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Research Article

ANALYTICAL STANDARDIZATION AND SAFETY EVALUATION OF *USHNA VAAYU MENI CHOORANAM*: A SIDDHA POLYHERBAL FORMULATION

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ABSTRACT

Traditional herbal medicines remain widely used in global healthcare, and the World Health Organization emphasizes the need for scientific standardization to ensure their safety, quality, and efficacy. Polyherbal Siddha formulations require validated analytical approaches due to their complex composition. *Ushna Vaayu Meni Chooranam* is a traditional formulation lacking comprehensive standardization. **Methods:** UVMC was prepared as per classical methods. Standardization included physicochemical evaluation (ash values, extractives, loss on drying, pH), preliminary phytochemical screening, High-Performance Thin Layer Chromatography (HPTLC) fingerprinting at 254nm and 366nm, microbial load testing, aflatoxin estimation, and powder microscopy. **Results:** Physicochemical parameters were within acceptable limits, with total ash (11.63% w/w), acid-insoluble ash (7.65% w/w), loss on drying (5.80% w/w), and pH (5.46). Water extractive (12.18% w/w) exceeded alcohol extractive (7.34% w/w), indicating predominance of polar constituents. Phytochemical analysis confirmed phenolics, phytosterols, quinones, and reducing sugars. HPTLC profiling showed 7 peaks at 254nm (major peak at Rf 0.89; 56.68%) and 11 peaks at 366nm, indicating diverse phytoconstituents. Microbial load and aflatoxin levels (5.3 ppb) were within permissible limits. Powder microscopy revealed diagnostic features confirming authenticity. **Conclusion:** Study drug demonstrated acceptable quality parameters, absence of contamination, and a characteristic HPTLC fingerprint. These findings provide scientific validation for the safety, authenticity, and reproducibility of UVMC, supporting its integration into evidence-based traditional medicine and future pharmacological investigations.

INTRODUCTION

Traditional medical systems have relied heavily on herbal treatments, which remain vital to global health. The World Health Organization (WHO) reports that a significant proportion of the world's population, especially in developing nations, receives primary medical care from traditional and herbal medicines [1,2]. This pervasive reliance highlights the continued applicability of plant-based treatments in contemporary medicine. The usage of traditional herbal medicines has significantly increased globally in recent years due to rising incidence of chronic illnesses, greater understanding of natural products, and concern about the adverse effects of modern medications. Therefore, standardization should be carried out to validate traditional medicine scientifically in order to emphasize the safety, efficacy, and quality of the formulations [3]. The WHO strongly advocates for the use of standardized evaluation techniques, such as pharmacognostic, physicochemical, and chromatographic tests, to ensure the quality, safety, and repeatability of herbal formulations[4]. High-Performance Thin Layer Chromatography (HPTLC) has become a dependable and affordable method among contemporary analytical techniques for creating distinctive fingerprint profiles and assuring batch-to-batch consistency [5].

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More than 45,000 plant species are thought to exist in India, of which 7,000 – 8,000 are used for medicinal purposes [6]. The Siddha system of medicine, one of the oldest traditional systems practiced primarily in South India, makes extensive use of polyherbal formulations, which are known for their synergistic therapeutic effects and reduced toxicity. The growing acceptance of Siddha and other traditional systems worldwide necessitates detailed scientific validation and standardization of their formulations. *Ushna Vaayu Meni Chooranam* (UVMC), a Siddha polyherbal preparation that contains *Acalypha indica* L., *Cuminum cyminum* L., and *Saccharum officinarum* L. *Cuminum cyminum* is said to have carminative, antioxidant, and antimicrobial properties, which are primarily due to its volatile oil constituents[7]. The traditional use of *Acalypha indica* for respiratory and inflammatory diseases is supported by its anti-inflammatory and antimicrobial properties[8]. *Saccharum officinarum* contributes to palatability and may increase the bioavailability of active substances by acting as a demulcent and supporting agent [9].

As polyherbal drugs have several bioactive ingredients, they are intrinsically complicated, making it challenging to assess their quality. The current study intends to establish standardization parameters for a Siddha polyherbal formulation UVMC using physicochemical analysis, preliminary phytochemical screening, HPTLC fingerprinting, and microbial evaluation in order to ensure their identification, purity, and consistency as well as to enhance their credibility and acceptance into evidence-based healthcare.

MATERIAL AND METHODS

Preparation of the drug

The formulation of *Ushna Vaayu Meni Chooranam* (UVMC) comprises *Cuminum cyminum* L. (seeds), *Acalypha indica* L. (whole plant), and *Saccharum officinarum* L. (sugar). All raw drugs, except sugar, were thoroughly cleaned to remove extraneous matter and shade-dried. The dried materials were pulverized separately and passed through a cloth sieve to obtain a fine powder. Precisely weighed quantities of each powdered drug (35gm each) were mixed with 105gm of sugar and triturated in a mortar for 1 hour to ensure uniform blending. The prepared *Chooranam* was then stored in an airtight container for further analysis.[10]

Physico-chemical parameters

- **Percentage Loss on Drying**
  Dry an evaporating dish under specific conditions for 30 minutes to find the moisture content. Weigh 5 to 10 grams of powdered medication precisely in a tared dish. Weigh every 30 minutes while drying at 105°C for three hours or until the weight change is less than 0.25%. Results should be reported as a percentage w/w. [11]
- **Determination of Total Ash**
  In a platinum or silica dish, burn two to three grams of the *Chooranam* at a maximum temperature of 600° until all carbon is eliminated. Weigh the sample after 30 minutes of cooling in a desiccator. If carbon-free ash is not obtained, use hot water to wash the burned mass, filter and burn the residue and filter paper, evaporate the filtrate until it is dry, and then ignite at 600°. The percentage of ash is computed as a percentage w/w of the air-dried medication. [11]
- **Determination of Acid Insoluble Ash**
  Fill the crucible with total ash and add 25ml of diluted hydrochloric acid. Use Whatman No. 41 ashless filter paper to filter out the insoluble material, then rinse with hot water until it becomes neutral. After returning the residue to the crucible, dry it on a hot plate and fire it until its weight remains constant. After 30 minutes of cooling in a desiccator, weigh right away and determine the percentage of acid-insoluble ash in relation to the air-dried medication. [11]
- **Determination of Alcohol Soluble Extractive**
  For 24 hours, macerate 5 grams of coarsely powdered, air-dried drug in 100ml of alcohol of the optimum strength while shaking for the first six hours. At 105°C, filter and evaporate 25ml of the filtrate until it is dry and has a consistent weight. Determine the alcohol-soluble extractive percentage using the medication that has been air-dried. For methanol-soluble extractive, use methanol rather than alcohol, and report the results as a percentage w/w. [11]
- **Determination of Water-Soluble Extractive**
  To determine the alcohol-soluble extractive, use a solution of chloroform water (2.5ml chloroform in 1000ml purified water) instead of ethanol, with the result expressed as a percentage w/w. [11]
- **Determination of pH**
  The pH value of an aqueous liquid is defined as the logarithmic reciprocal of the concentration of hydrogen ions in grams per liter. In pharmacopoeia, pH is measured with a standardized pH meter that replicates values down to 0.05 pH units using a sensitive indicator electrode and a reference electrode. Proper standardization involves selecting two buffer solutions with a pH difference of no more than four units and adjusting the meter at a controlled temperature to ensure the results match the reported pH. Rinsing with buffer solutions, checking for electrode defects, and preparing a test sample at a 5% w/v concentration using dilutions made with carbon dioxide-free water are all steps in

the procedure. Stabilization time is crucial for readings. [11]

Preliminary phytochemical tests

Detection of Alkaloids

a) Dragendroff's Test

A few drops of acetic acid, 2ml of diluted hydrochloric acid, 1ml of Dragendorff's reagent (potassium bismuth iodide solution), and vigorous shaking were added to approximately 2ml of UVMC extract. The formation of an orange-red precipitate indicates that alkaloids are present. [12]

b) Wagner's test

Wagner's reagent (1.27gm of iodine and 2gm of potassium iodide in 100ml of water) was added to about 2ml of UVMC extract along with 3–5 drops. The presence of alkaloids was confirmed by looking at the reddish-brown precipitate. [12]

Detection of Carbohydrates

a) Molish's test

Few drops of Molisch's reagent were added to 2ml of UVMC extract and added 2ml of concentrated sulfuric acid along the sides of the test tube; then allowed to stand for 2-3 minutes. Formation of a red or violet color at the interphase of the two layers confirms the presence of carbohydrates.[12]

Detection of Reducing sugars:

a) Fehling's test

About 2ml of UVMC extract was mixed with Fehling's solution A and B and examined for the appearance of red coloration indicates the presence of sugars.[12]

b) Benedict's test

A volume of 0.5ml of the filtrate was mixed with 0.5ml of Benedict's reagent and heated in a boiling water bath for 2 minutes. The appearance of green, yellow, or brick-red coloration indicated the presence of reducing sugars, whereas the solution remaining blue was considered a negative result. [13]

Detection of Glycosides

a) 10% NaOH

To 1ml of dilute sulfuric acid, 0.2ml of the extract was added and the mixture was boiled for 15 minutes. After cooling, the solution was neutralized with 10% sodium hydroxide. Subsequently, 0.2ml each of Fehling's solution A and Fehling's solution B were added and the mixture was heated. The formation of a brick-red precipitate indicated the presence of glycosides. [13]

Detection of Cardiac glycosides

a) Keller-Killani test

To 1ml of the filtrate, 1.5ml of glacial acetic acid and one drop of 5% ferric chloride solution were added. Concentrated sulfuric acid was then carefully added along the side of the test tube to form a separate layer. The appearance of a blue color in the acetic acid layer indicated the presence of cardiac glycosides. [13]

Detection of Protein and Amino acids

a) Ninhydrin test

About 2ml of UVMC extract was treated with 2-5 drops of ninhydrin solution placed in a boiling water bath for 1-2 minutes and observed for the formation of purple color indicates the presence of amino acids and proteins.[12]

Detection of Flavonoids

a) Alkaline reagent test

About 2ml of UVMC extract was treated with few drops of 20% sodium hydroxide solution. Formation of intense yellow color, which becomes colorless on addition of diluted hydrochloric indicates the presence of flavonoids.[12]

b) Lead acetate test

To 1ml of the UVMC, a few drops of 10% lead acetate solution were added. The formation of a yellow precipitate indicated the presence of flavonoids. [13]

Detection of Phenolic Compounds

a) Ferric chloride test

To the 2ml of UVMC extract, a few drops of aqueous 5% ferric chloride solution was added and formation of blue or black color confirms the presence of phenols.[12]

2.3.1 Detection of Tannins

To 0.4ml of the UVMC extract, 4ml of 10% sodium hydroxide solution was added and the mixture was shaken well. The formation of an emulsion indicated the presence of hydrolysable tannins. [13]

Detection of Phytosterols

a) Salkowski's test

To the filtrate, a few drops of concentrated sulfuric acid were added. The mixture was shaken well and allowed to stand. The development of a red color in the lower layer indicated the presence of phytosterols. [13]

Detection of Cholesterol

To 2ml of the extract, 2m; of chloroform was added, followed by 10 drops of acetic anhydride. Subsequently, 2–3 drops of concentrated sulfuric acid were added carefully. The development of a red to rose-colored solution indicated the presence of cholesterol. [13]

Detection of Terpinoides

To 2ml of chloroform, 5ml of the plant extract was added and the mixture was evaporated to dryness on a water bath. The residue was then treated with 3ml of concentrated sulfuric acid and heated on a

water bath. The formation of a grey-colored solution indicated the presence of terpenoids. [13]

Detection of Triterpenoids

a) Salkowski's test

To the filtrate, a few drops of concentrated sulfuric acid were added, and the mixture was shaken well and allowed to stand. The appearance of a golden yellow layer at the bottom indicated the presence of triterpenoids. [13]

Detection of Quinones

a) Concentrated HCl Test

Concentrated hydrochloric acid was applied to the UVMC extract. Quinones were detected by the formation of a green coloring [13].

Detection of Anthocyanins

a) HCl test

To 2ml of the plant extract, 2ml of 2 N hydrochloric acid was added. The formation of a pink to red solution indicated the presence of anthocyanins. Upon the addition of a few milliliters of ammonia solution, the color changed to blue-violet, further confirming the presence of anthocyanins. [13]

Detection of Carboxylic acid

a) Effervescence test:

To 1ml of the UVMC extract, 1ml of sodium bicarbonate solution was added. The appearance of effervescence due to the evolution of carbon dioxide indicated the presence of carboxylic acids. [13]

Detection of Gums and Mucilage's

a) Alcohol test

About 100mg of the extract was dissolved in 10ml of distilled water. To this, 25ml of absolute alcohol was added with constant stirring. The formation of a white or cloudy precipitate indicated the presence of gums and mucilage's. [13]

Detection of Resins

a) Turbidity test

One milliliter of the plant extract was dissolved in acetone and then poured into distilled water. The development of turbidity indicated the presence of resins. [13]

Detection of Fixed oils and Fat

a) Saponification test

After adding a few drops of phenolphthalein indicator and 0.5N alcoholic potassium hydroxide to the extract, it was boiled for two hours. The presence of fixed oils and fats was detected by the production of soap or partial alkali neutralization. [13]

High Performance Thin Layer Chromatography

In thin-layer chromatography (TLC), a solute is distributed between a liquid mobile phase and a stationary phase by adsorption. Separation can be based on either partition or a mix of partition and adsorption, usually using a thin layer of a dry powdered substance on a substrate. Identification can be done by comparing spots from known and unknown samples on the same plate. Test solutions are applied on prepared plates after a tank has been saturated with a mobile phase. The solvents are then allowed to move to a designated line before being visualized using UV light. The distance between each spot and its application point is divided by the mobile phase's travel distance to determine the retention factor (Rf value). [11]

Microbial contamination by pour plate method

The study tested the microbial quality of commercial and homemade herbal medicines by isolating and identifying pathogenic bacteria in accordance with WHO standards (2007). Samples were homogenized with peptone water, diluted, and analyzed for microbial content using the pour plate method on specific agar mediums. Bacterial screening occurred at 37°C for 24 to 48 hours, while fungal screening took place at 25°C for 48 to 72 hours. Colony-forming units per gram (CFU/g) were calculated, and samples exceeding 105 CFU in 1 g were deemed unsatisfactory according to WHO guidelines.[14]

For bacterial isolation and identification, samples were diluted and homogenized before transferring 1ml aliquots into 9ml of peptone broth for culturing. All tests were done in triplicate. Media used for investigating *Escherichia coli*, Salmonella spp., *Pseudomonas aeruginosa*, and *Staphylococcus aureus* included EMB agar, MacConkey agar, deoxycholate citrate agar, cetrimide agar, and mannitol salt agar. After incubation, pathogenic isolates were characterized based on colony morphology, Gram staining, and biochemical tests including oxidase, gas, and catalase production. [14]

Aflotoxin Assay by TLC (B1, B2, G1, G2)

Aflatoxins, toxins produced by *Aspergillus flavus* and *Aspergillus parasiticus*, can be detected using the AflaTest method, which quantitatively measures B1, B2, G1, G2, M1, and M2. The procedure involves mixing 5gm of UVMC with 1gm of sodium chloride in a methanol: water solution (60:40 v/v), vortexing, filtering, and diluting the extract. The diluted extract is then passed through the AflaTest WB column, washed, and eluted with methanol before mixing with AflaTest Developer. The concentration is then read using a VICAM fluorometer after 60 seconds. [14,15]

Powder Microscopic studies

To analyze take 5g coarsely powdered sample, mix it with 50ml of water and gently warm until fully dispersed. Centrifuge, decant the supernatant, and

wash the sediment with distilled water, followed by another centrifugation. Mount a few mg of the sediment in glycerine; additionally, for lignified cell identification, mount more in a watch glass with phloroglucinol and concentrated hydrochloric acid. Capture photomicrographs with a microscope camera.[11]

RESULTS

Physicochemical Parameters

The physicochemical analysis of UVMC showed that it complied with standard quality parameters. The presence of inorganic and siliceous materials was indicated by the formulation's total ash value of 11.63% w/w and acid-insoluble ash value of 7.65% w/w. Low moisture content is suggested by the drying loss of 5.80% w/w. The formulation's pH of 5.46 revealed that it was quite acidic. The extractive values indicated a predominance of polar components, with a higher water-soluble extractive (12.18% w/w) than an alcohol-soluble extractive (7.34% w/w). The bulk density was 0.6845g/cm^3, indicating appropriate physical properties. (Table 1)

Table 1: Physicochemical parameters of UVMC

S. No.	Parameter	Result
1	Total ash (% w/w)	11.63%
2	Acid-insoluble ash (% w/w)	7.65%
3	pH	5.46
4	Loss on drying (% w/w)	5.80%
5	Water extractive (% w/w)	12.18%
6	Alcohol extractive (% w/w)	7.34%
7	Bulk density (g/cm^3)	0.6845 g/cm^3

Preliminary Phytochemical Screening

Both alcohol and hydroalcoholic extracts included specific bioactive components, according to phytochemical screening. Fehling's test found reducing sugars, while Benedict's test turned out negative. Quinones, phytosterols (identified by the Salkowski test), and phenolic compounds were all present, with the hydroalcoholic extract exhibiting greater concentrations. There were no other phytoconstituents such tannins, terpenoids, alkaloids, flavonoids, glycosides, or fixed oils (Table 2).

Table 2: Preliminary phytochemical screening of UVMC

S.No.	Phytochemical Test	Alcohol Extract	Hydroalcohol Extract
1	Alkaloids		
	a) Dragendroff's Test	–	–
	b) Wagner's test	–	–
2	Carbohydrates	–	–
3	Reducing sugars		
	a) Fehling's test	+	+
	b) Bendict's test	–	–
4	Glycosides	–	–
5	Cardiac glycosides	–	–
6	Proteins and amino acids	–	–
7	Flavonoids		
	a) Alkaline reagent test	–	–
	b) Lead acetate test	–	–
8	Phenolic compounds	+	++
9	Tannins	–	–
10	Phytosterols	+	++
11	Cholesterol	–	–

12	Terpenoids	–	–
13	Triterpenoids	–	–
14	Quinones	+	++
15	Anthocyanins	–	–
16	Carboxylic acids	–	–
17	Gums and mucilage's	–	–
18	Resins	–	–
19	Fixed oils and fats	–	–

*(+ = Present; ++ = Strongly present; – = Absent)

HPTLC Fingerprint Analysis

The alcohol extract of UVMC was analyzed using High-Performance Thin Layer Chromatography (HPTLC), which confirmed the presence of several phytoconstituents by revealing well-resolved chromatographic profiles at both 254 and 366nm. 7 different peaks at Rf values of 0.03, 0.33, 0.51, 0.59, 0.74, 0.89, and 0.97 were seen at 254nm. The highest area percentage (56.68%) was shown by the peak at Rf 0.89, which was followed by peaks at Rf 0.51 (13.47%) and Rf 0.74 (10.98%), suggesting that non-polar and moderately polar ingredients predominate. Figure 2a–c displays the appropriate densitometric scan, Rf profile, and fingerprint chromatogram. 11 peaks with Rf values of 0.01, 0.03, 0.22, 0.30, 0.38, 0.47, 0.51, 0.66, 0.74, 0.79, and 0.89 were seen at 366 nm. The presence of fluorescent phytoconstituents was indicated by major peaks at Rf 0.03 (28.34%), Rf 0.01 (17.84%), Rf 0.79 (15.20%), and Rf 0.89 (14.99%). Figure 3a–c displays the densitometric profile and fluorescence chromatogram.

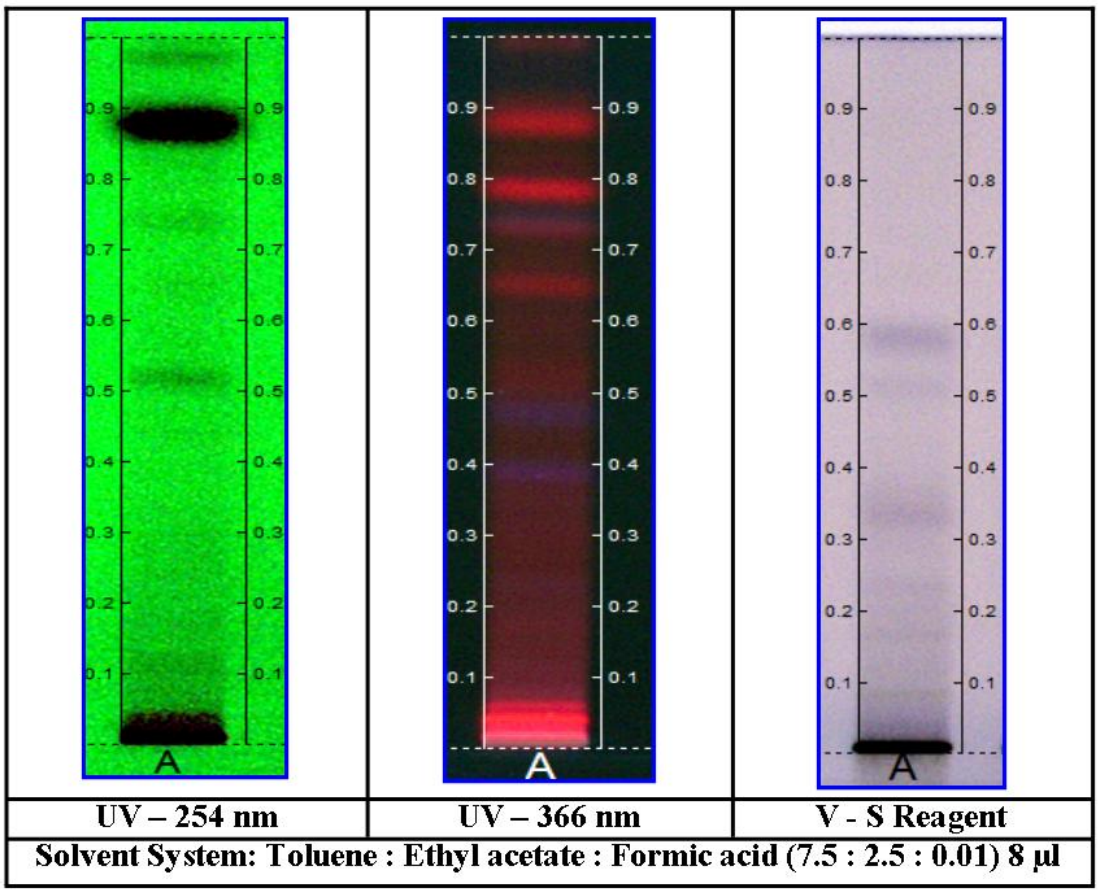
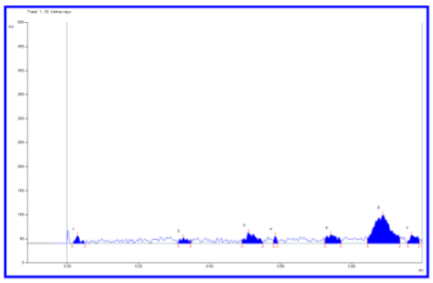


Figure 1: HPTLC fingerprint profile of UVMC (Alcohol extract)

a



b

Track 1, ID: Ushna vayu

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	0.0 AU	0.03 Rf	15.3 AU	9.52 %	0.05 Rf	0.0 AU	166.1 AU	4.22 %
2	0.31 Rf	0.0 AU	0.33 Rf	11.7 AU	7.27 %	0.35 Rf	8.1 AU	191.3 AU	4.86 %
3	0.49 Rf	6.2 AU	0.51 Rf	23.5 AU	14.59 %	0.55 Rf	1.6 AU	530.4 AU	13.47 %
4	0.58 Rf	4.6 AU	0.59 Rf	15.2 AU	9.41 %	0.59 Rf	3.6 AU	92.6 AU	2.35 %
5	0.73 Rf	8.4 AU	0.74 Rf	18.1 AU	11.23 %	0.77 Rf	6.5 AU	432.3 AU	10.98 %
6	0.85 Rf	10.0 AU	0.89 Rf	60.0 AU	37.21 %	0.94 Rf	11.3 AU	2231.7 AU	56.68 %
7	0.96 Rf	3.1 AU	0.97 Rf	17.3 AU	10.76 %	0.99 Rf	7.6 AU	292.8 AU	7.44 %

c

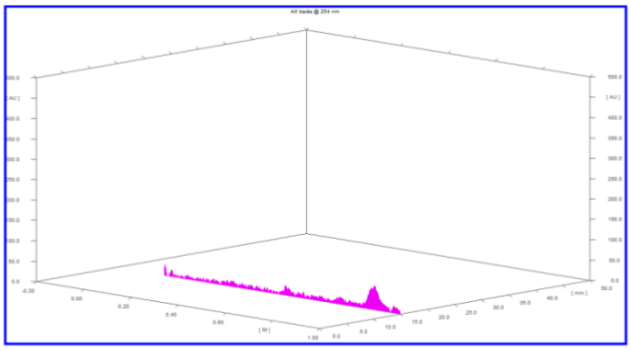
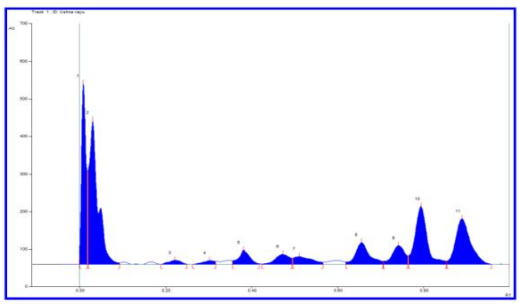


Figure 2. HPTLC profile of the alcohol extract at 254nm: (a) fingerprint chromatogram, (b) Rf values, and (c) densitometric chromatogram (absorbance mode)

a



b

Track 1, ID: Ushna vayu

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	24.7 AU	0.01 Rf	481.3 AU	35.52 %	0.02 Rf	47.0 AU	4181.3 AU	17.84 %
2	0.02 Rf	249.5 AU	0.03 Rf	383.5 AU	28.30 %	0.09 Rf	3.8 AU	6642.6 AU	28.34 %
3	0.19 Rf	0.2 AU	0.22 Rf	11.3 AU	0.83 %	0.25 Rf	0.2 AU	265.6 AU	1.13 %
4	0.26 Rf	0.3 AU	0.30 Rf	10.2 AU	0.75 %	0.32 Rf	8.0 AU	229.0 AU	0.98 %
5	0.36 Rf	9.6 AU	0.38 Rf	38.2 AU	2.82 %	0.42 Rf	2.0 AU	795.1 AU	3.39 %
6	0.42 Rf	1.2 AU	0.47 Rf	26.7 AU	1.97 %	0.49 Rf	16.1 AU	737.9 AU	3.15 %
7	0.50 Rf	16.1 AU	0.51 Rf	21.0 AU	1.55 %	0.57 Rf	5.1 AU	727.0 AU	3.10 %
8	0.62 Rf	6.0 AU	0.66 Rf	58.0 AU	4.28 %	0.71 Rf	8.2 AU	1558.1 AU	6.65 %
9	0.71 Rf	8.3 AU	0.74 Rf	49.9 AU	3.68 %	0.76 Rf	22.5 AU	1225.7 AU	5.23 %
10	0.77 Rf	22.6 AU	0.79 Rf	153.9 AU	11.36 %	0.85 Rf	8.9 AU	3561.9 AU	15.20 %
11	0.85 Rf	9.0 AU	0.89 Rf	121.2 AU	8.94 %	0.96 Rf	0.1 AU	3512.2 AU	14.99 %

c

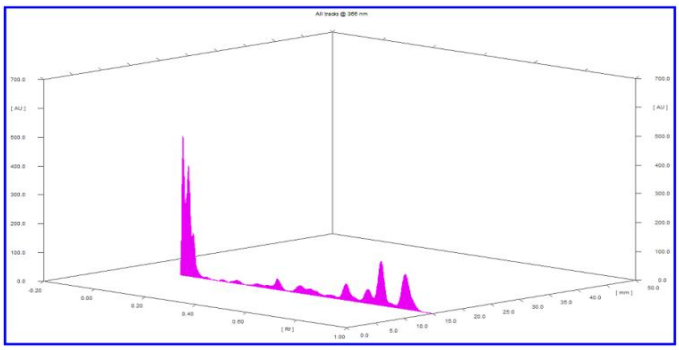


Figure 3. HPTLC profile of the alcohol extract at 366 nm: (a) fingerprint chromatogram, (b) Rf values, and (c) densitometric chromatogram (Fluorescence mode)

Microbial Load Analysis

The microbial evaluation indicated that the total bacterial count was 1.1×10^4 CFU/g, and the total fungal count was < 8 CFU/g, both within WHO permissible limits. Pathogenic organisms including Escherichia coli, Salmonella spp., Staphylococcus aureus, Pseudomonas aeruginosa, and Enterobacteriaceae were absent (Table 3).

Table 3: Microbial load analysis of UVMC

S.No.	Parameter	Result	Remark
1	Total bacterial count	1.1×10^4 CFU/g	Within permissible limits
2	Total fungal count	< 8 CFU/g	
3	Enterobacteriaceae	Absent	Safe
4	Escherichia coli	Absent	
5	Salmonella spp.	Absent	
6	Staphylococcus aureus	Absent	
7	Pseudomonas aeruginosa	Absent	

Aflatoxin Analysis

The total aflatoxin content (B1, B2, G1, G2) was found to be 5.3 ppb, which is within WHO permissible limits (0.5–15 ppb), indicating safety from mycotoxin contamination (Table 4).

Table 4: Aflatoxin content of UVMC

S.No.	Parameters	Method / Reference	Results
1.	Total Aflatoxin B1+B2+G1+G2	Vicam Aflatest Fluorometer Instruction Manual	5.3 ppb

(Note: Detection Limit - 1ppb)

Powder Microscopy

The distinctive diagnostic characteristics of the powdered formulation were identified through microscopic analysis of its components. Both simple and glandular trichomes were observed; simple trichomes were found to be uniseriate and up to 300µm long, whereas glandular trichomes had multicellular stalks with globose heads up to 80µm in diameter. There were noticeable calcium oxalate crystal druses up to 50µm in size.

In surface view, multicellular trichomes and epidermal cells with paracytic stomata, a feature of *Acalypha indica* L. (*Kuppaimeni*), were recognized. Vittae, which emerge as whole or fragmented structures up to 250µm in width and taper towards the ends, were also noted as distinguishing characteristics of *Cuminum cyminum* L. (*Seeragam*). Aleurone grains and micro-rosette crystals were found in the fairly thick-walled parenchyma of endosperm cells. The identification and purity of each individual constituent in the formulation are verified by these microscopic features. (Figure 4-6).

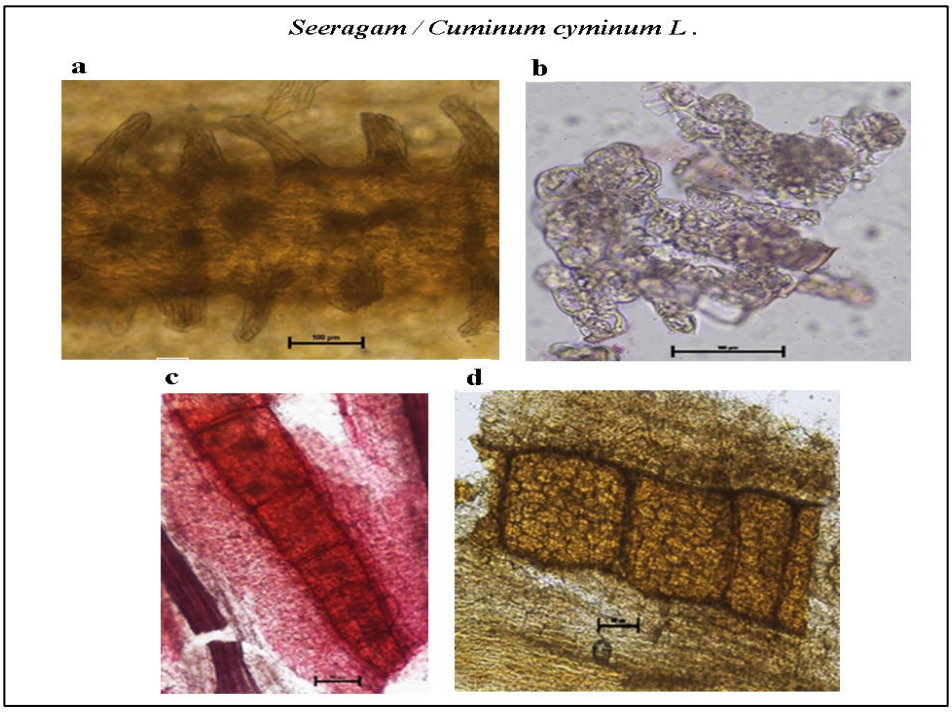


Figure 4: Powder microscopy of *Acalypha indica* L. (a) Trichomes, (b) & (c) Vittae, (d) Endosperm cells

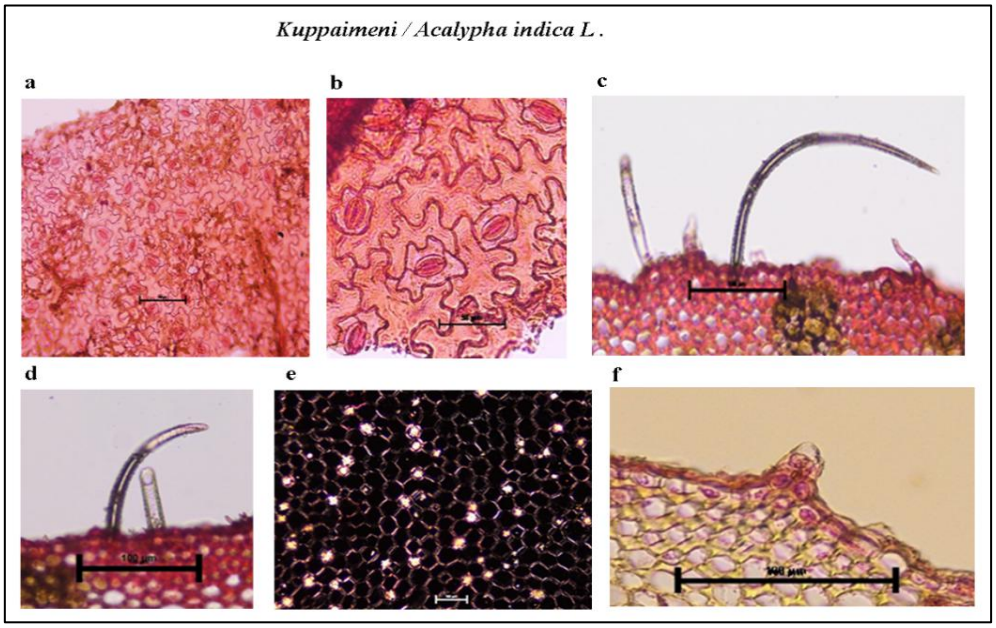


Figure 5: Powder microscopy of *Cuminum cyminum* L. (a) & (b) Paracytic Stomata, (c) & (d) Multicellular Trichome, (e) Druses of Calcium oxalate crystals. (f) Glandular Trichome

DISCUSSION

The current study provides a detailed standardization profile for *Ushna Vaayu Meni Chooranam* (UVMC) utilizing physicochemical, phytochemical, chromatographic, microbiological, and microscopic assessments. According to recent standardization studies, a multifaceted strategy is crucial for ensuring the quality, safety, and reproducibility of traditional herbal formulations. The formulation's physicochemical properties show stability and proper processing. Longer shelf life is ensured by regulating moisture, which is essential for preventing microbial growth and the deterioration of active ingredients. Ash readings additionally serve as markers of inorganic content and possible extraneous material contamination. The obtained parameters are within acceptable pharmacopoeial limits, indicating adherence to established manufacturing techniques and high-quality raw materials [16,17].

Extractive values provide key details about the formulation's chemical composition. The abundance of water-soluble elements indicates the presence of polar bioactive chemicals, which are frequently linked to therapeutic efficacy. This finding is consistent with the nature of plant-derived formulations, in which hydrophilic chemicals like phenolics play a substantial role in pharmacological activity[18]. Phytosterols, quinones, and phenolic compounds all of which are known to have a variety of biological activities, were found using phytochemical screening. While phytosterols help stabilize membranes and regulate inflammatory pathways, phenolics are well known for their anti-inflammatory and antioxidant properties. Conversely, quinones are linked to cytoprotective and antibacterial qualities [19-21]. The identification of these components validates the traditional use of UVMC in the treatment of head and nose disorders, fistula-in-ano, and other conditions associated with oxidative stress and inflammatory processes.

Multiple peaks spanning a broad range of Rf values under both detection wavelengths demonstrated a complex phytochemical composition in the HPTLC fingerprinting of UVMC (Figures 2 and 3). The dominant peak at Rf 0.89 (56.68%) (Figure 2b–c) at 254 nm, which detects UV-absorbing compounds, indicates the presence of major non-polar constituents, probably phytosterols and terpenoid fractions from *Cuminum cyminum* L. Phytosterols like β-sitosterol are well known for their immunomodulatory and anti-inflammatory effects[22]. The presence of volatile oil components, such as cuminaldehyde, which has antibacterial and antioxidant properties, is shown by the peaks at Rf 0.74 (10.98%) and Rf 0.97 (7.44%) (Figure 2b)[23]. Flavonoids and quinone derivatives may be responsible for the peaks seen in the mid-Rf region, especially at Rf 0.51 (13.47%) and Rf 0.59 (2.35%) (Figure 2 b–c). These substances, which are frequently found in *Acalypha indica* L., have strong anti-inflammatory and free radical scavenging activities[24]. The existence of phenolic chemicals, which enhance antioxidant potential, is further suggested by the peak at Rf 0.33 (4.86%). The chromatogram showed additional fluorescent bands at 366nm (Figure 3a–c), which are indicative of substances like phenolic derivatives, coumarins, and flavonoids. Terpenoid and sterol fractions are confirmed by the significant peaks at Rf 0.79 (15.20%)

and Rf 0.89 (14.99%) (Figure 3b–c), which are consistent with the results at 254nm. These substances have antioxidant, antibacterial, and anti-inflammatory properties[25]. Sugar derivatives and glycosidic chemicals from *Saccharum officinarum* may be responsible for the strong peaks at low Rf values (0.01 and 0.03) at 366nm (Figure 3b). The solubility and bioavailability of active substances may be improved by these components [26].

Significantly, several peaks, such Rf 0.51, 0.74, and 0.89, appear clearly at both wavelengths (Figures 2b and 3b), showing compounds that are both fluorescent and UV-absorbing, proving their importance as major phytoconstituents. Overall, the HPTLC fingerprint profile shows the presence of phenolics, flavonoids, quinones, terpenoids, and phytosterols and creates a distinctive chromatographic pattern for UVMC. This profile can be used as a reliable analytical tool for formulation batch uniformity, authentication, and quality control.

The formulation complies with the World Health Organization's allowed limits, suggesting appropriate sanitary handling and processing, as reported by microbiological examination. The absence of harmful bacteria further confirms the formulation's safety for consumption[14]. Furthermore, aflatoxin analysis showed levels within permissible bounds, reducing the risk of mycotoxin contamination, a crucial issue in the safety of herbal medicines [27].

The microscopic features observed in this study are reliable diagnostic indicators for the formulation's authentication and quality evaluation. The inclusion of *Acalypha indica* in the formulation is confirmed by the presence of uniseriate simple trichomes and glandular trichomes with multicellular stalks and globose heads. The authentic nature of this plant material is further supported by the finding of paracytic stomata[16]. *Cuminum cyminum*, which is known to possess secretory canals (vittae) rich in volatile oils responsible for its therapeutic action, is characterized by the identification of vittae, endosperm cells containing aleurone grains, and micro-rosette crystals[28]. Another crucial taxonomic characteristic that aids in the identification of crude drugs is the presence of calcium oxalate druses. Because they support in identifying adulteration and substitution as well as ensuring batch-to-batch consistency of herbal formulations, their unique microscopic characteristics are commonly acknowledged as key parameters in pharmacognostic standardization. In addition to physicochemical and chromatographic investigations, powder microscopy offers a quick, cost-effective, and reliable technique to verify the identification and purity of polyherbal formulations [14,17].

Overall, this study's results are consistent with earlier standardization research on polyherbal formulations, which highlights the significance of combining various analytical techniques ensures quality and safety. The long-term reliability of UVMC as a traditional therapeutic formulation is highlighted by the presence of bioactive phytoconstituents and compliance with physicochemical and microbiological standards.

CONCLUSION

Through physicochemical, phytochemical, chromatographic, microbiological, and microscopic analyses, the current study effectively creates a thorough standardized profile for UVMC. The formulation showed satisfactory quality characteristics, no microbiological contamination, and adherence to aflatoxin safety limits. Its traditional therapeutic use is supported scientifically by the identification of bioactive components such phenolics, phytosterols, and quinones. The HPTLC fingerprint profile produced in this investigation can be used as a reference for batch consistency and quality assurance. Together, these results confirm the legitimacy, safety, and quality of UVMC and provide a scientific basis for its usage in traditional medicine. Further studies involving advanced phytochemical characterization and pharmacological evaluation are recommended to explore its full therapeutic potential.

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