

Physicochemical and Phytochemical Profiling with Antimicrobial Activity of Selected Kushtaghna Mahakashaya Drugs

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ABSTRACT

Objectives: To do the profiling of Physical, Phytochemical parameters and to assess the antimicrobial activity of selected *kushtaghna mahakashaya* drugs. **Materials and Methods:** Raw drugs were authenticated and subjected to physicochemical, phytochemical, and High-Performance Thin-Layer Chromatography (HPTLC) analysis using standard procedures. Antimicrobial activity of the aqueous decoction was assessed against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans* using broth microdilution in 96-well plates to determine Minimum Inhibitory Concentration (MIC). **Results:** The kwatha churna showed characteristic physicochemical, phytochemicals and HPTLC markers consistent with its herbal components. It demonstrated significant antimicrobial activity, effectively inhibiting *S. aureus* and *C. albicans* at 1.60% (vol/vol) and *P. aeruginosa* at 3.13% (vol/vol), indicating promising therapeutic potential. **Conclusion:** The kwatha churna demonstrated acceptable physicochemical quality and a rich phytochemical profile, including flavonoids and tannins relevant for skin health. HPTLC profiling confirmed key marker compounds, while the aqueous extract exhibited notable antimicrobial activity against *S. aureus* and *C. albicans*. These findings substantiate its traditional use and highlight its potential for dermatologic and antimicrobial applications.

Keywords: Antimicrobial activity, HPTLC, MIC, Physicochemical Evaluation, Phytochemical Analysis.

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INTRODUCTION

The skin, the body's outermost layer, serves as a protective barrier for underlying tissues and organs. Skin disorders account for approximately 1.79% of all diseases worldwide (Deshmukh and Kshirsagar, 2024). Multiple factors contribute to the development of these conditions, often categorized under the term "*Kushta*" in Ayurvedic literature, which may result from environmental changes and lifestyle factors, such as altered work schedules that increase stress. Traditionally, a group of ten medicinal plants, known as *Kushthaghna Mahakashaya*, has been used to treat skin ailments. These herbs are believed to possess anti-inflammatory, immunomodulatory, and antioxidant properties that can help manage skin disorders (Saini *et al.*, 2022). From this group, five plants, *Khadira* (*Acacia catechu* Willd), *Haritaki* (*Terminalia*

chebula Retz), *Amalaki* (*Emblica officinalis* Gaertn.), *Haridra* (*Curcuma longa* Linn.) and *Vidanga* (*Embelia ribes*)-were selected based on their high therapeutic utility. These plants were evaluated for their physical and phytochemical properties, as well as the antimicrobial activity of their combined preparation, Kwatha Churna (powder).

MATERIALS AND METHODS

Raw drugs of *Khadira* (*Acacia catechu* Willd), *Haritaki* (*Terminalia chebula* Retz) Fruit, *Amalaki* (*E. officinalis* Gaertn.) Fruit, *Haridra* (*C. longa* Linn.) Rhizome, *Vidanga* (*Embelia ribes*) seed were collected from KLE GMP Certified Pharmacy, Belagavi, Karnataka. The authentication and identification of raw drugs were done at the AYUSH-Approved ASU Drug Testing Laboratory, Belagavi. Dry drugs were used to study the physical, phytochemical parameters, and antimicrobial activity (Patel *et al.*, 2019).

Ethical Statement

This *in vitro* study did not involve human or animal subjects; thus, ethical clearance was not required.



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Statistical Analysis

The obtained data were analyzed descriptively based on physicochemical, phytochemical, High-Performance Thin-Layer Chromatography (HPTLC), and antimicrobial observations. Results were presented in tabular and graphical form wherever applicable. No advanced statistical analysis was performed, as the study primarily focused on qualitative and observational evaluation of the selected Kushtaghna Mahakashaya drugs.

Reagents and Instruments

Every chemical reagent and solvent utilized in the investigation was of analytic quality. watch glass, microscopic slide, Simple microscope, compound microscope, flask shaker, sonicator, hot-water bath, muffle furnace and other common glassware were used in this experiment. Chemical reagents like Dragendorff's, Millon's, and Benedict's were used for physicochemical studies

Macroscopy

Organoleptic characters like color, texture, form, and odor were assessed by observing the dried raw drugs.

Microbial Load

The microbial load of *Khadira* (*Acacia catechu* Willd.), *Haritaki* (*Terminalia chebula* Retz) fruit, *Amalaki* (*E. officinalis* Gaertn.), *Haridra* (*C. longa* Linn.), and *Vidanga* (*Embelia ribes*) was evaluated using Nutrient Agar, MacConkey Agar, Sabouraud dextrose Agar, Soybean Casein Digest Agar, Mannitol Salt Agar, cetrimide Agar, Xylose Lysine Deoxycholate Agar (XLD), buffer, and Soybean Casein Digest Medium broth at the Microbiology Laboratory of the AYUSH-Approved ASU Drug Testing Laboratory, Belagavi.

Physicochemical Testing

Physicochemical characteristics, including foreign organic matter, total ash, acid-insoluble ash, alcohol-and water-soluble extractives, and loss on drying, were measured using established techniques. All of these quantitative experiments employed air-dried Fine powder (40 #) samples. Using ethanol and water, extractive values were calculated.

Phytochemical Assessment

Standard protocols were used for phytochemical screening. Alkaloids, glycosides, phenols, flavonoids, and other phytoconstituents were detected using chemical assays on the alcohol-soluble floral extract, whereas proteins, carbohydrates, and saponins were detected using an aqueous extract (Khandelwal, 2013).

HPTLC Analysis

Chemical and reagents

The HPTLC plate used measured 4×10 cm and was precoated with silica gel 60 F254 TLC (0.2-mm thickness) on an aluminum sheet support (E. Merck). A sample application was performed using a CAMAG Linomat V Automatic Sample Spotter (Camag, Muttenz, Switzerland) with a 100-μL Hamilton syringe. The plate was developed in a CAMAG glass twin-trough chamber (10×10 cm), and densitometric analysis was carried out using a CAMAG TLC Scanner 3 connected to WINCATS software. The mobile phase consisted of chloroform: ethyl acetate: formic acid (5:4:1).

Preparation of Test Sample

1 g of the sample was weighed. For the aqueous extract, the extract was initially prepared with 5 mL of water, and the solution was evaporated to dryness. The residue was then further extracted with 5 mL of methanol. For the alcoholic extract, the sample was extracted with 5 mL of ethanol, evaporated to dryness, and the residue was subsequently extracted with 5 mL of methanol. The resulting methanol solution was used for HPTLC. 5 μL of the extract was applied to the HPTLC plate and developed to a distance of 8 cm using a solvent system of chloroform: ethyl acetate: formic acid (5:4:1). After development, the plate was air-dried and observed under UV light at 254 and 366 nm.

Method

The plate was developed in a twin-trough chamber that had been presaturated with the solvent system for 30 min. The syringe was rinsed twice with methanol before use. After development, the plate was dried and placed in the scanner. A new file was opened, and all scanning, integration, and spectral parameters were set. For the absorption-reflection mode, the plate was scanned at 425 nm using a tungsten lamp. Each track was scanned individually, and the UV spectrum of every track was recorded. The fingerprint profile of each track was obtained, and the UV spectral spots were compared using the spectrum display.

Antimicrobial Study

Kashaya (decoction) of compound drugs – comprising *Haridra*, *Amalaki*, *Vidanga*, *Haritaki*, and *Khadira* was prepared from coarse powder (40#). 2g of each coarse powder was added to 160 ml of purified water and allowed to soak for 30 mins. The mixture was then boiled on medium flame until the part, that is, 20 mL. After the reduction, the decoction was allowed to cool and filtered. volume reduced to 1/8 th of its initial volume (20ml), after which it was filtered.

Microorganisms and Media

The sensitivity test was carried out against Gram-negative and fungal species, namely *Staphylococcus aureus*, *Pseudomonas*

aeruginosa, and *Candida albicans*, respectively. In 10 different concentrations.

Serial dilutions of the test compounds were prepared in different rows of sterile 96-well microplates and were exposed to fixed concentrations of standardized suspension of the test organism overnight. If the test compounds were able to inhibit the bacterial growth, then the solution in the test wells would remain clear in the respective dilutions. If there were no bacterial growth inhibition, then the growth medium in the test wells would be turbid. The last well in each row that shows no growth (remains clear) is considered the Minimum Inhibitory Concentration (MIC) value of the test product against the targeted organism. This could be confirmed by the addition of an indicator dye such as resazurin sodium.

RESULTS

Macroscopic Description

The form was kwatha churna, the color was brownish yellow, and the odor was aromatic.

Physicochemical Studies

Moisture content at 110°C was found to be 9.8 and 0.52%, respectively. The total ash and acid-insoluble ash values were found to be 4.3 and 0.61%, respectively. The water-soluble extractives and alcohol soluble extractives values were found to be 17.9 and 20.6%, respectively. Physicochemical studies results are shown in Table 1.

Phytochemical Evaluations

It has been demonstrated that the alcoholic extract contains reducing sugar, monosaccharides, proteins, flavonoids, and tannins, whereas the aqueous extract contains carbohydrates, monosaccharides, reducing sugars, and proteins. Table 2 displays preliminary phytochemical results.

HPTLC Analysis

HPTLC profiling of the aqueous and alcoholic extracts revealed the presence of multiple phytoconstituents with distinct banding patterns at different R_f values. The chromatographic patterns observed under UV 254 and 366 nm demonstrated characteristic fingerprints for the extracts. The HPTLC chromatograms

Table 1: Physicochemical parameters.

Parameters	Values (in%)
Moisture content	9.8
Foreign matter	0.52
Total ash	4.3
Acid insoluble ash	0.6
Water soluble extractives	17.9
Alcohol soluble extractives	20.6

recorded at 254 and 366 nm are presented in Figures 3 and 4, respectively. The corresponding R_f values and peak details obtained from HPTLC analysis are summarized in Tables 3 and 4 for the aqueous and alcoholic extracts, respectively. Densitometric analysis further showed multiple peaks corresponding to different chemical constituents, confirming the phytochemical complexity of the extracts. The densitograms of the aqueous and alcoholic extracts are shown in Figures 1-4, respectively.

Antimicrobial Activity (MIC Determination)

The minimum inhibitory concentrations of the Kashaya (Aqueous extract) were evaluated against Gram-positive, Gram-negative and fungus. The extract exhibited greater potency, showing inhibition of *S. aureus* at 1.60% (vol/vol), *P. aeruginosa* at 3.13% (vol/vol) and *C. albicans* at 1.60% (vol/vol). *P. aeruginosa* (Gram-negative) remains relatively less susceptible. Results are shown in Table 5.

DISCUSSION

The physicochemical evaluation of the churna reveals parameters that collectively affirm its quality, purity, and suitability for medicinal use. The moisture content of 9.8% is within acceptable limits for powdered herbal formulations, minimizing the risk of microbial growth and ensuring stability during storage. Excessive moisture can promote microbial contamination and degradation of active constituents; thus, the observed value suggests good handling and drying practices during preparation. The foreign matter content was found to be 0.52%, indicating minimal contamination with extraneous materials such as soil, sand, or plant debris. This low value reflects adherence to proper collection, cleaning, and processing protocols, aligning with pharmacopeial standards for crude drug materials. The total ash content was determined to be 4.3%, reflecting the total mineral residue remaining after incineration. This value encompasses both physiological ash (natural plant mineral content) and any extraneous inorganic matter. The relatively moderate ash

Table 2: Test for preliminary phytochemical analysis.

Test for	Water extract	Alcohol extract
Carbohydrates	Positive	Negative
Reducing sugar	Positive	Positive
Monosaccharides	Positive	Positive
Pentose sugar	Negative	Negative
Nonreducing sugar	Negative	Negative
Hexose sugar	Negative	Negative
Proteins	Positive	Negative
Amino acids	Negative	Negative
Steroids	Negative	Negative
Flavonoids	Positive	Positive
Alkaloids	Negative	Negative
Tannins	Positive	Positive

level suggests acceptable purity and minimal adulteration. The acid-insoluble ash value of 0.6% further confirms the low presence of siliceous matter such as sand or silica, which is particularly significant in assessing sample cleanliness and proper processing. High acid-insoluble ash would indicate contamination with insoluble minerals, but the current low value signifies good quality control (Government of India, 1990-2008). The phytochemical screening of the compound kwatha churna revealed the presence of flavonoids, tannins, reducing sugars, monosaccharides, and proteins, many of which are pharmacologically relevant to skin health. Flavonoids and tannins, present in both aqueous and alcoholic extracts, contribute antioxidant, anti-inflammatory, and antimicrobial effects that support their use in managing skin conditions (Kumar and Pandey, 2013; Trease and Evans, 2009). The presence of simple sugars and proteins may further enhance hydration and skin barrier function. These findings support the therapeutic potential of the formulation in dermatologic applications.

The formulation was analyzed through both aqueous and alcoholic extracts, revealing distinct chemical signatures. The alcoholic

extract exhibited seven peaks, including a prominent peak at R_f 0.93 (18.26% area) (Ansari *et al.*, 2005), which is suggestive of curcumin, the principal bioactive of *C. longa*. Curcumin has been shown to possess potent anti-inflammatory, antioxidant, and antimicrobial effects, supporting its widespread use in acne, eczema, psoriasis, and wound healing (Vijayakaran *et al.*, 2025).

A shared peak near R_f 0.51 in aqueous extracts indicates the presence of ellagic acid, commonly found in *E. officinalis* and *T. chebula*. These compounds exhibit antioxidant, photoprotective, and melanogenesis-inhibiting activities, making them beneficial for skin brightening and antiaging effects (Aggarwal *et al.*, 2007; Naik *et al.*, 2003). The strong antioxidant capacity of Amalaki, particularly due to ascorbic acid and polyphenols, enhances collagen biosynthesis, a crucial factor in maintaining skin elasticity and preventing premature aging (Sharma *et al.*, 2009).

The peak at $R_f 0.62 \pm 0.02$ (1.09% area) in aqueous extract and a may represent embelin, the key active of *Embelia ribes*. (KPR Vijayan *et al.*, 2021). Embelin possesses broad-spectrum antimicrobial

Table 3: Shows High-Performance Thin-Layer Chromatography (HPTLC) Result of HAVAK Alcoholic extract.

Peak	Start R_f	Start height	Max R_f	Max height	Max %	End R_f	End height	Area	Area %
1	-0.07	1.2	-0.03	587.5	60.62	0.02	37.3	14,559.1	55.00
2	0.02	37.4	0.05	73.2	7.56	0.08	1.7	1,970.6	7.44
3	0.39	2.7	0.43	59.1	6.10	0.48	0.1	1,621.4	6.13
4	0.49	0.5	0.51	18.7	1.93	0.54	1.0	357.2	1.35
5	0.62	2.8	0.65	13.2	1.36	0.68	1.8	289.6	1.09
6	0.75	2.4	0.80	54.9	5.66	0.85	35.5	2,838.6	10.72
7	0.89	29.4	0.93	162.7	16.78	0.96	0.6	4,834.1	18.26

Track 2, ID: Havak (Alcoholic extract)

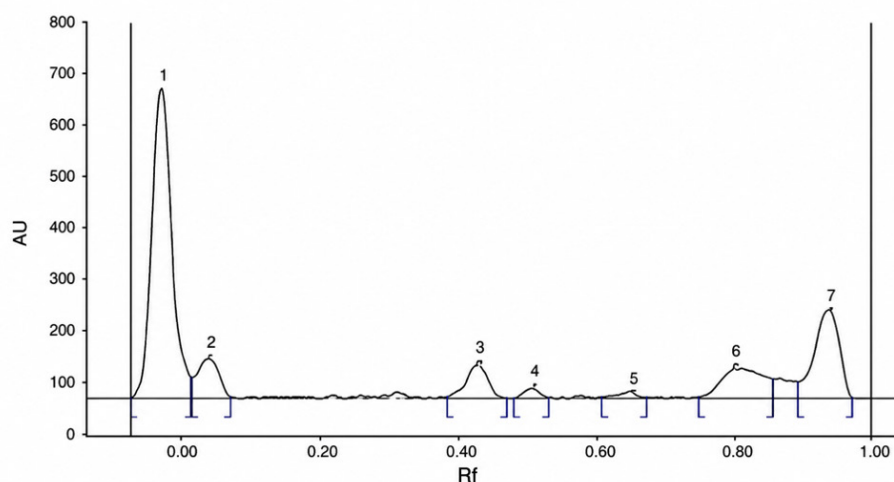


Figure 1: High-Performance Thin-Layer Chromatography (HPTLC) densitogram of Havak (alcoholic extract) showing the chromatographic peaks obtained after development in the solvent system chloroform: ethyl acetate: formic acid (5:4:1) and scanning at 425 nm. Multiple peaks indicate the presence of different phytochemical constituents in the extract.

Track 1, ID: Havak (aqueous extract)

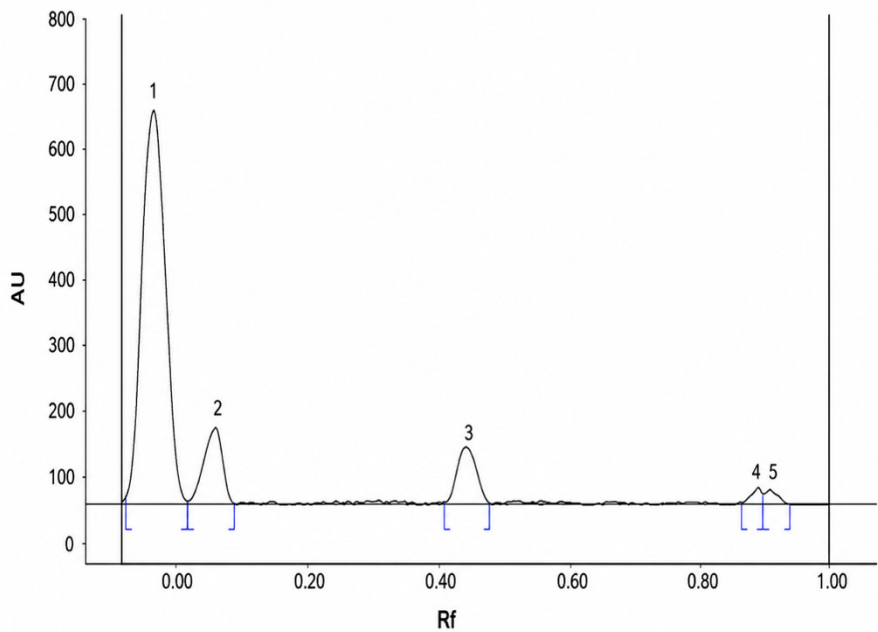


Figure 2: High-Performance Thin-Layer Chromatography (HPTLC) densitogram of Havak (aqueous extract) showing chromatographic peaks obtained after development in the solvent system chloroform: ethyl acetate: formic acid (5:4:1) and scanning at 425 nm, indicating the presence of different phytochemical constituents.

Table 4: Shows High-Performance Thin-Layer Chromatography (HPTLC) Result of HAVAK Aqueous extract.

Peak	Start R _f	Start height	Max R _f	Max height	Max %	End R _f	End height	Area	Area %
1	-0.06	27.2	-0.02	565.0	72.28	0.02	33.7	1,589.0	74.90
2	0.02	33.9	0.05	98.8	12.64	0.08	2.2	2,447.0	11.83
3	0.40	3.5	0.44	78.8	10.08	0.47	6.1	2,043.5	9.88
4	0.88	3.0	0.90	19.9	2.54	0.91	18.7	330.1	1.60
5	0.91	18.7	0.92	19.1	2.45	0.95	0.1	370.1	1.79

Table 5: Antimicrobial results of Kashaya.

Kashaya												
	50%	25%	12.50%	6.25%	3.13%	1.60%	0.20%	0.10%	0.05%	0.02%	NC	PC
<i>Staphylococcus aureus</i>	S	S	S	S	S	S	R	R	R	R	NG	G+
<i>P. aeruginosa</i>	S	S	S	S	S	R	R	R	R	R	NG	G+
<i>Candida albicans</i>	S	S	S	S	S	S	R	R	R	R	NG	G+

S: Sensitivity, R: resistance, NC: negative control, PC: positive control, NG: no Growth, G+: growth. Note. cannot decrease the Table numbers as each Table contains specific results. HPTLC.

and antifungal activity, contributing to the formulation’s role as a krimighna (antimicrobial) agent (Scartezzini and Speroni, 2000). in Ayurvedic dermatology. (Sharma V *et al.*, 2022)

Acacia catechu, represented likely by peaks around R_f 0.49 and 0.54, contains quercetin and catechin (Srivatsav *et al.*, 2024), which demonstrate anti-inflammatory and antioxidant effects. These flavonoids also enhance microvascular protection and inhibit

matrix metalloproteinases, both critical in the prevention of dermal degradation in inflammatory and aging skin conditions (Poojari *et al.*, 2013).

The Kashaya showed concentration-dependent antimicrobial activity against *S. aureus*, *P. aeruginosa*, and *C. albicans*. Higher concentrations (50-3.13%) exhibited significant antimicrobial effects, whereas lower concentrations showed resistance,

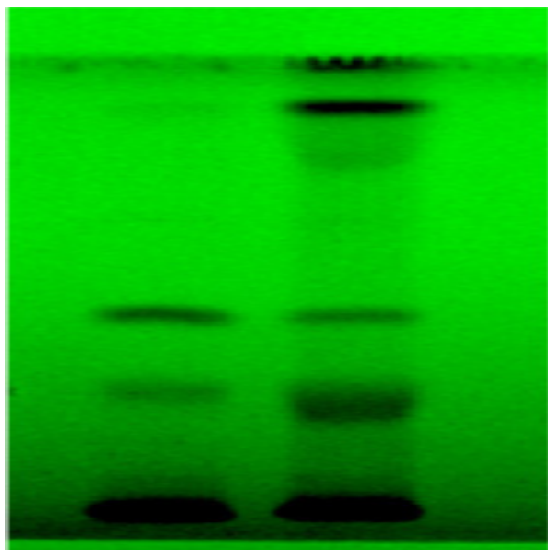


Figure 3: High-Performance Thin-Layer Chromatography (HPTLC) chromatogram of the aqueous showing distinct fluorescent bands under UV illumination, indicating the presence of multiple phytoconstituents. Prominent bands observed at different R_f values suggest the occurrence of characteristic marker compounds, confirming the chemical complexity and phytochemical richness of the formulation.

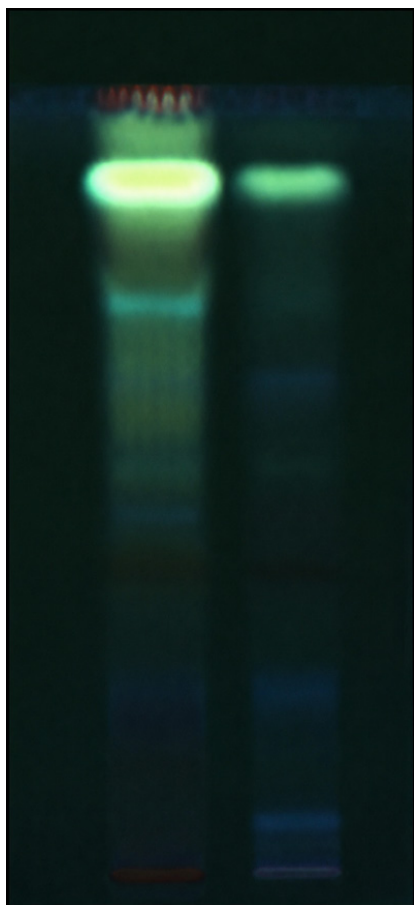


Figure 4: High-Performance Thin-Layer Chromatography (HPTLC) fingerprint profile of the extract observed under UV light, showing characteristic fluorescent bands corresponding to different phytochemical constituents. The intense fluorescent band indicates the presence of major bioactive compounds, while variations in band intensity and migration pattern confirm the chemical diversity of the sample.

indicating reduced activity due to lower phytochemical concentrations (Cowan, 1999). The kashaya also demonstrated antifungal activity against *C. albicans*, suggesting the presence of bioactive compounds capable of disrupting fungal cell membranes and metabolic pathways (Silva *et al.*, 2012). Overall, the results suggest that the Kashaya possesses broad-spectrum antimicrobial activity, likely due to phytochemicals such as flavonoids, tannins, phenols, and alkaloids present in the formulation.

CONCLUSION

The prepared kwatha churna exhibited satisfactory physicochemical characteristics. Was found to possess a diverse range of bioactive phytoconstituents, particularly flavonoids and tannins known for their therapeutic relevance in skin disorders. HPTLC analysis established the presence of important phytochemical markers, confirming the authenticity and chemical consistency of the formulation. Furthermore, the aqueous extract demonstrated significant antimicrobial efficacy. The overall findings scientifically support the traditional application of the formulation and indicate its promising potential as a natural dermatologic and antimicrobial agent.

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ABBREVIATIONS

PhD: Doctor of Philosophy; **AYUSH:** Ayurveda, Yoga and Naturopathy, Unani, Siddha and Homeopathy; **ASU:** Ayurveda, Siddha and Unani; **GMP:** Good Manufacturing Practice; **SDA:** Sabouraud Dextrose Agar; **SCDA:** Soybean Casein Digest Agar; **MSA:** Mannitol Salt Agar; **XLD:** Xylose Lysine Deoxycholate Agar; **SCDM:** Soybean Casein Digest Medium; **HPTLC:** High-Performance Thin-Layer Chromatography; **MIC:** Minimum Inhibitory Concentration; **UV:** Ultraviolet; **R_f :** Retention Factor; **HAVAK:** Haridra, Abhaya, Vidanga, Amalaki, and Khadira.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

1st Author: literature review, experimental work, data collection, analysis, interpretation of results, and manuscript preparation. **2nd Author:** Supervised the research work, provided scientific guidance, reviewed the manuscript, and approved the final version for submission. Competing Interests.

SUMMARY

This paper focused on the physicochemical, phytochemical, fluorescence, High-Performance Thin-Layer Chromatography (HPTLC), and antimicrobial evaluation of selected Kushtaghna Mahakashaya drugs. Standard analytic methods were used to profile the raw drugs and aqueous decoction. The study also assessed antimicrobial activity against selected bacterial and fungal strains to explore the therapeutic relevance of these formulations.

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