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STANDARDIZATION OF A CLASSICAL SIDDHA POLY HERBAL FORMULATION "NANNARI MATHIRAI" THROUGH ORGANOLEPTIC CHARACTER, PHYSIOCHEMICAL AND PHYTOCHEMICAL ANALYSIS

Monika T.*[1], Ilavarasi L.[1], Abinaya T.[2], Saravanadevi M. D.[3], Karolin Daisy Rani R.[4], Meenakumari R.[5]

[1]Post Graduate, Department of Gunapadam (Pharmacology), Government Siddha Medical College, Arumbakkam, Chennai, Tamil Nadu, India.

[2]B.A.M.S, Under Graduate, Government Ayurvedha Medical College, Kottar, Nagerkovil, Tamil Nadu, India.

[3]Professor, Department of Gunapadam (Pharmacology), Government Siddha Medical College, Arumbakkam, Chennai, Tamil Nadu, India.

[4]Lecturer, Department of Gunapadam (Pharmacology), Government Siddha Medical College, Arumbakkam, Chennai, Tamil Nadu, India.

[5]Head of the Department, Department of Gunapadam (Pharmacology), Government Siddha Medical College, Arumbakkam, Chennai, Tamil Nadu, India.

***Corresponding Author: Prof. Monika T.**

Post Graduate, Department of Gunapadam (Pharmacology), Government Siddha Medical College, Arumbakkam, Chennai, Tamil Nadu, India.

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ABSTRACT

Organoleptic character, physiochemical and phytochemical analysis of a *Siddha* poly herbal drug *Nannari mathirai* which is widely used to treat Hepatic diseases such as Jaundice etc. Many infectious diseases were cured by single herbs as well as poly herbal remedies throughout the history of mankind. Standardization of the drug brings the validation to be used as a medicine by subjecting the drug to many analyses and determining its quality and effectiveness. Standardization includes many studies such as its organoleptic properties, physical characteristics, physicochemical, phytochemical properties and also to assess the active principles and elements present in the drug. In the preparation of *Nannari mathirai* each drug has the action to treat against Jaundice. This action is due to the presence of various compounds includes glycosides, tannins, alkanes, amides, alkyl halides, nitro compounds, aromatics, aliphatic amines etc., Due to the change in our lifestyle, we are all exposed to many unknown diseases. *Siddha* medicine includes Herbal, Metals, Minerals, Marine living organisms. Nowadays Standardisation of traditional medicines is much important for establishing the biological activity. The results are indicative of active ingredients responsible for therapeutic effect of *Nannari mathirai*, therefore this study leads to the evidence for future clinical studies.

KEYWORDS: *Siddha*, Herbs, *Nannari mathirai*, Physicochemical, Phytochemical and Standardize.

1. INTRODUCTION

Siddha medicine consist of large numbers of herbs with medicinal and pharmacological importance. *Siddha* medicine consist of 32 internal and 32 external medicines.[1] In those days, *Siddhar*s invented the methodology of drugs scientifically and developed well-defined pharmacopoeias. In that pharmacopeia they describe about plants, animal parts, minerals and metals for treating purpose. Most of the metabolic and physiological processes of our body as well as the detoxification of various drugs and xenobiotic chemicals occur in the liver. It acts as both secretory and excretory organ and so is described as the central laboratory of the body as it participates in the maintenance of homeostasis by controlling all types of metabolism.[2] While liver undergoing this process, the reactive chemical intermediates damage the liver cells significantly. There are several commercially drugs were available, during the consumption of these drugs it results in idiosyncratic drug reaction mediated hepatotoxicity. Drug induced hepatotoxicity is a burning problem due to this regard several drugs are withdrawn from the market due to their hepatotoxic nature. Since ancient time, Indian society depends on traditional medicinal systems to cure and prevent acute and chronic diseases. Introduction of allopathic drug during British era and neglecting Indian traditional medicine by British ruler are responsible for significant erosion of Indian traditional medicine.[3] High scientific progress in allopathic medicine and modern facilities also resists the growth of traditional medicine. Thus, standardization plays a major role in the traditional *Siddha* formulations. So that the author is interested to standardize the *Nannari mathirai* by oraganoleptic, phytochemical and physiochemical evaluations in

accordance with AYUSH regulations which has been further used for the treatment of various hepatic ailments.

2. MATERIALS AND METHODS

2.1. Drug Selection

This present study, the Herbal formulation *"NANNARI MATHIRAI"*[4] was taken as the compound drug preparation for *Kaamaalai (Jaundice)* mentioned in the classical *Siddha* literature *"Kannuswamy Parambarai Vaithiyam"* written by *Kannuswamy* pillai, published by Thirumagal Vilasa acchagam, Chennai, pg.no:141, Edition year:1948.

2.2. Ingredients of *Nannari mathirai*

1.Purified Nannari	(*Hemidesmus indicus)*	(12 grams)
2. Purified Seeragam	(*Cuminum cyminum)*	(12 grams)
3. Purified Elam	(*Elettaria cardamomum*)	(12 grams)
4.Purified Perunseeragam	(*Foeniculum vulgare)*	(24 grams)
5.Purified Sevvagathi	*(Sesbania grandiflora)*	Required quantity

2.3. Collection of the Plant materials

All the raw materials were bought from the Ramasamy Mudhaliyar Store, Parry's corner, Chennai.

2.4. Identification and Authentication of the drug

The raw materials were identified and authenticated by the experts of *Gunapadam*, Government *Siddha* Medical College, Arumbakkam, Chennai- 106. The specimen sample of each raw material has been kept in the PG *Gunapadam* department individually for future reference.

2.5. Purification of the drugs

Purification process was done as per classical *Siddha* literature.[5]

2.6. Preparation of the Trial Drug *Nannari mathirai*

2.6.1. Procedure

All the ingredients such as *Root bark of Nannari, Seeragam, Elam,* were purified and dried in the shade until complete evaporation of the moisture content. Required quantity of fresh *Sevvagathi* flower juice and Root bark of Nannari juice was taken. Except *Sevvagathi* flower juice all other ingredients are taken and powered separately. Then all the powders were mixed together.

Finally, the mixture was ground well which favours the homogenous preparation. Then the mixture of the powder was sieved through the thin clean white cloth. After that by adding required quantity of root bark of *Nannari* juice and *Sevvagathi* juice then it was ground well and made into a *karkam*. Finally it was made into a Pills of 130mg weight and dried in the shade. Atlast the end product was obtained, which was kept in an air tight container and labelled as *"Nannari mathirai"(NM)*.

2.6.2. Storage of the Drug

The prepared test drug was stored in a clean, air tight glass container. The contents were inspected frequently to avoid moisture and insects.

2.6.3. Administration of the Drug

Form of the medicine : *Mathirai*
Route of administration : Enteral
Dose : 130mg.1 tab twice a day
Adjuvant : Water.
Indication : *Kaamaalai* (Jaundice).

Fig no: 1 *Nannari mathirai.*

Table no 1. Traditional test for pill.

SL.NO	Character	Inference
1.	Non sticky on rolling	+
2.	No cracks over the surface after drying	+
3.	Shall be rolled uniformly over the plane surface	+
4.	Shining surface	+

3. ORGANOLEPTIC CHARACTER[6]

The organoleptic characters of the sample were evaluated which include evaluation of the formulation by its colour, odour, taste, texture etc. Ten tablets were taken into watch glasses and positioned against white back ground in white tube light. Its colour was observed by naked eye and results are noted.

3.1. Colour

A sample of Mathirai were taken in watch glasses and placed against white back ground in white tube light. The *Mathirai* were observed for its color by naked eye.

3.2. Odour

Ten numbers of tablets were smelled individually.

Mathirai were smelled, the time intermission between two smelling was kept 2 minutes to nullify the effect of previous smelling.

3.4. Taste
A sample of about *Mathirai* was tasted and the taste was reported.

3.5. Size
The diameter of ten tablets was measured by Vernier caliper. The mean value of diameter was noted.

4. PHYSIOCHEMICAL ANALYSIS OF *NANNARI MATHIRAI*
Physicochemical- studies of the trial drug have been done according to WHO guidelines. Physico chemical studies like total ash, water soluble ash, acid Insoluble ash, water and alcohol soluble extract, loss on drying at 105°C and pH were done at, Dr. MGR University, Chennai.[7]

4.1. Solubility Test
A pinch of sample (*NM*) was taken in a dry test tube and to it 2 ml of the solvent was added and shaken well for about a minute and the results are observed. The test was done for solvents like distilled water, Ethanol, Chloroform and the results are observed individually.

4.2. pH value
Potentiometrically, pH value is determined by a glass electrode and a suitable pH meter. The pH of the ***Nannari mathirai*** was written in results column.

4.3. Loss on Drying
An accurately weighed 1g of ***Nannari mathirai*** was taken in a tarred glass bottle. The crude drug was heated at 105^0C for 6 hours in an oven till a constant weight. The Percentage moisture content of the sample was calculated with reference to the shade dried material.

4.4. Determination of total ash
Weighed accurately 2g of ***Nannari mathirai*** was added in crucible at a temperature 600^0C in a muffle furnace till carbon free ash was obtained. It was calculated with reference to the air-dried drug.

4.5. Determination of acid insoluble ash
Ash above obtained, was boiled for 5min with 25ml of 1M Hydrochloric acid and filtered using an ash less filter paper. Insoluble matter retained on filter paper was washed with hot water and filter paper was burnt to a constant weight in a muffle furnace. The percentage of acid insoluble as was calculated with reference to the air-dried drug.

4.6. Determination of water-soluble ash
Total ash 1g was boiled for 5min with 25ml water and insoluble matter collected on an ash less filter paper was washed with hot water and ignited for 15min at a temperature not exceeding 450^0C in a muffle furnace. The amount of soluble ash is determined by drying the filtrate.

4.7. Determination of water-soluble Extractive
5gm of air-dried drug, coarsely powered ***Nannari mathirai*** was macerated with 100ml of distilled water in a closed flask for twenty-four hours, shaking frequently. The Solution was filtered and 25 ml of filtrated was evaporated in a tarred flat bottom shallow dish, further dried at 100^0 C and weighted. The percentage of water-soluble extractive was calculated with reference to the air- dried drugs.

4.8. Determination of alcohol soluble extractive
1 gm. of air-dried drugs, coarsely powdered ***Nannari mathirai*** was macerated with 20 ml. alcohol in closed flask for 24 hrs. With frequent shaking. It was filtered rapidly taking precaution against loss of alcohol. 10ml of filtrate was then evaporated in a tarred flat bottom shallow dish, dried at 100^0C and weighted. The percentage of alcohol soluble extractive was calculated with reference to air dried drug.

5. PHYTOCHEMICAL SCREENING ANALYSIS OF NANNARI MATHIRAI
The preliminary phytochemical screening test was carried out for each extract of ***Nannari mathirai*** as per the standard procedure.[8]

5.1. Detection of alkaloids
Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

5.1.1. Mayer's Test: Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow colored precipitate indicates the presence of alkaloids.

5.2. Detection of carbohydrates
Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

5.2.1. Molisch's Test: To 2 ml of plant sample extract, two drops of alcoholic solution of α- naphthol are added. The mixture is shaken well and few drops of concentrated sulphuric acid is added slowly along the sides of test tube. A violet ring indicates the presence of carbohydrates.

5.3. Detection of glycosides
Extracts were hydrolyzed with dil. HCl, and then subjected to test for glycosides.

5.3.1. Modified Borntrager's Test: Extracts were treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution.

Formation of rose-pink color in the ammonical layer indicates the presence of anthranol glycosides.

5.4. Detection of saponins

5.4.1. Foam Test: 0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

5.5. Detection of phenols Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black color indicates the presence of phenols.

5.6. Detection of tannins Gelatin Test: The extract is dissolved in 5 ml of distilled water and 2 ml of 1% solution of Gelatin containing 10% NaCl is added to it. White precipitate indicates the presence of phenolic compounds.

5.7. Detection of Flavonoids

5.7.1. Alkaline Reagent Test: Extracts were treated with few drops of sodiummhydroxide solution. Formation of intense yellow color, which becomes colorless on addition of dilute acid, indicates the presence of flavonoids.

5.8. Detection of proteins

5.8.1. Xanthoprotein Test: The extracts were treated with few drops of conc. Nitric acid. Formation of yellow color indicates the presence of proteins.

5.9. Detection of aminoacids

5.9.1. Ninhydrin Test: To the extract, 0.25% w/v ninhydrin reagent was added and boiled for few minutes. Formation of blue color indicates the presence of amino acid.

5.10. Detection of diterpenes Copper Acetate Test

Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green color indicates the presence of diterpenes.

5.11. Gum and Mucilage

To 1ml of extract add 2.5ml of absolute alcohol and stirring constantly. Then the precipitate was dried in air and examine for its swelling properties. Swelling was observed that will indicate presence of gum and mucilage.

5.12. Test for Quinones

Extract was treated with sodium hydroxide blue or red precipitate indicates the presence of Quinones.

5.13. Test for Fixed oils and Fats

5.13.1. Spot test: A small quantity of extract is pressed between two filter papers. Oil stain on the paper indicates the presence of fixed oils.

RESULTS AND DISCUSSION

Table: 2. Organoleptic characters of *Nannari mathirai.*

Colour	Dark brown
Odour	Pleasant
Taste	Sweet
Texture	Powder in integrated form
Particle size	Completely pass through sieve no 88 when powdered

Table 3. Physicochemical Analysis of *Nannari mathirai.*

S.no	Parameters	Percentage
1	PH	5.45%
2	Loss on drying	2.88%
3	Total ash value	9.5%
4	Acid insoluble ash	6%
5	Water soluble ash	3%
6	Water soluble extraction	16%
7	Alcohol soluble extraction	8%
8	Solubility	
	Distilled water	Soluble
	Chloroform	Soluble
	Ethanol	Soluble

The physicochemical analysis of the drug (*NM)* result reveals pH, Loss on drying, Total ash value, Acid insoluble ash and Water-soluble ash. The interpretation of the result was given below.

Interpretation

1. pH

It is a measure of hydrogen ion concentration. It is the measure of the acidic or alkaline nature. 7.0 is neutral, above 7.0 is alkaline and below is acidic. The pH of the drug *Nannari mathirai* is 5.45 which is acidic in nature.

Acidic drug is essential for its bioavailability and effectiveness. Acidic drugs are better absorbed in stomach.[9]

2. Moisture (Loss on drying)

The total amount of volatile content and moisture present in the drug was established in loss on drying. Moisture content of the drug reveals the stability and its shelf-life.High moisture content can adversely affect the active ingredient of the drug. Thus, low moisture content could get maximum stability and better shelf life. Loss on drying of *Nannari mathirai* is 2.88.[10]

3. Total Ash

Ash constitutes are the inorganic residues obtained after complete combustion of a drug. Thus, Ash value is a validity parameter to describe and to assess the degree of purity of a given drug. Total ash value of plant material indicated the amount of minerals and earthy materials present in the drug. The total ash value of *Nannari mathirai is* 9.5 % which determine the absence of inorganic content.

5. Acid insoluble ash

The acid insoluble ash value of the drug denotes the amount of siliceous matter present in the plant. The quality of the drug is better if the acid insoluble value is low. Acid insoluable ash value of *Nannari mathirai* is 6 %.

6. Water soluble ash

Water-soluble ash is the part of the total ash content, which is soluble in water. Decreased water soluble ash value indicates easy facilitation of diffusion and osmosis mechanism. Water soluble ash value of *Nannari mathirai* is 3%.

7. Solubility

Solubility is the major factor that controls the bioavailability of a drug substance. It is useful to determine the form of drug and processing of its dosage form. The most frequent causes of low oral bioavailability are attributed to poor solubility and low permeability.[11] *NM* is soluble in major solvents and sparingly soluble in some solvents proves that its efficiency of solubility in the stomach indirectly, increasing the bio availability.

Phytochemical analysis

Tab 4. phytochemical *Nannari Mathirai* result were given below.

S.No.	Phytochemicals	Test Name	H2O Extract
1	Alkaloids	Mayer's Test	+ve
2	Saponins	Foam Test	+ve
3	Phenols	Ferric Chloride Test	+ve
4	Flavonoids	Alkaline Reagent Test	+ve
5	Proteins	Xanthoprotein Test	+ve
6	Amino acids	Ninhydrin Test	+ve
7	Diterpenes	Copper Acetate Test	+ve
8	Gum & Mucilage	Extract + Alcohol	+ve
9	Quinones	NAOH + Extract	+ve

The above stated phytochemical properties for the given sample certified to be present.

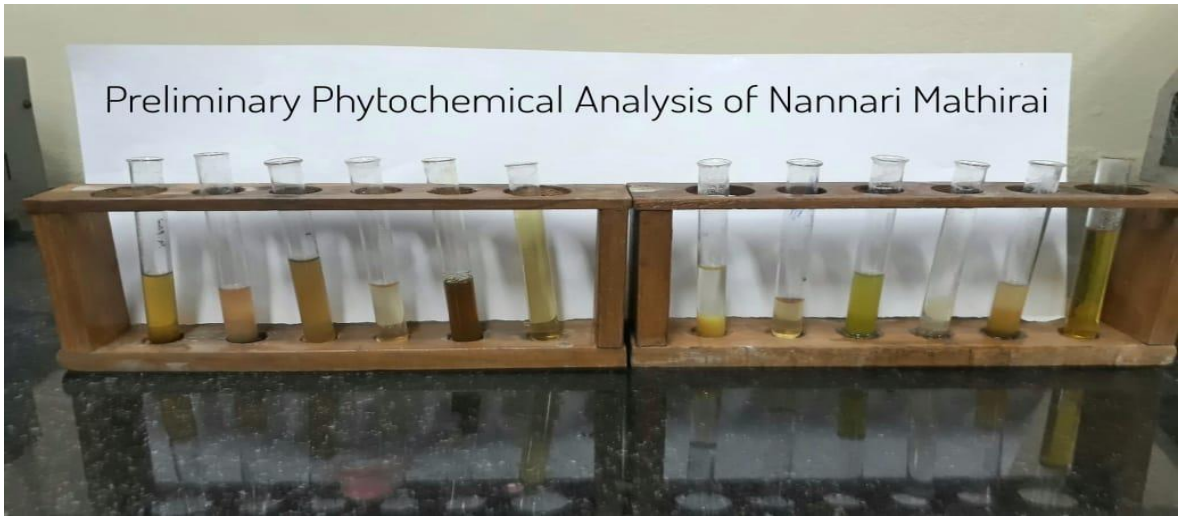

Fig 2: Phytochemical analysis of *Nannari Mathirai.*

The phytochemical analysis of the drug (*NM*) result reveals Alkaloids, Saponins, Phenols, Flavanoids, Proteins, Amino acids, Diterpenes, Gum & Mucilage and Quinones. The interpretation of the result was given below.

Interpretation

Alkaloids

Alkaloids possess antispasmodic, analgesic, bactericidal effects.Alkaloids are the active principles producing many essential effects in protecting the body.[12]

Saponins

Saponins include, supporting kuffer cells in the liver and encouraging normal detoxification. In the digestive tract, saponins produce an emulsification of fat-soluble molecules. Saponins bind with bile acids and helps to eliminate them from the body, preventing cholesterol from being reabsorbed.Saponins can boost the immune system, have an antioxidant effect and may even support bone strength.[13]

Phenols

Phenols possess rich anti-oxidant property and protect the body from oxidative stress. Phenols inhibit the LDL cholesterol levels. Phenols reduces cell death and it regulate glucose metabolism. Phenols increase the vasodilation of blood vessels to promote circulation. It is a Effective anti-hyperglycaemic agent.[14]

Flavanoides

It is the most important group of polyphenolic compounds in plants. Flavonoids can exert their anti-oxidant activity by scavenging the free radicals, by chelating metal ions or by inhibiting enzymatic systems responsible for free radical generation. Flavanoids are immunomodulator. It also possesses anti-microbial activity which is confirmed by the various anti-microbial assays.[15]

Protein and amino acids

Proteins are very useful in the liver regeneration and energy production. They boost glutathione production to protect the liver. Protein is an amalgamation of amino acids. It is an important component of every cell in the body. Body uses protein to build and repair tissues.[16]

Diterpenes

Diterpenes has an anti-oxidant effect. Diterpenes helps to cure hypertension. It also has tumour inhibitory properties as well as a stimulating effect on the immune system. It is used widely as a stomachic.[17]

Gum & Mucilage

It is used as a bulk laxative. Gum and mucilage are used for their demulcent properties for cough suppression.[18]

CONCLUSION

Standardization becomes highly mandatory as it evident the physiochemical, phytochemical as well as the bioactive component profile of the siddha preparations. Organoleptic property of the NM with respect to its Dark brown color, pleasant odour, sweet taste and Powder in integrated form which is Completely pass through sieve no 88 these characters justifies the purity and quality of the finished formulation. The results obtained from the physicochemical and phytochemical analysis of *Nannari mathirai* which might be responsible for the safety and potent therapeutic activity of this pill. But in future work, we are in position to prove its safety in animal models and also in clinical trials.

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