

RESEARCH ARTICLE

A Comparitive Pharmaceutico - Analytical Standardisation of *Vachalasunadi Taila* with *Murchitha* and *Amurchitha Tila Taila*

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ABSTRACT

BACKGROUND: In Ayurveda Classics, *Sneha Kalpanas* constitutes to be the most widely used and preferred formulations as it incorporates both water and lipid soluble active constituents of the medicine adding to its therapeutic efficacy. *Vachalasunadi Taila (VT)* is a potent poly herbal formulation explained in the context of Ear Diseases in *Sahasrayogam*. No research work has been carried out till date and hence an attempt is made to evaluate the Pharmaceutico -analytical standardization of VT with special reference to *Murchita (MTT)* and *Amurchitha tila taila (ATT)*.

METHODOLOGY: *VT* was prepared as per the reference of *Sharangadhara Samhita*. The sample was subjected to different phytochemical parameters like pharmacognostical, physico-chemical, and instrumental method of analysis comparing *Murchitha* (MTT) and *Amurchitha* (ATT) *Tila Taila*.

RESULT: Results for parameters includes specific gravity (0.89, 0.91 gm/ml), Refractive Index (1.486, 1.495), acid value (0.44, 0.45), iodine value (111.81, 113.67%), saponification value (187.09, 190.65), ester value (186.65, 190.2). Instrumental analysis with HPTLC and GC-MS for *MTT* and *ATT* also showed a slight difference.

CONCLUSION

The Pharmaceutico-Analytical standardization of samples reveals an improved stability data in terms of longer shelf life for VT prepared with MTT and VTA showed significant results in all other assessed organoleptic and physico chemical parameters.

KEYWORDS

Sneha Kalpana, Pharmaceutico-Analytical, Standardization, Vachalasunadi Taila



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INTRODUCTION

Sneha kalpana is an important formulation in Ayurveda. The etymology of sneha itself denotes its irreplaceable niche in the medical industry where Sneha derives to be the tailadi rasa bhedaand kalpana to be *kripu Samarthya*, which denotes any fat or fatty material capable enough to generate power in any desired matter.Before preparing any sneha kalpana the sneha is subjected to Murchana process with a view to remove gandha dosha mentioned in Bhaishajya kalpana. VT refers to a compound Ayurveda formulation prepared by using Tila Taila, Vacha, Lashuna, Haridra and Vilwa Patra Swarasa (Table 1).It is a formulation mentioned in Sharangadhara Samhitha, Madhyama Khanta and Sahasrayogam in the context of *karna roga*¹. It is a very famous poly herbal formulation frequently used by different Ayurvedic physicians effectively in ear injuries, pus collection, ear discharge and related ear problems in the form of karna Pooranam and Shiro Pichu.

The quality of a drug and looking at the effectiveness of the formulation of VT, there is a high need in the light of scientific evaluation. In the present era in order to establish the safety concern, the prepared drugs have to be understood well and interpreted with the help of modern

technology backed by proper scientific validation and this in turn will add to the scientific basis and credibility of the Ayurveda drugs and formulations in this pharmaceutical era. The use of readily available and genuine ingredients ensures the potency and efficacy of the formulation. Hence, a comparative pharmaceuticalanalytical study of VT prepared with Murchita (MTT) and Amurchitha Tila Taila (*ATT*) as per standard operating procedures (SOP) was attempted using the analytical methodologies encompassing raw material analysis, phytochemical screening. organoleptic parameters, HPTLC and GC-MS for making a preliminary data of the formulation.

AIMS AND OBJECTIVES

1. To prepare *Vacha Lashunadi Taila* with *Murchita* and *Amurchita Tila Taila* as per Standard Operating Procedures (SOP).

2. To observe the Comparative Pharmaceutico - Analytical Standardization of Vacha Lashunadi Taila prepared with Murchitha Tila Taila and Amurchitha Tila Taila.

MATERIALS AND METHODS

The raw materials for the preparation of *Vacha Lashunadi Taila* were collected from reliable sources and the study was

conducted at MIAMS, Rasa Bhaishajya Practical hall, Manipal, Karnataka. All the raw drugs were recognized and their purity was established. The ingredients and the part used are mentioned in (Table 1).

Fable 1 Ing	gredients of Vac	cha Lashunadi Taila			
S. No.	DRUG	BOTANICAL NAME	PART USED	PART	QUANTITY
1	Vacha	Acorus calamus	Rhizome)
2	Lashuna	Alium sativum	Bulb }	1 (Kalka)	≻75gm
3	Dosha	Curcuma longa	Kanda J		J
4	Bilva	Aegle marmelos	Swarasa	16	1.2 L
5	Tila taila	Sesamum indicum		4	300 ml

Method of preparation of VT:

The SOP for the preparation of *VT* involves following steps:

• Preparation of *MTT*: The reference

from Ayurvedic Formulary of India² was

Table 1.1 Ingredients for the	e murchana of tila taila
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followed for *murchana* of *tila taila*. Ingredients and part used are mentioned in table 1.1

Labic	1.1 Ingredients for				
S1.	INGREDIENTS	SCIENTIFIC NAME	PARTS USED	RATIO	QUANTITY
No					
1	Manjishta	Rubia cordifolia L.	Stem	1/16 of Sneha	93.75 gms
2	Haridra	Curcuma longa L.	Rhizome	1/4 of Manjishta	23.4 gms
3	Lodhra	Symplocos racemosaRoxb.	Stem bark	1/4 of Manjishta	23.4 gms
4	Mustha	Cyperus rotundus L.	Rhizome	1/4 of Manjishta	23.4 gms
5	Nalika	Cinnamomum verum J. Presl.	Leaves	1/4 of Manjishta	23.4 gms
6	Amalaki	Emblica officinalis Gaertn.	Pericarp	1/4 of Manjishta	23.4 gms
7	Harithaki	Terminalia chebula Retz.	Pericarp	1/4 of Manjishta	23.4 gms
8	Bhibhithaki	Terminalia bellerica Roxb.	Pericarp	1/4 of Manjishta	23.4 gms
9	Vatankura	Ficusbenghalensis L.	Rhizopods	1/4 of Manjishta	23.4 gms
10	Hribera	Coleus Zeylanicus	Whole plant	1/4 of Manjishta	23.4 gms
11	Kethaki	Pandanus odoratissimus L.	Root	1/4 of Manjishta	23.4
•	Preparation of	Bilva Swarasa: Fresh	samples VT	M and VTA	respectively.

• Preparation of *Bilva Swarasa*: Fresh Juice obtained from the macerated leaves of *Aegle marmelos* was considered as *Bilva Swarasa*.

• Preparation of *Kalka:* Each *kalka dravya* was taken in a vessel and mixed, followed by addition of sufficient amount of water until a uniform paste was obtained.

• Preparation of *VT*: The reference from *Sharangadhara Samhita*³wasfollowed for the preparation of *VT*, for obtaining the

Ingredients and parts used are mentioned in Table 1. Four parts of *MTT/ATT (300 ml)* was taken in a stainless steel vessel, directly heated on *Mandagni*. One part of (*Vacha*, *Lasuna*, *Haridra*) kalka and later 16 parts of *Bilva patra* swarasa was added and mixed well. This mixture was heated on *Mandagni* with continuous slow stirring for proper mixing. On obtaining all siddha lakshanas (optimal features), the heating was discontinued and both samples of *VT* were derived from *MTT* and *ATT* and was filtered through a clean cloth, transferred to Amber colored plastic bottle containers, labeled and stored.

Organoleptic Evaluation

• Analytical study for standardization was carried out on basis of classically illustrated organoleptic tests. Color, odor, taste and consistency were analyzed.

Physico-Chemical Evaluation

VTsubjected was to physicochemical study in order to develop analytical profiles. Parameters of physicochemical properties like loss on drying⁴. refractive index⁴. value⁴. acid value⁴. iodine Saponification value⁴. peroxide value⁴, specific gravity⁴ (melted), ester value⁴, kries test for rancidity⁴, shelf life study⁴were conducted.

• Qualitative tests were carried out for glycosides, saponins alkaloid, flavonoid, tannin, steroid. This task is undertaken to evaluate and to compare the formulation with the available physicochemical parameters.

Instrumental Method of Analysis:

• For HPTLC analysis, methanol extract of the samples were used to develop HPTLC. Stationary phase used was silica gel 60F 254 HPTLC Plates with the solvent system Toluene:Ethylacetate:Hexame (6:3:1). Curcumin a Bio-Marker of *Haridra* is used as a standard marker for qualitative analysis and quantitative estimation in the samples.

• GC MS Study was performed by Agilent GCMS 5975 C with FID using HP-5 capillary column using a Shimadzu 17A gas chromatograph fitted with a split-split less injector and a DB-5 fused silica capillary column. The spectra of the compounds were matched with NIST and Wiley library and their structures were defined by the % similarity values.

RESULTS

1. Organoleptic Characters

VT prepared by ATT was dark brown with greenish yellow tinge whereas MTT exhibited a dark brown colour with a reddish yellow tinge. All the samples had characteristic odor due to Vacha and Lashuna, Katu, Tikta rasa and consistency oily liquid (Table2)

PARAMETERS	VTM	VTA
Colour	Brownish with	Brownish
	reddish yellow	with greenish
	tinge	yellow tinge
Odor	Characteristic	Characteristic
	smell	smell
Taste	Katu, Tikta	Katu, Tikta
Consistency	Liquid, Oily	Liquid, Oily

 Table 2. Organoleptic Characters of VT

2. **Physico Chemical Parameters**

Both the samples were tested for basic physico Chemical parameters to assess the quality (Table 3). In the present study the following observations were ruled out after careful evaluation of the Physico-Chemical Parameters.

Table 3 Physico Chemical Parameters of VT

PARAMETERS	VTM	VTA
Loss on drying	0.883	0.708
Specific gravity	0.91	0.90
Refractive Index	1.488	1.495
Acid value	2.60	1.67
Iodine value	95.7	100.51
Saponification value	318.27	334.30
Viscosity	66.5cp	53.71cp
Ester value	315.67	332.6
Peroxide value	2.8908	16.9321
Rancidity-Shelf life	-ve	ve
HDTI C Analysia		

HPTLC Analysis

Table 4 Physico Cl	e 4 Physico Chemical Parameters of VT		
PARAMETER S	BATC H	RESULT (%CURCUMIN	
HPTLC Finger print	VTM) 0.45%	
HPTLC Finger print	VTA	0.28%	

It was observed that both samples of VTcontain curcumin in a quantity 0.45 % and 0.28 % respectively (Table 4). Higher amount curcumin in VTM sample may be due to the murchana $process^{5,6,7}$

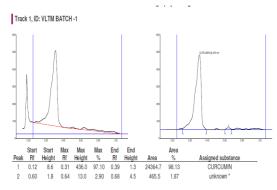
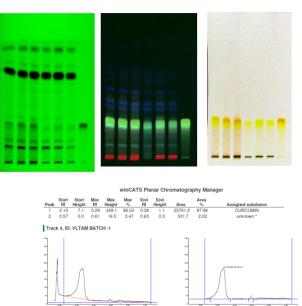


Figure 1 Highest peak value of VT

At 254nm At 366nm White R



End Rf 0.35

% 97.87 Area 11364.6

Start Height 7.7 0.7 Max Rf 0.27 0.59 Max Height 187.1 Max % 94.46 5.54 Figure 2 HPTLC photo documentation of sample of Curcumin fraction of VT Comparing both samples, Elaidic acid/ oleic acid and linoleic acid where the major fatty acids present which constitute about 42.40%, 34.42% in VTM and 42.355, 34.64% in VTA respectively. Apart from this, stearic acid and palmitic acid where observed in all the samples in considerable addition amount. In Myristic acid. Palmitoleic acid, Margaric acid, Arachidic acid. Eicosenoic acid. Behenic acid. Lignoceric acid and 11-Octadecanoic acid were observed in traces in both the samples

DISCUSSION

The present study was planned with the consideration of the fact that Taila Kalpana is the widely used medicinal preparation⁸, useful in several vata disorders. It is one of the best drug delivery systems adopted in Ayurveda. Since the study needed comparison between Murchita and Amurchitha taila preparation, the pharmaceutical study was done in these stages. As a prerequisite, the tests for purity of all the raw drugs were ensured. At the end of Taila Murchana, it was considered that there was a loss of about 7.3% in the final volume of the oil. There was also a loss of about 11.6% To 16.6% in the final volume of the taila in VTM sample whereas it was about 6.6-10 % loss in VTA final sample. Similar changes in colour and odor were observed in the product on gradually heating the samples but the prolonged loss was about 6.4 % on low heating and that was minimal among the samples. This that the standard operative indicates procedures were perfectly practiced by the time. Both the samples of VT were subjected to Organoleptic, Physiochemical and instrumental method of analysis. The sample of taila prepared by ATT shows a dark brown with greenish yellow tinge. The greenish color is the attribution of chlorophyll from Bilva patra swarasa and that prepared by using MTT were dark brown in colour with reddish yellow tinge. The reddish yellow colour may be due to the process of Murchana. Here the greenish colour is probably masked by other ingredients used for Murchana purpose.

The VTM and VTA samples were tested for basic Physico Chemical Parameters to assess the quality. Loss on Drying indicates the moisture content⁹ which is very critical. Moisture content should be minimal in order to prevent the decomposition of medicaments due to the chemical change or rancidity^{10, 9}. Both the evaluated samples denote an average values within the acceptable limits. Specific gravity is one of the important parameter for oil¹¹. In the case of VT samples prepared by MTT and ATT specific gravity was 0.91 and 0.708 respectively. According to Ayurveda, the specific gravity could be compared to the Guru (heavy for preservation of Puran Ghee was well done and digestion) and Snigdha (slimy, soft or fatty) quality of the formulation and this in turn indicates the increased density of molecules or solute content. Refractive indices of both the samples were stable with an average 1.488 and 1.495 respectively. Refractive index is an important parameter to assess the quality of oil or for identifying a substance in order to determine its purity or concentration. Both the samples reveal a negligible difference indicating marginally lesser concentration of the turbid materials.

Acid value indicates the amount of free fatty acids present in oils and fats¹² and in the present study samples, MTT and ATT showed an average acid value of 2.60 and

1.67 respectively. The changes observed in acid values suggest that the MTT is more saturated when compared to ATT. That is higher the free fatty acid concentration greater the rancidity and this helps in deciding the shelf life of the taila. The acid value for MTT was found to be good, indicating the longer shelf life of taila. Determination of iodine value is useful for determining the quality of oil¹³ or its freedom from adulteration. Iodine value is the degree of unsaturation in fat, also reflecting the susceptibility to oxidation. Higher the degree of unsaturation, greater is the possibility of rancidification due to absorption and atmospheric oxidation. Also an increase in iodine value detects a fair increase in the number of unsaturated fatty acid bonds which can better be absorbed when compared to saturated fatty acids. In the current study, MTT and ATT samples reveals an iodine value of 95.7 and 100.51 respectively. VT prepared with ATT was found to be fairly good indicating the less rancidity. The saponification value indicates the measure of fatty acid present as esters in the given fat¹⁵. It gives an idea about the molecular weight of oils or chain length of all fatty acids. Longer the chain of fatty acids. lower the value of saponification value and rate of absorption. In the current study the saponification values of MTT and ATT was found to be

318.27 and 334.30 respectively and there is also a greater probability of increasing this value indicative of more and more short chain fatty acids are generated as the time advances. Viscosity is the resistance offered by the surface to the flow of a liquid. Higher the viscocity of the taila, greater is its resistance to flow and lesser the rate of absorption. In the current study, viscosity of MTT and ATT was found to be 66.5 cp and 53.71 indicating that the VT prepared with ATT was found to be fairly good in its absorption indices.

Ester value is the number of milligram of potassium hydroxide required to saponify the ester in 1gm of the substance¹⁶. It is similar to saponification value hence same observation of saponification value could be seen here also and the value was found to be 186.65 and 190.2 respectively for MTT and ATT. Peroxide value indicates the value of degree of rancidification of oils. The increase in the value of peroxide number indicates that the oils have turn rancid or spoiled. The normal limit is 10. In the present study MTT samples showed stable and less peroxide value with an average of 2.8908 in the case of ATT sample VTA had an exceeding peroxide value of 16.932 an average peroxide value was 7.3733 indicatively more tendency of the sample for rancidity. In the present study the stability data of VT was found to

be greater in that prepared with MTT. Real time stability data was collected mainly organoleptic parameters and some physico chemical parameters (already described) are considered. Both the sample remained stable with their organoleptic character like colour, odour, taste, consistency till 4th month. During 5th month slight fungal growth appeared in both the sample, which slightly got increased during 6th month. When saponification value and peroxide value are considered VTM samples were found to be more stable however there were no difference between the samples when organoleptic characters are considered. Rancidity test of samples were carried out with the help of Kries test. All the samples turned rancid with an appearance of pinkish colour in 5th month however it can be said that both the samples were stable (VTM and VTA) till 4 months.

Plane chromatography widely used for both qualitative and quantitative analysis of drugs is very useful in standardization of herbal products High Performance Thin Layer chromatography is an advance automated form of Thin layer chromatography. HPTLC is an invaluable quality assessment widely accepted for the evaluation of botanical raw materials it allows for the analysis of broad number of compound efficiently. When compared to other tools of chromatography, HPTLC is

the most reliable and cost-effective technique while considering analysis of botanical herbal drugs. In VTM a prominent peak with maximum Rf value between 0.29 and 0.31 (Figure 1) were observed which corresponds to standard curcumin with max Rf of 0.61. Similar compounds were observed in VTA samples however VTA showed an additional compound to the R_f value of 0.59 and 0.61 may be due to Lashuna. Standard bio marker curcumin was estimated in all the samples. It was observed that VT samples showed curcumin in a quantity varying from 0.44 % to 0. 046% making an average 0.45%. of The HPTLC photo documentation of sample of curcumin fraction of VT is shown in Figure 2. The samples of VT prepared by ATT also showed the presence of curcumin varying from 0.20% to 0.037% with an average of 0.28%. Higher amount curcumin in MTT samples may be due to murchana process. Gas chromatography also known as gas liquid chromatography is a technique of separation of mixtures into component by a process which depends on the redistribution

process which depends on the redistribution of compounds between a stationary phase or a support material into form of oil, solid or combination of both and a gaseous mobile phase. In both the samples (Table 5), Elaidic acid / oleic acid and linoleic acid where the major fatty acids present which constitute about 75% of total fatty acids.

Table 5 Physico Chemical Parameters ofVT

VI		
PARAMETERS		
FATTY ACID	VTM	VTA
PROFILE		
Myristic acid	0.05%	0.02%
Palmitic acid	11.08%	11.66%
Palmitoleic acid	0.17%	0.19%
Margaric acid	0.06%	0.06%
Stearic acid	8.88%	8.46%
Elaidic acid/Oleic	42.40%	42.35%
acid		
11-Octadecanoic	1.06%	0.97%
acid		
Linoleic acid	34.42%	34.64%
Linolenic acid	0.33%	0.31%
Arachidic acid	1.0%	0.84%
Eicosenoic acid	0.16%	0.14%
Behenic acid	0.23%	0.32%
Lignoceric acid	0.15%	0.04%

Apart from this, stearic acid and palmitic acid where observed in all the samples in considerable amount. VTM sample in addition were identified with Myristic acid, Palmitoleic acid, Margaric acid, Arachidic acid, Eicosenoic acid, Behenic acid, Lignoceric acid and 11-Octadecanoic acid in small amount and similar components were also observed in VTA. These are the regular fatty acid components of sesame oil. Oleic acid is a mono unsaturated omega-a fatty acid capable of enhancing the immune systems and can combat infections. It also has antioxidant, anti-inflammatory and wound healing effects¹⁷. However oleic acid in excess can cause the blockage by using Linolenic acid which is secondary major fatty acid found in the current study.

The oils that are high in linoleic acid have antibacterial properties, so that they clean much deeper and can help get rid of various clinical manifestations. Palmitic-acid-9hydroxy-stearic acid (9-PAHSA) is another signaling molecule that exerts antiinflammatory effects. Several derivatives of palmitic acid function as cell signaling molecules — meaning that they bind to cell receptors and trigger specific effects. Palmitic acid is also beneficial in killing any microbial contamination in the ear From these evidences it can be canal. predicted that Elaidic acid, linoleic acid and palmitic acid etc present in VT are having health beneficial effects which are assigned to be the benefits of Vachalasunadi Taila. Time factor might have played a great role in changing the physico-chemical profile of VT samples. Present work has evaluated the comparative Pharmaceutico-Analytical standardization of samples prepared with VTM and VTA.

CONCLUSION

The Pharmaceutico-Analytical standardization of samples revealed an improved stability in terms of longer shelf in *Vachalasunadi taila* prepared with *murchitha tila taila* whereas all other organoleptic characters and physico chemical parameters predicted significant results in VT prepared with *Amurchitha tila taila.* The analytical studies including HPTLC have helped to generate preliminary standards of data. In addition detailed compositional analysis by GCMS has also contributed significantly in assessing to its parameters.

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