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Research Article

PHARMACEUTICAL EVALUATION OF VACHA CHURNA (ACORUS *CALAMUS* LINN) AND MUSTA CHURNA (CYPERUS *ROTUNDUS* LINN)

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ABSTRACT

Aim of study to analyze the samples by using different Physico chemical parameters, Samples by using qualitative method & to develop the TLC & HPTLC profile. Materials and methods content the rhizome powder of Vacha (*Acorus calmus* Linn.) and Musta (*Cyperus rotundus* Linn.) .Rhizome powder were evaluated for loss on drying, ash value, Acid insoluble ash, water soluble extractive, Methanol soluble extractive and pH. Qualitative test for various functional groups along with HPTLC study were also studied with methanol extract and densitometric study. Loss on drying, ash value, water soluble extract, acid insoluble ash, methanol soluble extract, pH and Chromatographic Parameter were vary while Qualitative Test were similar in Vacha churna and Musta churna.

Keywords: Pharmaceutical Evaluation, Vacha Churna *(Acorus calamus* Linn.) & Musta Churna (Cyperus *rotundus* Linn.)

INTRODUCTION

The Pharmaceutical studies of these drugs done by making use of various parameters help in standardizing the drug and authenticate it. It is essential to gratify the international standards and quality control of the drug used by convincing the drug regulatory authorities. The present study was carried out to evaluate the phyto-chemical parameters of test drug- *Vacha Churna (Acorus calamus* Linn*.)* and Control drug- *Musta Churna (Cyperus rotundus* Linn*.)* were analysed according to the available facilities. Phytochemical study deals with materials and methods content the rhizome powder of Vacha (*Acorus calmus* Linn.) and Musta (*Cyperus rotundus* Linn.) .Rhizome powder were evaluated for loss on drying, ash value, Acid insoluble ash, water soluble extractive, Methanol soluble extractive and pH. Qualitative test for various functional groups along with HPTLC study were also studied with methanol extract and densitometric study.

AIMS AND OBJECTIVES:-

The analysis of the *Vacha Churna* (*Acorus calamus* Linn.) and *Musta Churna* (*Cyperus rotundus* Linn.) samples were undertaken with the following aims and objectives.

- To analyze the samples by using different Physico chemical parameters.
- To analyze the Samples by using qualitative method.
- To develop the TLC & HPTLC profile.

MATERIALS AND METHODS

As described earlier the samples i.e. *Vacha Churna* (*Acorus calamus* Linn.) and *Musta Churna* (*Cyperus rotandus* Linn.) are procured and authenticated pharmacognostically. The dried samples were powdered (fine powder) and were used for the present study. The analyses of the samples were carried out by using different organoleptic, physicochemical and chromatographic methods. Following parameters were employed[1-3].

1. Organoleptic Parameter
2. Physico-Chemical Parameter
   - Loss on drying at 110° C
   - Total Ash
   - Acid insoluble ash
   - Water soluble extractive
   - Methanol soluble extractive.
   - pH value**.**
3. Qualitative Test for various Functional Groups.
4. Chromatographic Parameter -TLC & HPTLC profiles.

ORGANOLEPTIC PARAMETER

The organoleptic characters of Ayurvedic drugs are very important and give the general idea regarding the genuinity of the sample. It is done with the help of Panchaganendriya Pariksha. The characters like colour, odour and taste.

PHYSICO-CHEMICAL PARAMETER

1) **Loss on drying (LOD)**
The loss on drying of the samples were determined by taking 1 gm, accurately weighed sample and drying in an oven at 110 °C till constant weight. The weight after drying was noted and from the weight loss on drying was calculated and expressed as % w/w.

2) **Ash Value**
This test was conducted to evaluate the percentage of inorganic salts, naturally occurring in the drug or adhering to it or deliberately added as a form of adulteration. 1 gram accurately weighed sample was taken in a pre weighed dried crucible. It was incinerated in a muffle furnace up to 450°C. The crucible was taken out, self cooled and weighed immediately. From the weight of the ash, the ash value was derived with reference to the air dried drug. It was calculated and expressed as % w/w.

3) **Acid insoluble ash**
The ash obtained was further analysed for acid insoluble particles in ash. The ash of the drugs were boiled with 25 ml of 6N HCl for 5 min. and then solution was filtered through Whattman filter paper no. 41. It was washed with hot water, dried in oven, ignited and weighed. The percentages of acid insoluble ash were calculated with reference to the sample.

4) **Water soluble extractive**
About 5 gm, accurately weighed, powder sample was taken in a conical flask, 100 ml of distilled water was added to it, shaken and was kept overnight. Next day it was filtered. 20 ml of filtrate was taken in a previously dried and weighed, porcelain evaporating dish and evaporated on a hot water bath. They were dried to constant weight in an oven and weighed. From the weight of the residue the water soluble extractive percentage were calculated on the basis of air dried sample.

5) **Alcohol soluble extractive**
Macerate 5 g of the air dried drug, coarsely powdered, with 100 ml of Alcohol of the specified strength in a closed flask for twenty-four hours, shaking frequently during six hours and allowing to stands for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tired flat bottomed shallow dish, and dry at 105º, to constant weight and weigh. Calculate the percentage of alcohol-soluble extractive with reference to the air-dried drug.

6) **pH Value :**
This test is carried out to determine the pH of the sample drug with the help of pH meter. 5gm of test drug sample was weighted and taken in a conical flask. Then add 50 ml accurately measured water and stirred well for few minutes; kept this solution for some time and then filtered it through filter paper. Take the filtered solution in a beaker. Standardize the pH meter and electrodes with buffer solution of known pH i.e.7pH. Rinse the electrodes with distilled water and introduce into the test solution contained in a small beaker. Read the pH value of solution**.**

QUALITATIVE TEST FOR VARIOUS FUNCTIONAL GROUPS

1) Alkaloids:
(a) With Mayer's reagent-
The methanol extract of the sample was taken in a watch glass, solvent was evaporated. It was added with 2N HCl and few drops of Mayer's reagent when alkaloids gave a white precipitate.
(b) With Dragendroff's reagent
The methanol extract of the sample was evaporated. On addition of a few drops of dilute 2N HCl and Dragendroff's reagent alkaloids gave orange precipitate.

2) Saponin:
Water was added to the sample and was shaken vigorously. A stable froth with honeycomb structure was observed which indicate presence of Saponin.

3) Tannin :
(a) With 5% lead acetate solution tannins gave precipitate which turns red on addition of KOH solution on excess addition precipitates were dissolved.
(b) To aqueous extract of sample, very dilute solution of ferric chloride was added, blue colour was obtained, which changes to olive green by the addition of more amount of ferric chloride.
(c) With iodine solution sample gave red coloration due to the presence of tannins.
(d) 2ml extract was taken in test tubes. To it add equal volume of lead acetate. Presence of tannin gave white precipitate.

4) Steroids:
(a) Salkowski reaction:
2 ml extract was taken in a test tube and add 2 ml chloroform and 2 ml conc. H_2So_4. Presence of steroid gave red appearance to Chloroform layer and red appearance to the acid layer.

5) Tests for Volatile oil:
(a) Volatile oils have characteristic odour.
(b) Filter paper is not permanently stained with volatile oil.

THIN LAYER CHROMATOGRAPHIC STUDY

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.

Thin layer chromatographic study of the samples were carried out by using different conditions to develop suitable chromatographic patterns so that the TLC profiles will be useful for evaluating the quality of the drugs. The details will be mentioned separately.

Sample: Methanolic extracts of *Vacha Churna & Musta 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

HPTLC

High performance thin layer chromatography is a sophisticated and automated form of TLC. It is a valuable tool for quality assessment and evaluation of plant based drugs. The separations obtained through HPTLC have high resolutions. Compact starting spots allow a number of samples which may be applied to the HPTLC plate. It allows for the analysis of broad number of compounds both efficiently and cost effectively. More samples can be run in a single analysis thus reducing the analytical time. The same analysis can be viewed in different wavelengths of light there by providing a complete profile of the plant.

Steps involved in HPTLC:

- Selection of chromatographic layer.
- Sample and standard preparation.
- Layer pre-washing, Layer pre-conditioning.
- Application of sample and standard.
- Chromatographic development.
- Detection of spots.
- Scanning.
- Documentation of chromatic plate.

HPTLC of sample were carried out by employing different conditions as mentioned.

Chromatographic Conditions:
Track-1 : Methanolic extract of *Musta Churna*;
Track-2: Methanolic extract of *Vacha Churna* .
Stationary phase: Precoated Silica gel GF $_{254}$ Plates(E-merck)
Application mode: CAMAG Linomat V Hamilton Syringe
Mobile phase: Toluene: Ethyl acetate: : 9.3 :0.7
Development chamber: CAMAG Twin trough chamber (20 x 10 cm^{-})
Chamber saturation: 30 min
Scanner: CAMAG Scanner III
Scanning mode: Linear at 254 nm and 366 nm
Spray Reagent: Vanillin: Sulphuric acid.
Detection: 1) Deutarium lamp, Mercury lamp
2) In day light after Spraying with
Vanillin: Sulphuric Acid
Data system: WINCATS software (Ver. 3.17)
Drying device: Oven
U.V. Spectrum: 200 nm to 700 nm

DENSITOMETRIC EVALUATION OF HPTLC PLATE

Densitometric scanning was performed with a Camag T.L.C. scanner III in reflectance absorbance mode at 254nm and 366nm under control of CATS software (V 3.15 Camag). The slit dimension was 6 mm x 0.45 mm and the scanning speed was 10 mm^{-1}.

ORGANOLEPTIC CHARACTERS

Vacha *Churna* (*Acorus calamus* Linn.) and *Musta Churna* (*Cyperus rotundus* Linn.) are having following organoleptic characters and the presented below.

Table 1: Organoleptic character of *Vacha Churna* (*Acorus calamus* Linn.) & *Musta Churna* (*Cyperus rotundus* Linn.)

Organoleptic character	*Vacha Churna* (*Acorus calamus* Linn.)	*Musta Churna* (*Cyperus rotundus* Linn.)
Colour	Buff	Creamish -Brown
Odour	Aromatic	Pleasant
Taste	Pungent, Bitter	Pungent, Bitter

PHYSICO-CHEMICAL PARAMETERS

The Ayurvedic pharmacopoeia of India has mentioned different physico-chemical parameters in the various monographs. Hence, both the samples were analysed for those commonly prescribed parameters and the data has been presented in (Table-2)

Table 2: Analytical data of *Vacha Churna* (*Acorus calamus* Linn.) and *Musta Churna* (*Cyperus rotundus* Linn.)

Sr. No.	Parameters	Values	
		Vacha Churna (*Acorus calamus*L)	*Musta Churna* (*Cyperus rotandus*.L)
1	Loss on drying at 110° C	10.26% w/w	1.4% w/w
2	Total Ash value	6.3% w/w	0.55% w/w
3	Acid insoluble ash	0.88% w/w	0.33% w/w
4	Water soluble extractive	28.15% w/w	10.00% w/w
5	Methanol soluble extractive	42.02% w/w	40% w/w
6	pH value	4.79	5.4

The Table **2** the loss on drying value of the *Vacha Churna* (*Acorus calamus* Linn.) Churna and *Musta Churna* (*Cyperus rotundus* Linn.) was 10.26% W/W and 1.4% W/W. Ash value indicates the presence of inorganic constituents in the sample and it is found that there are more inorganic constituents in *Vacha Churna* (6.3 % W/W) than *Musta Churna* (0.55 % W/W). Acid insoluble ash value of *Vacha Churna* (0.88 % W/W) greater than *Musta Churna* (0.33 % W/W). Water soluble extractive value of *Vacha Churna* (28.15 % W/W) greater than *Musta Churna* (10.00 % W/W). Methanol soluble extractive value of *Vacha Churna* (42.02 % W/W) greater than *Musta Churna* (40 % W/W).

QUALITATIVE TEST:

Both *Vacha Churna* (*Acorus calamus* Linn.) and *Musta Churna* (*Cyperus rotundus* Linn.) are containing various functional groups in Table **-3.**

Table 3: Qualitative test for various functional groups

Sr. No.	Functional groups	*Vacha Churna* (*Acorus calamus* Linn.)	*Musta Churna* (*Cyperus rotundus* Linn.)
1.	Alkaloids	+	+
2.	Saponin	+	+
3.	Tannin	+	+
4.	Steroids	+	+
5.	Volatile oil	+	+

+ = Present, - = Absent

Various functional groups like Alkaloids, Saponin, Tannin, Steroids and Volatile oil present in both the *Vacha Churna* and the *Musta Churna*..

Thin Layer Chromatography :(Plate-4.1a&b)
Track-1:*Vacha Churna*
Track-2:Musta Churna

Table 4: R_f values for *Vacha Churna* (*Acorus calamus* Linn.) and *Musta Churna* (*Cyperus rotundus* Linn.)

Visualization	*Vacha Churna* (*Acorus calamus* Linn.)		*Musta Churna* (*Cyperus rotundus* Linn.)	
	No of spots	R_f values	No of spots	R_f values
In long UV 366nm	6	0.051	5	0.034
		0.152		0.153
		0.220		0.356
		0.373		0.390
		0.441		0.542
		0.559		
After Spraying with Vanillin Suphuric Acid	3	0.15	3	0.15
		0.22		0.45
		0.49		0.52

In (Table-**4**) T.L.C. analysis(on UV 366nm) six separated components resembles in *Vacha Churna* while five separated components resembles in *Musta Churna*. But after spraying (Vanillin : Sulphuric acid) in day light three separated components resembles in both *Vacha Churna* and *Musta Churna*.

HPTLC STUDY & DENSITOMETRIC STUDY
(Plate-4.1c,d,e& Plate-4.2)
Results of HPTLC study of Methalolic extracts *Musta Churna* & *Vacha Churna*:
Track 1: - *Musta Churna*
Track 2: - *Vacha Churna*
Both tracks were scanned under 254 nm & 366 nm, maximum R_f values are mentioned in table.

Table 5: Maximum R_f values of *Musta Churna* & *Vacha Churna*

Extract	Solvent system	Visualization	*Vacha Churna*		*Musta Churna*	
			No. of Spots	R_f	No. of Spots	R_f
Methanol Extract	Toluene : Ethyl acetate : : 9.3 :0.7	UV 254 nm	2	0.04, 0.60	2	0.03, 0.10
		UV 366 nm	14	0.06, 0.15, 0.18, 0.25, 0.32, 0.38, 0.46, 0.50, 0.53, 0.56, 0.62, 0.64, 0.69, 0.74	25	0.03, 0.10, 0.16, 0.18, 0.20, 0.21, 0.23, 0.24, 0.28, 0.30, 0.32, 0.35, 0.36, 0.41, 0.43, 0.45, 0.48, 0.50, 0.53, 0.54, 0.56, 0.57, 0.60, 0.64, 0.67,
		After Spray with Vanillin sulphuric acid	4	0.06, 0.20, 0.53, 0.86	-	-

In chromatographic finger printing and densitometric analysis, two separated components resembles to each other on UV 254 nm ,while in UV 366 nm 14 separated components resembles in *Vacha Churna* and 25 separated components resembles in *Musta Churna*. After spraying (Vanillin : Sulphuric acid) four separated components resembles in *Vacha Churna* while no any separated components resembles in *Musta Churna*.

Plate 4.1

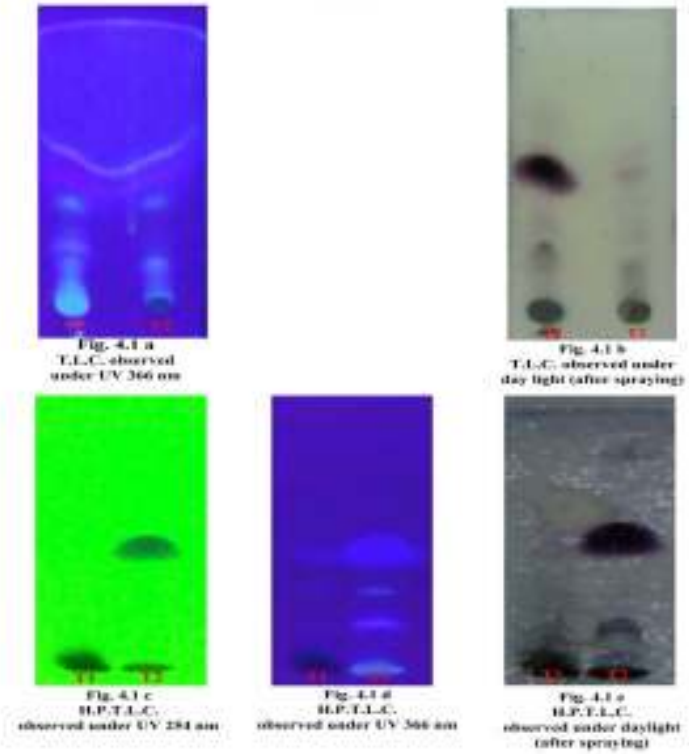

Fig. 4.1 a
T.L.C. observed under UV 366 nm

Fig. 4.1 b
T.L.C. observed under day light (after spraying)

Fig. 4.1 c
H.P.T.L.C. observed under UV 254 nm

Fig. 4.1 d
H.P.T.L.C. observed under UV 366 nm

Fig. 4.1 e
H.P.T.L.C. observed under daylight (after spraying)

Plat No. 4.2
Photomicrograph of Densitometric Scan

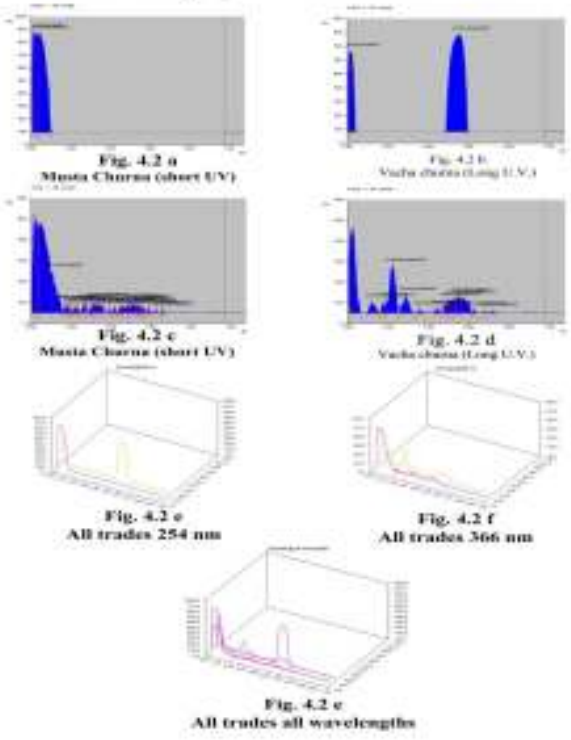

Fig. 4.2 a
Musta Churna (short UV)

Fig. 4.2 b
Vacha churna (Long U.V.)

Fig. 4.2 c
Musta Churna (short UV)

Fig. 4.2 d
Vacha churna (Long U.V.)

Fig. 4.2 e
All trades 254 nm

Fig. 4.2 f
All trades 366 nm

Fig. 4.2 e
All trades all wavelengths

CONCLUSION

Colour of Organoleptic character of Vacha (*Acorus calmus* Linn.) churna showed buff in colour, aromatic in odour while Musta (*Cyperus rotundus* Linn.) churna showed creamish – brown in colour, pleasant in odour but in taste is same in both powder . Loss on drying value of the Vacha (*Acorus calmus* Linn.) Churna and Musta (*Cyperus rotundus* Linn.) churna were 10.26% W/W and 1.4% W/W. Ash value indicates the presence of inorganic constituents in the sample and it is found that there are more inorganic constituents in Vacha churna (6.3 % W/W) than Musta churna (0.55 % W/W). Acid insoluble ash value of Vacha churna (0.88 % W/W) greater than Musta churna (0.33 % W/W). Water soluble extractive value of Vacha churna (28.15 % W/W) greater than Musta churna (10.00 % W/W). Methanol soluble extractive value of Vacha churna (42.02 % W/W) greater than Musta churna (40 % W/W).Thus result shwoed Vacha churna is more soluble than Musta churna in Acid soluble extractive, Water soluble extractive and Methanol soluble extractive. The pH value indicates the potential hydrogen ions available in particular substance. Both test samples are having slight acidic pH that is in Vacha churna sample- (4.79) and Musta churna sample (5.4). Thus result shwoed Vacha churna is more Acidic than Musta churna. Various functional groups like Alkaloids, Saponin, Tannin, Steroids and Volatile oil present in both the Vacha churna and the Musta churna. In T.L.C. analysis(on UV 366nm) six separated components resembles in Vacha churna while five separated components resembles in Musta churna.But after spraying (Vanillin : Sulphuric acid) in day light three separated components resembles in both Vacha churna and Musta churna.In chromatographic finger printing and densitometric analysis, two separated components resembles to each other on UV 254 nm ,while in UV 366 nm 14 separated components resembles in Vacha churna and 25 separated components resembles in Musta churna.After spraying (Vanillin : Sulphuric acid) four separated components resembles in Vacha churna while no any separated components resembles in Musta churna.

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