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ANALYSIS AND STANDARDIZATION OF RASANJANADI TAILA: A STEP TOWARDS QUALITY CONTROL AND GLOBAL ACCEPTANCE

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ABSTRACT

Analysis of *Ayurveda* formulations is crucial to identify the bioactive compounds, their concentrations, and interactions which affect their therapeutic efficacy and safety.

Standardisation of these formulations is essential to ensure consistency in their composition, manufacturing process, and quality control, thereby guaranteeing their safety and efficacy. The quality and effectiveness of *Ayurveda* formulations can vary significantly due to the lack of standardised manufacturing processes and analytical methods. Inadequate analysis and standardisation of *Ayurveda* formulations can result in adverse effects, interactions, and contamination, posing a risk to patient safety. Furthermore, the absence of standardisation can impede the development of new *Ayurveda* formulations and restrict their global acceptance. *Rasanjanadi Taila*, a traditional *Ayurveda* formulation, is specifically cited by *Acharya Vangasena* in the *Nasarogadhikara* chapter for the treatment of *Pratishyaya* (Rhinitis), which comprises *Rasanjana*, *ShodithaAtivisha*, *Musta*, and *Devadaru*. The formulation was prepared in accordance with the AFI standards for *Taila* preparation. These drugs are very potent individually, and the *Taila* contains many *Teekshna Dravya* in it. There are no analytical standards available for *Rasanjanadi Taila* in AFI to check its quality. In this study, an effort has been made to prepare *Rasanjanadi Taila* and analyze its physicochemical properties, phytochemical constituents, and microbial load to ensure

its quality and efficacy.

Keywords: *Rasanjanadi Taila, Pratishyaya*, Standardization, Analysis, Therapeutic Efficacy

INTRODUCTION

Sneha Kalpana is a pharmaceutical process in *Ayurveda* that involves the preparation of oleaginous medicaments from various substances. These substances include *Sneha (Ghrita or Taila), Kalka, Kwatha* or *Drava Dravya*. These substances are taken in proportions and subjected to unique heating patterns and duration to fulfil specific pharmaceutical parameters[1]. Among *Sneha Kalpana, Taila* holds a significant place due to its therapeutic efficacy and versatility. *Rasanjanadi Taila* is one such polyherbal formulation, mentioned by *Acharya Vangasena* in the context of *Nasarogadhikara* for *Pratishyaya Chikitsa* which contains *Rasanjana (Daruharidra* – Coscinium fenestrated), *ShodithaAtivisha* (Aconitum heterophyllum), *Musta* (Cyperus rotundus) and *Devadaru* (Cedrus deodara)[2]. The quality and efficacy of *Rasanjanadi Taila* can vary significantly depending on factors such as source of raw materials, manufacturing processes and storage conditions. Standardisation is crucial to establish the authenticity and consistency of the formulations, which is essential for acceptance in modern healthcare systems. However, the analysis and standardisation of *Rasanjanadi Taila* pose significant challenges due to the presence of multiple bioactive compounds and the variability of their chemical composition.

This study aims to present the analysis and standardisation of *Rasanjanadi Taila*, including its phytochemical profiling, physicochemical characterisation and biological evaluation. The study employed an advanced analytical technique – HPLC, to identify and quantity the bioactive compounds present in the formulation.

METHOD OF PREPARATION OF RASANJANADI TAILA: The ingredients of *Rasanjanadi Taila* were procured from an authenticated source and identified by a Botanist. (Table No.1) (Fig No 1 – 4)

Table No 1 –Quantities of ingredients for the preparation of 2litres of *RasanjanadiTaila*

Sl.No	Ingredients	PartUsed	Quantity
1.	***Kwatha Dravya***		**8 litres**
	Rasanjana	-	1 kg
	ShodithaAtivisha	*Moola*	1 kg
	Musta	*Moola*	1 kg
	Devadaru	*Kanda*	1 kg
	Jala	-	64 ltrs
2.	***Kalka Dravya***		**500 gm**
	Rasanjana	-	125 gm
	ShodithaAtivisha	*Moola*	125 gm
	Musta	*Moola*	125 gm
	Devadaru	*Kanda*	125 gm
3.	***Tila Taila***		**2 ltrs**

Preparation of *Rasanjana*[3]:

2 kgs of coarsely powdered *Daruharidra Kanda* (Seive No. 10/44)[4] was added with 32 litres of water and reduced to 1/4th quantity (8 liters)[5]. Then to that *Kashaya* (decoction), an equal quantity of cow milk was added and boiled till it gets into a semi-solid consistency. (Fig No 5 -7)

Shodana* (purification) of *Ativisha[6]:

Ativisha was tied in a cloth and prepared *pottali* (a packet) and then kept in *Dola Yantra* (device used in *shodhana* process), filled with the decoction of cow dung. The *pottali* should not touch the bottom of the vessel. Then it was boiled for 3hours and dried in the sunlight. (Fig No 8)

Preparation of *Rasanjanadi Taila*[7]:

Coarsely powdered *ShodithaAtivisha, Musta* and *Devadaru*, along with *Rasanjana*, were taken, and *Kashaya* (decoction) was prepared by adding 64 litres of water and reduced to 1/4th quantity (8 liters). Then fine powders of *Rasanjana, ShodithaAtivisha, Musta* and *Devadaru* (Seive No. 85)[4]were taken, and *Kalka* (paste) prepared. After this, in a wide mouth vessel, *Tila Taila* was added and heated till foam started appearing. To the *Taila*, freshly prepared *Kashaya* (decoction) and *Kalka* (paste) were mixed and heated in *Mandagni* (low flame) with frequent stirring. Heating was continued till we get *Taila Siddhi Lakshana (Mrudu Paka)*. Then, it was filtered through a clean cloth in another vessel and stored in a clean container. Finally, *Rasanjanadi Taila* was packed in a sterile 50ml container and sealed. Then, labelling was done. (Fig No. 9 -15)

Taila Siddhi Pariksha[8]: *Sneha Kalka* was soft to the touch, possible to prepare *Varti* (wick) and produced a crackling sound when kept on fire.

ANALYSIS OF *RASANJANADI TAILA:*

The analytical study was carried out in JSS Drug Testing Laboratory, A Unit of JSS College of Pharmacy, Sri Shivarathreeshwara Nagara, Mysuru.(Table No 2)

Table No 2: Observations of Analytical Study of *RasanjanadiTaila*

Sl.No	Test	Observations
1.	Organoleptic Characters	Colour : Light Brown Odour : Characteristic Appearance: Viscous Liquid Taste: Astringent
2.	Weight	1.05 kg/m^3
3.	Refractive Index	1.442 m/sec
4.	Viscosity	112 s/cm^2
5.	SaponificationValue	121.93
6.	Iodine Value	64.66 g
7.	Peroxide value	1
8.	Free Fatty Acid	1.9635
9.	Total Fatty Matter	35%
10.	Acid Value	1.9635

ESTIMATION OF BERBERINE IN RASANJANADI TAILA BY HPLC METHOD

Materials and methods

Instrumentation:

The Shimadzu UFLC was employed for the study, which has an integrated vacuum degasser, automatic sample manager, ultra-performance binary solvent manager.AC-18 stationary phase (Shimadzu, Shimpack C-18, 150×4.6mm, 5µm) was used for chromatographic separation and detection was carried by a Photodiode array detector.

Method:

The mobile phase was made up of 0.3%Formicacid in water as(A) and Methanol asanorganic modifier (B), and it was delivered at a flow rate of 1.0ml/min in the binary gradient mode wherein mobile phase (A) was delivered at a concentration of 95% as compared to B for a run time of5 minutes. The sample was injected in a volume of 10µL. The column oven temperature was maintained at 30˚C throughout the chromatographic run. Detection was carried out at an UV wavelength of 266 nm.

CHROMATOGRAPHIC CONDITIONS:(TableNo3)

TableNo3: Chromatographic conditions used for *RasanjanadiTaila*

Sr.no	Parameters	Conditions
1.	Instrument Model	Shimadzu UFLC
2.	Column	ShimpackC-18Column(150×4.6mm, 5µm)
3.	Mobile phase	0.3%Formicacid:Methanol
4.	Run time	5 minutes
5.	Flowrate	1.0 ml/min
7.	Injection volume	10µl
8.	Columnoventemperature	30˚C
9.	Detector	Photo diodearray detector

Preparation of solutions:

Preparation of standard stock(1000ug/mL):

Weighed 100.03 mg of Berberine standard into a 100 mL volumetric flask, added methanol and sonicated to dissolve. Further made up the volume with methanol to 10 mL.

Preparation of working standard solutions: Respective dilutions of the standard stock of berberine prepared the working standard solutions in a concentration ranging from 0.5-100ug/mL.

Preparation of sample solution:

Transferred 1ml of the sample (*Rasanjanadi Taila*) into a 10 ml volumetric flask, added 5ml of methanol, sonicated and further made up the volume to 10 ml with methanol.

Linearity study: Linearity was established for berberine in the range of 0.5-100ug/mL with a correlation coefficient (R2) 0.999.

Determination Of Berberine Concentration in Sample: (FigNo16-18)

The concentration of berberine in the sample was determined from the calibration curve using the regression equation: y = 53242x – 11346

Where y(Peak area response of berberine in sample)=16833.0

x= unknown concentration of berberine in sample

y =53242x-11346

x=16833.0-11346/53242 =0.103ug/mL

Report: The concentration of Berberine in the *Rasanjanadi Taila* is 0.103ug/mL.

DISCUSSION

Despite the advancements in science, standardising *Ayurveda* formulations remains a challenge. To gain global acceptance and achieve desired results, it is essential to standardize both the drugsand their manufacturing processes. The primary goal of standardizing medicinal plants is to ensure their therapeutic efficacy. By adhering to standard guidelines, we can gather valuable information for the proper identification of phytoconstituents and their types. The development of advanced analytical techniques can serve as a valuable tool in herbal drug research, enabling manufacturers to establish quality standards and specifications. Analytical study provides the objective parameters to fix up the standards for quality of finished products.

The properties of drugs used in *Rasanjanadi Taila* are mentioned in Table No.4

Table No 4: Properties of drugs used in *RasanjanadiTaila*

Sl. No	Drug Used	Botanical-Name& Family	*Rasa*	*Guna*	*Vi-rya*	*Vipaka*	*Doshagnata& Karma*
1.	*Da-ruharidra*	*Coscini-umFenestra-tum Menispermaceae*	*Tikta Kashaya*	*Laghu Ruksha*	*Ushna*	*Katu*	*Kapha- pittashamakaShophahara-KanduharaVishaharaAnabhishyandi*
2.	*Ativi-sha*	*Aconitum het-erophyllum Ranunculaceae*	*Tik-taKa-tu*	*Laghu Ruksha*	*Ushna*	*Katu*	*Tridosha- shamaka Krimighna Stambhana Amapachana Lekhana*
3.	*Musta*	*Cyperus rotundus Cyperaceae*	*Tikta-KatuKash aya*	*Laghu Ruksha*	*Shee-ta*	*Katu*	*Kapha- pittashamakaShotahara Lek-hana*
4.	*Devada ru*	*Cedrusdeo-daraPinaceae*	*Tikta*	*Laghu Snigdha*	*Ushna*	*Katu*	*Kapha- vatashamakaShotahara Krimighna Kaphanissaraka Lekhana*
5.	*Tila*	*Sesa mum indi-cum Pedaliaceae*	*Madhura Kashaya Tikta*	*Gu-ruSnig dha*	*Ushna*	*Ma-d-hura*	*Vatashamaka Snehana Deepana Ra-sayana Balya*

The ingredients of *Rasanjanadi Taila* contains Berberine, Atisine, Hetidene, a-Cyperone, Cedrin, Atlantoneetc. As Berberine is the main constituent, analysis had been done through HPLC, which states that the concentration of Berberine in the *Rasanjanadi Taila* is 0.103ug/mL. Berberine has shown anti-inflammatory and antihistamine effects by inhibiting the production of pro-inflammatory cytokines and reducing inflammation in various diseases.[9] It also has antioxidant properties, which can help to protect against oxidative stress and cell damage.[10] It has also been found to have anti-cancer properties, inhibiting the growth of cancer cells and inducing apoptosis (cell death).[11].

CONCLUSION

This study aimed to formulate and standardize *Rasanjanadi Taila*. The developed and validated HPLC methods are simple, precise, and accurate, enabling the quantification of solvents in herbal raw materials and their formulations. These quality-control parameters and HPLC methods can be valuable tools for scientific organisations and manufacturers to develop standards.

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Fig No1: Daruharidra

FigNo2 : Ativisha

Fig No3 : Musta

Fig No4: Devadaru

FigNo5 :DaruharidraKashaya

FigNo6: DaruharidraKashaya+Ksheera

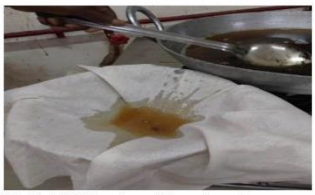
Fig No7 : Rasanjana

FigNo8 : Shodana of Ativisha

FigNo9:Kashaya of Rasanjana, Ativisha, Musta, Devadaru

FigNo10 : Shodana of Kashaya

FigNo11: Preparation of Rasanjanadi Taila



FigNo12: Filtration of Rasanjanadi Taila

Fig No13: Prepared Rasanjanadi Taila

Fig No 14:Filling in Sterilized Container

FigNo15: Rasanjanadi Taila

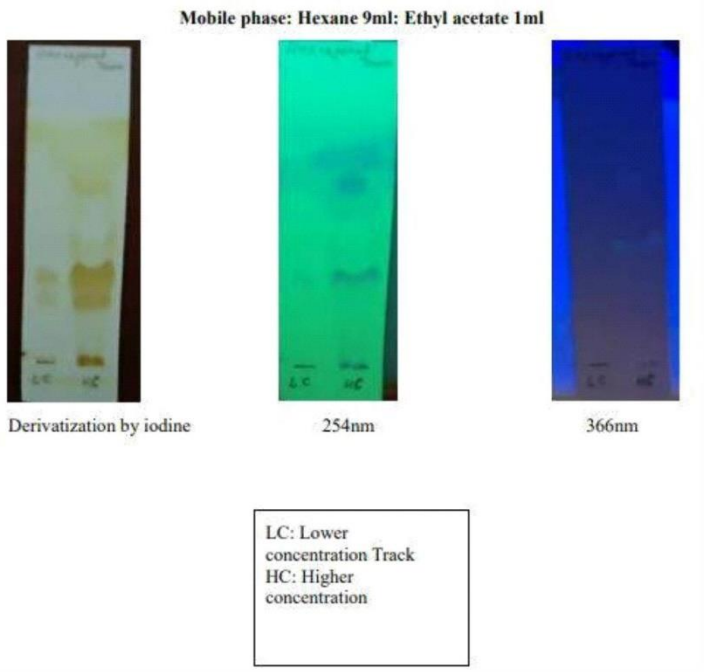
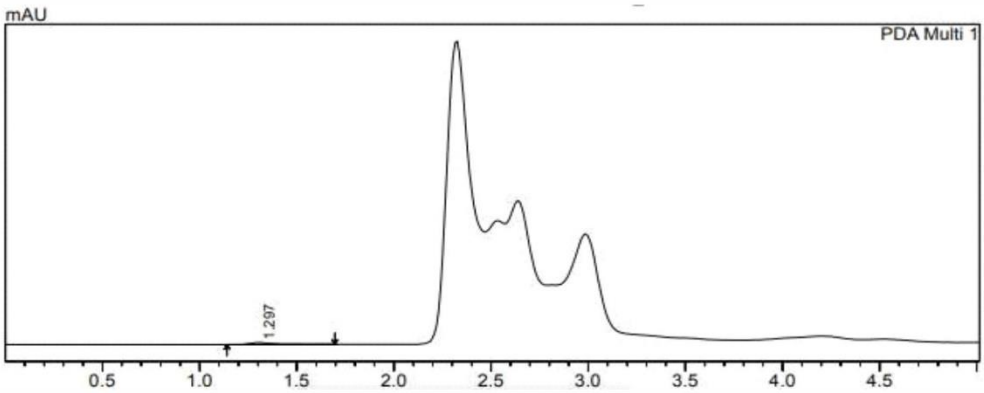
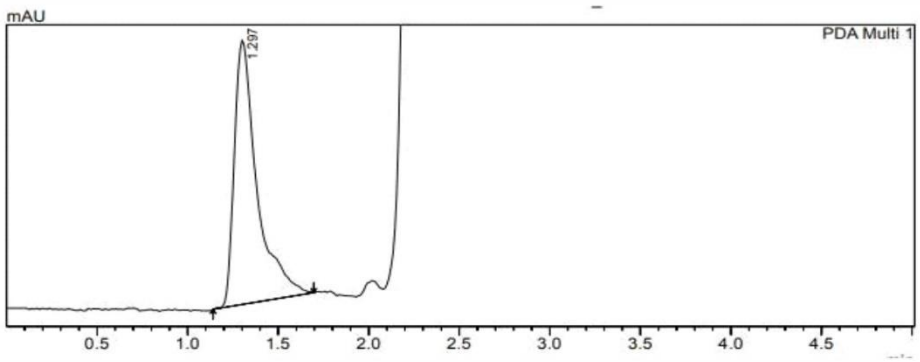


Fig No 16: TLC Profile of *Rasanjanadi Taila*



FigNo 17:HPLC Chromatogram of *Rasanjanadi Taila*



FigNo 18: Maximized viewof the sample peak at RT : 1.297