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### Original Research article

## Ingredients identification, physico-chemical and hptlc evaluation of *masha taila* – a polyherbal formulation

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#### ABSTRACT:-

**Background:** *Masha Taila* is mentioned in *Ayurvedic* classics as a therapeutic formulation to treat *Avabahuka* (Frozen shoulder). *Masha Taila* contains 24 ingredients and base is *Tila Taila*. All the ingredients have *Vata* pacifying properties. **Materials and Methods:** Powders of all ingredients were evaluated for their pharmacognostical study and finished product which is *Masha Taila* was evaluated for pharmaceutical

analysis. **Results:** Some typical microscopic characters of *Erandamoola*, *Katuki*, *Gokshur*, *Kapikachchoo*, *Karpasasthi* etc were found. Results obtained in pharmaceutical parameters of *Masha Taila* like loss on drying 0.085 w/w %, Acid value 5.38 %, Refractive index 1.485, Iodine value 98, Saponification value 190.16, Specific gravity 0.92 etc., are within limit mentioned by Ayurvedic Pharmacopoeia of India. High performance thin layer chromatography profile of *Masha Taila* showed similarities in number of spots. **Conclusion:** From this study, developed data can be espoused for laying down the standards for *Masha Taila*.

**KEYWORDS:** HPTLC, *Masha Taila*, Pharmacognosy, Pharmaceutics.

## INTRODUCTION:

In Ayurveda, *Avabahuka* is considered as *Vata Vyadhi*<sup>(i)</sup>. In *Avabahuka*, there is restricted movement of shoulder joint, stiffness and muscle wasting. These symptoms are shown in frozen shoulder. Thus, we can correlate this disease with Frozen shoulder in modern science.

When *Vata* localised in the region of the shoulder causes wasting of local musculature, ligaments, constricts the *Siras*(veins) present there and produces *Avabahuka*<sup>(ii)</sup>. Vagbhatta has stated similar symptoms along with loss of sensation in arms.<sup>(iii)</sup> Worldwide prevalence of *Avabahuka* is 2 to 5% and it affects at peak age 50s.<sup>(iv)</sup> *Nasya* with *Masha Taila* is indicated in Bhavaprakash Samhita<sup>(v)</sup> Acharya Vagbhatta mentioned *Brimhan Nasya* for *Avabahuka*<sup>(vi)</sup>.

*Masha Taila* contains *Masha*, *Atasi*, *Yava*, *Kurantaka*, *Erandabija* etc. Most of the drugs are having *Brimhan* property. Pharmacognostical work has not been done till the date. Thus, to maintain the therapeutic activity of the drug standardization is very much necessary for clinical trial. During the last decades, herbal medicines pointed out in *Ayurveda* are getting gratitude globally. In view of severe undesirable side effects of drug, there is growing focus to follow systematic research methodology and to provide scientific basis for the traditional herbal medicines. With the help of identity, purity, content, and other chemical, physical, or biological properties, or by the manufacturing processes quality can be defined as the status of a drug.

Different chromatographic analysis is routinely used and plays an important role in the quality control of complex herbal medicines. High performance thin layer chromatography (HPTLC) can provide an electronic image of the chromatographic fingerprint and a densitogram to detect the presence of marker compounds in a plant sample. The advantage of HPTLC in the analytical testing of herbal products is that it provides positive identification as well as visualization of the separated fractions of the sample component and helps in quantitative, qualitative analysis with the same system. With this background the present study was done to establish the authenticity of all the ingredients of *Masha Taila*. Till today no any standard quality parameters had been tested. In this study, identification of ingredients of dry samples macroscopically and microscopically, preliminary analysis of physic-chemical parameters including developing the HPTLC(High Performance Thin Layer Chromatography) profile of *Masha Taila* was done.

**MATERIALS & METHOD****Collection of Raw Drug**

All the raw drugs of *Masha Taila* were collected from Pharmacy, Gujarat Ayurveda University (GAU), Jamnagar, India and all these drugs were identified and authenticated in Pharmacognosy Laboratory, Institute for Postgraduate Teaching and Research in Ayurveda (IPGT & RA), GAU, Jamnagar, India. [Table No.1]

**Table No. 1: Ingredients of *Masha Taila*** <sup>(vii)</sup>

Drug name	Botanical Name	Part used	Quantity	
<i>Masha</i>	<i>Phaseolus mungo</i> Linn.	Seeds	1 part	} <i>Kwatha</i>
<i>Atasi</i>	<i>Linum usitatissium</i> Linn.	Seeds	1 part	
<i>Yava</i>	<i>Hordeum vulgare</i> Linn.	Seeds	1 part	
<i>Kurantak</i>	<i>Barleria prionitis</i> Linn.	Whole plant	1 part	
<i>Kantakari</i>	<i>Solanum surattense</i> Burm.	Whole plant	1 part	
<i>Gokshur</i>	<i>Tribulus terrestris</i> Linn.	Fruit	1 part	
<i>Tuntuka</i>	<i>Oroxylum indicum</i> Vent.	Stem bark	1 part	
<i>Kapikachchhu</i>	<i>Mucuna prurita</i> Hook.	Seeds	1 part	
<i>Karpasaasthi</i>	<i>Gossypium herbaceum</i> Linn.	Seeds	1 part	
<i>Shanabija</i>	<i>Crotalaria verrucosa</i> Linn.	Seeds	1 part	
<i>Kulattha</i>	<i>Dolichous biflorus</i> Linn.	Seeds	1 part	} <i>Kalka</i>
<i>Kola</i>	<i>Zizyphus sativa</i> Geartn	Fruits	1 part	
<i>Sunthi</i>	<i>Zingiber officinale</i> Roscoe.	Rhizomes	1 part	
<i>Pippali</i>	<i>Piper longum</i> Linn.	Fruits	1 part	
<i>Shatapushpa</i>	<i>Anathum sova</i> Kurz.	Fruits	1 part	
<i>Erandamoola</i>	<i>Ricinus communis</i> Linn.	Root	1 part	
<i>Punarnava</i>	<i>Boerhaavia diffusa</i> Linn.	Whole plant	1 part	
<i>Trivritta</i>	<i>Ipomea turpethum</i> R.Br.	Root	1 part	
<i>Rasna</i>	<i>Pluchea lanceolata</i> Oliver & Hiern.	Root	1 part	
<i>Bala</i>	<i>Sida cordifolia</i> Linn.	Root	1 part	
<i>Amrita</i>	<i>Tinospora cordifolia</i> Willd.	Stem	1 part	} <i>Kalka</i>
<i>Katuki</i>	<i>Picrorhiza kurrow</i> Royle ex.	Root	1 part	
<i>Tila taila</i>	<i>Sesamum indicum</i> Linn.	Seeds	8 times of <i>kalka</i>	
<i>Bast</i>	<i>Capra aegagrus hircus</i>	<i>Mansarasa</i>	30 liters	
<i>Mansarasa</i>				

### **Microscopical evaluation of powdered raw drugs of *Masha Taila*:**

It is possible to analyze the finished products for the pharmacognosy i.e. compound formulations like *Vati* (tablet), *Choorna* (powder), *Kalka* (paste) etc. but it is difficult to analyze the *Taila* to find out the cellular level of raw drugs. In this study as *Masha Taila* was made from *Kwatha* (decoction) & *Kalka*(paste) of above mentioned drugs, thus raw drugs powder was studied separately with and without staining. The micro pictures were taken under Carl zeis microscope attached with camera.<sup>(viii)</sup> [Table No.2]

### **Preparation of *Masha Taila*:**

*Masha Taila* was prepared in RSBK (*Rasashastra* and *Bhaishajya Kalpana*) department, IPGT & RA, GAU, Jamnagar, India. All identified drugs were washed and dried properly. *Kwatha* was prepared by adding 8 times water in equal amount of all drugs and then it was boiled in low flame to decrease it to 1/4th of total water.<sup>(ix)</sup> *Kalka* was prepared by adding adequate amount of water in above mentioned drugs. For preparation of *Masha Taila* 1: 4: 16 of *Kalka*, *Taila* and *Kwatha* respectively were taken as per classical reference.<sup>(x)</sup> After preparation of *Kalka* and *Kwatha*, *Taila* was measured and poured into a vessel with thick base on medium flare. The *Kwatha*, *Kalka* and *Mansa Rasa* were also poured into the vessel, and the mixture was boiled in medium flame with continuous stirring and monitoring of *Paka*. The boiling was stopped and the *Taila* was sieved by using a washed and dried white filter cloth when *Madhyama Paka*<sup>(xi)</sup> was attained.

### **Organoleptic study of prepared drug**

Organoleptic studies of prepared *Masha Taila* are endangered for various sensory characteristics like odour, colour etc. were carefully distinguished down. [Table No. 3]

### **Physico-chemical analysis**

Physico-chemical analysis of *Masha Taila* was done by using various standard physico-chemical parameters such as Acid value<sup>(xii)</sup>, Refractive Index value<sup>(xiii)</sup>, Saponification value<sup>(xiv)</sup>, Iodine value<sup>(xv)</sup>, and Specific gravity<sup>(xvi)</sup> at Pharmaceutical chemistry laboratory, IPGT and RA, Jamnagar, India. Physico-chemical analyses were carried out by following standard procedure mentioned in API (Ayurvedic Pharmacopeia of India). [Table No. 4]

### **HPTLC (High Performance Thin Layer Chromatography) evaluation** <sup>(xvii)</sup>

Sample was prepared by diluting 1 ml *Masha Taila* with 2 ml Hexane and it was used for spotting. Prepared sample of *Masha Taila* was spotted on pre-coated silica gel aluminium plate as 6 mm bands by means of a CAMAG Linomat V sample applicator fitted with a 100 µL Hamilton syringe. Then alcoholic KOH was applied on same spotted area and plate was heated at 110 oc on TLC plate heater for 10 minutes. Hexane: Diethyl Ether (7:3) was used for *Masha Taila* as a mobile phase. The development time was 30 minutes. After development, Densitometry scanning was performed with a CAMAG TLC scanner III in reflectance absorbance mode at 254 nm and 366 nm under control of Win CATS software (V1.3.4 CAMAG). Then the plate was dipped in 10% H<sub>2</sub>SO<sub>4</sub> followed by heating and then visualized in day light. The R<sub>f</sub> values and colour of resolved spots were noted.

## RESULTS

### Microscopic Characters

Powder microscopy characters of individual herbal drugs of *Masha Taila* were observed as below [Table No. 2] and microphotographs are placed at respective plate.

**Table No. 2 Microscopic character of drugs**

Sr.No.	Drug	Identified Microscopic Characters
1	<i>Masha</i>	Epidermal cells
2	<i>Atasi</i>	Lignified epidermal cells
3	<i>Yava</i>	Unicellular trichoms
4	<i>Kurantaka</i>	Border pitted vessels
5	<i>Kantakari</i>	Epicarp cells
6	<i>Gokshur</i>	Stone cells
7	<i>Tuntuka</i>	Lignified parenchyma
8	<i>Kapikachchhu</i>	Simple starch with hilum
9	<i>Karpasaasthi</i>	Scariform vessels
10	<i>Shanbija</i>	Epicarp cells
11	<i>Kulaththa</i>	Simple starch grains
12	<i>Kola</i>	Oil globule
13	<i>Pippali</i>	Starch grains
14	<i>Sunthi</i>	Olioresin content
15	<i>Shatpushpa</i>	Epidermal cells
16	<i>Erandmoola</i>	Brown content
17	<i>Poonarnava</i>	Lignified fibers
18	<i>Trivrit</i>	Pitted vessels
19	<i>Rasna</i>	Simple fibers
20	<i>Bala</i>	Prismatic crystals
21	<i>Amrita</i>	Simple compound starch
22	<i>Katuki</i>	Fragments of fibers

**Organoleptic Characters:**

Organoleptic characters of prepared *Masha Taila* carefully observed and distinguished as below.

**Table no 3: Organoleptic characters of *Masha Taila***

No.	Organoleptic Characters	Results
1	Color	Blackish Brown
2	Taste	Bitter
3	Odor	Sweet
4	Touch	Sticky

**Physico-chemical results:**

Physico-chemical findings of prepared *Masha Taila* are given in below table.

**Table No. 4. Physico-chemical findings of prepared *Masha Taila***

No	Parameter studied	Results
1	Acid value	5.38 % w/w
2	Refractive Index	1.485
3	Iodine value	98 % w/w
4	Saponification Value	190.16 % w/w
5	Specific Gravity	0.92
6	Loss on drying	0.085 % w/w
7	pH value	4.5

**Table No. 5: Results of HPTLC of *Masha Taila***

	Visualize under short UV (254 nm)	Visualize under short UV (366 nm)
<b>No. of spot separated</b>	10	3
<b>Rf values</b>	0.01,0.03,0.06,0.10,0.13,0.34, 0.60,0.78,0.89,0.95	0.01,0.03,0.89

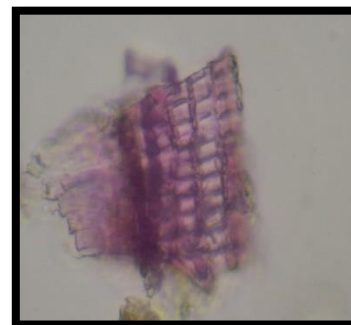
**Plate 1: Microscopic characters of *Masha Taila* raw drugs**



**Starch grain with oil globule of *Masha***



**Oil globules of *Atasi***



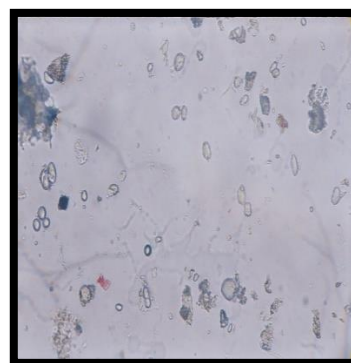
**Border pitted vessels of *Bala***



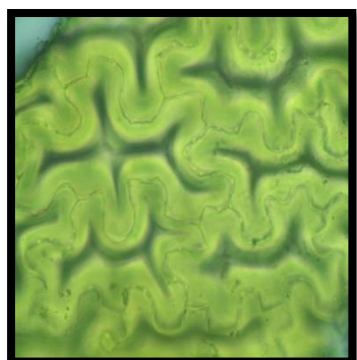
**Prismatic crystals of *Erandmoola***



**Stone cells of *Gokshura***



**Compound starch of *Guduchi***



**Epicarp cells of *Kantakari***



**Stone cells of *Kapikachchhu***



**Spiral vessels of *Karpasaasthi***



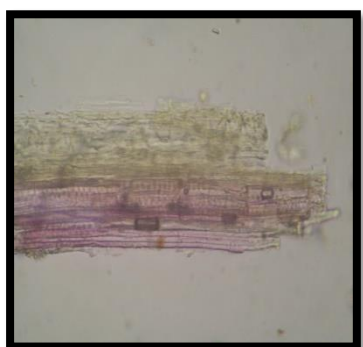
**Cork cell of *Katuki***



**Oil globule of *Kola***



**Fibers of *Kulaththa***



**Pitted fibers of *Kurantaka***



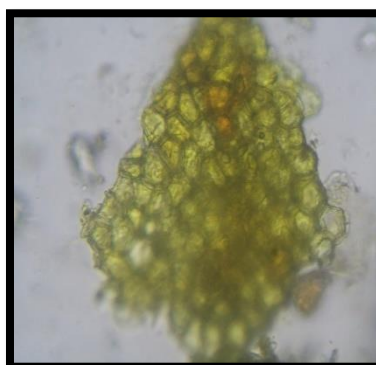
**Fibers of *Pippali***



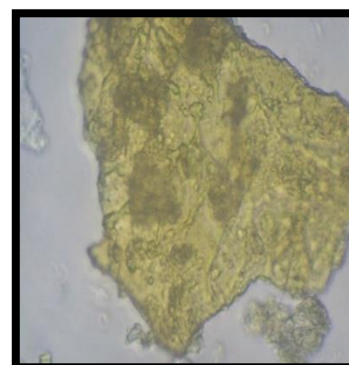
**Lignified fibers of *Punarnava***



**Stone cell of *Rasna***



**Epicarp cells of *Shanabija***



**Epidermal cells of *Shatapushpa***

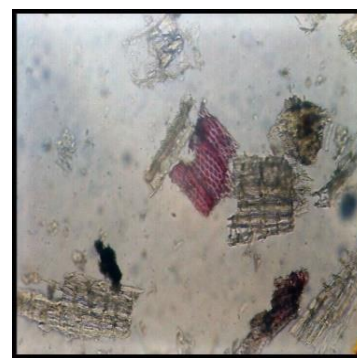




Lignified paranchyma of *Shyonaka*



Simple fibers of *Sunthi*

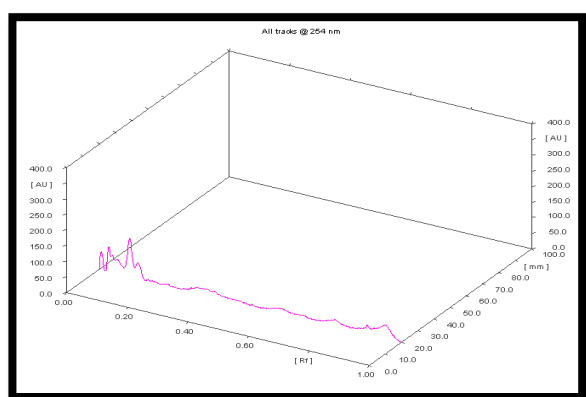


Pitted vessels of *Trivritta*

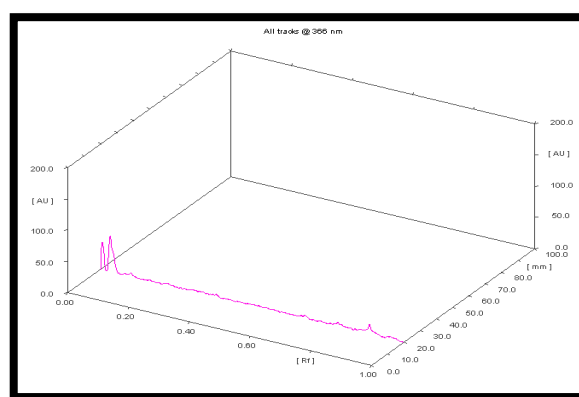


Unicellular trichoms of *Yava*

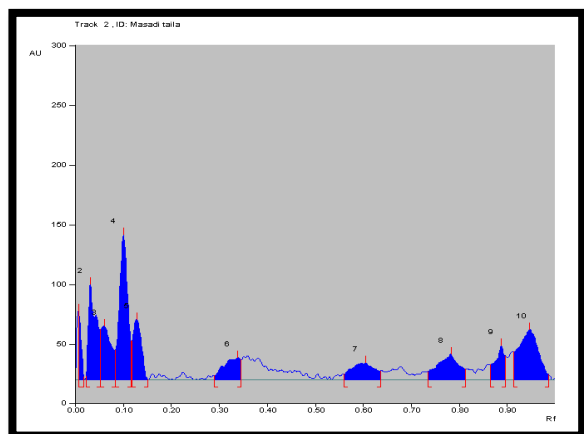
**Plate 2: HPTLC evaluation of *Masha Taila*:**



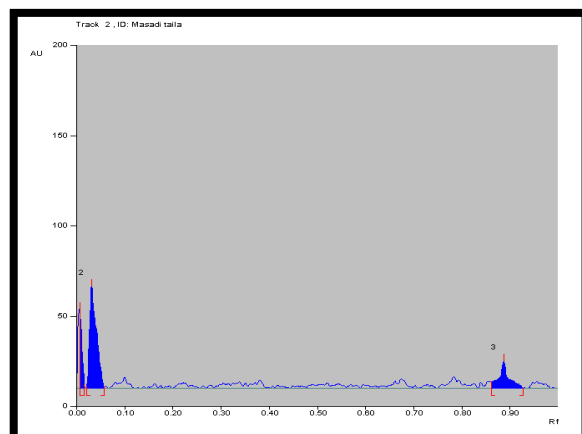
**3D Graph: 254nm of *Masha Taila***



**3D Graph: 366nm *Masha Taila***



**Chromatographic Results (Peak display) of *Masha Taila* short ultra violet (254 nm)**



**Chromatographic Results (Peak display) of *Masha Taila* short ultra violet (366nm)**

## DISCUSSION

The Pharmacognostical and pharmaceutical study exposes authentication of individual raw drugs of *Masha Taila* and it is cross verified in *Ayurvedic Pharmacopeia of India (API)*. The pitted vessels, oil globules, brown contents, starch grains, prismatic crystals, fibres etc. were observed in raw drugs. It is effective in frozen shoulder as *Nasya* (nasal drops). In physicochemical analysis, Loss on drying, Refractive Index, Saponification value, specific gravity, pH, Acid value, Iodine value were assessed. In this study, the quality groundwork for the standardization is covered. Additional analysis and investigations are required for the identification of the test drug to substantiate the clinical efficacy.

In this study, *Masha Taila* is well separated compact symmetrical bands in favour of chromophore sensitive component (Sterol, phytosterol, stigmasterol etc.) indirectly due to prechromatographic derivatization of oil sample directly. By visualization under short UV there were 10 spots and while under long UV exposure 3 spots.

## CONCLUSION

It is concluded that the formulation meets maximum qualitative standards based on physico-chemical parameters. The separation pattern of VG is documented with help of prechromatographic derivative method in context of  $R_f$  & densitogram. Pharmacognostical findings from this study will provide systemic evaluation and also serve as a master document to control the quality of *Masha Taila* formulation. The study results may be used as the standard reference in further research undertakings of its kind.

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