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Establishing phyto-pharmacognostical quality standards and HPTLC marker-based analysis of Panchavalkal Kwatha Churna-An Ayurvedic formulation

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Panchavalkal Kwatha Churna (PKC) is a traditional Ayurvedic poly herbal formulation referenced in classical Ayurvedic texts, is intended for the treatment of various ailments related to women's especially uterine diseases, cervical erosions, leucorrhea etc., The aim of this study is to set quality standards for Panchavalkal Kwatha Churna though a comprehensive quality control analysis, which included macro-microscopical studies, qualitative, quantitative assessments of its markers, aflatoxin testing, microbial load assessment and elemental analysis by following established protocols. High-performance thin layer chromatography (HPTLC) analysis brought to light the presence of caffeic acid and gossypol phytochemical marker compounds in Panchavalkal Kwatha Churna. In quantitative HPTLC analysis, caffeic acid and gossypol were quantified (mobile phase-water:acetic acid: n-butanol-1:2:7 v/v/v), yielding a concentration of 0.06941% and 1.186% respectively. In order to facilitate global utilization, Panchavalkal Kwatha Churna is being integrated with innovative scientific approaches to deal with concerns regarding standardization, safety and efficacy. The quality parameters developed for Panchavalkal Kwatha Churna can serve as a valuable quality control resource for Ayurvedic practitioners and stakeholders.

Keywords: Gossypol, HPTLC, Panchavalkal Kwatha Churna, Powder microscopy

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Panchvalkal Kwatha Churna (PKC), is an Ayurvedic classical formulation mentioned in ancient Nighantu's like Charak Samhita, Bhavaprakasha Nighantu, Sharangadhara Samhita and Dhanavantari Nighantu, intended for the treatment of women's diseases, especially leucorrhea, uterine diseases, cervical erosions, wound healing and dental care[1]. The term Panchavalkal denotes a set of 04 *Ficus* and one *Thespesia* species stem barks i.*e., F. benghalensis* L.-*Vata*, *F. religiosa* L.-*Ashwatha*, *F. glomerata* Roxb.-*Udumbara*, *F. lacor* Buch.-Ham.- *Plaksha*, *T. populnea* [L.] Sol. ex Corrêa- *Parisha*[2]. Scientific studies indicate that panchvalkal kwath stimulates the growth of pancreatic β-cells and boosts the efficacy of the glibenclamide[3]. PKC has been reported to exhibit wound healing[4], anti-bacterial[5,6], anti-oxidant[7], antineoplastic, immunomodulatory[8], healing action in cervical erosion[9], anti-inflammatory[10], antimicrobial[11] activities and recuperation from leucorrhea[12]. Despite of high therapeutic importance of this classical formulation, quality standards have not been reported. Hence the present study aimed for establishment of phytochemical, pharmacognostical standards followed by quantitative marker analysis for PKC.

Materials and Methods

Collection of raw drugs

One batch of individual crude drugs (taxonomist Dr. Vendrapati R. Rao, C.A.R.I, Bangalore authenticated) were collected from Kanakapura, Ramanagara District, Karnataka, India and its voucher Specimen Numbers are 21306-*Vata*; 21307-*Udumbara*; 21308-*Ashwatha*; 21309- *Plaksha*; 21310-*Parisha*. Two batches of crude drug samples were procured from local market and further authenticated by pharmacognostical analysis.

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Preparation of PKC formulation

Prepared three batches of PKC formulations by taking each 100 g of *Vata*, *Ashwatha*, *Udumbara*, *Plaksha*, *Parisha* stem barks and were mixed thoroughly to obtain a homogenous blend with the help of mass mixer, obtained coarse powder (sieve number 22) was used for further studies[13].

Organoleptic studies

The three batches of PKC formulations were subjected to organoleptic examination as per standard procedures, where their color, texture was observed and recorded[14,15].

Powder microscopy

From the macerated powder of three batches of PKC formulations, a small amount of the sample was placed on a glass slide, phloroglucinol reagent (two drops) was added and covered with a cover slip. The digital camera attached to the trinocular microscopic unit was used to capture key cellular characters at multiple magnifications[16,17].

Physicochemical studies and safety parameters

The physicochemical evaluation of three batches of PKC formulations was carried out in accordance to established protocols prescribed in the Ayurvedic Pharmacopoeia of India (API), including determination of total ash (Appendix 2.2.3), acid-insoluble ash (Appendix 2.2.4), alcohol-soluble extractives (Appendix 2.2.7), water-soluble extractives (Appendix 2.2.8), elemental analysis (Appendix 2.2.12), safety parameters *i.e.*, pesticide residue analysis (Appendix 2.2.13), aflatoxin analysis(Appendix 2.2.14), microbial load (Appendix 3.1.3) and specific pathogens analysis (Appendix 3.1.5)[18,19].

Phytochemical screening

Dilutions of the ethanolic and aqueous extracts from the three batches of PKC formulations were prepared with their corresponding solvents and a small portion of the test sample was exposed to chemical reagents to detect a range of phytochemicals such as phenols, flavonoids, alkaloids, and glycosides. For major secondary metabolites like tannins, phenols and flavonoids, quantitative analysis was performed for three batches of PKC formulations[20,21].

Qualitative HPTLC analysis

PKC of each batch (3 g of batch-I, II and III) were immersed in 30 mL of ethanol overnight, followed by boiling, filtering and adjusting the volume to 10 mL. 10 µL of batch- I, II and III were applied on track-1, 2 and 3 respectively and 2 mg of standard caffeic acid was dissolved in 10 mL of ethanol and 3 µL of this standard solution is applied on track-4 using Linomat-IV applicator. About 4g of *Vata*, *Ashwatha*, *Udumbara*, *Plaksha and Parisha* were immersed in 40 mL of ethanol overnight, followed by boiling, filtering and adjusting the volume to 10 mL and 10 µL of each were applied on tracks-5, 6, 7, 8 and 9 respectively. 2 mg of standard gossypol was dissolved in 10 mL of ethanol and 2 µL of this standard solution is applied on track-10. The TLC plate (Merck's silica gel 60 F_{254} aluminum plate) was developed in formic acid:ethyl acetate: toluene (0.2: 3.5: 6.5v/v/v) mobile phase, upto 90 mm and dried. The plate was examined through visualizer at UV-254 nm and 366 nm, photos were captured. Further the plate was submerged in vanillin-sulphuric acid reagent and placed in oven at 105^{o}C until the spots exhibited color[22,23].

Quantitative HPTLC analysis

10 mg of caffeic acid and gossypol (Procured from Natural Remedies Pvt. Ltd., Bangalore, India) were dissolved separately in 10 mL of ethanol in a volumetric flask. From this stock solution, working concentrations of caffeic acid (25-400 ng per spot) and gossypol (2-14 µg per spot) were applied on precoated TLC plate (Merck's silica gel 60 F_{254} aluminum plate). Similarly, 20 µL of the test solutions were applied on precoated TLC plate and developed the plate in water: acetic acid: n-butanol-1:2:7 v/v/v solvent system. Scanned densitometrically at 254 nm and 366 nm and recorded the peak area of the chromatograms. The amount of caffeic acid and gossypol present in the PKC formulation was estimated from the calibration curves[24,25].

Results

Organoleptic studies

PKC formulation is cream in color, coarse powder with small pieces of bark (Fig. 1) having woody odor with spicy, astringent taste.

Powder microscopy

PKC formulation showed thick-walled polygonal cork cells with no intercellular spaces, few cork cells filled with brown tannin masses. Thin-walled quadrangular shaped cortex cells were filled with cell contents. Stone cells of different shape and sizes are present either single or in group. Pitted vessels,

phloem fibers, plenty of prismatic calcium oxalate crystals, few rosette crystals, elongated fragments of medullary rays and pitted sclereides were observed. Results are represented in (Fig. 2).

Fig. 1 — Marphology of PKC Formulation

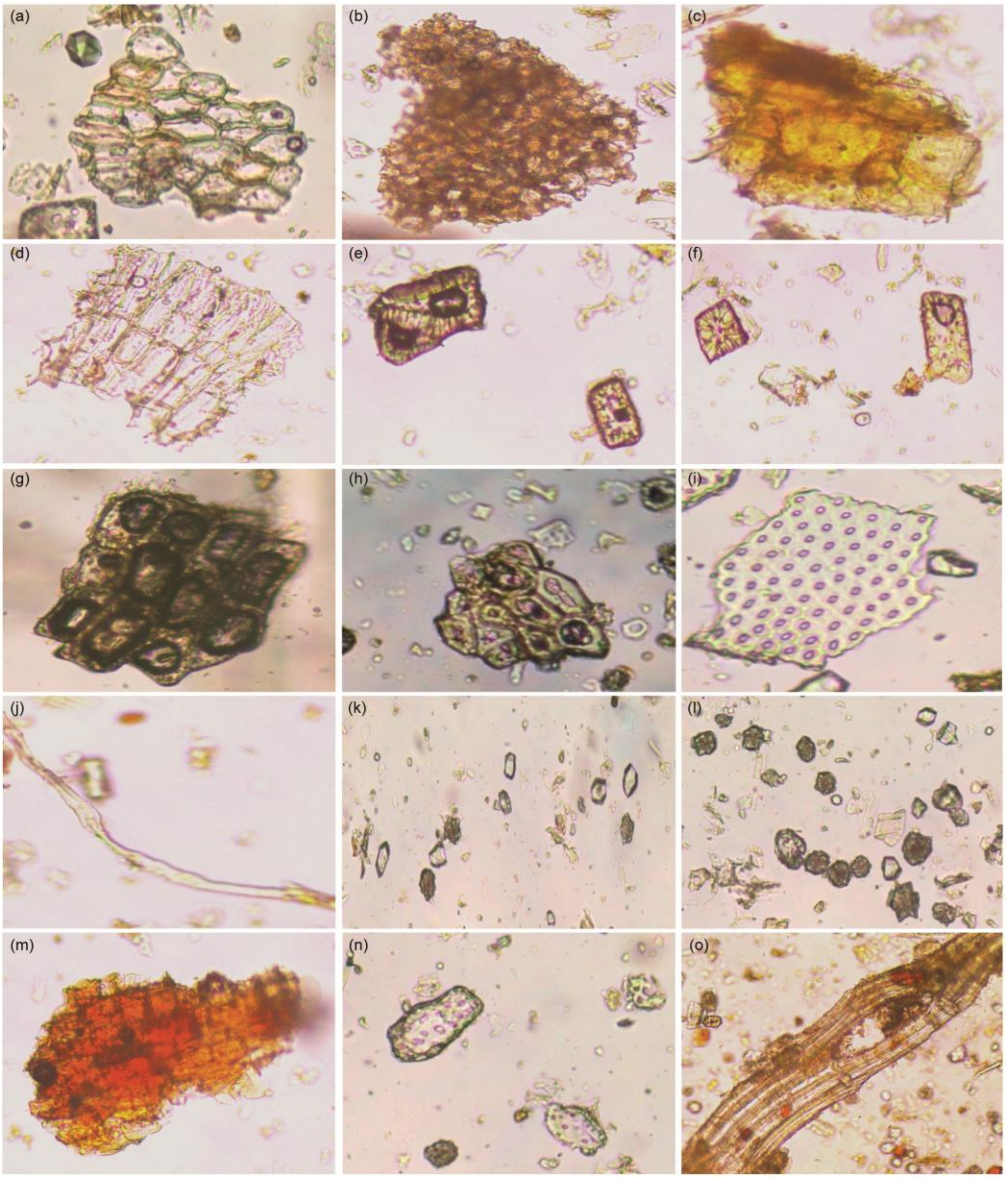

Fig. 2 — Powder microscopy of PKC formulation, (a-c) Cork cells; (d) Cortex cells; (e-h) Stone cells; (i) Pitted vessels; (j) Fiber; (k) Prismatic calcium oxalate crystals; (l) Rosette crystals; (m) Tannin contents; (n) Pitted sclerides; (o) Medullary rays)

Physicochemical analysis

The results were represented in (Table 1).

Heavy metal analysis and safety parameters

The PKC formulation was evaluated for heavy metal content (lead, arsenic, mercury and cadmium), pesticide residues, aflatoxins (B1, B2, G1, G2) and other safety parameters specified in the Ayurvedic Pharmacopoeia of India, *i.e.*, microbial load and specific pathogens (*Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*) to assess its compliance with the prescribed quality standards. The heavy metal levels were found to be within the permissible limits specified in the API. The results of the tested safety parameters also complied with the prescribed API specifications, indicating that PKC formulation met the established safety requirements. (Supplementary Table S1-S5)

Elemental analysis

The elemental analysis revealed that potassium (7582.86±470 mg/kg) constitutes the major portion of PKC formulation, followed by sodium (527.9±17 mg/kg), iron (116.12±9 mg/kg), zinc (104.54±12 mg/kg) and strontium (75.64±7 mg/kg).

Phytochemical analysis

It revealed that ethanolic extract of PKC is rich in phenols and flavonoids. Saponins, tannins, proteins and Carbohydrates were present only in aqueous extract.

Estimation of secondary metabolites

The quantitative estimation of total tannins, flavonoids and phenolic compounds was carried out using standard methods. The contents of total tannins, flavonoids and phenols were found to be 3.35±0.95% w/w, 8.91±1.05% w/w, 29.03±2.35% w/w, respectively. Among the estimated phytoconstituents, total phenolic content was found to be the highest, followed by flavonoids and tannins.

HPTLC analysis

In PKC formulation TLC pattern showed six visible bands at UV 254 nm, out of which, band at R_f 0.12 is comparable with standard caffeic acid, band at R_f 0.29 is comparable with standard gossypol. Similarly at UV 366 nm, 11 bands were appeared and out of which band at R_f 0.11 is comparable with standard caffeic acid and R_f 0.28 is comparable with standard gossypol. Further, comparison of R_f values of individual crude drugs with PKC at 254 nm and 366 nm confirmed the presence of *Vata*, *Udumbara*, *Ashwatha*, *Plaksha* and *Parisha* in PKC formulation. Results are represented in (Table 2-3 & Fig. 3-5).

Quantitative HPTLC analysis

The linearity of caffeic acid, gossypol is confirmed by the use of an equation for linear regression and correlation coefficient. The curve used for calibration demonstrated linearity within the concentration range of 25-400 ng/band, with r^2 of 0.999513 for caffeic acid and 2-12 µg/band, with $r^2$of 0.999569 for gossypol. Amount of caffeic acid, gossypol present in the PKC formulation was effectively measured using the established HPTLC technique. The quantifiable amount of caffeic acid, gossypol was found to be 69.41 µg/100 mg and 1.186 mg/100 mg

Table 1 — Physicochemical properties of PKC formulation

Sl. No.	Test Parameter	Result (in % W/W)			
		Batch-I	Batch-II	Batch-III	Mean ±SD
1.	Loss on Drying	10.02	10.45	10.52	10.33±0.22
2.	Total Ash	5.17	4.90	5.58	5.21±0.27
3.	Acid insoluble ash	0.492	0.396	0.395	0.427±0.04
4.	Alcohol soluble extractive	13.20	13.55	13.74	13.49±0.22
5.	Water soluble extractive	8.43	7.64	9.33	8.47±0.69
6.	pH (10% Aqueous suspension)	4.97	5.15	5.45	5.19±0.19
7.	Volatile oil	0.18	0.04	0.10	0.10±0.05

Table 2 — R_f Values of PKC-I, II & III, Markers and ingredients at 254 nm

Track 1	Track 2	Track 3	Track 4	Track 5	Track 6	Track 7	Track 8	Track 9	Track 10
PKC-I	PKC-II	PKC-III	Caffeic acid	*Vata*	*Ashwatha*	*Udumbara*	*Plaksha*	*Parisha*	Gossypol
0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	--	--
0.20	0.20	0.20	--	0.20	0.20		0.20	--	--
0.24	0.24	0.24	--	0.24	0.24	0.24	--	--	--
0.29	0.29	0.29	--	--	--	--	--	0.29	0.29
0.78	0.78	0.78	--	--	--	--	0.78	--	--
0.90	0.90	0.90	--	0.90	0.90	0.90	0.90	--	--

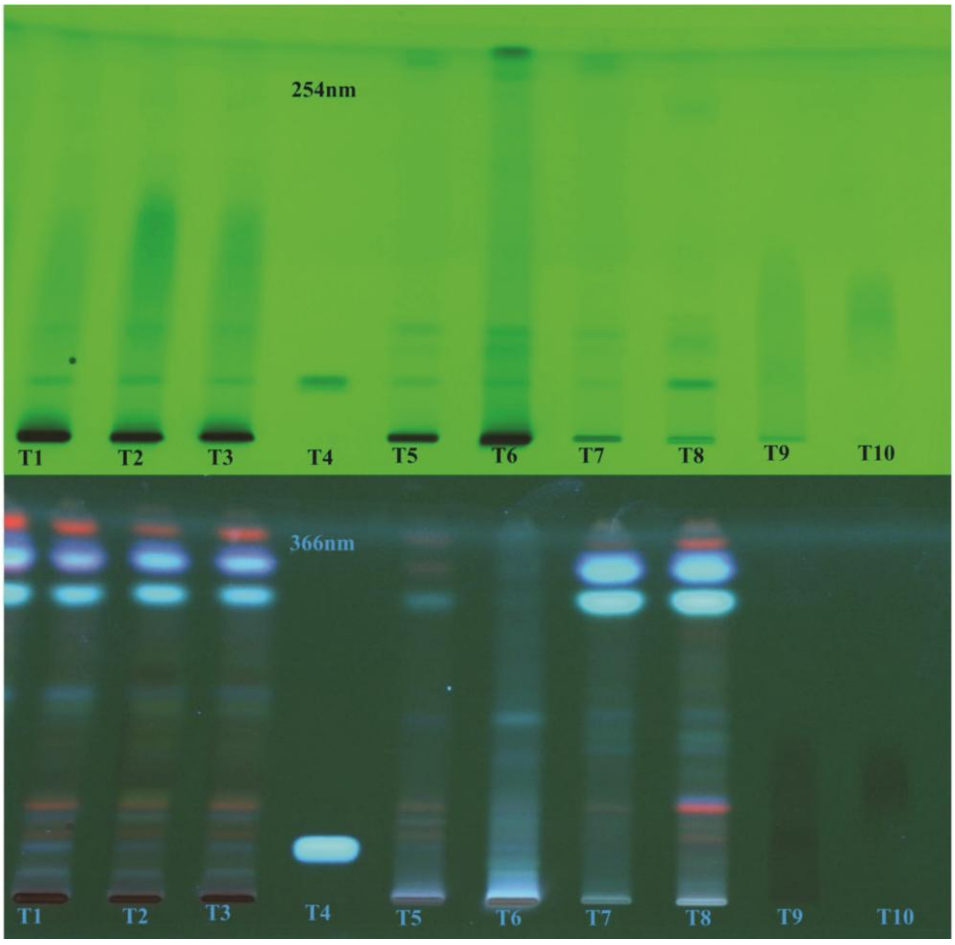

Fig. 3 — Comparative HPTLC of PKC formulation, raw drugs and markers (Solvent system: toluene: ethyl acetate: formic acid-6.5:3.5:0.2 v/v/v); Track-1 to 3: PKC (Batch-I to III)-10 µL, Track-4:Caffeic acid-3 µL, Track-5: *Vata*-10 µL, Track-6: *Ashwatha*-10 µL, Track-7: *Udumbara*-10 µL, Track-8: *Plaksha*-10 µL, Track-9: *Parisha*-10 µL, Track-10: Gossipol- 3 µL

Table 3 — R_f Values of PKC-I, II & III, Markers and ingredients at 366 nm

Track 1	Track 2	Track 3	Track 4	Track 5	Track 6	Track 7	Track 8	Track 9	Track 10
PKC-I	PKC-II	PKC-III	Caffeic acid	*Vata*	*Ashwatha*	*Udumbara*	*Plaksha*	*Parisha*	Gossypol
0.05	0.05	0.05	--	--	--	--	0.05	--	--
0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	--	--
0.14	0.14	0.14	--	0.14	--	--	0.14	--	--
0.19	0.19	0.19	--	0.19	--	--	0.19	--	--
0.22	0.22	0.22	--	0.22	--	0.22	0.22	--	--
0.28	0.28	0.28	--	--	--	--	--	0.28	0.28
0.51	0.51	0.51	--	0.51	0.51	0.51	0.51	--	--
0.76	0.76	0.76	--	0.76	0.76	0.76	0.76	--	--
0.83	0.83	0.83	--	0.83	--	--	--	--	--
0.85	0.85	0.85	--	--	--	0.85	0.85	--	--
0.93	0.93	0.93	--	0.93	--	0.93	0.93	--	--

respectively. Results are represented in (Supplementary Fig. S1-S2).

Discussion

Panchvalkal Kwatha Churna (PKC), is a classical Ayurvedic formulation used for treatment of women experiencing diseases *i.e.,* leucorrhea, endometriosis, vaginitis, female infertility and wound healing etc., Nevertheless, to date, comprehensive data for quality control studies pertaining to this formulation remain unavailable. Therefore, the objective of the present study is to establish quality standards for PKC formulation.

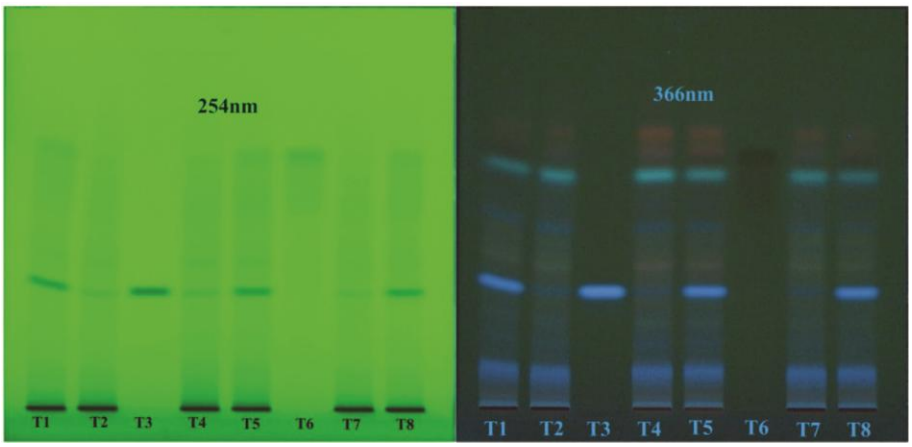

Fig. 4 — HPTLC of PKC formulation and markers (Solvent system:water:acetic acid: n-butanol-1:2:7 v/v/v); Track-1: PKC-I-10 µL, Track-2: PKC-I-5 µL, Track-3: Caffeic acid-3 µL; Track-4: PKC-II-5 µL, Track-5: PKC-II-10 µL, Track-6: Gossypol-3 µL; Track-7: PKC-III-5 µL, Track-8: PKC-III-10 µL

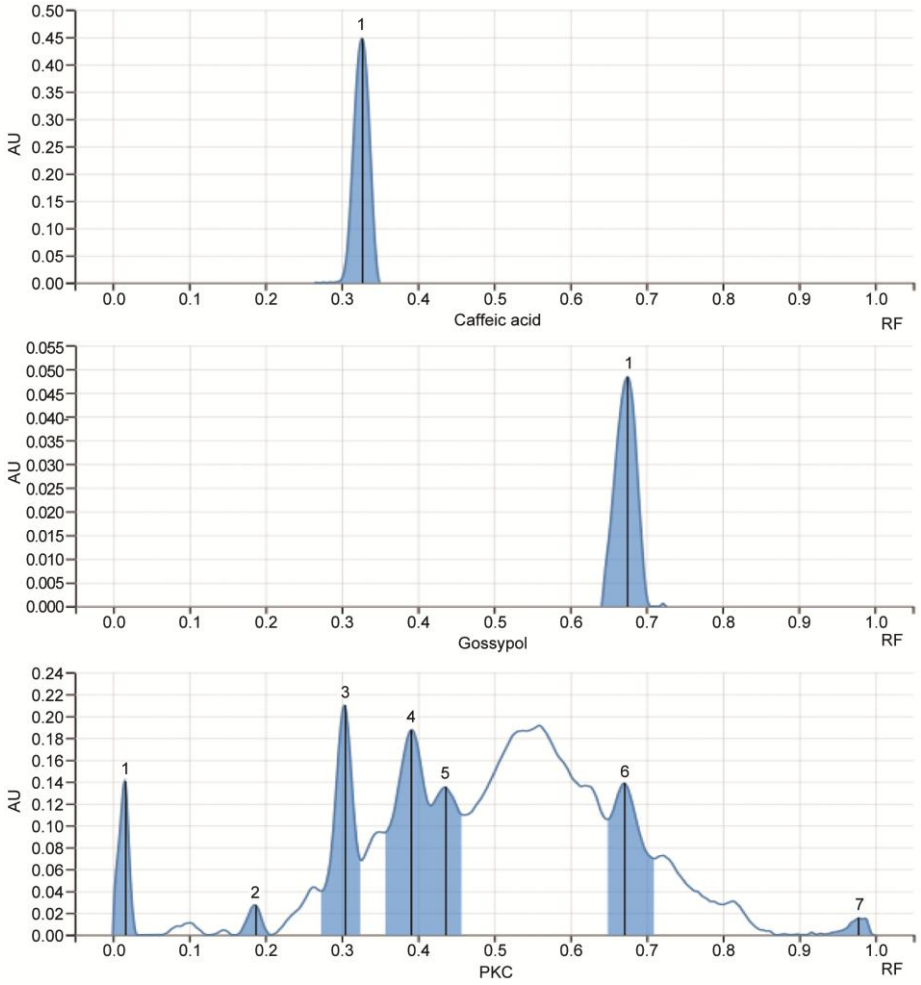

Fig. 5 — HPTLC chromatograms of PKC formulation with markers at 254 nm; (Solvent system: water: acetic acid: n-butanol-1: 2:7 v/v/v)

The observed powder microscopical characters in PKC-batch-I, II and III formulations are almost comparable with powder microscopical characters of raw drugs of *Ashwatha* (stone cells, sclereids) *Plaksha* (hypodermal cells in surface view embedded with small-size rosette crystals), *Vata*- (pitted vessels**)**, *Udumbara* (group of

pitted stone cells), *Parish*a (rosette crystals of calcium oxalate) which are mentioned in Ayurvedic Pharmacopoeia of India (API). Hence it is confirmed that all the three batches of PKC formulations are containing above mentioned drugs. These powder microscopy studies provided key diagnostic features that support for confirming the presence of the constituent drugs and for detecting adulteration. The physicochemical observations indicated the presence of more polar compounds in the extractive values (water-soluble, alcohol-soluble extractives were found to be 8.46% w/w, 13.47% w/w). The pH measurement of 10% aqueous suspension revealed that it is weak acidic in nature. Preliminary phytochemical analysis confirmed that PKC is rich source of phenols, flavonoids. HPTLC analysis was conducted to confirm the presence of *Vata, Ashwatha, Udumbara, Plaksha* and *Parisha* in PKC formulation and also to identify marker compounds namely caffeic acid, which is identified by a blue color band and gossypol, is identified by alight black color band at UV 366 nm, each exhibiting distinct R_f values in the HPTLC profile. The presence of these markers was further validated through HPTLC chromatograms, comparing the peaks of standard compounds with test chromatograms. At UV 366 nm, standard caffeic acid peak with R_f 0.327, standard gossypol peak with R_f 0.706 is comparable with the peaks at same R_f values in PKC. Additionally, fingerprints of individual drugs were developed and compared with the PKC formulation to create corresponding fingerprints for the verification of phytocomponents by correlating them with the separated bands in the HPTLC profiles. Quantitative HPTLC analysis revealed that in PKC, gossypol (1.186%; n=3) is present in major proportions followed by caffeic acid (0.06941%; n=3). Elemental analysis revealed that potassium constituted the major elemental component, followed by sodium, iron, zinc, and strontium. The presence and relative distribution of these elements provide additional information regarding the elemental profile of PKC formulation and it is useful as a supplementary quality-control parameter and can contribute to establishing a comprehensive analytical profile of PKC formulation. In addition to identity and chemical characterization, the safety profile of PKC formulation was evaluated through assessment of heavy metals, aflatoxins, microbial load, specific pathogens, and pesticide residues. The results for all these parameters were found to be within the prescribed permissible limits of Ayurvedic Pharmacopoeia of India. The absence of microbial contamination and specific pathogens at levels exceeding the specified limits, together with compliance for aflatoxins, pesticide residues, and heavy metals, indicates that the formulation meets the evaluated safety requirements.

Conclusions

The present study established comprehensive quality-control and standardization parameters for Panchavalkal Kwatha Churna. Powder microscopy revealed key diagnostic characters supporting the authentication of the formulation, while preliminary phytochemical screening demonstrated a diverse phytochemical profile, with phenols and flavonoids prominent in the ethanolic extract. HPTLC analysis confirmed caffeic acid and gossypol as identifiable markers, and their quantification provided additional parameters for assessing formulation consistency. Collectively, these findings mark the inaugural effort to document quality standards for Panchavalkal Kwatha Churna, and the methodologies established herein can serve as a valuable resource for its authentication, quality control and batch-to-batch consistency.

Supplementary Data

Supplementary data associated with this article is available in the electronic form at https://nopr.niscpr.res.in/jinfo/ijtk/IJTK_25(9)(2026)862-869_SupplData.pdf

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Author Contributions

CVN, SM, RB, YG conceptualization, drafting. SV, DD, MD, DD assessment and refinement. AKM, GB, RS, NS, RA project management.

Conflicts of Interest

No conflict of interest.

Ethics approval

Not applicable.

Use of artificial intelligence (AI)

The authors declare that Artificial Intelligence (AI) assisted tools were not used for the generation, analysis or interpretation of research data, nor for drawing scientific conclusions.

Data Availability

The information utilized to substantiate the findings of this study is accessible through the corresponding author and can be shared upon reasonable request.

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