

PHARMACEUTICO - ANALYTICAL STUDY OF GANDHAKA KALPA

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ABSTRACT

Herbo-mineral formulations occupy a significant seat in Ayurvedic pharmaceuticals. Nearly 70% of formulations include a combination of one or more metallic/minerals with several herbs which have a supporting role in treating the disease. *Gandhaka* is a primary substance and a fundamental part of the Ayurvedic laboratory. *Gandhaka, being the first among the Uparasa Varga*, plays a vital role in the preparation of *Chaturvidha Rasa-Rasayanas; Sagandha Yogas* are considered the most productive among all the herbo-mineral preparations and hence can be used for longer duration without any complications. *Gandhaka Kalpa*¹ is a herbo mineral formulation explained in *Rasayoga Sagara*, which includes the *bhavana* (trituration) of *shuddha Gandhaka* with *Amalaki Swarasa* for seven days. The study aims to prepare *Gandhaka Kalpa* as per the standard manufacturing procedure mentioned in classical texts and to analyse the sample for purity and quality by utilising suitable Analytical parameters. Results -The analysis of *Gandhaka Kalpa* shows an increase in weight after seven days of *Bhavana*; the Presence of a low amount of moisture content of *Gandhaka Kalpa* (2.88%) leads to decreased decomposition and enhanced shelf life and therapeutic value. Low Acid insoluble Ash determines the presence of soft adherent dirt and sand particles. Heavy metal tests and microbial analysis showed results within limits, which refers to the samples' safety profile and the drug's stability. Retention factor values in HPTLC confirmed the multi-polarity index of the compounds and a wide range of active principles.

Key words: Bhavana, *Gandhaka Kalpa*, HPTLC, Physico-chemical analysis

INTRODUCTION

Sulphur is known as *Gandhaka* in Ayurveda literature². It is known for its wide range of therapeutic efficacy. It is the first mineral among *Uparasa*. It has a peculiar smell, hence the name *Gandhaka*³. *Gandhaka* is an integral part of various herbo-mineral medicines used in therapeutics. The purified *Gandhaka* can be administered as a single drug with proper adjuvant. Such preparations are known as *Gandhaka kalpa*. *Sa-gandha Yogas* are considered the most productive among all the herbo-mineral preparations and hence can be used longer without any complications. *Gandhaka Kalpa* is one such herbo-mineral formulation mentioned in *Rasayogasagara*. Where etymology states that the drug contains *Gandhaka* as the main ingredient, which is to be triturated with *Amalaki swarasa* for seven days, which is best in *Kushta Haryana*, various other yogas in the name of *Gandhaka Kalpa* contain different prefixes, ingredients, indications, and doses. Any medicine should be of the highest quality, and to check the quality of the finished products, it becomes necessary to perform analytical studies of the products. With the analysis of the drug, the research of a drug is complete. Thus, the pharmaceutical processing of *Gandhaka Kalpa* was carried out according to classical references, and later, the final product was subjected to classical and modern analytical tests.

Aim & objectives: Preparation of *Gandhaka Kalpa* as per the Standard Manufacturing procedure mentioned in the classical texts and to analyse its purity and quality.

MATERIAL AND METHODS

1. PHARMACEUTICAL STUDY

Collection and authentication of raw drugs.

- Raw material, *Gandhaka*, was procured from M/s. S P Kajrekar, Belgaum.
- Other ingredients like *Amalaki*, milk, and ghee were procured from the local market of Ballari.
- *Gandhaka* was authenticated as per *Grahya Lakshana* (acceptable qualities) mentioned in *Rasa classics*. *Gandhaka Kalpa* was prepared at the Department of Post Graduate Studies in *Rasashastra* and *Bhaishajya Kalpana*, TGAMC, Ballari.

2. ANALYTICAL STUDY

Analysis related to the standardisation of *Ashodhita Gandhaka* (AS), *Shoditha Gandhaka* (SG), and *Gandhaka Kalpa* was done at Quality control lab A.L.N. Rao. Ayurvedic Medical College, Koppa. HPTLC for GK was done at the S.D.M. Centre for Research in Ayurveda and Allied Sciences, Udupi.

PHARMACEUTICAL STEPS INVOLVED IN THE PREPARATION OF GANDHAKA KALPA

1. *Gandhaka shodhana*
2. Extraction of *amalaki swarasa*
3. Preparation of *Gandhaka Kalpa*

PRACTICAL No 1 - *Gandhaka Shodhana*⁴ Materials Required

- I. *Ashodhita Gandhaka* 500g × 6 batches = 3000g
- II. Cow's milk 2 L × 3 × 3 = 18 L
- III. Cow's Ghee 20g × 3 × 3 = 180g
- IV. The hot water Quantity is sufficient.

Type of procedure: *Dhalana*

Procedure: Unpurified *Gandhaka* was made into a fine powder with the help of a mortar and pestle and weighed appropriately. Obtained amount of *Gandhaka* powder divided into six equal parts. Cow's milk was boiled in a stainless-steel vessel and kept for Lukewarm condition. The mouth of the ship was closed by a clean cotton cloth smeared with cow's ghee and tied with the help of thread. The first batch of powdered unpurified *Gandhaka* was kept in a stainless-steel vessel containing cow's ghee. Then, it was melted at mild heat with continuous stirring. Completely melted *Gandhaka* was poured immediately into the warm milk vessel through ghee-smeared cotton cloth. The cloth covering was removed from the ship, the milk part was discarded, and *Gandhaka* poured it into another hot water vessel. It was washed thoroughly several times until milk and ghee particles were removed. Dried it in the shade, then powdered and weighed. The same procedure was carried out thrice per batch and replaced milk every time. 6 batches were subjected to the same method, and finally, the purified *Gandhaka* was weighed. The product obtained after these processes was called *Shuddha Gandhaka*.

PRACTICAL No 2 - Extraction of *Amalaki swarasa*⁵

Equipment: Mixer, cloth, measuring jar, spoon, weighing machine

Drugs used: Fresh *Amalaki* fruits.

Procedure:

- Fresh fruits of *Amalaki* were taken and cleaned well with portable water.
- Seeds were removed and cut into small pieces.

The pieces of *Amalaki* were made into paste with the help of the mixer, and *swarasa* was obtained by squeezing through a clean cloth.

PRACTICAL No 3-Preparation of *Gandhaka Kalpa*

Ingredients

Shodhita Gandhaka –2 kgs (4 batches)

Amalaki swarasa – q.s

Method –*Bhavana*

Equipment – *Khalva Yantra, Spoon, Tula Yantra.*

Procedure: *Shodhita Gandhaka*, as shown in fig 1, 2 & 3, is taken in a clean *khalwa yantra*, finely powdered to this *amalaki swarasa* was added such that it gets immersed completely, and *Bhavana* is carried out for seven days, 8 hours per day. After completion

of the *Bhavana*, the *Gandhaka* is dried, weighed and stored in an airtight container. This compound is called *Gandhaka Kalpa*.

OBSERVATION AND RESULTS

Gandhaka Shodhana: The *Gandhaka* melted quickly in the pan and gained an orange colour upon melting. After filtration, a layer of fatty medium, which was somewhat brownish, was found over the *Godugdha Gandhaka*, which was coarse powder, had collected in a mass form in the vessel in milk media, with many spiky projection-like structures and slimy. In the end, from 3000gms *Ashuddha Gandhaka*, we got 2768gm *Shuddha Gandhaka*, and there was a 7.74% loss in the whole procedure.

Gandhaka Kalpa: *Shuddha Gandhaka* became completely wet within 30 minutes of trituration with *bhavana dravya*. The quantity of *Bhavana dravya* utilised in subsequent *bhavana*s decreased, and after the process was completed, the final product's weight was increased. (*sh Gandhaka*- 2000gms, after *bhavana* - 2520gms)

RESULTS OF ANALYTICAL STUDY

1. Organoleptic characters of AG, SG and GK

Table No.1: Showing organoleptic characters of AG, SG and KG

| | AG | SG | GK |
|---------|----------------------|------------------|------------------|
| Colour | Yellow | Pale Yellow | Light Grey |
| Odour | Odorous (sulfur) | Odorous (sulfur) | Odorous (sulfur) |
| Taste | Tasteless | Tasteless | Sour, Astringent |
| Texture | Powder (crystalline) | Fine powder | Fine powder |

Table No.2 shows the analytical results of the test below.

| Parameters | AG | SG | GK |
|-------------------------------------------|-----------|-------------|------------|
| pH ⁶ | 4.85+0.10 | 5.41 + 0.10 | 3.14+ 0.10 |
| Loss on Drying at 105°C ⁷ | 0.53 | 0.75 | 2.88 |
| Total ash ⁸ | 0.08 | 0.14 | 1.47 |
| Acid insoluble ash ⁹ | 0.00 | 0.11 | 0.22 |
| Water soluble ash ¹⁰ | 0.00 | 0.02 | 0.32 |
| Alcohol soluble extractives ¹¹ | 9.41 | 14.62 | 17.24 |
| Water soluble extractives ¹² | 9.07 | 30.76 | 35.46 |

3: HPTLC¹³

1g of *Gandhaka kalpa* was suspended in 10 ml of alcohol, warmed in a water bath, and filtered. 3, six and 9µl of the above extract were applied on a pre-coated silica gel F254 on aluminium plates to a bandwidth of 7 mm using a Linomat 5 TLC applicator. The plate was developed in Toluene: Ethyl ace-

tate (1.0: 1.0). The developed plates were visualised under short UV, long UV and then derivatised with vanillin sulphuric acid and scanned under UV 254nm, 366nm and 620nm (after derivatisation). R_f, the colour of the spots and the densitometric scan were recorded.

Table No.3: R_f values of a sample of GK

Fig 1a. At 254nm

Track 7, ID: Gandhaka kalpa

| Peak | Start Position | Start Height | Max Position | Max Height | Max % | End Position | End Height | Area | Area % |
|------|----------------|--------------|--------------|------------|---------|--------------|------------|------------|---------|
| 1 | 0.00 Rf | 5.0 AU | 0.04 Rf | 737.9 AU | 32.58 % | 0.05 Rf | 14.9 AU | 15119.0 AU | 20.38 % |
| 2 | 0.05 Rf | 717.0 AU | 0.06 Rf | 724.8 AU | 32.00 % | 0.10 Rf | 05.8 AU | 18239.6 AU | 24.58 % |
| 3 | 0.10 Rf | 505.9 AU | 0.16 Rf | 597.0 AU | 26.36 % | 0.26 Rf | 14.0 AU | 36196.2 AU | 48.79 % |
| 4 | 0.27 Rf | 10.6 AU | 0.28 Rf | 11.7 AU | 0.52 % | 0.31 Rf | 1.3 AU | 187.9 AU | 0.25 % |
| 5 | 0.39 Rf | 4.7 AU | 0.45 Rf | 22.7 AU | 1.00 % | 0.46 Rf | 18.5 AU | 494.8 AU | 0.67 % |
| 6 | 0.46 Rf | 18.8 AU | 0.49 Rf | 63.8 AU | 2.82 % | 0.50 Rf | 41.6 AU | 1112.4 AU | 1.50 % |
| 7 | 0.50 Rf | 41.9 AU | 0.53 Rf | 85.8 AU | 3.79 % | 0.59 Rf | 0.4 AU | 2615.4 AU | 3.53 % |
| 8 | 0.89 Rf | 1.8 AU | 0.91 Rf | 21.3 AU | 0.94 % | 0.92 Rf | 3.8 AU | 228.2 AU | 0.31 % |

Fig 1b. At 366nm

Track 7, ID: Gandhaka kalpa

| Peak | Start Position | Start Height | Max Position | Max Height | Max % | End Position | End Height | Area | Area % |
|------|----------------|--------------|--------------|------------|----------|--------------|------------|----------|----------|
| 1 | 0.01 Rf | 0.5 AU | 0.04 Rf | 16.3 AU | 100.00 % | 0.06 Rf | 0.3 AU | 259.3 AU | 100.00 % |

Fig 1c. At 620nm

Track 7, ID: Gandhaka kalpa

| Peak | Start Position | Start Height | Max Position | Max Height | Max % | End Position | End Height | Area | Area % |
|------|----------------|--------------|--------------|------------|---------|--------------|------------|-----------|---------|
| 1 | 0.01 Rf | 2.3 AU | 0.03 Rf | 71.9 AU | 51.12 % | 0.05 Rf | 48.0 AU | 866.3 AU | 44.19 % |
| 2 | 0.05 Rf | 48.2 AU | 0.06 Rf | 68.7 AU | 48.88 % | 0.11 Rf | 3.6 AU | 1094.0 AU | 55.81 % |

Results of heavy metal test¹⁴: Lead, Cadmium, Arsenic, and Mercury are not detected.

Microbial analysis¹⁵: Total aerobic bacterial count: 88 cfu / g

Total yeast mould coconut < 10 cfu / g

DISCUSSION

Gandhaka Shodhana: The total loss of *Gandhaka* during shodhana procedure was 7.74 % because of the below precautions; *Mandagni* was given to avoid burning Sulphur. Cloth was smeared with Ghee to prevent sticking of *Gandhaka* to the fabric. Here, finely powdered *Gandhaka* facilitates its easy dissolution in *Goghrita* under *Mandagni* intensity in a quicker duration due to its minute particle size. This uniform dissolving of the *Gandhaka* helps it filter easily through the cloth. Few blackish substances remained

over the fabric, considered physical impurities in sulphur. After filtration, a layer of fatty medium, which was somewhat brownish, was found over the *Godugdha*. This can be viewed as separating the *Visha Guna* of *Gandhaka* from it and coming out into the *Goghrita*, remaining at the surface of *Godugdha*. Loss may be due to the adhering of *Gandhaka* to the cloth and its impurities. ***Gandhaka Kalpa:*** *GK* is a *Kharaliya Rasa Yoga* in which the *Shoditha Gandhaka* was given *bhavana* with *Amalaki swarasa* for seven days; *Bhavana* that can be correlated with wet triturating in modern concept, which was given benefits such as

finer particle size, eliminates the dust hazards, produce low speed, consume less power, the minute particles of the materials come in close contact to the liquid media.

Bhavana Drava was prepared daily, and triturate continued till it dried on the same day to prevent microbial growth, reduce time factor, and ensure medicinal value; after seven days of *Bhavana*, the mass was dried to observe weight gain.

Analytical discussion.

1. The pH of AG, SG and GK is 4.85, 5.41 and 4.2, respectively. Values indicate the acidic nature of the drugs. A drug's solubility, stability, activity, and absorption depend upon its pH. The pH of *Gandhaka Kalpa* is more acidic than AG and SG, which might be due to *bhavana dravya*. In the present study, amalaki swarasa was used as *bhavana dravya*, which is acidic. The degree of ionisation and lipid solubility of a drug are two crucial factors that determine the rate of absorption of drugs from the GI tract and their passage through cellular membranes easily¹⁶. Drugs that are weak organic acids are primarily in unionised form and are soluble in lipids and absorbed rapidly through cellular membranes compared to solid acids or bases.
2. **Loss on drying:** LOD of AG, SG and GK is 0.53, 0.75% and 2.88% respectively. Hence, it can be stated that GK has significantly less moisture content and a scarce chance of bacterial and fungal growth. The drug has minor hygroscopic activity with less chance of medication contamination.
3. **Total Ash:** AG, SG and GK were evaluated for ash value, which was found to be 0.8%, 0.14% and 1.47%, respectively. As the ash value is within permeable limits, samples are not contaminated, showing the drug's genuineness.
4. **Water soluble ash:** Water Insoluble ash indicates selective media of drug administration. AG, SG and GK were estimated for water-insoluble ash, which was 0.00%, 0.02% and 0.32%, respectively.

5. **Acid insoluble ash:** The human metabolic process and pharmacokinetics depend upon the purity and human-acceptable forms of the drug. When the AG, SG, and GK ash were treated with hydrochloric acid, 0.00%, 0.11%, and 0.22%, respectively, acid-insoluble ash was detected. Which signifies the genuineness of the product and suggests it is best in terms of solubility and absorption.
6. **Water soluble and alcohol soluble extractives** were shown in higher % (62.18 % w/w & 63. 12 % w/w) in value, which can be seen more in active principles in the sample and their quality standards.
7. **HPTLC:** GK was shown in 254nm and 366nm, 8 & 1 peak & after derivatisation in 620nm wavelength two peaks or spots in retention factor (Rf) values that separation of spots confirmed about the multi-polarity index of compounds.
8. **Heavy metal test:** Lead, Cadmium, Arsenic and Mercury were not detected in the GR sample, which refers to the safe profile of the drug as an internal medication.
9. **Microbial analysis** of GK was shown within the limit results, which can say the safety and stability of the drugs.

CONCLUSION

The Pharmaceutico-analytical study of *Gandhaka Kalpa* confirmed the purity and genuineness of the drug. It showed all ingredients used were genuine and had no impurities. Summer is the best season to manufacture *Gandhaka Kalpa* to prevent fungal growth and help in early drying. *Bhavana dravya* is vital in potentiating purified *Gandhaka* with medicinal properties that increase *Gandhaka kalpa's* efficacy. The SOPs and SMPs adopted with conventional methods under GMP guidelines for preparing GK should lay a strong foundation for its validation and standardisation. The data obtained from GK's physicochemical analysis can be considered a reference for its standardisation.

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Fig.1Raw Gandhaka Fig.2 Powder raw Gandhaka Fig.3 Melting

Gandhaka



Fig.4 Pouring of melted Gandhaka Fig.5 colour of the milk after pouring Molten Gandhaka Fig.6 Decanting water



Fig -7 Shodhita Gandhaka Fig 8- Amalaki Swarasa Fig 9 -Gandhak Kalpa



Fig.10 Final product of Gandhaka Kalpa