

Analytical Standardization and Safety Assessment of Gulabapaka: HPTLC Fingerprinting, Heavy Metal Profiling, Physicochemical Evaluation, and In Vitro Antibacterial Activity

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ABSTRACT

Background: In contemporary times, the quality, safety, and therapeutic efficacy of classical Ayurvedic formulations are being established by using modern techniques. Gulabapaka is a semi-solid classical herbo-mineral formulation which is composed of rose petals, herbal ingredients and processed mineral preparations. It is a traditional formulation indicated under Balya (strength promoter) and Hridya (cardio-protector). Nonetheless, there is a lack of extensive analytical validation of this formulation.

Aim: The Aim of the study is to analysis the HPTLC fingerprint of Gulabapaka, examine the physicochemical characteristics, the safety of heavy metal and in vitro antibacterial activity.

Materials and Methods: The formulations were prepared through classical ayurvedic procedures resembling rose-flavored gulabapaka and was evaluated organoleptically and physicochemically. The process of HPTLC fingerprinting involved the application of samples and standards on silica gel 60 F254 plates, employing toluene:ethyl acetate:formic acid in the ratio of 5:4:1 (v/v/v) as the mobile phase. Densitometric scanning was subsequently executed at wavelengths of 254 and 366 nm. Additionally, an approved laboratory Ministry of AYUSH executed heavy metal analysis of Pb, Cd, As and Hg and elemental profiling as per the Ayurvedic Pharmacopoeia of India. The cylinder plate method was used for the assessment of antibacterial activity against Staphylococcus aureus ATCC 6538 and Escherichia coli ATCC 8739.

Results: HPTLC analysis gave reproducible chromatographic fingerprints having 7-8 peaks at 254 nm and 6-7 peaks at 366 nm. Heavy metal concentrations remained within acceptable limits of API. The formulation showed antibacterial action against both test organisms.

Conclusion: The conclusion indicated reproducible chromatographic features with acceptable heavy metal safety and reasonable antibacterial activity of gulabapaka. Thus gulabapaka is a drug with great potential for quality standardization for medicinal use with further pharmacological studies of this marketable drug as its lacunae.

Keywords: Herbo-mineral formulation; Gulabapaka; HPTLC fingerprinting Report; Physicochemical standardization; Heavy metal analysis Report; Antibacterial activity Report; Safety evaluation Assay.

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INTRODUCTION

Ayurveda is the most popular complementary medicine

system worldwide and has a storehouse of single drugs and compound formulations that have been clinically proven over thousands of years.[1] Of these, the classical preparations containing the herbo-mineral ingredients are high level formulation wherein the interactions that take place due to processing technology between the organic and inorganic ingredients cause modification in their efficacy and bioavailability.[2] The Gulaabapaaka referred to in Ayurvedic classics like Bhaishajya Ratnavali, Chakradatta is a semi-solid confectionary preparation comprising chiefly of rose petals (*Rosa damascena/centifolia*), ghee, sugar, aromatic herbs and the processed mineral preparations Vanga Bhasma (tin) and Abhraka Bhasma (mica).[3] Traditionally, the formulation is indicated for Balya (strength and vitality promoting), Hridya (cardioprotective), Vrishya (aphrodisiac) and regulating the Rakta Dhatu (blood tissue).[4]

Most noteworthy - Bhasma: The bhasma possesses special properties and characteristics due to the special process of preparation involving incineration or calcination. These may entail nano-particulate form with exceptionally high surface area, crystalline and. Scientific characterization of Gulabapaka, although documented for traditional use and used in Ayurveda clinical practice, is poorly documented by validated modern analytical techniques. Bhasma-based herbo-mineral formulations need safety and quality assessment due to the presence of metal derived ingredients which when not prepared in conformance with the guidelines may result in heavy metal accumulation.[5,6] As per the Ministry of AYUSH, Government of India regulation, a formulation belonging to these categories must comply with the limit of heavy metal contaminants as per the API definition. As a result, safety profile should form part of any standardization study.[7]

High-Performance Thin Layer Chromatography (HPTLC) has become the analytical method of choice for fingerprinting various complex herbal and herbo-mineral matrices due to its high resolution, the ability to process multiple samples simultaneously, and the capacity for densitometric quantification as per specifications of pharmacopoeias.[8,9] When accompanied by screening for antibacterial bioactivity,

the analytical evaluation of Gulabapaka achieves significance from a regulatory and pharmacological perspective, especially in light of emerging evidence for the antibacterial activity of constituents from various *Rosa* species and Ayurvedic mineral preparations.[10,11]

In view of the above the present investigation was undertaken on Gulabapaka to generate a detailed analytical profile which would comprise of HPTLC-based chromatographic fingerprinting along with densitometric peak area analysis, physicochemical and preliminary phytochemical analysis, safety profiling for heavy metals as per API guidelines and in vitro assessment for antibacterial activity using standard micro-organisms.

2. AIM AND OBJECTIVES

The comprehensive analytical framework of Gulabapaka was designed and standardised in this study.

Objectives

1. To prepare the formulation using authenticated ingredients and classical procedures.
2. To analyse characterised for the organoleptic and physicochemical properties.
3. HPTLC fingerprint developed along with the densitometric profiling.
4. The heavy metal and elemental status evaluated with respect to API limits.
5. To assess in vitro antibacterial activity was evaluated using reference microbial strains, which will help in lessening the already present quality assurance problems.

3. MATERIALS AND METHODS

3.1 Procurement and Authentication of Raw Materials

The authenticated Ayurvedic pharmacies and local markets supplied the raw materials for preparation of Gulabapaka. Freshly picked *Rosa damascena / centrifolia* petals were botanically authenticated. Vanga Bhasma (incinerated tin) and Abhraka Bhasma (incinerated mica) were obtained from a GMP-certified Ayurvedic pharmaceutical manufacturer and were confirmed to have been prepared as per classical Shodhana (purification) and Marana (incineration) procedure. The Ingredient of Gulabpaka is presented in Table 1.

Table 1: Ingredient Composition of Gulabapaka

Sr. No.	Drug Name	Botanical Name	Family	Part Used
1	Gulaba	<i>Rosa centrifolia L.</i>	Rosaceae	Flower petals
2	Ela	<i>Elettaria cardamomum (L.) Maton</i>	Zingiberaceae	Seeds
3	Tamala patra	<i>Cinnamomum tamala (Buch.-Ham.) T.Nees & Eberm.</i>	Lauraceae	Leaves
4	Nagakesara	<i>Mesua ferrea L.</i>	Calophyllaceae	Stamens
5	Guduchi Sattva	<i>Tinospora cordifolia (Willd.) Hook.f. & Thomson</i>	Menispermaceae	Starch extract

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6	Lavanga	<i>Syzygium aromaticum (L.) Merr. & L.M.Perry</i>	Myrtaceae	Flower buds
7	Karpura	<i>Cinnamomum camphora (L.) J.Presl</i>	Lauraceae	Processed extract
8	Vanga Bhasma	- (<i>Incinerated tin</i>)	-	Mineral
9	Abhraka Bhasma	- (<i>Incinerated mica</i>)	-	Mineral
10	Sharkara	-	-	Crystalline sugar
11	Ghrita	-	-	Clarified butter
12	Kshaudra	-	-	Honey

3.2 Preparation of Gulabapaka

According to classical formulation as mentioned in the Ayurvedic pharmacopoeia texts, Gulabapaka was prepared. [4,12] Taken About one thousand fresh Gulaba petals were cleaned and weighed. After that, controlled heating was done with Ghrita until the attainment of Paka Siddhi Lakshana which is a sign of Gulabapaka. In this stage, gulabapaka got formation of semisolid mass which did not stick to the vessel and was consistent with thread formation. The next step involved adding sharkara (1 adhaka 1.92 kg) with continuous stirring for its uniform distribution achieved.

After sufficient cooling, powdered drug admixtures of Ela, Tamala patra, Nagakesara and Guduchi Sattva (each around 24-48 g as per classical text mentioned) were added. After that Lavanga and Karpura were added in a measured quantity and mixed properly followed by the addition of Vanga Bhasma and Abhraka Bhasma. Lastly, Kshaudra (honey, about 1 Prastha (768 mL) was used at room temperature in order to protect its thermolabile ingredients. The prepared formulation was tied in a clean and dry amber glass container at a room temperature.

3.3 Organoleptic Evaluation

Organoleptic characterisation was performed by trained sensory assessment of colour, odour, taste, texture, and overall appearance using standardised descriptors consistent with Ayurvedic pharmacopoeial guidelines.

In trained sensory assessment of colour, odour, taste, texture and overall appearance using the standardised method which are in tune with the Ayurvedic pharmacopoeia guidelines. Evaluation was carried out on the freshly prepared batch and repeated at 30-day intervals to assess stability.[7]

3.4 Physicochemical Analysis

Physicochemical parameters including Loss on Drying (LOD), Total Ash content, Acid Insoluble Ash (AIA), Water-Soluble Extractive Value (WSEV), Alcohol-Soluble Extractive Value (ASEV), and pH were determined in triplicate following standard procedures described in the API and Indian Pharmacopoeia 2018.[7,13] All results were expressed as mean $\pm$ standard deviation. LOD was determined at 110°C to constant weight; ash values were obtained by incineration in a muffle furnace at 550°C; extractive values were measured gravimetrically after exhaustive solvent extraction; and pH was determined at 10% w/v aqueous dispersion using a calibrated digital pH meter.

3.5 Preliminary Phytochemical Screening

A hydroalcoholic extract (70% ethanol, maceration 48 h) of *Gulabapaka* was prepared and used for systematic phytochemical screening. Detection of alkaloids was performed using Dragendorff's and Mayer's reagents; flavonoids were identified using the alkaline reagent test (NaOH) and Shinoda test; tannins were detected using ferric chloride solution;

cardiac glycosides were screened using the Keller-Kiliani and Borntrager's tests; saponins were assessed by the persistent froth test; terpenoids were identified using the Salkowski reaction with concentrated sulphuric acid; and phenolic compounds were detected using ferric chloride test. All tests were performed in triplicate and results recorded as positive '+' or negative '-'. [14]

3.6 HPTLC Fingerprinting

HPTLC fingerprinting was carried out at Vasu Research Centre, Vadodara (Ministry of AYUSH Approved Ayurvedic Testing Laboratory; License No. GATL/08), using a validated CAMAG HPTLC system. The analytical report was generated under AR No. VARS/RS/26/04/023 (Report Date: 23 March 2026).

Sample preparation: Accurately weighed *Gulabapaka* (1.0 g) was transferred to a 25 mL volumetric flask. Methanol (HPLC grade, 10 mL) was added and the mixture was sonicated for 15 minutes at ambient temperature using a calibrated ultrasonic bath. The resulting extract was filtered through Whatman No. 1 filter paper and the clear filtrate was used directly for application.

Chromatographic conditions: Pre-coated aluminium-backed silica gel 60 F254 plates (Merck, 100 × 100 mm) were used as the stationary phase. Sample volumes of 5.0, 10.0, 15.0, and 20.0 μ L were applied as spray bands (application position Y: 8.0 mm, band length: 8.0 mm) using a CAMAG Linomat 5 automatic sample applicator (Serial No.: 280008; dosage speed: 150 nL/s; pre-dosage volume: 0.20 μ L). The mobile phase was toluene:ethyl acetate:formic acid (5:4:1,v/v/v). Chromatographic development was performed in a twin-trough chamber pre-saturated with mobile phase vapours for 20 minutes using a saturation pad, allowing solvent migration to 80 mm. Post-development, plates were air-dried and subjected to densitometric scanning.

Detection and scanning: Plates were scanned using a CAMAG TLC Scanner 4 (Serial No.: 271118) at 254 nm (deuterium lamp) and 366 nm (mercury lamp). Scanning was performed in absorption-reflectance mode at a speed of 100 mm/s with a data resolution of 100 μ m/step and a slit dimension of 6 × 0.45 mm (micro). Data acquisition and peak integration were performed using CAMAG visionCATS HPTLC software (Version 3.2.23095.1).

Phytochemical identity note: No authenticated reference standard compounds were co-chromatographed in the present study. Consequently, direct phytochemical identification of individual chromatographic peaks is not claimed. Tentative classification of peaks into polar (low R_f), semi-polar (mid R_f), and non-polar (high R_f) fractions is proposed for comparative purposes only, pending confirmation by co-chromatography with authenticated reference standards and post-

chromatographic derivatisation.

3.7 Heavy Metal Analysis

Quantitative determination of heavy metal contaminants - Lead (Pb), Cadmium (Cd), Arsenic (As), and Mercury (Hg) - along with elemental profiling for Calcium, Magnesium, Sodium, Manganese, Copper, Zinc, Potassium, and Iron was performed at Vasu Research Centre, Vadodara (AYUSH-approved; License GATL/08; AR No. VARS/RS/26/04/023; Report Date: 30 April 2026). Analysis was conducted by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) following acid digestion. Results were compared against API permissible limits for heavy metals in Ayurvedic preparations.

3.8 In Vitro Antibacterial Activity

In vitro antibacterial screening was performed at Vasu Research Centre, Vadodara (AR No. VARS/RS/26/04/023; Report Date: 30 April 2026) following the Cylinder Plate (agar well diffusion) Method as per Indian Pharmacopoeia 2014, Chapter 2.2.10.15

Test microorganisms: Standardised reference strains of *Staphylococcus aureus* ATCC 6538 (Gram-positive) and *Escherichia coli* ATCC 8739 (Gram-negative) were used.

Culture preparation: Fresh slant cultures of both organisms were prepared and individually washed with 10 mL of sterile normal saline to obtain bacterial suspensions.

Media preparation: Mueller Hinton Agar (MHA; Hi-media; Lot No. 0000659664) was prepared as per instructions, autoclaved at 121 °C and 15 lbs pressure for 20 minutes and cooled to about 55 °C.

Sample preparation: About 5.0 grams of

Gulabapaka was weighed accurately and quantity transferred into a 150 mL volumetric flask. In the systems of mixed solvents Dimethyl sulfoxide (DMSO; 1 mL), methanol (5 mL), and distilled water was added (10 mL) and sonicated for ten minutes. The suspension was then refluxed on a water bath at 90°C for 1 hour, followed by filtration through Whatman filter paper, with the resulting filtrate being evaporated to 5 mL and used in test extract.

Procedure: It was carried out by adding 10 µL of respective bacterial suspension to pre-cooled MHA and pouring it in labelled plates. A sterile borer was used at equal distanced positions and uniform wells were created after solidification. The appropriately labeled wells received the solvent blank (MeOH:DMSO in equivalent proportions) and Gulabapaka extract. The plates were kept at 35 degrees Celsius for 24 hours after complete sample diffusion. The diameter of the zone of inhibition was taken in millimetres.

4. RESULTS

4.1 Organoleptic Evaluation

Gulabapaka has been characterized is dark Pink colour attributable to the pigments of processed rose petals. The smell was fragrant and largely due to the volatile components of Ela and Karpura. The taste was sweet with a slight astringency. The texture was smoothly semi-solid and homogenous without any visible particulate matter According to the pharmacopoeia, these organoleptic characteristics conform to their respective classical organoleptic descriptions that suggest proper pharmaceutical processing.

4.2 Physicochemical Analysis

Table 2: Physicochemical Parameters of Gulabapaka

Sr. No.	Parameter	Result (Mean ± SD)	API/IP Reference Limit
1	Loss on Drying (% w/w, 110°C)	11%	As per API for semi-solid preparations
2	Total Ash (% w/w)	6%	As per API
3	Acid Insoluble Ash (% w/w)	1%	As per API
4	Water-Soluble Extractive Value (% w/w)	38%	As per API
5	Alcohol-Soluble Extractive Value (% w/w)	20%	As per API
6	pH (10% w/v aqueous dispersion)	5	Not specified (reference range)
7	Water-soluble ash	3%	
8	Total sugar	48%	
9	Reducing Sugar	18%	

4.3 Preliminary Phytochemical Profile

Table 3: Preliminary Phytochemical Profile of the Hydroalcoholic Extract of Gulabapaka

Sr. No.	Phytoconstituent	Test Applied	Result
1	Alkaloids	Dragendorff's; Mayer's	+
2	Flavonoids	Alkaline reagent; Shinoda	+

3	Tannins	Ferric chloride	+
4	Cardiac Glycosides	Keller-Kiliani; Borntrager's	+
5	Saponins	Persistent froth test	+
6	Terpenoids	Salkowski	+
7	Phenolic compounds	Ferric chloride	+

Note: Present: '+' Absent: '-'

4.4 HPTLC Fingerprinting

Under HPTLC analysis, chromatographic fingerprints of Gulabapaka were consistent at 254 and 366 nm irrespective of application volume. Position of peaks were stable indicating good reproducibility. R_f values were obtained from CAMAG visionCATS software whereas the phytochemical identities were not assigned owing to non-availability of reference standards.

Table 4: R_f Values of Gulabapaka at 254 nm in HPTLC

Peak No.	5.0 µL	10.0 µL	15.0 µL	20.0 µL
1	0.087	0.097	0.089	0.089
2	0.129	0.138	0.149	0.149
3	0.178	0.181	-	0.319
4	0.326	0.318	0.374	0.444
5	0.451	0.440	0.497	0.524
6	-	0.526	0.592	0.592
7	-	0.842	0.843	0.826
8	-	0.885	0.935	0.871

Table 5: HPTLC R_f Values of Gulabapaka at 366 nm Across Application Volumes

Peak No.	5.0 µL	10.0 µL	15.0 µL	20.0 µL
1	0.086	0.096	0.120	0.120
2	0.129	0.138	0.160	0.170
3	0.326	0.319	0.370	0.370
4	0.496	0.474	0.500	0.500
5	-	0.526	0.520	0.520
6	-	0.622	0.670	0.670
7	-	-	0.830	0.830

Table 6: HPTLC Densitometric Peak Area Data for Gulabapaka at 254 nm (20.0 µL Track)

Peak No.	R _f Start	R _f Max	R _f End	Height %	Area %
1	0.056	0.089	0.128	6.36	4.49
2	0.132	0.149	0.167	1.29	0.40
3	0.197	0.319	0.371	8.52	12.71
4	0.374	0.444	0.497	27.74	31.08
5	0.497	0.524	0.592	13.15	11.60
6	0.769	0.826	0.843	19.34	16.27
7	0.844	0.871	0.935	23.61	23.45

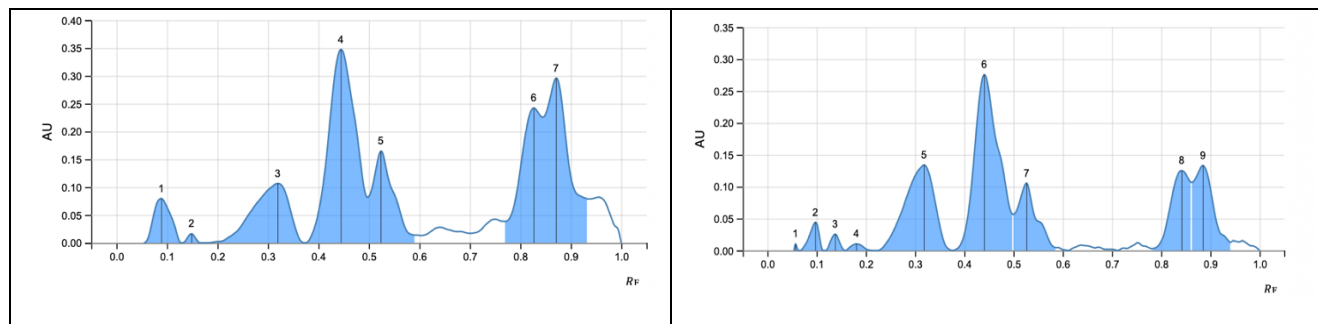


Figure 1: Representative HPTLC densitometric scan of Gulabapaka at 254 nm (20.0 μ L track) showing seven resolved peaks. Peaks six and seven (R_f 0.769-0.935) together constitute around 39.72% of the total chromatographic area. Thus, they are the major non-polar ingredients of the formulation.

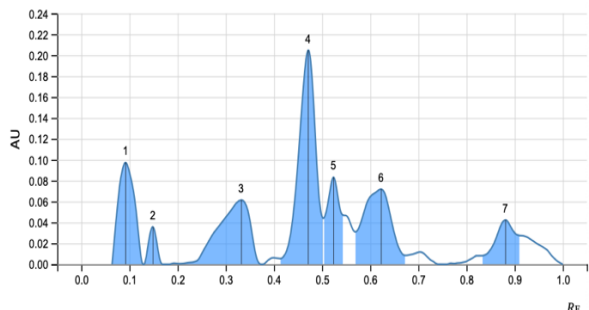


Figure 2: HPTLC densitometric scan of Gulabapaka at 254 nm (10.0 μ L track) indicating the presence of six resolved peaks which are found to possess consistent R_f positions of those of the track of 20.0 μ L.

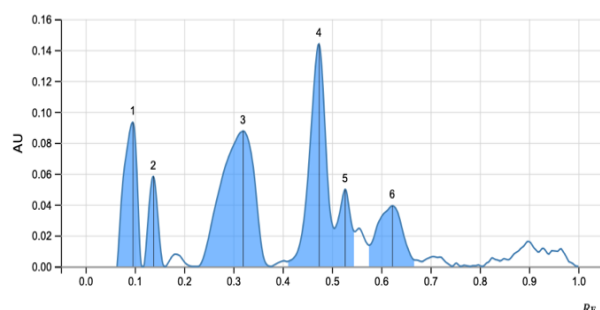


Figure 3: Densitometric scan of Gulabapaka at 366 nm (20.0 μ L track) showing seven peaks where peaks 3 (R_f ~0.370) and 4 (R_f ~0.500) are the main signals in the UV-fluorescent region

Figure 4: The HPTLC densitometric scan of Gulabapaka at 366 nm (10.0 μ L track) showing six fluorescent peaks in figure 4 confirms the reproducibility of chromatogram at lesser load.

4.5 Heavy Metal Analysis

Table 7. Heavy Metal Content and Compliance with API Limits in Gulabapaka

Sr. No.	Heavy Metal	Result (ppm)	API Limit (ppm)	Compliance Status
1	Lead (Pb)	4.176	NMT 10.0	COMPLIANT (41.8% of limit)
2	Cadmium (Cd)	0.016	NMT 0.3	COMPLIANT (5.3% of limit)
3	Arsenic (As)	0.103	NMT 3.0	COMPLIANT (3.4% of limit)
4	Mercury (Hg)	0.127	NMT 1.0	COMPLIANT (12.7% of limit)

Abbreviations: NMT, Not More Than; ppm, parts per million; API, *Ayurvedic Pharmacopoeia of India*. Heavy metal estimation was performed at the AYUSH-approved Vasu Research Centre, Vadodara (License No. GATL/08; AR No. VARS/RS/26/04/023).

Table 8: Elemental Profile of Gulabapaka

Sr. No.	Element	Result	Remarks
1	Calcium	Not Detected	Absence notable given Sharkara content
2	Magnesium	Not Detected	-
3	Sodium	Not Detected	-
4	Manganese	0.004%	Trace; not pharmacologically significant
5	Copper	0.0003%	Well within safe range
6	Zinc	0.010%	May relate to Vanga Bhasma composition
7	Potassium	Not Detected	-
8	Iron	Not Detected	Absence notable given Abhraka Bhasma

Gulabpaka		AR No.	VARs/RS/26/04/023
		Report Date	30.04.2026
Name of Scholar	Dr. Upasana Pawar, Parul Institute of Ayurved & Research, Vadodara		

CERTIFICATE OF ANALYSIS

Sr. No.	Parameters	Result	Limit as per API
HEAVY METAL ANALYSIS			
1	Lead	4.176 ppm	NMT 10 ppm
2	Cadmium	0.016 ppm	NMT 0.3 ppm
3	Arsenic	0.103 ppm	NMT 3 ppm
4	Mercury	0.127 ppm	NMT 1 ppm
ELEMENTAL ANALYSIS			
1	Calcium	Not Detected	NA
2	Magnesium	Not Detected	NA
3	Sodium	Not Detected	NA
4	Manganese	0.004 %	NA
5	Copper	0.0003 %	NA
6	Zinc	0.010 %	NA
7	Potassium	Not Detected	NA
8	Iron	Not Detected	NA
Key-Word: API – Ayurvedic Pharmacopoeia of India, ppm - Parts per millions, NA – Not Applicable.			

Figure 5. Certificate of heavy metal and elemental analysis of Gulabapaka obtained from Vasu Research Centre, Vadodara (AR No. VARs/RS/26/04/023), demonstrating compliance with Ayurvedic Pharmacopoeia of India permissible limits.

4.6 In Vitro Antibacterial Activity

Table 9. Antibacterial Activity of Gulabapaka Evaluated by the Cylinder Plate Method

Sr. No.	Sample	ZOI vs <i>S. aureus</i> (mm)	ZOI vs <i>E. coli</i> (mm)	Inference
1	Blank (MeOH:DMSO solvent control)	NZI	NZI	No solvent activity
2	Gulabapaka extract (5 g/5 mL equivalent)	17 mm	9 mm	Good; Moderate

ZOI: Zone of Inhibition; NZI: No Zone of Inhibition; *S. aureus* ATCC 6538; *E. coli* ATCC 8739. Method: Cylinder Plate Method per Indian Pharmacopoeia 2014, Chapter 2.2.10. Incubation: Mueller Hinton Agar, 35°C, 24 h.

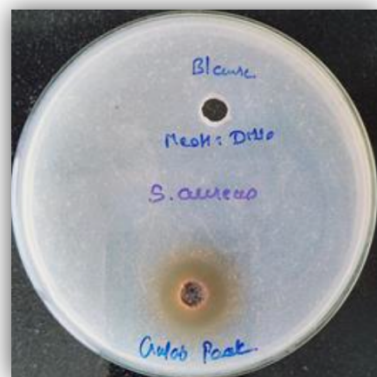
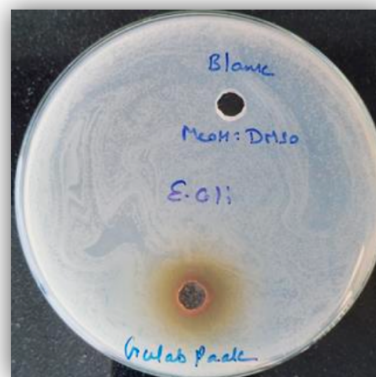
Observations:***S.aureus******E.coli***

Figure 6. Representative antibacterial activity of Gulabapaka against *Staphylococcus aureus* ATCC 6538 and *Escherichia coli* ATCC 8739 determined by the Cylinder Plate Method. Gulabapaka exhibited zones of inhibition of 17 mm and 9 mm against *S. aureus* and *E. coli*, respectively, while the solvent control (MeOH:DMSO) showed no inhibitory activity.

5. DISCUSSION**5.1 Physicochemical and Phytochemical Findings**

The prepared Gulabapaka showed the organoleptic characteristics like Dark pink colour, aromatic odour, and semisolid consistency almost similar to classical pharmacopoeial description. Paka Siddhi stage is completed successfully along with proper pharmaceutical processing as indicated by these properties.

The assessment of the physicochemical parameters is essential to establishing batch-to-batch reproducibility and detection of adulteration/degradation of complex semisolid Ayurvedic preparations. Physicochemical characterization offers baseline information for future stability studies for herbo-mineral formulations. Analysis of the formulation obtained in the present study gave a Total Ash value of 6 and Acid-Insoluble Ash value of 1. The values are higher than the standard limits usually prescribed for purely botanical semi-solid preparations; however, they are scientifically proven and expected for this composition. Gulabapaka contains Vanga Bhasma and Abhraka Bhasma. The natural mineral composition of these bhasmas will essentially increase the residual ash content after incineration thus it serves as an analytical proof for the successful cementation of these substances. The hydroalcoholic extract was confirmed to contain flavonoids, tannins, glycosides, saponins, terpenoids, phenolic compounds and alkaloids on prephytochemical screening. It is well known that this assortment of constituent classes corresponds well to Rose's known chemical substance classes (phytochemicals), exemplified by the presence of flavonoids like quercetin and kaempferol derivatives, tannins, anthocyanins and volatile terpenoids. Moreover, the confirmation of presence

of alkaloids indicates the co-extraction of the complex multi-ingredient herbal profile probably due to particular herbal admixtures like Guduchi Sattva, Nagakesara and Tamala patra used in the preparation.

The finding of saponins and glycosides supports earlier claims of balya and hridya activities, as the compounds with these activities are known to have cardioprotective and adaptogenic properties in experimental animals.[19,20]

5.2 HPTLC Fingerprinting

The HPTLC fingerprint of Gulabapaka exhibited similar profiles with a multi-peak response at both detection wavelengths for four application volumes. The reliability of R_f positions in different tracks with varying sample loads is a quality attribute in HPTLC fingerprinting since it indicates reproducibility of the chromatogram irrespective of concentration.[8,9] The reproducibility which we observed in the study with peak to peak R_f variation mostly within limit 0.02 in the various tracks confirms reliability of this fingerprints to act as identity marker for Gulabapaka.

At the wavelength of 254 nm, seven to eight peaks were recorded, which were chromatographically resolved. The major chromatographic contribution at this wavelength originates from two high R_f peaks (R_f 0.769 - 0.935) which together accounted for about 39.72% of total peak area in the 20.0 µL track. The wide spread of R_f (low-R_f polar region: Peaks 1-3; mid-R_f semi-polar region: Peaks 4-6; high-R_f non-polar region: Peaks 7-8) indicates the presence of phytoconstituents of a wide polarity range, which is in accordance with its multi-ingredient botanical and mineral composition.[21] The 10.0 µL track at 254 nm gave rise to six resolved peaks with their R_f values directly matching with those obtained from the 20.0 µL track, thereby confirming the reliability of the chromatographic method.

At 366 nm, we can see six to seven distinct peaks. The major fluorescent signals appeared as peaks in the mid-Rf region. The presence of peaks at higher application volumes (15.0 μ L and 20.0 μ L) at 366 nm, particularly in the high-Rf region (Rf 0.830), suggests that there are fluorescent compounds present in low concentrations. These compounds can only be detected at high levels of application volume which can play an important role.

It is relevant to mention that the presented data are not based on co-chromatography with any reference standard nor do they use any post-chromatographic derivatisation reagents. As a result, it is not possible to assign phytochemical classes to individual peaks, for instance, Rf zones related to flavonoids versus coumarins versus terpenoids. The positions and area contributions of chromatographic peaks can be described in the same way. Future research will also benefit from reconciling results with authenticated reference standards, derivatisation with NP/PEG reagent (for flavonoids) or Anisaldehyde-sulphuric acid (for terpenoids) to allow for definitive constituent assignment and expand the utility of this fingerprint.[22]

5.3 Heavy Metal Safety Profile

The examination of the heavy metals of Gulabapaka found all four protected metals to be within limits as per API. The whole compliance profile is especially important as it is a herbo-mineral formula containing Vanga Bhasma and Abhraka Bhasma, which are two Bhasma preparations that have a recognised safety profile regarding heavy metals with regulatory agencies and scientific scrutiny.[5,6,23]

The relative metal burden of Lead at 4.176 ppm was the highest among the four metals studied and represented 41.8% of the API permissible limit. Though this figure is entirely compliant with regulatory standards, it does emphasize the need for contextual consideration in scenarios that expose patients to high doses or long time periods. Lead contamination in ayurvedic preparations is thought to arise from environmental agricultural accumulation in their botanical raw materials, as well as their processing equipment, and sometimes their own Bhasma preparations due to improper incinerations.[5,24] Multi-batch validation studies would help in assessing if Lead levels in this formulation is of consistent batch and not variable-source contamination.

The four major heavy metal contaminants in traditional preparations include cadmium, arsenic, and mercury. All three were present in trace amounts far below their permissible limits. Specifically, cadmium was found at 0.016 ppm (5.3% of limit), arsenic at 0.103 ppm (3.4% of limit) and mercury at 0.127 ppm (12.7% of limit).

An elemental profiling showed the presence of Manganese (0.004%), Copper (0.0003%), and Zinc (0.010%). The presence of Zinc is not surprising and it may be due to Vanga Bhasma. In the past, some

Bhasmas made of tin have been found along with trace amounts of Zinc. It is surprising not to find Iron, which is an important part of a mica, Abhraka Bhasma. It can be an indication of the effectiveness of the processing conditions to bring about a complete inorganic transformation. Alternatively, it is also possible that the reason is the sensitivity of the method used for analysis. The absence of detectable Calcium, despite the presence of *Sharkara*, suggests that Calcium may be present below the detection limit or in a form not amenable to the extraction protocol used. These elemental findings are of interest for further mechanistic characterisation of the Bhasma transformation process.

5.4 In Vitro Antibacterial Activity

The in vitro antibacterial evaluation of *Gulabapaka* revealed meaningful inhibitory activity against both test organisms. A zone of inhibition of 17 mm against *S. aureus* ATCC 6538 indicates good antibacterial activity, while a 9 mm zone against *E. coli* ATCC 8739 represents moderate activity. The absence of inhibitory activity by the solvent blank (MeOH:DMSO) confirms that the observed antibacterial effects are attributable to constituents of the formulation rather than to the extraction solvent.

The differential susceptibility pattern - with stronger activity against the Gram-positive *S. aureus* than the Gram-negative *E. coli* - is consistent with patterns documented for *Rosa* species-derived preparations, where phenolic compounds and flavonoids demonstrate preferential activity against Gram-positive organisms.[25] The structural barrier of the outer membrane lipopolysaccharide layer in Gram-negative bacteria such as *E. coli* is widely recognised as conferring greater intrinsic resistance to hydrophilic phenolic antimicrobials.[26] The observed activity against *E. coli*, while moderate, may reflect the contribution of non-polar constituents (potentially terpenoids or volatile oils from *Ela*, *Lavanga*, and *Karpura*) that exhibit broader-spectrum activity.

It must be emphasised that the Cylinder Plate Method employed is a qualitative in vitro screening assay and that the zones of inhibition reported do not in themselves establish clinical therapeutic efficacy. To make therapeutic antibacterial claims, it would be essential to determine minimum inhibitory concentration (MIC) values, conduct time-kill assays, and in vivo infection model studies. The present data strongly demonstrate a meaningful preliminary bioactivity signal. And, it provides scientific basis for the traditional use of *Gulabapaka* in inflammatory and microbial aetiology.

5.5 Integrative Perspective

The analysis of *gulabapaka* has a sound scientific basis with a cognition incorporating several constituent multi-functional pharmacological actions. The wide-ranging HPTLC fingerprint

collaboration with multiple phytochemical classes known for their antioxidant, anti-inflammatory and antimicrobial activities, as well as proven in vitro antibacterial activity and confirmation of heavy metal safety, may lend credence to the contemporary relevance of this classical formulation.[27]

The classic texts in Ayurvedic pharmaceutical science recognize the importance of Samskara (pharmaceutical processing) in influencing the bioavailability and synergistic efficacy of multi-ingredient formulations. The herbal and Bhasma elements made in Gulabapaka are consistent with the classical principle of enhancing delivery through anupana (incorporation of mineral preparations to alter absorption and enhance therapeutic effect at the tissue level).[4,12] The current analytical data can be utilized for pharmacokinetic and clinical validation studies.

6. CONCLUSION

The study presents multi-parametric analytical profile of a classical ayurvedic herbo-mineral formulation Gulabapaka. The profile is generated with the help of HPTLC fingerprinting, physicochemical analysis, heavy metal safety profiling and in vitro antibacterial assay. The analysis of HPTLC afforded a well-resolved and reproducible chromatographic fingerprint at 254 nm 366 nm. The track 20.0 μ L showed the presence of seven distinct peaks at 254 nm. The peaks 6-7 at high R_f (R_f 0.769-0.935) have contributed approximately 39.72% of the area of the total. Peak-maximum R_f values from visionCATS chromatographic data will be used in which phytochemical identities will be assigned. Pending Co-Chromatography with reference standards.

The formulation is considered safe for human consumption at classical therapeutic doses and the heavy metal analysis has confirmed it complies with all permissible limits as prescribed by API.

The antibacterial assay demonstrated inhibitory activity against both test microorganisms. *Gulabapaka* produced inhibition zones of 17 mm against *Staphylococcus aureus* ATCC 6538 and 9 mm against *Escherichia coli* ATCC 8739, indicating stronger activity against the Gram-positive organism.

The established analytical fingerprint, heavy metal singularity, and bioactivity data all together would serve as a reproducible quality mark for Gulabapaka, which may be used for regulatory, research, and clinical quality assurance. Upcoming studies featuring the determination of the MIC, co-chromatography with the reference standard, multi-batch reproducibility assessment, and in vivo pharmacological validation will strengthen the evidentiary basis of this formulation.

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CONFLICT OF INTEREST

The author declares no conflict of interest.

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