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Original Article

Comparative Physicochemical Profiling and Antibacterial Screening of Patolakaturohiniyadi Kashaya: An Analytical Study

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ABSTRACT

Background: Kashaya (decoction) is a traditional Ayurvedic dosage form known for its rapid therapeutic action and effective bioavailability of active constituents. With the increasing commercialization, ready-to-use bottled Kashaya preparations have become widely available, raising concerns regarding their standardization, quality, and equivalence to freshly prepared formulations described in classical texts. In this context, the present study was undertaken to evaluate two commercially available samples of Patolakaturohiniyadi Kashaya and to determine their compliance with standards by comparing their physicochemical parameters, qualitative and quantitative characteristics, with those of a freshly prepared sample. **Materials and Methods:** Three samples of Patolakaturohiniyadi Kashaya were examined: two were procured GMP Certified companies and one was prepared freshly. Organoleptic properties, pH, total solids, extractive values, and phytochemical screening and quantification were among the analytical factors evaluated. **Results:** The comparison between commercially available market Samples and freshly prepared Kashaya showed clear physiochemical and phytochemical variation. The freshly manufactured Sample exhibit brown colour whereas market sample were dark brown in colour. the freshly made Kashaya was shown to be alkaline in nature by pH analysis i.e. 6.34. Where the market samples tended to be acidic Sample A is having 5.12 pH whereas sample is having 5.04 pH. **Conclusion:** All the three sample contained carbohydrates flavonoids and tannins according to qualitative screening however the freshly made sample had a more fluctuation in flavonoids and phenolic content suggesting higher level of phytochemical richness only the freshly made sample showed signs of microbial and fungal growth, although counts stay within the acceptable pharmacopeial limits.

Keywords: Patolakaturohiniyadi Kashaya, Phytochemical and Physiochemical Analysis, Standardisation.

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INTRODUCTION

Ayurveda, the traditional system of Indian medicine, emphasises the use of freshly made formulations for best therapeutic results. Kashaya (decoction) stands out due to its high extractive properties, fast absorption, and potency. According to historical writings such as Sharngadhara Samhita and Bhaishajya Ratnavali, Kashaya is made by boiling the necessary herbal ingredients in water until the volume is reduced to one-eight which ensures the release of active phytoconstituents into the medium.

The pharmaceutical industry has created ready-made bottled Kashaya formulations for consumer convenience in response to the growing demand for Ayurvedic medicines worldwide. However, maintaining consistency, stability, and therapeutic efficacy has become more difficult as a result of these formulations' commercialisation. Their chemical makeup and pharmacological action can be greatly impacted by variations in raw material quality, processing techniques, preservatives, and storage time.

Patolkaturohinyadi Kashaya is one the most potent classical Ayurvedic Formulation. This decoction is prepared with Patola (*Trichosanthes dioica*), Katurohini (*Picrorhiza kurroa*) Chandan (*Santalum album*), Murva (*Marsdenia tenacissima*) Guduchi

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(*Tinospora cardifolia*) and Patha (*Cissampelos pareira*) it helps in alleviating Kapha And Pitta disorders, skin diseases (Kustha), Fever (Jawara), Poisoning (Visha), Vomiting (Vami), Loss of taste (Arochak) and jaundice (kamala) and other Pitta disorders due to its Tikta rasa(bitter taste) and sheet veerya (cool potency) and pitta- kaphashamka properties (Phartale, 2025; Paradakar, 2002).

Because of the formulation's therapeutic value, it is crucial to make sure that commercial Kashaya preparations meet quality requirements as of freshly made decoctions. According to the Ayurvedic Pharmacopoeia of India (API) criteria, this study intends to do a comparative evaluation of two marketed samples and one freshly prepared sample of Patolakaturohiniyadi Kashaya, with an emphasis on physicochemical and phytochemical standardisation characteristics.

MATERIALS AND METHODS

Sample Collection

Three samples of Patolakaturohiniyadi Kashaya were selected from different sources to ensure a comparative evaluation. Sample A was obtained as a market sample from a GMP certified company, while sample B comprised a market formulation sourced from another GMP certified company. Sample C was freshly prepared in house strictly in accordance with classical textual method, at Pathaya unit of KAHER's shri BMK Ayurveda Mahavidyalaya, Belagavi.

Method of Preparation

Decoction was prepared using 1 part of patolakaturohiniyadi Kwatha churna (course powder) and 16 parts of water was added and then boiled on medium flame (95-105°C) till it get reduced to 1/8th part. Then Kashaya (decoction) was filtered and submitted for the analysis. (Sharangdhara, 2004).

Analytical Parameters

All samples were evaluated for organoleptic parameters (form, colour, odour, and taste) and physicochemical parameters (pH, specific gravity, and total solids) (Ayurvedic Pharmacopoeia of India [API], 2008). Preliminary phytochemical screening was carried out to detect carbohydrates, sugars, proteins, amino acids, steroids, flavonoids, alkaloids, tannins, and glycosides (Khandelwal, 2012). Further analysis included Thin Layer Chromatography (TLC) profiling, quantification of total flavonoid (Laoung-On *et al.*, 2021), and phenolic content (Singleton and Rossi, 1965), microbial count assessment, and evaluation of antibacterial activity to assess quality, safety, and bioactivity of the samples.

RESULTS

Organoleptic Parameters

The following characters are constantly reported in the macroscopic description of PatolkaturohinyadiKashaya. Each of the three samples is in the form of Kashaya and has a characteristic odour, which refers to the natural scent of the herbal component. Tests A and B Both are dark brown in colour however; Sample C is brown. The taste of all three samples is bitter.

Thin Layer Chromatography

The alcoholic extract of Patolakaturohinyadi Kashaya was chromatographed using a mobile phase of toluene: ethyl acetate (7:3). The existence of several phytochemical components was confirmed by the three samples' numerous resolved bands under short-wave UV. R_f values for Sample A, Sample B, and Sample C were 0.18, 0.44, 0.60, and 0.84, 0.15, 0.36, 0.58, and 0.82, respectively. Sample B displayed a single notable band at 0.63 under long-wave UV, while Sample A displayed three more bands at 0.12, 0.27, and 0.40. Sample C did not exhibit any long-wave bands. Patolakaturohinyadi Kashaya's distinctive chromatographic fingerprint was established by the TLC profiles of all samples, which generally showed similar patterns with slight differences.

Microbial count

All the three of the Patolkaturohinyadi Kashaya tested Sample met the Qualitative microbiological requirements, demonstrating the absence of, *E. coli, Staphylococcus Aureus* and *Pseudomonas aeruginosa* in 100 mL of the formulation. Sample A and B did not exhibit any bacterial or fungal growth in the quantitative microbiological limit test, staying below the permissible limits of 50-500 cfu/mL of bacterial count and 10-100 cfu/mL for total fungal count. Sample C showed a total fungal count of 15 cfu/mL and a total bacterial count of 45 cfu/mL, both of which were within the allowed limit. Overall. Every Sample satisfied the standards of microbiological quality

Anti-Bacterial Activity

Patolkaturohiniyadi Kashaya: Antibacterial activity against *Escherichia. Coli, Staphylococcus Aureus, Streptococcus pyogens and Pseudomonas aeruginosa* was determined using the cup-plate diffusion method at doses ranging from 5 to 100 mg/mL (Bauer *et al.*, 1966; Indian Pharmacopoeia Commission, 2018).

Sample A showed zones of inhibition against *Staphylococcus aureus* and *Streptococcus pyogens* with the greatest inhibition at 100 mg/mL measuring 25 mm and 30 mm respectively. At 75 mg/mL, zones of inhibition of 18 mm for *Staphylococcus aureus* and 25 mm for *Streptococcus pyogens* were reported, while *Streptococcus pyogens* alone showed inhibition of 18 mm at 50 mg/mL. At doses as low as 25 mg/mL, no inhibitory action was found.

Sample B showed antibacterial activity against *Escherichia coli, Staphylococcus pyogens* at 100 mg/mL, with zones of inhibition of 28 mm, 28 mm and 32 mm respectively. At 75mg/mL, inhibition zones of 2 0 mm (*E. coli*), 22 mm (*S. aureus*) and 28 mm (*S. pyogens*) were detected, while at 50 mg/mL inhibition was only noted against *Staphylococcus aureus* (19 mm) and *Streptococcus pyogens* (20 mm).

Sample C showed antibacterial activity against *Staphylococcus aureus* and *Streptococcus pyogenes* with zones of inhibition of 22 mm and 24 mm at 100 concentrations, 20 mm at 75 and 18 mm and 19 mm at 50 indicating dose dependent activity. No zone of inhibition was observed at 25,10, and 5 concentrations. The formulation showed no antibacterial activity against *Escherichia coli* and *Pseudomonas aeruginosa* at any concentration. The control group showed no zone of inhibition

DISCUSSION

The present study demonstrated noticeable differences between freshly prepared and commercially available samples of Patolakaturohiniyadi Kashaya, highlighting the influence of ingredient composition, processing methods, and storage conditions on the overall characteristics of the formulation. Although similarity in organoleptic properties confirmed the identity of the formulation, variations in colour among the samples indicate the possible impact of manufacturing practices and duration of storage.

Physicochemical evaluation revealed that the freshly prepared Kashaya exhibited a nearly neutral pH and lower total solid content, which is consistent with the classical Ayurvedic principle that freshly prepared decoctions possess better therapeutic potential. In contrast, the marketed samples showed a comparatively acidic pH and higher total solids, which may be attributed to concentration during large-scale manufacturing, addition of preservatives, and prolonged storage.

Preliminary phytochemical screening confirmed the presence of major constituents such as flavonoids and tannins in all samples. However, variations in certain secondary phytochemicals suggest differences in raw material quality, ingredient composition, and manufacturing procedures. Label comparison further revealed variations in ingredients, as *Santalum album* was present in Samples A and C but absent in Sample B, while *Marsdenia tenacissima*/*Cissampelos pareira* was present in Sample A but absent in Samples B and C. These compositional differences may have contributed to the qualitative and quantitative phytochemical variations observed.

The comparatively lower flavonoid content observed in Sample C may be attributed to differences in the proportion or quality of flavonoid-rich raw drugs, possible degradation of flavonoids during processing or storage, and variations in extraction

Table 1: Ingredients of Patolakaturohiniyadi Kashaya.

Sl. No.	Sample A (Market)	Sample B (Market)	Sample C (Fresh)
1	Murva *Marsdenia tenacissima* (Roxb) Moon	Agaru *Aquilaria agallocha* Roxb.	Patha *Cyclea peltata* Hook. Fil and Thoms
2	Guduchi *Tinospora cardifolia* (Thunb.) Miers	Guduchi *Tinospora cardifolia* (Thunb.) Miers	Guduchi *Tinospora cardifolia* (Thunb.) Miers
3	Patha *Cissampelos pareira* L.	Murva Chonemorpha fragrans (Moon) Alston	Murva Chonemorpha fragrans (Moon) Alston
4	Patola *Trichosanthes dioica* Roxb.	Patola *Trichosanthes lobata* Roxb.	Patola *Trichosanthes cucumerina* Roxb.
5	Katuki *Picrorhiza kurroa* Royle ex Benth	Katuki *Picrorhiza kurroa* Royle ex Benth	Katuki *Picrorhiza kurroa* Royle ex Benth
6	Chandana *Santalum album* L.	Patha *Cyclea peltata* Hook. Fil and Thoms	Chandana *Santalum album* L.

Table 2: Physiochemical Parameters.

Tests	Sample A	Sample B	Sample C
Specific Gravity	1.045	1.053	1.0128
pH	5.14	5.02	6.34
Total solids	15.021%	16.836%	4.149%

Table 3: Phytochemical Screening of Patolakaturohinyadi Kashaya samples.

Extracts	Sample A	Sample B	Sample C
Carbohydrates	+	+	+
Reducing Sugar	+	+	+
Monosaccharides	+	+	+
Pentose sugar	-	-	-
Non reducing sugar	-	-	+
Hexose Sugar	-	-	-
Proteins	-	-	-
Amino Acids	+	-	+
Steroids	-	-	-
Flavonoids	+	+	+
Alkaloids	-	-	-
Tannins	+	+	+
Cardiac Glycosides	+	-	+
Anthraquinone Glycosides	-	-	-
Saponin Glycosides	+	+	-

Table 4: Quantification of Phenolic and Flavonoid Content of Patolakaturohiniyadi Kashaya samples.

Sample	Mean Phenolic Content (mg GAE/mL)	Mean Flavonoid Content (ug QE/mL)
Sample A	3.86	851.30
Sample B	4.53±0.00	957.71±4.08
Sample C	0.89±0.01	95.11±6.55

efficiency during preparation, as flavonoids are known to be sensitive to heat, light, and oxidation.

Thin Layer Chromatography (TLC) profiling further supported the authenticity of the formulations despite the compositional differences. The higher phenolic and flavonoid content observed in commercial Kwatha compared to the freshly prepared sample may be due to concentration during large-scale manufacturing, improved extraction efficiency, and the use of dried and stored raw materials that facilitate the release of phenolic constituents. The increased phenolic and flavonoid content in marketed samples may also contribute to enhanced antimicrobial activity, as reflected by the detectable antibacterial effects (Tables 1-4).

Patolakaturohiniyadi Kashaya is traditionally indicated in the management of Kushtha, Twak Vikara, Jwara, and Pitta-Kapha predominant disorders, where its Tikta-pradhana dravyas contribute to detoxification, anti-inflammatory, and antimicrobial actions. Therefore, maintaining the quality and phytochemical consistency of this formulation is essential to ensure its therapeutic effectiveness in clinical practice.

Overall, the findings highlight that variations in ingredient composition, processing methods, and storage conditions play a significant role in determining the physicochemical and phytochemical characteristics of both freshly prepared and commercially available formulations.

CONCLUSION

Freshly prepared Patolkaturohiniyadi Kashaya aligns more closely with traditional Ayurvedic principles, whereas commercial formulations exhibit better stability and antibacterial activity. Variations in ingredient composition and processing highlight the need for proper standardization to maintain therapeutic consistency between classical and marketed preparations.

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ABBREVIATIONS

API: Ayurvedic Pharmacopoeia of India; **CFU:** Colony Forming Units; **GAE:** Gallic Acid Equivalent; **GMP:** Good Manufacturing Practices; **KAHER:** KLE Academy of Higher Education and Research; **pH:** Potential of Hydrogen; **QE:** Quercetin Equivalent; **SD:** Standard Deviation; **TLC:** Thin Layer Chromatography; **UV:** Ultraviolet.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

SUMMARY

The authors gratefully acknowledge the AYUSH Approved Drug Testing Laboratory for ASU Drugs, KAHER's Shri B.M.K. Ayurveda Mahavidyalaya, Belagavi, for extending laboratory infrastructure and technical assistance during the conduct of this study.

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