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Research Article

STANDARDIZATION OF A POLYHERBAL SIDDHA FORMULATION ASUVAATHI CHOORANAM FOR MANNUN VELUPPU NOI (IRON DEFICIENCY ANEMIA)

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A B S T R A C T

In India wide range of medicinal systems are available. SIDDHA system of medicine was part of Tamil civilization, is receiving more attention in global health care circle, which is unknown to many people. The word 'Siddha' comes from 'Siddhi' which means an object to be attained or perfection of heavenly bliss. Thus Siddha becomes one of the oldest systems of health care dealing with curative, preventive, preservative aspects and longevity of life in a simplest way. Iron deficiency anemia (Mannun veluppu noi) is a major and global health problem that affects particularly infants, young children, and women of childbearing age in developing countries. Asuvaathi Chooranam is a reputed drug mentioned in the ancient books of siddha medicine for the treatment of Mannun veluppu noi. In the present study an attempt has been made to standardize the Asuvaathi chooranam. Most of the traditional systems of medicine are effective but lack of standardization, so, there is a need to develop a standardization technique. According to PLIM Guidelines, Standardization of drugs confirms identity, determination of quality, purity and detection of adulteration by various parameters. The review article will help to provide details of information about physicochemical analysis, pesticide residues, aflatoxin, sterility test and heavy metals of herbo mineral ingredients of Asuvaathi Chooranam.

INTRODUCTION

The siddha system of medicine is mainly practiced in the southern part of India. The word Siddha has its origin in the Tamil word Siddhi which means "an object to be attained" or "perfection bliss". Sidhars, mainly hailing from Tamil Nadu were spiritual masters who possessed the ashtama siddhis (eight siddhis) or unique powers. Siddhars laid the foundation for Siddha system of medicine. Hence, it is called Siddha medicine.

The basic structure of siddha medical system is in no way differences from logic system of modern science. Like logic system, siddha system of medicine is based on three factors of Observation, Influence and Hypothesis. Moreover, siddha medicinal treatment is based on Inferences like Thesis, Anti-thesis and Synthesis.

According to siddha system of medicine, total diseases are 4448 in numbers. Of these, 108 diseases are said to occur during childhood. BalaVaagadam is a specialized branch in siddha medicine. It deals with the treatment of the diseases of children up to 12 years of age. The diseases of children are broadly classified into two categories.

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Agakkarana noigal (diseases that occurs in the intrauterine period) Purakarana noigal (diseases that occurs after child birth, due to extrinsic factors). Mannun veluppu noi is most commonly affecting Paediatric age group. Mannun veluppu noi in siddha system is more or less equal to Iron deficiency anaemia.

Iron deficiency anaemia is the most common type of anaemia, and it occurs when your body doesn't have enough of the mineral iron. Iron is a nutrient that's essential to your child's growth and development. In developed countries, iron deficiency is the only frequent micronutrient deficiency. The American Academy of Paediatrics (AAP) advises feeding your baby only breast milk for the first 6 months. But breast milk does not have a lot of iron, so infants that are breastfed only, may not have enough iron. Older infants and toddlers may not get enough iron from their diets.

Iron deficiency anaemia (IDA) affects approximately two billion people worldwide and most of them reside in low- and middle- income countries. In these countries, additional causes of anaemia include parasitic infections like malaria, other nutritional deficiencies, chronic diseases, hemoglobinopathies and lead poisoning. In India the prevalence of Iron deficiency anaemia in children (6-59 months) is reported as 56%., most common by due to nutritional iron deficiency. Anaemia was found in 44.4% of children, of which 46.0% had Iron deficiency anaemia.

Asuvaathi Chooranam is a reputed drug mentioned in the ancient books of siddha medicine for the treatment of Mannun veluppu noi. In the present study an attempt has been made to standardize the Asuvaathi chooranam.

Standardization of drugs confirms identity, determination of quality, purity and detection of adulteration by various parameters. It also maintains consistency of claimed efficacy of the product and its batch to batch reproducibility. The review article will help to provide details of information about physicochemical analysis, pesticide residues, aflatoxin and sterility test, heavy metals of herbo mineral ingredients of Asuvaathi Chooranam.

MATERIALS AND METHODS

Drug Selection

Herein, the compound herbal formulation "Asuvaathi Chooranam" for "Mannun veluppu noi" was taken, mentioned in the classical Siddha literature "Agathiyar vaithiya chuthiram 650" complied by S.S.Mathrubootheswaran published by Narmadha padhipagam page no: 105-107.

Collection of the raw materials

All the raw drugs are collected in the Vallalar naattu marundhu kadai, Town, Tirunelveli.

Identification and Authentication

All the ingredients of Asuvaathi Chooranam were identified and authenticated by Department of Gunapadam in Government Siddha medical college and hospital, palayamkottai, Tirunelveli-627002.

Purification and preparation

Take Amukkara kilangu is purified in milk, dried and powdered. Take the equal quantities of all the drugs chukka, milagu, thippili, jadhikai, jadhipathri, adhimadhuram, kirambu, kadukurohini and kurosani omam were roasted and grounded into a fine powder. The powder was sieved through a clean white cloth to get a uniform particle size of Chooranam. Then equal amount of powdered sugar added to it. The drug is stored in clean dry air-tight container.

Table 1 Ingredients of Asuvaathichooranam

S.No	Ingredients	Botanical Name/ Family	Parts Used	Quantity
1.	Amukkara Kilangu	*Withania somnifera*/Solanaceae	Tuber	27 kalanju
2.	Chukku	*Zingiber officinales*/Zingiberaceae	Rhizome	3 kalanju
3.	Milagu	*Piper nigrum*/Piperaceae	Fruit	3 kalanju
4.	Thippili	*Piper longum*/Piperaceae	Fruit	3 kalanju
5.	Saathikai	*Myristica fragrans*/Myristicaceae	Fruit	3 kalanju
6.	Saathipaththiri	*Myristica fragrans*/Myristicaceae	Aril	3 kalanju
7.	Athimathuram	*Glycyrrhiza glabra*/Fabaceae	Root	3 kalanju
8.	Kirambu	*Syzygium aromaticum*/Myrtaceae	Flower bud	3 kalanju
9.	Kadugurohini	*Picrorhiza Scrophulariiflora*/Scrophulariaceae	Root	3 kalanju
10.	Kurosani Omam	*Hyoscyamus niger*/Solanaceae	Seed	3 kalanju
11	Seeni	*Sugar*		54 kalanju

Administration of the drug

Form of medicine: Chooranam
Route of administration: Internal
Dosage: 500 mg/ twice a day.
Duration: 48 days.
Adjuvant: Fresh buffalo whey
Indication: Mannun veluppu noi.
Reference: AGASTHIYAR VAIDHYA CHUTHIRAM 650 (105-107)

Authentication of final trial drug

The prepared drug was authenticated by the trained experts from Gunapadam department of Government siddha medical college, palayamkottai for its completion.

Organoleptic Characters

State, Nature, Odor, Consistency, Appearance of the drug were noted. The analysis was done by Noble research solutions pvt, Ltd, Chennai, India.

Table 2 organoleptic characters

State	Solid
Nature	Moderately fine
Odor	Aromatic
Touch / Consistency	Soft
Flow Property	Non- free flowing
Appearance	Pale brownish

Physicochemical Analysis of Asuvaathi Chooranam

Table 3 Solubility Profile

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

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.

Table 4 physicochemical analysis of Asuvaathi Chooranam

S.No	Parameter	Mean (n=3) SD
1.	Loss on Drying at 105 °C (%)	11.7 ± 1.85
2.	Total Ash (%)	25.97 ± 0.70
3.	Acid insoluble Ash (%)	2.42 ± 0.37
4.	Alcohol Soluble Extractive (%)	11 ± 1.30
5.	Water soluble Extractive (%)	32.37 ± 2.15

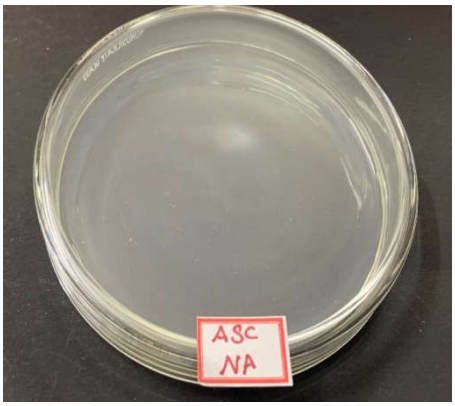
STERILITY TEST BY POUR PLATE METOD

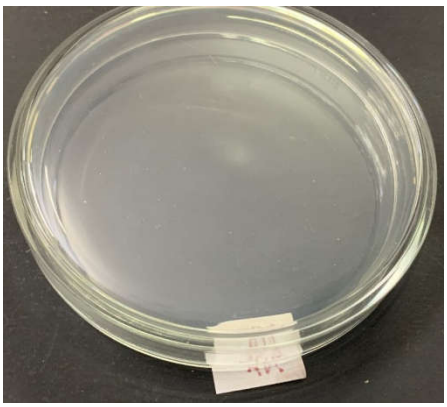
Objective

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 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 and further extended for 72 hrs for fungal growth observation. Grown colonies of organism was then counted and calculated for CFU.





Observation

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

RESULT

No growth / colonies was observed in any of the plates inoculates with the test sample.

Table 5 sterility test

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

Heavy Metal Analysis By AAS

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 inthe 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 HNO_3.

Standard reparation

As & Hg – 100 ppm sample in 1 mol/L HCL
Cd &Pb – 100 ppm sample in 1mol/L HNO_3

Table 6 heavy metals

Name of the Heavy Metal	Absorption Max Λ max	Result Analysis
Lead	217.0 nm	41.44
Arsenic	193.7 nm	8.11
Cadmium	228.8 nm	4.34

Pesticide Residue

Extraction

Test sample were extracted with 100ml of acetone and followed by homogenization for brief period. Further filtration was allowed and subsequent addition of acetone to the test mixture. Heating of test sample was performed using a rotary evaporator at a temperature not exceeding 40ºC until the solvent has almost completely evaporated. To the residue add a few milliliters of toluene and heat again until the acetone is completely removed. Result ant residue will be dissolved using toluene and filtered through membrane filter.

Table 7 pesticide residue

Pesticide Residue	Sample ASC	AYUSH Limit (mg/kg)
I.Organo Chlorine Pesticides		
Alpha BHC	BQL	0.1mg/kg
Beta BHC	BQL	0.1mg/kg
Gamma BHC	BQL	0.1mg/kg
Delta BHC	BQL	0.1mg/kg
DDT	BQL	1mg/kg
Endosulphan	BQL	3mg/kg
II.Organo Phosphorus Pesticides		
Malathion	BQL	1mg/kg
Chlorpyriphos	BQL	0.2 mg/kg
Dichlorovos	BQL	1mg/kg
III. Organocarbamates		

Carbofuran	BQL	0.1mg/kg
III.Pyrethroid		
Cypermethrin	BQL	1mg/kg

BQL- Below Quantification Limit

Result: The results showed that there were no traces of pesticides residuessuchas Organochlorine, Organophosphorus, Organocarbamates and pyrethroids in the sample provided for analysis.

Aflatoxin Assay BY TLC (B1, B2, G1, G2)

Standard

Aflatoxin B1
Aflatoxin B2
Aflatoxin G1
Aflatoxin G2

Solvent

Standard sample was dissolved in a mixture of chloroform and acetonitrile (9.8: 0.2) to obtain a solution having concentrations of 0.5 µg per ml each of aflatoxin B1 and aflatoxin G1 and 0.1 µg per ml of aflatoxin B2 and aflatoxin G2.

Procedure

Standard aflatoin was applied on to the surface to pre coated TLC plate in the volume of 2.5 µl. 5 µl, 7.5 µl and 10 µl. similarly, the test sample was placed and allow the spots to dry and develop the chromatogram in an unsaturated chamber containing a solvent system consisting of a mixture of chloroform, acetone and isopropyl alcohol (85:10:5) until the solvent front has moved not less than 15 cm from the origin. Remove the plate from the developing chamber, mark te solvent from and allow the plate to air-dry. Locate the spots on the plate by examination under UV light at 365 nm.

Table 8 Aflatoxin

Aflatoxin	Sample ASC	AYUSH Specification Limit
B1	Not Detected - Absent	0.5 ppm
B2	Not Detected - Absent	0.1 ppm
G1	Not Detected - Absent	0.5 ppm
G2	Not Detected - Absent	0.1 ppm

RESULTS

The results shown that there were no spots were being identified in the test sample loaded on TLC plates when compare to the standard which indicates that the sample were free from Aflatoxin B1, Aflatoxin B2, Aflatoxin G1, Aflatoxin G1.

CONCLUSION

The achieved results of physicochemical, organoleptic property, sterility test, heavy metals, pestiside residue and aflatoxin will be useful tool for authentication, standardization profile and quality control assessment of the poly herbal formulation.

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