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ORIGINAL ARTICLE

Comparative Pharmaceutical Analytical Evaluation of *Krimighna Arka* and *Krimighna* Suspension with HPTLC

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ABSTRACT

Helminth infections (*Krimi Roga*) are a serious health problem and *Ayurveda* has developed a number of *Krimighna* (anthelmintic) formulations for their management. The present study was undertaken to carry out comparative pharmaceutical and analytical evaluation of two dosage forms of a classical formulation of *Krimighna* called *Krimighna Arka* (KA) prepared by different methods as per *Arka Prakasha* i.e. by distillation *(arka yanta)* as *Krimighna Arka* and by aqueous Soxhlet extraction followed by incorporation in a hydrophilic polymer base as *Krimighna Suspension* (KS). The organoleptic properties (colour, odour, taste), physicochemical properties (pH, specific gravity, total solids, alcohol content, refractive index) and phytochemical fingerprinting by High Performance Thin Layer Chromatography (HPTLC) of both the preparations were compared. The results showed that Krimighna Arka had pale yellow colour and was clear while Krimighna Suspension was brownish and turbid. The Krimighna Arka had lower specific gravity and total solids content as compared to suspension. In case of phytochemical analysis, spots were visualized in both the preparations at 254 nm and 366 nm when observed under UV light. However, the chromatogram of Krimighna Arka was simpler as compared to the suspension which had spots at two Rf values (0.55 and 0.81) at 254 nm. Thus, it can be concluded that the physicochemical and phytochemical profiles of the two dosage forms of *Krimighna Arka* were different and the suspension showed a comparatively more complex chromatographic profile than *arka* which may help in comparative standardization of the formulations based on the method of preparation.

Keywords: *Krimighna Arka*, distillation, *Krimighna* Suspension, *Arka Prakasha, Arka Kalpana*, HPTLC, *Ayurveda*

INTRODUCTION

In *Ayurveda*, parasitic infections are named as *Krimi Roga* (helminthic infestations). Helminthic disease infestations pose an important health hazard worldwide and are especially frequent in developing countries and in children causing nutritional deficiency or health deterioration. Current concerns about undesirable effects and resistances towards synthetic drugs promote the need of herbal antihelmintic drugs or formulations termed as *krimighna*. *Arka Prakasha* treatise on distillation was authored by *Ravana*, the follower of *Lord Shiva*. It is a part of the series of *Sanskrit* manuscripts authored by *Ravana*. The treatise comprises exhaustive conversation between *Ravana* and his consort *Mandodari* disclosing the process of preparation of *Arka* (distillation), details of apparatus used and variety of ingredients used for preparation of *Arka*[1]. *Arka prakasha* describes various *Arka* Kalpanas; *Arka Kalpana* refers to *Ayurvedic* liquid dosage form prepared by distilling volatile and water-soluble components from the drug. *Krimighna Arka* contains drugs such as Embelia ribes

(*Vidanga*), Hyoscyamus niger (*Khurasani Ajavayana),* Caesalpinia bonducella (*Latakaranja*), Zingiber officinale (*Sunthi*), Piper nigrum (*Maricha*) and Piper longum (*Pippali*) and it helps in treating helminthic infections[1]. The present work was designed to compare *Krimighna Arka* (KA) with *Krimighna* Suspension (KS) by pharmaceutically and analytically evaluating the two preparations with special reference to phytochemical profile using HPTLC analysis.

MATERIALS AND METHODS

Table 1: Raw Materials

S. No.	*Ayurvedic Name*	Botanical Name	Family	Part Used
1	*Khurasani Ajavayana*	*Hyoscyamus niger*	Solanaceae	Seeds
2	*Vidanga*	*Embelia ribes*	Myrsinaceae	Fruit
3	*Latakaranja*	*Caesalpinia bonducella*	Caesalpiniaceae	Seeds
4	*Sunthi*	*Zingiber officinale*	Zingiberaceae	Rhizome
5	*Maricha*	*Piper nigrum*	Piperaceae	Fruit
6	*Pippali*	*Piper longum*	Piperaceae	Fruit

Raw materials were procured from authenticated sources. Botanical identity was confirmed according to the standards of the Ayurvedic Pharmacopoeia of India[2].



Fig. 1: Raw material

Preparation of *Krimighna Arka* (KA)

According to API, the process of preparation of *Arka* by simple distillation is described as follows:

The whole drug was cleaned and coarsely powdered. Each raw drug (*Vidanga, Khurasani Ajavayana, Latakaranja, Sunthi, Maricha* and *Pippali*) was taken in a quantity of 10 g, giving a total batch size of 60 g. The mixed coarsely powdered *(Yavakuta churna*) was taken in clean container and soaked in purified water in the ratio of 1:10 (drug:water) on overnight basis. This helps in leaching out of all essential materials upon boiling so that the drug becomes soft and releases all its constituents when boiled[12].

On the next day, whole of this is taken into *Arka Yantra* for distillation. The condensate collected is divided into *Prathama Bhaga* (first portion), *Madhyama Bhaga* (middle portion), and *AntimaBhaga* (last portion). Initially, the distillate is just steam with no drugs and is discarded. Towards the end of distillation process, the distillate may not contain any active principle and is also discarded. However, the portions of distillate collected in between contain the active principles which are mixed together to obtain *Arka* with uniform concentration.

The obtained *Arka* is filtered using a fine muslin cloth to remove any suspended matter. It is then stored in an airtight container away from light and heat to avoid loss of volatile principles. The yield of KA was 340 mL from 60 g of mixed raw drug soaked in 600 mL of purified water. The sample was stored at room temperature for further testing[3].



Fig. 2: *Arka Yantra* setup (distillation apparatus)

Method of Preparation *of Krimighna* Suspension by Aqueous Soxhlet Extraction Using Polymer

All raw materials were cleaned, shade-dried, and coarsely powdered. The powders were passed through a 40-mesh sieve to ensure uniformity suitable for extraction.

Aqueous Soxhlet Extraction

The amount of mixed herbal powder taken for extraction was weighed (60 g; 10 g of each drug) and introduced into a cellulose thimble, and the thimble was inserted into the extracting chamber of the Soxhlet apparatus.

Distilled water was taken as an extracting solvent to carry out an aqueous extraction. Extraction was performed at a temperature of 90-95 °C for 6-8 hours until a clear solution without colored content in the siphon tube was obtained. The obtained aqueous extract was concentrated on a water bath to remove excess water. The resulting concentrated solution was allowed to cool to room temperature and

filtered. From 60 g of mixed powder and 600 mL of distilled water, 420 mL of aqueous extract was obtained, which was concentrated to 55 mL.

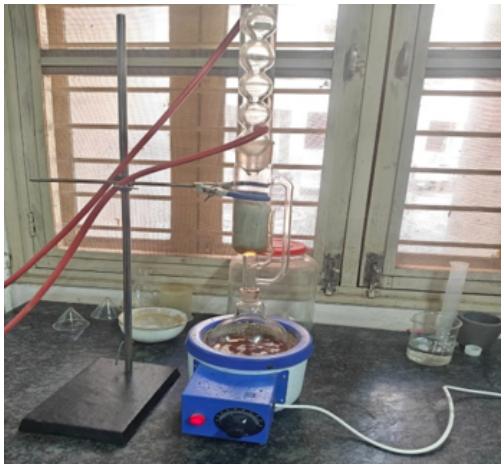


Fig. 3: Soxhlet extraction setup



Fig. 4: Magnetic stirring for suspension

Preparation of Polymer Base

A hydrophilic polymer (sodium carboxymethyl cellulose) at a concentration of 1% w/v (0.55 g in 55 mL) was added drop-wise to a beaker containing purified water with a magnetic stirrer to avoid clumps formation. The polymer suspension was stirred until a viscous homogeneous mixture was obtained[2].

Formulation of *Krimighna* Suspension

The diluted aqueous solution of the Soxhlet extract was added to the prepared polymer base under continuous stirring. The obtained suspension was homogenized. The freshly prepared KS was placed in the glass medicinal containers with a stopper and stored at room temperature. No organic solvents, alcohol, or chemical preservatives were used during the preparation[2].

Analytical Evaluation

Table 2: Organoleptic and Physicochemical Parameters

Parameter	*Krimighna Arka*	*Krimighna* Suspension
Colour	Pale yellow, clear	Brownish, opaque
Odour	Aromatic	Spicy, pungent
Taste	Bitter-aromatic	Bitter-pungent
pH	5.8	6.4
Specific gravity	0.986	1.074
Total solids (%)	1.2	7.8
Alcohol content (%)	0.95	Nil
Refractive index	1.334	1.372

Colour: KA appeared pale yellow and clear whereas KS appeared brownish and turbid. It was observed that KS retained the particles of the herbs due to the presence of the suspension. On the other hand, KA was in the form of a distillate.

Odour: KA had an odour of the type of an herb. However, KS had a strong spicy scent. It was inferred that KS had greater retention of volatile and non-volatile phytoconstituents.

Taste: KA had a bitter-aromatic taste whereas KS had a bitter-pungent flavour. It was argued that KS had more pungent taste due to the presence of intact herbal materials.

pH Value: It was found that KA had a pH of 5.8 whereas KS had a pH of 6.4. Both preparations were considered to be stable for consumption as they met the standard requirements for herbal preparations.

Specific Gravity: KS had greater specific gravity than KA. It was found that KS had a specific gravity of 1.074 whereas KA had 0.986. This implies that KS had a higher concentration of dissolved and suspended herbal materials.

Total Solids: KS had greater total solids than KA. KS had 7.8% total solids whereas KA had 1.2%. It was concluded that KS had a higher retention of phytochemical constituents.

Alcohol: There was a trace of alcohol present in KA but not in KS. In KA, there were present 0.95% alcohol. The amount of alcohol found in KA should be taken with a grain of salt, since its presence requires confirmation by the appropriate tests.

Refractive Index: KS had a higher refractive index than KA. It was found that KS had a refractive index of 1.372 whereas KA had 1.334. This implies that KS had a higher concentration of dissolved phytochemical constituents.

HPTLC Analysis

Sample Preparation: Methanolic extracts of the two formulations were spotted on a HPTLC plate as Track 1-2 (Krimighna Arka, 5 µL and 10 µL) and Track 3-4 (Krimighna Suspension, 5 µL and 10 µL) using Linomat 5 applicator (Camag).

Chromatographic Conditions: Merck HPTLC Silica gel 60 F254 plate of size 100 x 100 mm was used. The mobile phase consisted of Toluene : Ethyl acetate : Glacial acetic acid (5:2:0.5 v/v/v). The chamber was saturated for 20 min and development was performed up to 80 mm. The developed plate was scanned at TLC Scanner 4, Camag. The scan was performed at 254 nm (absorbance, deuterium lamp) and 366 nm (fluorescence, mercury lamp, K400 filter).

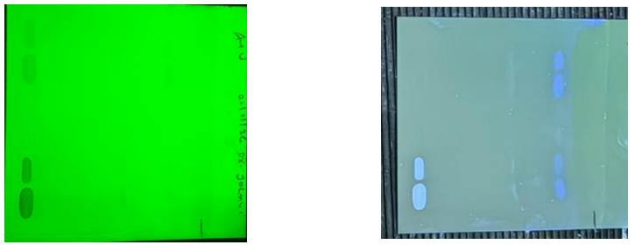


Plate @254 nm *Plate @366 nm*

Fig. 5: HPTLC plate images of *Krimighna Arka* and *Krimighna* Suspension tracks

HPTLC *at 254 nm*

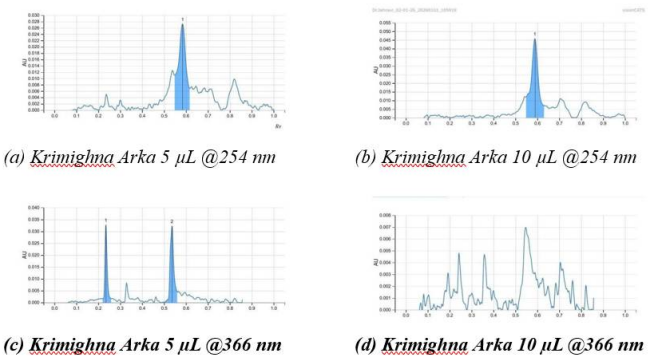
KA showed one prominent peak in the range of Rf 0.585–0.589, indicating the presence of simple phytochemical constituents. In contrast, KS showed two clearly visible peaks (approximately Rf 0.55 and 0.81) indicating that besides the common phytochemicals, the suspension also contains additional ones.

HPTLC at 366 nm

The fluorescence analysis showed that KA had lower phytochemical constituents than KS, which had two more fluorescent spots than the *arka*.

Densitometric Chromatograms

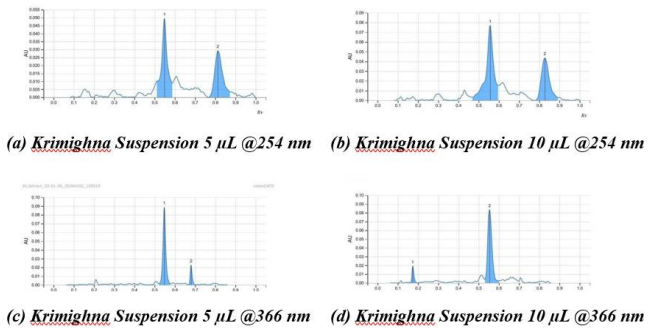
The scans of the developed thin layer chromatography are shown below (Camag TLC Scanner 4, visionCATS software). The peak(s) used to calculate the Rf value is marked in blue.



(a) Krimighna Arka 5 µL @254 nm *(b) Krimighna Arka 10 µL @254 nm*

(c) Krimighna Arka 5 µL @366 nm *(d) Krimighna Arka 10 µL @366 nm*

Fig. 6: Densitometric chromatogram of methanolic *Krimighna Arka* extract



(a) Krimighna Suspension 5 µL @254 nm *(b) Krimighna Suspension 10 µL @254 nm*

(c) Krimighna Suspension 5 µL @366 nm *(d) Krimighna Suspension 10 µL @366 nm*

Fig. 7: Densitometric chromatogram of methanolic *Krimighna* Suspension extract

Table 3: Rf Value Summary

Sample	Major Peaks @254 nm	Rf Value (s) @254 nm	Major Peaks @366 nm	Rf Value (s) @366 nm
Krimighna Arka (5 µL)	1	0.585	2	0.235, 0.536
Krimighna Arka (10 µL)	1	0.589	0	No distinct peak
Krimighna Suspension (5 µL)	2	0.549, 0.813	2	0.547, 0.681
Krimighna Suspension (10 µL)	2	0.557, 0.828	2	0.174, 0.554

The Rf values were obtained through automated Gauss (legacy) peak detection with Savitzky-Golay smoothing (window 7) and lowest-slope baseline correction, using vision CATS HPTLC software.

DISCUSSION

The comparative evaluation of KA and KS revealed that the method of the preparation influences the physicochemical and phytochemical properties of the final formulations. Though the preparations were achieved from the same set of

crude drugs, the differences were noticed in the physicochemical parameters like total solid content, specific gravity, refractive index, and alcohol content. The HPTLC analysis further revealed the significant difference in the phytochemical fingerprints of the preparations.

It can be postulated that KA, being prepared by simple distillation of the ingredients, contained more volatile and less polar phytoconstituents. This is evident from the low total solid content (1.2%), low specific gravity (0.986) and low refractive index (1.334). The trace amount of alcohol (0.95%) was detected in the preparation that can be explained by the fact that during the process of distillation of mixture the distillate collected initially *(Prathama and Antya Bhaga)* was discarded and only the middle fraction of the distillate was retained for use as per the description[1, 3]. As expected, the HPTLC profile of the *Arka* also showed minimal complexity with one major peak (98.09% area) observed at Rf value 0.585–0.589 at 254 nm for both 5 µL and 10 µL track indicating the homogeneity of the sample. On the contrary, the KS showed higher total solid content (7.8%), higher specific gravity (1.074) and higher refractive index (1.372) with absence of alcohol. This suggests that the Suspension has higher amount of non-volatile phytochemicals compared to *Arka*. This was also evident from the HPTLC analysis showing complexity of the profile with two prominent peaks at 254 nm with 5 µL track (Rf 0.549 and 0.813; area 55.5% and 44.5%) and two fluorescent peaks at 366 nm. The 10 µL track showed similar peak positions (Rf 0.557 and 0.828) with increased peak heights corresponding to 62.1%-37.9% area (254 nm). Thus, the linear response of the detector (consistency in the peak height and area) was achieved. It is also evident that the qualitative nature of the HPTLC profile of KA was not consistent between 5 µL and 10 µL, particularly for the 366 nm detection. A single intense peak was observed at 254 nm when using 10 µL of the sample compared to the 5 µL track, whereas the fluorescent peak (366 nm) was almost absent on the 10 µL track, suggesting that KA contained only a trace amount of fluorescent/polar phytochemicals[11].

It can be speculated that the two prominent absorbing peaks of 254 nm observed consistently for 5 and 10 µL tracks of KS in this study (Rf 0.55 and 0.81–0.83) corresponded to the markers of the constituent drugs. For example, embelin (a naphthoquinone derivative) and piperine (an alkaloid) were reported to be the active principles of *Vidanga* and *Maricha/Pippali*[13, 14], respectively, while the gingerols and shogaols are the pungent principles of *Sunthi* with anti-parasitic, antioxidant, and anti-inflammatory activities[1]. However, as no attempts were made in this study to perform co-chromatography with the authentic markers by HPTLC, these speculations were not confirmed . Further studies using hyphenated technique may be required to identify the bioactive markers.

Therefore, based on the findings of this study, it can be concluded that the different pharmaceutical preparations of *Ayurveda (Kalpanas)* obtained from the same drug combination demonstrated distinct physicochemical and chromatographic profiles and therefore should not be assumed to be analytically equivalent solely on the basis of their common raw-drug composition. The *Arka* being the distillate of the mixture contained more volatile phytochemicals and less polar material compared to the Suspension that retained more non-volatile substances. Whether these differences influence pharmacological activity or dose requirement needs further pharmacological evaluation. Thus, the findings of this study can contribute to the standardization of these preparations for their physicochemical and phytochemical properties.

CONCLUSION

KA and KS were found to be different concerning the physicochemical and chromatographic properties. The former has a simple fingerprint and exhibited reduced total solid content, while the latter showed higher specific gravity and total solid content and complex HPTLC fingerprint. In this regard, it may be concluded that, in terms of phytochemical retention, KS showed a comparatively higher retention of phytochemicals and a more complex chromatographic profile than KA, while KA represents the distilled preparation that is rich in isolated volatile components. Stability and safety of both preparations remain to be established, and further comparative standardization studies should involve KS due to its higher content of phytochemical compounds.

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