmacognostical evaluation, Phytochemical studies, *Vacha*

INTRODUCTION

Vacha (Acorus calamus Linn.) commonly called as 'Sweet flag' of family Araceae, is a semi-aquatic, perennial, aromatic herb with creeping rhizomes, sword shaped leaves and spadix inflorescence. *Acorus calamus* grows either as wild or cultivated crop throughout India ascending upto 1800 m in the Himalayas.(1)

The rhizomes are considered to possess anti-spasmodic, carminative, anthelmintic, aro-

matic, expectorant, nauseant, nervine, sedative, stimulant properties and also used for the treatment of epilepsy, mental ailments, chronic diarrhea, dysentery, bronchial catarrh, intermittent fevers and glandular and abdominal tumors. In Ayrvedic system of medicine the powder of this drug is being used to produce therapeutic emesis i.e. *Vamana*, one of the Panchakarma specialized therapeutic procedures of Ayurveda. The use of paste of the rhizhome in children (chanting) to improve / rectify the speech defect and improving the memory power is in vogue in most of the rural areas of southern India. They are also employed for kidney and liver troubles, rheumatism, sinusitis, and eczema. This medicine is also being used in Unani, Sowa-rigpa and Siddha systems of medicine in various disease conditions. Some of the Ayurvedic formulations with this drug are *Vachadi taila, Vachalasunadi taila, Sarasvata churna, Sarsvatarishta, Chandraprabha vati, Khadiradi vati, Hinguvachadi churna* etc.[(2)]

Rhizome is the main useful part in *Vacha*. The main chemical constituent are Asarone, β-asarone, calamenol, calamine, calamenone, eugenol, methyl eugenol, α-pinene and camphene, various fatty acids, calamol, calamine acoradin, azulene, two selinane type sesquiterpenes- acolamone and isoacolamone, sugars, glucosides-acorin, calameon, calamusenone, a flavone-luteolin-6, 8-C-diglucosides, new natural products acoramone. *Alpinia officinarum* Hance *and Alpinia galanga* Willd., are adulterants of *Vacha* and being sold in the name of *Bach* and *Ghorbach* in the local market apart from the genuine Calamus rhizomes.[(3)]

Therefore, the present paper attempts to evaluate the pharmacognostical, physicochemical parameters, preliminary phytochemical screening and heavy metal analysis of the rhizome for identification of the drug in dry form and control the adulterants.

AIM AND OBJECTIVES

Aim:

Evaluate the pharmacognostical and phytochemical studies of *Vacha* rhizome

Objectives:

1. To study of *Panchendrya pareeksha*(Organoleptic study), Macroscopic and Microscopic evaluation in *Vacha*,
2. To study of Phytochemical and Physicochemical studies in *Vacha*.

MATERIALS AND METHODS

Plant Material:

Rhizomes of *Vacha* which was cultivated under test conditions is collected from Bhavamishra vatika, (Herbal garden), Mahatma Gandhi Ayurved College, Hospital and Research Centre, Salod (H), Wardha. *Vacha* plant was authenticated by Botanical survey of India, Pune. The rhizomes were allowed to shade dry, avoiding damage by insects, flies and other contaminants. Later they were grinded into coarse and fine powder and stored in an air-tight container for further study. Fresh rhizomes were used for macroscopical and microscopical study.

Organoleptic study *(Panchendriya pareeksha)*: Organoleptic study has been done by means of sense organs.

Macroscopical evaluation: The dried rhizome of *Vacha* was subjected to macroscopical studies which comprised of organoleptic characteristics of the drug viz., size, colour, odour, taste, shape.

Microscopical evaluation: Fresh rhizomes of *Vacha* were used for taking sections. Thin sections were taken of different parts of rhizomes by sharp razor blade and put into watch glass containing water. A thin uniform and entire section was selected and transferred on to a clean glass slide with the help of a brush. Section was mounted with 1-2 drops of 50% glycerin and covered with a clean cover glass. Excess glycerin was removed by blotting paper. The sections were treated with reagents like phloroglucinol, concentrated hydrochloric acid were before examining. Slide was observed under microscope.

Powder microscopy: Studies were done on unstained powder to identify different structures present in powder such as trichomes; epidermal cells etc. and powders were stained with phloroglucinol (1% W/V in 90% alcohol) and conc. HCl to identify lignified tissues.

Physicochemical studies:

a. Foreign matter: *Vacha* rhizomes and there powder were spread into thin layer. It was examined for the presence of foreign matter like mud, leaves etc with the help of hand lens.

b. Moisture content: Accurately weighed 10g of the coarsely powder of rhizomes of *Vacha* was taken in a dried, weighed porcelain dish. It was kept in hot air oven at 105^{o}C for five hours after which it was taken out, cooled in desiccators and weighed. Drug was weighed at each one hour interval and the drying was continued till constant weight was obtained.

c. Ash Value

1) Total ash value: 2 gm of accurately weighed sample were taken and transferred to the cleaned, dried and weighed silica crucibles and were subjected to ignition using electric furnace at 450^{0}C for 3 hour. Silica crucibles was taken out from the furnace and allowed to cool in dessicator and weighed. Weighed silica crucibles were again subjected to ignition using electric furnace at 450^{0}C for an hour. They were taken out from the furnace and allowed to cool in dessicator and weighed. Procedure was repeated up to the constant weight was obtained. After cooling the weight of the ash obtained was calculated with reference to the air dried drug.

2) Acid insoluble ash: The ash obtained was digested with 25 ml dil. HCL for 5 min, then filtered through whatsman's paper and was washed with warm water. The residue and the filter paper was taken in a crucible and heated gently until vapors cease & then more strongly until all carbon has been removed, then it was cooled, the residue was weighed and the percentage of acid insoluble ash was calculated with reference to air dried drug.

3) Determination of water soluble ash: The ash was boiled for 5 minutes with 25ml of water, the insoluble matter was collected in an ash less filter paper, then it was washed with hot water and ignited for 15 minutes at a temperature not exceeding 450^{0}C. The weight of the insoluble matter was subtracted from the weight of the ash, the difference in the weight was represents the water soluble ash. The percentage was calculated of water soluble ash with reference to the air dried drug.

d. Extractive Value

1) Water soluble extractive: 5 gm Powder of *Vacha* rhizomes was taken in 100ml of Water. It was kept in a closed flask for 24 hours and then it was shaken for the first 6 hours with rotary shaker and then allowed to settle for 18 hours. Then mixture was filtered through Whatman's filter paper, taking precaution against loss of water. Then filtrate was evapo-

rated to 75% and the remaining 25% filtrate was poured in weighed petri dish and dried at 105°C in oven. It was later cooled in a dessicator and weighed.

2) Alcohol Soluble Extractive: 5 gm Powder of *Vacha* rhizomes was taken in 100ml of eathanol. It was kept in a closed flask for 24 hour and then the solution was shaked in rotary shaker for shaking frequently during the first 6 hours and then allowed to stand for 18 hours. Then mixture was filtered through Whatman's filter paper, taking precaution against loss of water. Blank petri dish was weighed. Then filtrate was evaporated to 75% and the remaining 25% filtrate was poured in petri dish and dried at 50°C in oven. It was later cooled in a dessicator and weighed. This procedure was done for methanol extractive value also.

e. Determination of P^H value: The different buffer solution (4, 7, and 9 (buffer solutions) were taken in the beaker. Then p^H meter was switched on. It was left for some time unless or on the board requirement of different p^H solution appears. The electrode was dipped in it. The same procedure was carried out for another buffer solution also, after washing the electrode thoroughly with distilled water. Then 1gm of the *Vacha* rhizomes powder was taken in 100ml distilled water and 10 gm of powder in 100 ml (for solution preparations 1% and 10% aqueous solution) distilled water. It was shaken for two hours and stabilized for 24 hours, the electrode was dipped in it and p^H value was noted.

Phytochemical evaluation:

Method of extraction: 5g of the powder of *Vacha rhizomes* was weighed and transferred to a 100ml methanol, 100ml distilled water,100ml chloroform and 100ml ethyl acetate graduated different conical flasks and the flask was corked and set aside for 24hrs with frequent shaking (Maceration). After 24 hrs it was filtered using a filter paper, 25ml of the total filtrate collected was kept aside for the determination of extractive values.

a. Procedure for organic compounds[4]

1) Test for carbohydrates:

Molisch's test (general test): 2 ml. aqueous extract taken in test tube and few drops of alpha-napthol solution (in alcohol) was added, it was shaken and Conc. H_2So_4 was added from the sides of the test tube.

Test for reducing sugars:

a) Fehling's test: 1ml. Fehling's A and Fehling's B solutions was mixed and then boiled for one minute. Equal volume of test solution was added in it. It was heated in boiling water bath for 5-10 min.

b) Benedict's test: Equal volume of Benedict's reagent and test solution was mixed in test tube. It was heated in boiling water bath for 5 min.

2) Test for Proteins:- Biuret test (general test): To 3ml test solution was taken in test tube. 4% NaOH and a few drops of 1% $CuSO_4$ solution was added in it.

3) Test for steroids:- Salkowski reaction: 2ml of extract was taken, 2ml chloroform and 2 ml conc. H_2SO_4 was added in it and shaken well.

4) Test for Flavanoids:

a) Shinoda test: 5ml extract was taken in test tube, 95% ethanol was added and then few drops conc. HCl and 0.5gm magnesium turnings were added in it.

b) Alkaline reagent test: In extract, few drops of sodium hydroxide solution were added.

5) Test for alkaloids:

a) Dragendroff's reagent: 2ml filtrate was mixed with few drops Dragendroff's reagent.
b) Mayer's test: 2ml filtrate was mixed with few drops Mayer's reagent.
c) Hager's test: 2ml filtrate was mixed with Hager's reagent.
d) Wagner's test: 2ml filtrate was mixed with few drops Wagner's reagent.

6) Test for Tannins and phenolic compounds; In 2ml extract, few drops of following reagents were added.

a) 5% $Fecl_{3:}$ deep blue black colour.
b) Lead acetate solution: white ppt.
c) Dilute iodine solution: transient red colour.
d) Dilute $HNO_{3:}$ reddish to yellow colour

7) Test for Glycosides:
I) Killar-Killiani test (K.T):- (Cardiac glycosides): 5ml extract of each sample was mixed with 0.4ml glacial acetic acid containing a trace of $FeCl_{3.}$ Conc.H_2SO_4 was added from sides of test tube.
II) Guignard test (G.T):- (Cyanogenetic glycosides): Extract was added with 1% of picric acid and 10% Na_2CO_3.
8) Test for Saponins:- Foam test: A drop of sodium bicarbonate was added in 5ml of extract of rhizomes. Then little water was added in the mixture, was vigorously shaken and left for about 3 min.
9) Test for Terpenoids:- Salkowski test: 3ml of conc.H_2SO_4 was added from sides of the tube to 5ml extract and 2 ml of chloroform.
b. Procedure for inorganic compounds:[5]

The ash of drug material was prepared. 50% HCl v/v or 50% HNO_3 v/v was added to ash. It was kept for 1 hr. Then it was filtered and with filtrate the following tests were performed.

1. Tests for calcium: To 10ml filtrate, 1 drop NH_4OH & saturated ammonium oxalate was added.
2. Tests for Magnesium: The calcium ppt obtained in test for calcium was filtered and separated. The filtrate was heated and cooled; in this solution of sodium phosphate in dilute ammonia was added.
3. Test for Sodium: To 2 ml test solution, little uranyl magnesium acetate reagent was added, it was shaken well and kept for few minutes.
4. Test for Potassium: To 3 ml of Test solution, few drops of sodium cobalt nitrate solution were added.
5. Test for Iron: In 5 ml test solution, few drops of 2% Ferrocyanide were added.
6. Test for Sulphate: In 5ml filtrate, few drops of 5% $Bacl_2$ solution were added.
7. Test for Phosphate: In 5ml test solution was prepared in HNO_3; to which few drops of 10% $AgNO_3$ solution was added.

Thin Layer Chromatographic (TLC) study: [6,7]
Chromatography is the separation of a mixture into individual components using a stationary phase and mobile phase. Thin Layer Chromatography is a method based on adsorption chromatography. The adsorbent such as silica gel-G is coated to a thickness at 0.3mm or clean TLC plates using commercial spreader, the plates are activated at 105°C for 30 minutes and used; the selection of mobile phase depends upon the type of constituents to be analyzed. After the development of chromatogram by ascending technique, the resolved spots are revealed by spraying with suitable detecting agents.

Sample : Methanolic extracts of *Vacha Churna*
Adsorbent layer: Silica gel G.
Solvent system: Toluene: Ethyl acetate: 9.3:0.7
Spray Reagent : Vanillin: Sulphuric acid.
Detection : 1) UV long wave (366 nm). 2) In day light after Spraying with Vanillin: Sulphuric Acid

OBSERVATION AND RESULTS:

MACROSCOPICAL STUDIES:

The *Vacha* rhizome are brownish in colour with 10-14cm in length and 1- 2cm thick, cylindrical and branched shape, aromatic odour, pungent/bitter in taste. (Figure.1).

The organoleptic evaluation of *Vacha* rhizome were showed in (Table1).

Figure 1: Dried rhizomes of *Vacha* (*Acorus calamus*)

Organoleptic study:

Table 1: Results of Organoleptic study of *Vacha* rhizome

Sr.No.	Test	Rhizome of *Vacha (Acorus calamus)*
1	*Shabda* pariksha (Fracture)	*Abhangur(Short)*
2	*Sparsha pariksha (Texture)*	*Khar(Rough)*
3	*Rupa pariksha (Colour)*	Externally-Light brown,Internally-Buff
4	*Rasa pariksha (Taste)*	*Tikta, Katu,(Pungent,Bitter)*
5	*Gandha pariksha(Smell/Odour)*	Sweet Aromatic

Microscopical Study of *Vacha* rhizome and rhizome powder:

Table 2: Results of microscopic study of *Vacha* rhizome and rhizome powder

Sr.no.	Study	Structures
1	T.S. of Rhizomes	Epidermis
		Endodermis
		Cortical region
		Stelar region
		Vascular bundles
		Vessels
		Parenchyma cells
		Ground tissue
		Cortex
		Fibres
		Cell content:-Oil, Oleoresin, Starch grains, Crystal
2	Powder microscopy(Buff coloured)	Fibres
		Vessels
		Oil globules

Figure 2: T.S. of rhizome of *Vacha*

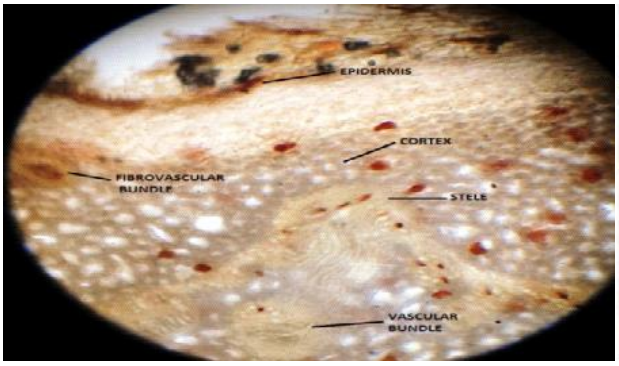
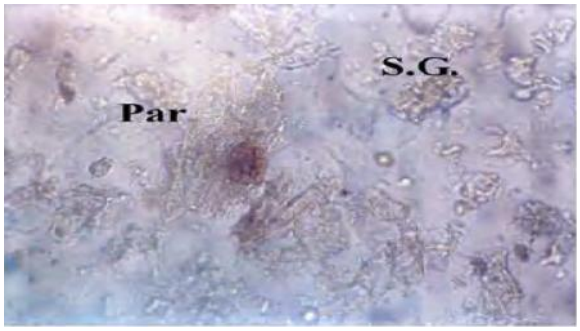


Figure 3: Powder microscopy of *Vacha* rhizome: Vessel

Figure 4: Powder microscopy of *Vacha* rhizome: Starch grain, parenchymatous cells



PHYSICOCHEMICAL STUDIES:

Table 3: Results of physicochemical studies of *Vacha* rhizome

Parameters	Observed value	Standard value(API)
Total % of foreign matter	Nil	Not more than 1%
Moisture content at 105° C	7.75%	
Total ash value	6.3%	Not more than 7%
Acid insoluble ash	0.85%	Not more than 1%
Water soluble ash	0.09%	
Water soluble extractive	28.18%	Not less than 16%
Alcohol soluble extractive	13.96%	Not less than 9%
Methanol soluble extractive	42.4%	
p^H	6.05%	

PHYTOCHEMICAL STUDIES:

Table 4: Results of chemical analysis Organic compounds in *Vacha* rhizomes

Sr.No.	Organic compound	Test	Water extract	Methyl Alcohol extract	Ethyl acetate extract	Chloroform extract
1	Carbohydrate	Molisch's	+ve	+ve	+ve	-ve
		Fehling's	+ve	-ve	-ve	+ve
		Benedict's	-ve	+ve	+ve	+ve
2	Proteins	Biuret test	+ve	+ve	+ve	+ve
3	Steroids	Salkowski	-ve	+ve	+ve	+ve

		reaction				
4	Flavanoids	Shinoda test	+ve	+ve	+ve	+ve
		Alkaline re-agent test	+ve	+ve	+ve	+ve
5	Glycosides	Keller killiani test	+ve	+ve	+ve	+ve
6	Saponins	Foam test	+ve	+ve	+ve	+ve
7	Alkaloids	Mayer's	+ve	+ve	+ve	+ve
		Hager's	+ve	+ve	+ve	+ve
		Wagner's	+ve	+ve	+ve	+ve
		Dragendroff's	+ve	+ve	+ve	+ve
8	Tannins and Phenolics	5% $FeCl_3$	+ve	+ve	+ve	+ve
		Lead acetate	+ve	-ve	+ve	-ve
		Dilute iodine	+ve	+ve	+ve	-ve
		Dilute HNO_3	-ve	+ve	-ve	-ve

Table 5: Results of Chemical analysis Inorganic compounds in *Vacha Rhizome*.

Sr.no.	Inorganic constituents
1	Calcium
2	Magnesium
3	Sodium
4	Potassium
5	Iron
6	Sulphates
7	Phosphates

Thin Layer Chromatography (TLC) of methanolic extract:

Table 6: R_f values for *Vacha Churna* (*Acorus calamus* Linn.)

Visualization	*Vacha churna (Acorus calamus* Linn.*)*	
	No. of spots	R_f values
In long UV 366 nm	6	0.051
		0.152
		0.220
		0.373
		0.441
		0.559
After spraying with Vanillin Sulphuric Acid	3	0.15
		0.22
		0.49

In (Table-6) T.L.C. analysis (on UV 366nm) six separated components resembles in *Vacha Churna*. But after spraying (Vanillin: Sulphuric acid) in day light three separated components resembles in *Vacha Churna.*

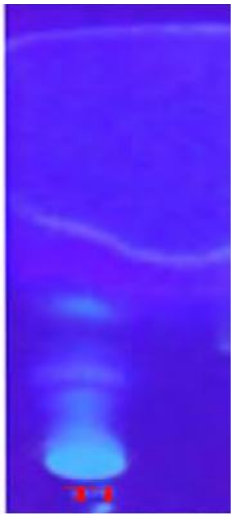


Figure 5: TLC observed under UV 366nm

Figure 6: TLC observed under day light(after spraying)

DISCUSSION

Rhizome of *Vacha (Acorus calamus* Linn.*)* was collected and analysed as per various standardisation parameters. Macroscopy studies of *Vacha* rhizome are brownish in colour with 10-14cm in length and 1- 2cm thick, cylindrical and branched shape, aromatic odour, pungent/bitter in taste. Microscopical analysis of the rhizome presence of epidermis, stellar region, vascular bundles, annuler vessels and fibre. The physical constant evaluation of a rhizome is an important parameter in detecting adulteration. Preliminary physicochemical parameters showed that water soluble extractive value is more than alcohol soluble extractive value, which indicates the presence of more water soluble contents in the rhizome. The test drug has pH 6.05 indicating its weak acidic nature. Chemical analysis of methanolic extract showed the presence of carbohydrate, proteins, steroids, flavanoids, glycoside, saponins, alkaloids, tannins and phenolics.

CONCLUSION

A systematic approach is necessary in pharmacognostical and phytochemical study which helps in confirmation and determination of identify, purity and quality of crude drug. Hopefully, the parameters which have been evaluated as per the standard norms and presented in this pharmacognostical and phytochemical study of *Vacha* rhizomes (*Acorus calamus* Linn.) will provide valuable information for future research work.

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