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# COMPARITIVE ANALYTICAL EVALUATION OF *KOKILAKSHADI KASHAYA* AND *KOKILAKSHADI GHANAVATI*

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## ABSTRACT

Ayurvedic medicine standardisation and quality control concepts can be traced all the way back to ancient times. Vaidyas used to collect herbs based on their organoleptic characteristics, such as taste, texture, smell, and colour, and use them in the preparation of medications. Drug manufacturing principles and desired features of final medications have also been discussed. Even if the principles were formed based on the scientific parameters in use at the time, they must be assessed and answered in light of the current state of science and technology. The *Kashaya* formulation is also made in the form of *Ghanavati* (Tablet) to compare it analytically and check which is more durable with medicinal value maintained for a longer period, easy to administer and store. The present study has been undertaken to prepare *Kokilakshadi Kashaya* and *Kokilakshadi Ghanavati* and compared analytically.

## INTRODUCTION

Research in Ayurveda has been in practice since ages. The explanation of Tantra Yukti is an example of research protocols and advice for search of knowledge in depth. In the present scenario research is to establish create evidence for the science with timeless concrete foundations. Research is a necessary practise for the resuscitation and advancement of any science.

*Bheshaja*(medicines) is an inevitable and most important part of *Chikitsa Pada Chatushtaya*.[1] The *Panchavidha Kashaya Kalpanas*[2] (five basic dosage forms) play a major role in the manufacturing process of all other formulations. *Kashaya Kalpana*(Decoctions) is one of the *Kalpanas* that is frequently utilised in general practise. Many patients, however, do not appreciate its palatability. As a result, in this modern period, there is a need to make the *Kashaya* form more appealing while maintaining its usefulness.

In the present era of science and technology, a solid back up of research is essential for the development of new drugs and herbal industry. Pharmacognostical, analytical and biological assays help to develop a rational relationship between the active principles of drugs and the therapeutic effects they generate. The scientific foundation which forms the backbone of herbal industry will make the ancient science of Ayurveda acceptable worldwide.

*Kokilakshadi Kashaya*[3] is a composition mentioned in *Vataraktadhikara* of *Bhaishajya Rathnavali*. It is a combination of *Kokilaksha* (*Asteracantha longifolla Nees. Syn. Hygrophila spinosa T.Anders*) and *Guduchi (Tinospora cordifolia* (Willd.) Miers.) This is a widely practiced formulation for the diseases such as rheumatoid arthritis and inflammatory diseases. Though it is very effective clinically, the patient compliance persists as it lacks palatability, mobility, and shelf life. Therefore the same formulation consisting of two herbs was prepared into a *Ghana vati (*Tablet). There is always a need to evaluate a new drug dosage form for various criteria like potency, applicability, stability, quickly absorbable, pleasant and safe.

The growth of research and sophisticated technology has made it possible to create a standard database of herbs, minerals, metals, and even finished goods. This would not only give Ayurvedic drugs a scientific foundation and respectability, but it will also aid in the globalisation of Ayurveda.

Physico-chemical analysis gives objective parameters for establishing quality standards for both raw and finished pharmaceuticals. Analytical studies can be used to standardise plants and drugs, as well as differentiate adulterants, which is a necessity in today's world. Analytical study is the application of a process or a series of processes in order to identify and authenticate or quantify a substance, the components of a solution or mixture, or the determination of the structures of chemical compounds. Prior to pharmacological examination, phytochemical investigations are critical because they provide a basic understanding of the potential pharmacological active ingredients.

Physicochemical characteristics are highly valuable for transforming a single raw medication into a dosage form since they provide vital information about the nature of the drug, whether it is a simple or compound formulation, its likely shelf-life, how to store the sample, and so on. Physico-chemical parameters and HPTLC tests are used to examine *Kokilakshadi Kashaya* and *Kokilakshadi Ghanavati* in this study.

## MATERIALS AND METHODS

1. Preparation of *Kokilakshadi Kashaya*

**Table 1: Showing ingredients of *Kokilakshadi Kashaya.***

| S. No. | *Dravya* | Latin Name | Part Used | Condition | Quantity (g) |
|---|---|---|---|---|---|
| 1 | *Kokilaksha* | *Asteracantha longifolia (Linn.) Nees* | Roots, seeds, leaves | Dry | 3 kg |
| 2 | *Guduchi* | *Tinospora cordifolia (Willd.) Miers ex Hook.f.Thoms* | Stem | Fresh | 3 kg |
| 3 | Potable Drinking Water | | | | 96 L |

The raw drug was collected from KVG Ayurveda Pharma & Research Centre, Sullia, Dakshina Kannada district, Karnataka and authenticated by Quality Control department of KVG Ayurveda Pharma & Research Centre, Sullia, Dakshina Kannada. The raw drugs were pounded into coarse powder. 3 Kg each of *Kokilaksha* and *Guduchi* were taken in a wide mouthed stainless steel vessel. It was added with 96 L of water and heated in a gas stove. Constant mild heat was applied to facilitate the evaporation and intermediate stirring was carried out till reduced to 1/8th of the initial quantity. After desirable reduction in volume (1/8th – 12.2 L), the *Kashaya* was filtered through four folded cotton cloth and collected in a separate vessel for further processing. The residue remained above cloth were discarded.

It was observed that initially the liquid was light brown in colour and bitter in taste. The prepared *Kashaya* had the odour of the drugs added. Evaporation was started at 70 º C, which was aggravated on stirring. The *Kashaya* on boiling was light brown colour in the initial stage, which was gradually turned to dark brown in colour. The maximum temperature during boiling stage was found in the liquid in between 90°C-95°C. The residue after preparation was odourless and tasteless.

**2. Preparation of *Kokilakshadi Ghanavati***

**Table 2: Showing details of ingredients for *Kokilakshadi Ghanavati.***

| Sr. No. | Ingredients | |
|---|---|---|
| 1 | *Kokilakshadi Kashaya* | 11.2 L |

Previously prepared *Kashaya* was taken in a stainless steel vessel. The *Kashaya* was heated on Gas stove and temperature was maintained in between 70°C-75°C. Continuous stirring of *Kashaya* was done to avoid burning of *Kashaya*. After that it was converted into semisolid stage and heating process was stopped. The material was taken into stainless steel tray and kept in oven for drying in 45°C-50°C until it was of the consistency of rolling into pills. After complete drying, the material was rolled into tablets of the size of approximately 0.5 g weight.

It was observed that after 3 hours of boiling mild sticky nature was observed when rubbed between two fingers. After 5.5 hours of heating stickiness of the liquid and adhesiveness to the vessels was increased. Thickness of liquid and adhesiveness to vessel was gradually increased. After keeping in the oven dark brown coloured, semi-solid material was converted into blackish brown coloured solid material.

**Table 3: Showing result obtained during preparation of *Kokilakshadi Ghanavati.***

| Parameters | |
|---|---|
| Total time taken for preparation of *Ghana* | 6 hours |
| Total time for drying | 26 hours |
| Final quantity of dried *Ghana* obtained | 210 g |

**3. Analytical study**

The following parameters were employed for this study, which is mentioned in Ayurvedic Pharmacopoeia of India.

1. Organoleptic parameters
2. Physicochemical analysis

The following parameters were compared of *Kokilakshadi Kashaya* and *Kokilakshadi Ghanavati.*

a) pH value[4]
b) Total Solids[5]
c) Loss on drying[6]
d) Alcohol soluble extractive[6]

Viscosity and Specific gravity of the decoction was checked, while the disintegration time, hardness, friability of the tablet, total ash and acid insoluble ash was checked to determine the stability of the dosage form.

### 3. Chromatographic Study

e) Aflatoxin.[7]

f) Heavy metals – Lead, Cadmium, Mercury, Arsenic.[8]

g) Organo chlorine pesticides and Organo phosphorous pesticides.[9]

h) HPTLC

## RESULTS

### 1. Organoleptic characters

**Table 4: Organoleptic characters of *Kokilakshadi Kashaya* and *Kokilakshadi Ghanavati.***

| S. No. | Parameter | *Kokilakshadi Kashaya* | *Kokilakshadi Ghanavati* |
|---|---|---|---|
| 1 | Colour | Dark brown | Blackish Brown |
| 2 | Odour | Characteristic | Characteristic |
| 3 | Appearance | Liquid | Hard, Solid, Round shaped |
| 4 | Taste | Bitter | Bitter |

### 2. Physicochemical analysis

**Table 5: Physicochemical analysis of *Kokilakshadi Kashaya* and *Kokilakshadi Ghanavati.***

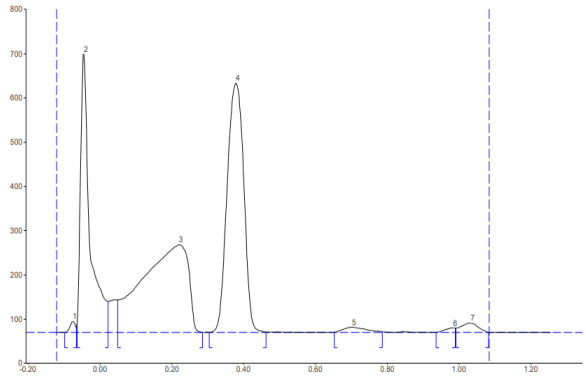
| S. No. | Parameter | *Kokilakshadi Kashaya* | *Kokilakshadi Ghanavati* |
|---|---|---|---|
| 1 | pH | 5.74 | 5.5 |
| 2 | Total solid content (% by mass) | 3.17% | 86.28% |
| 3 | Loss on Drying/ Moisture (% by mass) | 96.83% | 13.72% |
| 4 | Alcohol Soluble Extractive (% by mass) | 1.56% | 0.35% |
| 5 | Specific Gravity at 25°C | 1.02 $kg/m^3$ | * |
| 6 | Viscosity | 0.8 cP | * |
| 7 | Disintegration time (minutes) | * | 18 min |
| 8 | Hardness ($kg/cm^2$) | * | 2.5 $kg/cm^2$ |
| 9 | Friability (% w/w) | * | 0.1 % w/w |
| 10 | Total Ash | * | 22.43% |
| 11 | Acid Insoluble Ash | * | 0.56% |

### 3. Chromatographic study

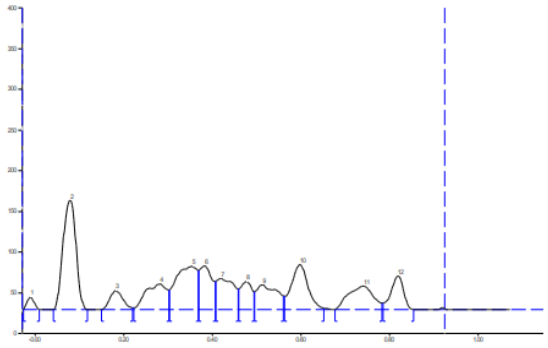
**Table 6: Chromatographic analysis of *Kokilakshadi Kashaya* and *Kokilakshadi Ghanavati*.**

| S. No. | Parameter | *Kokilakshadi Kashaya* | *Kokilakshadi Ghanavati* |
|---|---|---|---|
| 1 | Aflatoxin by TLC | Not detected | Not detected |
| 2 | Lead (mg/kg) or ppm | *BQL (QL: 0.5) | *BQL (QL: 0.5) |
| 3 | Cadmium (mg/kg) or ppm | *BQL (QL: 0.05) | *BQL (QL: 0.05) |
| 4 | Mercury (mg/kg) or ppm | *BQL (QL: 0.005) | *BQL (QL: 0.005) |
| 5 | Arsenic (mg/kg) or ppm | 0.23 mg/kg | 0.47 mg/kg |
| 6 | Organo Chlorine Pesticides | Not detected | Not detected |
| 7 | Organo Phosphorus Pesticides | Not detected | Not detected |

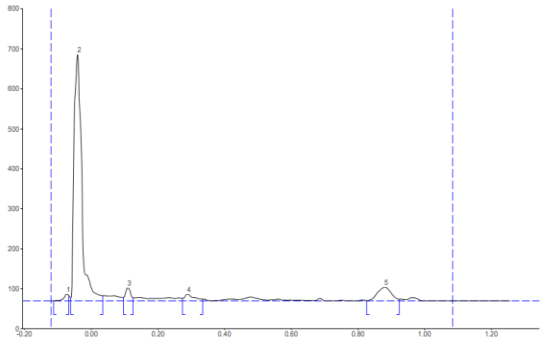
High performance thin layer chromatography(HPTLC)



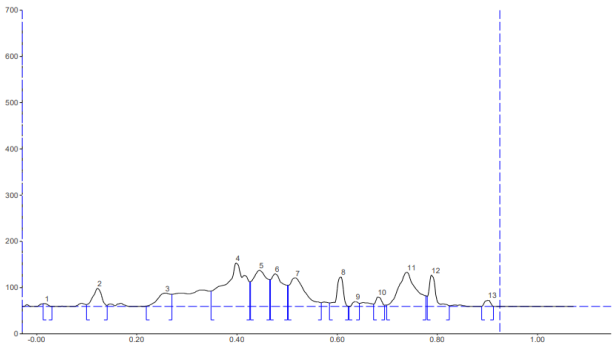
**Graph no.1 *Kokilakshadi Kashaya* at 254 nm**



**Graph no.2 *Kokilakshadi Ghanavati* at 254 nm**



**Graph no.3: *Kokilakshadi Kashaya* at 366 nm.**



**Graph no.4: *Kokilakshadi Ghanavati* at 366 nm.**

**Table 7: Showing Rf value & % of area of *Kokilakshadi Kashaya* at 254nm.**

| PEAK NO | Rf VALUE | AREA(AU) | % AREA(AU) |
|---|---|---|---|
| 1 | -0.1 | 260.2 | 0.55 |
| 2 | -0.06 | 11081 | 23.34 |
| 3 | 0.05 | 17074.6 | 35.96 |
| 4 | 0.31 | 17588.9 | 37.04 |

| | | | |
|---|---|---|---|
| 5 | 0.65 | 553 | 1.16 |
| 6 | 0.94 | 207.7 | 0.44 |
| 7 | 0.99 | 716.4 | 1.51 |

**Table 8: Showing Rf value & % of area of *Kokilakshadi Ghanavati* at 254nm.**

| PEAK NO | Rf VALUE | AREA(AU) | % AREA(AU) |
|---|---|---|---|
| 1 | -0.01 | 238.5 | 1.62 |
| 2 | 0.05 | 3440.3 | 23.38 |
| 3 | 0.14 | 600.8 | 4.08 |
| 4 | 0.22 | 1120.5 | 7.62 |
| 5 | 0.3 | 3015.1 | 20.49 |
| 6 | 0.39 | 959.5 | 6.52 |
| 7 | 0.44 | 734.3 | 4.99 |
| 8 | 0.49 | 979.8 | 6.66 |
| 9 | 0.55 | 1459.1 | 9.92 |
| 10 | 0.65 | 817.3 | 5.56 |
| 11 | 0.78 | 502.4 | 3.42 |
| 12 | 0.87 | 844 | 5.74 |

**Table 9: Showing Rf value & % of area of *Kokilakshadi Kashaya* at 366 nm.**

| PEAK NO | Rf VALUE | AREA(AU) | % AREA(AU) |
|---|---|---|---|
| 1 | -0.11 | 173.6 | 1.51 |
| 2 | -0.06 | 9612.2 | 83.83 |
| 3 | 0.1 | 359.1 | 3.13 |
| 4 | 0.27 | 343 | 2.99 |
| 5 | 0.83 | 977.8 | 8.53 |

**Table 10: Showing Rf value & % of area of *Kokilakshadi Ghanavati* at 366 nm.**

| PEAK NO | Rf VALUE | AREA(AU) | % AREA(AU) |
|---|---|---|---|
| 1 | 0.01 | 46.1 | 0.36 |
| 2 | 0.1 | 537.1 | 4.18 |
| 3 | 0.22 | 625.6 | 4.86 |
| 4 | 0.35 | 3099.8 | 24.1 |
| 5 | 0.43 | 1800 | 14 |
| 6 | 0.47 | 1387.6 | 10.79 |
| 7 | 0.5 | 1538.8 | 11.97 |
| 8 | 0.59 | 655.9 | 5.1 |
| 9 | 0.63 | 103.9 | 0.81 |
| 10 | 0.67 | 195.4 | 1.52 |
| 11 | 0.7 | 2019.8 | 15.71 |
| 12 | 0.78 | 722.2 | 5.62 |
| 13 | 0.89 | 128.3 | 1 |

## DISCUSSION

The organoleptic characters of *Kokilakshadi Kashaya* was dark brown liquid, characteristic odour and bitter in taste. The organoleptic characters of *Kokilakshadi Ghanavati* were dark

brown in colour, dry solid and hard to touch. The similarity of both samples was that of colour and its bitter taste.

pH was taken of *Kokilakshadi Kashaya* and *Kokilakshadi Ghanavati*. The pH of *Kashaya* was 5.74 and pH of *Kokilakshadi Ghanavati* was 5.5. *Kashaya* and *Kokilakshadi Ghanavati* does not have much difference in the pH, and was inferred that both *Kashaya* and *Ghanavati* is acidic in nature.

The total solids accounted to the solid mass by means of percentage in the given sample. This pharmaceuticaly could be correlated to the micro-granular content available in the sample. 10 gm each of the sample gave different results. The *Kokilakshadi Kashaya* had total solids at a value of 3.17% while that of *Kokilakshadi Ghanavati* had 86.28%. This evidently proved that total solid was less in *kashaya* as the liquid portion in the sample was higher.

The moisture content accounted for the watery element persisting in the given sample. This was high in liquid sample and low in solid sample. *Kokilakshadi Kashaya* got a higher value of 96.83% wheras in *Kokilakshadi Ghanavati*, the value was 13.72% which was five times lower compared to the *Kashaya*, which showed that *Ghanavati* was more stable as a product. The presence of excess moisture was conducive to the detoriation and spoilage of drug.

Specific Gravity implied to the increase in its value after the duration of heating based on the presence of dissolved substances. Specific gravity of *Kokilakshadi Kashaya*. The result observed was 1.02 kg/m3

Viscosity of the sample referred to the fluid resistance to flow a column with reference to speed of water as standard. The higher the viscosity, the greater was the resistance to flow. The viscosity of *Kokilakshadi Kashaya* was observed to be 0.8 cP.

Extractive value represented the quantification of the phytoconstituents and materials that are soluble in the respective solvent. It was also related to the availability of drugs in different medium in body through different carriers. Any change in the value showed change in the process of extraction of the phytoconstituents. In *Kokilakshadi Kashaya* the alcohol soluble extractive was 1.56 % wheras in *Ghanavati* it was 0.35 %. There was not a larger variation as the *Ghanavati* was only the crude form of the *Kashaya*. The phytoconstituents absorbed during the preparation of *Kashaya* existed in the *Ghanavati* also. Alcohol soluble extractives showed presence of polar substances like phenols, tannins, glycosides and flavonoids.

Aflatoxin testing was carried out to analyse the presence of aflatoxins B1, B2, G1 and G2 in any material of plant origin in the prepared *Kokilakshadi Kashaya* and *Ghanavati*. Their presence were not detected. This proved the safety of the drug.

Disintegration time referred to the solubility and absorption of the tablet in the body. The disintegration time of *Kokilakshadi Ghanavati* was 18 min. Generally non coated tablets disintegrate in 30 mins and thus *Ghanavati* could be standardized.

Friability was checked to analyse the ability of tablets to withstand abrasions during packing, handling and transit. The loss of less than 0.8 % was considered to be the acceptable limit. *Kokilakshadi Ghanavati* had a friability of 0.1 % w/w which showed that the sample had friability within the limits.

Hardness of the tablet was checked to analyse the structural integrity and strength during packing and transit. *Kokilakshadi Ghanavati* was observed to have 2.5 kg/cm2 as its hardness which was within the acceptable limit.

Ash value showed the adulteration of the inorganic materials and it had greater importance in the QC and standardization. The higher the ash value, higher the inorganic material present in it. The *Kokilakshadi Ghanavati* had ash value of 22.43 %, which indicated the presence of inorganic material present which could have been due to plant constitutes and physical impurities.

Acid insoluble ash represented the presence of siliceous content and was present in the value of 0.56 % in *Kokilakshadi Ghanavati*.

Heavy metal estimation was done for Lead, Cadmium, Mercury & Arsenic in both the prepared *Kokilakshadi Kashaya* and *Ghanavati*. The value of the metals, Lead, Cadmium and Mercury in both the samples were below the limit of detection. Arsenic was detected in *Kashaya* with a value of 0.23 mg/kg and in *Ghanavati* 0.47 mg/kg. Though it could be detected, the value was seen well below the acceptable limit and therefore complies to be used as medicine, without any adverse effects of heavy metal poisoning.

The *Kokilakshadi Kashaya* and *Ghanavati* were checked for residual pesticide content. The residual pesticides could create many harmful and adverse effects on the body. This content

was analysed to make the sample safe to use. In the samples of *Kashaya* and *Ghanavati*, the pesticide content was non detectable.

Chromatographic Study (HPTLC) showed comparison of *Kokilakshadi Kashaya* with *Ghanavati* at 254 nm, total numbers of spots observed were 7 and 12 respectively and after observing the tracks at 366 nm, showed 5 and 13 spots respectively. Similar peaks at Rf 0.05 and 0.65 was observed at 254 nm. Similar peaks at Rf 0.1 was observed at 366 nm.

## CONCLUSION

Preparation of *Kokilakshadi Kashaya* was comparatively easy. Preparation of *Kokilakshadi Ghanavati* involves longer duration and fuel. But it was observed that the *Ghanavati* could be preserved for a longer period without the addition of any preservatives. This has a positive score from the pharmaceutical point of view.

Analytically, both *Kokilakshadi Kashaya* as well as *Kokilakshadi Ghanavati* have some similar characteristics but some alterations are noted in the peaks in H.P.T.L.C.

Study revealed modification in the form of medicine from *Kashaya* to *Ghanavati* adds the advantages of prolonged and sustained action.

Further clinical evaluation can add weightage to such research getting converted to industrial making.

## PREPARATION OF *KOKILAKSHADI KASHAYA*

**Pic.1 Asteracantha longifolia Plant Specimen.**

**Pic.2 Asteracantha longifolia Dry Drug- Kokilaksha**

**Pic 3: Tinospora cordifolia Plant Specimen**

**Pic 4: Tinospora cordifolia Fresh Drug Sample- Guduchi**

**Pic 5: Yavakuta Choorna Preparation**

**Pic 6: Guduchi - Yavakuta Choorna**

**Pic 7: Kokilaksha - Yavakuta Choorna**

**Pic 8: *Kashaya* Choorna soaked in water**

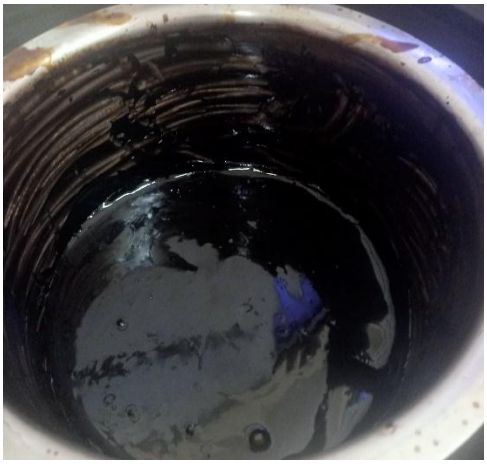
**Pic 9: Boiling *Kashaya***

**Pic 10: Filtered *Kashaya***

## PREPARATION OF *KOKILAKSHADI GHANAVATI*

**Pic 11: Filtered *Kashaya***

**Pic 12: *Kashaya* turning semisolid**



**Pic 13: *Kashaya* turning semisolid**

**Pic 14: *Kashaya* turning sticky solid mass**

**Pic 15: Rolled into pill**

**Pic 16: *Ghanavati***

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