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Standardization of *Shadbindu Taila*: An Ayurvedic oil based medicine

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Abstract

Shadbindu Taila (ST) is an Ayurvedic formulation used as a remedy for loosening of tooth, weakness of the eyesight, loss of hair, diseases of head, etc., Present study is an attempt to develop some newer approaches for the quality control and standardization of ST. Standardized operating procedure for the preparation of ST was developed in accordance with Ayurvedic Formulary of India. Preliminary phytochemical, physicochemical, and chromatographic evaluation of ST was carried out. Safety of ST was evaluated in terms of skin irritation test and presence of heavy metals. Chemical characterization of ST was done on the basis of kaempferol using validated -High Performance Thin Layer Chromatographic (HPTLC) method. ST did not show presence of any of the heavy metals analyzed and was found non-irritant on rabbit skin. The quality control parameters resulted after scientific evaluation of ST can be used as reference standard for quality control/assurance laboratory of a pharmaceutical firm in order to have a proper quality check over its preparation and processing.

Key words: High performance thin layer chromatography, kaempferol, safety, *Shadbindu Taila*, standardization

Introduction

Siddha Taila (medicated/processed 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.[1] Thus, *Taila Paka Vidhi* (traditional method of *Taila* preparation) assures the enrichment of *Sneha dravya* with the active principles of the ingredients.

Shadbindu Taila (ST) is a polyherbo-mineral Ayurvedic formulation prescribed widely for several conditions such as *Danta Chalana* (loosening of tooth), *Drishti Daurbalya* (weakness of the eyesight), *Keshashata* (loss of hair), *Siroroga* (diseases of head), etc., Following are the ingredients used for the preparation of ST:[2]

- *Sneha Dravya*: *Murcchita Krishna Tila Taila* (MKTT) (processed oil from black seeds of *Sesamum indicum* L.)
- *Drava Dravya: Bhringa Rasa* (juice obtained from macerated whole plant of *Eclipta alba* (L.) Hassk.) and *Aja Paya* (goat milk)
- *Kalka Dravya:* A fine paste obtained from the powdered mixture of *Eranda* (*Ricinus communis* L.), *Tagara* (*Valeriana wallichii* DC.), *Shatahva* (*Anethum sowa* Roxb.), *Jivanti* (*Leptadenia reticulata* [Retz.] Wight and Arn.), *Rasna* (*Alpinia galanga* (L.) Swartz), *Bhringa* (*Eclipta alba* (L.) Hassk.), *Vidanga* (*Embelia ribes* Burm.f.), *Yashti* (*Glycyrrhiza glabra* L.) and *Shunthi* (*Zingiber officinale* Roscoe.) and *Saindhava Lavana*: Rock salt.

Quality control of ST remains an unexplored issue despite of its increase in the popularity. Thus, in the present work an attempt has been made to use some newer approaches for the standardization of ST with following objectives:

- To develop Standardized Operating Procedure (SOP) for the preparation of ST.
- To evaluate the physicochemical, phytochemical and safety profile of ST.
- To carry out chemical characterization of ST on the basis of active principle(s) of the ingredients, using validated HPTLC method.

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Materials and Methods

Selection, processing and quality evaluation of the raw materials

As identification of the raw materials used to prepare any finished product is a must, all ingredients procured through local market were authenticated by Department of Botany (HRL/Auth/10/14-33). Raw materials complying pharmacopoeial quality were further used for ST preparation. Plant raw materials were dried in oven at 45°C, powdered, sieved (BSS sieve, 85-mesh) and stored in air tight containers.

Preparation of ST

The SOP for the preparation of ST involved following steps:

- Preparation of MKTT: The reference from Ayurvedic Formulary of India[2] was followed for *Murcchana* of *Krishna Tila Taila* (KTT). Ingredients and parts used are mentioned in Table 1
- Preparation of *Bhringa Rasa*: Fresh juice obtained from macerated whole plant of *E. alba* was considered as *Bhringa Rasa*
- Preparation of *Kalka*: Each *Kalka Dravya* (19.0 g powder) was taken in a vessel and mixed, followed by addition of sufficient amount of water until a uniform paste was obtained
- Preparation of ST: As per Table 2, MKTT (0.768 l) was indirectly heated on a mild flame (by placing a pan between burner and vessel to avoid direct heating) with *Bhringa Rasa* (3.072 l), *Saindhava Lavana* (19.0 g powder) and *Kalka* obtained from *Kalka Dravya*. Mixture was stirred intermittently till it became slimy. The heating was stopped and *Aja Paya* (3.072 l) was added. The mixture was kept standing overnight. Next day, the heating was continued till the mixture attained *Sneha Siddhi Lakshana* (completion test for chief desired characteristics) like *Gandha-Varna-Rasotpatti* (desired smell, color and taste), *Shabdahinata* (no cracking sound), *Phenodgama* (appearance of froth) and *Vartivat Kalka* (rolling of paste of herbal drugs between fingers). Finally, the mixture was filtered when hot through muslin cloth and stored in amber colored bottle until use. ST was prepared in three batches.

Quality evaluation of ST

Quality of ST was analyzed by employing following quality control methods:

Table 1: List of ingredients for the *Murchana* of *Krishna Tila Taila*

Sanskrit name	Description	Part used	Quantity
Jala	Water	-	6.144 l
Manjishtha	*Rubia cordifolia* L.	Root	96.0 g
Haritaki	*Terminalia chebula* Retz.	Pericarp	24.0 g
Bibhitaki	*Terminalia bellerica* Roxb.	Pericarp	24.0 g
Amalaki	*Emblica officinalis* Gaertn.	Pericarp	24.0 g
Bala	*Coleus vettiveroides* K. C. Jacob.	Root	24.0 g
Haridra	*Curcuma longa* L.	Rhizome	24.0 g
Jaladhara (*Mushtha*)	*Cyperus rotundus* L.	Rhizome	24.0 g
Lodhra	*Symplocos racemosa* Roxb.	Stem bark	24.0 g
Suchipushpa (*Ketaki*)	*Pandanus odoratissimus* L.	Root	24.0 g
Vatankura (*Nyagrodha*)	*Ficus benghalensis* L.	Leaf bud	24.0 g
Nalika (*Naluka*)	*Cinnamomum verum* J. Presl.	Stem bark	24.0 g
Krishna Tila Taila	*Sesamum indicum* L.	Sesame oil	1.536 l

Table 2: List of ingredients for the preparation of *Shadbindu Taila*

Sanskrit name	Description	Part used	Quantity
Murcchita Krishna Tila Taila	*Sesamum indicum* L.	Processed sesame oil	0.768 l
Bhringa Rasa	*Eclipta alba* (L.) Hassk.	Juice of whole plant	3.072 l
Eranda	*Ricinus communis* L.	Root	19.0 g
Tagara	*Valeriana wallichii* DC.	Rhizome	19.0 g
Shatahva	*Anethum sowa* Roxb.	Fruit	19.0 g
Jivanti	*Leptadenia reticulata* (Retz.) Wight and Arn.	Root	19.0 g
Rasna	*Alpinia galanga* (L.) Swartz	Root	19.0 g
Bhringa	*Eclipta alba* (L.) Hassk.	Whole plant	19.0 g
Vidanga	*Embelia ribes* Burm.f.	Fruit	19.0 g
Yashti	*Glycyrrhiza glabra* L.	Root	19.0 g
Shunthi	*Zingiber officinale* Roscoe.	Rhizome	19.0 g
Saindhava Lavana	Rock salt	-	19.0 g
Aja Paya	Goat milk	-	3.072 l

- Preliminary phytochemical tests for major secondary metabolites[3]
- Physicochemical analysis[4]: Comparative evaluation of ST with KTT and MKTT.
- Extraction of phytochemical constituents from ST:
  For extraction 5 ml ST was mixed with 10 ml of 90% aqueous methanol solvent. The mixture was stirred for 1h by using a magnetic stirrer. Then it was stored in deep freezer at –20°C for 2 days. After 2 days, mixture was filtered through Whatman filter paper no. 41 and filtrate was used for HPTLC profiling.
- HPTLC profile for estimation of kaempferol and method validation.[5]
  Equipment details: Sample spotter (CAMAG Linomat 5 with syringe [Hamilton]), Visualizer (CAMAG UV Cabinet), Phodocumentation unit (CAMAG Reprostar 3), Scanner (CAMAG Scanner 4 with winCATS planar chromatography manager software version 1.4.7), Wavelength (254 nm), Stationary phase (Merck silica gel 60 F_{254} HPTLC pre-coated plates of 0.2 mm thickness with aluminium sheet support), Mobile phase (n-hexane: ethyl acetate: formic acid (6.0:4:0.2, v/v/v)].
- Safety evaluation: Heavy metal analysis using - Inductively Coupled Plamsa-Optical Emission Spectroscopic (ICP-OES) technique and skin irritation test in animals.[6,7]

Statistical analysis

Microsoft Excel-2007 was used to determine mean, standard deviation, relative standard deviation and mean difference during the analysis.

Results

A bio-analytical approach was employed for the quality control and standardization of ST. SOP for the preparation of ST was developed and ST was prepared in three batches. Tables 3-8 provide the results of the analysis carried out on ST.

Discussion

Quality assurance is an integral part of all systems of medicine to ensure the quality medicament. Various multi-disciplinary and bio-analytical approaches such as development of SOP,[8] preliminary phytochemical and physicochemical evaluation,[9] chromatographic evaluation,[10] safety evaluation,[11] efficacy evaluation[12] etc., have been employed and reported for the scientific evaluation of various traditional formulations. In the present research work, only suitable and available techniques were selected for the quality evaluation of ST.

SOP of ST was prepared and ST was subjected to various quality methods to standardize for further studies and utility. *Murchhana* of KTT and preparation of ST were carried out till the achievement of desired *Sneha Siddhi Lakshana*. *Murcchana* resulted in the elimination of undesired smell and incorporation of color in KTT (pale yellow color of KTT changed to orange-red because of *Manjishtha*) and the entire process took about 40-42 h with the average recovery of 79.75 ± 0.08%. All three batches of ST were prepared in between 21 h and 22 h with the average recovery of 80.26 ± 1.01%. ST was found more viscous than MKTT, probably because of goat milk.

During the *Taila Murcchana* and preparation of ST, mild and indirect heating (peak temperature of 90°C-95°C) was carried out along with the continuous stirring for proper extraction, to lessen the possible chances of degradation of some active constituents, which may be decomposed due to hydrolysis and to facilitate the natural circulation evaporation.

Qualitative tests are used to detect the presence of some major phytochemicals which play a very important role in the expression of biological activity.[13] Almost all the phytochemical groups analyzed were found to be available in ST except resins and steroids. Presence of such secondary metabolites into ST assures the traditional process of medicating the oils.

Table 3: Results of preliminary phytochemical analysis of *Shadbindu Taila*

Major phytochemicals	Results
Flavonoids	+
Essential oils	+
Tannins	+
Glycosides	+
Alkaloids	+
Resins	–
Steroids	–

+: Present, –: Absent

Table 4: Results of physicochemical analysis of *Krishna Tila Taila, Murcchita Krishna Tila Taila* and *Shadbindu Taila*

Parameters	Results (mean±SD, *n*=3)		
	KTT	MKTT	ST
Refractive index	1.468±0.018	1.475±0.010	1.463±0.017
Specific gravity (g/mL)	0.912±0.006	0.908±0.003	0.909±0.005
Optical rotation (°)	2.403±0.095	1.351±0.094	1.354±0.142
Unsaponifiable matter (%)	1.398±0.030	3.562±0.124	4.439±0.098
Acid value (mg KOH/g)×10^{-2}	54.50±1.153	67.00±1.418	65.80±0.656
Saponification value (mg KOH/g)	189.20±1.833	195.60±0.794	195.50±1.637
Iodine value (g I_2/100 g)	103.40±1.212	97.90±1.000	98.50±1.442
Peroxide value (Meq KOH/g)×10^{-1}	73.01±1.454	32.47±1.072	22.82±1.256

KTT: *Krishna Tila Taila*, MKTT: *Murcchita Krishna Tila* Taila, ST: *Shadbindu Taila*, KOH: Potassium hydroxide

The physicochemical parameters of KTT, MKTT and ST were compared. In comparison with KTT, acid value, saponification value and unsaponifiable matter of MKTT and ST were increased while the iodine and peroxide values were decreased. There was no change observed in the refractive index and specific gravity of KTT, MKTT and ST; while variations in optical rotation were observed. The results were in compliance with some recently published reports on physicochemical analysis of some Ayurvedic *Taila*.[8,11] These observations suggest that *Taila Paka Vidhi* affects physicochemical parameters of *Sneha Dravya* during processing.

The ingredients of ST possess potential therapeutic agents such as kaempferol, luteolin, embelin, lupeol, β-sitosterol, etc., Presence of these markers in ST was evaluated using HPTLC technique. Extraction of these markers from ST was found to be a daunting task because of its complex oil-based matrix and herbo-mineral nature. This created a major problem during development of phytochemical fingerprint and detection/separation of marker compound(s). Hence, a reported method for the extraction of phytochemical constituents from *Taila*[1] was modified and optimized in order to develop phytochemical fingerprint and to separate active principles from ST.

As, kaempferol is reported to possess anticancer, antioxidant, anti-inflammatory, analgesic and antimicrobial activity,[14] a HPTLC method was developed in order to separate it from other interfering phytochemical constituents of ST. This was achieved on HPTLC plates using n-hexane: ethyl acetate: formic acid (6.0:4:0.2, v/v/v) as a mobile phase. In HPTLC of ST, five major spots were observed at

Table 5: Results of HPTLC evaluation of *Shadbindu Taila*

Spot no.	R_f	Color
1	0.32	Dark brown
2	0.43	Light brown
3	0.54	Dark brown
4	0.67	Light brown
5	0.72	Light brown

HPTLC: High Performance Thin Layer Chromatographic

Table 6: Results of HPTLC method validation for the estimation of kaempferol

Parameters	Results
Specificity	Specific
LOD (µg/mL)	2.0
LOQ (µg/mL)	5.0
Linear working range (µg/mL)	5.0-125.0
Regression equation	y=11.689x+42.606
Correlation coefficient (r^2)	0.999
Instrumental precision (% RSD, n=7)	0.61
Repeatability (% RSD, n=5)	0.75
Intra-day precision (% RSD, n=3)	0.91-1.21
Inter-day precision (% RSD, n=3)	0.95-1.29
Average recovery (% mean±SD, n=3)	99.11±0.980
Assay (content of kaempferol in ST, mg/mL)	0.0845±0.0032
Ruggedness	Rugged

LOD: Limit of detection, LOQ: Limit of quantification, RSD: Relative Standard Deviation, ST: *Shadbindu Taila*, HPTLC: High Performance Thin Layer Chromatographic

Table 7: Results of heavy metal analysis of *Shadbindu Taila*

Heavy metal	Results	Limit of detection of the instrument (mg/kg)
Lead (Pb)	Not detected	1.0
Arsenic (As)	Not detected	1.0
Cadmium (Cd)	Not detected	1.0
Mercury (Hg)	Not detected	1.0

Table 8: Results of skin irritation potential of *Shadbindu Taila* on rabbit skin

Rabbit id no.	Total erythema+edema						Average
	24 h		48 h		72 h		
	Control	Test	Control	Test	Control	Test	
R-01	0	0	0	0	0	0	0
R-02	0	0	0	0	0	0	0
R-03	0	0	0	0	0	0	0
Mean	0	0	0	0	0	0	P. I. I.=0

P. I. I.: Primary Irritation Index

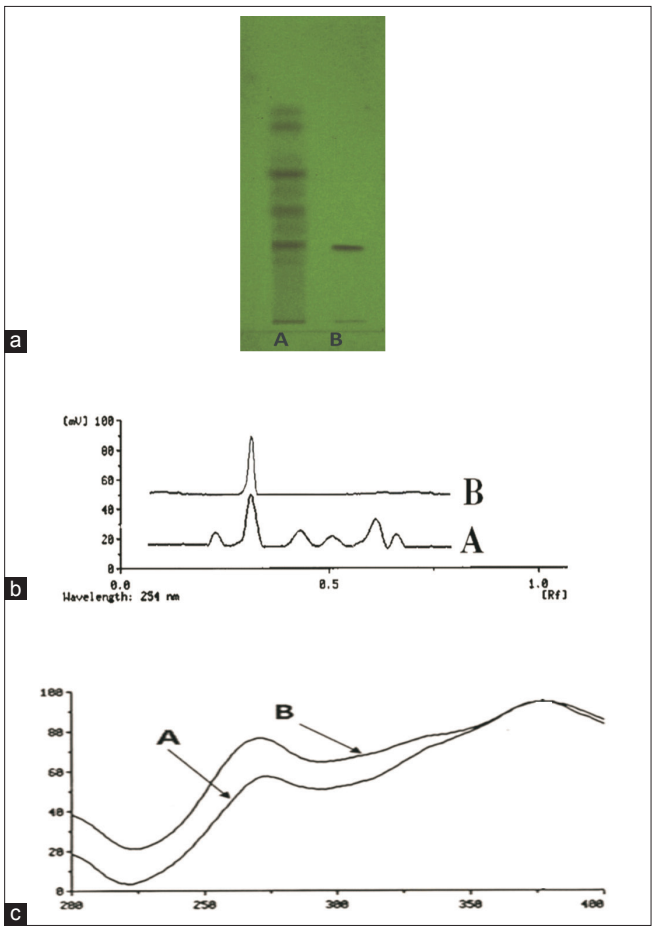

Figure 1: Kaempferol in *Shadbindu Taila* (ST) at UV-254 using HPTLC. (a) Plate photo (b) Overlay (c) absorption spectra of ST (A) and kaempferol (B)

254 nm [Table 5, Figure 1] indicating its possible compounds of the matrix which may be responsible for its therapeutic activity. Among these spots, kaempferol was detected at R_f = 0.32 and its identity in *Taila* matrix was confirmed by overlay and its UV absorption spectra with that of the standard [Figure 1]. Using the regression equation, the exact content of kaempferol was determined. The method was found rapid, specific, precise, sensitive and rugged during validation experiment. The developed HPTLC fingerprint of the drug along with the presence of kaempferol in it within the specified limits can be useful to verify the quality and purity of ST. This method can be applied to various polyherbal formulations of any matrices containing kaempferol.

ST was analyzed for the presence of some heavy metals which were found to be below the detection limits of the instrument. Topical application of ST on rabbit skin did not show any adverse effects and dermal irritation which was well supported by the value of primary irritation index. Heavy metal analysis and skin irritation study revealed that ST has adequate safety margin to be used on human skin.

The quality control parameters resulted after scientific evaluation of ST can be used as reference standard for quality control/assurance laboratory of a pharmaceutical firm in order to have a proper quality check over its preparation and processing. Use of such scientific techniques routinely may lead to standardization of ST to a certain extent and would definitely help in building confidence in its use.

Conclusion

It can be concluded that the bio-analytical approaches used in the present study is useful in the quality control and standardization of ST. As ST contains a wide range of phytochemical components, it is needed to validate its therapeutic utility through preclinical and clinical studies.

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हिन्दी सारांश

षडबिंदु तैल का मानकीकरण – एक तैलीय आधारयुक्त आयुर्वेदिक औषधि

सुनीता शैलेजन, शशिकुमार एन. मेनन, भावेश आर. तिवारी, आशिष एस. सिंह

आयुर्वेदिक औषधि षडबिंदु तैल का उपयोग दंतचलन, दृष्टिदौर्बल्य, केशशत, शिरोरोग इत्यादि व्याधियों में किया जाता रहा है । वर्तमान अध्ययन षडबिंदु तैल के गुणवत्ता नियंत्रण और मानकीकरण हेतु कुछ नए दृष्टिकोणों को विकसित करने के लिए एक प्रयास है । षडबिंदु तैल के निर्माण हेतु मानकीकृत संचालन प्रक्रिया को भारतीय आयुर्वेदिक फॉर्म्युलरी के आधार पर विकसित किया गया । षडबिंदु तैल का प्रारंभिक पादप–रासायनिक, भौतिक–रासायनिक और वर्णलेखन विश्लेषण किया गया । षडबिंदु तैल की सुरक्षा का मूल्यांकन त्वचा की उत्तेजना/जलन परीक्षण और भारी धातुओं की उपस्थिति के आधार पर किया गया । षडबिंदु तैल का रासायनिक परीक्षण केम्फेरॉल के आधार पर मानक एच.पी.टी.एल.सी. प्रक्रिया द्वारा किया गया । उपरोक्त प्रयोगों के आधार पर षडबिंदु तैल में किसी भी प्रकार की भारी धातुओं की उपस्थिति नहीं पाई गई और इस तैल द्वारा खरगोश की त्वचा पर किसी भी प्रकार की उत्तेजना जन्य जलन उत्पन्न नहीं हुई । षडबिंदु तैल के वैज्ञानिक मूल्यांकन के पश्चात निर्मित इन गुणवत्ता नियंत्रण के मापदंडों का उपयोग संपर्क मानक के रूप में किसी भी औषधि उद्योग के गुणवत्ता नियंत्रण/आश्वासन प्रयोगशाला में इस तैल के निर्माण और प्रसंस्करण की प्रक्रिया के दौरान उचित गुणवत्ता की जाँच हेतु किया जा सकता है ।