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# PHYSICOCHEMICAL AND PHYTOCHEMICAL ANALYSIS OF AYURVEDIC MEDICINE *SIMHYADI KASHAYA* GRANULES AND ITS STANDARDIZATION BY HPTLC METHOD

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## ABSTRACT

**Aim:** In different health-care field, priority was given to the usage of herbal products and herb-based medicines as the treatment measure. This is an endeavor to establish the scientific basis of one of the herbal formulations in Ayurveda *"Simhyadi Kashaya* Granules*"*. Physico-chemical constants like pH, Acid insoluble ash values, Water soluble extract, Alcohol soluble extractive values, Moisture content, total ash were found to be in acceptable range as per Ayurvedic Pharmacopoeia of India Part l, Vol I. Analysis showed the presence of phenol, saponins and carbohydrate. The macro-microscopical study, physico-chemical and phyto-chemical analysis, HPTLC study results obtained confirmed the effectiveness and stability of the formulation. These studies will definitely help to set a standard for this traditional medicine.

**Keywords: Phytochemical, Physico-chemical, High performance thin layer chromatography, *Simhyadi Kashaya* Granules**

## 1. INTRODUCTION:

The usage of herbal medications continues to expand rapidly among the population. Majority of the public population now prefer herbal products as the prime health care **[1]**. The World Health Assembly – in its various resolutions highlights a sequel of tests for estimating the quality of medicinal plant materials **[2]**. As the demand for herbal medicines is increasing tremendously, it is the cardinal responsibility of the regulatory authorities to ensure that the public gets medications, which are pure, safe, potent, and efficacious. To fulfill all these criteria, it is essential to standardize herbal medicines with the help of available technology. Standardization measures include the evaluation of various parameters like phytochemical, and physiochemical measures. This is an attempt to establish the scientific basis of herbal formulations in Ayurveda *"Simhyadi Kashaya* Granules*"*. Phytochemical screening is the scientific process of investigating, extracting, testing, and thus recognizing different classes of phytoconstituents present in various parts **[3]**. High performance thin layer chromatography (HPTLC) helps to detect the phytochemical constituents and can deliver an electronic copy of the chromatographic fingerprint and a densitogram to spot the presence of marker compounds in a plant sample **[4]**. These studies will enable to set a standard for this traditional medicine.

## 2. MATERIAL AND METHODS:

### 2.1. *Simhyadi Kvatha Churna* [5]:

**Table: 1 showing the Ingredients of *Simhyadi Kvatha Churna***

| S. No. | Ingredient | Part | Quantity |
|---|---|---|---|
| 1. | ***Kantakari* (*Solanum xanthocarpum* Schradt.Wendl)** | **Plant (whole)** | **1 part** |
| 2. | ***Nisha* (*Curcuma longa* linn)** | **Rhizome** | **1 part** |
| 3. | ***Vasa* (*Adhathoda vasica* Nees.)** | **Leaves** | **1 part** |
| 4. | ***Guduchi* (*Tinospora cordifolia* Wall.)** | **Stem** | **1 part** |
| 5. | ***Sunti* (*Zingiber officinale* Roxb)** | **Rhizome** | **1 part** |
| 6. | ***Pippali* (*Piper longum* Linn.)** | **Fruit** | **1 part** |
| 7. | ***Bharangi* (*Clerodendrum serratum* Linn)** | **Root** | **1 part** |
| 8. | ***Musta* (*Cyperus rotundus* Linn.)** | **Rhizome** | **1 part** |

The prepared *Kvatha* (decoction) as in **Table 1** should be consumed with *Pippali* and *Marica Churna*. This pacifies *Shwasa* (Bronchial Asthma) as clouds pacify fire.

### 2.2 Method of preparation of *Kashaya* Granules [9]:

1. Check the raw materials as per formula

2. Pulverize the *Simhyadi Kashaya Chooranam* (decoction powder) in a pulverizer
3. Sieve the *Chooranam* (powder) in hand sifter with # 40
4. Mix the *Chooranam* got from step 3 with Gum acacia manually.
5. Add sufficient qty of distilled mineral water in to it.
6. Mix them well manually to make it into wet lumps.
7. Pass the wet lumps through mess no. 8 to get wet granules.
8. Put the wet granules in Stainless Steel trays and keep it in the drier
9. Dry the granules in the drier at the temperature between 50°C to 60°C
10. Pass the dried granules through mess no. 14
11. Pack the granules in a HDPE (High Density Polyethylene) container.

**2.3. Analytical Parameters:**

**2.3.1. Macroscopic Study**:

For Macroscopic study evaluation of the drug for the confirmation of its identity, determination of quality and purity and detection of adulteration was done by visual appearance by the naked eyes. Other sensory characteristics like odour, taste, and feel of the drug to the touch were also observed.

**2.3.2. Microscopic Study:**

For microscopic studies the plant material was subjected to various sections as well as subjected to various stains and studied as per standard procedures.

**2.3.3. Physico-chemical Analysis:**

Physico-chemical constants **(Table 2)** were determined as per methods given in Ayurvedic Pharmacopoeia of India (API Part l, Vol I) **[6]** and Indian Pharmacopoeia (IP-2010) **[7]**.

**Table 2: Physico-chemical Analysis**

| S. No. | Table showing the Physico-chemical Analysis Constants |
|---|---|
| 1. | Determination of pH Values [8] |
| 2. | Total ash [8] |
| 3. | Acid-insoluble ash [10] |
| 4. | Determination of Alcohol Soluble Extractive [11] |
| 5. | Water Soluble Extract [11] |
| 6. | Determination of Moisture Content [11] (Loss on Drying) |

**2.3.4. Phytochemical Analysis:**

The air-dried powdered plant material is extracted in Soxhlet assembly with various solvents (ethanol, methanol). Finally, the drug is macerated with chloroform water and dried in hot air oven below $50^0$C. thus obtained extract is subjected to phytochemical

screening for the detection of various plant constituents **[12] (Table 3)**.

**Table 3: Showing Phytochemical Analysis**

| Test Done | Phytochemical |
|---|---|
| Dragendorff's Test | Alkaloids |
| Shinoda Test | Flavonoids |
| Picric Acid Test | Glycosides |
| Folin Ciocalteu Reagent Test | Phenol |
| Froth Formation Test | Saponins |
| Ferric Chloride Test | Tannins |
| Benedict's Solution Test | Carbohydrates |

**2.4. HPTLC**

The air-dried powdered material of the ingredients in *Simhyadi Kashaya* Granules (100 g) was extracted with methanol and water solvent (1:1 v/v) using the Soxhlation process with the help of a Soxhlet apparatus. Excess solvent was then dispersed in a water bath at 50– 100 °C to get the crude form and stored in airtight containers.

**2.4.1. Instrumentation**

A HPTLC system (CAMAG) equipped with applicator (LINOMAT 5) fitted with 100 µl syringe, Scanning was carried out with the Planar Chromatography manager (win CATS), TLC scanner (CAMAG) and software (win CATS) was used.

**2.4.2. Development - Glass tank:**

The plate was developed using Toluene: Chloroform: Methanol (8: 3: 1) in the (CAMAG) twin trough chamber 20 x 10cm, previously saturated with the solvent for 5 minutes. The mobile phase was decided after testing on various solvent systems of different polarity. After development, the plates were dried in an oven at $60^0$C and scanned using a (CAMAG) TLC scanner in absorption mode.

**2.4.3. Post-Chromatographic Derivatization**

Hot air oven is used to dry the developed plates. Spot is visualized after the process of derivatization. For this purpose, 5% ANISALDEHYDE SULPHURIC ACID solution is used. Further, the plates were dried at 120ºC in hot air oven for 20 min. Using (CAMAG) visualizer, the bands were visualized. Later the images are captured in 254 nm (short UV) and 366 nm (long UV) wavelengths under white light. UV-active compounds will undergo fluorescence extinction and looks as dark spots on a bright background, when exposed to short-wave UV light of 254 nm **[13]**. On the other hand, mixtures that absorb 366 nm UV light will look as bright spots on a dark background.

## 3. RESULTS AND DISCUSSION:

**Result of Macro- Microscopic Study of *Simhyadi Kashaya* Granules:**

**3.1. Macroscopy:** The powder is greenish yellow in colour. It is present in form of fine powder and small round granules **(Figure 1)**. It has a characteristic odour and taste **(Table 4)**.

**3.2. Microscopy:** Microscopy of *Simhyadi Kashaya Granules* shows **A** -fragments of xylem vessels with bordered pits (*Guduci* **[20]**, *Bharang* **[15]**); **B**- fragments of vessels with reticulate thickening (*Nisa* **[16]**, *Vasa* **[19]**, *Sunthi* **[18]**); **C**- fragments of annular xylem vessels (*Nisa*, *Vasa* **[19]**); **D**- fragments of simple pitted vessels (*Kantakari* **[17]**, *Vasa*); **E**- fragments of spiral xylem vessels (*Kantakari*, *Nisa*, *Musta* **[14]**, *Vasa*), are shown. Brown coloured testa **(P)** and pollen grain**(G)** (*Kantakari*); oil globules **(H)** (*Kantakari*, *Vasa*, *Pippali* **[15]**); prismatic crystal **(I)** of calcium oxalate (*Kantakari*, *Guduci*, *Bharangi*, *Vasa*) are found. Lignified, pitted sclereid **(J)** with lumen (*Kantakari*, *Guduchi*); golden brown coloured cell contents; a fragment of cork **(L)** (*Bharangi*, *Vasa*, *Sunthi*); non-lignified, pitted trachiedal fiber **(M)** (*Guduchi*); unicellular, aseptate short and long trichomes **(N)** are seen. Lignified crystal fibers **(Q)** with large number of crystals (*Guduchi*); lignified stone cells **(R)** (*Guduchi*); oval- elongated non-lignified stone cells (*Kantakari*, *Pippali*[18]); lignified group of fibers **(S)** (*Bharangi*, *Kantakari*, *Sunthi*); surface view of epidermal cells **(T)** is also found. Starch grains **(U)** of round to oval in structure are found abundantly. Simple starch grains (*Musta* **[17]**, *Nisa*, *Sunthi*, *Pippali*, *Kantakari*, *Bharangi*) and compound starch grains with 2-3 components (*Guduchi*); non-lignified phloem fiber **(V)** with wide lumen (*Bharangi* **[15]***)*; stomata **(W)** are seen.

**3.3. Physio-chemical Analysis:**

pH (10 % aqueous solution) was found to be 5.91 as per API Part l, Vol I. Acid insoluble ash percentage was found to be 0.79 as per IP 2018. Water soluble extract percentage was found to be 19.83 as per IP 2018. Alcohol soluble extract percentage was found to be 8.09 as per IP 2018. Loss on drying percentage was 8.32 according to API Part I, Vol I. Total Ash percentage was 6.85 as per IP 2018.

**3.4. Phyto-chemical Screening Analysis:**

Alkaloids done by Dragendorff's reagent Test were found to be absent. Flavonoids done by Shinoda test were found to be present. Glycosides were absent which is done by Picric acid test. Phenol was present by Folin ciocalteu reagent test. Saponins were present done by Foam test. Tannins were absent done by Ferric chloride test. Carbohydrate was present done by Benedict solution test.

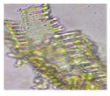
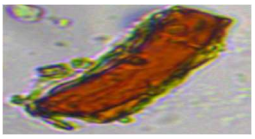
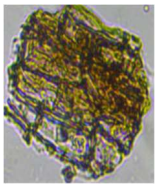
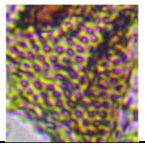
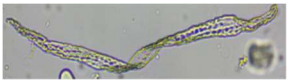
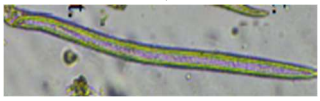
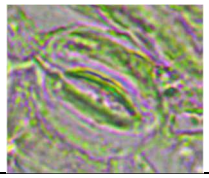
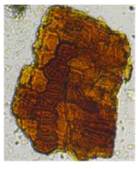
**3.5. HPTLC Analysis:**

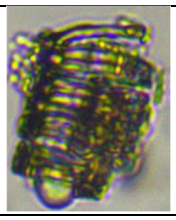
The HPTLC analysis of *Simhyadi Kashaya* Granules revealed the presence of various phytochemicals as illustrated in the figures and tables below **(Figure 3, 4, 5) (Table 5 and Table 6)**.


**Figure 1: Macroscopy of *Kashaya* Granules**

**Table 4: showing macroscopic study of *Simhyadi Kashaya* Granules**

| Organoleptic Characteristics | |
|---|---|
| **Colour** | **greenish yellow** |
| **Taste** | **Characteristic** |
| **Appearance** | **present in form of fine powder and small round granules** |
| **Odour** | **Characteristic** |

| Xylem Vessels with broad Pits | Vessels With Reticulate Thickening | Coloured Content | Cork |
|---|---|---|---|
| **A**  | **B**  | **K**  | **L**  |
| **Annular Xylem Vessels** | **Simple pitted vessels** | **Trachiedal Fiber** | **Long Trichomes** |
| **C**  | **D**  | **M**  | **N**  |
| **Spiral Xylem Vessels** | **Stomata** | **Short Trichomes** | **Testa** |
| **E** | **F**  | **O** | **P**  |



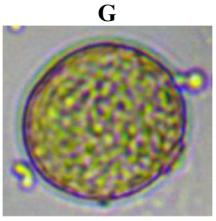
**Pollengrains**

**Oil globules**


**Crystal Fibers**

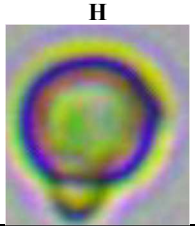
**Stone cells**

**G**



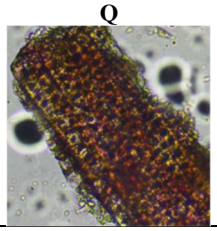
**Crystals**

**H**



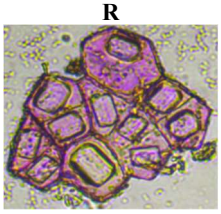
**Sclereids**

**Q**



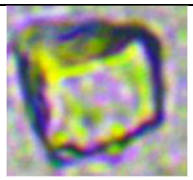
**Fibers**

**R**



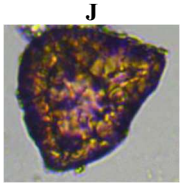
**Epidermal Cells**

**I**



**Starch Grains**

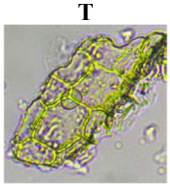
**J**



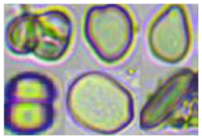
**Phloem Fibers**

**S**

**T**



**U**



**V**

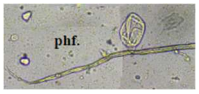



**Figure 2: Showing microscopic features of ingredients in *Simhyadi Kashaya Granules***

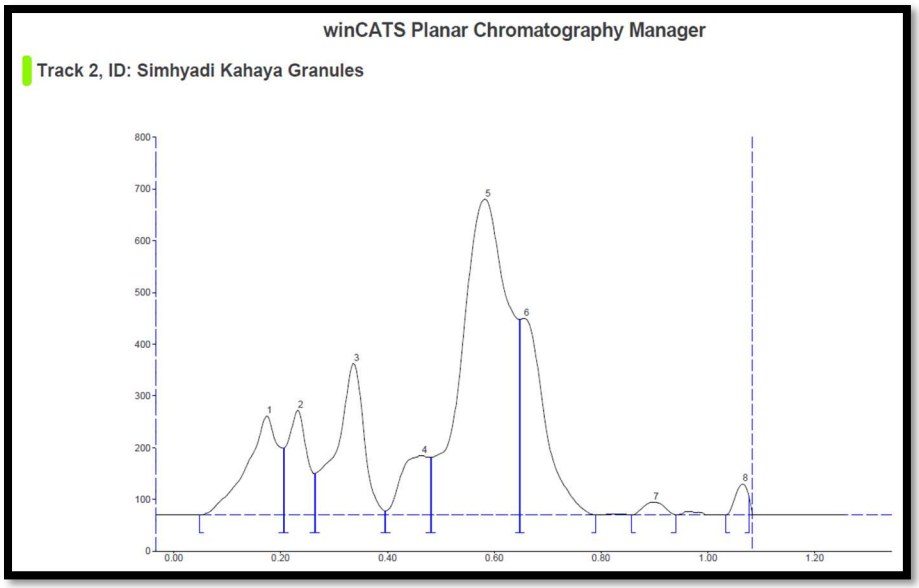



**Figure: 3: (UV 254 nm) chromatogram of *Simhyadi Kashaya* Granules**

**Table 5: Showing Rf values (UV 254nm)**

| Peak | Start Rf | Start Height | Max Rf | Max Height | Max % | End Rf | End Height | Area | Area % | Assigned Substance |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.05 | 0.2 | 0.17 | 191.8 | 10.20 | 0.21 | 129.7 | 7707.4 | 10.09 | Unknown* |
| 2 | 0.21 | 130.1 | 0.23 | 202.9 | 10.79 | 0.26 | 80.9 | 4997.4 | 6.54 | Unknown* |
| 3 | 0.27 | 81.0 | 0.34 | 292.9 | 15.58 | 0.40 | 8.4 | 10141.6 | 13.28 | Unknown* |
| 4 | 0.40 | 8.6 | 0.47 | 115.2 | 6.13 | 0.48 | 111.7 | 4037.9 | 5.29 | Unknown* |
| 5 | 0.48 | 111.8 | 0.58 | 610.9 | 32.50 | 0.65 | 378.6 | 36128.6 | 47.30 | Unknown* |
| 6 | 0.65 | 378.6 | 0.66 | 380.3 | 20.23 | 0.79 | 0.8 | 11751.1 | 15.39 | Unknown* |
| 7 | 0.86 | 0.2 | 0.90 | 25.7 | 1.37 | 0.94 | 0.4 | 682.7 | 0.89 | Unknown* |
| 8 | 1.04 | 0.2 | 1.07 | 60.2 | 3.20 | 1.08 | 36.5 | 932.1 | 1.22 | Unknown* |

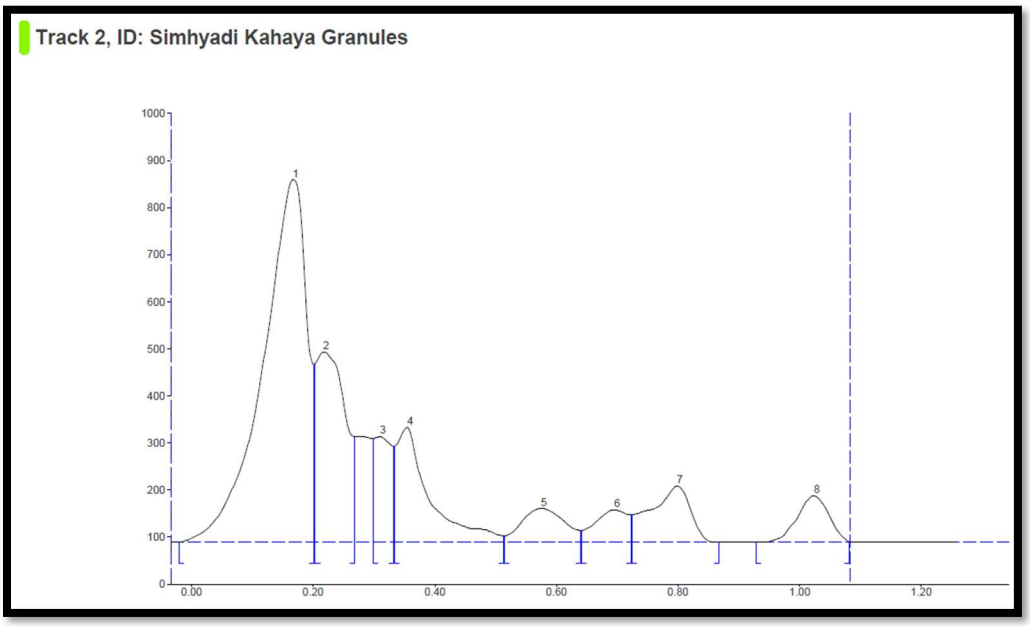



**Figure: 4: (UV 366 nm) chromatogram of *Simhyadi Kashaya* Granules**

**Table 6: Showing Rf values (UV 366nm)**

| Peak | Start Rf | Start Height | Max Rf | Max Height | Max % | End Rf | End Height | Area | Area % | Assigned Substance |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | -0.02 | 0.1 | 0.17 | 770.1 | 38.45 | 0.20 | 378.0 | 37332.8 | 47.28 | Unknown* |
| 2 | 0.20 | 379.8 | 0.22 | 404.9 | 20.22 | 0.27 | 225.0 | 13297.9 | 16.84 | Unknown* |
| 3 | 0.30 | 221.7 | 0.31 | 223.6 | 11.17 | 0.33 | 204.0 | 4565.9 | 5.78 | Unknown* |
| 4 | 0.33 | 204.3 | 0.36 | 244.3 | 12.20 | 0.51 | 14.3 | 9015.9 | 11.42 | Unknown* |
| 5 | 0.52 | 14.3 | 0.58 | 71.9 | 3.59 | 0.64 | 25.1 | 3457.3 | 4.38 | Unknown* |
| 6 | 0.64 | 25.2 | 0.70 | 69.1 | 3.45 | 0.72 | 58.1 | 2525.6 | 3.20 | Unknown* |
| 7 | 0.73 | 58.3 | 0.80 | 119.9 | 5.99 | 0.87 | 0.0 | 5278.4 | 6.68 | Unknown* |
| 8 | 0.93 | 0.0 | 1.03 | 98.8 | 4.93 | 1.08 | 1.1 | 3487.2 | 4.42 | Unknown* |

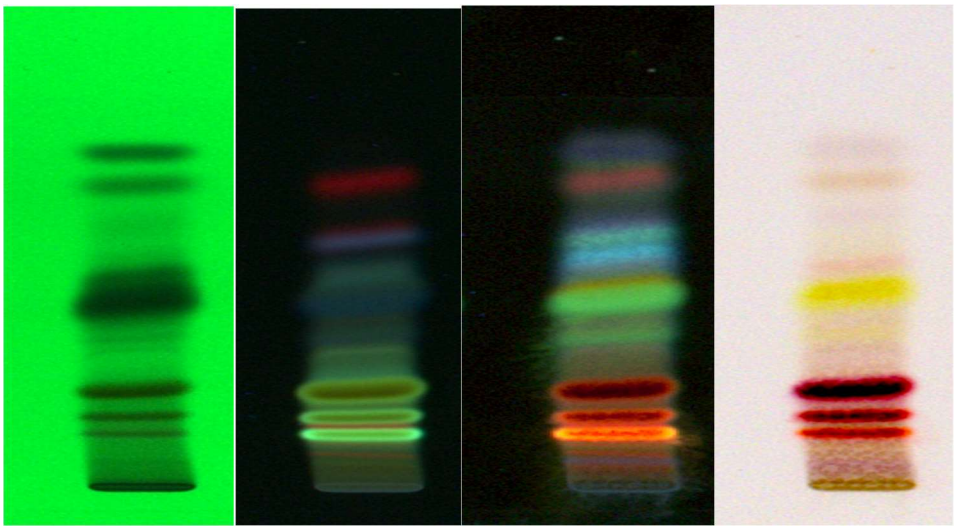


**Figure 5: showing the Visualizer Document of *Simhyadi Kashaya* Granules**

## 4. CONCLUSION:

Asthma is a chronic inflammatory disease of the airway characterized by airway eosinophilia, goblet cell hyperplasia with mucus hypersecretion, and hyperresponsiveness to inhaled allergens and nonspecific stimuli. In addition, increased oxidative stress is related to severity of asthma, propagation of inflammatory response, and reduction of responsiveness to corticosteroids. Above said Phyto-chemicals such as Saponins, Flavonoids and Phenol compounds seen in *Simhyadi Kashaya* Granules helps to reverse the pathological process involved in Bronchial Asthma. Thus, step-by-step approach and descriptions of analytical study on *Simhyadi Kashaya* Granules helps to understand the mode of action at each level against the disease pathogenesis.

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