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Research Article

STANDARDIZATION OF SIDDHA HERBAL FORMULATION - *VAASATHI KASHAYAM* ACCORDING TO PLIM GUIDELINES

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ABSTRACT

Despite the fact that the global market for herbal and traditional medicines has surged, the rise in demand has brought about a threat for adulteration/substitution of raw drugs, clamoring consumers' faith into skepticism with the ensuing implications. To evaluate and avoid substandard herbal medicine manufacture, the Indian government has taken stringent measures, notably the establishment of PLIM (Pharmacopoeial Laboratory of Indian Medicine - Protocol for testing AYUSH pharmaceuticals). The purpose of this research is the standardization of Siddha herbal decoction formulation *'Vaasathi kashyam'* encompassing the leaves of *Justicia adhatoda*.L (Acanthaceae) and dry fruits of *Vitis vinifera*.L (Vitaceae) procured from the classic Siddha text *'Agathiyar 2000'* specifically indicated for systemic hypertension (*Raththa kothippu noi*). The physicochemical parameters, phytochemical analysis, and powder microscopy of *Vaasathi kashayam* revealed an acidic pH and demonstrated the presence of phytochemicals such as tannins, phenols, terpenoids, alkaloids, flavonoids, and carbohydrates. HPTLC fingerprinting illustrates phytochemical spikes. Furthermore, the number of heavy metals, pesticide residues (organochlorine, organophosphorus and pyrethroids) and aflatoxins in VK was trace (BQL) or nonexistent, indicating its safety for therapeutic use. The total aerobic bacterial count and yeast/mold growth reported no growth/colonies, implying that the sample is free of aerobic microorganisms. Standardization of VK entailed authenticating along with evaluating the safety and quality of the prepared VK sample. These standardized characteristics could be deployed as a reference standard to guide subsequent VK qc evaluations.

INTRODUCTION

The shift in public perception toward traditional and complementary medicine necessitates the recourse of standard, evidence-based drugs to cater for the general public's medical needs. The World Health Organization (WHO) has consistently promoted the incorporation of traditional medicine practices and products into national health systems, provided they adhere to established standards of quality, safety, and efficacy.

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Contrary to common assumptions, traditional medicine products are not universally safe and may pose potential health risks. A total of 170 countries have reported on the utilization of traditional medicine[1]. Considering the extensive global use of these practices, monitoring their safety is a crucial and prioritized area of focus [2]. Although around 6000 higher plant species are used in diverse folkloric healthcare systems, only 2400 medicinal plants have been recognized by the codified Indian medical system[3,4]. Furthermore, a considerable number of cottage level herbal companies, both recognized and unregistered, rely on medicinal plant supplies, with only 10% coming from cultivated sources [3,5]. However, barely 10% of the supply is met from cultivated sources, the remaining obtained are from collection of naturally

occurring populations [3,4]. Collections of plants from the wild were often plagued by adulterations either intentionally or unintentionally (substitution) [3,6]. Adulteration is considered as an intentional addition of foreign substances to increase the weight of the product or to decrease its cost. It may be due to confusion in vernacular names, lack of knowledge about authentic plants, non-availability, similarity in morphology, activity, aroma, careless collection and other unknown reasons [7]. To warranty the quality of herbal pharmaceuticals and traditional remedies, the Government of India has enacted regulations for the Siddha sector. The Government of India has notified Good Manufacturing Practice under Schedule 'T' of the Drugs and Cosmetics Act of 1940 to ensure to enhance the quality of ASU medicines. It also assures that the supplies used in medicine manufacturing are trustworthy, of the requisite quality, and free of contamination[2]. The Department of AYUSH entrusted the Pharmacopoeia Laboratory for Indian Medicine in Ghaziabad with developing a protocol for evaluating ASU medicines. The current volume of the protocol for testing Ayurveda, Siddha, and Unani products encompasses various parameters for testing different categories of single and compound formulations, parameters for heavy metals, microbial load, pesticide residue, and aflatoxins, and methods to determine the quality and safety parameters. These guidelines were tailored from different official pharmacopoeia, including API, IP, BP, USP, EP, and Chinese pharmacopoeia[8]. Standardization of formulations from Siddha classical texts, as well as these evidence-based, time-tested pharmaceuticals, must be accomplished to combat AYUSH's propelling responsibility in public healthcare.

MATERIALS AND METHODS

Trial drug - *Vaasathi kashayam* (Decoction)

Vaasathi kashayam is a fresh decoction made from *Justicia adhatoda*.L leaves and dried *Vitis vinifera* L fruits. extracted from the classical Siddha text 'Agathiyar 2000'[9], and is particularly indicated for systemic hypertension *(Raththa kothippu noi).* The analytical parameters provided in the PLIM guidelines were only stipulated for *Kashaya Chooranam* (coarsely grounded powder); therefore these were adhered to for this study.

Collection of raw materials and authentication

The leaves and black raisins were sourced dust-free from Thakkalai town, Kalkulam Taluk, Kanyakumari district, in June, and identified and authenticated by Medicinal Botanist, The Department of Postgraduate Gunapadam (Pharmacology), Government Siddha Medical College, Palayamkottai, Tirunelveli District, on June 12, 2023.

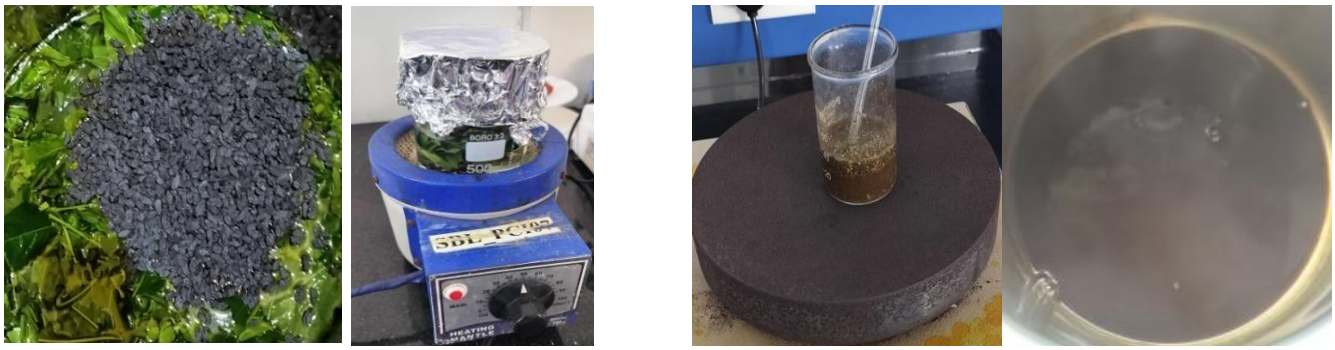
The ingredients of *Vaasathi kashayam* are as given below (Figure 1a-b).

(a) Leaves of *Justicia adhatoda*.L. (Acanthaceae)

(b) Dried fruits of *Vitis vinifera*.L (Vitaceae)

Figure 1: (**a**) Tamil name: Adhathodai, Family: Acanthaceae, Parts used: Leaves (**b**) Tamil name: Trakshai palam, Family: Vitaceae, Parts used: Dry fruits

Preparation of *Vaasathi kashayam*: Drugs that have been refined are immersed in eight times as much as water over night. Equivalent quantities of *Justicia adhatoda* leaves and *Vitis vinifera* dry fruits (5 grams each) were pulverized and stirred with approximately 240ml of water. This mixture was boiled down to 30ml with a hot plate/heating mantle encased in aluminum foil, and the fresh decoction extract was prepared upon straining the residues through a strainer/Whatman filter paper as given in figure 2 a-d.



(a) (b) (c) (d)

Figure 2: Preparation of *Vaasathi kashayam*: (a) Mixing of ingredients for the preparation of VK concoction (b) Usage of the hot plate and stirrer (c) Usage of the heating mantle (d) Prepared decoction of *Vaasathi kashayam*

Organoleptic Characters: Characteristics such as state, nature, odour, touch, flow property, appearance of the drug were noted. Along with this, the following phytochemical, physicochemical parameters and HPTLC profiling were conducted at Siddha regional Research Institute, Poojapura, Trivandrum, Kerala.

Physicochemical Evaluation

Loss on drying at 105°C: The loss on drying test evaluates the moisture content and volatile oil content of the medication. i.e., volatile oil and water evaporate from the medication. A 100ml beaker is correctly weighed. Approximately 4gm of VK (about 3mm thick) is placed in the beaker and weighed properly. Placed in the oven, the beaker is dried for 5 hours at 105°C. Cooled in a desiccator, then weighed. The procedure until a steady weight is achieved. The percentage of weight loss in the sample is assessed. [10-15]

Percentage of Loss on drying at 105°C =

$$\frac{\text{Loss in weight of the sample} \times 100}{\text{Weight of the sample taken}}$$

Total ash: The total ash method is intended to determine the total amount of material remaining after ignition. This contains both "physiological ash" (produced from plant tissue) and "non-physiological" ash (residue of extraneous materials (e.g., sand and dirt) sticking to the plant surface). A silica crucible is ignited, then cooled and weighed. Around 2g of VK is placed in the crucible and accurately weighed. In a muffle furnace, VK is incinerated until carbon has been eliminated, then cooled and weighed. The total ash (%) is calculated.

Percentage of Total ash=

$$\frac{\text{Weight of ash} \times 100}{\text{Weight of sample taken}}$$

Acid-insoluble ash: After boiling total ash with weak hydrochloric acid and burning the insoluble particles, the residue left is called acid-insoluble ash. This determines the amount of silica present, specifically in sand and siliceous earth. The complete ash is collected in the crucible. 25 cc of 6N hydrochloric acid is added and heated for five minutes. Filtered using ash-free filter paper and rinsed with hot water until the filtrate is acid-free (some filtrate from the funnel is removed and a silver nitrate solution is added). If no precipitate forms, the filtrate is acid-free. Following filtration, filter paper containing the insoluble particles is put into the same crucible and burnt either in an electric Bunsen or in a muffle furnace to constant weight.

Percentage of Acid insolube ash =

$$\frac{\text{Weight of acid insoluble residue} \times 100}{\text{Weight of sample taken}}$$

Water-soluble ash: Water-soluble ash is the weight difference between total ash and residue after it has been treated with water. Place the entire ash in the crucible. 25ml of water is added and heated for five minutes. Filtered with ash-free filter paper and rinsed in hot water. The filter paper containing the insoluble materials is transferred to the same crucible and burnt at a consistent weight. The weight of water-soluble ash is calculated by subtracting the water insoluble residue from the overall ash content. The percentage of water-soluble ash with respect to the air-dried medication is computed.

Percentage of Water solube ash =

$$\frac{\text{Weight of water-soluble ash} \times 100}{\text{Weight of sample taken}}$$

Sulphated ash: The total ash is collected in the crucible. The ash is moistened with a little amount (1ml) of sulfuric acid and burned in a muffle furnace at 600°C until the charred ash is totally incinerated. The crucible is cooled and weighed (%).

Percentage of Sulphated ash =

$$\frac{\text{Weight of sulphated ash} \times 100}{\text{Weight of sample taken}}$$

Alcohol-soluble extractive: This approach calculates the amount of chemical components extracted using ethyl alcohol from a given amount of medicinal plant material. About 4g of VK is extracted and precisely weighed. It is transferred to a glass-stoppered conical flask, and 100ml of alcohol (about 95%) is added. Occasionally, the mixture is shaken for 6 hours before allowing standing for 18 hours. Filter fast. A 50ml beaker is weighed, and 25ml of filtrate is pipetted into it. Treated on a water bath and placed in an oven at 105°C for 6 hours to dry, cooled in a desiccator for 30 minutes before weighing.

Percentage of Solubility in alcohol =

$$\frac{\text{Weight of extract x 100 x 100}}{\text{25 x Weight of sample taken}}$$

Water-soluble extractive: This technique calculates the amount of chemical components extracted with water from a specific amount of medicinal plant material. Approximately 4gm of VK is precisely weighed and transferred to a glass stoppered conical flask. 100ml of distilled water is added and shaken intermittently for 6 hours. Allow to stand for 18 hours and filter quickly. A 50ml beaker is weighed, and 25ml of filtrate is pipetted into it. At 105°C for 6 hours, the filtrate is dried after evaporated in water bath, cooled in a desiccator for 30 minutes before weighing.

Percentage of Solubility in water=

$$\frac{\text{Weight of extract x 100 x 100}}{\text{25 x Weight of sample taken}}$$

pH of water extract: The pH of an aqueous liquid is often computed as the common logarithm of the reciprocal of the hydrogen ion concentration, given in grams per liter. The pH value reflects whether the water extract of the medication is acidic, neutral, or alkaline. A value of less than 7 is acidic and greater than 7 is basic. A liquid's pH can be determined potentiometrically using a glass electrode, a reference electrode, and a digital pH meter.

Volatile oil: 1 litre R. B. flask comprising of 20gm of VK is taken. 300ml of water and a few porous pieces are introduced which is attached to a volatile oil equipment (Clevenger apparatus). The contents of the flask are now heated and boiled for two hours, or until the distillation is complete. The flask is turned occasionally to remove any debris that has adhered to its sides. The device is allowed to cool for 10 minutes before the volume is measured. The percentage volatile oil is calculated.

Percentage of Volatile oil =

$$\frac{\text{Volume of volatile oil x 100}}{\text{Weight of drug taken}}$$

Preliminary Phytochemical Analysis

Test for Saponins: Saponins are tested by adding a few milligrams of extract to distilled water and shaking thoroughly. The appearance of foam suggests the presence of saponin.

Test for Tannin: Tannin test involves adding 5% alcoholic ferric chloride to a substance in water. The dark blue tint indicates the presence of tannin.

Test for Terpenoids: Add conc. H_2SO_4, to a few mg of extract in chloroform. The presence of dark brown precipitate implies terpenoids.

Test for Phenol: To test for phenol, add 5% alcoholic ferric chloride to the substance in water. A dark blue or green color indicates the presence of phenol.

Test for Steroids (Lieberman Burchard Test):

Steroid Test (Lieberman Burchard Test): In a dry test tube, add a few milligrams of the extract and 2ml of chloroform. A few drops of acetic acid are added, heated, and then a few drops of acetic anhydride and two drops of concentrated sulfuric acid are introduced. The green tint shows the presence of a steroid.

Test for Quinones: Quinones can be tested by adding a few drops of strong sulfuric acid to a few milligrams of extract. The appearance of red indicates the presence of quinone.

Test for Glycosides: Glycoside testing involves treating a substance with anthrone and strong sulfuric acid. When heated over a water bath, the appearance of green indicates the presence of glycoside.

Test for Carbohydrates: To the sample solution, add few drops of α-naphthol and 2-3ml of concentrated H2SO4. A reddish violet or purple ring at the intersection of two liquids shows the presence of carbohydrates.

Test for Alkaloids (Dragendorff's Test): A few milligrams of extract were heated in a separate test tube with 2% sulfuric acid for two minutes. It was then filtered in a separate test tube, and a few drops of Dragendorff's reagent were added. Orange-red precipitates suggest the presence of alkaloids.

Test for Flavonoid: Add 10% NaOH or ammonia to the alcohol-based material. A dark yellow color suggests the presence of flavonoids.

Test for Proteins (Biuret test): In a test tube, add sodium hydroxide solution, followed by a few drops of extremely weak (1%) copper II sulphate solution. Gently mix. The appearance of purple indicates the presence of protein.

HPTLC Profiling

Developing solvent system: A number of solvent systems were tried and a system which gave the maximum resolution was selected as the solvent system for the extract. The optimum separations of constituents were achieved using the solvent system: Toluene: Ethyl acetate: Formic acid (5:2: 0.1), Volume applied: Track 1-5 µl: Track 2-7µl.[16, 17]

Sample application: The extracts were applied on different tracks at different concentrations of width 8mm each on silica gel 60 F254 pre-coated aluminium sheets through CAMAG micro litre syringe using Automatic TLC Sampler 4 (ATS4).

Development of chromatogram: After sample application the plate was introduced vertically in a CAMAG developing chamber (10cm×10cm) pre-saturated with the mobile phase selected.

Documentation: The generated chromatogram was air dried to remove solvents from the plate, then placed in the CAMAG Visualizer, and pictures were acquired using UV light at 254 and 366nm.

Densitometry*:* The plate was scanned at 254 and 366nm with the TLC Scanner 4, and the finger print profiles were captured. The Rf values and fingerprint data were captured using the scanner's associated Win CATS program.

Post chromatographic derivatization: The plate was derivatized with vanillin-sulphuric acid reagent and heated at 105°C on a CAMAG TLC plate heater until coloured bands emerged. The plate was then viewed with white light, and the chromatograms were recorded. The plate was scanned at 575nm, and the Rf values and fingerprint data were recorded.

Test for heavy metals: The tests were performed in The Tamilnadu Test House, Chennai, hereinafter.

Lead and Cadmium: AOAC 19th Edn, 2019; 971.21 methods were adopted. [18,19]

Principle: Test parts are dried and ashed at 450°C with a progressive increase (≤50°C/h) in temperature. 6M HCl (1+1) is added, and the solution is evaporated until dry. The residue is dissolved in 0.1M HNO_3 and the analytes are analysed using the flame and graphite technique. Pb and Cd are typically determined using atomic absorption spectrophotometry in a graphite furnace.

Atomic absorption spectrometry (AAS) is a prominent and reliable method for screening metals and metalloids in environmental materials. The total heavy metal content of the sample was determined using Atomic Absorption Spectrometry (AAS) Model AA 240 series. The following procedures were employed. For **Arsenic:** AOAC 19th Edn, 2019; 973.34 and Mercury: AOAC 19th Edn, 2019; 971.21

Test for Aflatoxins: Aflatoxins A1, A2, G1 and G2 were tested using procedures from Ayurveda Pharmacopeia. [20]

Test for pesticide residues: Polychlorinated biphenyls, organochlorine pesticides and organophosphorus pesticides were tested for in the given VK sample using APHA6630 and EPA8081B protocols respectively. [21,22]

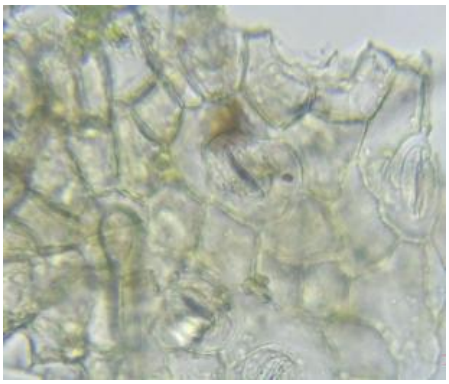
Microbial contamination: Pour plate methods were utilized to determine the sterility of the product. When a contaminated/unsterile sample (formulation) comes into contact with a nutrition-rich medium, it encourages the growth of the organism, which is detected by a particular pattern of colonies after the necessary period of incubation. The colonies are known as Colony Forming Units (CFUs).

Methodology: The test sample was placed on a sterile petri dish, and about 15ml of molten agar at 45°C was added. The agar and sample were well combined by tilting and rotating the plate. The agar was allowed to gel completely without being disturbed. (Around ten minutes). Plates were then inverted and incubated at 37°C for 24-48 hours, then extended for 72 hours to observe fungal growth. CFU were calculated from grown organism colonies. For total aerobic bacterial count and yeast– Mould count the procedures IS5402:2012RA2018 and IS5403: 1999RA2005 were followed respectively. [23,24]

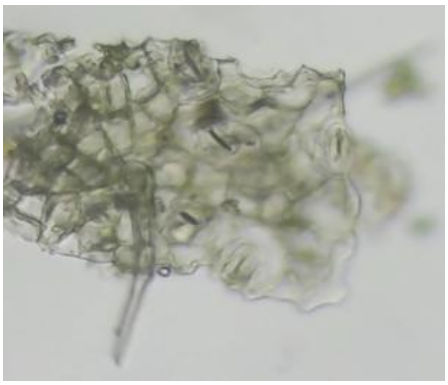
Test for specific pathogens: Specific pathogens such as E. coli, Salmonella sps, Pseudomonas aeruginosa and Staphylococcus aureus were examined in the given sample of VK using the following techniques IS5401:2012RA2018, IS5887 (Part 1): RA2018, IS5887 (Part 2): 1976RA2018 and IS5887 (Part 3): 1999RA2018 respectively.

RESULTS

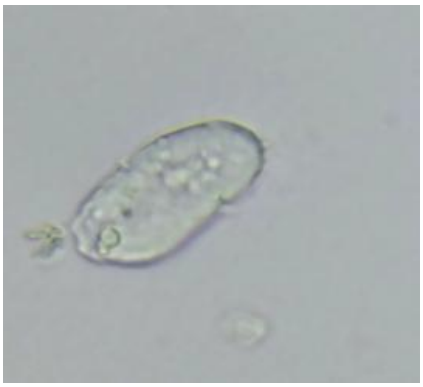
Powder microscopy: Organoleptic characters of *Vaasathi chooranam*



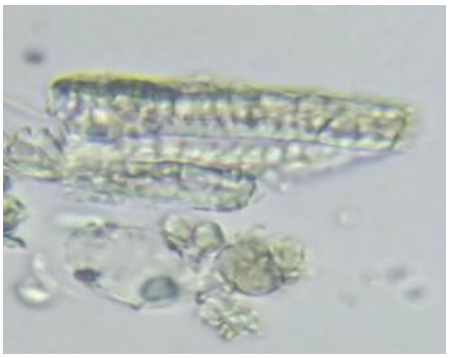
Diacytic stomata



Diacytic stomata and trichome

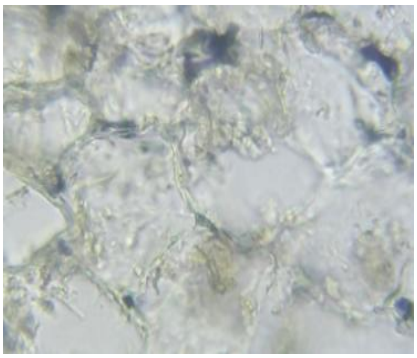


Simple starch grain

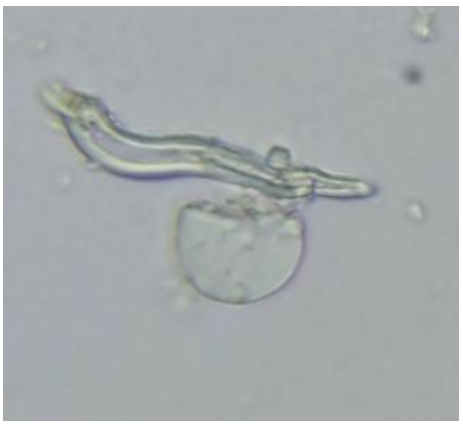


Pitted vessel

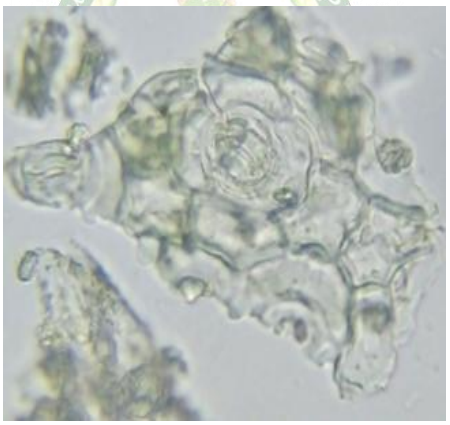
Stone cell with brown content



Thin walled parenchyma cells

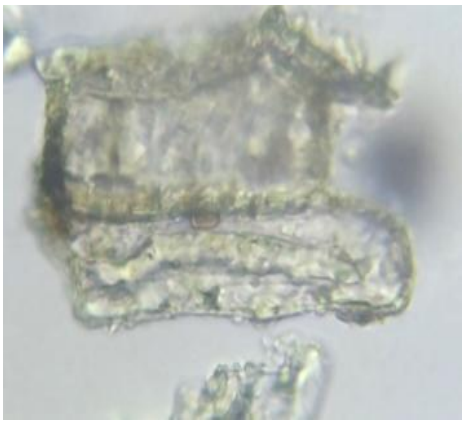


Uniseriate trichome

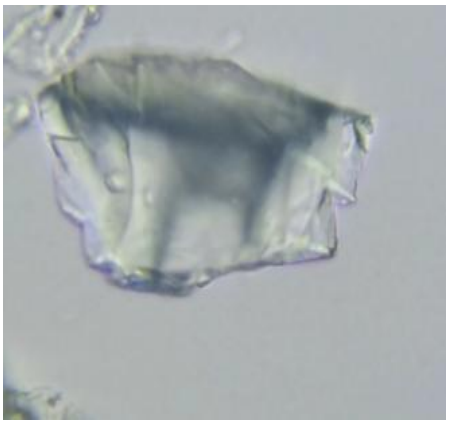


Diacytic stomata

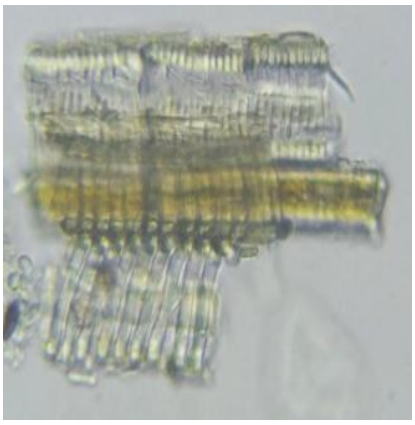
Thick walled fibre with narrow lumen



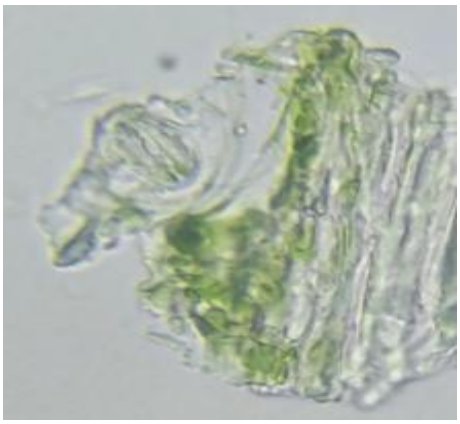
Group of stone cells



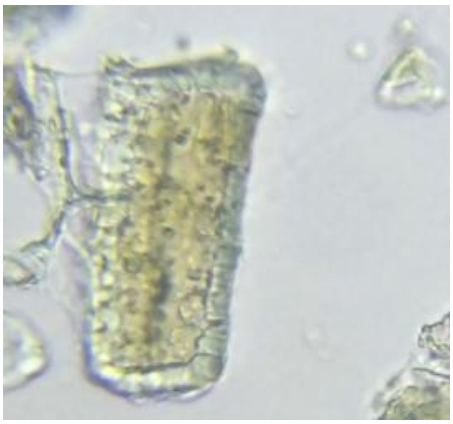
Calcium oxalate crystal



Annular and pitted vessels



Isolated stomata

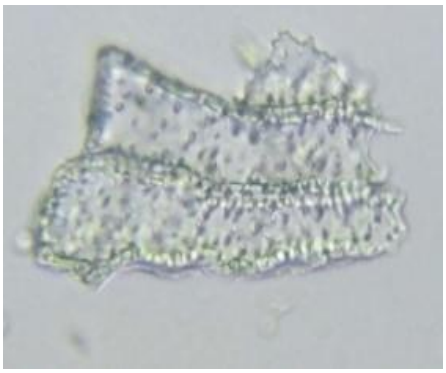


Stone cell

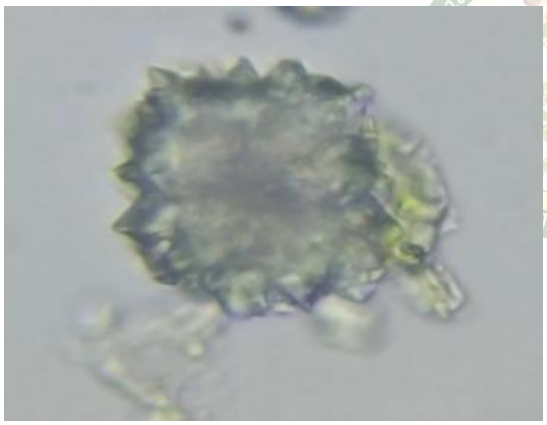
Fragment of pitted vessels

Annular thickening

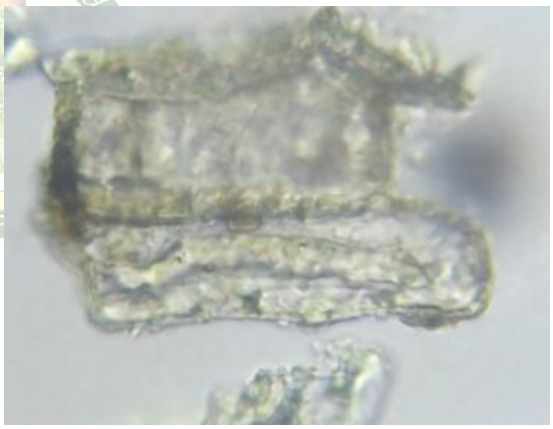
Longitudinal view of parenchyma cells



Fragment of pitted vessels



Rosette calcium oxalate crystals



Group of stone cells

The descriptive microscopic characteristics of VK are enlisted in the below table 1.

Powder microscopy characteristics: Annular thickening, stone cell, simple starch grain, longitudinal view of parenchyma, thick-walled fibre with narrow lumen, diacytic stomata, rosette calcium oxalate crystal, pitted vessels, uniseriate trichomes (unicellular and multicellular with blunt end) were seen.

Table 1: Organoleptic characteristics of *Vaasathi kashayam*

Specification	**Character**
State	Solid
Nature	Granular
Odour	Characteristic odour
Touch	Rough
Flow Property	Non-Free flowing
Appearance	Brown in colour
Taste	Bitter in taste

Physicochemical parameters

The results were depicted below in table 2 showing 13.85 - LOD at 105°C, 5.25 - Total Ash, 0.31 - Acid insoluble ash, 2.15 - water soluble ash, 8.42 – sulphated ash, 5.37 – pH of water extract, 27.30 – Alcohol soluble extractives and 36.83 – Water soluble extractives.

Table 2: Results of Physicochemical parameters of *Vaasathi kashayam*

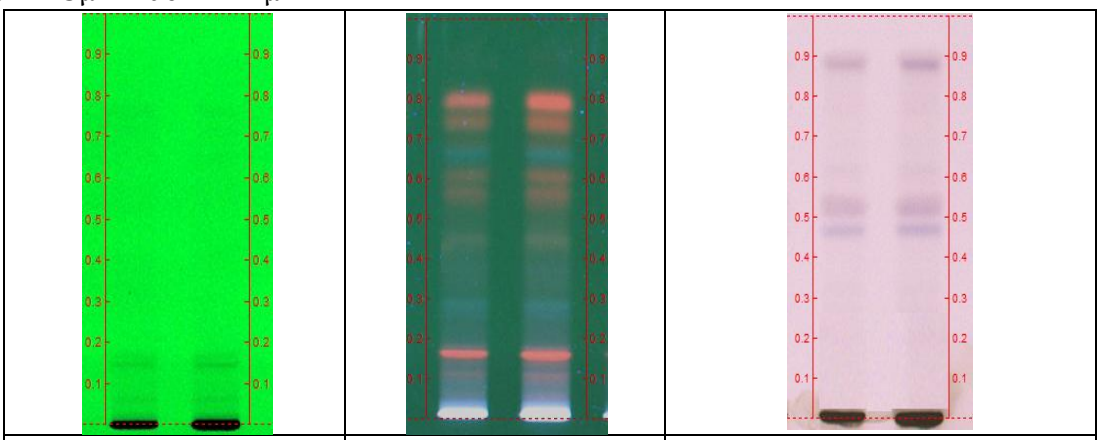
Physicochemical Parameters	***Vaasathi Kashayam***
LOD at 105°C	13.85
Total Ash	5.25
Acid insoluble ash	0.31
Water soluble ash	2.15
Sulphated ash	8.42
pH of water extract (4% aqueous solution)	5.37
Alcohol soluble extractives	27.30
Water soluble extractives	36.83
Volatile oil	Nil

Phytochemical tests: The preliminary phytochemical tests on VK revealed the presence of tannins, phenols, terpenoids, alkaloids, flavonoids and carbohydrates as given in table 2.

Table 3: Results of phytochemical tests on *Vaasathi kashayam*

Phytochemical Tests	***Vaasathi Kashayam***
Saponins	-
Tannins	+
Phenols	+
Terpenoids	+
Alkaloids	+
Flavonoids	+
Steroids	-
Glycosides	-
Carbohydrates	+
Quinones	-
Proteins	-

HPTLC profiling: Alcohol extract: Solvent system: Toluene: Ethyl acetate: Formic acid (5:2: 0.1), Volume applied; Track 1- 5µl: Track 2 – 7µl

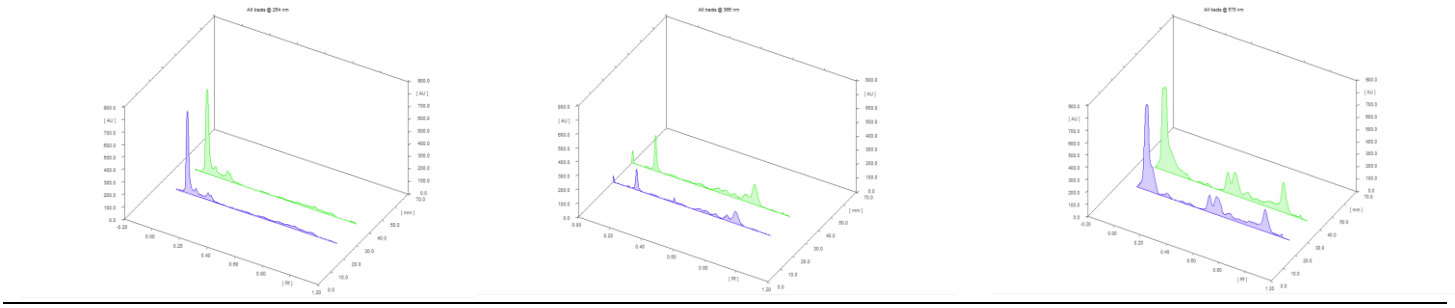


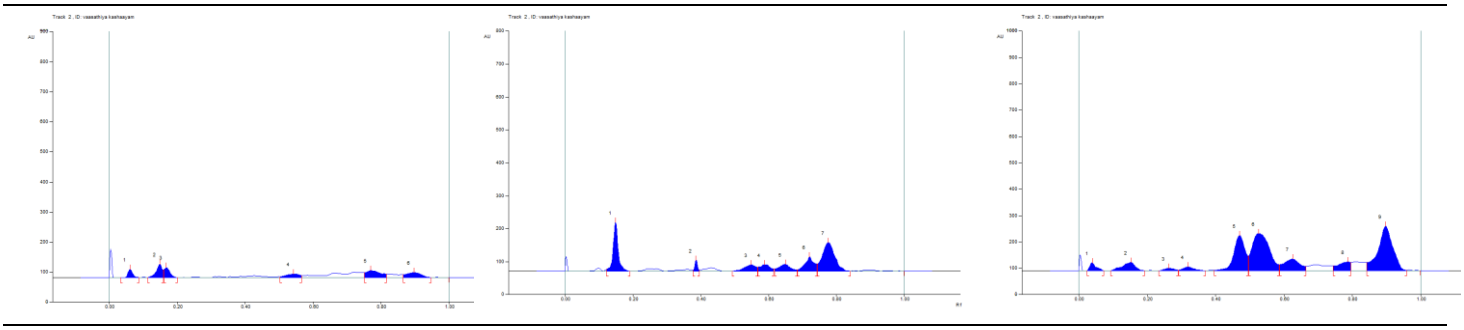
Under UV short | Under UV long | Under white light after derivatization

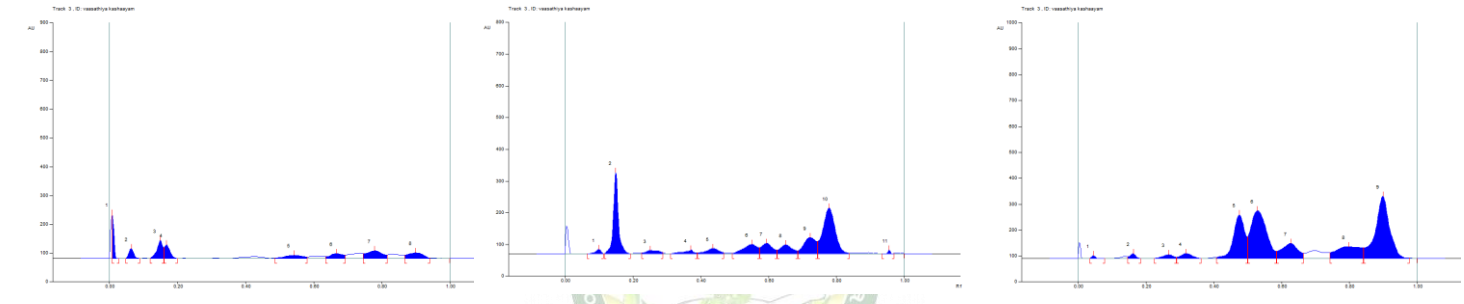
Figure 3 HPTLC results of VK a-Under UV short, b-Under UV long and c-Under white light after derivatization. The plate was derivatised using vanillin-sulphuric acid reagent, heated at 105°C by placing on CAMAG TLC plate heater till the colour of the bands appeared.

The R_f values under 254nm, 366nm and 575 nm are tabulated in the figure 4 given below.

254 nm **366nm** **575 nm**







Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.03 Rf	0.0 AU	0.06 Rf	27.6 AU	17.53 %	0.09 Rf	0.2 AU	269.0 AU	9.73 %
2	0.11 Rf	0.4 AU	0.15 Rf	43.8 AU	27.82 %	0.16 Rf	25.7 AU	549.3 AU	19.87 %
3	0.16 Rf	26.8 AU	0.17 Rf	33.2 AU	21.12 %	0.20 Rf	0.1 AU	386.9 AU	14.00 %
4	0.50 Rf	2.5 AU	0.54 Rf	12.1 AU	7.71 %	0.57 Rf	7.2 AU	337.7 AU	12.22 %
5	0.75 Rf	16.7 AU	0.77 Rf	23.3 AU	14.81 %	0.82 Rf	11.9 AU	712.1 AU	25.76 %
6	0.87 Rf	9.6 AU	0.90 Rf	17.3 AU	11.00 %	0.95 Rf	0.4 AU	509.1 AU	18.42 %

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.12 Rf	6.1 AU	0.15 Rf	149.3 AU	39.96 %	0.19 Rf	0.1 AU	1533.7 AU	25.11 %
2	0.38 Rf	2.9 AU	0.39 Rf	33.4 AU	8.94 %	0.40 Rf	3.6 AU	185.6 AU	3.04 %
3	0.49 Rf	0.1 AU	0.55 Rf	19.8 AU	5.29 %	0.57 Rf	12.4 AU	464.6 AU	7.61 %
4	0.57 Rf	12.5 AU	0.59 Rf	19.5 AU	5.23 %	0.62 Rf	6.8 AU	418.6 AU	6.85 %
5	0.62 Rf	6.9 AU	0.65 Rf	20.8 AU	5.58 %	0.69 Rf	4.2 AU	489.6 AU	8.02 %
6	0.69 Rf	4.3 AU	0.72 Rf	43.4 AU	11.61 %	0.74 Rf	22.1 AU	808.0 AU	13.23 %
7	0.75 Rf	22.2 AU	0.78 Rf	87.3 AU	23.38 %	0.84 Rf	0.3 AU	2207.6 AU	36.15 %

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.03 Rf	0.9 AU	0.04 Rf	30.7 AU	5.04 %	0.07 Rf	0.1 AU	387.8 AU	2.27 %
2	0.09 Rf	0.3 AU	0.15 Rf	31.7 AU	5.20 %	0.19 Rf	0.1 AU	841.5 AU	4.92 %
3	0.23 Rf	0.1 AU	0.26 Rf	10.2 AU	1.68 %	0.29 Rf	2.6 AU	196.2 AU	1.15 %
4	0.29 Rf	2.7 AU	0.32 Rf	14.5 AU	2.38 %	0.37 Rf	0.2 AU	317.2 AU	1.86 %
5	0.40 Rf	2.0 AU	0.47 Rf	134.1 AU	22.00 %	0.50 Rf	56.0 AU	3064.4 AU	17.92 %
6	0.50 Rf	56.9 AU	0.53 Rf	141.5 AU	23.20 %	0.59 Rf	23.3 AU	4950.1 AU	28.95 %
7	0.59 Rf	23.3 AU	0.63 Rf	44.2 AU	7.26 %	0.67 Rf	16.8 AU	1453.0 AU	8.50 %
8	0.75 Rf	17.9 AU	0.79 Rf	33.7 AU	5.53 %	0.80 Rf	32.2 AU	855.1 AU	5.00 %
9	0.84 Rf	31.0 AU	0.90 Rf	169.1 AU	27.72 %	0.96 Rf	2.1 AU	5031.3 AU	29.43 %

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	152.9 AU	0.01 Rf	152.9 AU	41.28 %	0.03 Rf	0.0 AU	629.0 AU	13.83 %
2	0.05 Rf	0.3 AU	0.06 Rf	34.3 AU	9.27 %	0.09 Rf	0.1 AU	337.1 AU	7.41 %
3	0.12 Rf	0.5 AU	0.15 Rf	61.7 AU	16.65 %	0.16 Rf	37.7 AU	742.8 AU	16.33 %
4	0.16 Rf	38.3 AU	0.17 Rf	46.8 AU	12.65 %	0.20 Rf	0.3 AU	524.2 AU	11.53 %
5	0.49 Rf	1.0 AU	0.54 Rf	11.0 AU	2.97 %	0.58 Rf	5.0 AU	405.4 AU	8.92 %
6	0.64 Rf	6.4 AU	0.67 Rf	17.5 AU	4.73 %	0.69 Rf	11.4 AU	444.9 AU	9.78 %
7	0.75 Rf	16.2 AU	0.78 Rf	26.5 AU	7.15 %	0.82 Rf	12.2 AU	855.8 AU	18.82 %
8	0.87 Rf	11.3 AU	0.90 Rf	19.7 AU	5.31 %	0.94 Rf	1.0 AU	608.3 AU	13.38 %

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.06 Rf	0.0 AU	0.10 Rf	13.6 AU	2.24 %	0.11 Rf	0.2 AU	152.8 AU	1.41 %
2	0.12 Rf	0.5 AU	0.15 Rf	256.6 AU	42.36 %	0.19 Rf	0.0 AU	2618.3 AU	24.11 %
3	0.23 Rf	2.5 AU	0.25 Rf	10.7 AU	1.76 %	0.29 Rf	4.1 AU	279.4 AU	2.57 %
4	0.31 Rf	0.2 AU	0.37 Rf	11.4 AU	1.89 %	0.39 Rf	2.4 AU	237.5 AU	2.19 %
5	0.39 Rf	2.5 AU	0.44 Rf	16.2 AU	2.67 %	0.47 Rf	3.6 AU	422.3 AU	3.89 %
6	0.49 Rf	1.3 AU	0.55 Rf	29.4 AU	4.85 %	0.57 Rf	19.1 AU	838.5 AU	7.72 %
7	0.57 Rf	19.3 AU	0.60 Rf	32.8 AU	5.42 %	0.63 Rf	12.1 AU	723.7 AU	6.66 %
8	0.63 Rf	12.2 AU	0.65 Rf	28.4 AU	4.69 %	0.69 Rf	8.7 AU	688.9 AU	6.34 %
9	0.69 Rf	9.2 AU	0.72 Rf	51.5 AU	8.50 %	0.75 Rf	38.5 AU	1267.4 AU	11.67 %
10	0.75 Rf	38.9 AU	0.78 Rf	143.9 AU	23.76 %	0.84 Rf	1.4 AU	3574.1 AU	32.91 %
11	0.94 Rf	0.4 AU	0.96 Rf	11.3 AU	1.86 %	0.97 Rf	1.0 AU	58.2 AU	0.54 %

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.04 Rf	0.2 AU	0.05 Rf	10.5 AU	1.38 %	0.08 Rf	0.2 AU	79.0 AU	0.36 %
2	0.15 Rf	6.4 AU	0.16 Rf	18.4 AU	2.43 %	0.19 Rf	0.1 AU	211.7 AU	0.96 %
3	0.22 Rf	0.0 AU	0.27 Rf	14.2 AU	1.87 %	0.29 Rf	4.6 AU	298.9 AU	1.36 %
4	0.29 Rf	4.6 AU	0.32 Rf	18.8 AU	2.48 %	0.36 Rf	0.3 AU	427.3 AU	1.94 %
5	0.41 Rf	3.2 AU	0.48 Rf	168.2 AU	22.19 %	0.50 Rf	83.4 AU	3902.4 AU	17.72 %
6	0.50 Rf	84.7 AU	0.53 Rf	184.5 AU	24.35 %	0.58 Rf	27.6 AU	5903.1 AU	26.80 %
7	0.59 Rf	27.7 AU	0.63 Rf	58.1 AU	7.67 %	0.67 Rf	20.9 AU	1932.2 AU	8.77 %
8	0.74 Rf	22.3 AU	0.80 Rf	45.3 AU	5.98 %	0.84 Rf	41.6 AU	2331.3 AU	10.58 %
9	0.84 Rf	41.6 AU	0.90 Rf	239.8 AU	31.64 %	0.98 Rf	1.5 AU	6939.6 AU	31.51 %

Figure 4. Under 254nm, 366nm, and 575nm, the Rf was plotted on a graph and tabulated. The extracts were applied as various tracks of varied concentrations with a width of 8 mm each on silica gel 60 F254 pre-coated aluminium sheets using a CAMAG micro-litre syringe and the Automatic TLC Sampler 4. The plate was scanned at 254 and 366 nm with the TLC Scanner 4, and the finger print profiles were captured. The Rf readings and finger print data were captured using the scanner's accompanying Win CATS program. After derivatisation, the plate was photographed under white light, and the chromatograms were recorded. The plate was scanned at 575 nm, and the Rf values and fingerprint data were recorded.

Heavy metal analysis: The fresh decoction of *Vaasathi kashayam* is tested for heavy metals such as lead, cadmium, arsenic, and mercury by AAS in accordance with PLIM recommendations. Table 4 displays the results, which reveal that the aforementioned heavy metals are below the quantification limit in the trial drug.

Table 4: Results of heavy metal analysis

Parameter	Method	Result	Limit (Unit - ppm)
Lead	AOAC 19th Edn, 2019; 999.11	BQL (LOQ:0.01)	NMT 10
Cadmium	AOAC 19th Edn, 2019; 999.11	BQL (LOQ:0.01)	NMT 1.0
Arsenic	AOAC 19th Edn, 2019; 973.34	BQL (LOQ:0.01)	NMT 0.3
Mercury	AOAC 19th Edn, 2019; 971.21	BQL (LOQ:0.01)	NMT3

Test for aflatoxins: The fresh decoction of *Vaasathi kashayam* is tested for aflatoxins such as A1, A2, G1, and G2. Table 5 shows that the aforementioned aflatoxins are within the measurement limit in the experimental medicine.

Table 5: Results of test for aflatoxins

Parameter	Method	Result	Limit *Unit-ppb*
Total Aflatoxins	Ayurvedic Pharmacopoeia	BQL (LOQ0.001)	5.0
AflatoxinsB1	Ayurvedic Pharmacopoeia	BQL (LOQ:0.001)	2.0
AflatoxinsB2	Ayurvedic Pharmacopoeia	BQL LOQ:0.05)	-
AflatoxinsG1	Ayurvedic Pharmacopoeia	BQL (LOQ:1.0)	-
AflatoxinsG2	Ayurvedic Pharmacopoeia	BQL (LOQ:0.001)	-

Test for Pesticide residue: Non-dioxin-like polychlorinated biphenyls were identified in trial drug decoction of *Vaasathi kashayam*, with values below the quantification limit. OC-Pesticides and OP-Pesticides - L,G were also determined to be below the quantification limit, as shown in tables 6 and 7.

Table 6: Results of test for pesticide residues (PCBs, Organochlorine - pesticides)

Pesticide Residues	Results	Pesticide Residues	Results
Non-Dioxin Like PCBs	Method: APHA6630 (mg/kg)	delta-HCH	BQL (LOQ0.001)
Polychlorinated biphenyls PCB	BQL (LOQ:0.00005)	Dicofol	BQL (LOQ0.001)
OC-Pesticides	Method: EPA8081B (mg/kg)	Dieldrin	BQL (LOQ0.001)
2,4'-DDE	BQL(LOQ0.001)	Endosulfan Sulfate	BQL (LOQ0.001)
2,4'-DDT	BQL(LOQ0.001)	Endrin	BQL (LOQ0.001)
2,4'-DDD	BQL(LOQ0.001)	Fen valerate and Esfenvalerate	BQL (LOQ0.001)
4,4'-DDD	BQL(LOQ0.001)	Heptachlor	BQL (LOQ0.001)
4,4'-DDE	BQL (LOQ0.001)	Heptachlor epoxide	BQL (LOQ0.001)
4,4'-DDT	BQL (LOQ0.001)	Hexachlorobenzene	BQL (LOQ0.001)
Aldrin	BQL (LOQ0.001)	Hexachlorocyclohexane	BQL (LOQ0.001)
alpha-Endosulfan	BQL (LOQ0.001)	Lindane(gamma-HCH)	BQL (LOQ0.001)
alpha-HCH	BQL (LOQ0.001)	Methoxychlor	BQL (LOQ0.001)
beta-Endosulfan	BQL (LOQ0.001)	Pyrethrins	BQL (LOQ0.001)
beta-HCH	BQL (LOQ0.001)	Chlordane (cis & trans)	BQL (LOQ0.001)
Chlorothalonil	BQL (LOQ0.001)		

Table 7: Results of test for pesticide residues (Organophosphorus pesticides)

Pesticide residues	Results	Pesticide residues	Results
OP Pesticides-G	Method: EPA8081B (mg/kg)	Glufosinate ammonium	BQL(LOQ0.001)
4-Brono-2-Chlorphenol	BQL (LOQ0.001)	Glyphosphate	BQL(LOQ0.001)
Chlorfenvinphos	BQL (LOQ0.001)	Iprobenfes	BQL(LOQ0.001)
(cis & trans)	BQL (LOQ0.001)	Malaoxon	BQL(LOQ0.001)
Chlorpyrifos methyl	BQL (LOQ0.001)	Malathion	BQL(LOQ0.001)
Diazinon	BQL (LOQ0.001)	Methamidophos	BQL(LOQ0.001)
Dichlorvos	BQL (LOQ0.001)	Metribuzin	BQL(LOQ0.001)
Ethion	BQL (LOQ0.001)	Monocrotophus	BQL(LOQ0.001)
Fenitrothion	BQL (LOQ0.001)	Omethoate	BQL(LOQ0.001)
Fenthion	BQL (LOQ0.001)	Parnoxan Methyl	BQL(LOQ0.001)
Formothion	BQL (LOQ0.001)	Phenthoate	BQL(LOQ0.001)
Parathion Methyl	BQL (LOQ0.001)	Phorate sulphonoxide	BQL(LOQ0.001)
Quinaphos	BQL (LOQ0.001)	Phosalone	BQL(LOQ0.001)
Disulfoton	BQL (LOQ0.001)	Phosphamidon	BQL(LOQ0.001)
Fenamiphos	BQL (LOQ0.001)	Pirimiphosethyl	BQL(LOQ0.001)
Methidathion	BQL (LOQ0.001)	Pirimiphos methyl	BQL(LOQ0.001)
OP Pesticides-L	BQL (LOQ0.1)	Profenophos	BQL(LOQ0.001)
Acephate	BQL (LOQ0.1)	Sulfotep	BQL(LOQ0.001)
Azinphos ethyl	BQL (LOQ0.1)	Temephos	BQL(LOQ0.001)
Azinphos methyl	BQL (LOQ0.1)	Thiometon	BQL(LOQ0.001)
Coumaphos	BQL (LOQ0.1)	Thionazin	BQL(LOQ0.001)
Dimethoate	BQL (LOQ0.001)	Triazophos	BQL(LOQ0.001)
Acephate	BQL (LOQ0.001)	Trichlorfon	BQL(LOQ0.001)
Azinophos Ethyl	BQL (LOQ0.001	Triethion	BQL(LOQ0.001)
Azinophos methyl	BQL (LOQ0.001	Chlorthion	BQL(LOQ0.001)
coumaphos	BQL (LOQ0.001)	Edifenphos	BQL(LOQ0.001)
Dimethoate	BQL (LOQ0.001)	Ethephos	BQL(LOQ0.001)
Dimeton O	BQL (LOQ0.001)	Etrimphos	BQL(LOQ0.001)

Microbial contamination: Total bacterial count and yeast-mould count were assessed revealing <10 CFU/ml as depicted in table 8.

Table 8: Results of microbial analysis

Microbiology Analysis				
Total Aerobic Bacterial Count	IS5402:2012RA2018	CFU/ml	**<10**	NMT1000
Yeast and Mould Count	IS5403:1999RA2005	CFU/ml	**<10**	NMT100

Test for specific pathogens: Specific pathogens such as E. coli spp, Salmonella spp, P.aeuginosa, S.aureus were absent in the decoction of *Vaasathi kashayam* as tabulated in table 9.

Table 9: Results of test for specific pathogens

E.Coli spp.	IS5401:2012RA2018	Per ml	Absent
Salmonella Spp.	IS5887(Part1):RA2018	Per ml	Absent
Pseudomonas aeruginosa	IS5887(Part2):1976RA2018	Per ml	Absent
Staphylococcus aureus	IS5887(Part3):1999RA2018	Per ml	Absent

DISCUSSION

The growing integration of traditional and complementary medicine into mainstream healthcare systems emphasizes the importance of stringent requirements and evidence-based validation for these approaches [25]. Standardization of *Vaasathi kashayam* indicated for hypertension is vital for ensuring quality, effectiveness, and safety that comply with contemporary pharmacological standards. The organoleptic attributes of *Vaasathi kashayam* reveal a solid, granular form with a distinct odor and rough texture, which aids in its identification and quality evaluation. The microscopic examination clarifies the formulation's composition by highlighting the presence of diacytic stomata, stone cells, and calcium oxalate crystals. These findings are consistent with the features of plants from the Acanthaceae and Vitaceae [26,27], emphasizing the need of recognizing cellular structures to assure the authenticity of herbal products. The physicochemical investigations offer intriguing insights into the composition of *Vaasathi kashayam.* The loss on drying (LOD) at 105°C is 13.85%, indicating moderate moisture content, which is essential for preserving the decoction's stability. The total ash value (5.25%) estimates the inorganic matter, which, together with the acid-insoluble ash (0.31%), provides information about the purity and potential contamination of the raw materials. The water-soluble extractives (36.83%) and alcohol-soluble extractives (27.30%) indicate a diverse phytochemical profile, emphasizing the potential bioactive chemicals present. The pH of 5.37 suggests that the aqueous extract of *Vaasathi kashayam* is relatively acidic, which may influence the stability and bioavailability of its active ingredients. This finding lines up with the concept that weak acids exist predominantly in their un-ionized state and are better substrates for passive diffusion at the pH of the stomach [28]. The phytochemical analysis of *Vaasathi kashayam* revealed the presence of tannins, phenols, terpenoids, alkaloids, flavonoids, and carbohydrates. These compounds have been identified for an array of pharmacological activities, including antioxidant, anti-inflammatory, and antihypertensive effects. Flavonoids possess cardio-vasculo protective effects and could delay the onset or progression of many cardiovascular diseases, particularly hypertension[29], and phenols can suppress ROS, reducing oxidative stress to biomolecules within cells[31-33], exerting various biological activities including antioxidant, anticancer, anti-diabetic, anti-inflammatory, and antihypertensive[33-35], which may contribute to the formulation's intended effects on hypertension. The absence of saponins, steroids, glycosides, and proteins indicate a distinct profile that may be unique to *Vaasathi kashayam*, implying that the therapeutic benefits are most likely mediated by the previously identified bioactive chemicals rather than the lacking classes. The HPTLC evaluation yields a precise fingerprint of the bioactive components in *Vaasathi kashayam*. The solvent system utilized (toluene: ethyl acetate: formic acid) was efficient in accomplishing the required segregation of ingredients, facilitating the identification of individual compounds based on Rf values. The visualization of bands under different light conditions (UV and white light) following derivatization on two distinct tracks (1: 1-5µl, 2: 2-7µl) demonstrated that on 254nm (UV short), a total of 6 peaks with Rf values ranging from 0.03 to 0.87 and 8 peaks with Rf values ranging from 0.01 to 0.87. At 366nm (UV long), 7 spikes (Rf ranging from 0.12 to 0.75) and 11 peaks (Rf ranging from 0.06 to 0.94) were seen. On 575nm (after derivatization), track 1 has 9 peaks with Rf values ranging from 0.03 to 0.84, whereas track 12 has 9 peaks with Rf values ranging from 0.04 to 0.84. This fingerprinting generates a comprehensive profile that may be utilized for quality control and future pharmacological studies. Heavy metal screening verified that *Vaasathi kashayam* is devoid of lead, cadmium, arsenic, and mercury, which is critical for patient safety. The thorough analysis for aflatoxins, which revealed levels below the quantification limit, emphasizes the trial drug's quality and safety. The pour plate technique sterility tests demonstrate that *Vaasathi kashayam* is free of microbial contamination, with a total bacterial count of <10 CFU/ml, as well as yeast and mold counts, proving the formulation's safety. The absence of specific pathogens such as E. coli, Salmonella, Pseudomonas

aeruginosa, and Staphylococcus aureus increases the assurance of the formulation, ensuring its safety.

CONCLUSION

This research has established a comprehensive standardization framework for the Siddha herbal formulation *Vaasathi kashayam*, aligning with the PLIM guidelines. The findings emphasize the critical importance of quality, safety, and efficacy assessments. The organoleptic and microscopic evaluations confirmed the formulation's identity and integrity, while physicochemical analyses provided valuable insights into its stability and potential therapeutic profile. The diverse phytochemical composition, particularly the presence of bioactive compounds such as flavonoids and phenols, underscores *Vaasathi kashayam's* potential in managing hypertension and its associated cardiovascular risks. The HPTLC fingerprinting technique has produced a detailed profile of the formulation, facilitating quality control and paving the way for future pharmacological investigations. Additionally, rigorous safety assessments confirmed the absence of harmful heavy metals, aflatoxins, and microbial contamination, reinforcing the formulation's suitability for clinical application. Collectively, these findings support the integration of *Vaasathi kashayam* into modern therapeutic regimens, encouraging further research to elucidate its mechanisms of action and enhance animal and clinical trials.

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REFERENCES

1. WHO, Traditional medicine, August 2023, https://www.who.int/news-room/questions-and-answers/item/traditional-medicine
2. The science of holistic medicine, Siddha system of medicine, Ministry of Ayurveda, Yoga & Naturopathy, Unani, Siddha and Homoeopathy (AYUSH) Government of India New Delhi www.ayush.gov.in 2019
3. Ved, D. K., & Goraya, G. S. (2007). Demand and supply of medicinal plants in India. NMPB, New Delhi & FRLHT, Bangalore, India, 18(85), 210-52.
4. Santhosh Kumar, J. U., Krishna, V., Seethapathy, G. S., Ganesan, R., Ravikanth, G., & Shaanker, R. U. (2018). Assessment of adulteration in raw herbal trade of important medicinal plants of India using DNA bar coding. 3 Biotech, 8(3), 135. https://doi.org/10.1007/s13205-018-1169-3
5. Seethapathy GS, Ganesh D, Santhosh Kumar JU, Senthilkumar U, Newmaster SG, Ragupathy S, Uma Shaanker R, Ravikanth G. Assessing product adulteration in natural health products for laxative yielding plants, Cassia, Senna, and Chamaecrista, in Southern India using DNA barcoding. Int J Legal Med. 2014; 129: 693–700. doi: 10.1007/s00414-014-1120-z.
6. Srirama R, Senthilkumar U, Sreejayan N, Ravikanth G, Gurumurthy BR, Shivanna MB, Uma Shaanker R. Assessing species admixtures in raw drug trade of Phyllanthus, a hepato-protective plant using molecular tools. J Ethnopharmacol. 2010;130:208–215. doi:10.1016/j.jep.2010.04.042.
7. Rajeev kumar, *Kurele, Rohit, K., Gajana, P., Abdulah, D., JP, S., & Srinivasulu, B. (2018). A Comprehensive Review on Adulteration Of Raw Materials Used In Asu Drug Manufacturing. International Journal of Ayurveda and Pharma Research, 6(3). Retrieved from https://ijapr.in/index.php/ijapr/article/view/ 889
8. Lohar DR. Protocol for testing: Ayurvedic, Siddha and Unani Medicines. Pharmacopoeial Laboratory for Indian Medicine, Ghaziabad
9. Dr. S.Venkatrajan. L.I.M, Agathiyar 2000, 2014, pg no. 218

10. K. Megala, S. Janani, V. Velpandian et.al. Standardization of siddha formulation "gandhagapoora parpam" by using modern techniques. Int J Health Sci Res. 2022; 12(5): 228-237. DOI: https:// doi.org/10.52403/ijhsr. 20220524
11. Saravanan K, Venugopal S and Shilpa R: Standardization of Amukkara Chooranum-Siddha drug. Int J Pharm Sci & Res 2023; 14(6): 3101-09. doi: 10.13040/IJPSR.0975-8232.14(6). 3101-09.
12. Kannaiyan, N., & Muthu Kanniyakumari. (1). Standardization Of Siddha Herbo-Mineral Drug As Nano Medicine Through Ftir, ICP-OES And Sem. International Journal of Ayurveda and Pharma Research, 8(2), 58-63. Retrieved from https://ijapr.in/index.php/ijapr/article/view/1 366
13. Saraswathy, Ariamuthu. (1994). Standardisation of Siddha drugs. Ancient science of life. 14. 53-60.
14. Anonymous, Pharmacopoeial Standards for Ayurvedic Formulations, Central Council for Research in Ayurveda and Siddha, Ministry of Health & Family Welfare, Govt. of India, New Delhi, (1987).
15. J Kumar, S Heamavathi, M D Saravana Devi et.al. Physicochemical evaluation and standardization of siddha formulation ratthi naagara rasa mezhugu by modern techniques. Int J Health Sci Res. 2022; 12(5): 238-250. DOI: https://doi.org/10.52403/ijhsr.20220525
16. Chaitali Das, Rajlakshmi Poi and Ashim Chowdhury, HPTLC Determination of Vasicine and Vasicinone in Adhatoda vasica, Phytochemical Analysis Phytochem. Anal. 16, 90–92 (2005) Published online in Wiley Interscience (www.interscience.wiley.com). DOI: 10.1002.pca.817
17. Lei, M., Wang, L., Olatunde, O.O. et al. UPLC–ESI–QTOF–MS profiling, antioxidant, anti-diabetic, antibacterial,anti-inflammatory, antiproliferative activities and in silico molecular docking analysis of Barleria strigosa. Chem. Biol. Technol. Agric. 10, 73 (2023). https://doi.org/ 10.1186/s40538 -023-00451-2
18. AOAC official method 999.11, first action 1999, NMLK-AOAC method, 2002, AOAC International.
19. AOAC: Official Methods of Analysis (Volume 1), Association of official analytical chemists Inc. 15th edition, 1990
20. The Ayurvedic Pharmacopoeia of India Part - II (Formulations) Volume - II First Edition, The Controller of Publications Civil Lines, Delhi – 110054, ISSBN no: 978 – 81 905952 – 0 – 9
21. Standard Methods: 6630 C: Organochlorine Pesticides in Water by GC, https://www.nemi.gov/methods/method_summary/4717/
22. Method 8081B Organochlorine Pesticides by Gas Chromatography, https://www.epa.gov/sites/default/files/2015-12/documents/8081b.pdf
23. Food Hygiene, Safety Management and Other Systems Sectional Committee, FAD 15, April 2012, IS 5402: 2012, ISO 4833: 2003
24. Food Microbiology Sectional Committee, FAD 46, May 1999, IS 5403: 1999
25. Patwardhan B. (2015). Public perception of AYUSH. Journal of Ayurveda and integrative medicine, 6(3), 147–149. https://doi.org/10.4103/0975-9476.166389
26. Ettiyappan, Munuswamy & Krishnan, V. & Amerjothy, S. (2018). Micromorphology of Calcium Oxalate Crystals in Selected Medicinal Plants. World Journal of Pharmaceutical Research. 7. 751-758. 10.20959/wjpr201811-12377.
27. Lukšić, K.; Zdunić, G.; Mucalo, A.; Marinov, L.; Ranković-Vasić, Z.; Ivanović, J.; Nikolić, D. Microstructure of Croatian Wild Grapevine (Vitis vinifera subsp. sylvestris Gmel Hegi) Pollen Grains Revealed by Scanning Electron Microscopy. Plants 2022, 11, 1479. https://doi.org/10.3390/plants11111479
28. Koen Boussery, Frans M. Belpaire, Johan Van de Voorde, Chapter 23 - Physiological Aspects Determining the Pharmacokinetic Properties of Drugs, Editor(s): Camille Georges Wermuth, David Aldous, Pierre Raboisson, Didier Rognan, The Practice of Medicinal Chemistry (Fourth Edition), Academic Press, 2008, Pages 539-559, ISBN 9780124172050, https://doi.org/10.1016/B978-0-12-417205-0.00023-7.
29. Maaliki, D., Shaito, A. A., Pintus, G., El-Yazbi, A., & Eid, A. H. (2019). Flavonoids in hypertension: a brief review of the underlying mechanisms. Current opinion in pharmacology, 45, 57–65. https://doi.org/10.1016/j.coph.2019.04.014
30. Yu, M., Kim, H. J., Heo, H., Kim, M., Jeon, Y., Lee, H., & Lee, J. (2022). Comparison of the Antihypertensive Activity of Phenolic Acids. Molecules (Basel, Switzerland), 27(19), 6185. https://doi.org/10.3390/molecules27196185

31. Saibabu, V., Fatima, Z., Khan, L. A., & Hameed, S. (2015). Therapeutic Potential of Dietary Phenolic Acids. Advances in pharmacological sciences, 2015, 823539. https://doi.org/10.1155/2015/823539
32. Bahare Salehi, Sanja Vlaisavljevic, Charles Oluwaseun Adetunji, Juliana Bunmi Adetunji, Dorota Kregiel, Hubert Antolak, Ewelina Pawlikowska, Yadav Uprety, Ksenija S. Mileski, Hari Prasad Devkota, Javad Sharifi-Rad, Gitishree Das, Jayanta Kumar Patra, Arun Kumar Jugran, Antonio Segura-Carretero, Maria del Mar Contreras, Plants of the genus Vitis: Phenolic compounds, anticancer properties and clinical relevance, Trends in Food Science & Technology, Volume 91, 2019, Pages 362-379, https://doi.org/10.1016/j.tifs.2019.07.042.
33. Nassiri-Asl M, Hosseinzadeh H. Review of the pharmacological effects of Vitis vinifera (Grape) and its bioactive compounds. Phytother Res. 2009 Sep; 23(9): 1197-204. doi: 10.1002/ptr.2761. PMID: 19140172
34. Sun, J., Chu, Y. F., Wu, X., & Liu, R. H. (2002). Antioxidant and antiproliferative activities of common fruits. Journal of agricultural and food chemistry, 50(25), 7449–7454. https://doi.org/10.1021/jf0207530
35. Krishnaiah D, Sarbatly R, Nithyanandam R. A review of the antioxidant potential of medicinal plant species. Food Bioprod Process. 2011; 89: 217–33.

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