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Physicochemical Standardization of Siddha Herbal Drug Formulation - Vallarai Nei

Dr. K. Ajitha Bala[1], Dr. A. M. Amala Hazel[2], Dr. M. Meenakshi Sundaram[3], Dr. R. Meenakumari[4]

[1]PG Scholar, Department of Kuzhandhai Maruthuvam, National Institute of Siddha, Chennai – 47, *kajithabala[at]gmail.com*

[2]Guide & Associate Professor, Department of Kuzhandhai Maruthuvam, National Institute of Siddha, Chennai – 47, India

[3]Head of the Department, Department of Kuzhandhai Maruthuvam, National Institute of Siddha, Chennai – 47, India

[4]Director, National Institute of Siddha, Chennai – 47, India

Abstract: ***Siddha system of medicine is a system blended with Tamil culture. Among the various system of Traditional Medicine, Siddha medical science is the oldest and excellent one. Siddha drugs belong to herbal, metal, mineral or herbomineral and animal origin. World over there is renaissance for herbal drugs. Herbs maintain a natural ability within the body even in times of disharmony and diseases. Systematic protocols for standardization of Vallarai Nei is not available, hence it was decided to evaluate the qualitative and quantitative analysis for Vallarai Nei scientifically to prevent its adulteration. The drug Vallarai Nei is in the consistency of semisolid with Dark greenish in colour with Non- free flowing property, bitter in taste. The analytical report was as below Specific Gravity -0.8564, Viscosity at 50°C (Pa s) - 97.79, Weight per ml - 0.5015, Acid Value mg KOH/g - 0.8976 and Peroxidase Value mEq/kg - 4.84.Results of the present investigation have clearly shows that the sample has no traces of heavy metals such as Mercury, Lead, Arsenic and Cadmium.***

Keywords: siddha herbal drug**,** Vallarai Nei and standardization

1. Introduction

Siddha system of medicine is a system blended with Tamil culture. Among the various system of Traditional Medicine, Siddha medical science is the oldest and excellent one. It's based on the combination of medicinal practices and spiritual disciplines. It's a system of medical practice developed by 18 Siddhars many centuries ago.[1]

Siddha drugs belong to herbal, metal, mineral or herbomineral and animal origin. World over there is renaissance for herbal drugs. Herbs maintain a natural ability within the body even in times of disharmony and diseases.[2]

Thus, the present study deals with standardization of Siddha herbal preparation, Vallarai Nei mentioned in siddha text "Athmarakshamirtham ennum vaidhiya sarasangiragam", has been used for the treatment of Suram (Fever), Irumal (Cough), Pandu/VeluppuNoi (Anemia) and Kamalai (Jaundice).[3]

Systematic protocols for standardization of Vallarai Nei is not available, hence it was decided to evaluate the qualitative and quantitative analysis for Vallarai Nei scientifically to prevent its adulteration. For the standardization of this drug organoleptic properties and physicochemical parameters, were carried out. In addition, residue analyses such as heavy metal analysis, microbial load, and Aflatoxin analysis were also examined to strength the standardization process.

Aim and Objective

The aim of this study is to do physicochemical analysis, Heavy metal analysis, TLC and HPTLC analysis for the drug 'Vallarai Nei'.

2. Materials and Methods

Collection of raw drugs

The required drugs were purchased from Ramaswamy Chettiyar country drug shop, Kandhaswamy Kovil Street, Paris, Chennai.

Authentication

Raw drugs were authenticated by the Medicinal Botanist in National Institute of Siddha, Chennai. The trial drug was prepared in Gunapadam lab, National Institute of Siddha, Chennai-47.

Table 1: Ingredients of Vallarai Nei[3]

DRUG NAME	BOTANICAL NAME	ENGLISH NAME	FAMILY	QUANTITY
Vallarai	*Centella asiatica*	Indian pennywort	Apiaceae	2 padi (2.6 litre)
Seenthil	*Tinospora cordifolia*	Heart leaved moonseed	Menispermaceae	1\2 padi (650ml)
Kadukkai	*Terminalia chebula*	Ink nut	Combretaceae	2 varagan edai (8.4grams)
Thanrikkai	*Terminalia bellirica*	Beleric myrobalans	Combretaceae	2 varagan edai (8.4grams)
Nellikkai	*Phyllanthus emblica*	Indian gooseberry	Euphorbiaceae	2 varagan edai (8.4grams)
Chukku	*Zingiber officinale*	Dried ginger	Zingiberaceae	2 varagan edai (8.4grams)
Milagu	*Piper nigrum*	Black pepper	Piperaceae	2 varagan edai (8.4grams)

Thippili	*Piper longum*	Long pepper	Piperaceae	2 varagan edai (8.4grams)
Kirambu	*Syzygium aromaticum*	Clove tree	Myrtaceae	2 varagan edai (8.4grams)
Thalisapathiri	*Abies spectabilis*	Himalayan fir	Pinaceae	2 varagan edai (8.4grams)
Athimathuram	*Glycyrrhiza glabra*	Indian liquorice	Fabaceae	2 varagan edai (8.4grams)
Kostam	*Costus speciosus*	Costus root	Zingiberaceae	2 varagan edai (8.4grams)
Siruthekku	*Clerodendrum serratum*	Blue flower glory tree	Verbenaceae	2 varagan edai (8.4grams)
Kadukurohini	*Picrorhiza kurroa*	Picrorhiza	Scrophulariaceae	2 varagan edai (8.4grams)
Naruku moolam	*Piper longum*	Long pepper root	Piperaceae	2 varagan edai (8.4grams)
Nannari	*Hemidesmus indicus*	Indian sarasaparilla	Asclepiadaceae	2 varagan edai (8.4grams)
Sitramutti	*Sida cordifolia*	Country mallow	Malvaceae	2 varagan edai (8.4grams)
Seeragam	*Cuminum cyminum*	Cumin seeds	Apiaceae	2 varagan edai (8.4grams)
Perichampazham	*Phoenix dactylifera*	Date palm	Arecaceae	2 varagan edai (8.4grams)
Munthiripazham	*Vitis vinifera*	Dry grapes	Vitaceae	2 varagan edai (8.4grams)
Karkandu	*Saccharum officinarum*	Candy sugar	Poaceae	2 palam (70 grams)
Nei	-	Ghee	-	1 padi (1.3litre)

Purification of the drugs: [4,5]

Vallarai -It's should be washed in distilled water
Seenthil-The outer epidermal layer is to be peeled off
Kadukkai-The inner seeds are to be removed
Thanrikkai-The inner seeds are to be removed
Nellikkai-The inner seeds are to be removed
Chukku-It's to be soaked in lime water.
Milagu -It's to be soaked in sore butter milk for 1 to 11/4 hours and then fried
Thippli-It's soaked to be lemon juice and then fried
Kirambu -It's should be dried in sunlight
Thalisapathiri -It's should be dried in sunlight
Athimathuram -It's should be washed well in water and then the outer epidermal layer is removed and cut into small pieces and then dried
Kostam -It's should be dried in sunlight
Siruthekku-The outer epidermal layer is to be removed and cut to small pieces and dried in sunlight
Kadugurohini- It's to be soaked in neem leaf juice for 3 hours and then dried in sunlight
Narukumoolam -The nodes are to be removed and dried
Nannariver-It's should be washed in distilled water
Sitramuttiver -It's should be washed in distilled water
Seeragam -It's to be dried in sunlight for 6 hours and then dried

Preparation of Vallarai Nei

Kadukkai, Nellikkai, Thanrikkai, Chukku, Milagu, Thippili, Kirambu, Thalisapathiri, Athimathuram, Kostam, Siruthekku, Kadugurogini, Narukumoolam, Nannariver, Sitramuttiver, Perichampazham, Munthiripazham. The above ingredients are grinded to fine powder. Juice of *Centella asiatica*, Juice of *Tinospora cordifolia* and Ghee is to be added to the above Choornam (Fine Powder) and kept in flame. When it reaches its consistency it's kept off the flame and powdered sugar candy is sprinkled over it and preserved in a glass container.

Standardization Parameters:

The various standardization parameters organoleptic properties, Physicochemical analysis, TLC and HPTLC analysis and Heavy metal analysis were studied.

I. Organoleptic Evaluation:

Organoleptic evaluation refers to evaluation of the formulation by colour, odour, taste and texture etc.

II. Physico-chemical evaluation:[6 to 8]

The physicochemical analysis of the test drug Vallarai Nei was carried out as per WHO guidelines (Anonymous 1998). The test procedures were done at Noble Research Solutions. ISO 9001-2015 certified company.

Physico-chemical studies of the plant drugs are necessary for standardization, as it helps in understanding the significance of physical and chemical properties of the substance being analyzed in terms of their observed activities and especially for the determination of their purity and quality. The analysis includes the determination of specific gravity, Iodine value, saponification value, Viscosity value, Refractive Index, Weight per ml, Acid Value, and Peroxide value.

1) **Determination of specific gravity:**
   Fill the dry sp. gravity bottle with prepared samples in such a manner to prevent entrapment of air bubbles after removing the cap of side arm. Insert the stopper, immerse in water bath at 50OC and hold for 30 min. Carefully wipe off any substance that has come out of the capillary opening. Remove the bottle from the bath, clean and dry it thoroughly. Remove the cap of the side and quickly weigh. Calculate the weight difference between the sample and reference standard.
2) **Determination of Iodine value:**
   About 20 gm of test sample was transferred into Iodine flask. To which 10 ml of chloroform was added and warmed slightly and cooled for 10 minutes. Followed by this about 25 ml of Wiji's solution was added in the same flask and shaken well. The flask was allowed to stand for 30 mins and refrigerated for an hour. T about 10 ml of KI solution was added to this and titrated against 0.1 N Sodium thiosulphate solutions until the appearance of yellow colour. 1 ml of starch indicator was added and again titrated against the sodium thiosulphate solution from the burette. Disappearance of blue colour indicates end point. Repeat the above procedure without taking sample and note the corresponding reading for blank titration.
3) **Determination of saponification value:**
   About 2 gm of test sample was transferred into the round bottomed flask. To this about 20 ml of 0.5 N alcoholic KOH solutions was added to the round bottomed flask. Repeat the same procedure without taking the sample for blank titration. Reflux both sample and blank round bottomed flasks for 1 hour. After reflux, allow both the round bottomed flasks to cool. Titrate the samples using 0.5 N HCl with

phenolphthalein indicator. The disappearance of pink indicates the end point.

4) **Determination of Viscosity value:**
Viscosity determination were been carried out using Ostwald viscometers. Measurement of viscosity involves the determination of the time required for a given volume of liquid to flow through a capillary. The liquid is added to the viscometer, pulled into the upper reservoir by suction, and then allowed to drain by gravity back into the lower reservoir. The time that it takes for the liquid to pass between two etched marks, one above and one bellow the upper reservoir, is measured.

5) **Determination of Refractive Index:**
Determination of RI was carried out using Refractometer.

6) **Determination of Weight per ml:**
Weight per ml was determined using the comparative weight calibration method, in which the weight of 1ml of the base of the formulation was calculated and then weight of 1 ml of finished formulation were been calculated. The difference between weight variations of the base with respect to finished formulation calculated as an index of weight per ml.

7) **Acid Value:**
Accurately 5 g of test sample was weighed and transferred into a 250 mL conical flask. To this, a 50 mL of neutralized alcohol solution was added. This mixture was heated for 10 min by heating mantle. Afterwards, the solution was taken out after 10 min and 1 or 2 drops of phenolphthalein indicator was added. This solution was titrated against KOH solution from the burette. The appearance of pink colour indicated the end point. The volume of consumed KOH solution was determined and the titration of test sample was carried out in triplicate and the mean of the successive readings was used to calculate the acid-value of the respective sample by following expression.
Acid value = Titter Value X 0.00561X 1000 / Wt of test sample (g)

8) **Peroxide value:**
5 g of the substance being examined, accurately weighed, into a 250-ml glass-stoppered conical flask, add 30 ml of a mixture of 3 volumes of glacial acetic acid and 2 volumes of chloroform, swirl until dissolved and add 0.5ml volumes of saturated potassium iodide solution. Allow to stand for exactly 1 minute, with occasional shaking, add 30 ml of water and titrate gradually, with continuous and vigorous shaking, with 0.01M sodium thiosulphate until the yellow colour almost disappears. Add 0.5 ml of starch solution and continue the titration, shaking vigorously until the blue colour just disappears (a ml). Repeat the operation omitting the substance being examined (b ml). The volume of 0.01M sodium thiosulphate in the blank determination must not exceed 0.1 ml.
Peroxide value =10 (a-b) w.

III. TLC and HPTLC Analysis

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.[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.[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.

IV. Heavy metal analysis

Standard:
Hg, As, Pb and Cd – Sigma

Methodology
Atomic Absorption Spectrometry (AAS) is a very common and reliable technique for detecting metals and metalloids in environmental samples. The total heavy metal content of the sample was performed by Atomic Absorption Spectrometry (AAS) Model AA 240 Series. In order to determination the heavy metals such as mercury, arsenic, lead and cadmium concentrations in the test item.

Sample Digestion
Test sample was digested with 1mol/L HCl for determination of arsenic and mercury. Similarly, for the

determination of lead and cadmium the sample were digested with 1mol/L of HNO3.

Standard reparation
As & Hg- 100 ppm sample in 1mol/L HCl Cd &Pb- 100 ppm sample in 1mol/L HNO3.

3. Results and Discussion

I. Organoleptic Evaluation:

Figure 1: Sample Description

Table 2: Organoleptic characters of Vallarai Nei

State	Semisolid
Odor	Characteristic
Touch	Greasy
Flow Property	Non- free flowing
Appearance	Dark greenish

Inference
The drug Vallarai Nei is in the consistency of semisolid with Dark greenish in colour with Non- free flowing property, bitter in taste.

II. Physico-chemical evaluation:

Table 3: Physico-chemical Analysis of Vallarai Nei

S.No	Parameter	Vallarai Nei - VN
1	Specific Gravity	0.8564
2	Viscosity at 50^{o}C (Pa s)	97.79
3	Refractive index	1.44
5	Iodoine value (mg I2/g)	77.47
6	Saponification Value (mg of KOH to saponify 1gm of fat)	156.61
7	Weight per ml	0.5015
8	Acid Value mg KOH/g	0.8976
9	Peroxidase Value mEq/kg	4.84

Inference
Physico-chemical analysis was done as preliminary evalution of Vallarai Nei. The analytical report was as below Specific Gravity -0.8564, Viscosity at 50^{o}C (Pa s) - 97.79, Weight per ml - 0.5015, Acid Value mg KOH/g - 0.8976 and Peroxidase Value mEq/kg - 4.84.

The refractive index is a measure of purity of a sample. It is a ratio of velocity of light in vacuum. If any adulteration is present in the sample the refractive index will increase or decrease, which is very helpful in determination of unsaturation. Refractive index increases with increase in unsaturation. Since the refractive index is 1.44 it interprets that there is no adulteration in the sample.

Iodine value, Saponification value is used to measure the relative degree of unsaturated fatty acid in the sample. Smaller the molar weight of the fat higher the saponification value. The saponification value indicates the mean molecular weight of fatty acid and triglycerides comprising of fat. Lower the saponification value larger the molecular weight of fatty acids and triglyceride vice versa. Since the Iodine value (12mg/g) is 77.47 & saponification value (mg of KOH to saponify 1gm of fat) is 156.61 the observation shows medium chain fatty acid or triglycerides as the main component. Medium chain triglycerides passively diffuse from the GI Tract to the portal system. It facilitate easily absorbed and metabolization of the trial drug.

III. TLC and HPTLC Analysis

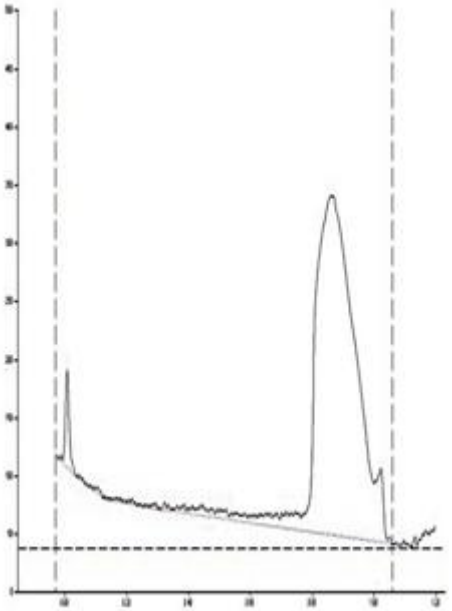

Figure 2: TLC Visualization of VN - TLC plate visualization at 366 nm

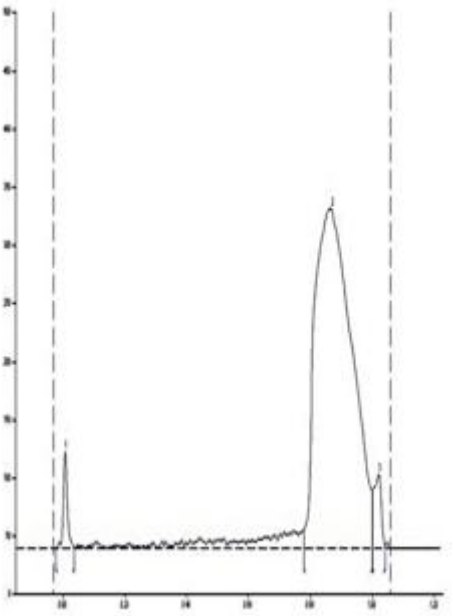

Figure 3: HPTLC finger printing of Sample VN

Table 4: Peak Value

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	-0.02	1.1	0.01	83.6	19.04	0.04	1.4	818.9	2.90
2	0.78	16.2	0.87	291.7	1.00	1.00	50.2	26199.3	92.83
3	1.00	50.6	1.02	63.8	1.04	1.04	2.8	1204.2	4.27

Report:
HPTLC finger printing analysis of the sample reveals the presence of two prominent peaks corresponds to presence of two versatile phytocomponents present within it. Rf value of the peaks ranges from 0.78 to 1. Further the peak 2 occupies the major percentage of area of 92.83which denotes the abundant existence of such compound.

Heavy metal analysis

Table 5: Heavy Metal Analysis of Vallarai Nei

Name of the Heavy Metal	Absorption Max Λ max	Result Analysis	Maximum Limit
Mercury	253.7 nm	BDL	1 ppm
Lead	217.0 nm	BDL	10 ppm
Arsenic	193.7 nm	BDL	3 ppm
Cadmium	228.8 nm	BDL	0.3 ppm

BDL- Below Detection Limit

Report and Inference
Results of the present investigation have clearly shows that the sample has no traces of heavy metals such as Mercury, Lead, Arsenic and Cadmium.

4. Conclusion

The present study on physicochemical parameters, TLC and HPTLC analysis and Heavy metal analysis provides important information which can be used as a fingerprint of herbal Siddha medicine Vallarai Nei.

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