

COMPARATIVE PHARMACEUTICO ANALYTICAL STUDY OF VISHATINDUKADI VATI WITH SHUDDHA KUCHALA SHODHIT BY THREE DIFFERENT MEDIA

[Kadale Jagruti¹](#), [Dongre Sushma²](#)

¹PG Scholar, Department of Rasashastra and Bhaishyakalpana, Government Ayurved College, Osmanabad, Maharashtra - 413501, India

²PG Guide & Assistant Professor, Department of Rasashastra and Bhaishyakalpana, Government Ayurved College, Osmanabad, Maharashtra – 413501, India

Corresponding Author: jrsagbhor@gmail.com

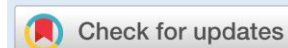
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ABSTRACT

Vishatindukadi vati (VTV); is a *Vishakalpa* containing *Visha dravya* i.e. *Kuchala* which has therapeutic potential is mentioned in *Agnimandya*, *Ajirna*, *Jeerna Vataroga*, *Pakshaghata*, *Nadi Daurbalya* etc. *VTV* is prepared by using *Kanji Shodhita*, *Godugdha Shodhita* and *Gomutra Shodhita Kuchala churna*. Current research is the first effort to document the qualitative and quantitative parameters of this formulation. **Aim:** To prepare *Vishatindukadi vati* by using *Shuddha Kuchala (Shodhita)* by three different media – *Kanji*, *Godugdha* and *Gomutra*) and evaluate its Analytical characteristics. **Material & Method:** *VTV* was prepared as per the classical reference of *Rasatantrasara Siddhaprayog Sangrah*. Its Physico-chemical parameters, analytical parameters, Qualitative test for functional groups & Chromatographic estimation were investigated. **Result & Discussion:** The organoleptic study of all three samples is the same except *Gandha* (odour) is different in all three samples. HPTLC study shows Strychnine % is lower in *VTV (SK Gm)* and Brucine % is lower in *VTV (SKK)*. Functional groups tannin, saponin, alkaloid, mucilage was present. **Conclusion:** There is uniformity among the result of observed test parameters, among three samples. Results of the Pharmaceutical study, Physico-chemical tests, HPTLC profile in the present study have set some preliminary pharmaceutical and analytical parameters for *Vishatindukadi Vati* which may be used as reference standards for further study.

Keywords: *Vishatindukadi Vati*, Pharmaceutical-analytical test, HPTLC

INTRODUCTION

Pharmaceutical study of any *Rasaushadhis* is quite a strenuous job as one needs to take a lot of precautionary measures while handling these poisonous substances. Also, analytical parameters must be studied to assure its quality. The therapeutic efficacy of any drug or formulation mainly depends on quality control of pharmaceutical processes by standard operating procedures and when it comes to the handling of poisonous drugs, one needs to remain cautious regarding every step of processing. The toxicity and adverse effect of *Rasaushadhis* occur mainly due to shortcut processing or compromising the classical pharmaceutical guidelines. Modern pharmaceutical science also gives stress on the application of SOP (Standard Operating Practice) in drug manufacturing^[1]. *Vishatindukadi vati* is one such formulation that is mentioned in *Rasatantrasara Siddhaprayog Sangrah*. It has remarkable therapeutic potential as it has been used in *Agnimandya* (Loss of appetite), *Ajirna* (Indigestion), *Jeerna Vataroga*, *Pakshaghata* (Hemiplegia), *Nadi Daurbalya* (Nervine debility), *Hruday Daurbalya* (Heart weakness), *Atisara* (Diarrhoea), *Klaibya* (Infertility), *Dhatu Kshinata* (General debility)^[2]. Its ingredients are *Kuchala* (*Strychnos nux vomica*), *Marich* (*Piper nigrum*), *Puga* (*Acacia catechu*), and *Amlika beeja* (*Tamarindus indica*). Its main constituent is *Kuchala* (*Strychnos nux vomica*) which is poisonous. There are four different methods of *Kuchala shodhana* which are mentioned as per their therapeutic context but in the market formulation of *Vishatindukadi vati* no specified *shodhana* technique is followed, so there need to assess the difference between *Vishatindukadi vati* prepared by using *Kuchala* which is *Shodhita* by various techniques so that its analytical characteristics can be studied. In this study *shodhana* of *Kuchala* is carried out by using *Kanji*, *Godugdha* and *Gomutra*. Preparation of *Vishatindukadi Vati* was done by using this *Kuchala* and its analytical study was carried out using *Vati* parameters along with HPTLC study.

Aim and Objectives:

Aim – To prepare *Vishatindukadi vati* by using *shuddha Kuchala* (*Shodhita* by three different media – *Kanji*, *Godugdha* and *Gomutra*) and evaluation of its Analytical characteristics.

Objectives –

1. Identification and Authentication of Raw material.
2. *Shodhana* of *Ashuddha Kuchala*.
3. Powdering of contents of *Vishatindukadi Vati*.
4. Preparation of *Vishatindukadi Vati*.
5. Analytical study of *Vishatindukadi vati*.

Material And Method: -

Material and methods used in the preparation of *Vishatindukadi vati* according to the classical texts of *Rasashastra*. In this study, *Shodhana* and preparation are based on availability, feasibility in a classical indication of *Rasashastra* and expert opinion.

Materials

There are various instruments (*yantra*) are used in the *Shodhana* procedure and the preparation of *Vishatindukadi vati* by 3 methods.

1. **The gas burner as a source of heat:** LPG cylinder is used to provide the required amount of heat.
2. **Dolayantra** – A thick earthen pot having a height of 25 cm, an upper diameter of 15 cm and a middle diameter was 30 cm used for *Kuchala shodhana*.
3. **Mortar and Pestle (Khalva yantra)** – Used for *Bhavna* purpose (Trituration) of *Vishatindukadi vati*. (Dimension – 16×10×10 (lbh) *angula*, 7 fingers deep, 2 feet thick. Pestle – 12 fingers.)
4. **Sieves** –80 no. the mesh was used in the present study.
5. **Cotton cloth** – Cotton cloth is used for *Pottali*, which is used for *Kuchala shodhana*. (Dimension – 30 × 30 cm)

Table 1: Contents of Vishatindukadi vati ^[3]:

| Sr. no | Ingredients | Latin name | Part used |
|--------|-----------------|----------------------|-----------|
| 1 | Shudhha Kuchala | Strychnos nux vomica | Seed |
| 2 | Puga phala | Piper nigrum | Fruit |
| 3 | Maricha | Areca catechu | Fruit |
| 4 | Chincha phal | Tamarindus indica | Seed |

Table 2: Contents of Vishatindukadi vati & its Pharmacodynamic (Rasa Panchaka) properties ^[4]:

| S. N. | Ingredients | Rasa | Guna | Virya | Vipaka | Dosha Karma |
|-------|-----------------|---------|----------------------|--------|---------|---|
| 1 | Shudhha Kuchala | Katu | Laghu, Grahi, Ushna | Ushna | Katu | Kapha-Vatashamak |
| 2 | Puga phala | Kashaya | Sheeta, Guru, Ruksha | Sheeta | Madhura | Mohakara, Deepak, Kapha-Pit-tashamaka |
| 3 | Maricha | Katu | Ruksha, Tikshna | Ushna | Madhura | Kapha-Vatashamaka, Agnideepaka, Shwashara, shoolahara |
| 4 | Chincha phal | Amla | Laghu | Sheeta | Madhura | Trishnahara, Rochaka, Vatashamaka, |

Methods

1) **Raw material Authentication:** Authentication of Raw material of Vishatindook Vati was done by the Dravyaguna experts.

2) Shodhana of Kuchala

Preparation of Kanji: -

Reference: – Ayurvedic Formulary of India Part 1-B (Parada Vidnyana)

Procedure: - 1.5 kg of cooked rice was taken in a ceramic pot and 4.5 litres of water was added to it. Stirred properly. The mouth of the pot was properly closed by the lid and sealed by mud smeared cloth to resist entry into the air. The pot was kept in a dark place for fifteen days for the process of fermentation. Tests for Acidic fermentation were performed, and Kanji was collected by straining and used for shodhan.

3) Shodhana in Kanji ^[5]:

(Nimajjana process)

Seeds were soaked in kanji for three days in an earthen pot. The Kanji was changed every 24 hrs. On the fourth day, seeds were washed with warm water. Then outer seed coat and embryo are removed, cotyledons are dried in sunlight and pulverised. Pulverised material was kept in an airtight container.

4) Shodhana in Godugdha ^[6]:

(Swedana process)

Swedana was done in Godugdha by Dolayantra method for three hours. Then outer covering was

scrapped with a knife, the seed coat and embryo are removed, cotyledons were dried in sunlight and pulverised.

5) Shodhana in Gomutra ^[7]: (Nimajjana process)

Seeds were soaked in Gomutra for seven days. Gomutra was changed every day by adding fresh Gomutra. Then outer covering was scrapped with a knife, the seed coat and embryo are removed, cotyledons were dried in sunlight and pulverised.

6) Powdering of Raw drugs for Vishatindukadi vati: -

Equipment: - Weighing machine, Mortal and pastel, Grinder, mixer, sieve no 80, Plate, spoon.

I) Powdering of Shuddha Kuchala: -

a) **Procedure** – Shuddha Kuchala was powdered with the help of a grinder and sieved in a pot through 80 No. mesh. After that powder was kept in an airtight container.

b) Precaution –

- ✓ Kuchala should be dried completely before the procedure of powdering.
- ✓ During the procedure of powdering, care must be taken, hence disposable hand gloves and a mask should be used.

II) Powdering of other raw drugs: -

Procedure – All the drugs were crushed separately with the help of Mortal and pastel. Then crushed drugs were powdered through a mixer separately. The

grounded part was sieved through mesh no. 80 to get a fine powder. Only sieved material was selected for further procedure.

III) Preparation of Vishatindukadi vati: -

- **Equipment:** - Weighing machine, *Khalwayantra*, Stainless steel plate, spoon, measuring glass beaker.
- **Ingredients:**

Table 3: Showing Ingredients of *Vishatindukadi vati*.

| Sr.No. | VTV | VTV | VTV | Quantity in gm. |
|--------|------------------------------|-------------------------------|-------------------------------|-----------------|
| 1 | <i>Shuddha Kuchala (SKK)</i> | <i>Shuddha Kuchala (SKGd)</i> | <i>Shuddha Kuchala (SKGm)</i> | 100 gm |
| 2 | <i>Puga</i> | <i>Puga</i> | <i>Puga</i> | 10 gm |
| 3 | <i>Maricha</i> | <i>Maricha</i> | <i>Maricha</i> | 9 gm |
| 4 | <i>Amlika beeja</i> | <i>Amlika beeja</i> | <i>Amlika beeja</i> | 6 Gm |

(VTV – *Vishatindukadi vati*, SKK- *Shodhita Kuchala* by Kanji, SKGd- *Shodhita Kuchala* by Godugdha, SKGm- *Shodhita Kuchala* by Gomutra)

Procedure:

- All raw drugs *churna* were taken in *Khalwayantra*.
- After that 100 ml of water was added to a mixture of *churna*, as a *Bhavana dravya*. And trituration (*mardana*) was done to form a homogenous mixture.
- Trituration (*mardana*) was done for about 8 hrs and water was added gradually.
- After that approximately 125 mg weighted *vatis* (Tablets) were manually prepared and shade dried.
- These *vatis* were stored in an airtight container and labelled.

● Precautions:

- ✓ *Khalva yantra* and all equipment were cleaned taken to avoid contamination.
- ✓ *Mardana* should not be done vigorously, to avoid loss of material.
- ✓ *Vati* should be dried well before doing the analytical study.

IV) Analytical study

It includes

- Organoleptic Parameters of Raw drugs
- Analytical Parameters of *Vati*

- Physicochemical parameters
- Qualitative and quantitative parameters

All physicochemical and quality parameters of *Vati Kalpana* were followed as per standard parameters mentioned in Ayurved Pharmacopeia of India and Indian Pharmacopeia.

V) HPTLC estimation of Strychnine and Brucine:

A CAMAG HPTLC system equipped with a sample applicator Linomat 5 sample applicator was used. Pure strychnine and Brucine were obtained. The mobile phase was Toluene: Ethyl acetate: Triethylamine (7:2:2 v/v). Peaks areas were noted, and their quantities were calculated.

Observation & Results

Raw Drug Analysis: The quality and standardisation of finished products is depending on the Raw material used. So, the Raw drugs are selected from the market as per guidelines given for identification and purity by classical text as well as by the analytical study carried out in Laboratory. The above results show that the raw material selected was authentic in Ayurvedic as well as modern Parameters. The type and part which is indicated in the text were used to prepare the *Vishatindukadi Vati*.

Observation of Shuddha Kuchala

Table 4: Showing Total yield of *Shuddha Kuchala* obtained by three different methods^[8]

| Sr. no. | Shodhana Dravya | Quantity of A. Kuchala | Wt. obtained after shodhana | Wt. loss After Shodhana | % Yield of S. Kuchala |
|---------|-----------------|------------------------|-----------------------------|-------------------------|-----------------------|
| 1. | Kanji | 400 gm | 250 gm | 260 gm | 62.5 % |
| 2. | Godugdha | 400 gm | 280 gm | 265 gm | 70 % |
| 3. | Gomutra | 400 gm | 260 gm | 240 gm | 65 % |

Finished product Analysis:

Comparison of Vishatindukadi Vati:

Table 5: Showing comparison of Organoleptic study of *Vishatindukadi vati*.

| Sr.no | Classical parameters | VTV (SKK) | VTV (SKGd) | VTV (SKGm) |
|-------|----------------------|-------------------|-------------------|-------------------|
| 1. | Shabda (Sound) | No specific sound | No specific sound | No specific sound |
| 2. | Sparsha (Touch) | Mrudu, | Mrudu, | Mrudu, |
| 3. | Rupa Colour | Brownish, | Brownish, | Brownish, |
| 4. | Rasa (Taste) | Katu, Tikta | Katu, Tikta | Katu, Tikta |
| 5. | Gandha (Odour) | Amla | Katu | Gomutra Gandhi |

Table 6: Showing Physico-chemical Parameters of *Vishatindukadi vati* and their results:

| Sr. no | Parameters | VTV (SKK) | VTV (SKGd) | VTV (SKGm) |
|--------|--------------------------------------|-------------|-------------|----------------|
| 1 | pH 5% | 5.68 | 5.91 | 5.11 |
| 2 | Moisture content @105 ⁰ c | 7.27%-8.49% | 4.5% -3.12% | 6.77% - 8.26 % |
| 3 | Total Ash Content | 2.14 | 2.27 | 1.47 |
| 4 | Acid insoluble ash | 0.083% | 0.084 % | 0.054 % |
| 5 | Water-insoluble ash | 1.29 % | 1.72 % | 1.03 % |
| 6 | Alcohol soluble extract | 12.19 % | 22.7 % | 22.16 % |
| 7 | Water-soluble extract | 16.67 % | 13.41 % | 13.08 % |

Table 7: Showing Analytical Parameters of *Vishatindukadi vati*: -

| Sr. no | Parameters | VTV (SKK) | VTV (SKGd) | VTV (SKGm) |
|--------|---------------------|-------------------------|-------------------------|-------------------------|
| 1. | Average weight | 0.1183 gm | 0.09285 gm | 0.10151 gm |
| 2. | Average diameter | 0.510 cm ² | 0.402 cm ² | 0.399 cm ² |
| 3. | Hardness | 1.93 kg/cm ² | 1.97 kg/cm ² | 1.96 kg/cm ² |
| 4. | Disintegration time | 15min20sec | 15 min48 sec | 15min 40 sec |
| 5. | Friability test | 0.01% | 0.01% | 0.01% |
| 6. | Dissolution time | 13 min | 18 min | 18 min |

Table 8: Showing Qualitative Assay for Functional groups

| Sr. no | Parameters | VTV (SKK) | VTV(SKGd) | VTV (SKGm) |
|--------|------------|-----------|-----------|------------|
| 1. | Alkaloid | + | + | + |
| 2. | Tannin | + | + | + |
| 3. | Saponin | + | + | + |
| 4. | Mucilage | + | + | + |

HPTLC Study

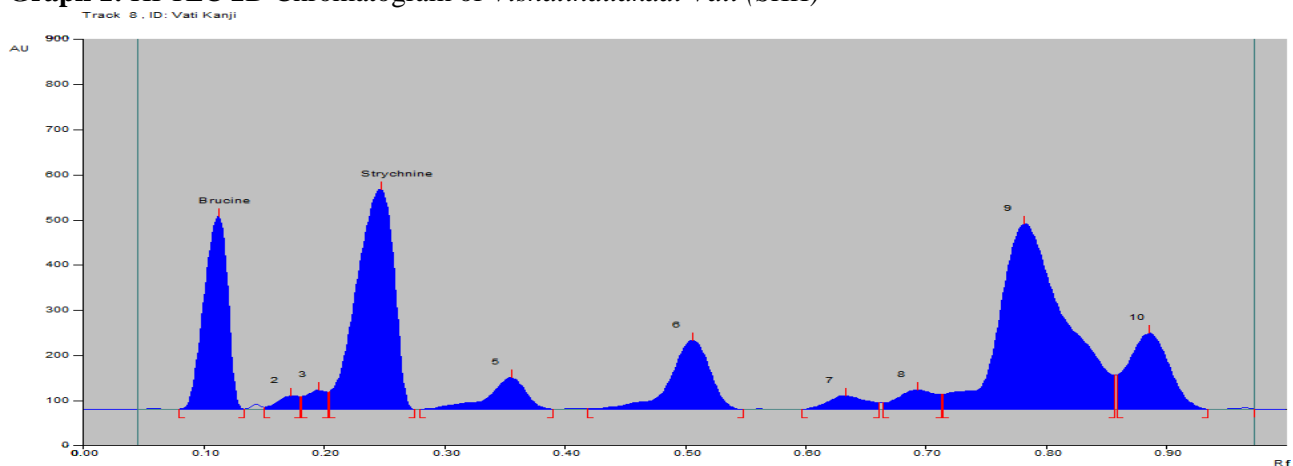
Table 9: Quantification of Strychnine by HPTLC:

| Parameters | Standard Strychnine | Kuchala (Impure) | VTV (SKGm) | VTV (SKGd) | VTV (SKK) |
|--------------|---------------------|------------------|------------|------------|-----------|
| Weight | 10.0 mg | 1.007 gm | 2.044 gm | 2.029 gm | 2.019 gm |
| Rf Value | 0.09 | 0.24 | 0.24 | 0.25 | 0.25 |
| AUC | 11136.2 | 14650.8 | 9548.5 | 9911.4 | 11988.9 |
| % Strychnine | --- | 0.827 % | 0.265 % | 0.278 % | 0.337 % |

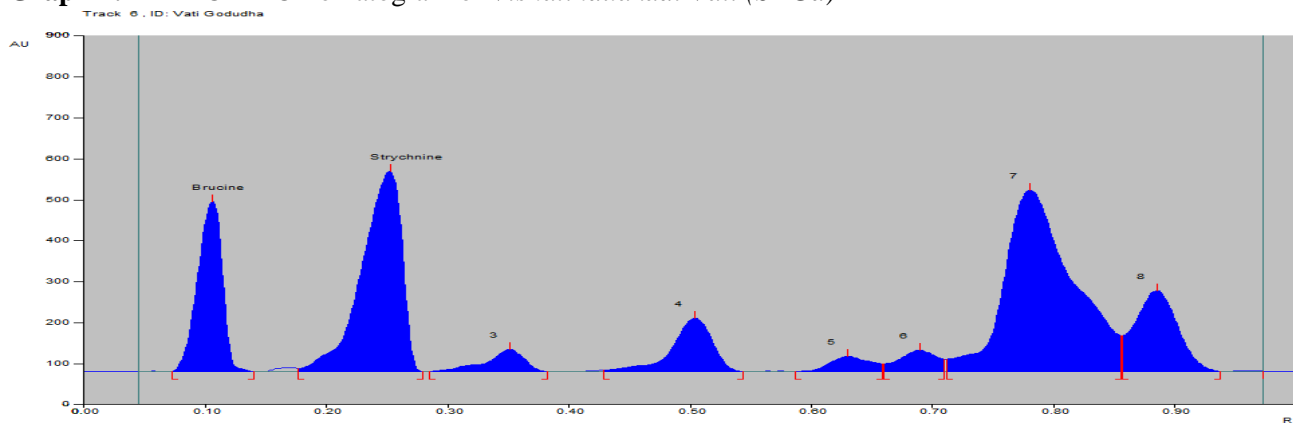
Table 10: Quantification of Brucine by HPTLC:

| Parameters | Standard Brucine | Kuchala (Impure) | VTV (SKGm) | VTV (SKGd) | VTV (SKK) |
|------------|------------------|------------------|------------|------------|-----------|
| Weight | 10.0 mg | 1.007 gm | 2.044 gm | 2.029 gm | 2.019 gm |
| Rf Value | 0.09 | 0.10 | 0.10 | 0.11 | 0.11 |
| AUC | 13935.5 | 8740.4 | 7201.5 | 6758.4 | 6655.5 |
| % Brucine | --- | 0.749 % | 0.300 % | 0.287 % | 0.280 % |

Graph 1: HPTLC 2D Chromatogram of Vishatindukadi Vati (SKK)



Graph 2: HPTLC 2D Chromatogram of Vishatindukadi Vati (SKGd)



Graph 3: HPTLC 2D Chromatogram of Vishatindukadi Vati (SKGm)

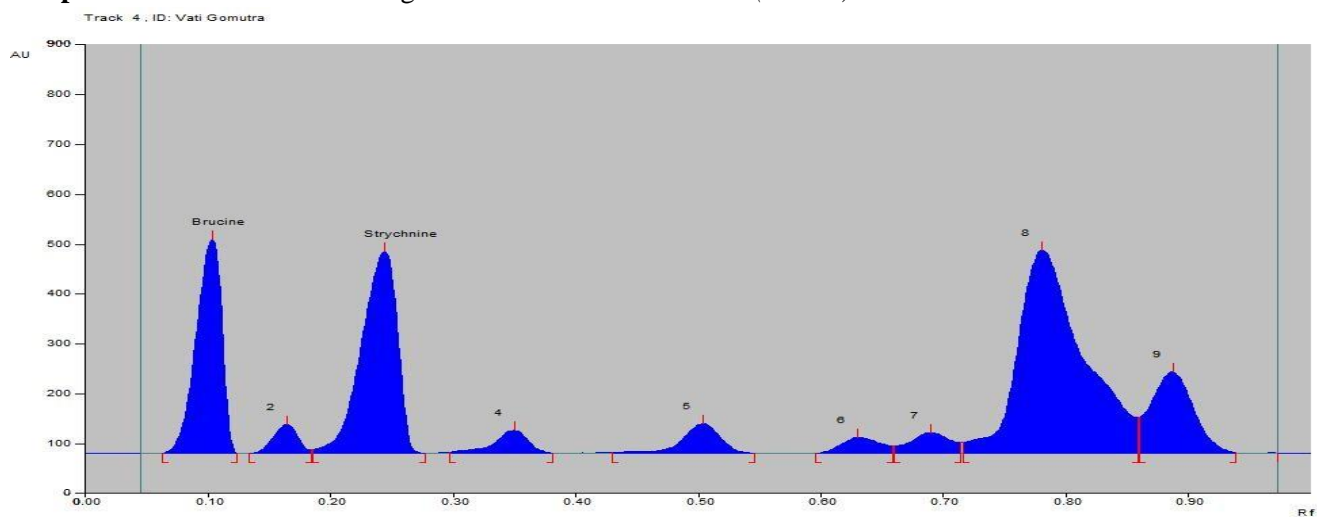


Table 11: Showing R_f value of Vishatindukadi vati

| Sample | No. of spot | R _f value |
|------------|-------------|--|
| Strychnine | 2 | 0.25, 0.50 |
| Brucine | 1 | 0.10 |
| VTV (SKK) | 5 | 0.10, 0.25, 0.35, 0.50, 0.78 |
| VTV (SKGd) | 6 | 0.10, 0.17, 0.25, 0.35, 0.50, 0.78 |
| VTV (SKGm) | 9 | 0.10, 0.17, 0.25, 0.35, 0.50, 0.63, 0.69, 0.78, 0.89 |

DISCUSSION

Ayurveda has the potential to convert a poisonous substance into a therapeutically useful drug after several processes like *Shodhana*, *Marana* etc. But if these methods are not followed strictly due to lack of proper SOP (Standard Operating Procedures), it may cause harmful effects. Preparation of *Vishatindukadi vati* as per standard operative procedure systematically is essential to ensure the quality of *Vishatindukadi vati*. *Shodhana* is done to remove toxic substances from toxic material. *Shodhana* of *Kuchala* in three different media i.e., *Kanji*, *Godugdha* and *Gomutra* has a specific therapeutic purpose hence it needs to set its standard by analytical techniques. The total yield of *shuddha Kuchala* after *shodhana* in *Godugdha* was highest i.e., 70% to that of *Gomutra* and *Kanji* which was 65% and 62.5% respectively (Table no 4). It may be due to less time duration of *shodhana* in *Godugdha*. Powdering of *Shuddha Kuchala* is a very tedious process because it is difficult to powder easily due to its sticky nature, hence it needs to paste in the coarse form in wet

form as soon as after its *shodhana*. Once it is dried then it was finely powdered for vati purpose. *Ayurvedic concept* of *Bhavana* is more important as *Subhavita Lakshana* impart to achieve the standards of *vati Kalpana* by *ayurvedic* and modern parameters. *Bhavana* is the important *sanskara* that helps to reduce particle size as well as impart properties of *Bhavana dravya*. In the Organoleptic study of *VTV*, all parameters were seen same in all three samples of *Vishatindukadi Vati* except *Gandha* which was different i.e., *VTV(SKK) Amla Gandhi*, *VTV(SKGd) Katu Gandhi* and *VTV(SKGm) Gomutra Gandhi* (Table no. 5). In Physico-chemical study of *VTV* (Table no. 6), the Moisture content of *VTV (SKK)* is 7.27% - 8.49%, *VTV (SKGd)* is 3.12% - 4.5% and *VTV (SKGm)* is 6.77% - 8.26%. Higher Moisture content or loss on drying in the studied sample may be due to the presence of mucilage. The pH Value of *VTV (SKK)* is 5.68, *VTV (SKGd)* is 5.91 and *VTV (SKGm)* is 5.11. It suggests its acidic nature. Total ash content of *VTV (SKK)* is 2.14%, *VTV (SKGd)* is 2.27% and *VTV (SKGm)* is 1.47%. Acid

insoluble ash in VTV (SKK) is 0.083%, VTV (SKGd) is 0.084% and VTV (SKGm) is 0.054%. Water-insoluble ash in VTV (SKK) is 1.29%, VTV (SKGd) is 1.72% and VTV (SKGm) is 1.03%. Alcohol soluble extract in VTV (SKK) is 12.19%, VTV (SKGd) is 22.7% and VTV (SKGm) is 22.16%. Water-soluble extract in VTV (SKK) is 16.67%, VTV (SKGd) is 13.41% and VTV (SKGm) is 13.08%. In an analytical study of VTV (Table no. 7) hardness of all the 3 samples of *Vishatindukadi Vati* was soft and not brittle. The average weight of VTV (SKK) is 0.1183 gm, VTV (SKGd) is 0.09285 gm and VTV (SKGm) is 0.10151 gm. Average diameter of VTV (SKK) is 0.510 cm², VTV (SKGd) is 0.402 cm² and VTV (SKGm) is 0.399 cm². which suggests quite a difference, this may be due to the manual preparation of *Vati*. Disintegration Time of VTV (SKK) is 15 min 20 sec, VTV (SKGd) is 15 min 48 sec and VTV (SKGm) is 15 min 40 sec. The increase in disintegration time hampers the absorption of the tablet. The dissolution Time of VTV (SKK) is 13 mins, VTV (SKGd) is 18 mins and VTV (SKGm) is 18 mins. Dissolution of a Drug is a prerequisite for absorption of the drug into the body and the dissolution rate is directly related to its bioavailability. In terms of Ayurvedic formulation, bioavailability is dependent on particle size, *Anupana*, time of drug administration, *Ag-nibala* of patients etc. Before and After the weight of tablets is recorded, there is 0.01 % friability seen in the prepared three samples of *Vishatindukadi Vati*. In the qualitative assay for the presence of the functional group (Table no.8), Alkaloids, Tannins, Saponin and Mucilage were present in all three samples of *Vishatindukadi Vati*. The presence of saponin suggests the foamy nature of *Kuchala*. The presence of Tannins suggests its therapeutic relevance as it is used in diarrhoea, skin eruptions, haemorrhoids and tonsillitis due to its astringent property and bitter taste. The presence of Mucilage suggests its sticky nature and more moisture carrying properties. Therapeutically it protects the gastric irritation by forming a protective film. In the HPTLC study, the Strychnine % was marked lower in *Vishatindukadi Vati* (SKGm) (Table no. 9) and the Brucine % was marked lower in *Vishatindukadi Vati* (SKK) (Table no. 10). There is three common R^f value

other than the R^f value of Strychnine & Brucine, which were observed i.e., 0.35, 0.50, 0.78 which suggest that these may be potent phytoconstituent of VTV which can be identified by further study (Table 11). The presence of two R^f Values in pure strychnine marker may be due to the impurity of the marker. There is a total of nine spots observed in VTV (SKGm) which is higher other than the two samples, which indicates more phytoconstituents are present, which may suggest the importance of *Gomutra* as a shodhana media in *Kuchala shodhana*.

CONCLUSION

The pharmaceutical study of *Vishatindukadi Vati* needs optimization of particle size of raw drugs, duration of trituration and uniformity of weight. HPTLC study indicates the importance of *Gomutra* as a *Shodhana* media for *Kuchala* which further needs to evaluate on a therapeutic basis. Also, more or less the Percentage of Strychnine and Brucine in VTV does not specify its importance on a therapeutic basis, it needs further preclinical and clinical evaluation to prove its therapeutic potential. This study has set some preliminary pharmaceutical and analytical parameters for *Vishatindukadi Vati* which may be used as reference standards for further study.

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