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Research Article

PHYSICOCHEMICAL AND PHYTOCHEMICAL ANALYSIS OF SIDDHA POLYHERBAL FORMULATION - *SAARANAI MOOLI KUZHAMBU*

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ABSTRACT

The standardization of Siddha polyherbal preparation using modern scientific methods to ensure safety, efficacy and consistency is now mandatory for global acceptance. *Saaranai Mooli Kuzhambu (SMK)* is one among the Siddha polyherbal preparations indicated for *Paandu* (anemia), which is referred in *"Pathinen Siddhargal Vaithiya Sillarai Kovai* Part II". The present study was designed to evaluate a comprehensive standardization profile for *SMK*, covering the physicochemical, phytochemical parameters, TLC/HPTLC fingerprinting and screening for aflatoxins, pesticide residue and microbial contamination. Physicochemical analysis showed a total ash of 2.40%, loss on drying of 65.95% and a pH of 4.85. Phytochemical screening confirmed the presence of phenols, tannins, flavonoids, triterpenoids, glycosides, quinones and anthraquinones. HPTLC analysis at 254nm and 520nm established a distinct fingerprint profile with 14 and 13 peaks, respectively, indicating the presence of various phytoconstituents. Safety evaluations revealed that aflatoxins (B1, B2, G1, G2), pesticide residues, and microbial counts were all within the permissible limits as per PLIM standards. From the outcome of results, this study shows how modern standardization methods can improve the reliability and acceptance of polyherbal formulations at the global level. The findings confirm that *SMK* is safe and nontoxic when administered at the recommended dose.

INTRODUCTION

The Siddha system consists of polyherbal, herbo-mineral and metallic preparations. The drug sources are obtained from plants, minerals, metals and animals. *Saaranai Mooli Kuzhambu (SMK)* is one such polyherbal formulation which is mentioned in classical *Siddha* literature, "*Pathinen Siddhargal Vaithiya Sillarai Kovai* Part II," and it is indicated for *Paandu* (anaemia). Anaemia is a common condition in which the body does not have enough red blood cells or haemoglobin to carry oxygen efficiently. According to WHO, anaemia affects about 1.9 billion people worldwide. Due to its high prevalence and impact on health, anaemia remains an important area of research and prevention.

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In this study, *SMK* was filtered for standardization procedures as per the respective testing guidelines. This present study aims to standardize *SMK* by assessing its organoleptic characters, physicochemical and phytochemical parameters, along with TLC/ HPTLC analysis, aflatoxin limit analysis, pesticides residue and microbial limit test were evaluated.

MATERIALS AND METHODS

Identification and Authentication

The raw drugs were purchased from the reputed drug store in Chennai. All the raw drugs were identified and authenticated by the *Gunapadam* experts, Government Siddha Medical College, Arumbakkam, Chennai. The specimen samples were numbered and kept in the Department for future reference.

Table 1. Ingredients of *SMK*

S.No	Ingredients	Botanical Name	Part used	Quantity
1	*Saaranai*	*Trianthema portulacastrum*	Root	2 *Palam* (70 g)
2	*Thippili*	*Piper longum*	Fruit	6 *Palam* (210 g)
3	*Inji Saaru* (juice of ginger)	*Zingiber officinale*	Rhizome	Qs
4	*Vellaattu Paal* (goat's milk)	*Capra hircus*	Milk	Qs

Purification of Ingredients

The ingredients in *SMK* are purified as per the method mentioned in the *Siddha* literature, *Sarakkugalin Suthi Sei Muraigal*[1].

Method of preparation

The *Saaranai* root was cleaned and cut into small pieces. It was then boiled in goat's milk for a sufficient duration to ensure proper processing. After boiling, the root was taken out and dried completely. Once dried, it was ground into a fine powder using an iron mortar and pestle. *Thippili* was also purified and ground into a fine powder. Both powders were mixed well. Then, ginger juice was slowly added and mixed until it became a semisolid consistency resembling *Kuzhambu* was obtained.

Drug Profile

- Form of the medicine: *Kuzhambu*
- Route of administration: Oral
- Dosage: *1 Kazhanju* (5.1 g)
- Indication: *Gunmam, Soolai, Vaadham, Kuthulaichal, Paandu.*

Standardization of *SMK*

The standardization and analytical evaluation of polyherbal formulations ensure their quality, safety and therapeutic effectiveness. The following parameters are commonly assessed;

1. Organoleptic characters
2. Physicochemical analysis
3. Phytochemical analysis
4. TLC/HPTLC
5. Aflatoxin limit analysis
6. Pesticide residue
7. Microbial limit test

Physicochemical analysis of *SMK*

In Siddha, physicochemical analysis is used to evaluate the physical and chemical properties of polyherbal formulation to ensure their quality, purity and consistency. All the following physicochemical parameters were carried out as per the standard test procedures (Lohar DR. protocol, PLIM; 2008)[2].

- Loss on drying
- Total ash
- Water-soluble ash
- Acid-insoluble ash
- Water-soluble extractive
- Alcohol soluble extractive
- pH

Phytochemical analysis of SMK

Phytochemical tests were carried out at Siddha Central Research Institute, Chennai. All the following phytochemical parameters were carried out as per the standard test procedures (Harborne JB. Phytochemical methods, Chapman and Hall, London, 1973)[3].

- Phenol
- Tannin
- Flavonoids
- Triterpenoids
- Proteins
- Glycosides
- Reducing sugar
- Quinones
- Anthraquinone
- Alkaloids
- Saponins
- Cardiac glycoside
- Steroids
- Coumarin
- Acids
  - TLC/HPTLC analysis
  - Aflatoxin limit analysis method
  - Pesticide residue analysis method
  - Microbial limit test

RESULT AND DISCUSSION

Organoleptic characters of *SMK*

In Siddha medicine, organoleptic characters are an important part of drug evaluation, mainly used for preliminary identification and quality assessment of raw drugs and formulations.

Table 2: Organoleptic Characters of *SMK*

S.no	Parameter	Result
1	State	Semisolid
2	Appearance	Brownish green
3	Nature	Viscous
4	Odour	Characteristic
5	Flow property	Non-free flowing

Physicochemical analysis of *SMK*

The physicochemical studies were carried out at Siddha Central Research Institute, Chennai.

The physicochemical analysis showed a loss on drying value of 65.95%, indicating the presence of moisture content or volatile compounds. The total ash value was 2.40%, which reflects the overall inorganic residue remaining after incineration and suggests a low level of total mineral content. The water-soluble ash and acid-insoluble ash were 0.38% and 0.75% respectively. The water-soluble extractive value was 7.18% and the alcohol soluble extractive value was 7.69%, indicating the presence of soluble constituents in both water and alcohol. The pH of the sample was 4.85, showing that it is slightly acidic. Overall, these parameters help in assessing the quality, purity and consistency of the sample.

Table 3: Results of Physicochemical Analysis

S. No	Experiment	I	II	Mean
1	Loss on drying	65.29	66.61	65.95
2	Total ash	2.35	2.46	2.40
3	Water-soluble ash	0.38	0.38	0.38
4	Acid insoluble ash	0.69	0.81	0.75
5	Water soluble extractive	7.14	7.23	7.18
6	Alcohol soluble extractive	7.72	7.66	7.69
7	pH	4.85	4.85	4.85

Phytochemical analysis of *SMK*

The phytochemical tests were carried out at Siddha Central Research Institute, Arumbakkam, Chennai. The phytochemical screening of the sample revealed the presence of phenols, tannins, flavonoids, triterpenoids, glycosides, quinones, and anthraquinones. These compounds are often associated with various biological activities. On the other hand, proteins, reducing sugars, alkaloids, saponins, cardiac glycosides, steroids, coumarins, and acids were found to be absent. This profile indicates that the sample mainly contains phenolic and glycosidic compounds, which may contribute to its therapeutic properties.

Table 4. Results of Phytochemical Analysis

S.No	Phytochemical test	Result
1	Test for phenol	Present
2	Test for tannin	Present
3	Test for flavonoids	Present
4	Test for triterpenoids	Present
5	Test for proteins	Absent
6	Test for glycosides	Present
7	Test for reducing sugar	Absent
8	Test for quinones	Present
9	Test for anthraquinone	Present

10	Test for alkaloids	Absent
11	Test for saponins	Absent
12	Test for cardiac glycoside	Absent
13	Test for steroids	Absent
14	Test for coumarin	Absent
15	Test for acids	Absent

TLC/HPTLC Analysis

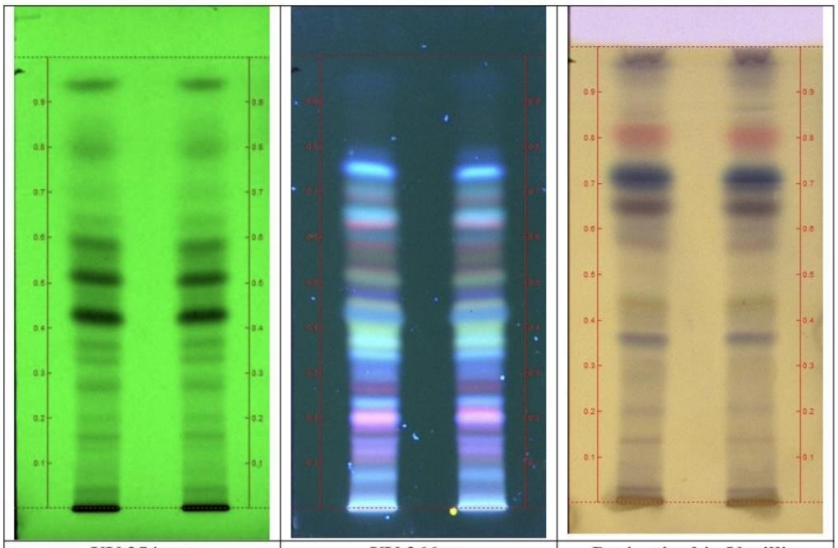
Sample preparation: 1gm of sample was dissolved in 10ml of ethanol and then sonicated for 15 minutes and filtered. The filtrate was used for HPTLC analysis.

Thin-layer chromatography Methodology

Applied 10µl of ethanol extract of *Saaranai mooli kuzhambu* on TLC plate using Camag's ATS4 applicator and developed by the mobile phase consisting of Toluene: Ethyl acetate: Formic acid (7.5:1.5:0.5, v/v/v) up to 8.5cm distance. After development, the plate was photo documented using Camag's TLC visualizer under UV 254nm and UV 366nm and then scanned using Camag's Scanner 4 at (D2 lamp/Absorption mode) finger print profiles of the extract were documented at 254nm. Then the plate was dipped in 5% vanillin sulphuric acid reagent followed by heating at 105°C until the development of colored spots. The plate was then photo documented in white light and scanned at 520nm for finger print profile.

Fig 1. TLC Photo documentation of *SMK*

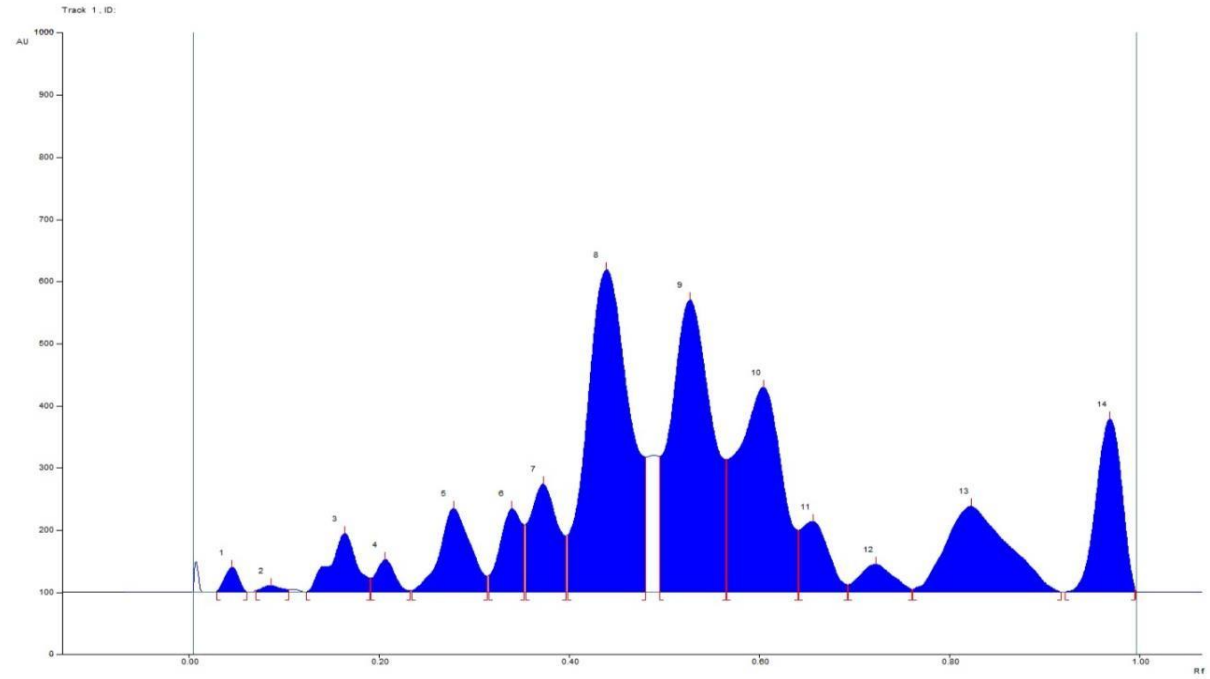
TLC Photodocumentation



UV 254 nm		UV 366nm		Derivatized in Vanillin Sulphuric acid	
Colour	R_f value	Colour	R_f value	Colour	R_f value
Green	0.05	Blue	0.08	Purple	0.03
Green	0.16	Purple	0.13	Pale Purple	0.14
Green	0.21	Pink	0.19	Blue	0.21
Green	0.28	Blue	0.23	Purple	0.36
Green	0.33	Red	0.27	Yellow	0.44
Green	0.37	Blue	0.32	Brown	0.56
Green	0.44	Green	0.37	Dark blue	0.63
Green	0.51	Fluorescent blue	0.44	Dark blue	0.70
Green	0.59	Purple	0.48	Rose red	0.80
Green	0.64	Green	0.51	Brown	0.86
Green	0.70	Green	0.55	Purple	0.94
Green	0.79	Red	0.58		
Green	0.92	Red	0.63		
		Fluorescent blue	0.66		
		Green	0.71		
		Fluorescent blue	0.76		
		Purple	0.92		

Fig 2. HPTLC fingerprint profile and Peak table at 254 nm

HPTLC finger print profile at 254 nm

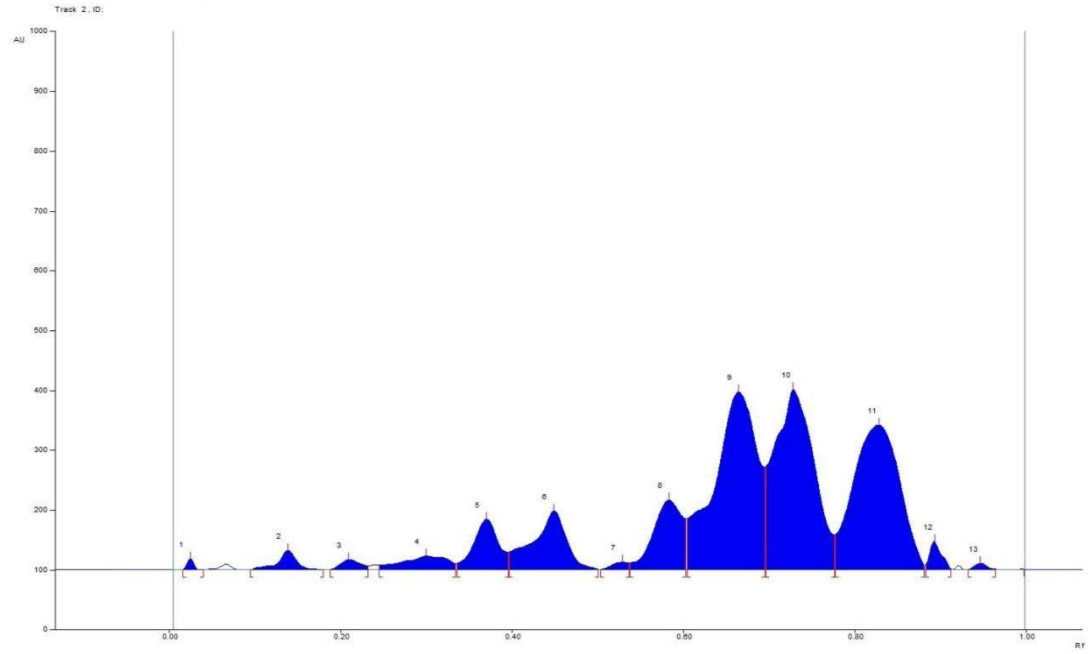


Peak table at 254 nm

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.03 Rf	0.6 AU	0.05 Rf	39.3 AU	1.55 %	0.06 Rf	0.1 AU	486.8 AU	0.58 %
2	0.07 Rf	1.9 AU	0.09 Rf	10.8 AU	0.43 %	0.11 Rf	3.9 AU	180.2 AU	0.21 %
3	0.12 Rf	0.1 AU	0.16 Rf	94.4 AU	3.73 %	0.19 Rf	22.3 AU	2301.5 AU	2.73 %
4	0.19 Rf	22.3 AU	0.21 Rf	51.8 AU	2.05 %	0.23 Rf	2.0 AU	881.1 AU	1.05 %
5	0.23 Rf	2.1 AU	0.28 Rf	134.2 AU	5.30 %	0.31 Rf	25.8 AU	3753.2 AU	4.46 %
6	0.32 Rf	26.5 AU	0.34 Rf	134.2 AU	5.30 %	0.35 Rf	08.2 AU	2686.3 AU	3.19 %
7	0.35 Rf	108.4 AU	0.37 Rf	174.0 AU	6.87 %	0.40 Rf	90.1 AU	4409.0 AU	5.23 %
8	0.40 Rf	91.1 AU	0.44 Rf	518.9 AU	20.49 %	0.48 Rf	17.3 AU	20137.2 AU	23.91 %
9	0.50 Rf	218.4 AU	0.53 Rf	470.2 AU	18.57 %	0.57 Rf	12.9 AU	17583.3 AU	20.87 %
10	0.57 Rf	213.1 AU	0.61 Rf	329.6 AU	13.02 %	0.64 Rf	99.2 AU	13413.8 AU	15.92 %
11	0.64 Rf	99.5 AU	0.66 Rf	113.6 AU	4.49 %	0.69 Rf	11.7 AU	2876.8 AU	3.42 %
12	0.69 Rf	11.8 AU	0.72 Rf	44.2 AU	1.75 %	0.76 Rf	3.9 AU	1355.0 AU	1.61 %
13	0.76 Rf	4.0 AU	0.82 Rf	138.0 AU	5.45 %	0.92 Rf	0.1 AU	7740.8 AU	9.19 %
14	0.92 Rf	0.2 AU	0.97 Rf	278.7 AU	11.01 %	1.00 Rf	7.9 AU	6427.6 AU	7.63 %

Fig 3. HPTLC fingerprint profile and Peak table at 520 nm

HPTLC finger print profile at 520 nm



Peak table at 520 nm

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.02 Rf	0.1 AU	0.03 Rf	18.0 AU	1.39 %	0.04 Rf	0.0 AU	123.3 AU	0.26 %
2	0.09 Rf	0.1 AU	0.14 Rf	31.8 AU	2.45 %	0.18 Rf	0.1 AU	587.3 AU	1.22 %
3	0.19 Rf	0.1 AU	0.21 Rf	16.5 AU	1.27 %	0.23 Rf	6.7 AU	324.1 AU	0.67 %
4	0.25 Rf	7.6 AU	0.30 Rf	22.7 AU	1.75 %	0.33 Rf	10.4 AU	1031.7 AU	2.14 %
5	0.34 Rf	10.6 AU	0.37 Rf	84.3 AU	6.50 %	0.40 Rf	29.0 AU	2120.7 AU	4.40 %
6	0.40 Rf	29.3 AU	0.45 Rf	98.1 AU	7.57 %	0.50 Rf	0.2 AU	3315.6 AU	6.89 %
7	0.50 Rf	0.1 AU	0.53 Rf	12.3 AU	0.95 %	0.54 Rf	11.6 AU	206.2 AU	0.43 %
8	0.54 Rf	11.8 AU	0.58 Rf	116.4 AU	8.98 %	0.60 Rf	85.2 AU	3426.2 AU	7.12 %
9	0.61 Rf	85.3 AU	0.67 Rf	296.7 AU	22.89 %	0.70 Rf	71.5 AU	12832.7 AU	26.65 %
10	0.70 Rf	172.2 AU	0.73 Rf	301.0 AU	23.22 %	0.78 Rf	58.5 AU	12030.5 AU	24.98 %
11	0.78 Rf	59.1 AU	0.83 Rf	241.9 AU	18.66 %	0.88 Rf	6.8 AU	11469.8 AU	23.82 %
12	0.88 Rf	7.1 AU	0.89 Rf	46.5 AU	3.59 %	0.91 Rf	0.7 AU	555.5 AU	1.15 %
13	0.93 Rf	0.0 AU	0.95 Rf	10.1 AU	0.78 %	0.97 Rf	0.7 AU	128.2 AU	0.27 %

Aflatoxin Limit Analysis[4,5]

The aflatoxin limit analysis for SMK has revealed that all tested subtypes (B1, B2, G1, and G2) were Below the Limit of Quantification (BLQ), specifically less than 0.25 ppb. The formulation complies with the Ayurvedic Pharmacopoeia of India (API) standards, which mandate limits of < 2 ppm for B1 and < 5 ppm for total aflatoxins, affirming the sample's safety regarding mycotoxin contamination.

Table 5. Aflatoxin limit results

Parameter	Observed level	Permissible Limit (API)
Aflatoxin B1	BLQ	< 2 ppb
Total Aflatoxins (B1+B2+G1+G2)	BLQ	< 5 ppb

Table 6. Test for Pesticide residue analysis[6]

Substance (s)	Results (In ppb)	Permissible Limit prescribed in API (In ppb)
Alachlor	3.716	20
Aldrin and Dieldrin (sum of)	4.719	50
Azinphos-methyl	111.652	1000
Bromopropylate	Not detected	3000
Chlordane (sum of cis-, Trans, and oxythlordane)	Not detected	50
Chlorfenvinphos	Not detected	500
Chlorpyrifos	Not detected	200
Chlorpyrifos-methyl	Not detected	100
Cypermethrin (Isomers)	15.242	1000
DDT (sum of p, p-DDT, o, p, p-DDE and p,p-TDE)	Not detected	1000
Deltamethrin	228.781	500
Diazinon	Not detected	500
Endosulfan (sum of isomers and endosulfan sulfate)	Not detected	3000
Endrin	3.635	50
Ethion	120.014	2000
Fenitrothion	2.288	500
Fenvalerate	1.502	1500
Fonofos	13.896	50
Heptachlor (sum of heptachlor and heptachlorepoxide)	Not detected	50
Hexachlorobenzene	Not detected	100
Lindane	3.789	600
Malathion	Not detected	1000
Parathion	10.528	500
Parathion-methyl	4.643	200
Permethrin	18.977	1000
Phosalone	86.362	100
Piperonyl butoxide	280.428	3000
Primiphos-methyl	Not detected	4000

Microbial limit Test

The microbial limit testing for SMK showed a Total Microbial Plate Count of $5.3x10^3$ cfu/g and a Total Yeast and Mold Count of $<10^1$ cfu/g. Specific pathogens including Escherichia coli, Salmonella spp., Staphylococcus aureus and pseudomonas aeruginosa were absent. These findings comply with the API standards (Part I, vol IX), confirming the microbiological safety and quality of the formulation for research and clinical application.

Table 7. Result of Microbial Limit Analysis

S.No	Parameters Tested	Result (cfu/g)	Permissible Limit
1	Total microbial plate count	$5.3*10^3$	Max. $1*10^5$(Topical:10^7)
2	Total yeast and mold count	$< 10^1$	Max. $1*10^3$
3	Escherichia coli	Absent	Absent
4	Salmonella spp.	Absent	Absent
5	Staphylococcus aureus	Absent	Absent
6	Pseudomonas aeruginosa	Absent	Absent

CONCLUSION

The standardization of *SMK*, a traditional *Siddha* polyherbal formulation indicated for *Paandu Noi* (anemia), demonstrates that the drug meets the rigorous quality and safety standards required for therapeutic use. Organoleptic and physicochemical assessments characterized the formulation as a brownish green, viscous semisolid with a slightly acidic pH of 4.85 and a total ash value of 2.40%. Phytochemical screening confirmed the presence of bioactive secondary metabolites, including phenols, flavonoids, tannins and glycosides, which likely contribute to its reported medicinal efficacy. Furthermore, HPTLC fingerprinting established a distinct chromatographic profile for identification, while safety analyses confirmed that aflatoxins, pesticide residues, and microbial counts remain well within the permissible limits prescribed by the PLIM standards. Collectively, these results validate the purity, consistency, and safety of *SMK*, providing a standardized baseline for its clinical application and further pharmacological research.

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