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Research Article

PHARMACEUTICAL STANDARDIZATION OF *AMAPACHAKA VATI*: A HERBO-MINERAL FORMULATION

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ABSTRACT

Introduction: Standardization of formulation is essential to certify their quality, purity and reproducibility. The standard of an Ayurvedic product can be assessed by analysing the analytical parameters of raw drugs, in-process materials, and the finished products. *Amapachaka Vati* is a well-known Ayurvedic herbo-mineral formulation which is mentioned by Chikitsapradip in *Agnimandhya* and *Ajirna.* Till date, no work has been done on pharmaceutical standardization of *Amapachaka Vati.* The present study was aimed to develop Standard Manufacturing Procedure (S.M.P.) of *Amapachaka Vati*. **Materials and methods:** In pharmaceutical process, 3 batches of *Amapachaka Vati* were carried out with the *Shodhana* procedure of required ingredients. Organoleptic and physicochemical analysis of raw, in-process and finished product were done. HPTLC and heavy metal analysis of finished product were also carried out. **Results:** In all three batches of *Amapachaka Vati* average 97.11% yield was found. Prepared *Vati* was greenish brown in color with characteristic odor. Physical parameters of *Vati* were in compliance with the general consideration of IP standards. HPTLC profile shows 6,5,8 peaks in 254, 366, and 540 nm. **Conclusion:** The preparation method of *Amapachaka Vati* carried out here can be considered as Standard Manufacturing Procedure (S.M.P.) as no previous standards are available for this preparation. Evaluated parameters for *Amapachaka Vati* can be used in future reference as a standard.

INTRODUCTION

Rasashastra is the branch of Ayurveda which mainly deals with herbo mineral formulations. *Amapachaka Vati* is a formulation in which include a combination of one or more metals or minerals along with herbal drugs which helps in increasing the potency and efficacy of the formulations. *Amapachaka Vati* is one among the herbo-mineral Ayurvedic formulations. It is described by Chikitsapradip in *Agnimadhya* and *Ajirna Roga.*[1] It contains total 8 ingredients with *Bhavana* (Levigation) of *Kumari* (*Aloe barbadensis* Mill.) *Svarasa.*

Amapachaka Vati is commonly sold by the name of *Aampachana Vati*. Many GMP certified pharmacies are preparing and manufacturing *Aampachana Vati* as per the reference of Bhaishajya Ratnavali where it is named as *Shoolaharana Yoga.*[2] *Shoolaharana Yoga* has same ingredients as of *Amapachaka Vati* but the different in ration of *Saindhava* and also *Bhavana* is not given.

Standardization of formulation is the process of prescribing a set of standards or inherent characteristics, constant parameters, and definitive qualitative and quantitative values that carry an assurance of quality, efficacy, safety, and reproducibility.[3] Though, there are two research works were found on standardization of *Shoolaharana Yoga*, till date no work was found on *Amapachaka Vati*. Thus, it is need of hour to develop its standardization to authenticate this useful formulation. So, the current study was aimed to develop Standard Manufacturing Procedure (S.M.P.) of *Amapachaka Vati* and its primary physico-chemical profile.

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MATERIAL AND METHODS

Amapachaka Vati is herbo-mineral formulation containing 8 ingredients and *Bhavana* of *Kumari Svarasa*. (Table No.1)

Table 1: Details of ingredients of *Amapachaka Vati* (Ref: Chikitsapradip, p.8)

No.	Name of ingredients	Latin name/ English name	Part used	Proportion
1.	*Haritaki*	*Terminalia chebula* Retz.	Pericarp	1 Part
2.	*Shunthi*	*Zingiber officinale* Rosc.	Rhizome	1 Part
3.	*Maricha*	*Piper nigrum* Linn.	Fruit	1 Part
4.	*Pippali*	*Piper longum* Linn.	Fruit	1 Part
5.	*Shuddha Kupilu*	*Strychnos nux-vomica* Linn.	Seed	1 Part
6.	*Shuddha Hingu*	*Ferula foetida* Regel.	Resin	1 Part
7.	*Shuddha Gandhaka*	Sulfur	Mineral	1 Part
8.	*Saidhava Lavana*	Rock salt	Mineral	2 Parts
9.	*Kumari Svarasa* (*Bhavana Dravya*)	*Aloe barbadensis* Mill.	Pulp	Q.S.

Procurement of raw material

Haritaki, Shunthi, Maricha, Kupilu, and *Saindhava* were procured from the Government Ayurved Pharmacy, Rajpipala, Gujarat. *Pippali, Hingu,* and *Gandhaka* were procured from the local market of Vadodara. Kumari was collected from Dhanvantari Udhyan of Government Ayurved College, Vadodara. *Godugdha, Goghrita*, and *Eranda Taila* were procured from the local market of Vadodara, Gujarat as per fssai standard. The samples were identified in Pharmacognosy Laboratory of Upgraded Department of Dravyaguna, Government Ayurved College, Vadodara.

Preparation of drug

All the batches of *Amapachaka* Vati were prepared in the Pharmaceutical Laboratory of Upgraded Department of Rasashastra and Bhaishajya Kalpana, Government Ayurved College, Vadodara, Gujarat.

To develop S.M.P., three batches of *Amapachaka Vati* were carried out in the following steps entitled;

1. *Kupilu Shodhana*
2. *Gandhaka Shodhana*
3. *Hingu Shodhana*
4. Preparation of *Churna* of *Haritaki, Shunthi, Maricha, Pippali,* purified *Kupilu,* purified *Hingu,* purified *Gandhaka*
5. *Kumari Svarasa*
6. Preparation of *Amapachaka Vati*

Table 2: Equipment specification

Sr.no.	Equipment	Specifications
1.	Electric weighing balance	Swisser (An ISO 9001:2008) Cls-3 Maximum capacity: 100 kg, Minimum capacity: 10 g
2.	Gas stove	Prestige Gas cook tops, Model no.: Gas Top GTM-01 Ignition mode- Electro Burner size supply by Gas
3.	Pyrometer	26ºC to 1200ºC capacity K type Diameter-19 mm × 450 mm
4.	Infrared thermometer	Fluke 64 max Maximum: 600° C, Minimum: -30° C
5.	S. S. spoon	Length-17 cm, width-4 cm
6.	S.S. vessel	Diameter -30 cm, depth - 7 cm
7.	Kitchen pincer	Length-32 cm, width-7 cm
8.	S.S. plate	Diameter -28 cm, depth-2 cm
9.	Knife	Length-9 cm

10.	Mixer grinder	Eassy cook Motor-2Hp with 50 Hz
11.	Measuring jar	500 ml
12.	Strainer	Material- stainless steel
13.	S.S. mortar and pestle	Mortar: depth-7.5 cm, diameter-16 cm, height-9 cm Pestle: length-18 cm, width-4 cm
14.	Sieve	Brass, 120#; S.S., 60#
15.	Measuring cylinder	500 ml
16.	Mortar and pestle	Material: Stone Mortar: Length-42 cm, width-23 cm, depth-17 cm Pestle: Length-26 cm

1. *Kupilu Shodhana*

Shodhana of *Kupilu* was done by the reference of R.T.S.S.P.S.[4] *Eranda Taila* was taken in s. s. vessel and heated. *Ashuddha Kupilu* was added to it and *Bharjana* was done in *Eranda Taila* till it puffed up. After that, it was taken out from the vessel; the testa and embryo were removed with the knife. *Shuddha Kupilu* was collected and stored in an airtight container. [Figure 1]

2. *Gandhaka Shodhana*

Gandhaka Shodhana process was done as per the reference of Rasatarangini.[5] For that, *Ashuddha Gandhaka* was taken and powder was prepared by mixer grinder. *Godugdha* was taken in steel vessel and its mouth was covered by cotton cloth smeared with *Goghrita*. *Goghrita* was taken in another steel vessel and *Gandhaka* powder was added into it. Mild heat was given until it melted completely. Then it was poured into a vessel containing *Godugdha*. *Gandhaka* was taken out of vessel and washed with warm water. Repeat this procedure for further two times in same manner. *Shuddha Gandhaka* was collected and stored in an airtight container. [Figure 2]

3. *Hingu Shodhana*

Hingu Shodhana was carried out as per the reference of Rasatarangini.[6] *Ashuddha Hingu* was taken and small pieces were made by pounding in a stainless-steel mortar and pestle. *Goghrita* was taken in s. s. vessel and heated. Pieces of *Hingu* were added in s. s. vessel and *Bharjana* was done. When *Hingu* became crispy it was removed carefully. Put and spread it on butter paper. After drying, *Shuddha Hingu* was collected and stored in an airtight container. [Figure 3]

4. Preparation of *Churna* of *Haritaki, Shunthi, Maricha, Pippali, Shuddha Kupilu, Shuddha Hingu* and *Shuddha Gandhaka*

Haritaki, Shunthi, Maricha, Pippali, Shuddha Kupilu, Shuddha Hingu and *Shuddha Gandhaka* were crushed in mortar and pestle individually and ground in the mixer grinder. *Haritaki, Shunthi, Maricha, Pippali,* and *Shuddha Gandhaka* were sieved through 120#, while *Shuddha Kupilu* and *Shuddha Hingu* sieved through 60#. Sieved fine powders were collected and packed in air tight container.

5. *Kumari Svarasa*

The leaves of *Kumari* were washed thoroughly with water. Then the thorny ridges, apex, and cuticle parts of *Kumari* leaves were cut by knife, and mucilaginous pulp was separated from the inner surface of leaves with the help of knife. After that pulp was churned with the grinder, then strained through a clean cotton cloth. Collected *Svarasa* were used for further process. [Figure 4]

6. Preparation of *Amapachaka Vati*

Amapachaka Vati was prepared as per the reference of Chikitsapradip.[7] Powder of *Haritaki, Shunthi, Maricha, Pippali, Shuddha Kupilu, Shuddha Hingu, Shuddha Gandhaka,* and *Saindhava* were taken in a mortar pestle and trituration was carried out till it became a homogenous mixture. After proper mixing, *Kumari Svarasa* was added little by little and levigated well till became doughy mass, then *Vati* was prepared out of it. *Vati* was shade dried, weighted, labelled, and stored in airtight container. [Figure 5]

Analytical evaluation

Raw materials, in process materials (*Shuddha Kupilu, Shuddha Gandhaka* and *Shuddha Hingu*) and finished product were analysed by using different organoleptic characters i.e., colour, odour, taste, texture and appearance; physico-chemical parameters i.e., pH,[8] loss on drying,[9] total ash,[10] acid insoluble ash,[11] water soluble extractive,[12] and alcohol soluble extractive.[13] Additionally, uniformity of weight, friability, hardness, and disintegration time of finished product were done. Organoleptic and preliminary physico-chemical analysis were conducted at Quality Testing Laboratory, Upgraded Department of Rasasashtra and Bhaishjya Kalpana, Government Ayurved College, Vadodara, Gujarat.

Amapachaka Vati was also evaluated for HPTLC and heavy metal analysis.

High Performance Thin Layer Chromatography (HPTLC) analysis

High Performance Thin Layer Chromatography (HPTLC) was conducted at Vasu Research Centre, Division of Vasu Healthcare PVT. LTD. Vadodara, Gujarat. HPTLC instrument CAMAG with 4 spotter Linomat 5, HPTLC Scanner 4 and Vision CATS software was used for chromatographic analysis. A twin trough chamber was used for the development of the HPTLC plate. A photo documentation cabinet fitted with High-Resolution camera was used for capturing images at different wavelengths. Approximately weighted 1 g of sample in a reflux flask. To it add 20 mL of methanol and reflux it for 20 minutes in water bath. On completion, the flask was removed from the water bath and filtered with the help of Whatsmann No.1. The test solution was used and obtained for HPTLC fingerprinting. 0.5 mL of Anisaldehyde is mixed with 10 mL Glacial acetic acid, followed by 85 mL Methanol and 5 mL Sulphuric acid (98%).

CAMAG Linomat 5 – applicator was used for application. The application start position (Y axis) was 10 mm to avoid edge effects. Linear ascending development to a distance of 80 mm with Toluene: Ethyl acetate: formic acid 5:15:0.5 (v/v) as mobile phase was performed in a twin-trough glass chamber previously saturated with mobile phase for 30 min. The plates were dried under TLC plate heater and visualized under 254 nm, 366 nm, and 540 nm for ultraviolet detection, and taken the fingerprints as evident.

Heavy metal analysis by ICP-OES

Heavy metal analysis was conducted at Vasu Research Centre, Division of Vasu Healthcare PVT. LTD. Vadodara, Gujarat. Inductive Coupled Plasma Optical Emission Spectrometer (ICP-OES), Make: Perkin Elmer Model: Optima 3300 RL was used. Reagents used for sample preparation are Deionized water, resistivity > 18.2 M ohm cm, Hydrochloric acid, 37% GR, Merck, and Nitric acid 69%GR, Merck. For acid digestion of sample, take 0.5 g sample and add 5 ml concentrated HNO_3: 1 ml concentrated HCL (3) in a closed vessel, followed by heating on plate. Allow it to cool, filter the solution into 25 ml volumetric flask and make up by deionized water up to mark. Prepare blank in similar way.

Analysis was done by calibrate using the blank and standard and then analyze blank and sample solution. Close the vessels tightly and keep on the turner. Detection was done by using calibration curve.

OBSERVATIONS AND RESULTS

1. *Kupilu Shodhana*

On addition of *Ashuddha Kupilu* in *Eranda Taila, Kupilu* turns into a black colour with the characteristic smell of *Eranda Taila*. After heating it for 15 min. *Kupilu* started to puffed up (146 ºC). Further, after 18 min. of heating, *Kupilu* fully puffed up and turned dark brown in color (179 ºC). It was observed that, testa and embryo were removed easily after *Shodhana*. *Shuddha Kupilu* was dark brown in colour and flat disc shaped. The results of *Kupilu Shodhana* are mentioned in Table no.3.

Table3: Results of *Kupilu Shodhana* process

Sr.no	Parameters		Results			
			B-1	B-2	B-3	Average
1.	Initial weight of *Ashuddha Kupilu* (g)		300	300	300	300
2.	Initial weight of *Eranda Taila* (g)		19	19	19	19
3.	Final weight of *Shuddha Kupilu*	(g)	244	248	245	245.67
		(%)	81.33	82.67	81.67	81.89
4.	Total loss	(g)	56	52	55	54.33
		(%)	18.67	17.33	18.33	18.11
5.	Reason for loss		Due to roasting and removal of testa and embryo			
6.	Total time taken for *Kupilu Shodhana* (hrs.)		9	9	9	9

2. *Gandhaka Shodhana*

On heating, *Gandhaka* was melted and turned into liquid form which looks like red colored oil. Characteristic smell of sulphur was perceived. When melted *Gandhaka* (121 ºC) was poured through cloth, solidified sulphur particles were seen on the surface of cloth. *Gandhaka* was turned into a granular mass after pouring in *Godugdha*. Traces of *Goghrita* with impurities was floated over *Godugdha*. After washing with hot water, the color of *Gandhaka* turned into dull yellow. The results of *Gandhaka Shodhana* were mentioned in Table no.4.

Table 4: Results of *Gandhaka Shodhana* process

Sr.no.	Parameters		Results			
			B-1	B-2	B-3	Average
1.	Initial quantity of *Ashuddha Gandhaka* (g)		250	250	250	250
2.	Initial quantity of *Goghrita* (g)		25	25	25	25
3.	Initial quantity of *Godugdha* (ml)		1500	1500	1500	1500
4.	Final weight of *Shuddha Gandhaka*	(g)	243	240	242	241.67
		(%)	97.2	96	96.8	96.67
5.	Total loss	(g)	7	10	8	8.33
		(%)	2.8	4	3.2	3.33
6.	Reason for loss		Due to removal of impurities and sticking with cloth & vessel			
7.	Total time taken for *Gandhaka Shodhana* (days)		2	2	2	2

3. *Hingu Shodhana*

The characteristic smell of *Goghrita* and *Hingu* were perceived throughout the *Shodhana*. After heating it for 7 min. Bubbles started to appear with the characteristic smell of *Goghrita* and *Hingu*. Further after heating for 21 min., *Hingu* turned crispy and dark brown in color (142 ºC). The results of *Hingu Shodhana* were mentioned in Table no.5.

Table 5: Results of *Hingu Shodhana* process

Sr.No.	Parameters		Results			
			B-1	B-2	B-3	Average
1.	Initial weight of *Ashuddha Hingu* (g)		300	300	300	300
2.	Initial weight of *Goghrita* (g)		300	300	300	300
3.	Final weight of *Shuddha Hingu* (g)		304	301	303	302.67
4.	Total gain	(g)	4	1	3	2.67
		(%)	1.33	0.33	1	0.89
5.	Reason for gain		Due to absorption of *Goghrita*			
6.	Total time taken for *Hingu Shodhana* (days)		8	8	8	8

4. Preparation of *Churna* of *Haritaki, Shunthi, Maricha, Pippali, Shuddha Kupilu, Shuddha Hingu* and *Shuddha Gandhaka*

Small particles of powder flew around the room after removing the lid from the mixer jar and during powdering. During powdering of *Shuddha Kupilu* and *Shuddha Hingu*, the powder was stuck in a mixer jar which had to scraped with spoon and the sieve was often blocked with fine powder which had to remove with brush. Details of powder of *Haritaki, Shunthi, Maricha, Pippali, Shuddha Kupilu, Shuddha Gandhaka* and *Shuddha Hingu* were given in Table No.6.

Table 6: Details of powder of the ingredients

No.	Ingredients	Initial weight (g)	Final weight (g)	Loss (g)	Yield (%)
1	*Haritaki*	900	562	336	62.44
2	*Shunthi*	900	844	56	93.78
3	*Maricha*	900	822	78	91.33
4	*Pippali*	900	660	240	73.33
5	*Shuddha Kupilu*	245.67	181.33	64.34	73.81
6	*Shuddha Gandhaka*	241.67	241.67	0	100.0
7	*Shuddha Hingu*	300.67	271	29.67	90.13

5. *Kumari Svarasa*

Kumari Svarasa was translucent in color and sticky on touch. Foaming was observed during the squeezing of pulp through cloth. The results of *Kumari Svarasa* were mentioned in Table no.7.

Table 7: Details of results of *Kumari Svarasa*

Sr.No.	Parameters	Results			
		B-1	B-2	B-3	Average
1.	Initial weight of *Kumari* Leaves (g)	400	400	400	400
2.	Final weight of *Kumari Svarasa* (ml)	210	200	200	203.33
3.	Volume yield (%)	52.5	50	50	50.83
4.	Total time taken for preparation of *Kumari Svarasa* (min.)	30	30	30	30

6. Preparation of *Amapachaka Vati*

For the preparation of *Amapachaka Vati*, 15 min. of trituration was carried out to prepare homogenous mixture and material was turned to dark cream colour. After addition of *Kumari Svarasa*, 1 hour of levigation was carried out till become doughy mass. Characteristic smell of *Pippali, Shunthi* and *Maricha* were felt during process. Dried *Vati* was greenish brown in colour. The results of *Amapachaka Vati* were mentioned in Table no.8.

Table 8: Results of *Amapachaka Vati*

Sr.no	Parameters		Results			
			B-1	B-2	B-3	Average
1.	Initial weight of total ingredients (g)		300	300	300	300
2.	Initial quantity of *Kumari Svarasa* (ml)		185	185	185	185
3.	Final weight of *Amapachaka Vati*	(g)	290	291	293	291.33
		(%)	96.67	97	97.67	97.11
4.	Total loss	(g)	10	9	7	8.67
		(%)	3.33	3	2.33	2.89
5.	Reason for loss		Due to sticking in mortar pestle			
6.	Total time taken for preparation of *Amapachaka Vati* (days)		3	3	3	3

Analytical evaluation:

Organoleptic characters:

The organoleptic characteristics of raw materials are tabulated in Table No.9.

Table 9: Organoleptic characters of raw materials

No	Samples	Observations				
		Color	Odor	Taste	Touch	Appearance
1.	*Ashuddha Kupilu*	Grey	Odorless	NA*	Smooth	Flat disc
2.	*Ashuddha Hingu*	Brown	Strong, characteristic	Astringent	Hard	Lump
3.	*Ashuddha Gandhaka*	Dull yellow	Odorless	NA*	Hard	Crystalline
4.	*Haritaki*	Yellowish brown	Characteristic	Astringent	Wrinkled	Ellipsoid to ovoid
5.	*Shunthi*	Creamish	Agreeable, Aromatic	Pungent	Wrinkled	Irregular
6.	*Maricha*	Black	Aromatic	Pungent	Wrinkled	Round
7.	*Pippali*	Greenish black	Aromatic	Pungent	Rough	Longitudinal
8.	*Saindhava*	White	Odorless	Salty	Hard	Crystaline
9.	*Kumari* pulp	Translucent	Characteristic	Bitter	Slimy	Gel like

* *Ashuddha* material can be toxic in nature so taste was not done.

The organoleptic characteristics of in-process materials are given in Table no.10.

Table 10: Organoleptic characters of in process material

No	Samples		Observations				
			Color	**Odor**	**Taste**	**Texture**	**Appearance**
1.	*Shuddha Kupilu*	B 1	Dark brown	Characteristic smell of *Eranda Taila*	Bitter	Hard	Flat disc
		B 2	Dark brown	Characteristic smell of *Eranda Taila*	Bitter	Hard	Flat disc
		B 3	Dark brown	Characteristic smell of *Eranda Taila*	Bitter	Hard	Flat disc
2.	*Shuddha Hingu*	B 1	Dark brown	Characteristic smell of *Goghrita* & *Hingu*	Astringent	Brittle	Small pieces
		B 2	Dark brown	Characteristic smell of *Goghrita* & *Hingu*	Astringent	Brittle	Small pieces
		B 3	Dark brown	Characteristic smell of *Goghrita* & *Hingu*	Astringent	Brittle	Small pieces
3.	*Shuddha Gandhaka*	B 1	Dull yellow	Characteristic smell of *Goghrita*	Tasteless	Hard	Granular
		B 2	Dull yellow	Characteristic smell of *Goghrita*	Tasteless	Hard	Granular
		B 3	Dull yellow	Characteristic smell of *Goghrita*	Tasteless	Hard	Granular

The organoleptic characteristics of *Amapachaka Vati* are given in Table no.11.

Table 11: Organoleptic characters of *Amapachaka Vati*

Sr.No.	Characteristic	Observations		
		B 1	**B 2**	**B 3**
1	Color	Greenish brown	Greenish brown	Greenish brown
2	Odor	Characteristic	Characteristic	Characteristic
3	Taste	Bitter, salty	Bitter, salty	Bitter, salty
4	Texture	Rough	Rough	Rough
5	Appearance	Spherical	Spherical	Spherical

Physico-chemical parameters:

The physico-chemical parameters of raw materials with their available API standard are tabulated in Table no.12.

Table 12: Physico-chemical parameters of raw material

Parameter	*Ashu. Kupilu*		*Ashu. Hingu*		*Ashu. Gandhaka*	*Haritaki*		*Shunthi*		*Maricha*		*Pippali*		*Saindhava*	*Kumari* pulp
	Results	API std.	Results	API std.	Results	Results	API std.	Results	API std.	Results	API std.	Results	API std.	Results	Results
Loss on drying (%w/w)	3.88	-	6.27	-	0.44	6.09	-	5.89	-	5.73	-	5.87	-	3.65	-
Total Ash (%)	0.92	NMT 2	1.33	NMT 15	3.89	1.7	NMT 5	3.86	NMT 6	3.63	NMT 5	4.54	NMT 7	2.78	-
Acid insoluble Ash (%)	0.11	NMT 0.2	0.34	NMT 3	1.02	0.59	NMT 5	0.52	NMT 1.5	0.41	NMT 0.5	0.39	NMT 0.5	0.69	-
Water soluble extractive (%)	18.3	NLT 12	52.65	NLT 50	0.072	66.78	NLT 60	22.48	NLT 10	10.21	NLT 10	34.72	NLT 7	0.052	-
Alcohol soluble extractive (%)	4.44	NLT 4	56.24	NLT 50	0.13	72.2	NLT 40	7.95	NLT 3	15.38	NLT 6	32.93	MLT 5	-	-
pH	5.81	-	7.88	-	-	4.07	-	6.65	-	6.77	-	6.29	-	7.52	4.15
Specific gravity	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.73
Total solid content	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.07

The physico-chemical parameters of in process materials are given in Table no.13.

Table 13: Physico-chemical parameters of in process material

No	Samples		Results					
			pH	Loss on Drying (%w/w)	Total Ash (%w/w)	Acid insoluble Ash (%w/w)	Water soluble extractive (%w/w)	Alcohol soluble extractive (%w/w)
1.	*Shuddha Kupilu*	B 1	4.38	7.89	0.89	0.14	16.62	2.40
		B 2	4.35	7.77	0.99	0.09	17.24	2.47
		B 3	4.41	7.79	0.94	0.09	17.75	2.31
		Average	**4.38**	**7.81**	**0.94**	**0.11**	**17.20**	**2.39**
2.	*Shuddha Hingu*	B 1	5.34	3.19	1.09	0.29	31.39	8.61
		B 2	5.38	3.17	1.19	0.24	30.54	8.68
		B 3	5.39	3.16	1.14	0.24	31.74	7.87
		Average	**5.37**	**3.17**	**1.14**	**0.26**	**31.22**	**8.38**
3.	*Shuddha Gandhaka*	B 1	6.07	0.80	4.02	1.03	0.23	0.23
		B 2	6.04	0.87	4.07	1.06	0.27	0.26
		B 3	6.08	0.83	4.10	1.08	0.25	0.30
		Average	**6.06**	**0.83**	**4.06**	**1.05**	**0.25**	**0.26**

The physico-chemical parameters of *Amapachaka Vati* are given in Table no.14.

Table14: Physico-chemical parameters of *Amapachaka Vati*

Sr.No.	Parameters	Observations			
		B 1	B 2	B 3	Average
1.	pH	4.26	4.3	4.35	4.3
2.	Loss on Drying (%w/w)	4.52	4.52	4.52	4.52
3.	Total Ash (%w/w)	21.26	22.43	22.46	22.05
4.	Acid insoluble Ash (%w/w)	3.6	3.9	3.85	3.78
5.	Water soluble extractive (%w/w)	35.92	36.79	36.45	36.38
6.	Alcohol soluble extractive (%w/w)	18.81	19.42	18.69	18.97
7.	Uniformity of weight (mg)	249.9±2.42	249.75±2.43	249.8±2.47	249.81±2.44
8.	Friability (%)	0.89	0.87	0.79	0.85
9.	Hardness (kg/cm^2)	1.95	1.8	1.9	1.88
10.	Disintegration time (min:sec)	13.71	13.72	13.83	13.75

High Performance Thin Layer Chromatography analysis (HPTLC)

6, 5 and 8 spots are found at different R_f value in *Amapachaka Vati* under 254, 366 and 540 nm UV light. (Table no.15) [Figure 6]

Table 15: Rf value and no. of spot found in *Amapachaka Vati* under 254, 366 and 540 nm

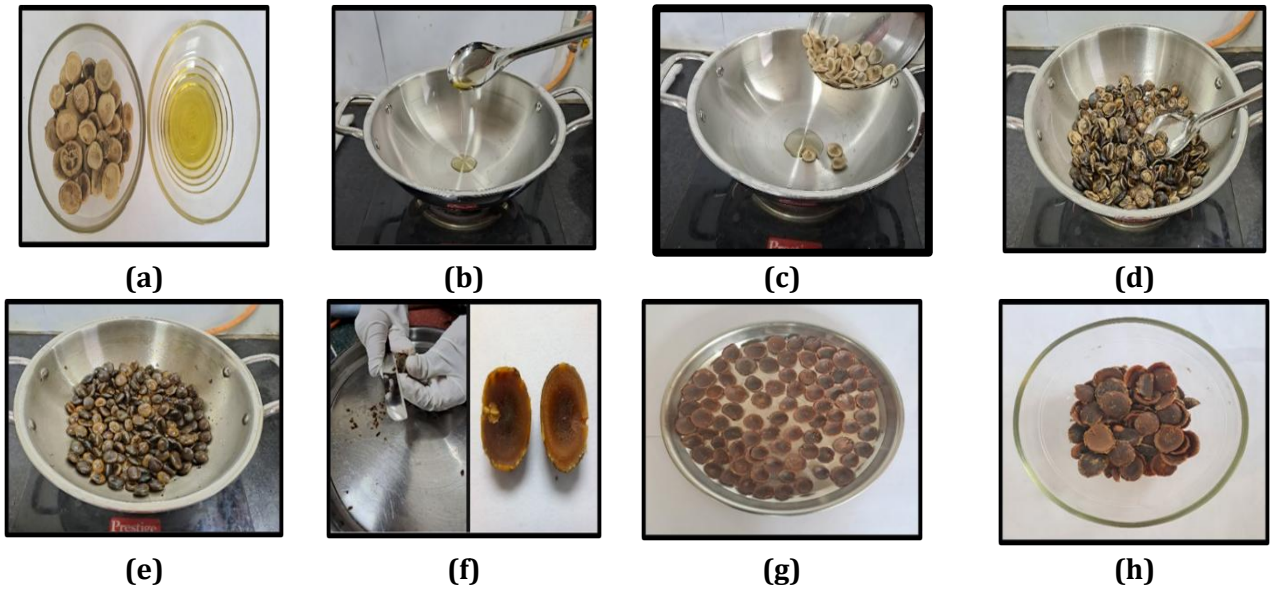
Solvent system (v/v)	Observed under UV light	No. of spots	Rf value
Toluene: Ethyl Acetate: Formic acid (5:15:0.5)	254 nm	6	0.14, 0.53, 0.68, 0.83, 0.88, 0.94
	366 nm	5	0.11, 0.19, 0.68, 0.83, 0.94
	540 nm	8	0.14, 0.32, 0.53, 0.68, 0.74, 0.83, 0.88, 0.94

Heavy metal analysis by ICP-OES

Heavy metal analysis was also carried out for *Amapachaka Vati*. (Tabel no.16)

Table 16: Heavy metal analysis of *Amapachaka Vati*

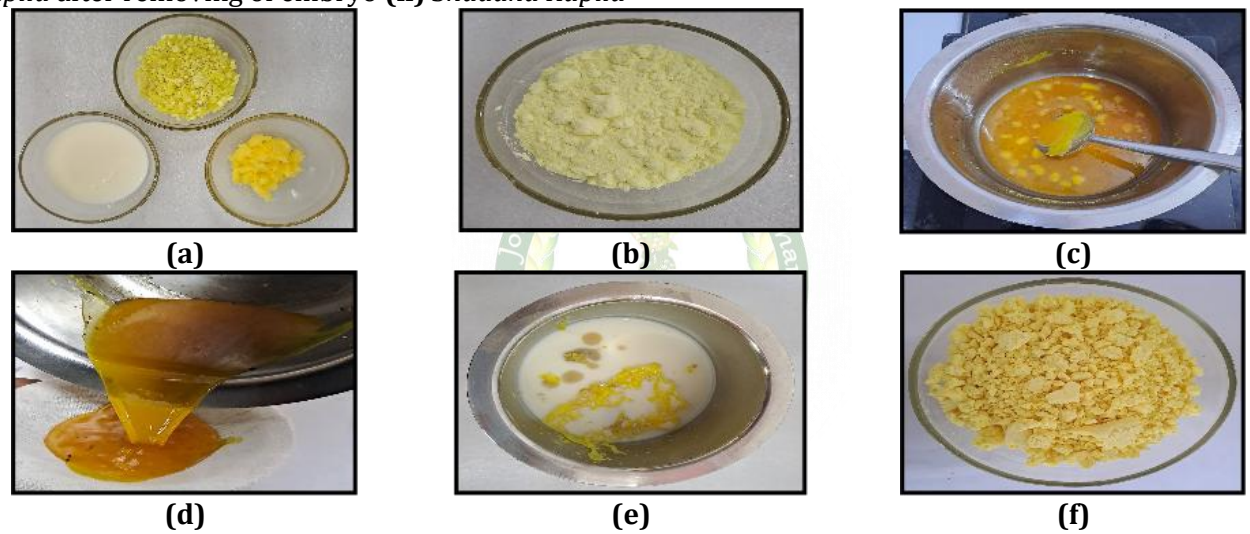
Sr.No	Heavy Metal Content	AV	Permissible limits as per API[14]
1	Lead	1.435 ppm	NMT 10 ppm
2	Cadmium	0.034 ppm	NMT 0.3 ppm
3	Arsenic	0.843 ppm	NMT 3 ppm
4	Mercury	0.107 ppm	NMT 1 ppm



(a) **(b)** **(c)** **(d)**

(e) **(f)** **(g)** **(h)**

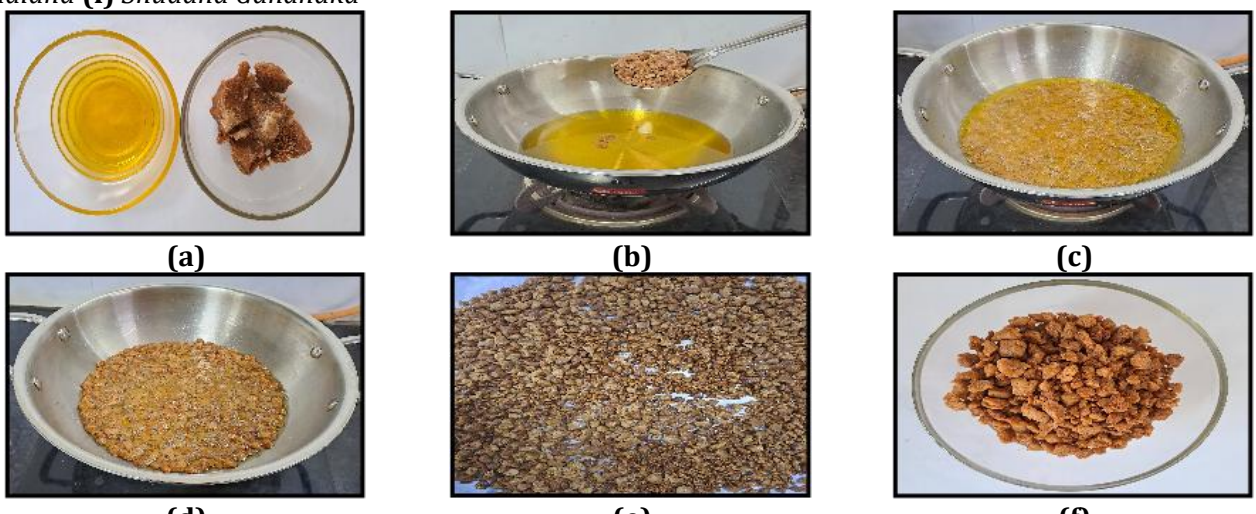
Figure 1: *Kupilu Shodhana*: (a) Ingredients **(b)** Taking *Eranda Taila* in s.s.vessel **(c)** Adding *Ashuddha Kupilu* in *Eranda Taila* **(d)** *Bharjana* of *Kupilu* **(e)** Puffed *Kupilu* **(f)** Removing testa and embryo **(g)** *Kupilu* after removing of embryo **(h)** *Shuddha Kupilu*



(a) **(b)** **(c)**

(d) **(e)** **(f)**

Figure 2: *Gandhaka Shodhana* (a) Ingredients **(b)** *Gandhaka* powder **(c)** Melted *Gandhaka* in *Goghrita* **(d)** Pouring of melted *Gandhaka* into vessel containing *Godugdha* **(e)** *Gandhaka* in *Godugdha* after *Dhalana* **(f)** *Shuddha Gandhaka*



(a) **(b)** **(c)**

(d) **(e)** **(f)**

Figure 3: *Hingu Shodhana* (a) Ingredients **(b)** Adding pieces of *Hingu* in melted *Goghrita* **(c)** *Bharjana* process **(d)** Brown coloured *Hingu* floated over *Goghrita* **(e)** Drying of *Hingu* **(f)** *Shuddha Hingu*

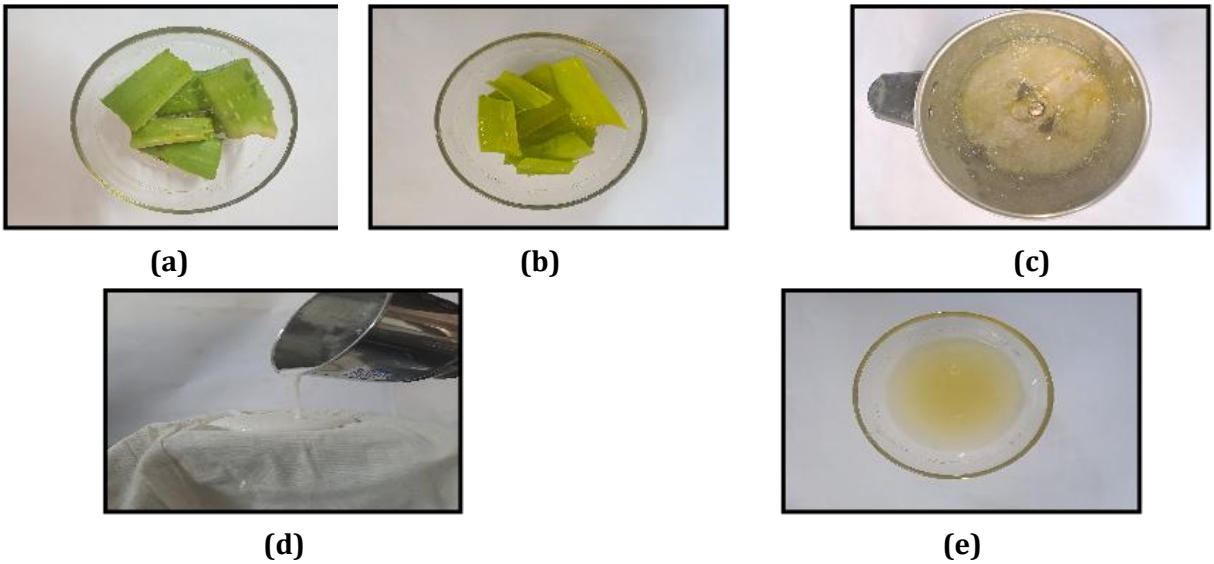
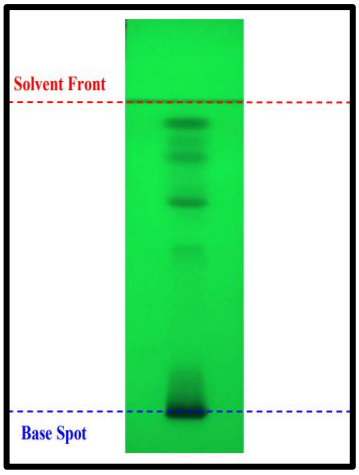
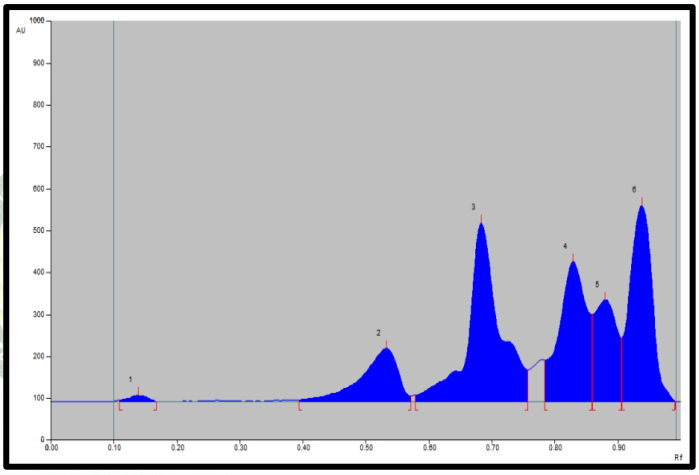


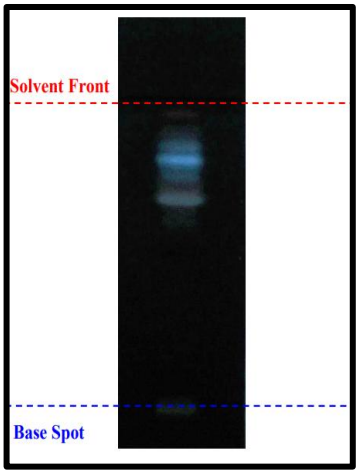
Figure.4: *Kumari Svarasa* (a) *Kumari* leaves **(b)** Cut thorny ridges, apex and cuticle parts of *Kumari* leaves **(c)** Griding pulp in mixer grinder **(d)** Straining through cloth **(e)** *Kumari Svarasa*



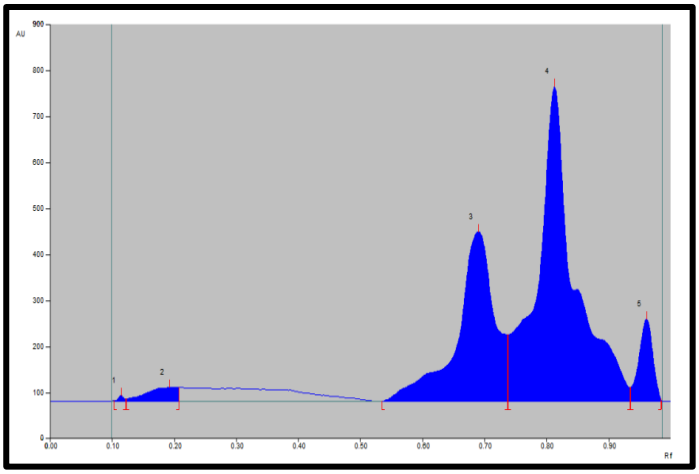
HPTLC plate @ 254 nm



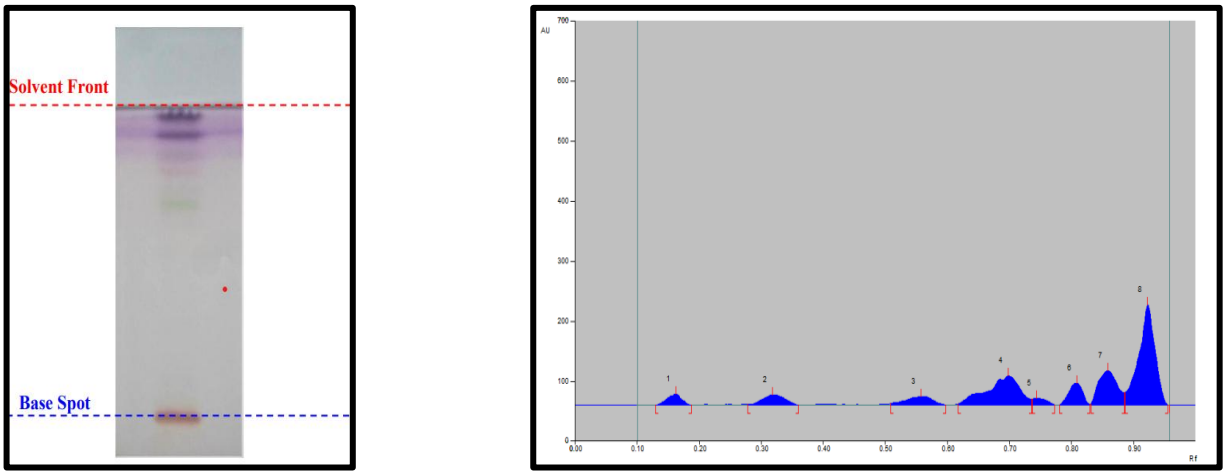
HPTLC chromatogram @ 254 nm



HPTLC plate @ 366 nm



HPTLC chromatogram @ 366 nm



HPTLC plate @ 540 nm **HPTLC chromatogram @ 540 nm**

Figure 6: HPTLC plate and chromatogram

DISCUSSION

This study has been conducted to develop Standard Manufacturing Procedure (S.M.P.) of *Amapachaka Vati* as per reference of Chikitsapradip. For the fulfilment of the aim, three batches of *Kupilu Shodhana, Gandhaka Shodhana, Hingu Shodhana* and *Amapachaka Vati* were prepared.

In *Kupilu Shodhana* process, 1/32nd to 1/16th part of *Eranda Taila* was mentioned.[15] So, two pilot batches were carried out to determine the quantity of *Eranda Taila*, to see whether testa and embryo were removed easily and changes in *Kupilu* during or after *Shodhana* including its yield percentage. In both pilot batches, 50 g of *Ashuddha Kupilu* was taken for the *Shodhana* procedure. In first pilot batch, 1/32nd part (1.5 g) and in the second pilot batch, 1/16th part (3 g) of *Eranda Taila* was taken and *Bharjana* was done till it puffed up. It was observed that, in the first pilot batch, difficulty was found to remove testa and embryo, and 3 hours were taken for procedure. In the second pilot batch testa and embryo were removed easily and 2 hours were taken for procedure. The yield obtained in the first and second pilot batches were 78% and 80% respectively. After observing the findings of both the pilot batches, it was decided to adopt 2nd pilot batch for the process of the main batch. Total 3 main batches were carried out to develop SMP. In that, 300 g *Kupilu* and 19 g *Eranda Taila* were taken for *Shodhana* in each batch. During *Shodhana*, *Kupilu* started to puffed up which may be due to the moisture content inside the seeds evaporates which causes thermal pressure which ultimately helps to separate cotyledons easily After *Shodhana*, *Kupilu* turns in dark brown color which is due to roasting of outer layer of seed. The same observations were found in all three batches. Average 81.89% yield of *Kupilu Shodhana* was found. l8.11% loss was found due to the removal of testa and embryo and also some contains may burnt during the process.

For *Gandhaka Shodhana*, one pilot batch was carried out to determine the ratio of *Godugdha* and *Goghrita* for *Shodhana*, and to decide the duration required for the whole procedure. For this, 100 g of powder *Gandhaka,* total 600 ml of *Godugdha* and 10 g of *Goghrita* were used. 96% yield for *Gandhaka Shodhana* was obtained. Based on pilot batch, total 3 main batches were carried out to develop SMP. For *Shodhana,* powdered *Gandhaka* was used because it increases the effective surface area which facilitates quick melting.[16] During filtering, stone and other physical impurities are filtered through cloth and fat-soluble impurities gets dissolve in *Ghrita* and *Godugdha* are removed with it.[17] After *Shodhana*, crystalline sulphur turned into amorphus sulphur which is easily absorbed by the body.[18] The same observations were found in all three batches. The average yield % of *Gandhaka Shodhana* was 96.67%. Loss was due to the removal of physical impurities and sticking to the vessel and cloth.

In *Hingu Shodhana*, two pilot batches were carried out to determine the size of *Hingu* pieces should take for *Shodhana*, and to decide the duration required for the whole procedure. In both pilot batches, 20 g of *Ashuddha Hingu* and 20 g *Goghrita* were taken. In the first pilot batch, powdered *Hingu* was taken and *Bharjana* was done till it turned crispy. It was taken out and put on butter paper and spread to absorb the extra *Goghrita* in *Hingu.* It was observed that *Ghrita* absorbed by powdered *Hingu* and it didn't get dry even after 23 days. So, the second pilot batch was carried out taking small pieces of *Hingu* instead of powder and *Shodhan* procedure was done in *Ghrita*. It got dry after 3 days. Thus, it was decided to adopt 2nd pilot batch for preparation of the main batch. Total 3 main batches were carried out to develop SMP. *Hingu* contains sulphur[19] and during heating, it may release various sulphur-containing compounds. During

Bharjana in *Goghrita,* the triglycerides in the fat break down into free fatty acids and other compounds.[20] This breakdown may produce a characteristic smell of *Goghrita*. After *Shodhana*, *Hingu* turns in dark brown color which may be due to *Bharjana* process. Ideal temperature for deep frying is 120ºC to 150ºc till turns crispy.[21] All the observations were found same in all three batches. The average yield of *Hingu Shodhana* is 302.87 gm (0.89% gain). this gain may be due to absorption of *Goghrita* by *Hingu*.

In powder preparation; *Haritaki, Shunthi, Maricha, Pippali,* and *Shuddha Gandhaka* were sieved through 120#. During sieving, *Shuddha Kupilu* and *Shuddha Hingu* don't pass through 120#. *Shuddha Kupilu* is hard in nature and contains trichome which is a fibrous structure in it[22] while *Shuddha Hingu* was sticky due to *Shodhan* in *Ghrita* which block the sieve. So, they sieved through 60#. 62.44%. 93.78%, 91.33%, 73.33%, 73.81%, 100%, and 90.13% yield were obtained in *Shunthi, Maricha, Pippali, Shuddha Kupilu, Shuddha Gandhaka* and *Shuddha Hingu* powder respectively.

For preparation of *Amapachaka Vati*, one pilot batch was carried out to decide the quantity of *Bhavana Dravya* and *Vati* size. For pilot batch, 45 g of powdered ingredients were taken and 28 ml of *Kumari Svarasa* was added and levigated. 350 mg size of *Vati* was prepared manually. 93.33% yield was obtained. The main batches of *Amapachaka Vati* were carried out by adopting the same methodology of pilot batch. *Pippali, Shunthi*, and *Maricha* contains volatile oil like piperine, gingerol, and piperine respectively.[23] All these are released during grinding and mixing. The heat generated during the trituration and levigation processes may enhance the volatilization of these compounds, intensifying their aroma. Average yield % of *Amapachaka Vati* was 97.11%. *Vati* was manually rolled so sticking in vessel and handing loss occurred.

An analytical study plays a significant role in Ayurveda since it helps in define criteria for final products and validates product perfection. Organoleptic characters, physico-chemical parameters, HPTLC, and heavy metal analysis were taken into consideration to develop the analytical profile of *Amapachaka Vati*.

Organoleptic and physicochemical parameters of *Haritaki, Shunthi, Maricha, Pippali, Kupilu, Hingu,* and *Kumari,* were done and they found compliance with the API standards.[24] While organoleptic and physicochemical parameters of *Gandhaka* and *Saindhava* were found compliance with previous research works.[25,26]

During *Shodhana*, toxic alkaloids like strychnine and brucine are reduced which are alkaline.[27] This may lead to decreases in the pH of *Kupilu* after *Shodhana*. *Shuddha Kupilu* seeds absorb some amount of *Eranda Taila*, which is a viscous, non-volatile substance.[28] This may play a role in increase the loss on drying. *Kupilu* seeds contain strychnine and brucine, which are partially water and alcohol-soluble.[29] During *Shodhana* with *Eranda Taila*, these alkaloids are decreased. This may reduce the water-soluble and alcohol-soluble extractive.

pH of *Hingu* was slightly decreased after *Shodhana* which may be due to the heating provided in *Shodhana* procedure vaporized it's volatile contains like ferulic acid and umbelliferone.[30] Also, *Goghrita* used here as a *Shodhana* media has 5.9 pH.[31] This may incorporate in decrease of pH. Due to evaporation of moisture content and volatile oil during *Shodhana* decreases the loss on drying value of *Shuddha Hingu. Hingu* contain ferulic acid and umbelliferone, which are partially water and alcohol soluble.[32] During *Shodhana* with *Goghrita*, these alkaloids are decreases, this may lead to decrease in the value of water-soluble and alcohol soluble extractive after *Shodhana*.

pH of *Gandhaka* was decreased after *Shodhana* which may be due to *Goghrita* and *Godugdha* used here as a *Shodhana* media contains fats, proteins, and small amount of organic acids such as lactic acid and butyric acid.[33] Both water and alcohol-soluble extractive of *Gandhaka* were slightly increased after *Shodhana*, it may be due to the chemical reaction involved in the *Shodhana* might alter the molecular structure of *Gandhaka*, making it more water and alcohol-soluble.

Amapachaka Vati was bitter and salty. It contains *Kupilu* and *Bhavana* of *Kumari* pulp which has prominent bitter taste and *Saindhava* in double proportion which imparts a salty taste.

Analytical profile is important in the pharmaceutics because they help to ensure the safety, quality, and efficacy of drug. Here analytical profile of *Amapachaka Vati* was done. Loss on drying at 105 ºC is one of the major factors responsible for the deterioration of the drug and formulation.[34] Lower value of loss on drying was desirable for higher stability of formulations. For, *Amapachaka Vati*, it is 4.52%. Total ash, acid insoluble ash, water soluble extractive (w/w%), and alcohol soluble extractive (w/w%) were 22.05%, 3.58%, 36.38%, and 18.97% respectively. Higher ash value indicates the presence of inorganic matter[35] which may due to presence of *Gandhaka* and *Sanidhava* in *Amapachaka Vati*.

As previous work was not found on this formulation, general considerations for tablets given in Indian Pharmacopeia were taken for comparison. Size of *Amapachaka Vati* was 250 mg. Its average uniformity of weight (mg) was 249.81±2.44 mg. All the *Vati* were found within the 5% deviation of weight as per Indian Pharmacopeia for 250 mg. The friability

(%), and disintegration time (min.) of *Amapachaka Vati* were 0.85% and 13.75 min respectively which was found within limits given in Indian Pharmacopeia.[36] The hardness (kg/m3) of *Amapachaka Vati* was 1.88 kg/m^3, which was lower than Indian Pharmacopeia standard maybe due to manually made pills. Increase of hardness may be a scope for further research work.

HPTLC study of *Amapachaka Vati* was also carried out. HPTLC analysis, allows simplification of quality control processes for herbal drugs, because from it, information on identity, purity and content can be obtained.[37] 6, 5 and 8 spots are found at different R_f value in *Amapachaka Vati* under 254, 366 and 540 nm UV light which indicates different phyto-chemical compound in product. 0.68, 0.83, and 0.94 spots were found common when observed under UV light of 254 nm, 366 nm, and 540 nm.

Data of heavy metal revealed that heavy metals like lead (Pb), cadmium (Cd), arsenic (As), and mercury (Hg) were detected in the sample of *Amapachaka Vati* which was found within the permissible limit specified in API.[38] As the formulation contains herbal material, the physicochemical properties of the soil may also affect the heavy metal concentration.[39]

Amapachaka Vati is one of the most important Ayurvedic formulations that is advised by the Ayurvedic scholars for GI tract disorders. Although, being administered by a vast community of Ayurvedic practitioners, there was no any study that are available on the physicochemical analysis and standardization of *Amapachaka Vati*. In the present study, *Amapachaka Vati* prepared by the Ayurvedic classical method complies with the standard parameters of tablet as mentioned in Indian Pharmacopoeia.

CONCLUSION

The preparation method of *Amapachaka Vati* carried out here can be considered as Standard Manufacturing Procedure (S.M.P.). The evaluated parameters for *Amapachaka Vati* can be used in future reference as a standard. Moreover, further works should be carried out to explore the therapeutic outcomes of this medication.

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