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Research Article

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Physico chemical and phytochemical analysis of Visnukandhi kudineer chooranam, a Siddha polyherbal formulation

Dr.K.Visithra[1], Dr.K.Vennila[2] , Dr. M.Meenakshi Sundaram[3] , Dr. N.J. Muthukumar[4]

[1] PG scholar, Department of Kuzhandhai Maruthuvam, National Institute of Siddha, Chennai-47
[2] Lecturer, Department of Kuzhandhai Maruthuvam, National Institute of Siddha, Chennai-47
[3] Prof & Head of the Department, Department of Kuzhandhai Maruthuvam, National Institute of Siddha, Chennai - 47
[4]Director (i/c), National Institute of Siddha, Chennai-47

Abstract

Pharmacognostical standardization of herbal formulation is essential in order to assess the quality of drugs, based on the concentration of their active principles. Visnukandhi kudineer chooranam is a poly herbal formulation mentioned in Siddha classical texts, consisting of five ingredients. It is commonly used for the treatment of fever with or without respiratory infection. The drug is prepared as per the method mentioned in the classic siddha literature. The drug is subjected to physicochemical analysis such as total ash, loss on drying, total sugar, reducing sugar, fat content, microbial load, Thin Layer Chromatography and High Performance Thin Layer Chromatography as per the Pharmacopoeial laboratory standards of Indian medicine. This information will be used for laying down the pharmacopoeial standards of Visnukandhi kudineer chooranam.

Keywords: Poly herbal siddha formulation, Visnukandhi kudineer chooranam, Physico-chemical analysis, phytochemical studies.

Introduction

The Siddha system of medicine is the ancient system of medicines and being practiced by a large population in Southern part of India. The reason for the popularity of the system is attributed to its effective cure with minimal side effects. Siddha preparations have been classified into different categories of medicinal forms as 32 internal and 32 external and chooranam is one among the internal medicinal form. Visnukandhi Kudineer chooranam is a classic siddha compound drug which is mentioned in Siddha textbook of Balavagadam. This drug is used for fever, cough particularly for kabasuram[1]. The current drug is further classified as kudineer chooranam which means a drug to be made into decoction and consumed. According to WHO guidelines, an herbal product needs to be standardized with respect to safety before releasing it into the market. In this paper an attempt was made to evaluate a Siddha formulation, Visnukandhi kudineer chooranam by analytical methods and chromatographic studies. Kudineer chooranam consists of coarse powders of drugs and is used for the preparation of decoction. Decoctions should be prepared whenever required and should not be stored for a long period, as molds develop in the decoction.

Aim and Objective

The aim of this study is to do Physico chemical analysis, phyto chemical analysis and HPTLC finger printing for the drug Visnukandhi kudineer chooranam.

Materials and Methods

Collection and Identification of plant materials

The herbal ingredients were authenticated by the Assistant Professor of Medicinal botany , National Institute of Siddha, Chennai.

Preparation of the drug Visnukandhi kudineer chooranam[2]

Ingredients:

Visnukandhi (*Evolvulus alsinoides)* - ¼ palam (8.75gm)
Chukku (*Zingiber officinale)* - ¼ palam (8.75gm)
Kadukkaithol (*Terminalia chebula*) - ¼ palam (8.75gm)
Indu (*Mimosa paniculatus*) - ¼ palam (8.75gm)
Valuzhuvai (*Celastrus paniculatus*) - ¼ palam (8.75gm)

Purification of raw drugs[3]

The raw drugs are purified as per the methods mentioned in the Siddha literatures .

Analytical methods

The analytical methodology includes determination of organoleptic characters, preliminary phytochemical analysis, physico- chemical analysis, TLC photo documentation and HPTLC finger print studies.

Organoleptic characters

The organoleptic characters such as colour, taste and odour were noted in table -1. Fig: 1

Extraction[4, 5]

The drug was extraction by using petroleum ether, chloroform, ethyl acetate and methanol. The extracts were concentrated under reduced pressure at room temperature. Fig: 2 & table 2

Physico-chemical parameters [6, 7]

The physico-chemical examinations include determination of total ash, acid insoluble ash, extractable matter in water and alcohol, loss on drying at 105˚C . All the physico-chemical parameters were determined by standard methods. Table - 3

Percentage Loss on Drying

Test drug was accurately weighed in evaporating dish. The sample was dried at 105^{o}C for 5 hours and then weighed.

Determination of Total Ash

Test drug was accurately weighed in silica dish and incinerated at the furnace a temperature 400 ºC until it turns white in color which indicates absence of carbon. Percentage of total ash will be calculated with reference to the weight of air-dried drug.

Determination of Acid Insoluble Ash

The ash obtained by total ash test will be boiled with 25 ml of dilute hydrochloric acid for 6mins. Then the insoluble matter is collected in crucible and will be washed with hot water and ignited to constant weight. Percentage of acid insoluble ash will be calculated with reference to the weight of air-dried ash.

Determination of Alcohol Soluble Extractive

Test sample was macerated with 100 ml of Alcohol in a closed flask for twenty-four hours, shaking frequently during six hours and allowing it to stand for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish, and dry at 105ºC, to constant weight and weigh. Calculate the percentage of alcohol-soluble extractive with reference to the air-dried drug.

Determination of Water Soluble Extractive

Test sample was macerated with 100 ml of chloroform water in a closed flask for twenty-four hours, shaking frequently during six hours and allowing it to stand and for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of

the filtrate to dryness in a tared flat bottomed shallow dish, and dry at 105ºC, to constant weight and weigh. Calculate the percentage of water-soluble extractive with reference to the air-dried drug. Fig 3 & Table- 4.

Sterility test by pour plate method

Objective

The pour plate techniques were adopted to determine the sterility of the product. Contaminated / un sterile sample (formulation) when come in contact with the nutrition rich medium it promotes the growth of the organism and after stipulated period of incubation the growth of the organism was identified by characteristic pattern of colonies. The colonies are referred to as Colony Forming Units (CFUs).

Methodology

Test sample was admixed with sterile distilled water and the mixture were been used for the sterility evaluation. About 1ml of the test sample was inoculated in sterile petri dish to which about 15 mL of molten agar 45^{o}C were added. Agar and sample were mixed thoroughly by tilting and swirling the dish. Agar was allowed to completely gel without disturbing it. (about 10 minutes). Plates were then inverted and incubated at 37^{o} C for 24-48 hours. Grown colonies of organism was then counted and calculated for CFU. Fig 4 & Table – 5.

Test for Specific Pathogen

Methodology

One part of the test sample was dissolved in 9 mL of sterile distilled water and the test sample was directly inoculated in to the specific pathogen medium (EMB, DCC, Mannitol ,Cetrimide) by pour plate method. The plates were incubated at 37^{o}C for 24 - 72h for observation. Presence of specific pathogen identified by their characteristic color with respect to pattern of colony formation in each differential media. Table -6,7 & Fig:5,6,7,8

Preliminary phytochemical analysis

Preliminary phytochemical analysis for phenols, terpenoids, steroids, flavonoids, quinones, coumarins, alkaloids, tannins, acids and glycosides were carried out by standard procedures. Table – 8

Thin layer chromatographic (TLC) and high performance thin layer chromatographic (HPTLC) analysis [8, 9]

HPTLC is an invaluable quality assessment tool for the evaluation of herbal drugs and is the simplest separation technique today available to the analyst.

TLC Analysis

Test sample was subjected to thin layer chromatography (TLC) as per conventional one dimensional ascending method using silica gel 60F254, 7X6 cm (Merck) were cut with ordinary household scissors. Plate markings were made with soft pencil. Micro pipette were used to spot the sample for TLC applied sample volume 10-micro liter by using pipette at distance of 1 cm at 5 tracks. In the twin trough chamber with the specified solvent system After the run plates are dried and was observed using visible light Short-wave UV light 254nm and light long-wave UV light 365 nm. Fig:9

High Performance Thin Layer Chromatography Analysis

HPTLC method is a modern sophisticated and automated separation technique derived from TLC. Pre-coated HPTLC graded plates and auto sampler was used to achieve precision, sensitive, significant separation both qualitatively and quantitatively. High performance thin layer chromatography (HPTLC) is a valuable quality assessment tool for the evaluation of botanical materials efficiently and cost effectively. HPTLC method offers high degree of selectivity, sensitivity and rapidity combined with single-step sample preparation. Thus this method can be conveniently adopted for routine quality control analysis. It provides chromatographic fingerprint of phytochemicals which is suitable for confirming the identity and purity of phytotherapeutics. Fig:10

Chromatogram Development

It was carried out in CAMAG Twin Trough chambers. Sample elution was carried out according to the adsorption capability of the component to be analyzed. After elution, plates were taken out of the chamber and dried.

Scanning

Plates were scanned under UV at 366nm. The data obtained from scanning were brought into integration through CAMAG software. Chromatographic finger print was developed for the detection of phytoconstituents present in each sample and their respective Rf values were tabulated.

Results and Discussion

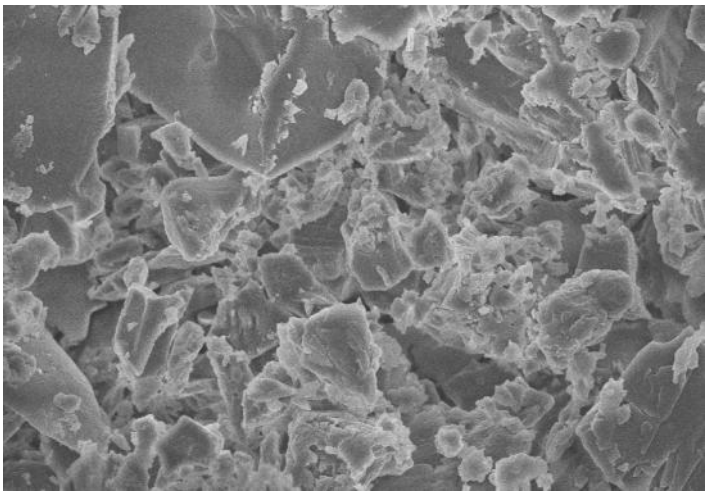
Organoleptic characters:

Fig :1Sample Description

Table :1- Organoleptic character

State	Solid	Decoction- Water Extraction
Appearance	Pale greenish	Brownish
Nature	Fibrous Coarse powder	Slight viscous
Odor	Strong Characteristic	Characteristic

Fig:2 Electron Microscopic Observation of Particle Size for the Test Sample- VKK



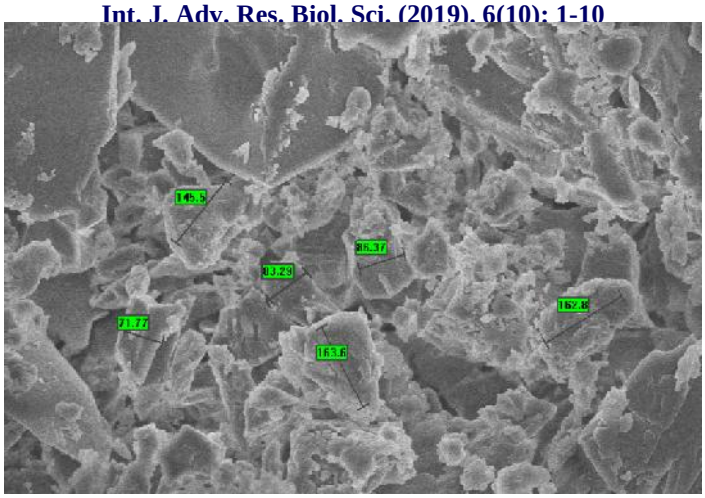


Table : 2

Mean	**118.3**
Std. Deviation	**42.77**
Std. Error	**17.46**

Report

Microscopic observation of the particle size analysis reveals that the average particle size of the sample was found to be 118.3± 42.77 µm

Table: 3

S.No	Parameter	Mean (n=3) SD
1.	**Loss on Drying at 105 °C (%)**	1.233 ± 0.15
2.	**Total Ash (%)**	4.478 ± 0.3977
3.	**Acid insoluble Ash (%)**	0.6467 ± 0.11
5.	**Alcohol Soluble Extractive (%)**	19.17 ± 1.77
6.	**Water soluble Extractive (%)**	37 ± 5.568

Fig: 3 Solubility Profile of VKK

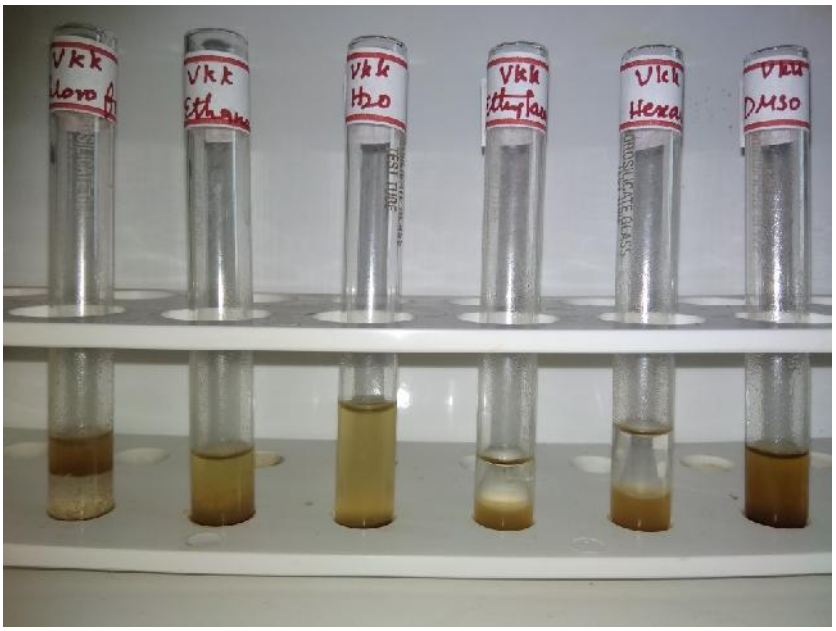
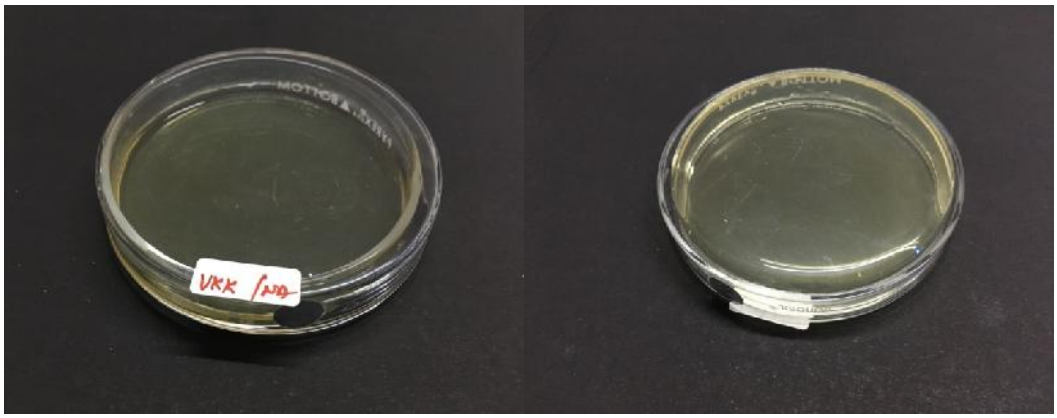


Table – 4

S.No	Solvent Used	Solubility / Dispersibility
1	Chloroform	Insoluble
2	Ethanol	Soluble
3	Water	Soluble
4	Ethyl acetate	Insoluble
5	Hexane	Insoluble
6	DMSO	Soluble

Fig: 4 Sterility test by pour plate method



Observation

No growth was observed after incubation period.
Reveals the absence of specific pathogen.

Result

Table 5:

Test	Result	Specification	As per AYUSH/WHO
Total Bacterial Count	Absent	NMT 10^5CFU/g	As per AYUSH specification
Total Fungal Count	Absent	NMT 10^3CFU/g	

Table: 6 Detail of Specific Medium and their abbreviation

Organism	Abbreviation	Medium
E-coli	***EC***	***EMB Agar***
Salmonella	***SA***	***Deoxycholate agar***
Staphylococcus Aureus	***ST***	***Mannitol salt agar***
Pseudomonas Aeruginosa	***PS***	***Cetrimide Agar***

Observation

No growth was observed after incubation period.
Reveals the absence of specific pathogen.

Result

Table: 7

Organism	Specification	Result	Method
E-coli	Absent	Absent	As per AYUSH specification
Salmonella	Absent	Absent	
Staphylococcus Aureus	Absent	Absent	
Pseudomonas Aeruginosa	Absent	Absent	

Fig: 5Culture plate with E-coli (EC) specific medium

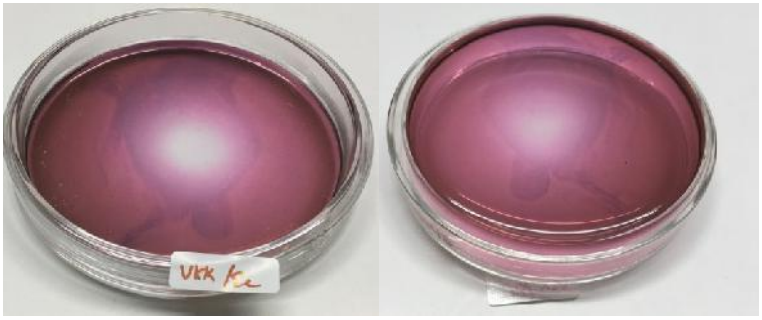


Fig: 6Culture plate with Salmonella (SA) specific medium

Fig: 7 Culture plate with Staphylococcus Aureus (ST) specific medium

Fig: 8 Culture plate with Pseudomonas Aeruginosa (PS) specific medium

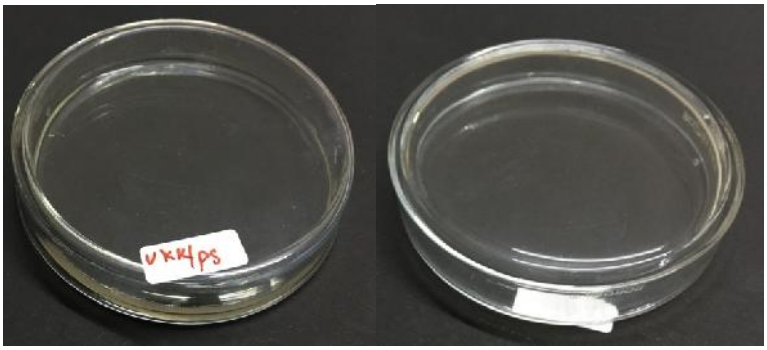


Table: 8 Preliminary phytochemical analysis of VKK

S.No	Test	Observation
1	Alkaloids	+
2	Flavanoids	+
3	Glycosides	+
4	Steroids	+
5	Triterpenoids	-
6	Coumarin	+
7	Phenol	+
8	Tanin	+
9	Protein	-
10	Saponins	+
11	Sugar	+
12	Anthocyanin	-
13	Betacyanin	-

Fig: 9 TLC Analysis

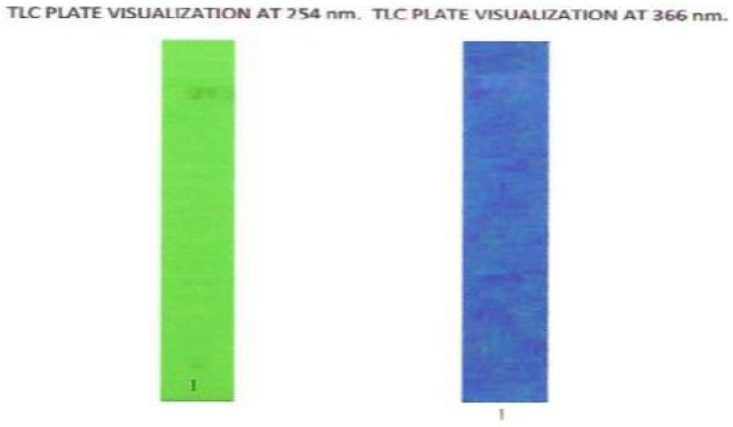


Fig:10 HPTLC finger printing of Sample VKK

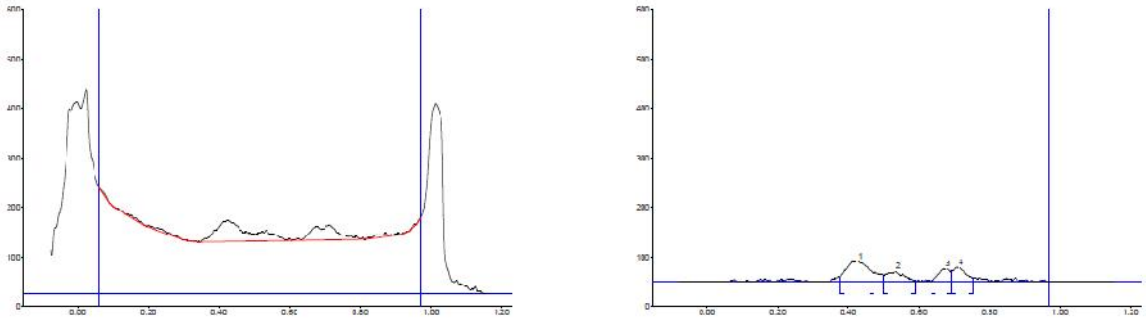


Table :9 Peak Table

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.37	9.4	0.43	41.9	34.52	0.47	18.4	1926.7	46.11
2	0.50	13.8	0.53	21.9	18.07	0.59	1.8	770.5	18.44
3	0.64	1.1	0.68	27.0	22.26	0.69	21.4	626.4	14.99
4	0.69	21.7	0.71	30.6	25.16	0.75	8.6	855.1	20.46

Report

HPTLC finger printing analysis of the sample reveals the presence of four prominent peaks corresponds to presence of four versatile phytocomponents present with in it.

Conclusion

Analysis of Visnukandhi kudineer has been carried out with a view to suggest standards for evaluating its quality and purity. The analytical parameters along with TLC photo documentation and HPTLC fingerprinting profile will be diagnostically important characters in fixing its pharmacopoeial standards. Therefore, the necessity of standardization of various herbal formulations will pave way to explore the therapeutic effects as claimed in Siddha literature and thereby improving the scientific credibility of Siddha medicine.

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