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Original Article

Phytochemical Profiling and Standardization of *Punarnavastak Kwath Ghanvati* Using Physicochemical Parameters and HPTLC

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ABSTRACT

Background: *Punarnavastak Kwath*, a classical Ayurvedic decoction described in the *Bhaishajya Ratnavali*, is renowned for its efficacy in managing *Udara Roga* (abdominal disorders) and *Shotha Roga* (oedema). To enhance patient compliance, this formulation is transformed into a concentrated tablet known as *Punarnavastak Kwath Ghanvati* (PAKG), prepared without any excipients. Despite extensive traditional use, scientific validation of PAKG through contemporary analytical techniques remains necessary to ensure consistent therapeutic effectiveness and quality control. **Purpose:** The present study aimed to conduct a comprehensive evaluation of PAKG through organoleptic examination, physico-chemical analysis, phytochemical screening, and High-Performance Thin-Layer Chromatography (HPTLC). **Materials and Methods:** The formulation was prepared as per the guidelines stipulated in the Indian Pharmacopoeia. Organoleptic characteristics (colour, taste, odour), along with moisture content, total ash, acid-insoluble ash, water-soluble ash, and extractive values (aqueous and alcoholic), were systematically assessed. Preliminary phytochemical screening was conducted to detect major bioactive groups such as alkaloids, flavonoids, tannins, saponins, and glycosides. Furthermore, HPTLC profiling was performed to identify and quantify specific phytochemical constituents, documenting characteristic retention factor (R_f) values essential for establishing reproducible quality standards. **Results:** The analysis revealed consistent physico-chemical parameters indicative of formulation stability and quality. Phytochemical screening confirmed the presence of active constituents, reinforcing traditional claims. HPTLC analysis provided reproducible fingerprints, enabling precise standardization of PAKG. **Conclusion:** This integrative scientific evaluation effectively bridges traditional Ayurvedic knowledge and contemporary analytical validation, ensuring therapeutic consistency, enhancing credibility, and facilitating wider acceptance of *Punarnavastak Kwath Ghanvati* in modern healthcare practice.

Keywords: *Punarnavastak Kwath Ghanvati*, Ayurvedic formulation, Phytochemical screening, Physico-chemical analysis, HPTLC fingerprinting, Standardization.

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INTRODUCTION

Punarnavastak Kwath is a classical Ayurvedic polyherbal formulation documented in the *Bhaishajya Ratnavali*, traditionally prescribed for conditions such as *sarvanga sotha* (generalized edema), *udara roga* (abdominal disorders), *kasa* (cough), *svasa* (dyspnoea), and *pandu* (anaemia).[1] Rooted in the principles of Ayurvedic formulation exemplifies the holistic approach of combining multiple herbs to enhance therapeutic efficacy through synergistic action.[2] Despite its longstanding traditional use, there is a paucity of comprehensive scientific studies validating the safety, efficacy, and quality control parameters of *Punarnavastak Kwath Ghanvati*. Preliminary phytochemical analyses have identified constituents such as alkaloids, tannins, flavonoids, and saponins, which are believed to contribute to its therapeutic effects. Furthermore, studies have demonstrated its hepatoprotective and antioxidant activities,[3] supporting some of its traditional claims. However, the complexity of herbal formulations necessitates rigorous analytical evaluation to ensure consistency and reproducibility. Modern analytical techniques, including organoleptic assessment, physicochemical analysis (such as moisture content, ash values, and extractive values), phytochemical screening, and High-Performance Thin-Layer Chromatography (HPTLC),[4] are essential tools in the standardization process. For instance, HPTLC fingerprinting has been effectively employed to assess the phytoconstituents of similar Ayurvedic formulations, aiding in their authentication and quality control.

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Aims and Objectives

This study aims to conduct a comprehensive analytical evaluation of *Punarnavastak Kwath Ghanvati* using modern scientific methodologies to substantiate its traditional claims and establish quality standards. The specific objectives are:

1. To authenticate the formulation through organoleptic and qualitative phytochemical assessments.
2. To determine physicochemical parameters, including moisture content, ash values, and extractive values, to ensure batch-to-batch consistency.
3. To perform HPTLC profiling for the identification and quantification of key phytoconstituents, facilitating the development of a standardized fingerprint for quality assurance.

By achieving these objectives, the study seeks to bridge the gap between traditional Ayurvedic knowledge and contemporary scientific validation, thereby enhancing the credibility and acceptance of *Punarnavastak Kwath Ghanvati* in modern healthcare.

MATERIALS AND METHODS

Drug review

पुनर्नवानिम्बपटोलशुण्ठीतिक्ताऽमृतादार्वभयाकषायः |
सर्वाङ्ग शोथोदरकासशूलश्वासान्वितंपाण्डुदंनिहन्ति ||
भैष्ज्यरत्नावली उदररोग चिकित्सा 40/33

Stepwise Detailed Method for the Preparation of *Punarnavastak kwath ghanvati*

Collection and Authentication of Raw Drugs

Selection of Ingredients: Each herbal ingredient (1 kg) was selected based on classical Ayurvedic texts *Sharngdhara samhita* (Table 1).

Authentication: All raw materials were authenticated as per the Ayurvedic Pharmacopoeia of India, ensuring correct identity, quality, and absence of contaminants.

Cleaning and Processing of Raw Drugs

Cleaning: All raw herbs were thoroughly washed with clean water to remove dust and impurities, then shade-dried for 2-3 days to preserve active constituents.

Cutting and Crushing: The dried materials were chopped into small pieces and coarsely crushed to facilitate decoction preparation.

Preparation of *Punarnavastak Kwath* (Decoction)

Measurement: A total of 8 kg of raw drugs (1 kg each) was taken, and 128 L (16 parts) of water was added.[5]

Decoction Process: The mixture was boiled on a mild flame (*Mridvagni*) until the volume was reduced to 32 L (one-fourth of original volume), with intermittent stirring.

Filtration: The decoction was filtered through a clean muslin cloth to remove solid residues, yielding *Punarnavastak Kwath*.

Preparation of *Ghanvati* (Tablet)

1. **Ghana Preparation:** The decoction (32 L) was further heated over a mild flame with continuous stirring until it reached a thick, semisolid consistency, yielding approximately 3.5 kg of Ghana (solid extract).[6]
2. **Drying Process:** The *Ghana* was dried in a hot air oven to eliminate residual moisture.
3. **Tablet Formation:** The dried extract was mixed with Acacia gum as a binder, and 500 mg tablets were prepared using a tablet punching machine. Tablets were then packed for therapeutic use.

Storage

1. **Container Selection:** Tablets were stored in sterilized, airtight plastic containers.
2. **Labelling:** Containers were labelled with the formulation name (*Punarnavastak Kwath Ghanvati*), batch number, date of manufacture, and storage instructions.

Table 1: Ingredients of Punarnavastak Kwath Ghanvati with Their Botanical Identity.

Sl. No.	Name of the sample	Botanical Name	Family	Useful Part
1	Punarnava	*Boerhavia diffusa*	Nyctaginaceae	Root (*Mool*)
2.	Sunthi	*Zingiber officinalis*	Zingiberaceae	Rhizome (*Kanda*)
3.	Haritaki	*Terminalia chebula*	Combretaceae	Fruit (*Phala*)
4.	Guduchi	*Tinospora cordifolia*	Menispermaceae	Stem (*Kanda*)
5.	Nimba	*Azadirachta indica*	Meliaceae	Bark (*Tvaka*)
6.	Patol	*Tricosanthes dioica*	Cucurbitaceae	Leaf (*Patra*)
7.	Katuki	*Picrohiza kurroa*	Scrophulariaceae	Root (*Mool*)
8.	Daruharidra	*Berberis aristate*	Berberidaceae	Rhizome (*Kanda*)

Table 2: Physico-chemical and Pharmacopoeial Parameters of *Punarnavastak Kwath Ghanvati*.

Sl. No.	Parametre	Value
1.	Loss on Drying at 110 c(%w/w)	8.35%
2.	Total Ash Value (%w/w)	14.22%
3.	pH Value	6.8
4.	Tablet Weight Variation	430 mg
5.	Tablet Highest Weight	442 mg
6.	Tablet Lowest Weight	408 mg
7	Tablet Highest Weight Value	2.79%
8	Tablet Lowest Weight Value	5.11%
9	Tablet Friability	0
10	Tablet Hardness	3.83 kg/cm2
11	Tablet Disintegration Start Time	10 sec
12	Tablet Disintegration End Time	8.05 min
13	Alcohol Soluble Extract	18.4%
14	Water Soluble Extract	14.3%

3. **Storage Conditions:** The containers were kept in a cool, dry place, protected from sunlight and moisture.

Quality Control and Testing

1. Organoleptic Characteristics.

➢Colour: Brown,

➢Odour: Bitter,

➢Consistency: Hard,

➢Taste: Bitter.

2. Physico-Chemical Parameters (Table 2).

3. Phytochemical screening (Table 3).

4. Phytochemical Analysis: HPTLC was carried out to identify and confirm the presence of key phytoconstituents, ensuring quality, consistency, and compliance with pharmacopeial standards.

High performance thin layer chromatography

HPTLC Fingerprint Analysis Procedure for Methanol Extract of *Punarnavashtaka Kwath Ghanavati*. The High-Performance Thin-Layer Chromatography (HPTLC) fingerprinting for the methanolic extract of *Punarnavashtaka Kwath Ghanavati* was performed as follows:

Sample Preparation

5 g of the sample was accurately weighed into a beaker, and 100 mL of methanol was added. The mixture was sonicated for 16 hr, after which the extract was filtered through standard filter paper, followed by filtration through a 0.45 µm membrane filter to obtain a clear solution.

Chromatography Setup

The filtered test solution was used for HPTLC analysis. Aliquots were applied as 15 mm wide bands on 10 × 10 cm TLC plates pre-coated with a 0.2 mm layer of silica gel F254 (Merck), using a Linomat 5 sample applicator (CAMAG, Switzerland).

Chromatogram Development

The plates were developed in a CAMAG chamber pre-saturated with vapours of the mobile phase consisting of Toluene: Ethyl acetate: Formic acid in the ratio 6:3:0.5 (v/v/v). The solvent front was run to a distance of 8.0 cm.

Detection and Documentation

Post-development, the plates were air-dried and then scanned at 254 nm and 366 nm using a CAMAG TLC scanner 3 equipped with winCATS 4 software. This facilitated the detection and documentation of the HPTLC fingerprint for the methanol extract of the formulation.

Figures 1 and 2 show the HPTLC Chromatograms of *Punarnavashtaka Kwath Ghanavati* at UV 254 nm and UV 366 nm respectively using Toluene: Ethyl acetate: Formic acid (6:3:0.5 v/v/v) and gives the R_f values of the same.

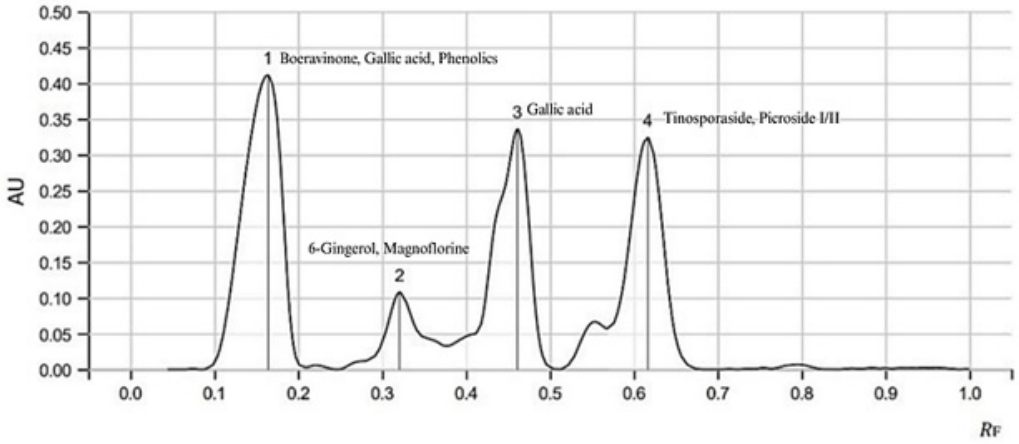
RESULTS

The HPTLC analysis revealed distinct spots, each corresponding to specific phytoconstituents present in the *Punarnavastak Kwath*. The identified R_f values and the likely phytoconstituents associated with each are summarized below (Table 4).

DISCUSSION

The present study provides a comprehensive evaluation of *Punarnavastak Kwath Ghanvati* (PAKG) through organoleptic, physicochemical, and phytochemical analyses, along with advanced chromatographic profiling using High-Performance Thin Layer Chromatography (HPTLC). These findings contribute valuable data to the scientific understanding and standardization of this classical Ayurvedic formulation. The organoleptic and physicochemical parameters of PAKG, including colour, taste, texture, pH, and extractive values, were consistent with Ayurvedic Pharmacopeial standards, suggesting good quality and acceptable formulation characteristics. These parameters play a vital role in identifying the authenticity and stability of herbal products, which is essential for patient safety and efficacy.

1(A)



1 (B)

Peak #	Start		Max			End		Area	
	R_F	H	R_F	H	%	R_F	H	A	%
1	0.087	0.0000	0.164	0.4107	34.89	0.208	0.0024	0.02197	37.00
2	0.249	0.0000	0.321	0.1074	9.13	0.376	0.0325	0.00536	9.04
3	0.376	0.0325	0.461	0.3353	28.49	0.508	0.0000	0.01672	28.16
4	0.569	0.0564	0.617	0.3236	27.49	0.685	0.0000	0.01532	25.80

1 (C)

Figure 1: HPTLC Chromatogram of *Punarnavashtak Ghanavati* at UV 254 nm (A=Fingerprint, B= Peak height, C= R_f value and area percentage).

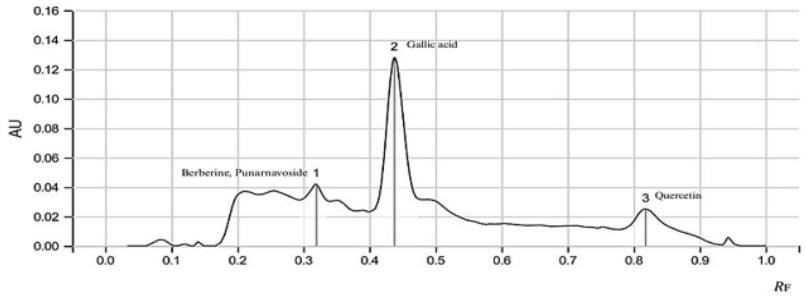
Phytochemical screening revealed the presence of important bioactive constituents such as alkaloids, flavonoids, saponins, glycosides, tannins, and phenolic compounds, which are known for their diverse pharmacological activities. These phytochemicals are likely responsible for the traditional therapeutic effects of PAKG, including its anti-inflammatory, diuretic, hepatoprotective, and antioxidant properties. The HPTLC fingerprinting showed distinct peaks corresponding to various phytochemical constituents, confirming the polyherbal nature and chemical complexity of the formulation. HPTLC proved to be an effective tool in detecting and documenting the marker compounds in PAKG, ensuring reproducibility and providing a scientific basis for its standardization.

Notably, major ingredients such as *Punarnava* and *katuki* have been extensively studied for their hepatoprotective, nephroprotective and anti-inflammatory properties, supporting their inclusion in this formulation. The observed phytochemical profile correlates well with the known traditional uses of these herbs, affirming the therapeutic rationale behind PAKG's formulation. The study also highlights the importance of integrating modern analytical techniques with traditional Ayurvedic knowledge to establish quality benchmarks and promote global acceptance of herbal medicines. Standardization through HPTLC not only enhances the scientific credibility of PAKG but also lays the foundation for its future clinical validation.

Table 3: Key Phytoconstituents and Pharmacological Activities of Individual Ingredients of *Punarnavashtaka Kwath Ghanavati*.

Sl. No.	Drug Name	Key Phytoconstituents	Pharmacological Activities
1	Punarnava.[7]	Punarnavine, Punarnavoside, Boeravinone, Lignans, Flavonoids, Phenolics.	Diuretic, Anti-inflammatory, Hepatoprotective, Antioxidant, Immunomodulatory, Antiallergic.
2	Sunthi (Ginger).[8]	6-Gingerol, 6-Shogaol, Zingerone, Gingerdiol, Paradol, Ginger oleoresin.	Antioxidant, Anti-inflammatory, Anti-emetic, Antimicrobial, Digestive aid.
3	Haritaki.[9]	Chebulic acid, Chebulagic acid, Chebulinic acid, Gallic acid, Tannins.	Antioxidant, Antidiabetic, Antimicrobial, Anti-inflammatory, Hepatoprotective, Neuroprotective.
4	Guduchi.[10]	Berberine, Jatrorrhizine, Tinosporide, Cordifolioside A, β-Sitosterol.	Immunomodulatory, Antioxidant, Antidiabetic, Hepatoprotective, Antimicrobial, Anti-inflammatory, Anticancer.
5	Nimba (Neem).[11]	Azadirachtin, Nimbin, Nimbidin, Nimbolide, Quercetin, β-Sitosterol.	Antimicrobial, Anti-inflammatory, Hepatoprotective, Antioxidant, Anticancer.
6	Patol.[12]	Tannins, Saponins, Alkaloids, Proteins, Triterpenes, Vitamin A.	Hepatoprotective, Antioxidant, Anti-inflammatory, Antidiabetic.
7	Katuki.[13]	Picroside I & II, Kutkoside, Apocynin.	Hepatoprotective, Antioxidant, Immunomodulatory, Anti-inflammatory, Antidiabetic.
8	Daruharidra.[14]	Berberine, Palmatine, Oxyacanthine, Aromoline.	Antimicrobial, Hepatoprotective, Antidiabetic, Anti-inflammatory, Antioxidant, Anticancer.

2 (A)



2 (B)

Peak #	Start		Max			End		Area	
	R_F	H	R_F	H	%	R_F	H	A	%
1	0.293	0.0312	0.319	0.0420	21.51	0.340	0.0302	0.00166	19.49
2	0.401	0.0230	0.438	0.1280	65.63	0.478	0.0314	0.00500	58.64
3	0.778	0.0111	0.818	0.0251	12.86	0.932	0.0007	0.00187	21.87

2 (C)

Figure 2: HPTLC Chromatogram of *Punarnavashtak Ghanavati* at UV 366 nm (A=Fingerprint, B= Peak height, C= R_f value and area percentage).

Table 4: Phytoconstituents Identified via HPTLC (Based on R_f values) and Their Plant Sources.

R_f Value	Phytoconstituent(s)	Plant Source(s)
0.16	Boeravinone (flavonoid),[15] Gallic acid, Phenolics.	*Boerhavia diffusa* (Punarnava),[16,17] *Terminalia chebula* (Haritaki).[17]
0.32	6-Gingerol,[18] Magnoflorine,[19] phenolic alkaloid.	*Zingiber officinale* (Sunthi),[20] *Tinospora cordifolia* (Guduchi),[21] *Trichosanthes dioica* (Patol).
0.31	Berberine,[22] Punarnavoside.	*Berberis aristata* (Daruharidra), *Boerhavia diffusa* (Punarnava).[23]
0.43	Gallic acid.[24]	*Terminalia chebula* (Haritaki).
0.50	p-Coumaric acid.	*Azadirachta indica* (Nimba), *Zingiber officinale* (Sunthi).[25-28]
0.61	Tinosporaside, Picroside I/II.[29]	*Tinospora cordifolia* (Guduchi),[30-32] *Picrorhiza kurroa* (Katuki).
0.81	Quercetin (flavonoid aglycone).[33]	*Azadirachta indica* (Nimba),[34,35] *Terminalia chebula* (Haritaki).[36]

CONCLUSION

This study highlights the therapeutic potential and quality assurance of *Punarnavastak Kwath Ghanvati* through comprehensive organoleptic, physicochemical, phytochemical, and HPTLC analyses. The identification of key bioactive compounds and a distinctive chromatographic fingerprint support its traditional Ayurvedic use and emphasizes the necessity of standardization in herbal formulations. These findings contribute significantly to ensuring the safety, efficacy, and consistency of Ayurvedic medicines. Future research should aim to explore its molecular mechanisms and validate its clinical efficacy through well-designed clinical trials. Overall, this study reinforces the value of integrating traditional knowledge with modern scientific approaches to broaden the therapeutic applications of classical formulations.

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ABBREVIATIONS

PAKG: Punarnavastak Kwath Ghanvati; **HPTLC:** High-Performance Thin-Layer Chromatography; **TLC:** Thin-Layer Chromatography; **R_f:** Retardation factor; **UV:** Ultraviolet; **% w/w:** Percentage weight by weight; **kg/cm²:** Kilogram per square centimeter; **β:** Beta; **min:** Minutes; **sec:** Seconds; **nm:** Nanometer; **API:** Ayurvedic Pharmacopoeia of India; **CAMAG:** Company for Analytical Measuring Systems (Chromatography equipment manufacturer).

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

SUMMARY

This study focuses on the scientific standardization of *Punarnavastak Kwath Ghanvati* (PAKG), a classical Ayurvedic formulation traditionally used in the treatment of abdominal disorders (Udara Roga), edema (Shotha), and related conditions. The formulation was prepared following traditional methods and evaluated through organoleptic, physicochemical, phytochemical, and HPTLC analyses.

The formulation was prepared using classical Ayurvedic procedures and analyzed through organoleptic, physicochemical, and phytochemical methods. Physicochemical parameters such as moisture content (8.35%), total ash (14.22%), and extractive values demonstrated acceptable quality and batch consistency. Phytochemical screening confirmed the presence of active constituents including alkaloids, flavonoids, tannins, glycosides, and saponins, which support its known therapeutic effects.

High-Performance Thin-Layer Chromatography (HPTLC) fingerprinting of the methanolic extract was carried out using Toluene: Ethyl acetate: Formic acid (6:3:0.5) as the mobile phase. Detection at 254 nm and 366 nm revealed distinctive R_f values corresponding to key phytoconstituents such as Boeravinone, 6-Gingerol, Berberine, Gallic acid, Picrosides, and Quercetin, confirming their presence in specific plant ingredients like *Boerhavia diffusa, Zingiber officinale, Tinospora cordifolia*, and *Terminalia chebula*.

The study scientifically validates the formulation's traditional use and establishes a reproducible standard, supporting its therapeutic consistency and promoting its integration into evidence-based Ayurvedic practice.

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