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ORIGINAL ARTICLE

Analytical Characterization and Chromatographic Fingerprinting of *Gandhaka* Based Lipid Formulations- A Comparative Study

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ABSTRACT

Gandhaka Ghrita and *Gandhaka Dhruti* are classical *Ayurvedic* sulphur-based lipid formulations used primarily for the management of dermatological disorders. To prepare *Gandhaka Ghrita* and *Gandhaka Dhruti* according to classical *Ayurvedic* references and to evaluate their physicochemical and HPTLC profiles. *Gandhaka Ghrita* and *Gandhaka Dhruti* were prepared following the procedures described in *Rasa Tantra Sara* and *Rasa Ratna Samuchaya* respectively. Organoleptic, physicochemical and HPTLC analyses were performed according to CCRAS guidelines using Toluene: Ethyl acetate: Acetic Acid: Methanol (8.5:1:0.5:0.5) as the mobile phase and scanned at 254 nm and 366 nm. Both formulations complied with standard physicochemical parameters and showed no rancidity. HPTLC analysis of *Gandhaka Ghrita* suggested the presence of Tyrosine, tryptophan and riboflavin whereas *Gandhaka Dhruti* exhibited phytochemicals such as 6-gingerol,6-shogaol, piperine and zingerone, indication distinct phytochemical fingerprinting. Although both formulations contain purified sulphur, differences in processing media resulted in distinct chemical profiles. *Gandhaka Ghrita* was enriched with milk-derived constituents, while *Gandhaka Dhruti* contained *Trikatu* derived bioactive compounds, supporting their different traditional therapeutic applications.

Keywords: *Gandhaka Ghrita, Gandhaka Dhruti*, Phytochemical evaluation

INTRODUCTION

Metals and minerals have been widely employed in therapeutics in traditional and modern system of medicines[1]. *Ayurveda*, an Indian system of medicine has an abundant collection of formulations rich in metals and minerals. '*Rasa Shastra'* is an ancient science prevailing in India which thoroughly deals with metal and mineral drugs[2]. These drugs are commonly known as '*Rasadravya'*. These *Rasa Dravya* are classified as *Maharasa, Uparasa, Sadharana Rasa, Dhatu, Updhatu, Ratna, Upratna etc* on its capability to react and potentiate mercury. *Gandhaka (Sulphur)* is a *Rasadravya* therapeutically considered as a major *Rasayana in Rasa shastra* and an important drug in contemporary system of medicine for various dermatological diseases, neurological pathologies, joint diseases etc. *Gandhaka ghrita* is one such lipid-based formulation described in *Rasa Tantra sara evum siddha Prayoga Sangraha*. *Gandhaka Dhruti*, is oil-based formulation of *gandhaka* which is indicated which have indication for *Kasa* (Respiratory problems), *Pandu* (Anaemia), *Pama* (Scabies), *Vicharchika* (Eczema), *Kshudra Kushta* (Skin diseases) and for

Rasayana (Rejuvenation) purpose as well. The base of *Dhruti* is *Tila taila* which is *ushna virya* added with *trikatu*, making it more *ushna* and specific for *Kapha* and *Ama Janya vikara*[3] *"Dru-Kitan"* means *Drutatva* (Liquefaction). *Dhruti* is a dosage form that is believed to have been utilized for both *Deha & Loha Siddhi*. *Dhruti Kalpana* is the process that converts hard and dense metals and minerals into a liquified state after processing with specific herbs and intense heat. It possesses characteristics such as *Nirlepatwam* (No stickiness), *Drutatvam* (Liquified state), *Tejastvam* (Lustrous)[4]. Among all *Dhruti's Gandhaka Dhruti* because of oil in it does not possess *Drutatvam, Tejastwam* characteristic. The administration of *Gandhaka Dhruti* is described as a systematic manner with the inclusion of Betel leaf, *Parada* (incinerated mercury) There are different references of *Gandhaka Dhruti* available in classics like *Rasa Ratna Samuchchaya, Rasa Tarangini, Rasendra Chintamani, Rasa Yoga Sagara and Ayurveda Prakasha*.

A detailed pharmaceutical guideline of *Gandhaka ghrita* and *Gandhaka Dhruti* and its analytical information is lacking and therefore the present work aims to prepare a recommendation on pharmaceutical phase of *Gandhaka Ghrita* and *Gandhaka Dhruti* followed by an analytical evaluation.

METHODOLOGY

Pharmaceutical Phase

A. Shodhana of *Ashuddha Gandhaka.*

B. Preparation of *Gandhaka Ghrita.*

C. Preparation of *Gandhaka Dhruti.*

(A) Preparation of *Shodhit Gandhaka*: Reference- *Rasa Ratna Samucchaya*[5]

Table 1: Ingredients for Gandhaka Shodhana

Sr. no.	Materials	Quantity
1.	*Ashuddha Gandhaka*	150 grams
2.	Cow's *Ghrita*	1050 grams
3.	Milk	Q. S
4.	Muslin Cloth	1 Unit
5.	Stainless Steel Vessel	1 Unit

Procedure:

Ashuddha gandhaka procured from the supplier was authenticated according to the characteristics of *gandhaka* which possess Sulphur Yellow color, Yellowish White Streak, Resinous Lustre, Brittle Tenacity *et al*[6]. *Ashuddha Gandhaka* was finely powdered in *Khalva yantra*.150 grams of *Ghrita* were taken in vessel and held over fire till hot and 150 grams of *Ashuddha Gandhaka* was poured into it. Once the Sulphur was completely melted it was poured (*Dhalana)* and filtered in the vessel containing milk through a muslin cloth on the vessel for shodhana. The whole process was repeated 7 times.



Fig. 1: *Gandhaka Shodhana* Procedure

Result:

Yellowish amorphous Sulphur was deposited at the bottom of the vessel and impurities of Sulphur were left above the cloth. *Shodhita Gandhaka* was collected, finely powdered and stored in an air tight vessel for further procedure. The weight of *Shuddha Gandhaka* obtained was 142 Grams after the *Shodhana* procedure.

(B) Preparation of *Gandhaka Ghrita*- Reference *RasaTantraSara evum Siddha Prayoga Sangraha*[7]

Table 2: Ingredients for *Gandhaka Ghrita*

Sr. no.	Materials	Quantity
1.	*Shodhita Gandhaka*	142 grams
2.	Cow's Milk	Q.S.
3.	Curd	200 grams
4.	Muslin Cloth	1 Unit
5.	Stainless Steel Vessel	1 Unit

Procedure:

Cow's milk was boiled. *Shodhita Gandhaka* was added and kept for 3-4 boils. After cooling curd was added to the milk. The mixture was allowed to ferment into curd overnight. On the next day the prepared curd was churned using a blender. During the process cold water was gradually added to aid separation. Butter with *gandhaka* was separated and rose to the surface, while *takra* (Buttermilk) remained below. The collected butter was placed in a heavy bottomed vessel and

heated over a mild flame. The heating continued till the froth subsided, melted *gandhaka* was left at the bottom and *ghrita* floating above. The obtained *Gandhaka ghrita* was filtered using muslin cloth to remove sediment of *gandhaka*.



Fig. 2: A) Procedure of Gandhaka Ghrita; B) Procedure of Gandhaka Ghrita

Result:

Liquid yellowish brown *gandhaka ghrita* with minute *gandhaka* particles sedimented below was obtained at last of the process. *Gandhaka Ghrita* was collected and stored in air tight container for further analytical process. The weight of *Gandhaka ghrita* obtained after the procedure was 80 ml.

(C) *Gandhaka Dhruti* Preparation- Reference *Ras Ratna Samucchaya*[8]

Procedure:

Shuddha Gandhaka powder was first triturated with *Trikatu Churna* in the proportion of 1/16th part of *Gandhaka* in a *khalva yantra* until a fine, uniform mixture was obtained. This homogenous blend was then evenly spread over the surface of a clean, rectangular cotton cloth of dimensions of one *Aratani* (10.5 inches) length and breadth, after which the cloth was rolled to form a *varti* (wick). The varti was tied securely with cotton thread to prevent disintegration during subsequent procedures. The prepared varti was then completely immersed in *Tila Taila* (sesame oil) contained in a stainless steel and allowed to remain soaked for three hours. After3 hours, the varti was removed from the oil, held firmly with forceps at one end. Then the varti was lightened using gas flame.

Table 3: Ingredients of *Gandhaka Dhruti*

Sr. no.	Materials	Quantity	Proportion
1.	*Shuddha Gandhaka(sulphur)*	1 part	16 grams
2.	*Sunthi (Zingiber officinale)*		
3.	*Maricha (Piper nigrum)*	1/16 part	1 gram
4.	*Pippali (Piper longum)*		
5.	*Til Taila (Sesame oil)*	Quantity sufficient	Q. S
6.	Muslin Cloth	1 Aratani (~ 42 cms)	-
7.	Stainless Steel Vessel	1 Unit	-
8.	Thread	Quantity sufficient	-



Fig. 3: Procedure of *Gandhaka Dhruti*

Result:

On burning thick, blackish, oily exudate drip from the burning end. This exudate was collected and was allowed to cool to room temperature. This viscous, dark-coloured, oily preparation with a distinct odour is designated as '*Gandhaka Druti'*. It was transferred into airtight glass bottles and subsequently stored in a cool and dry place.

Analytical Phase

1. Organoleptic Parameters: (a) Color, (b) Odor, (c) Taste, (d) Touch, (e) Appearance

2. Physicochemical Parameters: (a) Rancidity, (b) Specific gravity, (c) pH value, (d) loss on drying at 105°C, (e) Viscosity, (f) Iodine Value, (g) Saponification Value, (h) Unsaponifiable Matter, (i) Acid Value, (j) Free Fatty acids

3. HPTLC Analysis of *Gandhaka Ghrita* and *Gandhaka Dhruti*

RESULTS OF ANALYTICAL STUDY

Table 4: Organoleptic Characteristics of *Gandhaka Ghrita* and *Gandhaka Dhruti*

Formulation	Color	Odor	Taste	Appearance	Touch
Gandhaka Ghrita	Yellowish brown	Characteristic smell of *Gandhaka* and *Ghrita*	Tasteless	Ghrita form	Slimy and liquid
Gandhaka Dhruti	Reddish Black	Characteristic smell of *Gandhaka* and *Tila Taila*	Bitter	Oil form	Slimy

Table 5: Comparative Physico-chemical Analysis of *Gandhaka Ghrita* and *Gandhaka Dhruti*

Sr. No	Parameter	Result of Gandhaka Ghrita	Result of Gandhaka Dhruti
1.	Loss on drying at 105°C	0.017	0.010
2.	Specific Gravity at room temperature	0.9576	1.043
3.	Acid Value	2.91	1.16
4.	Saponification	210.5	45.28
5.	Iodine value	35.5	58.40
6.	pH	5.3	6
7.	Viscosity	80 cP	40 cP
8.	Unsaponifiable Matter	5.0–5.1%	0.8–1.5%
9.	Free Fatty Acids	0.1–0.5%	0.2–0.6%
10.	Rancidity	No rancidity	No rancidity

HPTLC Analysis

Stationary Phase- Merch HPTLC Silica Gel F_{254}

Mobile Phase- Toluene: Ethyl Acetate: Acetic Acid: Methanol (8.5:1:0.5:0.5)

Plate- Silica gel 60 F_{254}

Sample Solvent- Methanol

Detection Wavelength- 254 nm and 366 nm

Application Volume- 5µL and 10µL per sample

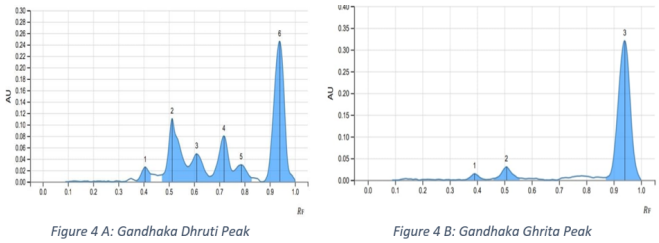


Figure 4 A: Gandhaka Dhruti Peak

Figure 4 B: Gandhaka Ghrita Peak

Fig. 4: *Gandhaka Dhruti* & *Gandhaka Ghrita* Peaks

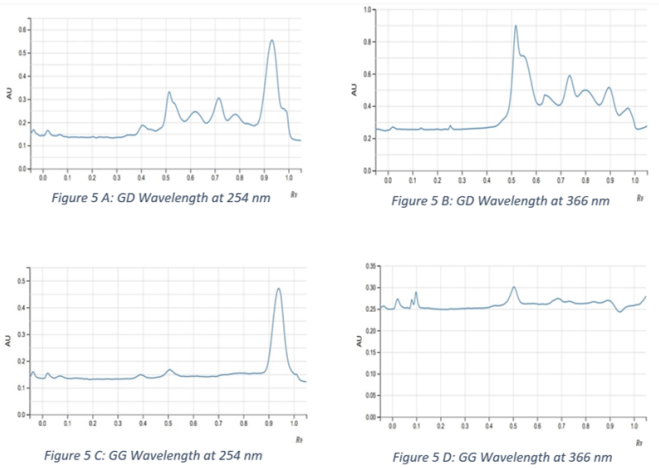


Figure 5 A: GD Wavelength at 254 nm

Figure 5 B: GD Wavelength at 366 nm

Figure 5 C: GG Wavelength at 254 nm

Figure 5 D: GG Wavelength at 366 nm

Fig. 5: *Gandhaka Ghrita* and *Gandhaka Dhruti* Wavelength at 254 nm & 366 nm

Table 6: HPTLC Profile Summary of *Gandhaka Ghrita* and *Gandhaka Dhruti*

Wavelength (nm)	*Gandhaka Ghrita* – Rf (Area%)	Probable Compound[9, 10]	*Gandhaka Dhruti* – Rf (Area%)	Probable Compound
254	0.390 (4.03%), 0.507 (8.34%), 0.940 (87.63%)	Tyrosine, Tryptophan, Riboflavin	0.939 (45.46%), 0.512 (20.42%), 0.718 (14.82%), 0.61 (9.04%), 0.786 (5.49%), 0.406 (4.78%)	6-Shogaol, Tryptophan, 6-Gingerol, Piperine, Gingerol derivative, Zingerone
366	0.099 (36.07%), 0.503 (63.93%)	Aromatic amino acids, Riboflavin	0.907, 0.644	6-Shogaol, Piperine

DISCUSSION

Gandhaka Ghrita and *Gandhaka Dhruti* embodies unique Sulphur-lipid transformations: *Gandhaka Ghrita* yields a smooth, stable dispersion suitable for internal use, while *Gandhaka Dhruti* forms complex organo-Sulphur compounds with potent redox potential-reflected in their distinct HPTLC fingerprints.

HPTLC interpretation of *Gandhaka Ghrita:* HPTLC analysis of *Gandhaka Ghrita* revealed three major peaks at 254 nm with Rf values of 0.390, 0.507, and 0.940, corresponding to area percentages of 4.03%, 8.34%, and 87.63%, respectively. At 366 nm, two prominent peaks were observed at Rf 0.099 and 0.503 with area percentages of 36.07% and 63.93%, respectively. The predominantly non-polar mobile phase (Toluene: Ethyl Acetate: Acetic Acid: Methanol; 8.5:1:0.5:0.5) allowed tentative identification of compounds based on their reported Rf values. The peak at Rf 0.390 may correspond to tyrosine, an aromatic amino acid derived from milk casein, while the peak at Rf 0.507 is suggestive of tryptophan, a whey protein constituent[11]. The prominent peak at Rf 0.940 may indicate the presence of riboflavin, a naturally occurring vitamin in milk. These findings suggest that the preparation process of *Gandhaka Ghrita* facilitates the incorporation of milk-derived amino acids and vitamins into the formulation.

Tyrosine is a non-essential aromatic amino acid synthesized from phenylalanine. It serves as a precursor for dopamine, norepinephrine, epinephrine, thyroid hormones, and melanin[12]. Due to its role in neurotransmitter synthesis, it contributes to normal brain function and is an essential component of many proteins and peptides[13]. Tryptophan is an essential aromatic amino acid required for protein synthesis. As it cannot be synthesized by the human body, it must be obtained through the diet[14]. It serves as a precursor for serotonin, melatonin, and vitamin B3 (niacin), contributing to neurological and metabolic functions. Riboflavin (Vitamin B2) is a water-soluble vitamin essential for energy metabolism. It functions as a coenzyme in the metabolism of carbohydrates, fats, and proteins and contributes to antioxidant defence and normal immune function[15].

HPTLC interpretation of *Gandhaka Dhruti:* HPTLC analysis of *Gandhaka Dhruti* using the mobile phase Toluene: Ethyl Acetate: Acetic Acid: Methanol (8.5:1:0.5:0.5) revealed six major peaks at 254 nm with Rf values of 0.939, 0.512, 0.718, 0.610, 0.786, and 0.406, corresponding to area percentages of 45.46%, 20.42%, 14.82%, 9.04%, 5.49%, and 4.78%, respectively. Based on literature and the formulation constituents, the dominant peak at Rf 0.939 (254 nm) and 0.907 (366 nm) was tentatively assigned to 6-shogaol, while the peaks at Rf 0.610 (254 nm) and 0.644 (366 nm) corresponded to piperine. Peaks at Rf 0.718 and 0.786 were suggestive of 6-gingerol, whereas Rf 0.406 was likely attributable to zingerone. The peak at Rf 0.512 may indicate tryptophan from milk-purified.

6-Gingerol is phenolic compound of *Zingiber officinale* (ginger). It exhibits significant anti-inflammatory, antioxidant, and anticancer activities, including inhibition of angiogenesis and induction of cytotoxic effects in cancer cells[16]. l6-Shogaol is a bioactive compound formed by dehydration of 6-gingerol during ginger drying. It possesses anti-inflammatory, antioxidant, and anticancer properties by inducing apoptosis, suppressing inflammatory mediators, and inhibiting NF signalling[17]. Zingerone is a non-volatile bioactive compound of ginger with antioxidant, anti-inflammatory, antimicrobial, and anticancer properties. Piperine is the principal bioactive alkaloid of *Piper nigrum* (black pepper). It exhibits anti-inflammatory, antioxidant, anticancer and is a bioavailability enhancer[18].

CONCLUSION

The therapeutic identity of *Gandhaka Ghrita* and *Gandhaka Dhruti* appears to depend on the chemical and physicochemical environment created around *Gandhaka* through *Samskaras*. In *Gandhaka Ghrita,* the lipid-rich matrix, together with milk derived constituents, may provide a relatively nutritive and metabolically supportive environment[19]. The chemical profile of *Gandhaka Dhruti* is more suggestive of rapidly active, inflammation modulating and bioavailability- enhancing potential. Ginger derived phenolics influences oxidative and inflammatory signalling

while piperine may enhance the systemic availability of accompanying bioactives. The lipid vehicle may favour solubilization and membrane partitioning of lipophilic constituents, potentially influencing their absorption and tissue distribution. Thus, the difference in *Rasa, Guna, Virya* of the processing media and accompanying drugs together with heat and lipid mediated chemical transformation during *Samskaras*, may shift the functional profile of the final formulation. A more nutritive and sustaining profile in *Ghrita* and a more bioavailability, inflammation and metabolism-oriented profile in *Dhruti*.

HPTLC provides an analytical window into these differences, giving contemporary chemical support to the classical principle that the therapeutic behaviour of a *Dravya* can be transformed by its pharmaceutical processing and accompanying media. Hence the classical principle of selecting a formulation according to *Dosa* and Disease stage gains a measurable modern dimension through its chemical fingerprint, supporting pathology specific rather than formulation interchangeable use.

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