

Pharmaceutico-Analytical study of *Shadbindu taila*

Research Article

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Abstract

Introduction: *Shadbindu taila* is an Ayurvedic herbo-mineral preparation used in a variety of clinical conditions especially in diseases of upper part of body. This *taila* is described as exceptionally beneficial for improving the strength of hands. Before a human trial it is essential to evaluate the quality and authenticity of the trial drug, Standardization is needed for assuring quality of any drug and it should be started with the identification and authentication of the drug. **Materials and method:** Pharmacognostical evaluation carried out at Pharmacognosy department, ITRA, Jamnagar, pharmaceutical and HPTLC study were done at Pharmaceutical chemistry department, ITRA, Jamnagar. Microbiological evaluation was carried out at department of Microbiology I.T.R.A., Jamnagar. **Results:** Organoleptic examination: Colour: Greenish Brown, Odour: Aromatic, Taste: Salty, Touch: Viscous, oily. **Pharmaceutical evaluation:** specific gravity 0.9177, density 0.8753 Refractive Index 1.4860, Acid Value 4.042, Saponification Value 132.43, Iodine Value 169.33 HPTLC at 254nm (short wave) showed mainly 6 major spot and at 366 nm (long wave) showed 2 major spots. No organisms isolated in aerobic and fungal culture, in microbiology evaluation. **Conclusion:** The trial drug is authentic and devoid of any adulteration. All quality control parameters were found to be within the permissible pharmacopeial limits and the stability and shelf life of the trial drug also will be more as per the pharmaceutical parameters.

Key Words: *Shadbindu taila*, *Madhyama paka*, Standardization of *taila*, Pharmaceutical study, *Sneha sidhi lakshana*, *Bhringaraja*.

Introduction

Ayurveda is a medical system highly depends on the availability of medicinal plants. This is an era of industrialization, where the scarcity of herbs is increasing day by day. When the demand of herbal medicine is not fulfilled by the available resources, adulterants were added to compensate it, which means the actual drug is being replaced by another drug of different botanical identity and property. This will eventually leads to a compromise in maintaining the proper standard of formulations. Due to these reasons, Standardization is needed for assuring quality of any drug and it should be started with the identification and authentication of the drug. An Ayurvedic formulation must confirm test for identity, potency, purity, safety and efficacy as per WHO guidelines (1), (2). Moreover in a clinical trial, human trial is situated at the end of the sequence, so that the trial drug should be evaluated in every aspect, from the beginning of the collection of raw drug, processing, preparation and after the preparation. Quality assurance of traditional

formulations relies upon good manufacturing practices with adequate batch to batch analysis and standardized method of preparation (3). For the analysis of the drug, a lot of sophisticated methods and tools are available now, and different branches of sciences can be utilized for the same.

Shadbindu taila is an Ayurvedic herbo-mineral preparation used in a variety of clinical conditions especially in diseases of upper part of body. This formulation is described in the context of *shiroroga* of Bhaishajya ratnavali (4). *Taila* will be *teekshna* in potency due to the presence of *saindhava* in a considerable amount and also it contain drugs like *Erandamoola*. May be because of that, the dose of *taila* mentioned as only 6 bindu as per classics, hence the name also came as *Shadbindu taila*. This *taila* is described as exceptionally beneficial for improving the strength of hands. Before a human trial it is essential to evaluate the authenticity of the trial drug, and quality control parameters should also be assessed. For that purpose physico-chemical parameters, pharmaceutical evaluation and HPTLC were evaluated here.

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Materials and Methods

All the drugs of *Shadbindu taila* as per API were obtained from Pharmacy, I.T.R.A, Jamnagar(5). *Krishna tila taila* is used classically for the preparation, which is obtained from the local market, Jamnagar. Goat's milk also purchased from the local market. The drugs were powdered separately, and they were subjected to

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pharmacognostical evaluation at Pharmacognosy laboratory, I.T.R.A., Jamnagar, Gujarat. The *taila* is prepared at Dept of Rasa shastra & Bhaishajya Kalpana Laboratory of I.T.R.A, Jamnagar. Once the formulation was ready, it was subjected to Pharmaceutical evaluation and HPTLC at pharmaceutical laboratory, I.T.R.A, Jamnagar. Microbiological evaluation was carried out at department of Microbiology I.T.R.A, Jamnagar.

Preparation of Shadbindu Taila

Taila Paka Vidhi (traditional method of *Taila* preparation) assures the enrichment of *Sneha dravya* with the active principles of the ingredients (6). medicated oil is prepared by protracted boiling of the *Sneha Dravya* (base oil) with prescribed *drava dravya* (liquid drug) and *Kalka Dravya* (drugs used as a fine paste) to dehydration or near dehydration. This process results in the transfer of some therapeutically active principles of the ingredients into the base oil (7). *Shadbindu Taila* was prepared in Rasa shastra & Bhaishajya Kalpana Laboratory of ITRA, Jamnagar, using quality raw material as per AFI (8)

The measured quantity of *Krishna tila taila* was taken in a stainless steel vessel and heated it over mild flame (80°C for 5 min) till it got evaporated moisture content, then specified quantity of *drava dravya (Bhringraj Swarasa)* was added, followed by fresh goat milk and bolus of *Kalka* were added to it. After thorough mixing of *Kalka*, the mixture was subjected to heat. Heating was maintained in a temperature between 95-100°C and stirring was continued. Heating was continued for 3 day till *Sneha Siddhi Lakshanas* were obtained (*Madyama paka*). On 3rd day, after obtaining desired *Sneha Siddhi Lakshanas*, the vessel was removed from the fire and then specified quantity of *saindhava* was added as *patrapaka*. After that oil was filtered through a two folded cotton cloth in its hot stage itself. The prepared oil (after cooling), was stored in a properly labelled air tight container.

Results

Pharmacognostical Study

Identification and authentication of herbal drugs is carried out by pharmacognostical study. A routine use of such scientific techniques will lead to standardization of the Ayurvedic product to a certain extent and would definitely help in building confidence in use of these products (9).

Table 1: The drugs of Shadbindu taila

| Drug | Scientific name | Part used |
|--------------------|---|-------------|
| <i>Eranda mula</i> | <i>Ricinus communis L</i> | Root |
| <i>Tagara</i> | <i>Valeriana wallichii DC</i> | Root |
| <i>Satahwa</i> | <i>Foeniculum vulgare (Mill.)</i> | Fruit |
| <i>Jivanti</i> | <i>Leptadenia reticulata(Retz) Wight& Arn</i> | Root |
| <i>Rasna</i> | <i>Pluchea lanceolata(C B Clarke)</i> | Root |
| <i>Bhringa</i> | <i>Eclipta alba(L)</i> | Whole plant |
| <i>Vidanga</i> | <i>Embelia ribes Brum.F</i> | Fruit |

| | | |
|----------------------------|----------------------------------|-------------|
| <i>Yashti</i> | <i>Glycorrhyza glabra(L)</i> | Root |
| <i>Shunti</i> | <i>Zingiber officinalis Rosc</i> | rhizome |
| <i>Saindhava</i> | <i>Rock salt</i> | — |
| <i>Krishna tila taila</i> | <i>Sesame oil</i> | — |
| <i>Aja paya</i> | <i>Goats milk</i> | — |
| <i>Bhringaraja swarasa</i> | <i>Eclipta alba(L)</i> | Whole plant |

There are two steps in raw drug analysis:

Organoleptic Study

This is the evaluation carried out with sense organs and it is very important for assessing the genuinity of ayurvedic drugs. The raw drugs and its powder were separately evaluated by organoleptic characters like colour, odour, taste and touch. Characteristics of the samples were identified and noted.

Powder microscopy

Powdered drug was studied microscopically and microscopic characters of individual drugs were noted. The powder of the drug was dissolved with water followed by microscopy of the sample without stain and after staining with Phloroglucinol + HCl. Microphotographs of the sample were also taken under Carl-zeiss trinocular microscope.

Observations

Every *taila* will have a characteristic colour and odour, relating to the herb used (10)hence by assessing the features we can find out the true quality of the formulation. Findings of Organoleptic examination of *Shadbindu taila* are tabulated below:

Table 2: Organoleptic properties

| | | |
|---|--------|----------------|
| 1 | Colour | Greenish Brown |
| 2 | Odour | Aromatic |
| 3 | Taste | Salty |
| 4 | Touch | Viscous, oily |

Powder microscopy

Observations on powder microscopy were listed below, and the micro photographs were also given

Table 3: Powder Microscopy

| Sl. No | Name of Drug | Powder microscopy results |
|--------|-----------------------------------|--|
| 1 | <i>Ricinus communis L</i> | Lignified Fibres Starch grains Pitted vessels Rosette crystals Group of fibres |
| 2 | <i>Valeriana wallichii DC</i> | Simple fibres Prismatic crystals Scleriform vessels Parenchyma |
| 3 | <i>Foeniculum vulgare (Mill.)</i> | Epicarp cells Fibres with oil globules Parenchyma with oil globules Oil globules |

| | | |
|---|--|---|
| 4 | <i>Leptadenia reticulata</i> (Retz) Wight& Arn | Border pitted vessels Cork in surface Lignified fibres Rosette crystals Prismatic crystals Starch with Hilum |
| 5 | <i>Pluchea lanceolata</i> (C B Clarke) | Cork cells Fibres Rosette crystals Stone cells Brown content Simple Starch grains |
| 6 | <i>Eclipta alba</i> (L) | Anisocystic stomata Pollen grains Oil globules Fibres Warty trichome with base |
| 7 | <i>Embelia ribes</i> Brum.F | Oil globules Olioresine content Scleroids Trichome Stone cells |
| 8 | <i>Glycorrhyza glabra</i> (L) | Crystal fibres Fibre with starch grains Lignified fibres Pitted vessels Rhomboidal crystal |
| 9 | <i>Zingiber officinalis</i> Rose | Cork cells Cork in surface Scalariform vessels Starch grains fibres |

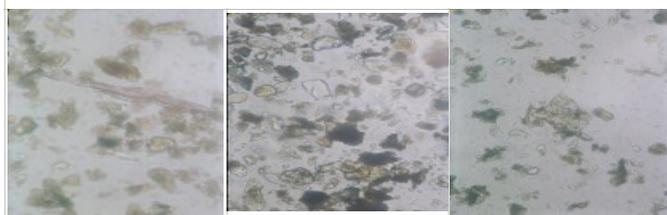
Micro-photographs of each ingredients

Erandamoola



1.1: Group of fibres 1.2: Lignified fibre 1.3: Pitted vessels

Tagara



2.1: Scleriform cells 2.2: Prismatic cells 2.3: Parenchyma

Shatapushpa



3.1: Epicarp cells 3.2: Parenchyma with oil globules 3.3: Fibres with oil globules

Jeevanti



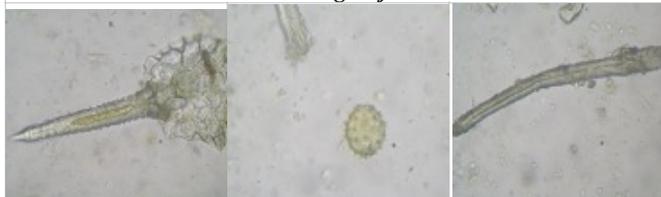
4.1: Border pitted vessel 4.2: Cork in surface 4.3: Lignified fibres

Rasna



5.1: Brown content 5.2: Stone cells 5.3: Rosette crystals

Bhringaraja



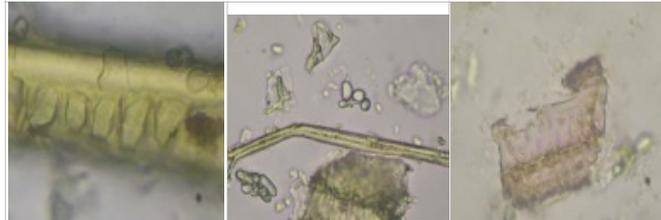
6.1: Warty trichome 6.2: Pollen grains 6.3: Fibres

Vidanga



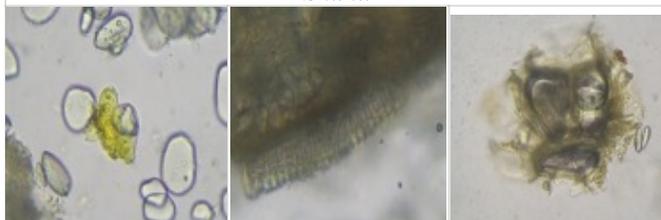
7.1: Scleroids 7.2: Stone cell 7.3: Oleo-resin content

Yashthimadhu



8.1: Crystal fibre 8.2: Fibre with starch grains 8.3: Pitted vessels

Shunti



9.1: Starch grains 9.2: Scalariform vessels 9.3: Cork in surface view

Pharmaceutical evaluation

Pharmaceutical evaluation is carried out for assuring the quality of the prepared drug, and it helps to calculate the shelf life also. As per API, Specific Gravity, Acid Value, Saponification value, Iodine value and Refractive Index are the parameters to be evaluated

for the quality assessment of a *taila*. The specific gravity of a liquid is the weight of a given volume of the liquid at 250 (unless otherwise specified) compared with the weight of an equal volume of water at the same temperature, all weighing being taken in air (11). The refractive index (η) of a substance with reference to air, is the ratio of the sine of the angle of incidence to the sine of the angle of refraction of a beam of light passing from air into the substance (12). The saponification value is the number of mg of potassium hydroxide required to neutralize the fatty acids, resulting from the complete hydrolysis of 1 g of the oil or fat (13). The Iodine value of a substance is the weight of iodine absorbed by 100 part by weight of the substance (14). The acid value is the number of mg of potassium hydroxide required to neutralize the free acids in 1 g of the substance (15).

The results of physico-chemical evaluation is listed below:

Table 4

| No. | Parameters/ Sample | Shadbindu taila |
|-----|----------------------|---------------------|
| 2 | Specific Gravity | 0.9177 at room temp |
| 3 | Acid Value | 4.042 |
| 4 | Saponification value | 132.43 |
| 5 | Iodine value | 169.33 |
| 6 | Refractive Index | 1.4860 |

High Performance Thin Layer Chromatography

TLC and HPTLC Chromatography is an analytical technique where the technique used is separation of closely related compounds from a mixture. This is an important analytical technique used for the analysis of herbal medicines. A fingerprint of the preparation was obtained by HPTLC for standardization purpose. The fingerprint has the potential to determine authenticity and reliability of chemical constituents of herbal drugs and formulations (16,17).

Steps involved in HPTLC

- a. Selection of chromatographic layer.
- b. Sample and standard preparation.
- c. Layer pre-washing,
- d. Layer pre-conditioning.
- e. Application of sample and standard.
- f. Chromatographic development.
- g. Detection of spots.
- h. Scanning.
- i. Documentation of chromatic plate

Rf. Value (Retention factor value):

The distance of each spot from the point of its application was measured and recorded and the Rf. Value was calculated by dividing the distance travelled by the spots by the distance travelled by the front of the mobile phase (18).

Results

In the photo documentation of *Shadbindu taila* at 254nm (short wave) observed mainly 6 major spot and Rf values are 0.06, 0.2, 0.23, 0.25, 0.3 and 0.68. At 366 nm (long wave) showed only 2 major spots. Rf

values are 0.06, 0.25. On densitometric scan at 254 nm, *Shadbindu taila* showed 6 peaks, peak with Rf value 0.3 contributed major area 34.4%. Densitometric scan at 366 nm showed 2 peaks in which the peak with Rf value 0.06 contributed major area 82.05 %.

Densitogram of Shadbindu taila

Image 10: Peak at 254nm

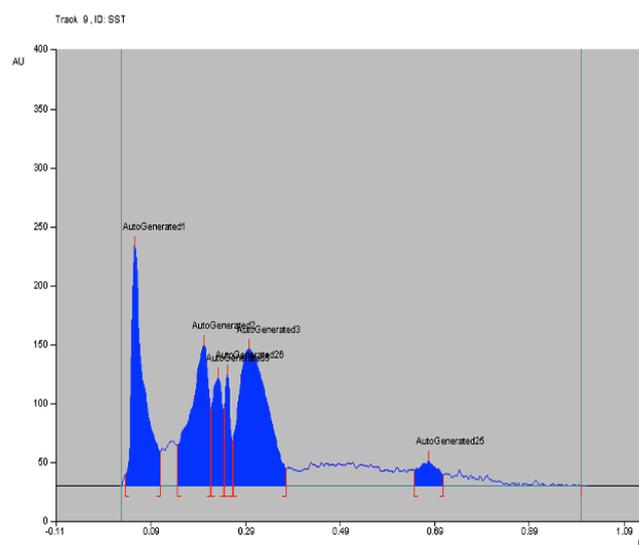
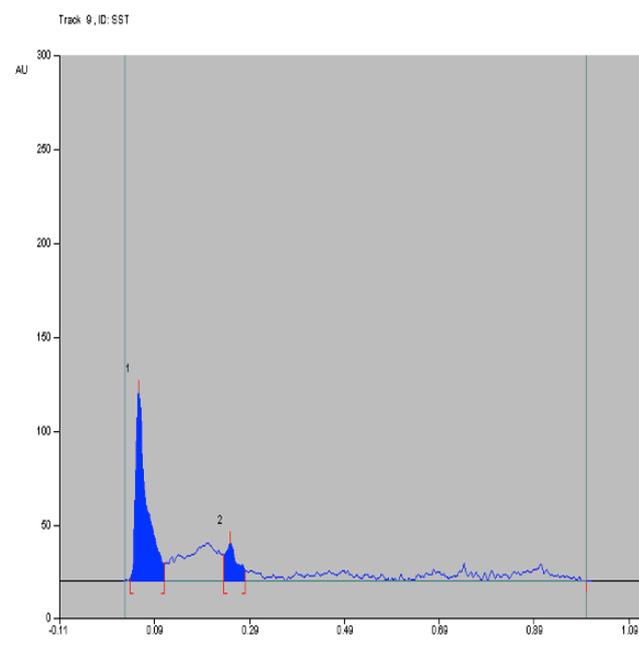


Image 11: Peak at 366nm



Microbiological Investigation

Microbial culture examination helps to find out the contamination of prepared drug by micro-organisms. This can happen at any stage of preparation, storage, or handling, hence before the administration of medicine to the trial subjects one must ensure that microbial presence in the sample is in the normal limit.

Aerobic culture report

No organisms isolated (after 48 hrs of incubation at 37°C)

Fungal Culture report

No fungal pathogen isolated (after 7 days of incubation at 37°C)

Discussion

Quality assurance is an integral part of all systems of medicine to ensure the quality medicament. Standardization is another essential factor for ASU preparations in order to assess their quality based on the concentration of chemical or bioactive marker (19,20). Modern bio-analytical techniques like HPTLC and HPLC are being used to achieve the aforesaid objectives (20). Here the trial drug is evaluated in various ways and the discussions based on that are given below.

As per Acharya Susruta the paka for Nasya is madhyama (21), and as per Acharya Sharmgadhara madhyama is considered as a paka suitable for all purposes (22). So here the *taila* is prepared to madhyama paka so as to extract maximum active chemical constituents (20). Colour of *taila* depends on the ingredients added to it and the proper paka attained. Here the colour is greenish brown probably due to the greenish appearance of *Bhringaraja swarasa*. Order is aromatic due to the addition of particular medicinal herbs to the *Tila Taila*. Powder microscopic examination helps to authenticate the drugs mentioned in the yoga and there by exclude adulteration. Refractive index indicate density of sample compared to air and liquid media; the value for *Shadbindu taila* was 1.4860. Specific gravity indicates the presence of solute content in the solvent, Specific gravity of plain *Tila Taila* is 0.90- 0.92. [23], here the value of *Shadbindu taila* is 0.9177. Free fatty acids are nascent and hence susceptible for formation of newer compounds in an attempt to get stabilized which has been seen in the form of changes in saponification value after Sneha Paka (19). The amount of alkali needed to saponify a given quantity of oil will depend upon the number of COH group present in it; the saponification value also indicates the average molecular weight /chain length of all fatty acids present or it is the amount of all free fatty acids present in a sample. Fatty acids with longer chains, have low saponification value, and the shorter chain fatty acids have high saponification value. Shorter chain fatty acids (high saponification value) have faster rate of absorption than longer chain fatty acids; saponification value of ST was found to be 132.43. The acid value indicates the presence of free fatty acids in the oil which are responsible of rancidity of the compounds; higher the free fatty acid the more is the rancidity. This will help to decide the shelf life of the oil; acid value for ST was 4.042. Iodine value indicates the degree of unsaturation of oil; greater the degree of unsaturation, higher will be the possibility of absorption and atmospheric oxidation leading to rancidity. The more the iodine number, the more unsaturated fatty acid bonds are present; unsaturated fatty acid is better absorbed than saturated fatty acids, the value of ST was found to be 169.33.

The HPTLC of *Shadbindu taila*, 6 major spots were observed at 254 nm and 2 major spots were observed at 366 nm indicating it's possible compounds in the matrix, which may be responsible for therapeutic activity of the same. Microbial culture examination helps to find out the contamination of prepared drug by micro-organisms, here the values were in normal limits.

Conclusion

By analysing the results it can be concluded that the trial drug is authentic and devoid of any adulteration. All quality control parameters were found to be within the permissible pharmacopeial limits and the shelf life of the trial drug also will be more as per the pharmaceutical parameters. The therapeutic efficacy and safety of the trial drug should be evaluated through the pre-clinical and clinical studies.

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