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## Research Article

# PHARMACEUTICAL STANDARDISATION AND PHYSICOCHEMICAL ANALYSIS OF KRIMIGHATINI VATI

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#### **KEYWORDS:**

Antihelminthic, Krimighatini vati, Pharmaceutical standardization.

## **ABSTRACT**

Standardization is the process for the establishment of standard for a particular or drug. Standardization in Ayurvedic formulation mainly deals with ensuring standards for the quality and purity of raw materials, quality control during the drug manufacturing process, production of a good quality finished product and storage and distribution to maintain the quality of the final product. Standard of an Ayurvedic product can be assessed only by analyzing the analytical parameters of raw drugs, drugs after preprocessing and the finished products. Krimighatini vati explained in Rasendra chintamani is an antihelminth preparation explained in Rasendra Chintamani krimirogadhikara. It contains Parada- 1part, Gandhaka- 2 parts, Ajamoda -3 parts, Vidanga bheeja- 4 parts, Palaasha bheeja- 5parts, and Kaarsakara beeja- 6 parts. It is similar to Krimimudgara rasa that is available in market but differs in proportion of Palaasha beeja and Karaskara beeja. Present study aims to standardize Krimighatini vati after preparing the medicine according to the method explained in Ayurveda samhithas. Preprocessing of the Gulika includes, Shodhana of Hingula, Gandhaka and Karaskara beeja according to the methods mentioned in Rasasastra books. Parada was extracted from *Hingula* by *Ordhwapaathana vidhi*. It was then triturated with *Shuddha* Gandhaka to get Kajjali. Fine powders of other drugs were mixed with Kajjali and rolled into pills with Honey. Study observed the physicochemical parameters of individual drugs and validated the *Gulika* by HPTLC method. Analysis of *Kajjali* was done using XRD so as to prove the complete formation of *Kajjali*. Pharmaceutical standardization helps in reproducibility of drug paving way for more studies on toxicity and efficacy of drug.

## **INTRODUCTION**

Intestinal worm infestation is a global health problem. Worm infestations are more prevalent in subtropical countries and occur where there is poverty and poor sanitation. Soil transmitted worms forms the most important group of intestinal worms affecting 2 billion people worldwide. About 27% of entire school age and preschool age children population in world are in need of antihelminthic treatment. Antihelminthics are used in mass deworming campaigns of school going children in many developing countries.

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Since Antihelminthics are mostly administrated in sensitive groups in the society, surety must be assured to the society regarding its safety. Krimighatini vati is an antihelminthic preparation mentioned in Rasendra chintamani, Krimiroga adhikara. [1] Rasendra chintamani is a book which was written in 15th century A.D. It was written by Acharya Shri Dundukanatha who was a shisya of Acharya Kalanatha. The book consists of total 9 chapters and is named as adhyaaya. 9th chapter is divided into many adhikaaras with several formulations that can be practically utilized. It includes Jwaradhikara, Jwara athisaaraadhikara etc. In Krimi rogaadhikara Acharya explained only one Antihelminthic preparation named Krimighatini vati. It contains Rasa, Gandhaka, Ajamoda, Krimighna, Palaasha bheeja and Kaaraskara in progressively increasing proportions. Krimighatini vati as such in not available in the market which might be due to the increased proportion of *Kaaraskara beeja* in

it. A similar preparation that is available in the market is *Krimimudgara rasa* which contain the same ingredients as that of *Krimighatini vati* but in different proportions. There is no doubt on the efficacy or antihelminthic activity of the *Krimighatini vati* as all the contents of the Vati possess proven antihelminthic action. The present study attempts in evaluating the physicochemical parameters and standardization of the same.

## MATERIALS AND METHODS

Relevant samhitas were referred to collect the details about the concept of Krimi and *Krimighatini* 

*vati* explained in Ayurveda books. Physico chemical analysis of the individual drugs, Kajjali and gulika were done.

## Krimighatini vati

Krimighatini vati is only antihelminthic preparation mentioned in Rasendra Chintamani in the context of Krimirogaadhikara. That which kills Krimi is Krimighatini. Krimighatini vati is indicated in all types of Krimi. According to Author, If one feels excessive thirst after consuming the vati then he should drink Mushta kashaya or Aakhu parni kashaya with sugar.

Table 1: showing ingredients of Krimighatini vati

Sl.no	Ingredients	Scientific name	Proportion mentioned in yoga
1	Parada	Mercury	1 part
2	Gandhaka	Sulphur	2 parts
3	Ajamoda	Trachyspermum roxburghianum	3 parts
4	Vidanga	Embelia ribes	4 parts
5	Palaasha beeja	Butea monosperma	5 parts
6	Karaskara beeja	Strychnos nuxvomica	6 parts

## Formulations similar to *Krimighatini vati*

# a) Krimimudgara rasa- Rasendra sara sangraha [2] Rasa, Gandhaka, Ajamoda, Vidanga, Visamushtika and seeds of Palaasha are taken in increasing proportions and compounded together into a fine powder. 2.5 g dose of this recipe is mixed with honey and taken. It destroys parasites and is Agni deepana if taken for 3 consecutive days.

## b) Krimighatini vati - Bhaisajya Ratnavali [3]

Sasilekha (Bakuchi), Haridra, Pippali, Kampilla, purified Swarna gairika, root of Trivrit, Shiva beeja, and seeds of Palaasha are taken in equal quantity and made into fine powder. It is triturated in water and made into pills of 4 ratti. It cures diseases like vomiting, depression, edema, colic pain, sneezing, sinus, head ache, fever, weakness, constipation and 20 types of Krimi.

# Preparation of Krimighatini vati

Hingulottha Parada was taken and triturated with purified Gandhaka to get Kajjali. On attaining Siddha lakshanas kajjali was then mixed the fine powders of Palasha Beeja, Vidanga, Ajamoda and Purified Karaskara beeja. After triturating well pulverized compound was mixed with honey and rolled into pills of 1 Ratti size. Dose of the Gulika is approximately 125 mg to 500 mg per day.

# Preparation includes following steps

#### Collection of raw materials

The contents of the formulation were collected from Ambuja Institute of Ayurvedic research and documentation and the quality of drugs were analysed in the Department of *Dravyaguna Vijana* and Department of *Rasasastra* and *Bhaishajya Kalpana*.

## Preparation of Gulika

Preparation of *Gulika* was done in following steps

• Shodhana of Karaskara [4]

**Method** of *shodhana* adopted: *Dolayantra swedana* in milk.

Reference: Rasa tarangini

## Procedure

- Mature seeds *of Karaskara* were taken and tied into a loose *Pottali*.
- *Pottali* was then subjected to *Dolayantra swedana* in cow's milk for 3 hours.
- On self-cooling *Pottali* was taken out from *Dolayantra*.
- *Karaskara* seeds were collected from *pottali* and washed in hot water thrice.
- Outer seed coat of the *Karaskara* seeds were scraped off.
- Then the seeds were cut longitudinally with knife to expose the plumule. And the plumule was removed.
- Then seeds were washed in hot water twice and rubbed with dry cloth to remove the moisture content and allowed to dry.
- Drying was continued for 3 weeks.
- Karaskara seeds were then weighed and kept in a sealed container.

## **Observations**

- Karaskara seeds became soft after Dolayantra swedana
- Milk after 3 hours of *Swedana* turned into light yellowish and thick in consistency
- *Karaskara* seeds were tied in loose *Pottali* before *Swedana*. After *swedana Karaskara* seeds were swollen making the *Pottali* full and tightly packed.
- Eventhough the seeds were soft and swollen after 3 hours of *Swedana*, the scraping of seed coat was a strenuous job.

## **Precautions**

- Handle *Karaskara* seeds only after wearing gloves.
- Altered sensations were experienced in fingers where the gloves got cut while removing the seed coat.

## Results

Weight of *Ashodhitha Karaskara* seeds: 330 grams Weight of *Shodhitha* and dried *Karaskara* seeds: 265 grams

Weight loss: 65 grams



Fig1: Karaskara beeja before shodhana



Fig 2: Karaskara beeja after Shodhana

• Shodhana of Hingula<sup>[5]</sup>

**Method of** *Sodhana* **adopted:** *Bhavana* in *Ardraka swarasa* for 7 times

Reference: Rasa tarangini

#### Procedure:

- 200 grams of *Hingula* was taken in a *Khalwa yantra*
- It was powdered well using mortar
- Ardraka was taken and its outer skin was peeled off and Swarasa was extracted.
- For first *Bhavana*, *Ardraka swarasa* was added in quantity so as to fully soak *Hingula*.

- *Bhavana* was done with pestle by applying uniform pressure and speed without spilling the contents.
- Bhavana was continued till all the liquid portion dried up.
- On complete drying of *Ardraka swarasa*, 2<sup>nd</sup> *Bhavana* was done.
- And procedure was continued till 7 bhavanas were completed.
- The dry powder obtained after 7 *bhavanas* was washed with warm water, dried and stored in suitable air tight container as *Suddha Hingula*.

#### Results

Quantity of Ashoditha Hingula taken: 200 grams Quantity of Hingula obtained after  $7^{\rm th}$  Bhavana: 205 grams

Total Weight gain: 5 grams



Fig 3: Hingula before Shodhana



Fig 4: Hingula after Shodhana

• Hingulotha parada nirmaana [6]

**Method of procedure adopted:** *Urdwa paathana vidhi* **Reference:** *Rasa ratna samucchaya* 

# Procedure

- 2 pots of equal sizes were taken.
- In the first pot freshly prepared Betel juice was smeared and allowed to dry. The process was repeated for 7 times. After smearing with Betel juice, the inner surface of pot turned greenish black in colour.
- In the second pot, Shodhita Hingula which was made into a paste with Ardraka swarasa was smeared.
- The second pot was then covered with the pot that was smeared with Betel juice. The junction of the mouths of the pots were then sealed thoroughly with a cloth smeared with Multani Mitti. And the apparatus was allowed to dry.

- The next day the apparatus was then placed over gas stove for 6 hours in high flame.
- In between the upper pot was cooled frequently using a towel dipped in cold water.
- After six hours the flame was put off and the apparatus was allowed to cool on its own.
- Next day the seal was broken and the pots were separated.
- Pots were scraped with cotton to obtain small globules of mercury

## **Observation:**

- The inner surfaces of the pots were seen coated with a black powder with shining micro globules of Mercury in between.
- The *Hingula* that was coated in the pots had already burned out.

## Method of collection of parada

The blackish powder with Mercury globules were scraped using a small brush and collected in a container. The mixture was then sieved through a cloth several times to separate the Mercury.

## **Precautions:**

Care must be taken while separating mercury.

#### **Results:**

Amount of Shuddha Hingula taken: 200 grams

Amount of Parada obtained: 86 grams

• Gandhaka shodhana [7]

Method of shodhana adopted: Daalana in cow's milk

Reference: Rasa Tarangini

#### **Procedure:**

- *Gandhaka* was finely powdered in a *Khalwa yantra* and taken in an iron *Darvi*.
- Equal amount of cows ghee was added to it and subjected to low flame in stove.
- The mixture of *Gandhaka* and Ghee was slowly stirred using a spoon.
- When it got completely melted the mixture was poured through a cloth into vessel containing milk.
   The impurities in *Gandhaka* were seen left behind in the cloth.
- After that, Gandhaka that was left inside milk was taken out and washed in hot water and allowed to dry.
- Procedure was repeated for 6 times.

#### Observation

- Colour of *Gandhaka* turned into brighter and deeper yellow.
- *Gandhaka* became much more brittle after each *Daalana*.

## Results

Weight of *Gandhaka* before *Shodhana*: 80 grams Weight of *Gandhaka* after *Shodhana*: 61 grams Weight loss: 19 grams



Fig 5: Gandhaka before Shodhana in milk



Fig 6: Gandhaka after Shodhana in milk

Preparation of Kajjali [8]

Table 2: Materials and quantity taken for preparing *Kajjali* 

<b>Materials required</b>	Quantity
<mark>Pa</mark> rada	25 grams
<b>G</b> andhaka	50 grams
Khalwa yantra	1 in number
Spoon	1 in number

**Method of preparation**: *Mardana* in *Khalwa yantra*.

Reference: Rasa Tarangini

## **Procedure**

- 25 grams of mercury was added to *khalwa yantra*
- To these 50 grams of finely powdered purified *Gandhaka* was added
- Using pestle, the mercury was triturated with *Gandhaka* slowly and uniformly. Trituration was continued till it all necessary properties of *Kajjali* were met.
- *Kajjali* was measured and stored in glass containers.

## **RESULTS:**

Amount of *Shuddha Gandhaka* taken: 50 grams Amount of *Shuddha Parada* taken: 25 grams Amount of *Kajjali* obtained: 71 grams

# Powdering of remaining drugs

Remaining drugs like *Ajamoda, Palaasha beeja* and *Vidanga* after confirming its quality and purity were finely powdered separately and sieved through a

double layered cloth. Purified *Kaaraskara seeds* after drying were also finely powdered and sieved thoroughly.

# • Preparation of Krimighatini vati

**Reference:** Rasendra Chintamani **Ingredients:** 

*Kajjali:* 6 grams

Ajamoda choorna: 6 grams Vidanga beeja choorna: 8 grams Palaasha beeja choorna: 10 grams

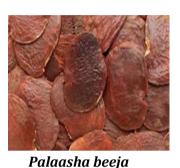
Shuddha Karaskara beeja choorna: 12 grams

Honey: Quantity sufficient

Kajjali, Ajamoda choorna, Vidanga beeja choorna, Palaasha beeja choorna and Shuddha Karaskara beeja choorna were taken in the specified quantity in a clean and dry Khalwa yantra. All the choornas were mixed well. Then sufficient quantity of honey was added to it slowly and little by little. The mixture was then triturated well with a pestle, till proper consistency was attained. After that it was rolled into Gulikaas of one Ratti size, and stored in dry and airtight containers.







yumou Ka



*Vidanga* 

<mark>Shuddha Karaskara beeja</mark>



Krimighatini vati

Fig 7: Ingredients of Krimighatini vati

# **Analytical Findings of Herbal Drugs**

Table 3: Raw drug analysis Shodhitha and Ashodhitha Kupeelu

Test parameter	Ashoditha kupeelu	Shoditha kupeelu
Description	Pale brown colour	Pale brown colour
LOD (%w/w)	2.89	4.19
Ash (%w/w)	2.92	2.65
Acid insoluble ash (%w/w)	BDL	BDL
Water soluble extractive (%w/w)	29.49	29.52
Alcohol soluble extractive(%w/w)	17.62	20.7

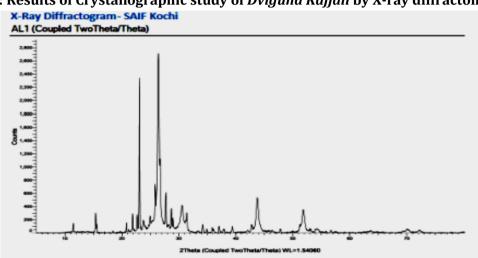
Table 4: Raw drug analysis of Palaasha beeja, Vidanga and Ajamoda

Test parameter	Palaasha beeja	Vidanga	Ajamoda
Description	Whitish brown powder	Dark brown powder	Brown powder
LOD (%w/w)	5.14	6.49	6.34
Ash (%w/w)	6.08	3.29	5.02
Acid insoluble ash (%w/w)	BDL	BDL	BDL
Water soluble extractive (%w/w)	60.78	8.33	27.95
Alcohol soluble extractive (%w/w)	16.64	31.25	18.25

# ANALYSIS OF KAJJALI

# • XRD analysis of Kajjali

Fig 8: Results of Crystallographic study of Dviguna Kajjali by X-ray diffractometer



X-Ray diffraction analysis is a technique that provides information about the crystallographic structure, chemical composition, and physical properties of a material. XRD spectra of *Dviguna Kajjali* shows concentration of HgS, HgO and S (Sulphur).

# Kajjali Pareeksha

Table 5: Kaijali pareeksha

Parameters	Findings	
Varna	Kajjalabha varna	
Shlakshantwa	Powdery and smooth	
Nischandratwa	No <i>Chandratwa</i> observed	
Rekhapurnatwa	Particles entered in furrows of finger	
Varitara	Particles of Kajjali floated in water	
Tamrapatra pariksha	No silver like coating was observed over Tamra Patra	

## ANALYSIS OF KRIMIGHATINI VATI

# • HPTLC analysis of krimighatini vati

Test solution: 1 g Krimighatini sample is weighed, extracted with 10ml Methanol and spotted as 15 microlitre.

Stationary phase: Merk, 1.05554.0007, TLC Silica gel 60 F<sub>254</sub>, 10x10 cm Aluminium sheet.

Mobile phase: Toluene: Ethyl acetate: Formic acid: Methanol (7:5:1:0.5)

**Development:** CAMAG 10 x 10 cm Twin trough chamber

**HPTLC instrumentation:** CAMAG Linomat 5

**Derivatization:** Iodine reagent.

The plates were dried in air and visualized under 254nm and 366nm for ultra violet detection and taken the finger prints. The results of HPTLC analysis are given in fig 9, fig 10, fig 11, fig12, fig 13 and fig 14.

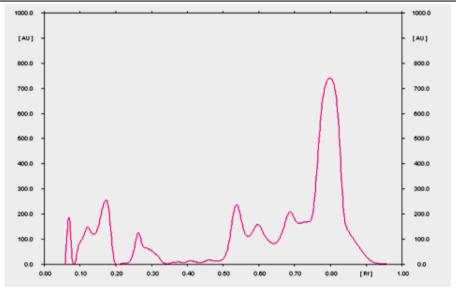


Fig 9: Over view graph of Krimighatini vati sample at 254nm

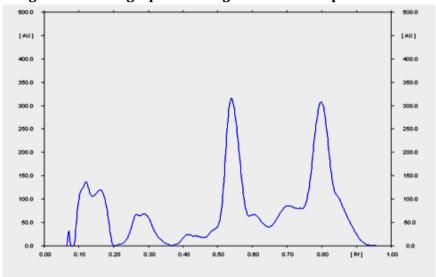


Fig 10 : Over view graph of Krimighatini vati sample at 366nm AT 254nm AT 366nm AT WHITE LIGHT

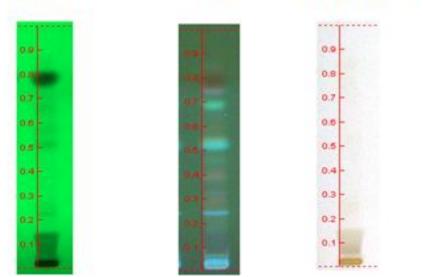
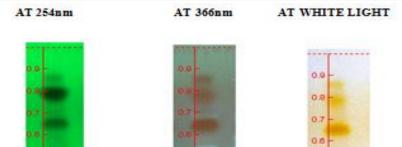


Fig 11: TLC Plate views of Krimighatini vati sample



0.5

Fig 12: Derivatized TLC Plate views of Krimighatini vati sample

Peak No	Rf Value	Area (AU)	% Area(AU)
1	0.07	1807.6	2.04
2	0.12	4347.2	4.91
3	0.17	7759.8	8.77
4	0.26	4335.2	4.90
5	0.46	383.0	0.43
6	0.54	yurve <b>8045.7</b>	9.09
7	0.60	6866.9	7.76
8	0.69	8638.6	9.76
9	0.80	46336 <mark>.5</mark>	52.34
Total P <mark>eak N</mark> o – 09 Tota <mark>l Area – 88520.5 (AU)</mark>			

Fig 13: Rf value & % area of Krimighatini vati sample at 254nm

Peak No	Rf Value	Area (AU)	% Area(AU)
1	0.12	4394.1	8.40
2	0.16	4033.7	7.71
3	0.27	1520.9	2.91
4	0.29	2358.2	4.51
5	0.41	636.6	1.22
6	0.54	13692.5	26.18
7	0.61	2482.5	4.75
8	0.71	4678.7	8.95
9	0.80	18498.2	35.37
Total Peak No – 09			
Total Area - 52295.4 (AU)			

Fig 14: Rf value & % area of Krimighatini vati sample at 366nm

# • Physicochemical Analysis of Krimighatini vati

Table 6: Physicochemical analysis of Krimighatini vati

Test parameter	Result
Description	Black round pill
LOD (% w/w)	1.26
рН	2.81
Ash (% w/w)	5.62

Acid insoluble ash (% w/w)	0.35
Water soluble ash (% w/w)	BDL
Water soluble extractive (% w/w)	20.84

#### DISCUSSION

*Krimighatini vati* is a herbomineral preparation that contains Parada- 1part, Gandhaka- 2 parts, Ajamoda -3 parts, Vidanga bheeja- 4 parts, Palaasha bheeja- 5parts, and Kaarsakara beeja- 6 parts. In a formulation if *Suddha gandhaka* is mentioned in more quantity than that of Suddha Parada, then Kajjali has to be prepared with specified excessive quantity of Sulphur and used.9 It is clearly mentioned in Bhavaprakasha that the bad/toxic effects of Ashodhita visha are minimized when these are subjected to Shodhana process. So Vishas should be subjected for *Shodhana* before being used in therapeutics. So the pre processing of Krimighatini vati that was required includes: preparation of Kajjali and Shodhana of Kaaraskara bheeja. As a part of Dviguna kajjali preparation, Parada was extracted from Hingula (Hingulaakrista parada) because according to Acharyas, Parada obtained from Hingula is devoid of Parada doshaas and can be used in all types of therapeutics without subjecting samskaaras.[10] Hingula was subjected to Bhaavana in Ardraka swarasa for 7 times and Hingulotha parada was extracted from it. Gandhaka after Shodhana in Cow's milk and ghee was tritutarated with *Parada* for hours to get Kajjali with Siddha lakshanas. Kaaraskara bheeja was subjected to Shodhana in Cow's milk and its plumule and outer seed coat was removed, washed well and dried to get Shodhita Kaaraskara bheeja. Shoditha Kaaraskara bheeja, Palaashabeeja, Vidanga, Ajamoda were powdered and mixed with Kajjali and rolled into pills of *Ratti* size using honey.

Presence of heavy metals and poisonous drugs in a formulation is always a matter of safety concern to the community. Here *Parada* and *Gandhaka* were used in form of *Kajjali. Kajjali* after grinding for hours attained the *Siddha lakshanas* thus making it therapeutically safe. The *Visha dravya, Kaaraskara seeds* were purified by *Dolayantra swedana* in milk one of the best purificatory methods to reduce the Strychnine and Brucine content in *Kaaraskara seeds*.

Analytical study was conducted as 3 sections, analysis of herbal drugs, analysis of *Kajjali* and analysis of gulika. Herbal drugs were analysed by parameters like Organoleptic characters, foreign matter, Loss of drying, determination of total ash and acid insoluble ash, determination of Alcohol soluble extractive and water soluble extractive. Organoleptic evaluation depicts a clear cut idea about the nature of the drug, its taste, smell, odour, feel of the drug to touch and occasionally sound or snap of its fracture. Absence of Foreign matter indicates the genuinity and quality of

drug. Loss on drying is designed to measure the amount of water and volatile matters present in a sample when dried under specified conditions. Determination of total ash helps in analysing the purity of a drug and gives information related to adulteration if any with inorganic matter. Acid insoluble ash may give the percentage of sand and impurities. It is to ensure the percentage of silica and oxalates which are introduced accidently at the time of collection and value might be arised due to improper washing of the crude drug. Generally lower the value of Acid insoluble ash, higher the purity. Water soluble extractive value plays an important role in evaluation of crude drugs. The alcohol soluble extractive value mainly represents the percentage of organic plant constituents such as alkaloids, phenols, flavanoids, volatile oils, resins, steroids, glycosides, carotenoids, terpenoids etc present in the drug. [11,12]

The analysis of raw drugs was done at QA department of Arya Vaidyasala Kottakkal. The results show that for Kaaraskara beeja, Loss on drying, Water soluble extractive and Alcohol soluble extractive increased after Shodhana while Ash value slightly decreased after Shodhana, Increased value of LOD in Shodhita Karaskara beeja may be due to the moisture content gained from *Dolayantra swedana* in milk for 3 hours during Shodhana. High percentage of water and alcohol soluble extractives of Kaaraskara bheeja after Shodhana confirms the presence of more active principles. Analytical results of Vidanga, Palasha beeja, Ajamoda and Karasakara beeja, were meeting the standards mentioned in API thus proving the authenticity of drug. Crystallographic analysis by X Diffractometer and Classical mentioned in Rasasastra books were done to assess Kajjali. Crystallographic study of Dviguna Kajjali by Xray diffractometer was done at STIC, CUSAT. According to the XRD study, majority of peaks corresponds to HgS (meta cinnabar) and Sulphur indicating complete formation of Kajjali after grinding. Mercury content in Kajjali was not in the form of free mercury which is considered as poisonous but as HgS which has got high safe therapeutic profile. Also, Kajjali meets the Siddha lakshanas explained like Vaaritaratva, Kajjalaabha Varna, Rekhapoornatva etc. Hence proving the safety of Kajjali. Gulika Krimighatini vati was analysed using phyisco chemical parameters like LOD, pH value, Total ash, Acid insoluble ash, water soluble ash etc. Physicochemical Analysis of Krimighatini vati was done at QC department Arya vaidya sala, Kottakkal. Loss on drying at 110°C gives the percentage of moisture content in a given sample. The value of LOD in *Krimighatini vati* is 1.26 indicating higher shelf life of *Gulika*. Mean pH value of *Krimighatini vati* was 2.81 suggesting the acidic nature of *Gulika*. Acidic pH facilitates the absorption of drug at the stomach level. Friability of the tablets are within the desirable range which validates its capacity to with-stand mechanical shocks during manufacturing, packing and transportation.

### CONCLUSION

Krimighatini vati is a herbomineral preparation meant for Krimi explained in Rasendra chintamani Krimirogadhikara. Ingredients of vati are Karaskara beeeja, Palaasha beeja, Vidanga, Ajamoda and Kajjali. The powder of these ingredients was rolled into pills using honey. The individual drugs in the formulations have got proven antihelminthic action. Pharmaceutical and analytical parameters of the vati are done and validated by HPTLC method. As we are done with the preliminary studies, further studies of toxicity and efficacy of Krimighatini vati can be performed. Antihelminthics being such an important group of drugs in pharmaceutics, studies on Krimighatini vati will be noteworthy.

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