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Research Article

PHARMACEUTICAL AND ANALYTICAL STANDARDISATION OF *KHANDA SHUNTHI AVALEHA*

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ABSTRACT

Background: To assure the therapeutic efficacy and safety of Ayurvedic formulation it is demand of time to standardise the Ayurvedic medicines. *Khanda Sunthi Avaleha* is Ayurvedic formulation used for the treatment of *Aamvata* described in *Bhavprakasha Samhita*. The present study was aimed that to standardise the *Khanda Shunthi Avaleha* by using the various testing parameters. **Methods:** Raw material used to prepare *Khanda Shunthi Avaleha* was procured from reliable sources, identified and authenticated by using different parameters as per API. Total 8 batches of *Avaleha* were prepared, different observations were noted down and finally analytical study of *Khanda Shunthi Avaleha* was carried out by using the. organoleptic characters, physico-chemical parameters. Other specified test like heavy metal test, microbial test, aflatoxins, pesticide residue, HPTLC etc. **Result:** Raw material was of standard quality which was used to prepare the *Khanda Shunthi Avaleha*. All 8 batches of *Khanda Shunthi Avaleha* has passed the confirmatory test of *Avaleha*. Organoleptic characters of all the batches were found similar. The findings of analytical parameter testing of *Avaleha* were within the limit as per the standards of Ayurvedic formulations mentioned in API. **Conclusion:** Current method followed for the preparation of *Khanda Shunthi Avaleha* can be considered as standard operative procedure in further studies and the test result obtained would serve as the tools for assistance to the scientific organisations, regulatory bodies and manufacturer for developing standard formulation of great efficacy.

INTRODUCTION

Now days, the fastest growing sector in world pharmaceutical market is Ayurvedic pharmaceutics. Standardisation of Ayurvedic drug is the demand of globalisation. The semisolid preparation in Ayurvedic pharmaceutics used for internal administration is *Avaleha Kalpana*. Acaharya Sharangdhar has considered *Avaleha Kalpana* as secondary preparation under the *Kwatha Kalpana*[1]. A semisolid preparation of herbal drugs prepared in the decoction or the extracts of different herbs by adding sweetening agents like jaggery, sugar or sugar candy is known as *Avaleha Kalpana*.

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Avaleha Kalpana is the *Upkalpana* of *Kwath Kalpana*. Different variety of *Avaleha* are described in various Ayurvedic classics and they are the most accepted varieties of Ayurvedic dosage forms due to its easy administration, palatability and long shelf life. Synonyms used for *Avaleha Kalpana* are *Rasakriya*, *Phanita, Avaleha, Khanda, Ghana*[2]. *Avaleha* can be administered along with specific *Anupana* as per the patient conditions.

Khanda Shunthi Avaleha is the Ayurvedic formulation used for the management of *Aamvata* disease. This is described by Acharya Bhavmishra in *Bhavprakasha Samhita* in *Aamvata Chikitsa*[3]. With collection, identification and authentication of raw drugs, pharmaceutical study along with its analytical study the present work was focus on first attempt to develop the pharmaceutical and analytical standards of *Khanda Shunthi Avaleha* on the basis of organoleptic characters, physico-chemical parameters, other specified tests along with High Performance Thin Layered Chromatographic evaluation.

METHODOLOGY

The raw material used to prepare the *Khanda Shunthi Avaleha* was *Shunthi (Zingiber officinalis), Maricha (Piper nigrum), Pippali (Piper longum), Ela (Elattaria cardamomum), Tvak* (Cinnamomnm zeylanicum), *Tejpatra (Cinnamomnm tamala), Khanda, Go dugdha* was procured from the local vendor of Ayurvedic herbs from Kurukshetra market. *Go ghrita* was procured from NDRI, Karnal. Raw drugs were identified by PG department of Dravyaguna, IASR, SKAU, Kurukshetra on the basis of their macroscopic characters mentioned in API. Authentication of raw drugs was on the basis of the parameters mentioned in AP[4] i.e., foreign matter, total ash, acid insoluble ash, loss on drying, water soluble extractive, alcohol soluble extractive, pH, TLC carried out in Government Drug Testing Laboratory, Kurukshetra and Statiate Research and Anatech Pvt. Ltd., Panchkula.

The eight batches of *Khanda Shunthi Avaleha* was prepared in Shri Krishna AYUSH Pharmacy with lic no. 1300-ISM-(HR), PG Department of Rasa Shastra evum Bhaishajya Kalpana, IASR, SKAU, Kurukshetra.

Table 1: Equipment specification for preparation of *Avaleha*

Name of Equipment	Nature of Material	Dimensions	
Mixer grinder with jar	Borosil electric grinder	Motor capacity	750 W
Sieve	Stainless Steel	No.	80
Muslin cloth	Muslin cloth	Length	1 mt
		Width	1 mt
Kadhai	Stainless steel	Depth	9.5 cm
		Diameter	26 cm
		Thickness	5 mm
		Weight	1280 gm
Plate	Stainless Steel	Depth	2 cm
		Diameter	17.5 cm
		Thickness	2 mm
		Weight	88 gm
Spoon	Satinless steel	Length-1	20 cm
		Weight-1	35 gm
		Length-2	16 cm
		Weight-2	33 gm
Spatula	Stainless steel	Length	40 cm
		Weight	150 gm
Thermometer	Digital thermometer	Max. Temp.	300 ^{0}C
		Min. Temp.	0 ^{0}C
Weighing balance	Electronic weighing balance	Max. capacity	10 kg
		Min. capacity	1 gm
Vessels for drug	Mealamine	Depth	4.5 cm
		Diameter	6.5 cm
		Weight	40 gm
Vessels for drug	Mealamine	Depth	3.5 cm
		Diameter	8.5 cm
		Weight	73 gm
Container for milk	Glass	Capacity	100 ml
Induction stove	Electric induction		
Finished product Storage container	Plastic	Depth	7.5 cm
		Diameter	5 cm
		Weight	15 gm

Table 2: Formula for *Khanda Shunthi Avaleha*

S.No	Ingredient	Family	Botanical name	Part used	Quantity
1.	*Shunthi*	Zingiberaceae	*Zingiber officinale*	Rhizome	16 Parts
2.	*Go-Ghrita*				40 Parts
3.	*Go-Dugdha*				64 Parts
4.	*Khanda*				60 Parts
5.	*Shunthi*	Zingiberaceae	*Zingiber officinale*	Rhizome	2 Parts
6.	*Maricha*	Piperaceae	*Piper nigrum*	Fruit	2 Parts
7.	*Pippali*	Piperaceae	*Piper longum*	Fruit	2Parts
8.	*Ela*	Zingiberaceae	*Elettearia cardamomum*	Seed	2 Parts
9.	*Twak*	Lauraceae	*Cinnamomum zeylanicum*	Bark	2 Parts
10	*Tejpatra*	Lauraceae	*Cinnamomum tamala*	Leaves	2 Parts

The raw drugs were dried, grind for the fine powder, sieved through sieve/muslin cloth. Firstly, put the required amount of *Ghrita* in *Kadhai* and kept the *Kadhai* on induction with minimum temperature. Heat the *Ghrita* till it became *Nishphena*. Note the time taken during the process and temperature at which *Ghrita* became *Nishphena*. Now, add the *Shunthi* in required amount and fried it, till it became brown in colour with specific odour of fried *Shunthi*. Note the time and temperature during the process. Add the milk in fried *Shunthi* and heat it with continuously stirring by spatula. Note the time and temperature during the process. Now, add the *Khanda* in said amount and mix it well in the above mixture till it attain the *Avaleha paka Lakshana.* Note the temperature and time during the process. Kept, the *Avaleha* for *Swangsheet* till it attains the temperature 60℃ and Note the time of process. After *Swangsheeta*, add the *Prakshepa drvyas* in required amount one by one so that, they mixed in *Avaleha* homogenously. Perform the confirmatory tests of *Avaleha* i.e., *Darvi praleptavam, Tantumatavam, Pidite Anguli Mudra, Apsumajjanam, Sthiratvam, Shlakshana, Patitstu na shiryte, Gandhavarnarasodbhava*[5,6,7] and store it in air tight containers.

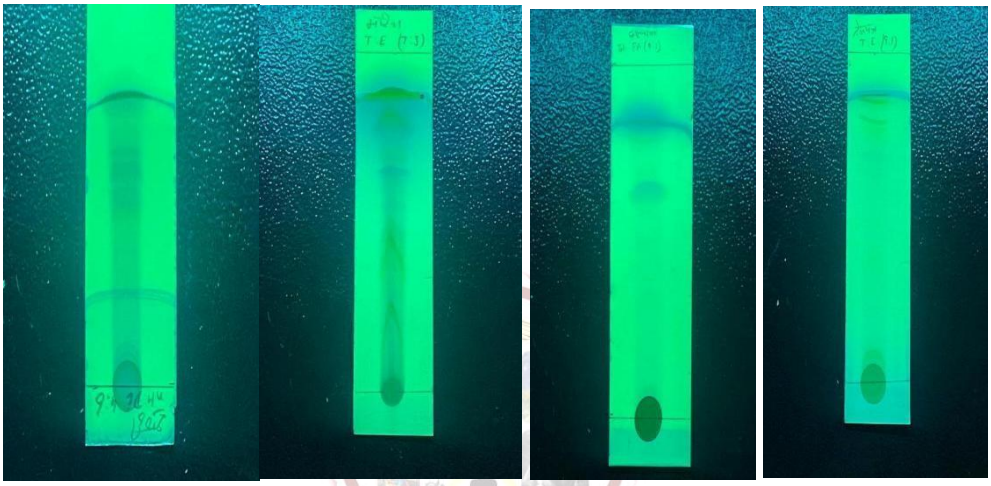
Analytical parameter tests of *Khanda Shunthi Avaleha*[8] included description, colour, odour, taste, consistency, composition, microbial staining test, total ash, acid insoluble ash, water soluble extractive, alcohol soluble extractive, reducing sugar, non-reducing sugar, total sugar, loss on drying, total solid, acid value, pH, heavy metal test, microbial test, aflatoxins, pesticide residue test, HPTLC was carried out at Government Drug Testing Laboratory, Kurukshetra, Interstellar Testing Centre, Panchkula, Vardan Envirolab, Gurugram.

RESULT AND OBSERVATIONS

Table 3: Analysis of Raw Drugs

		Shunthi	***Maricha***	***Pippali***	***Ela***	***Tvak***	***Tejpatra***
S.No.	**Parameters**	**Observation**	**Observation**	**Observation**	**Observation**	**Observation**	**Observation**
1.	Description	Laterally compressed rhizome	Black brown round fruit	Brown black cylindrical fruit	Green dried fruit	Brown dried bark	Green dried leaves
2.	Colour	Yellow	Black brown	Brownish black	Green	Brown	Green
3.	Odour	Aromatic and pleasant	Aromatic	Aromatic	Aromatic	Fragrant	Aromatic
4.	Taste	Agreeable and pungent	Pungent	Pungent	Astringent	Sweet	Slightly sweet
5.	Loss on drying at 105°C	7.3%	4.15%	6.2%	5.56%	4.50%	4.75%
6.	Acid insoluble ash	0.60%	0.22%	0.37%	0.48%	1.22%	0.29%

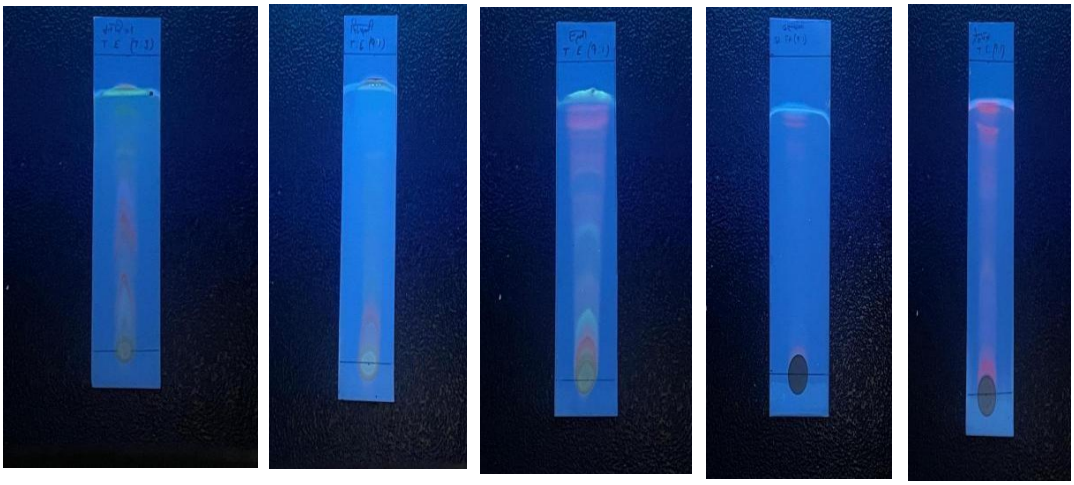
7.	Total ash	4.34%	4.55%	4.59%	5.03%	2.89%	3.60%
8.	Foreign matter	Nil	0.18%	Nil	Nil	0.29%	0.16%
9.	Water soluble extract	18.26%	14.35%	16.44%	26.91%	12.40%	22.73%
10.	pH (10% aq)	4.0		6.11	5.86	3.67	5.65
11.	Alcohol soluble extract	15.3%	10.8%	17.9%	8.7%	12.4%	18.5%
12	TLC Rf	0.54, 0.60, 0.63	0.08, 0.52, 0.27, 0.05, 0.57, 0.74, 0.97, 0.46, 0.66, 0.90	0.15, 0.26 0.04, 0.22	0.50, 0.76, 0.14, 0.18, 0.82	0.09, 0.58, 0.95, 0.75, 0.89	0.55, 0.26, 0.48, 0.95



Shunthi ***Maricha*** ***Tvak*** ***Tejpatra***

Figure 1 A **1B** **1C** **1D**

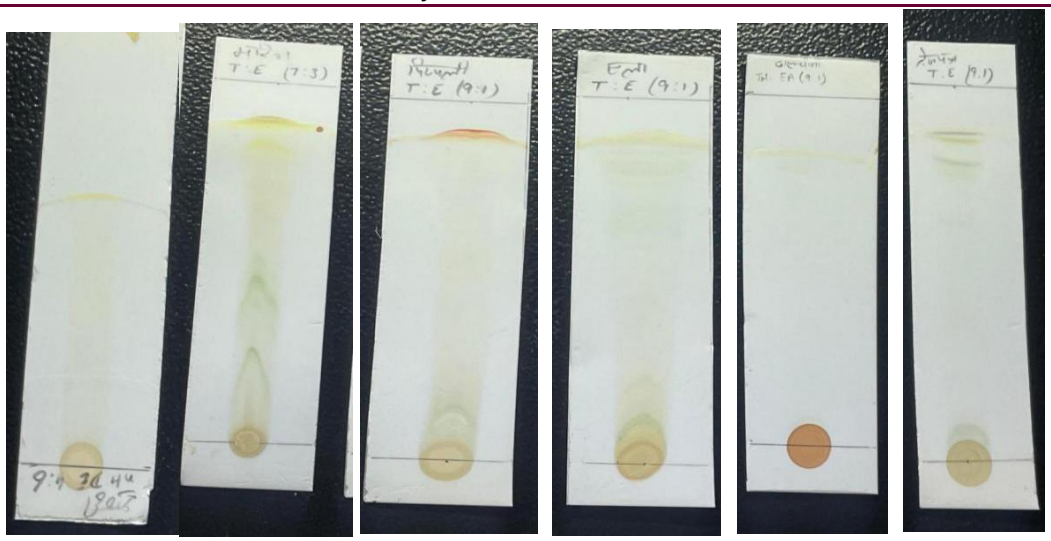
Figure 1: TLC Plates of Raw Drugs at 254nm



Maricha ***Pippali*** ***Ela*** ***Tvak*** ***Tejpatra***

Figure 2A **2B** **2C** **2D** **2E**

Figure 2: TLC Plates of Raw Drugs at 366nm



Shunthi ***Maricha*** ***Pippali*** ***Ela*** ***Tvak*** ***Tejpatra***

Figure 3A **3B** **3C** **3D** **3E** **3F**

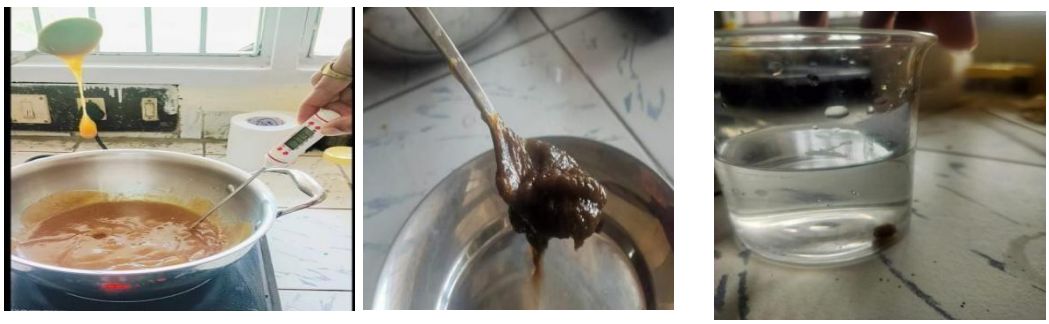
Figure 3: TLC plates of Raw Drugs at Visible light

Preparation of *Khanda Shunthi Avaleha*

Table 4: Observations during Preparations

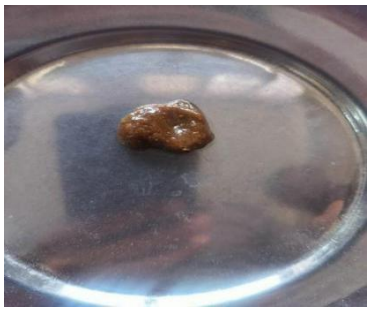
S.No.	Steps	Timing	Temperature
1.	*Ghrita Nishphena*	Start- 2:20 min	Room Temp- 60°C
2	*Shunthi Bhrajan*	2:20 min - 6:50 min	60°C- 100°C
3	*Dugdha addition*	6:50 min - 8:32 min	100°C- 101°C
4	*Khanda addition*	8:32 min- 12:30 min	101°C- 102°C
5	*Swangsheeta*	12:30 min- 21:00 min	60°C
	Prakshepa Dravyas		
1	*Shunthi*	21:00 min- 22:00 min	
2	*Maricha*	22:00 min- 23:00 min	
3	*Pippali*	23:00 min- 24:00 min	
4	*Ela*	24:00 min- 25:00 min	
5	*Tvak*	25:00 min- 26:00 min	
6	*Tejpatra*	26:00 min- 27:00 min	

All the 8 batches of *Khanda Shunthi Avaleha* pass the confirmatory tests of *Avaleha Kalpana* which was already mentioned. *Rasa* of *Khanda Shunthi Avaleha* was sweet, *Gandha* was pleasant and *Varna* was brown.



Tantumatvam ***Darvipralepam*** ***Apsumajjati***

Figure 4A **4B** **4C**



Pidite Anguli mudra

4D

Sthirtavam, Slakshana

4E

Figure 4: Confirmatory tests of *Avaleha*

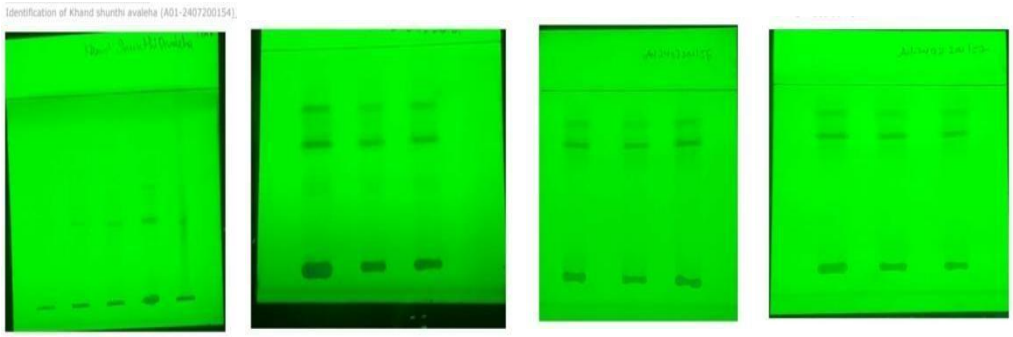
Analytical study of *Khanda Shunthi Avaleha.*

All batches of *Khanda Shunthi Avaleha* shows the similar organoleptic characters i.e., color brown, odour pleasant, taste sweet, consistency semisolid with no viable organism, animal body, tissue or any insect body part is detected into the sample.

Table 5: Physicochemical Parameters of *Khanda Shunthi Avaleha*

Batch	1st	2nd	3rd	4th	5th	6th	7th	8th
Total ash (% w/w)	1.12%	1.02%	1.04%	1.01%	1.01%	1.09%	1.10%	1.08%
Acid Insoluble ash (% w/w)	0.38%	0.14%	0.17%	0.10%	0.10%	0.09%	0.09%	0.07%
Water soluble extractive (% w/w)	61.14%	62.43%	60.76%	62.22%	62.47%	65.13%	66.27%	67.45%
Alcohol soluble extractive (% w/w)	74.26%	69.20%	71.82%	78.0%	79.33%	77.38%	76.80%	78.22%
Reducing sugar	5.16%	6.0%	5.40%	5.56%	5.82%	5.63%	5.87%	5.44%
Non-reducing sugar	26.84%	24.0%	25.23%	26.19%	27.30%	23.37%	24.13%	24.06%
Total sugar	32.0%	30.0%	30.63%	31.75%	33.12%	29.0%	30.0%	29.50%
Loss on drying (% w/w)	9.62%	9.30%	12.0%	10.78%	10.50%	10.0%	10.43%	11.25%
Total solid	90.38%	91.70%	88.0%	89.22%	89.50%	90.0%	89.57%	88.75%
Acid value (% by mass)	1.56	1.23	1.30	1.19	1.28	1.35	1.29	1.18
pH (10% aq)	6.28	6.34	6.37	6.72	6.72	6.70	6.75	6.75

Different chromatograms have been developed by using HPTLC techniques. Mobile phase used for this was Toluene:Ethyl acetate:Formic acid:Methanol (6:4:1:0.4), scanning wavelength 254nm, 366nm, 500nm. The Rf value obtained from the HPTLC was analysed, matched with the Rf of raw drugs used for preparation of *Khanda Shunthi Avaleha* and found that the raw drugs can be easily identified in finished product.



Batch 1st

Figure 5A

Batch 2nd

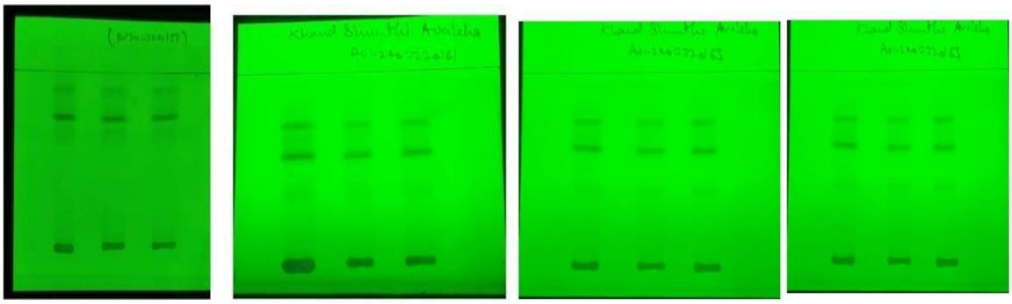
5B

Batch 3rd

5C

Batch 4th

5D



Batch 5th **Batch 6th** **Batch 7th** **Batch 8th**

Figure 5E **5F** **5G** **5H**

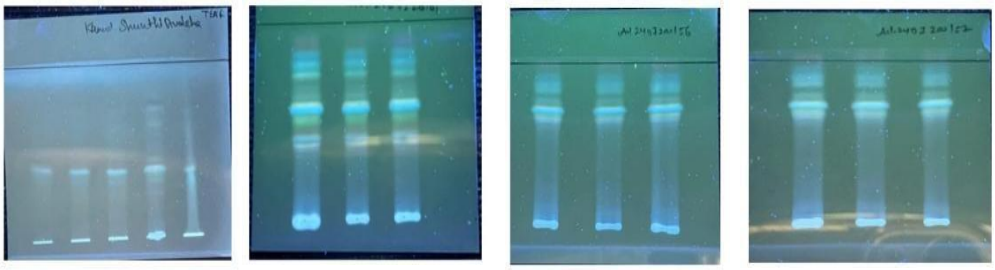


Figure 5: HPTLC Plates of *Khanda Shunthi Avaleha* at 254 nm

Batch 1st **Batch 2nd** **Batch 3rd** **Batch 4th**

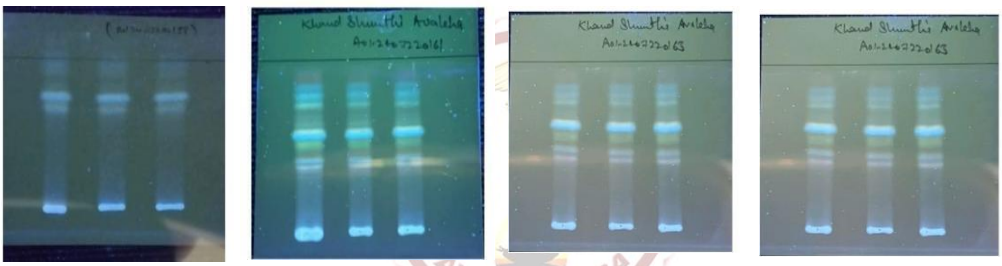
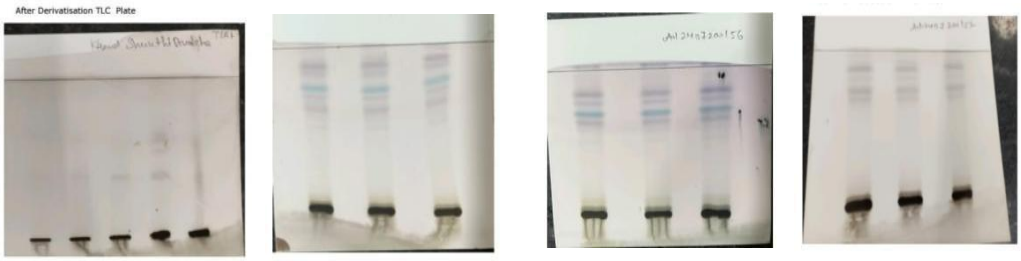


Figure 6A **6B** **6C** **6D**

Batch 5th **Batch 6th** **Batch 7th** **Batch 8th**

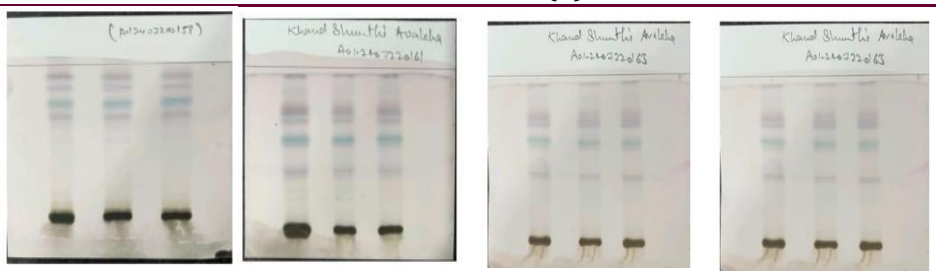
Figure 6E **6F** **6G** **6H**

Figure 6: HPTLC Plates of *Khanda Shunthi Avaleha* at 366 nm



Batch 1st **Batch 2nd** **Batch 3rd** **Batch 4th**

Figure 7A **7B** **7C** **7D**



Batch 5th	Batch 6th	Batch 7th	Batch 8th
Figure 7E	7F	7G	7H

Figure 7: HPTLC Plates of *Khanda Shunthi Avaleha* at 500nm

Lead, arsenic, cadmium, mercury was analysed in prepared batches of *Khanda Shunthi Avaleha*. The quantity of heavy metals present in the *Avaleha* was below the limit mentioned in API[9]. The amount of Lead present was less than 10 ppm i.e. 0.27ppm, 0.23ppm, 0.16ppm, 0.20ppm, 0.23ppm, 0.16 ppm, 0.19ppm, 0.25ppm from 1st batch to 8th batch respectively and other heavy metals i.e. arsenic, mercury, cadmium was below the limit of quantification in all batches.

Tests for microbial contamination[10] conducted in which total microbial count, total fungal count, E.coli, Salmonella, Pseudomonas aeruginosa, Staphylococcus aureus count was calculated and the values comes out was within the limit mentioned in API. Total microbial count was 30cfu/gm, 25cfu/gm, 20cfu/gm, 25cfu/gm, 20cfu/gm, 25cfu/gm, 25 fugm, 30cfu/gm from 1st batch to 8th batch respectively. Total yeast and mould count was <10cfu/gm. E.coli, Salmonella, S. aureus, Pseudomonas aeruginosa was found absent in all batches.

Pesticide residue was quantified in *Avaleha* batches and they were found below the detection limit. Tests for Aflatoxins[11] was performed for B1, B2, G1, G2 and these were not detected in *Khanda Shunthi Avaleha*.

DISCUSSION

The *Khanda Shunthi Avaleha* was prepared by utilising the authenticated and tested raw drugs. The *Khanda Shunthi Avaleha* was brown coloured, pleasant odour, sweet in taste with semisolid consistency. The total ash calculated in prepared *Avaleha* was 1.01-1.12%, less total ash content indicates its purity while acid insoluble ash was 0.07-0.38% indicate less silicecous impurities in *Avaleha*. Water soluble extractive was 60.76- 67.45% while alcohol soluble extractive was 69.20- 79.33%, shows the amount of active ingredients in *Khanda Shunthi Avaleha*. Total sugar was 29-33.12%, reducing sugar was 5.16- 6% while non-reducing sugar was 23.37-27.30%. Sugar act as natural preservative and increase the potency of medicine. Loss on drying was 9.30-12%. Less moisture content is desirable for stability and shelf life. Total solid was 88- 91.70% indicates the product is semisolid in consistency. Acid value was 1.18-1.56, indicate the quality of product while pH was 6.28-6.75 means the preparation is slightly acidic in nature indicates its beneficial effects in humans.

HPTLC fingerprinting of *Khanda Shunthi Avaleha* shows the presence of its ingredients in it. Heavy metals mainly cadmium, mercury, arsenic was found below the limit of quantification while lead was 0.16-0.27ppm. Total microbial plate count was 20-30cfu/gm, total yeast and mould count was <10cfu/gm while E.coli, Salmonella, Staphylococcus aureus, Pseudomonas aeruginosa was found absent. Pesticide residue was below the detection limit while Aflatoxins B1, B2, G1, G2 was not detected. The result obtained from the conducted tests found to be within the limit, as mentioned in guidelines given by CCRAS and Ayurvedic Pharmacopoeia of India.

CONCLUSION

Adopted procedure for the preparation of *Khanda Shunthi Avaleha* can be suggested as its SOP (Standard Operating Procedure) in further studies and the test result obtained would serve as the tools for assistance to the scientific organisations, regulatory bodies and manufacturer for developing standard formulation of great efficacy.

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