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Research Article

PHYSICOCHEMICAL AND PHYTOCHEMICAL ANALYSIS OF SIDDHA POLYHERBAL FORMULATION *INJI PODI*

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ABSTRACT

Aim: Standardization of Siddha polyherbal formulation is more important to ensure its safety, efficacy and also for global acceptance. This study aims at quality standards and the phytochemical estimation of *Siddha* polyherbal formulation *Inji Podi* (IP) through modern analytical screening. **Methods**: The standardization of *Inji Podi* was carried out in accordance with the guidelines prescribed by the Pharmacopeial Laboratory for Indian Medicine (PLIM). Physicochemical analysis, phytochemical analysis, pesticide residue, microbial load, aflatoxin analysis was carried out as per PLIM guidelines. HPTLC fingerprint profiling of *Inji Podi* (IP) was taken. **Result**: Physicochemical analysis of *Inji Podi* shows the loss on drying (5.21%), the total ash value (4.73%), while acid-insoluble ash (1.05%), water-soluble ash (1.60%). The extractive values, both water-soluble (17.05%) and alcohol-soluble (17.45%). The pH (4.95) indicates a mildly acidic nature. The microbial limit analysis showed that the total microbial plate count (5.9×10^3cfu/g) and total yeast and mold count (5.1×10^2 cfu/g) were within permissible limits for herbal formulations and absence of *Escherichia coli*, *Salmonella spp.*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa.* The pesticide residue analysis demonstrated that most of the tested pesticides were either not detected or present within the permissible limits prescribed by the Ayurvedic Pharmacopoeia of India (API). The aflatoxin analysis revealed that aflatoxins B1, B2, G1, and G2 were below the limit of quantification (BLQ). The HPTLC fingerprint profiling of *Inji Podi* at different wavelengths (254nm and 366nm) indicating the presence of diverse phytoconstituents. **CONCLUSION**: From the set of parameters, it can be concluded that the Siddha polyherbal formulation *Inji Podi* meets standard quality and safety parameters. Physicochemical and microbial analysis was within permissible limits, pesticide residues were acceptable, and aflatoxins were below quantification levels. The presence of key phytoconstituents and a characteristic HPTLC fingerprint support its identity and therapeutic potential. Overall, the formulation is safe, standardized, and suitable for medicinal use.

INTRODUCTION

The *Siddha* system of medicine represents an ancient medical tradition predominantly practiced in southern India. Historical documentation indicates that therapeutic applications of Siddha medicine have been in existence since prior of 10000–4000 BC. This system asserts that every atom comprises "96 principles," so as the human gross body. This system encompasses all dimensions of medicine like physical, physiological, psychological, and intellectual approach in treatment. The primary components of *Siddha* medical formulations are various plant parts, minerals, and animal products [1]. The World Health Organization (WHO) strongly advocates drug standardization to ensure consistent quality, safety, and therapeutic efficacy. Standardization of traditional medicine is important to ensure its safety and efficacy in the global market. Physicochemical analysis, phytochemical screening, microbial load, and pesticide residue were performed as per PLIM guidelines. Coronary heart

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disease is a condition resulting from an imbalance between myocardial oxygen supply and demand, most commonly due to atherosclerotic narrowing of the coronary arteries [2]. The prevalence of coronary heart disease (CHD) in India is estimated to be around 7–13% in urban populations and 2–7% in rural populations, affecting more than 30 million individuals. Multiple polyherbal or Herbo mineral formulations have been traditionally employed for the effective management of chronic diseases, particularly in long-term conditions and non-communicable diseases such as diabetes mellitus, respiratory diseases, skin diseases, coronary heart diseases, etc. One such siddha medicine is *Inji Podi* (IP) which is mentioned in *Theraiyar Tharu* is traditionally indicated for the management of *Maradaippu* referred in modern as coronary heart disease. The Aim of the study is to standardize *Inji Podi* as per PLIM guidelines formulated by the Central Council for Research in Ayurvedic Sciences (CCRAS), Ministry of AYUSH, Government of India.

MATERIALS AND METHODS

Selection of Drugs

The raw drugs were purchased from the reputed drug store in Chennai. All the raw drugs were identified and authenticated by *Gunapadam* experts; Government Siddha Medical College, Chennai and the voucher samples were kept in the department with respective authentication numbers for future reference.

Purification of drugs

All the raw drugs were purified as per the *Siddha* literature *Sarakkugalin Suthi Sei Muraigal* [3].

Table 1: Ingredients of *Inji Podi*

S.No	Vernacular Name	Botanical Name	Used Part	Quantity
1	*Inji*	*Zingiber officinale*	Rhizome	10 grams
2	*Kadugu*	*Brassica juncea*	Seed	20 grams
3	*Seeragam*	*Cuminum cyminum*	Dried fruit	20 grams
4	*Omam*	*Carum copticum*	Fruit	20 grams
5	*Kottam*	*Costus speciosus*	Rhizome	20 grams
6	*Chukku*	*Zingiber officinale*	Rhizome	20 grams
7	*Milagu*	*Piper nigrum*	Fruit	20 grams
8	*Thippili*	*Piper longum*	Fruit	20 grams
9	*Kunguma poo*	*Crocus sativus*	Dried stigma	20 grams
10	*Chittrarathai*	*Alpinia officinaurum*	Rhizome	20 grams
11	*Perungayam*	*Ferula asafoetida*	Dried latex	20 grams
12	*Adhimadhuram*	*Glycyrrhiza glabra*	Root	20 grams
13	*Musumusukai*	*Mukia maderasapatna*	Leaves	QS
14	*Moare*	*Cow's buttermilk*		QS

Standard operating procedure of *Inji Podi*

Ginger was skin peeled and shredded into pieces. *Musumusukkai* juice was taken from their leaves and the peeled ginger pieces was soaked in *Musumusukkai* juice. It was dried and ground into fine powder. All the other ingredients were soaked in *Pallaiaadu* (goat) buttermilk and then dried. They were ground into fine powder and mixed with the ginger powder that was prepared earlier. All the powders were mixed well and sieved to get a fine *Chooranam.* This *Chooranam* was processed by *Pittaviyal* method, dried well and stored in air tight container.

Physicochemical Analysis

Organoleptic Characters

Organoleptic characters are important parameter in drug standardization. Organoleptic characters include colour, odour, texture, taste, etc.

Physiochemical evaluation [4]

Loss on drying

The drug was weighed and kept in the evaporating dish dry at 105°C for 5 hours, and weigh. Continue the drying and weighing at one hour interval until difference between two successive weighing corresponds to not more than 0.25%.

Total Ash

Incinerate about 2 to 3g accurately weighed, of the ground drug in a tared platinum or silica dish at a temperature not exceeding 450°C until free from carbon, cool and weigh. If a carbon free ash cannot be obtained in this way, exhaust the charred mass with hot water, collect the residue on an ashless filter paper, incinerate the residue and filter paper, add the filtrate, evaporate to dryness, and ignite at a temperature not exceeding 450°C. Calculate the percentage of ash with reference to the air-dried drug.

Water soluble ash

Boil the ash for 5 minutes with 25ml of water; collect insoluble matter in a Gooch crucible, or on an ashless filter paper, wash with hot water, and ignite for 15 minutes at a temperature not exceeding 450°C. Subtract the weight of the insoluble matter from the weight of the ash; the difference in weight represents the water-soluble ash. Calculate the percentage of water-soluble ash with reference to the airdried drug.

Acid soluble ash

Boil the ash obtained from the total ash for 5 minutes with 25ml of dilute hydrochloric acid; collect the insoluble matter in a Gooch crucible, or on an ashless filter paper, wash with hot water and ignite to constant weight. Calculate the percentage of acid-insoluble ash with reference to the air-dried drug.

Water soluble extractive

Macerate 5gm of the air-dried drug, coarsely powdered, with 100ml chloroform water of the specified strength in a closed flask for twenty-four hours, shaking frequently during six hours and allowing to stand for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25ml of the filtrate to dryness in a tared flat bottomed shallow dish, and dry at 105°C, to constant weight and weigh. Calculate the percentage of water-soluble extractive with reference to the air-dried drug.

Alcohol soluble extractive

Macerate 5g of the air-dried drug, coarsely powdered, with 100ml of alcohol of the specified strength in a closed flask for twenty-four hours, shaking frequently during six hours and allowing to stand for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25ml of the filtrate to dryness in a tared flat bottomed shallow dish, and dry at 105°C, to constant weight and weigh. Calculate the percentage of alcohol-soluble extractive with reference to the air-dried drug.

pH

Operate the pH meter and electrode system according to the manufacturer's instructions. Standardise the meter and electrodes with 0.05M potassium hydrogen phthalate (pH 4.00) when measuring an acid solution, or with 0.05 M sodium borate when measuring an alkaline solution.

Phytochemical analysis [5]

Test for phenol (Ferric Chloride Test)

Take 1–2ml of extract. Add a few drops of 5% ferric chloride solution. Blue, green, or black coloration indicates phenols.

Test for tannin

Add 1–2ml of extract to water and boil, then filter. Add a few drops of ferric chloride solution. Blue-black or green precipitate indicates tannins.

Test for Flavonoids (Shinoda Test)

Add a small piece of magnesium ribbon to the extract. Add a few drops of concentrated HCl. Pink, red, or orange colour indicates flavonoids.

Test for Triterpenoids (Salkowski Test)

Mix extract with chloroform. Carefully add concentrated H_2SO_4 along the side of the test tube. Reddish-brown interface indicates terpenoids.

Test for proteins (Biuret Test)

Add 1ml of 10% NaOH to extract. Add a few drops of 0.1% $CuSO_4$. Violet colour indicates proteins.

Test for Glycoside

Hydrolyse extract with dilute HCl. Neutralize with NaOH. Perform Fehling's test. Brick-red precipitate indicates glycosides.

Test for Reducing sugar (Fehling's Test)

Mix extract with Fehling's solution A and B. Heat in a water bath. Brick-red precipitate indicates reducing sugars.

Test for Quinones

Add concentrated H_2SO_4 to extract. Red coloration indicates quinones.

Test for Anthraquinones (Borntrager's Test)

Boil extract with dilute H_2SO_4 and filter. Extract filtrate with chloroform. Add ammonia solution. Pink/ red/ violet colour in ammoniacal layer indicates anthraquinones.

Test for Alkaloids (Dragendorff's Test)

Acidify extract with dilute HCl. Add Dragendorff's reagent. Orange or reddish-brown precipitate indicates alkaloids.

Test for Saponins (Foam Test)

Shake extract vigorously with water. Stable persistent froth indicates saponins.

Test for Cardiac glycoside (Keller–Killiani Test)

Add glacial acetic acid containing $FeCl_3$ to extract. Add concentrated H_2SO_4 carefully. Brown ring at interface indicates cardiac glycosides.

Test for Steroids (Salkowski Test)

Mix extract with chloroform. Add concentrated H_2SO_4. Red or yellow coloration indicates steroids.

Test for Coumarins

Add NaOH to extract. Yellow fluorescence indicates coumarins.

Test for Acids

Dissolve extract in water. Add sodium bicarbonate. Effervescence (CO_2 release) indicates carboxylic acids.

Microbial Load [6]

The microbial limit test is performed to determine the total number of microorganisms and to check the absence of harmful pathogens in a sample. A measured amount of the sample is diluted with a

sterile medium and inoculated onto suitable culture media. For bacterial count, the sample is cultured on plate count agar and incubated at 30–35°C for 48–72 hours. For yeast and mold count, it is cultured on Sabouraud dextrose agar and incubated at 20–25°C for 5–7 days. To detect specific pathogens such as *Escherichia coli*, *Salmonella*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*, the sample is enriched and plated on selective media. The number of colonies is counted and expressed as cfu/g or cfu/mL. The sample is considered safe if microbial counts are within limits and pathogens are absent.

Pesticide Residue Analysis [7]

The pesticide residue test is used to detect and measure pesticide residues in food or herbal samples. A powdered or homogenized sample is extracted using acetonitrile. Magnesium sulfate and sodium chloride are added to separate the layers and remove water. The mixture is shaken and centrifuged. The upper organic layer is cleaned using sorbents (like PSA) to remove impurities. The cleaned extract is then analysed using gas chromatography (GC) or liquid chromatography (LC). The amount of pesticide residue is calculated and expressed in mg/kg (ppm) and compared with permissible limits.

Aflatoxin Limit Test [8]

The aflatoxin test is carried out to detect toxic aflatoxins (B_1, B_2, G_1, G_2) in herbal drugs. A powdered sample is extracted using a suitable solvent such as methanol–water. The extract is filtered and purified using clean-up methods like immunoaffinity columns. The purified extract is then analysed by thin-layer chromatography (TLC) or HPLC. In TLC, the sample is spotted on a plate, developed in a solvent system, and observed under UV light, where aflatoxins show fluorescent spots. In HPLC, aflatoxins are detected and quantified using a fluorescence detector. The amount of aflatoxin is calculated by comparing with standards and expressed in µg/kg. The sample passes the test if it is within limits:

- Aflatoxin B_1: Not more than 5 µg/kg
- Total aflatoxins: Not more than 10 µg/kg

Thin Layer Chromatography (TLC)

Sample preparation for TLC

1g 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 ethanol extract of *Inji Podi* on TLC plate using Camag's ATS4 applicator and developed by the mobile phase consists of Toluene: Ethyl acetate: Formic acid (7.5: 1.5:0.5 v/v/v) up to 8.5 cm 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 (D: lamp/Absorption mode) finger print profiles of the extract were documented at 254mm. Then the plate was dipped in 5% vanillin-sulphuric acid reagent followed by heating at 105°C till development of coloured spots. The plate was then photo documented in white light and scanned at 520nm for finger print profile.

RESULTS

Table 2: Organoleptic characters of *Inji Podi*

State	Solid
Appearance	Pale yellow
Nature	Moderately coarse powder
Odour	Characteristic
Flow property	Non free flowing

Table 3: Physicochemical analysis of *Inji Podi*

S.No	Name of the experiment	I	II	Mean
1	Loss on drying	5.45	4.95	5.21
2	Total Ash	4.55	4.92	4.73
3	Water soluble ash	1.55	1.85	1.60
4	Acid insoluble ash	1.3	0.82	1.05
5	Water soluble extractive	16.9	17.2	17.05
6	Alcohol soluble extractive	16.5	18.00	17.45
7	pH	4.95	4.95	4.95

Table 4: Microbial load

S.No	Drug Received	Parameters tested	Unit of Measurement	Result
1		Total microbial plate count	cfu/g	5.9×10^3
2		Total yeast and mould count	cfu/g	5.1×10^2
3	*Inji Podi*	*Escherichia coli*	Absent or Present/g	Absent
4		*Salmonella spp.*	Absent or Present/g	Absent
5		*Staphylococcus aureus*	Absent or Present/g	Absent
6		*Pseudomonas aeruginosa*	Absent or resent/g	Absent

Permissible limits for herbal extracts & powders

Total microbial plate count-Max. 1×10/g. Total yeast and mold count-Max. 1×10/g. *E. coli* Absent, *Salmonella spp*.-Absent, *Staphylococcus aureus* Absent and *Pseudomonas aeruginosa* Absent. For topical use, the limit is 10 per gram.

Permissible limits for plant materials which will be treated before use

Total microbial plate count Max. 1x10/g, Total yeast and mold count Max.1×10/g. E. Coli-Max. 10/g. *Salmonella spp*.-Absent, *Staphylococcus Aureus*-Absent and *Pseudomonas aeruginosa* Absent.

Table 5: Phytochemical analysis of *Inji Podi*

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	Present
8.	Test for Quinones	Present
9.	Test for Anthraquinone	Absent
10.	Test for Alkaloids	Absent
11.	Test for Saponins	Absent
12.	Test for Cardiac glycoside	Present
13.	Test for Steroids	Absent
14.	Test for Coumarin	Present
15.	Test for Acids	Absent

Table 6: Pesticide residue analysis of *Inji Podi*

Substance	Results (in ppb)	Permissible limit prescribed in API (In ppb)
Alachlor	13.241	20
Aldrin and Dieldrin (sum of	6.742	50
*Azinphos-methyl	909.762	1000
*Bromopropylate	Not detected	3000
Chlordane (sum of cis-, Trans-, and Oxythlordane)	Not detected	50
Chlorfenvinphos	Not detected	500
Chlorpyrifos	1.162	200
"Chlorpyrifos-methyl	Not detected	100
Cypermethrin (and Isomers)	34.125	1000
DDT (sum of p,p-DDT, o,p-DDT, p.p-DDE and p.p-TDE)	13.133	1000

Deltamethrin	77.292	500
Diazinon	Not detected	500
*Endosulfan (sum of isomers and endosulfan sulfate	Not detected	3000
*Endrin	5.629	50
Ethion	115.413	2000
Fenitrothion	2.296	500
Fenvalerate	13.463	1500
*Fonofos	35.210	50
Heptachlor (sum of Heptachlor and Heptachlorepoxide)	Not detected	50
Hexachlorobenzene	Not detected	100
*Lindane(x-Hexachlorocyclohexane)	Not detected	600
Malathion	Not detected	1000
*Parathion	18.942	500
*Parathion-methyl	5.775	200
Permethrin	53.056	1000
*Phosalone	67.041	100
Piperonyl Butoxide	242.118	3000
*Pirimiphos-methyl	Not detected	4000

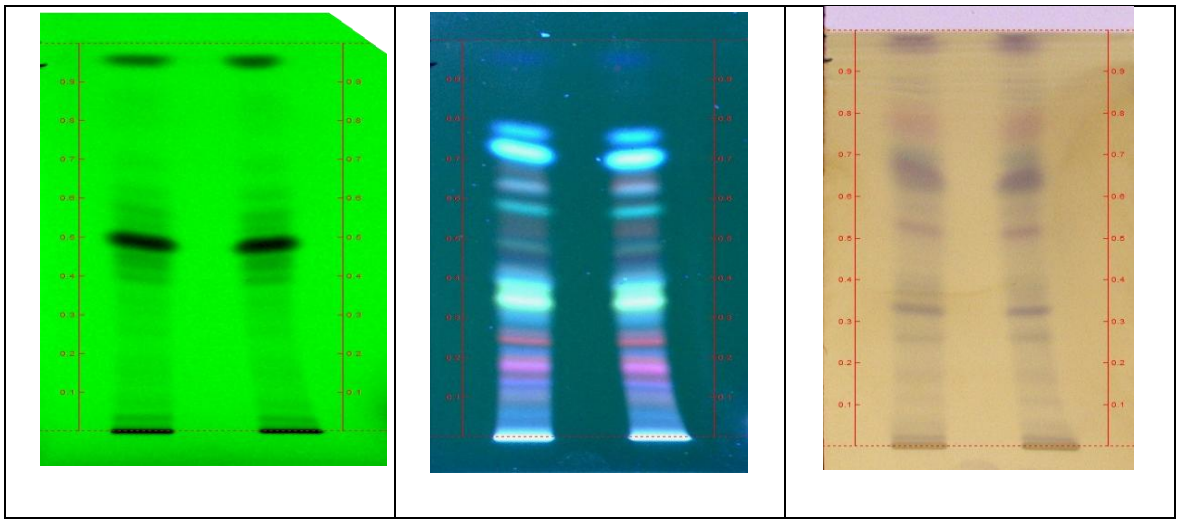
*Under non-NABL

Table 7: Aflatoxin Limit Analysis

S.No	Sample Name	Aflatoxin levels (ng/g)				Sample result
		B1	B2	G1	G2	
1	*Inji Podi*	BLQ	BLQ	BLQ	BLQ	Sample passes the aflatoxin limit test as per API

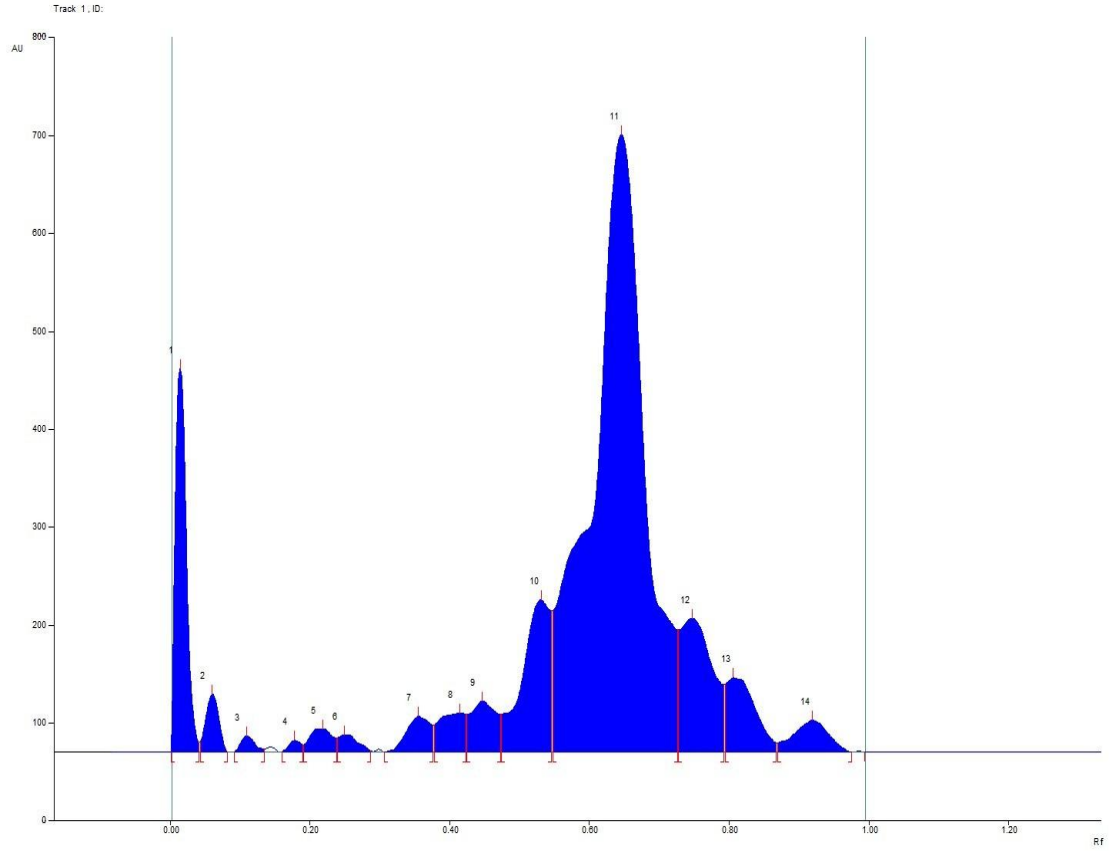
- BLQ: Below Limit of Quantification
- Limit of Quantification: 0.25 ppb for B1, B2, G1 and G2.
- Permissible Limits per API B1: <2 ppb and B1+B2+G1+G2+: <5 ppb

TLC Photo documentation



UV 254 nm		UV 366nm		Derivatized in Vanillin Sulphuric acid	
Color	**Rf value**	**Color**	**Rf value**	**Color**	**Rf value**
Green	0.03	Blue	0.05	Pale ash	0.11
Green	0.07	Blue	0.10	Pale ash	0.16
Green	0.39	Pink	0.18	Pale green	0.27
Green	0.43	Blue	0.22	Purple	0.33
Green	0.48	Red	0.25	Purple	0.51
Green	0.56	Blue	0.30	Pale green	0.56
Green	0.62	Fluorescent blue	0.34	Purple	0.64
		Blue	0.40	Green	0.70
		Green	0.48	Red	0.78
		Pale brown	0.52	Purple	0.95
		Green	0.57		
		Blue	0.64		
		Fluorescent blue	0.72		
		Fluorescent blue	0.76		
		Purple	0.94		

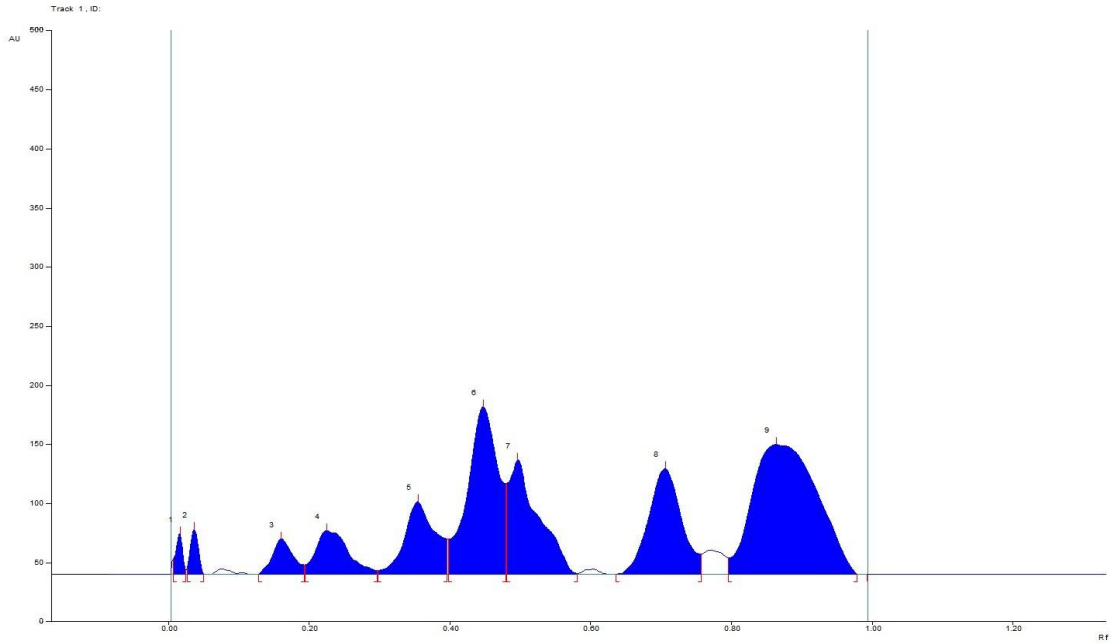
HPTLC Fingerprint profile at 254 nm



Peak table at 254 nm

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	0.0 AU	0.01 Rf	391.4 AU	23.31 %	0.04 Rf	9.4 AU	4425.1 AU	8.11 %
2	0.04 Rf	9.9 AU	0.06 Rf	58.7 AU	3.50 %	0.08 Rf	0.3 AU	756.2 AU	1.39 %
3	0.09 Rf	0.2 AU	0.11 Rf	16.5 AU	0.98 %	0.14 Rf	3.3 AU	228.3 AU	0.42 %
4	0.16 Rf	0.1 AU	0.18 Rf	11.8 AU	0.70 %	0.19 Rf	7.5 AU	139.0 AU	0.25 %
5	0.19 Rf	7.6 AU	0.22 Rf	23.7 AU	1.41 %	0.24 Rf	14.6 AU	533.7 AU	0.98 %
6	0.24 Rf	14.6 AU	0.25 Rf	17.7 AU	1.05 %	0.29 Rf	1.4 AU	335.2 AU	0.61 %
7	0.31 Rf	0.0 AU	0.35 Rf	36.4 AU	2.17 %	0.38 Rf	27.4 AU	853.2 AU	1.56 %
8	0.38 Rf	27.7 AU	0.41 Rf	39.9 AU	2.38 %	0.42 Rf	38.8 AU	1019.0 AU	1.87 %
9	0.43 Rf	38.8 AU	0.45 Rf	52.0 AU	3.10 %	0.47 Rf	38.9 AU	1333.8 AU	2.44 %
10	0.47 Rf	39.0 AU	0.53 Rf	155.3 AU	9.25 %	0.55 Rf	44.1 AU	4343.5 AU	7.96 %
11	0.55 Rf	144.3 AU	0.65 Rf	630.6 AU	37.56 %	0.73 Rf	24.8 AU	32815.0 AU	60.15 %
12	0.73 Rf	124.8 AU	0.75 Rf	136.7 AU	8.14 %	0.79 Rf	68.7 AU	4422.9 AU	8.11 %
13	0.80 Rf	69.2 AU	0.81 Rf	75.8 AU	4.52 %	0.87 Rf	9.3 AU	2234.0 AU	4.09 %
14	0.87 Rf	9.3 AU	0.92 Rf	32.4 AU	1.93 %	0.98 Rf	0.1 AU	1120.8 AU	2.05 %

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	0.01 Rf	13.0 AU	0.02 Rf	34.1 AU	5.35 %	0.03 Rf	3.4 AU	235.7 AU	1.11 %
2	0.03 Rf	5.0 AU	0.04 Rf	37.8 AU	5.93 %	0.05 Rf	0.5 AU	301.6 AU	1.41 %
3	0.13 Rf	0.0 AU	0.16 Rf	29.7 AU	4.67 %	0.19 Rf	7.9 AU	606.6 AU	2.85 %
4	0.19 Rf	8.0 AU	0.22 Rf	36.8 AU	5.78 %	0.30 Rf	3.0 AU	1188.5 AU	5.58 %
5	0.30 Rf	3.1 AU	0.35 Rf	61.3 AU	9.63 %	0.40 Rf	29.6 AU	1860.0 AU	8.73 %
6	0.40 Rf	29.6 AU	0.45 Rf	141.8 AU	22.26 %	0.48 Rf	76.8 AU	4293.7 AU	20.14 %
7	0.48 Rf	76.8 AU	0.50 Rf	96.5 AU	15.14 %	0.58 Rf	0.6 AU	2825.3 AU	13.25 %
8	0.64 Rf	0.2 AU	0.71 Rf	89.4 AU	14.03 %	0.76 Rf	17.1 AU	2894.3 AU	13.58 %
9	0.80 Rf	13.9 AU	0.87 Rf	109.7 AU	17.22 %	0.98 Rf	0.3 AU	7111.0 AU	33.36 %

DISCUSSION

The present study aimed to standardize and evaluate the quality, safety, and phytochemical composition of the Siddha polyherbal formulation *Inji Podi* using organoleptic, physicochemical, microbiological, phytochemical, pesticide residue, aflatoxin, and chromatographic analyses.

The organoleptic evaluation revealed that *Inji Podi* is a pale yellow, moderately coarse, non-free-flowing powder with a characteristic odour. These sensory attributes are important for preliminary identification and ensure batch-to-batch consistency of the formulation.

The physicochemical parameters provide essential information regarding purity and quality. The loss on drying (5.21%) indicates a low moisture content, which is favourable for preventing microbial growth and enhancing shelf life. The total ash value (4.73%) reflects the presence of inorganic components, while acid-insoluble ash (1.05%) suggests minimal contamination with siliceous matter such as sand and soil. Water-soluble ash (1.60%) indicates the presence of water-soluble inorganic salts. The extractive values, both water-soluble (17.05%) and alcohol-soluble (17.45%), suggest the presence of a considerable amount of polar and semi-polar phytoconstituents, which may contribute to therapeutic efficacy. The pH (4.95) indicates a mildly acidic nature, which may influence drug stability and absorption.

The microbial limit analysis showed that the total microbial plate count (5.9×10^3 cfu/g) and total yeast and mold count (5.1×10^2 cfu/g) were within permissible limits for herbal formulations. Furthermore, the absence of pathogenic microorganisms such as *Escherichia coli*, *Salmonella spp.*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* confirms the microbiological safety of the formulation. This indicates adherence to good manufacturing and handling practices.

The preliminary phytochemical screening revealed the presence of important bioactive compounds such as phenols, tannins, flavonoids, triterpenoids, glycosides, reducing sugars, quinones, cardiac glycosides, and coumarins. These phytoconstituents are known for their antioxidant, anti-inflammatory, cardioprotective, and antimicrobial activities, which may justify the traditional therapeutic use of *Inji Podi*. The absence of alkaloids, steroids, saponins, and anthraquinones suggests a selective phytochemical profile.

The pesticide residue analysis demonstrated that most of the tested pesticides were either not detected or present within the permissible limits prescribed by the Ayurvedic Pharmacopoeia of India (API). Although certain compounds such as azinphos-methyl, cypermethrin, and deltamethrin were detected, their concentrations were within acceptable safety limits. This indicates that the formulation is largely free from harmful pesticide contamination and safe for consumption.

The aflatoxin analysis revealed that aflatoxins B1, B2, G1, and G2 were below the limit of quantification (BLQ), confirming that the formulation complies with API standards. The absence of aflatoxins is crucial, as these toxins are potent carcinogens and can pose serious health risks.

The HPTLC fingerprint profiling of *Inji Podi* at different wavelengths (254nm and 366nm) and after derivatization showed multiple peaks with distinct Rf values, indicating the presence of diverse phytoconstituents. The variation in band colours and Rf values confirms the complexity of the formulation and serves as a characteristic fingerprint for identification and quality control. Such chromatographic profiling is essential for detecting adulteration and ensuring consistency in herbal formulations.

Overall, the results of the present study establish that *Inji Podi* possesses acceptable physicochemical properties, is microbiologically safe, free from aflatoxin contamination, and contains significant bioactive compounds. The HPTLC fingerprint further supports its identity and purity. These findings provide a scientific basis for the standardization and quality assurance of this *Siddha* formulation and support its safe therapeutic use.

CONCLUSION

The study confirms that *Inji Podi* meets standard quality and safety parameters. Physico-chemical and microbial analyses were within permissible limits, pesticide residues were acceptable, and aflatoxins were below quantification levels. The presence of key phytoconstituents and a characteristic HPTLC fingerprint support its identity and therapeutic potential. Overall, the formulation is safe, standardized, and suitable for medicinal use.

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