International Journal of Pharmaceutics and Drug Analysis

Content Available at www.ijpda.org ISSN: 2348:8948

STANDARDIZATION OF SIDDHA POLYHERBAL FORMULATION "VALLARAI CHOORANAM"

S.PRIYANKA*[1], S.SWATHI[1], K.SUDHAMATHI PUSHPARAJ[2], R.MENAKA[3], U.CHITHRA[3]

[1] PG Scholar, Department of PG Pothu Maruthuvam, Government Siddha Medical College, Chennai
[2]Head of Department, Department of PG Pothu Maruthuvam, Government Siddha Medical College, chennai
[3]Lecturer, Department of PG Pothu Maruthuvam, Government Siddha Medical College, Chennai

Received: 28 Dec 2025 Revised: 16 Feb 2026 Accepted: 24 Mar 2026

ABSTRACT

Standardization plays a crucial role in ensuring the authenticity, quality and safety of Siddha polyherbal formulations. Vallarai Chooranam is one of the Siddha herbal formulation which is traditionally prescribed in the management of urinary tract infection. The present study was carried out to standardize the formulation and to evaluate its microbial load and aflatoxin contamination in accordance with AYUSH protocols and WHO guidelines. The formulation showed characteristic physicochemical parameters and a well-defined HPTLC fingerprint profile, which may serve as a reference standard for identification and quality control. Preliminary phytochemical screening indicated the presence of important bioactive constituents such as carbohydrates, flavonoids, phenolic compounds, tannins, and phytosterols. Aflatoxinlevels were within the permissible limit and no pathogenic microbial contamination was detected. The findings of this study provide scientific baseline data for quality assessment and support the safe therapeutic use of Vallarai Chooranam.

Keywords: *Vallarai Chooranam, Siddha Medicine, Urinary Tract Infection, Standardization, HPTLC, Microbial load.*

***CORRESPONDING AUTHOR**

S. Priyanka

DOI: https://doi.org/10.47957/ijpda.v14i1.709

PRODUCED AND PUBLISHED BY

South Asian Academic Publications

INTRODUCTION

Siddha medicine is one among the ancient systems of medicine that is predominantly being practiced in South India, which involves a holistic approach to rejuvenate the body and mind. Vallarai chooranam is one of the internal medicines that is mentioned in the textbook "AgasthiyarVaithiya Rathina Churukkam"[1] which is indicated for its therapeutic application for the management of urinary tract infections. Urinary tract infection (UTI) is defined as the presence of microbial pathogens in the urinary tract [2]. The clinical features of UTI are abrupt onset of frequency of micturition and urgency, oliguria, haematuria, dysuria, incontinence, and suprapubic pain during and after voiding,Urine that may appear cloudy and have an unpleasant odor. Traditional formulations such as vallaraichooranam are widely utilized for their anti-inflammatory and anti-microbial activity. Hence, scientific validation and quality standardization are essential to ensure the safety, efficacy, and reproducibility of the formulation by determining the concentration of its active principles, particularly for preparations intended for internal use. Therefore, this drug needs to be standardized to ensure the safety profile and efficacy for internal use. Thus, the present study deals with the preliminary phytochemical screening, physicochemical analysis, High-performance Thin Layer Chromatography(HPTLC), Microbial load and Aflatoxin analysis based on the AYUSH [5] protocol and WHO[6] guidelines to Scientifically validate the siddha formulation "Vallarai Chooranam" to be used in the management of Urinary Tract infection in siddha system of medicine.

MATERIALS AND METHODS

Collection of Drug

Vallarai chooranam consists of 11 ingredients which are procured from a well reputed indigenous raw drug shop. These drugs were authenticated by the Department of Siddha Pharmacology, Government Siddha Medical College, Chennai. Raw drugs were purified as mentioned in "Sikitcha Ratna Deepam Enum Vaithiya Nool" [3] and "Marunthu sei Iyalumkalaium" [4]. Vallarai leaves were washed, steamed with milk, dried, and made into a fine powder. The raw drugs except sugar mentioned in Table 1 were mildly roasted and powdered separately. Sugar was powdered separately and mixed with the other powdered ingredients. The powdered ingredients were sieved in a fine cloth (vasthirakaayam) to obtain fine powder.

Table1: Ingredients of Vallarai Chooranam

Ingredients	Scientific Name	Ratio	Used Parts
Vallarai	Centella asiatica	10 palam	Leaf
Kirambu	Syzygium aromaticum	1 palam	Flower
Elam	Elettaria cardomomum	1 palam	Seed
Jathikkai	Myristica fragrans	1 palam	Fruit
Jathipathiri	Myristica fragrans	1 palam	Aril
Masikkai	Quercus infectoria	1 palam	Fruit
Thalisapathiri	Abies spectabilis	1 palam	Leaf
Kadukkai Thol	Terminalia chebula	1 palam	Fruit
Nellivatral	Phyllanthus emblica	1 palam	Fruit
Thaantrikkai	Terminalia bellirica	1 palam	Fruit
Sarkarai	Saccharum officinarum	Equal quantity (665gm)	

Standardization Parameters

Vallarai Chooranam was subjected to standardization Parameters including physicochemical analysis, HPTLC Finger printing, preliminary Phytochemical analysis, microbial load determination, and Aflatoxin estimation.

Physicochemical Investigations

Physicochemical analysis was performed to determine pH, Total ash, acid-insoluble ash, moisture content, water-soluble extractive value, and alcohol-soluble extractive Value according to AYUSH protocols.

HPTLC Fingerprinting

TLC Methodology

Weigh 4 g of the sample and add 40 mL of distilled methanol. Keep the mixture overnight with occasional shaking. Then, boil it for 10 minutes in a water bath, allow it to cool, and filter. Concentrate the filtrate and make up the volume to 10 mL in a volumetric flask.

Apply 10 µL of the sample solution onto an E. Merck aluminum plate pre-coated with silica gel 60 F254 using a CAMAG automatic sample applicator. Develop the plate in a solvent system composed of Toluene: Ethyl acetate: Formic acid (7.5:3.5:0.01) up to a distance of 80 mm. Dry the plate and observe it under UV light at 254 nm and 366nm using the CAMAG TLC Visualizer. Capture photographs and scan the developed plate.

Dip the plate in vanillin–sulphuric acid reagent and heat it in a hot air oven at 105 °C until colored spots appear. Place the plate in the CAMAG TLC Scanner. Scan all the tracks, integrate the data. Record the fingerprint of each track.

TLC plate was developed using Toluene: Ethyl acetate: Formic acid (7.5: 3.5: 0.01) as mobile phase. After development allow the plate to dry in air, record the finger print and densitometric chromatogram of the two batch samples of the single compound scanned at 254 and 366 nm.

Preliminary Phytochemical Analysis

Preliminary phytochemical analysis was carried out on Alcohol and hydroalcohol extracts using standard qualitative tests.

Microbial Analysis

Microbial Load Determination

The microbial quality, including the isolation and identification of pathogenic bacteria from commercial and homemade herbal medicines, was tested according to the regulations of the WHO standards (2007). The tests were used to quantify the number of bacteria and the fungi isolated that are able to grow aerobically in 1 g of sample. The sample were homogenized by mixing vigorously with H_2O. 1-gram of sample was transferred to 9 mL of peptone broth, then, serial dilutions were made to achieve an appropriate concentration. All microbial analyses were carried out in triplicate. Briefly, serial dilutions were made, and viability was assessed using the pour plate method on Casein soyabean digest agar and Sabouraud dextrose agar for bacterial counts and fungal identification. All dehydrated media were prepared according to the manufacturer's instructions and seeded and incubated at 37 °C for 24 to 48 hours for bacterial screening and at 25 °C for 48 to 72 hours for fungal screening. At the end of the incubation period, the number of colony-

CODEN (USA): IJPDBY

forming units per gram (CFU/g) was calculated by multiplying the average number of colonies by the dilution factor. The obtained CFU/g of sample was compared with WHO standards. Samples that presented bacterial growth greater than 10^5 CFU in 1 g of herbal medicine were considered unsatisfactory or inadequate according to WHO guidelines for aerobic bacteria.

Identification of Bacteria

For bacterial isolation and identification, the samples were diluted in water, and homogenized by vigorously mixing. The 1-mL aliquots were transferred to 9 mL of peptone broth and cultured at the recommended time and temperature. All microbial analyses were carried out in triplicate. For investigating *Escherichia coli*, *Salmonella spp*., *Pseudomonas aeruginosa* and *Staphylococcus aureus* EMB agar, MacConkey agar, Deoxycholate citrate agar, Cetrimide agar and Mannitol salt agar culture media were used respectively. At the end of the incubation period, pathogenic bacterial isolates were preliminarily characterized by colony morphology, Gram staining, and biochemical tests (oxidase, gas and catalase production).

Aflatoxin Test

Aflatoxins are a group of naturally occurring toxins produced by *Aspergillus flavus* and *Aspergillus parasiticus*, two common Mold species. AflaTest is a quantitative method for the detection of aflatoxin in B1, B2, G1, G2, M1, and M2. Five gram of Siddha formulation (Vallarai Chooranam) and 0.4 g of sodium chloride was mixed with methanol: 2% Tween 20 or phosphate buffer (60:40 v/v). Vortex the mixture of extract on high speed 3 minutes. Filter the extract through fluted filter paper. Add 10 ml of filtered extract in measuring cylinder, in that 20 mL purified water was added and vortex on high for 1 minute. Then filter the diluted extract through a pre-wet glass microfiber filter (1.5µm). Pass 10 mL of diluted extract through AflaTest WB column. Apply pressure to get 1-2 drops per second. Wash the column with 10 mL 2% Tween 20. Wash column with 10 mL purified water twice. Elute AflaTest WB columns by passing 1 mL HPLC-grade methanol (100%) through column, apply pressure to get 1 drop per second. Collect eluate in sterile VICAM cuvette. In that add 1.0 mL of AflaTest Developer and mix well, then immediately place in fluorometer (VICAM fluorometer-series 4EX). Fluorometer will read concentration after 60 seconds.

RESULTS

Thin Layer Chromatography

Physicochemical parameters

Table 2: Physicochemical analysis of VC

S.No.	Test Parameter(s)	Result(s)
1	**Ash** (%w/w)Total ash	11.0 %
2	**Acid-insoluble ash**	2.35 %
3	**pH**	5.12
4	**Loss on Drying**	5.01 %
5	**Water Extractive Value**	7.68%
6	**Alcohol Extractive Value**	8.39 %
7	**Bulk density**	0.4155g/cm^3

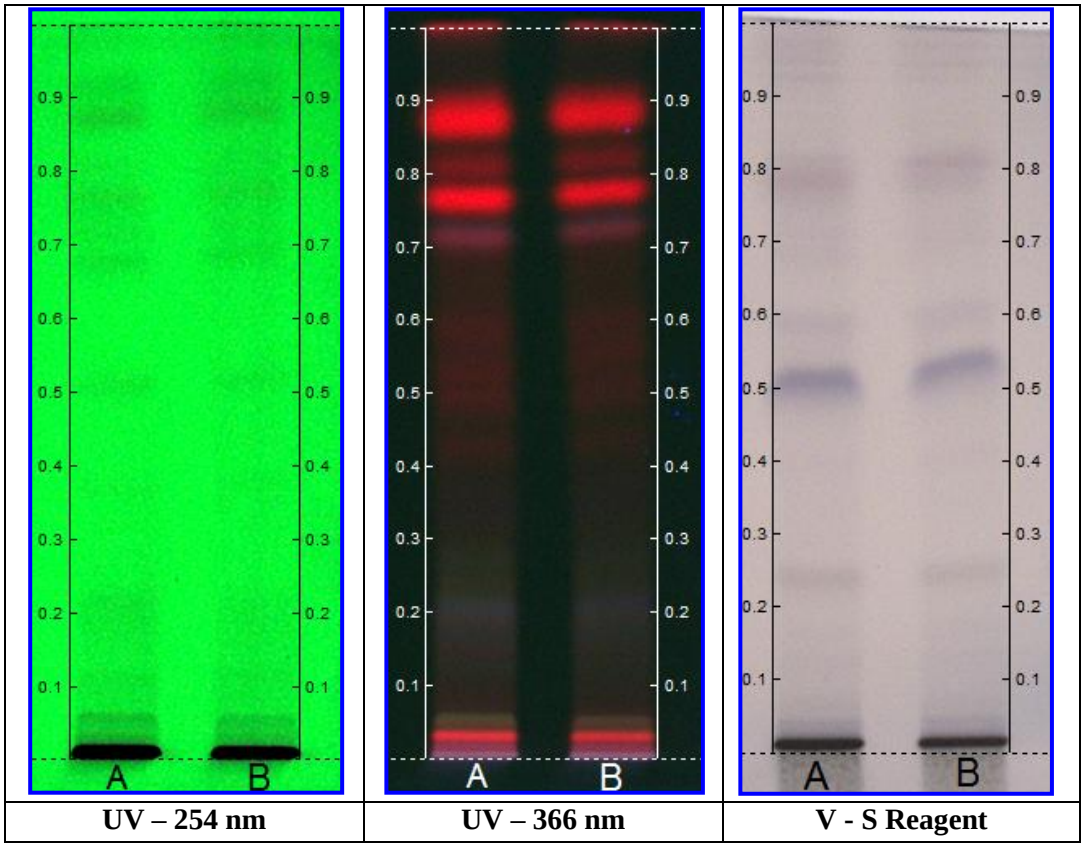
Preliminary Phytochemical Analysis

Table 3: Phytochemical findings of VC

S. No	Test	Alcohol Extract	Hydro Alcohol Extract
1	**Alkaloids** a. Dragendroff's Test b.Wagner's test	 - -	 - -
2	**Carbohydrates** Molish's test	 +	 ++
3	**Reducing sugars** Fehling's test Bendict's test	 + +	 + +
4	**Glycosides** 10% NaOH	 -	 -
5	**Cardiac glycosides** Keller-Killani test	 -	 -
6	**Protein and Amino acids** Ninhydrin test	 -	 -
7	**Flavanoids** a.Alkaline reagent test b.Lead acetate test	 + -	 + -
8	**Phenolic Compounds** a.Ferric chloride test	 +	 ++
9	**Tannins** **a)** NaOH	 +	 -
10	**Phytosterols** Salkowski's test	 +	 ++
11	**Cholesterol**	-	-
12	**Terpinoides**	-	-
13	**Triterpinoides** Salkowski's test	 -	 -
14	**Quinones** Conc.HCl test	 -	 -
15	**Fixed oils and Fat** Saponification test	 -	 -

CODEN (USA): IJPDBY

Fig 1: Solvent system: Toluene: Ethyl acetate: formic acid (7.5:3.5:0.01)10 µl



UV – 254 nm	UV – 366 nm	V - S Reagent

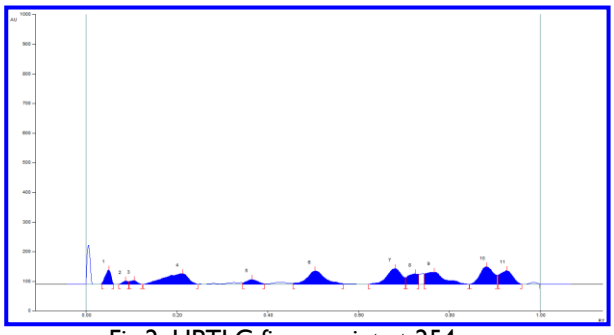


Fig 2: HPTLC finger print at 254 nm (Absorbance mode)

Table 4: R_f values at 254 nm (Absorbance mode)

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.03 Rf	1.4 AU	0.05 Rf	48.3 AU	11.98 %	0.06 Rf	1.5 AU	508.6 AU	4.94 %
2	0.07 Rf	0.0 AU	0.09 Rf	10.7 AU	2.66 %	0.09 Rf	9.2 AU	113.2 AU	1.10 %
3	0.10 Rf	9.3 AU	0.11 Rf	12.7 AU	3.15 %	0.12 Rf	0.0 AU	164.7 AU	1.60 %
4	0.13 Rf	0.2 AU	0.21 Rf	35.6 AU	8.83 %	0.25 Rf	0.6 AU	1609.5 AU	15.64 %
5	0.35 Rf	5.0 AU	0.37 Rf	16.3 AU	4.04 %	0.39 Rf	1.4 AU	337.7 AU	3.28 %
6	0.46 Rf	3.8 AU	0.51 Rf	45.4 AU	11.27 %	0.57 Rf	2.2 AU	1462.4 AU	14.21 %
7	0.62 Rf	0.2 AU	0.68 Rf	52.9 AU	13.14 %	0.70 Rf	22.3 AU	1432.7 AU	13.92 %
8	0.71 Rf	22.6 AU	0.73 Rf	35.0 AU	8.69 %	0.73 Rf	33.3 AU	663.3 AU	6.44 %
9	0.75 Rf	35.6 AU	0.77 Rf	41.0 AU	10.19 %	0.84 Rf	0.1 AU	1432.7 AU	13.92 %
10	0.85 Rf	0.2 AU	0.88 Rf	59.0 AU	14.66 %	0.91 Rf	30.9 AU	1493.2 AU	14.51 %
11	0.91 Rf	31.2 AU	0.93 Rf	45.8 AU	11.37 %	0.96 Rf	0.5 AU	1075.9 AU	10.45 %

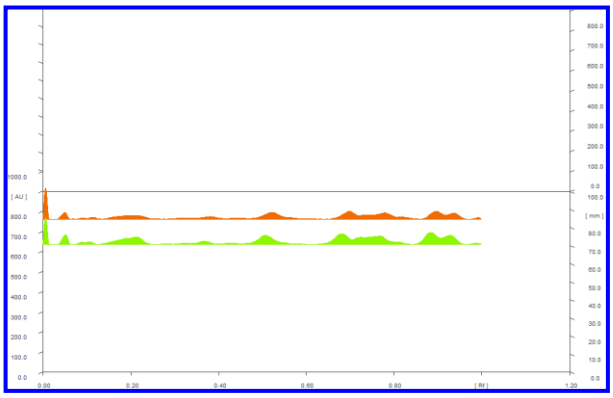


Fig 3: Densitometric chromatogram at 254 nm in Alcohol (Absorbance mode)

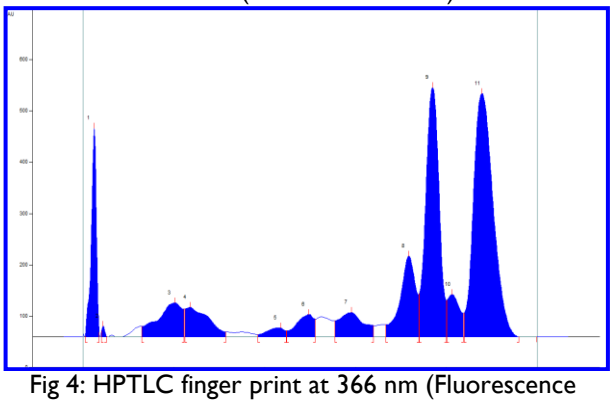


Fig 4: HPTLC finger print at 366 nm (Fluorescence mode)

Table 5: R_f values at 366 nm (Fluorescence mode)

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	0.6 AU	0.03 Rf	406.2 AU	21.85 %	0.04 Rf	8.8 AU	3751.3 AU	7.68 %
2	0.04 Rf	11.2 AU	0.04 Rf	20.5 AU	1.10 %	0.05 Rf	0.6 AU	109.0 AU	0.22 %
3	0.13 Rf	20.2 AU	0.20 Rf	66.1 AU	3.55 %	0.22 Rf	54.0 AU	2998.9 AU	6.14 %
4	0.22 Rf	54.1 AU	0.24 Rf	57.9 AU	3.11 %	0.32 Rf	9.7 AU	2451.9 AU	5.02 %
5	0.39 Rf	4.3 AU	0.44 Rf	17.6 AU	0.95 %	0.45 Rf	11.4 AU	563.9 AU	1.15 %
6	0.45 Rf	11.4 AU	0.50 Rf	43.5 AU	2.34 %	0.51 Rf	34.8 AU	1387.1 AU	2.84 %
7	0.56 Rf	31.2 AU	0.59 Rf	47.3 AU	2.55 %	0.64 Rf	22.2 AU	2152.3 AU	4.41 %
8	0.67 Rf	23.5 AU	0.72 Rf	157.7 AU	8.48 %	0.74 Rf	82.2 AU	4439.2 AU	9.09 %
9	0.74 Rf	84.1 AU	0.77 Rf	485.5 AU	26.12 %	0.80 Rf	70.9 AU	11982.9 AU	24.53 %
10	0.80 Rf	71.4 AU	0.81 Rf	82.6 AU	4.45 %	0.84 Rf	45.9 AU	1835.6 AU	3.76 %
11	0.84 Rf	46.5 AU	0.88 Rf	473.8 AU	25.49 %	0.96 Rf	0.0 AU	17186.9 AU	35.18 %

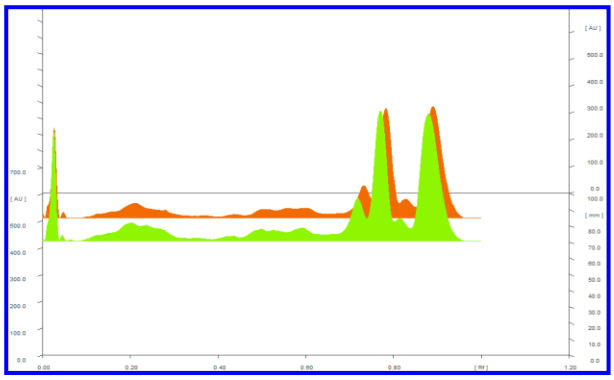


Fig 5: Densitometric chromatogram at 366 nm in Alcohol (Fluorescence mode)

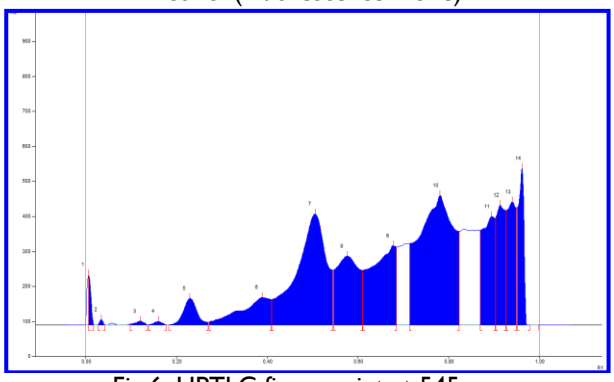


Fig 6: HPTLC finger print at 545nm (Absorbance mode)

Table 6: R_f values at 545 nm (Absorbance mode)

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	144.3 AU	0.01 Rf	144.3 AU	4.99 %	0.02 Rf	2.4 AU	670.7 AU	0.75 %
2	0.03 Rf	0.1 AU	0.03 Rf	15.7 AU	0.54 %	0.04 Rf	0.0 AU	93.8 AU	0.10 %
3	0.10 Rf	2.8 AU	0.12 Rf	11.4 AU	0.39 %	0.14 Rf	0.4 AU	177.2 AU	0.20 %
4	0.14 Rf	0.5 AU	0.16 Rf	10.1 AU	0.35 %	0.18 Rf	0.3 AU	151.6 AU	0.17 %
5	0.18 Rf	0.1 AU	0.23 Rf	76.4 AU	2.64 %	0.27 Rf	7.9 AU	1895.5 AU	2.11 %
6	0.27 Rf	8.0 AU	0.39 Rf	78.4 AU	2.71 %	0.41 Rf	73.2 AU	4299.9 AU	4.79 %
7	0.41 Rf	73.3 AU	0.51 Rf	316.8 AU	10.94 %	0.55 Rf	56.7 AU	17385.7 AU	19.35 %
8	0.55 Rf	156.9 AU	0.58 Rf	196.2 AU	6.78 %	0.61 Rf	56.5 AU	8263.7 AU	9.20 %
9	0.61 Rf	156.7 AU	0.68 Rf	225.9 AU	7.80 %	0.69 Rf	23.3 AU	10078.2 AU	11.22 %
10	0.72 Rf	232.7 AU	0.78 Rf	369.9 AU	12.78 %	0.82 Rf	67.4 AU	23572.5 AU	26.24 %
11	0.87 Rf	270.9 AU	0.90 Rf	309.4 AU	10.69 %	0.90 Rf	05.5 AU	7247.0 AU	8.07 %
12	0.91 Rf	307.0 AU	0.92 Rf	341.4 AU	11.79 %	0.93 Rf	27.2 AU	5591.0 AU	6.22 %
13	0.93 Rf	327.5 AU	0.94 Rf	351.3 AU	12.14 %	0.95 Rf	33.6 AU	5768.4 AU	6.42 %
14	0.95 Rf	334.6 AU	0.96 Rf	447.5 AU	15.46 %	0.98 Rf	0.0 AU	4653.3 AU	5.18 %

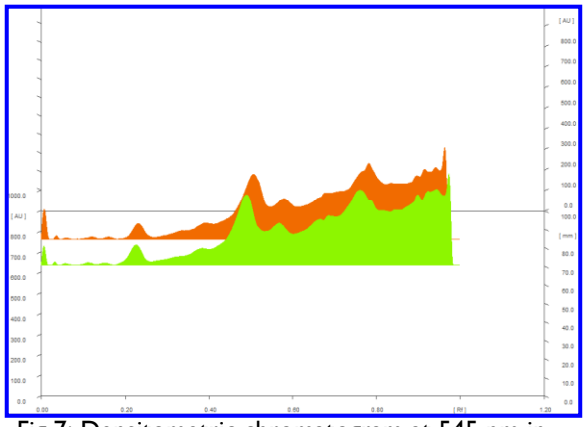


Fig 7: Densitometric chromatogram at 545 nm in Alcohol (Absorbance mode)

Microbial load

Table 7: Microbial load determination

S. No.	Parameters	Results	Remarks
1	Total Bacterial Count (TBC)	$1.1x10^4$cfu/g	Within permissible limits
2	Total Fungal Count (TFC)	Less than 5 cfu/g	
3	Enterobacteriaceae	Absent	
4	*Escherichia coli*	Absent	
5	Salmonella Spp	Absent	
6	*Staphylococcus aureus*	Absent	
7	*Pseudomonas aeruginosa*	Absent	

DISCUSSION

In the present study, Vallarai Chooranam was subjected to various standardization parameters including physicochemical evaluation, phytochemical screening, HPTLC, microbial load assessment, aflatoxin estimation in accordance with AYUSH and WHO guidelines.

Physicochemical Characteristics

The total ash value (11.0%) indicates the presence of inorganic components and helps assess the purity of the formulation. The acid-insoluble ash value (2.35%) represents the amount of silica, suggesting that the formulation is free from excessive inorganic impurities or adulterants. The pH value of 5.12 indicates a mildly acidic nature, which is favorable for stability and therapeutic action of the formulation. The loss on drying (5.01%) reflects low moisture content, thus preventing microbial growth and supporting shelf-life stability. The water and alcohol extractive values (7.68% and 8.39%, respectively) indicate the presence of water-soluble and alcohol-soluble phytoconstituents in the formulation. Bulk density of the formulation was 0.4155g/cm3, demonstrating its suitability for herbal pharmaceutical formulations.

Phytochemical Screening

Preliminary phytochemical screening revealed the presence of carbohydrates, flavonoids, phenolic compounds, tannins, and phytosterols. The presence of flavonoids and phenolic compounds may contribute to the antimicrobial activity of the formulation, which supports its traditional use in the management of urinary tract infections. Tannins are known for their astringent and antimicrobial effects, which may help reduce microbial proliferation in the urinary tract.

HPTLC Fingerprinting

The HPTLC profile, developed in the solvent system Toluene: Ethyl acetate: Formic acid (7.5:3.5:0.01), displayed distinct Rf values under UV 254 nm, UV 366 nm, and after derivatization with vanillin–sulphuric acid. In the present study, the HPTLC chromatogram of Vallarai Chooranam showed multiple peaks at different wavelengths, indicating the presence of several phytochemical constituents in the formulation.

The Rf values obtained in the chromatographic profile may serve as reference standards for future identification and quality assessment of this formulation.

Microbial Quality and Aflatoxin Levels:

The microbial load analysis demonstrated that the total bacterial count (1.1×10^4 CFU/g) and total fungal count were within the permissible limits as per WHO guidelines. Furthermore, pathogenic microorganisms such as Escherichia coli, Salmonella spp., Staphylococcus aureus, Pseudomonas aeruginosa, and Enterobacteriaceae were absent in the sample. This indicates that the formulation is microbiologically safe for internal consumption. Aflatoxin analysis revealed that the levels of aflatoxins were within permissible limits, indicating minimal fungal toxin contamination.

Overall, the results obtained from physicochemical evaluation, phytochemical screening, chromatographic fingerprinting, microbial load analysis, aflatoxin testing, and powder microscopy confirm the purity, safety, and quality of Vallarai Chooranam. These findings provide scientific evidence supporting the traditional use of this Siddha formulation in treating urinary tract infection.

CONCLUSION

The results of the present study establish scientific baseline data for the quality, purity, physicochemical, phytochemical parameters, microbial load contamination and safety of the formulation Vallarai Chooranam. These findings support the traditional therapeutic use of this Siddha formulation and may serve as a reference for future pharmacological studies and standardization of herbal formulations.

ACKNOWLEDGEMENT

The author expresses sincere gratitude towards the faculty of the Department of Pothu Maruthuvam, Government Siddha Medical College, Chennai who gave us the Great opportunity to carry out this Standardization of VC. The author would also like to thank the Regional Research Institute of Unani Medicine, Chennai for providing the necessary laboratory facilities to carry out this research work.

CONFLICT OF INTEREST

None

SOURCE OF FUNDING

Self- Funded

ETHICAL APPROVAL

Approved

AUTHOR CONTRIBUTION

Dr. K. Sudhamathi pushparaj designed and supervised the project, Dr. S. Priyanka collected and analyzed the data and prepared the manuscript. Dr. K. Menaka and Dr. U. Chithra reviewed the final version of the manuscript. All the authors approved the manuscript.

REFERENCES

1. Agathiyar Vaithiya Rathina Surukkam 360. Chennai: K.A. Madhuraimuthaliyar Publisher; 1937. 1st ed.
2. Muthulakshmi M, et al. International Journal of Community Medicine and Public Health. 2017 Oct;4:3915.
3. Kannusami Pillai. Sikicharathnadeepamennum Vaithiya Nool. 2014 ed. p. 28–31.
4. Directorate of Indian Medicine and Homoeopathy. Marunthu Sei Iyalum Kalayum. p. 283–287.
5. Lohar DR. Protocol for testing of Ayurvedic, Siddha and Unani medicines. Ghaziabad: Pharmacopoeial Laboratory for Indian Medicine.
6. World Health Organization. Quality control methods for medicinal plant materials. 1st ed. Geneva: World Health Organization; 1998. ISBN: 9241545100.