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Research Article

Pharmaceutico Analytical Study of Bhringaraj Taila & Its Modification Using Double Concentration of Bhringaraj W.S.R to Hptlc Analysis"

Lata Melkani[1], Dr. Shuchi Mitra[2], Dr. Usha Sharma[3], Dr. K.C. Sharma[4]

[1]MD. Scholar P.G. Deptt. Of Rasa Shastra and Bhaishajya kalpana Uttarakhand Ayurved University, Rishikul Campus
[2,3]Associate Professor P.G. Deptt. Of Rasa Shastra and Bhaishajya kalpana Uttarakhand Ayurved University, Rishikul Campus
[4]Professor & H.O.D. P.G. Deptt. Of Rasa Shastra and Bhaishajya kalpana Uttarakhand Ayurved University, Rishikul Campus

***Corresponding author- Lata Melkani**
MD. Scholar
P.G. Deptt. Of Rasa Shastra and Bhaishajya kalpana Uttarakhand Ayurved University, Rishikul Campus

ABSTRACT

In *Ayurvedic* therapeutics drug therapy has given primary importance, there is a very well developed sub-discipline completely devoted to drug formulations known as *'Bhaishajya Kalpana'* which deals with the pharmaceutics of a paramount product of excellent quality. In the present scenario, the demand for herbal medicines is increasing day by day. Traditional medical systems have always played a crucial role in the maintenance of health and longevity of humanity. Taila, taken in this study is processed with the addition of certain medicinal drugs and heated for a particular period. The study aimed at Pharmaceutical processing of *Bhringaraj Taila* & its modification with double concentration of *Bhringaraj*, & special reference to their HPTLC studies. The observations made during the preparation of the drug will be discussed & also the area estimation of *Bhringaraj Taila* by HPTLC fingerprinting technique will be analysed. The Rf value @540 nm spot 1of *Bhringaraj Taila* (sample I) SBT was found 0.16, area % was 8.71 & for (sample II) DBT area% of spot 1 was 11.47%.

Keywords: *Kalpana*, *Bhringaraj taila*, HPTLC fingerprinting, technique, Rf value.

INTRODUCTION

Although Taila *Kalpana* has not been included under primary five dosage forms, nevertheless it has its vast terrain. In the present study, an attempt is made to prepare *Bhringaraj Taila* as per classical & modified *Bhringaraj taila* with double concentration of *Bhringraj* to get better therapeutic efficacy. *Bhringaraj Taila* is one such formulation, quoted by *Acharya Chakrapani* in his vibrant classical text *Chakradatt* for treating *Palitya Roga.*[1] .To prove this on scientific grounds, chromotographical method was deployed. High performance liquid chromatography (HPTLC) is a method of chromatographic separation in which the

mobile phase is pumped into a column containing stationary phase by a high-pressure pump system. The test solution injected is carried into the column by the mobile phase. All the components are separated in the column & pass through the detector sequentially. The recorder, integrator or data acquisition system thus records the chromatographic signals. So the researcher has made an attempt to extract more active phytochemicals from double concentration pharmaceutical method and prove it through HPTLC studies.

NOTE- SBT- Single Concentration *Bhringaraj taila*
DBT- Double Concentration *Bhringaraj taila*

AIMS & OBJECTIVE

1. Pharmaceutical study of *Bhringaraj Taila*
2.Analytical study of *Bhringaraj Taila*

MATERIALS & METHODS

1. To procure the authentic raw materials.
2. Equipment Specification.
3. Pharmaceutical procedure.
4. Analytical study of *Bhringaraj Taila*

1. Raw Drug Authentication:

- **Procurement of Authentic Raw Drugs**
  - *Til taila* having *fssai* mark were procured from local market of Hardwar in order to prepare sample I, II respectively .
  - All dry herbs needed for the pharmaceutical procedure were collected from Shri Hans Ayurved Bhawan, Hardwar (Uttarakhand) and were subjected to authentication from *Dravya Guna* department of Rishikul Campus, Hardwar, UAU as per the API protocol.
  - Fresh *Bhringaraj* was collected from laksar belt Hardwar and to get fresh juice after authentication from *Dravya Guna* department of Rishikul Campus, Hardwar, UAU as per API protocol.
  - Fresh *Go-Dugdha* was procured from local milkman.

2..Equipment specification:

Table 1: List of equipment needed for the pharmaceutical procedure:

Equipment	Specification
Stainless steel vessel-01	Weight- 859gm,Circumference-27 inch , Diameter-10 inch
Stainless steel vessel-02	Weight- 422gm,Circumference-20 inch , Diameter-6.5 inch
Spatula	Weight -118gm, length-12 inch
Spoon	Weight -18gm, length-6.5 inch
Stainless steel tray	Weight- 455.5gm, length-20 inch , Width-10 inch
LPG and burner	14.2 kg capacity
Mortar & pestle	Length – 14.7 inch,depth- 5.5inch,Width-8.5inch
Thermometer	Lab thermometer, max. range- 360^0 C
Cotton cloth	1x1 meter
Mixer- grinder	Model-smart chef MX-101, 750 watts,rpm-12000-18000
Electronic weighing scale	Goldtech company, model GTET

3. Pharmaceutical Process

Manufacturing place:

The study was carried out in Hans Ayurved Bhawan pharmacy, Hardwar.

Bhringaraj Taila Paka procedure was conducted into four experiments.

Table 2: Detail of ingredients required for the preparation *Bhringaraj Taila*

Ingredients	SBT	DBT
Murcchit til taila	2000ml	2000ml
Mulethi Kalk	500gm	1000gm
Bhringaraj swarasa (Eclipta alba)	8000ml	1600ml
Go- dugdha	8000ml	8000ml

4.Analytical study of *Bhringaraj Taila*

High performance liquid chromatography (HPTLC) is a method of chromatographic separation in which the mobile phase is pumped into a column containing stationary phase by a high-pressure pump system.

Preparation of test solutions (T):

- Accurately weighed 2.0 g of sample individually in iodine flask and add 20 mL methanol to it. Reflux it for 15 min, Centrifuge (1000 RPM for 15 minutes) and collect supernatant layer and spot it.

Track T1: *Bhringaraj Taila* – SBT **Track T2:** *Bhringaraj Taila* – DBT

Preparation of Spray reagent [Anisaldehyde – sulphuric acid reagent]:

- 0.5 mL Anisaldehyde EP is mixed with 10 mL Glacial acetic acid AR, followed by 85 mL Methanol AR and 5 mL Sulphuric acid 98 % GR.

Table 3 :Chromatographic Conditions:

Application Mode	CAMAG Linomat 5 – Applicator
Stationary Phase	MERCK—TLC/ HPTLC silicagel60 F_{254} on Aluminum sheets
Application (Y axis) Start Position	10 mm
Development (Y axis) End Position	90 mm from plate base
Space Between Band	10 mm
Sample Application Volume	10 µL
Development Mode	CAMAG TLC Twin Trough Chamber
Chamber Saturation Time	30 minutes
Mobile Phase (MP)	Toluene : Ethyl Acetate : Formic Acid (7:2:1)
Visualization	@ 254 nm, @ 366 nm and @ 540 nm (after derivatization)
Spray reagent	Anisaldehyde Sulphuric acid reagent
Derivatization mode	CAMAG – Dip tank for about 1 minute
Drying Mode, Temp. & Time	TLC Plate Heater Preheated at 100 ± 50C for 3 minutes

OBSERVATIONS & RESULTS

Experiment-1: *Yavkut Churna*[2] Preparation & Kalka[3] for *Til Tail Murcchana*-

To prepare *Kalk* for *Til Tail Murcchana*, of two samples, namely SBT & DBT, the required amount of *Yavkut Churna*[4] of ingredients was taken from *Churna* obtained in experiment no. 01.

Table 4: Amount of ingredients and the successive quantity of coarse powder along with the % yield and % loss obtained in the experiment

Ingredients	Amount taken	Powder obtained	% yield	% loss	The required amount of *Churna* for *Kalk* preparation	
					SBT	DBT
Haritaki (Terminalia chebula)	150gm	130gm	86.66	13.34	46.87gm	46.87gm
Bibhitaka (Terminalia belerica)	150gm	126gm	84	16	46.87gm	46.87gm
Haridra (Curcuma longa)	150gm	120gm	80	20	46.87gm	46.87gm
Mustaka (Cyprus rotandus)	150gm	128gm	85.33	14.67	46.87gm	46.87gm
Lodhra (Symplocos racemosa)	150gm	134gm	89.33	10.67	46.87gm	46.87gm
Vatankur (Ficus bengalensis)	150gm	128gm	85.33	14.67	46.87gm	46.87gm
Ketaki (Pandanus tectorius)	150gm	135gm	90	10	46.87gm	46.87gm
Tagara (Valeriana officinalis)	150gm	135gm	90	10	46.87gm	46.87gm
Nalika (Nelumbo nucifera)	150gm	130gm	86.66	13.34	46.87gm	46.87
Manjishtha (Rubia cordifolia)	450gm	400gm	88.88	11.12	187.5gm	187.5gm
Water	-	-	-	Q.S	Q.S	Q.S

Experiment 02- Til Taila Murcchana[4]

- The detail of drug ingredients used for Til *(Seasemum Indicum)* Tail Murcchana were following. The raw til tail for sample SBT & DBT was 3000 ml each. *Murcchana Dravyas Kalk* was obtained from experiment no 1. Water used for both the samples was 3000 ml each.

The detail of observations of Tila *(Seasemum Indicum)* Taila *Murcchana* of sample I & II

- When the two samples SBT & DBT were observed at 11:00am & 12:00pm ; both were viscous & yellow coloured at 30^0C.
- The small bubbles were seen at the top of the taila when Sample (I) was observed at 11:15am & sample (II) at 12:10pm .The temperature noticed was 75^0C& 80^0C repectively.
- Fumes started to appear and the smell of Til Tai*l* was predominantly felt for sample(I) at 11:20am & sample (II) at 12:20pm .The temperature noticed was 100& 105^0C respectively.
- After addition of kalk bolus effervescences appeared at 11:35am for sample (I) & 12:35pm for sample (II) .The temperature at that time was 75^0C & 80^0C successively.
- Tail started to separate out through the mass of Kalka for sample (I) at 4:20pm & sample (II) at 4:40pm .The temperature noticed was 80^0C & 85^0C.
- The *kalk* could be rolled into varti space & no crackling sound was heard at 4:55pm for sample (I) & 5:15pm for sample (II) at the same temperature i.e 75^0C

The detail of % yield and % loss of *Murcchit Tail*

- During *murchana* of taila, the intial amount of both the samples were 3000ml each. The yield of sample (I)SBT was 2600ml i.e 86.66% & loss was 13.34% .In sample (II) DBT the yield was 2700ml i.e 90% & loss was 10%.

The% yield, % loss of *Yavkut Churna of Mulethi* & required amount for *Kalk* preparation

- The *Mulethi* was coarsely powdered for *kalk* preparation; Initial amount for both the sample were 1300gm, powder obtained in SBT was 1200gm & DBT was 1270gm. Therefore yield was 92.30% & loss was 7.69% in SBT & in DBT yield was 97.69% & loss was 2.30%.

Experiment 03: *Bhringaraj (Eclipta alba)* Swarasa[5] preparation

- Fresh *bhringaraj* taken for sample SBT was 17 kg which yielded 8 litres of *swarasa* .while for DBT was 39 kg which yielded 20 litre *swarasa*. The yield and loss of *Bhringaraj swarasa* for sample SBT were 47.05% & 52.95% & DBT were 51.28% & 48.72% respectively.

Experiment 04: *Bhringaraj Taila* preparation

Table5: Detail of observations during the preparation of *Bhringaraj Taila* of Sample Ist(SBT)

Observation	Day	Time	Temp
Small bubbles and fumes at the top of the *Tail*	D 1	11 am	100^0C
After addition of *Mulethi Kalk* effervescence appeared		11.15 am	85-90^0C
After addition of *Bhringaraj swarasa*, the color of taila turned greenish		11.20 am	85^0C
Boiling of *Bhringaraj swarasa*	D 2	12 pm	90^0C
The mixture became a little thicker.		12pm-04:30pm	90^0C
Greenish cream was appeared at the top	D 3	11 am- 1pm	85^0C
After addition of *Bhringaraj swarasa*, milk is added		1pm- 04 pm	90^0C
colour of *taila* changed to dark brown with a characteristic odour		10.10 am	80^0C
Vaporization of *milk* was seen		10.10 am-04pm	85-90^0C
Vaporization of *Bhringaraj swarasa* was seen	D 4	10 am-03pm	85-90^0C
The mixture became little viscous	D 5	10am-2 pm	85^0C
The bulk of the brown-coloured mass of *Kalk* was separated		2:15 pm	80^0C
The thick blackish slurry-like filtrate was heated		2:25-04 pm	80^0C
The mixture turned to blackish mass	D 6	10-12 pm	80^0C
Separation of *Tail* through the black tar-like mass of *Kalk*		12 pm-02pm	75-80^0C
Kalk could be rolled into *Varti*; no crackling sound was heard when *Tail* dipped cloth piece was put on fire		03:45 pm	75-80^0C

Table 6:Detail of observations during the preparation of *Bhringaraj Taila* of Sample IInd(DBT)

Observation	Day	Time	Temp.
Small bubbles and fumes at the top of the *Tail*	D 1	10.05 am	105^0C
After addition of *Mulethi Kalk* effervescence appeared		10.10 am	90-95^0C
Evaporation of water was seen		10.15 am-03 pm	90^0C

After addition of *Bhringaraj swarasa*, the colour of *Taila* turned greenish	D 2	10.10 am	90^0C
Boiling of *Bhringaraj swarasa* was seen, the mixture became thicker		10.40 am-02.20pm	95^0C
Greenish cream was appeared at the top	D 3	10 am-12 pm	85^0C
Milk was added in taila		12 pm- 03 pm	90^0C
After the addition of *milk*, the colour of *Tail* changed to dark brown.		03.10 pm	80^0C
Vaporization of *milk* was seen		3.10-04 pm	95^0C
Vaporization of *Bhringaraj swarasa* was seen	D 4	10 am-03 pm	90^0C
The mixture became little viscous	D 5	10am-12 pm	95^0C
The bulk of the brown-coloured mass of *Kalk* was separated		12:15 pm	80^0C
The thick blackish slurry-like filtrate was heated		12:25-04 pm	85^0C
The mixture turned to blackish mass	D 6	10-10.15 am	80^0C
Separation of *Tail* through the black tar-like mass of *Kalka*		10.40 am	75-80^0C
Kalk could be rolled into *Varti*; no crackling sound was heard when *Tail* dipped cloth piece was put on fire		01.20 pm	80^0C

The detail of % yield and % loss of *Bhringaraj Taila*

- The Intial amount of both the samples were 2000ml, Sample (I) SBT yielded 90% i.e 1800ml & sample (II) DBT yielded 81% i.e 1620ml . The loss of both the samples were 10% & 19% respectively.

HPTLC FRINGERPRINTING

Table 7: Results of test for by HPTLC Chromatogram (Rf) @254nm(Track 1- SBT, Track2- DBT)

SPOT NO.	Track-1	Track-2
1	0.31	0.31
2	0.29	0.29
3	0.49	0.49
4	0.59	0.58
5	0.66	0.66
6	0.81	0.81

Table 8:Results of test for by HPTLC Chromatogram (Rf) @366nm(Track 1- SBT, Track2- DBT)

SPOT NO.	Track-1	Track-2
1	0.59	0.59
2	O.67	0.67
3	0.78	0.78
4	0.85	0.85

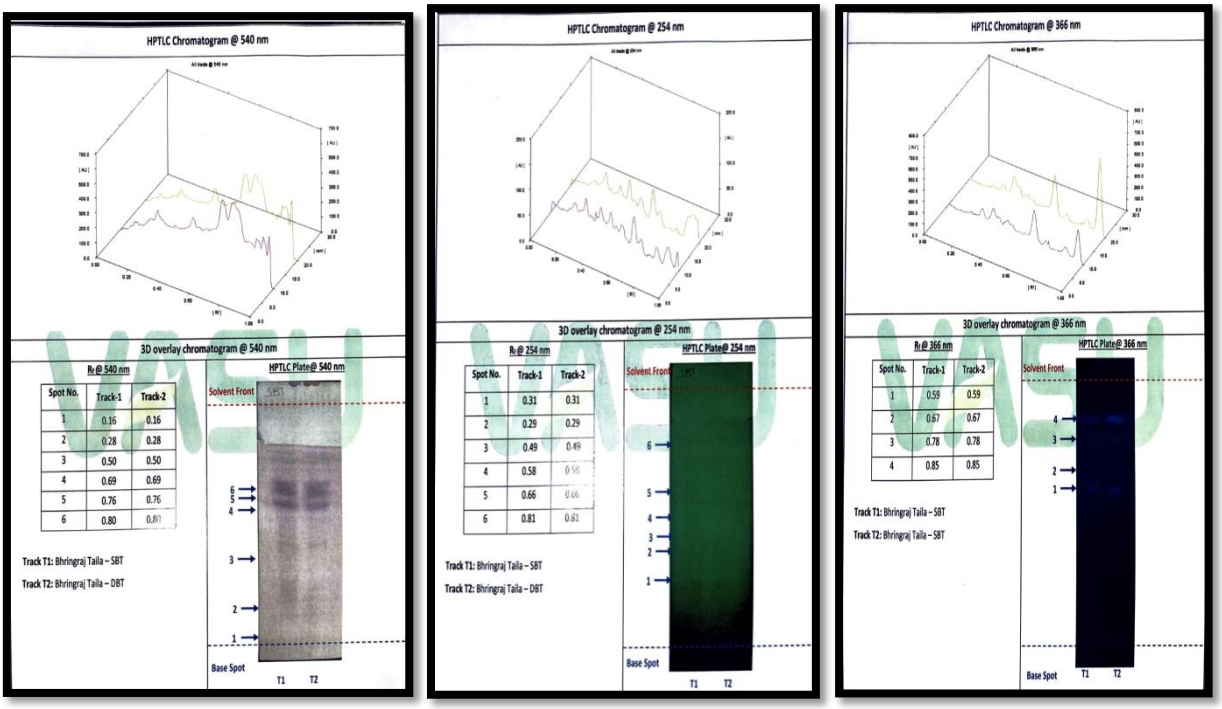
Table 9:Results of test for by HPTLC Chromatogram (Rf) @540nm(Track 1- SBT, Track2- DBT)

SPOT NO.	Track-1	Track-2
1	0.16	0.16
2	O.28	0.28

3	0.50	0.50
4	0.69	0.69
5	0.76	0.76
6	0.80	0.80

Table 10: HPTLC Fingerprinting Data (Area estimation)

SPOT NO.	Rf Value	*BHRINGARAJ TAILA*-SBT		*BHRINGARAJ TAILA*-DBT	
		Area	**%Area**	**Area**	**% Area**
1	0.16	41.2 AU	8.71%	43.2AU	11.47%
2	0.28	60.0 AU	12.70%	57.7AU	15.32%
3	0.50	38.6AU	8.16%	37.3AU	9.91%
4	0.69	66.1AU	13.99%	64.5AU	17.14%
5	0.76	10.4AU	7.46%	28.4AU	----
6	0.8	10.4AU	2.77%	26.7AU	5.64%



DISCUSSION

Before *Tail Pak*, *Murcchana* was done. *Murcchana* is a pre-procedure of *Tail Pak*, which is believed to remove *Ama*[6] & *Gandha Doshas* of Tail[7]. Murcchana *Samskara* imparts good colour & smell- *Haridra (Curcuma longa), Manjishtha (Rubia cordifolia)* and *Vatankur (Ficus bengalensis)* are supposed to be responsible for appealing colour, & *Nalika (Nelumbo nucifera) Ketaki Pushpa (Pandanus tectorius)* possess pleasant odour and supposed to remove *Gandha doshas* of Tail.

During Yavkut Churna preparation of *Murcchana* drugs between 10-20% loss was seen due to manual errors such as scattering during crushing. Drugs having more fibrous part showed more % loss. During *Murcchana* of SBT, 13.34% loss & of DBT 10% loss of *taila* was seen due to absorption of *taila* by *Kalka* of *Murcchana* dravya.

In order to extract the maximum active principle of ingredients & avoid any chances of burning of material, *Tail Pak* was done in *Manda-Madhyamagni* i.e. 60-105^{0}C. Continuous stirring was done during *Tail Pak* as it enhances the extraction process by weakening of bonds. It reduces the thickness

of Taila & disperses the concentration of solution homogenously. Marked Loss i.e. % Loss during *Bhringaraj swarasa* preparation was 45-50% due to its higher fibrous content. While during *Taila* preparation, in SBT 10% loss & in DBT 19 % loss was seen.

For authentication of the drug, HPTLC was performed to estimate the % of Wedalactone anti-oxidant present in *Bhringaraj,* in the samples of oil. Samples were simultaneously ran with Wedalactone Standard marker, and there R_f values were determined relatively. **The assay(% Wedalactone) in SBT was 0.42% & DBT was 0.75%.**

CONCLUSION

- During *Murcchana* 13.34% & 10% loss of *taila* was seen due to absorption of taila by Kalka of *Murcchana* dravya in SBT & DBT respectively.
- During *bhringaraj Taila* preparation, in SBT 10% & DBT 19% loss was seen.
- **HPTLC profile showed six spots at 254 nm,540nm and four spots at 366 nm respectively.**
- **The active constituent Wedalactone was found near to double in DBT & the concentration of other Phyto-constituents was also high in DBT.**

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