Research Article

www.ijrap.net (ISSN:2229–3566)

PHYSICOCHEMICAL STANDARDISATION OF SIDDHA TABLET PREPARATION JATHIKAAI MATHIRAI

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Received on: 07/02/21 Accepted on: 16/07/21

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DOI: 10.7897/2277-4343.1204112

ABSTRACT

The aim of this study is to standardize the herbal Mathirai preparation Jathikaai Mathirai based on qualitative and quantitative methods as per the analytical specifications of Tablet/Mathirai prescribed by the Protocol testing of ASU drugs by Pharmacopoeial laboratory for Indian Medicines. The Tablet is prepared as described in the text Bala Vagadam. The medicine is subjected to Physicochemical standardisation as per the pharmacopoeial laboratory standards of Indian medicine. The tablet is in solid form which is brown in colour. It is rigid with strong characteristic odor. The aflatoxin assay and pesticide residue revealed that the tablet is free of aflatoxins and pesticide residue. The formulation is free of microbial contamination and shows positive for the presence of steroids, alkaloids, coumarins, tannins, carbohydrates, glycosides etc. The heavy metals are below detectable limit. The result of HPTLC finger printing of the drug at UV 366 nm shows that the peak at Rf 0.02 constitutes 100% of the total area of the separated peaks, which denotes the abundant existence of the drug at minimum peak level itself. It indicates drug as phytochemicals. The result of the present study ensures the safety profile of the Jathikaai Mathirai – Siddha herbal Tablet intended for paediatric usage and indicative of presence of active phytoconstituents that are responsible for its efficacy in treating the Valippu Noi (Seizure disorder) in children.

Keywords: Jathikaai Mathirai, Valippu Noi, Seizure disorder, Physicochemical analysis, Phytochemical analysis.

INTRODUCTION

Siddha system of Medicine- the medical knowledge of Tamil people that has developed its root from southern most part of India. The term "Siddha" itself gives the meaning of completeness and hence the system of medicine is a complete medical system that not only deals with medicine but also describes the way of life in this world[1].

According to Siddha the health of human body is maintained by the three vital forces (Uyir Thathukkal) namely, Vatham, Pitham and Kabam which are functioned by the influence of Pancha boothams. Derangement of these kutram produces diseases[2].

Siddha system of medicine deals the diseases of children separately in a section Bala Vagadam. Seizure disorder which is called as Valippu noi[3] in siddha.

A seizure is a paroxysmal, time-limited change in motor activity and/or behaviour that results from abnormal electrical activity in the brain. Seizure is common in the pediatric age group and occurs in 10% of children. A few children also exhibit pseudoseizures of psychiatric origin. The cumulative lifetime incidence of epilepsy is 3%; more than half of cases begin in childhood[4].

One seizure does not signify epilepsy (up to 10% of people worldwide have one seizure during their lifetime). Epilepsy is defined as having two or more unprovoked seizures. Epilepsy is a chronic non communicable disease of the brain that affects people of all ages. According to WHO around 50 million people worldwide have epilepsy, making it one of the most common neurological diseases globally. Nearly 80% of people with epilepsy live in low-and middle -income countries. It is estimated that up to 70% of people living with epilepsy could live seizure - free if properly diagnosed and treated[5]. The risk of premature death in people with epilepsy is up to three times higher than for the general population. Three quarters of people with epilepsy living in low-income countries do not get the treatment they need. Many people with active epilepsy do not receive appropriate treatment for their condition leading to large treatment gap[6].

In Siddha medicine, many numbers of formulations are mentioned in various literatures to treat Seizure disorder. Most of the formulations are using metal and minerals as the main ingredient of the medicine. But there are formulations that are based on only with herbal raw drugs as their ingredients. One of such polyherbal formulation is "Jathikaai Mathirai" mentioned in the text "Balavagadam"[3]. In this study, "Jathikaai Mathirai" is selected to screen for its Physico chemical characteristics as a step to validate scientifically.

MATERIALS AND METHODS

As per the reference literature[3] the Jathikaai Mathirai is prepared with the following ingredients.

1. Jathikaai (*Myristica fragrans*) -1 varagan (4.2 grams)
2. Kirambu (*Syzygium aromaticum*) -1 varagan (4.2 grams)
3. Omam (*Carum copticum*) - 1 varagan (4.2 grams)
4. Karunseeragam (*Nigella sativa*) - 1 varagan (4.2 grams)
5. Chukku (*Zingiber officinale*) - 1 varagan (4.2 grams)
6. Thippili (*Piper longum*) - 1 varagan (4.2 grams)
7. Kasthurimanjal (*Curcuma aromatica*) -1 varagan (4.2 grams)

8. Vasambu (*Acorus calamus*) - 1 varagan (4.2 grams)
9. Perungayam (*Ferula asafoetida*) - 1 varagan (4.2 grams)
10. Vellai kaakanam verpattai (*Clitoria ternatea*) - 9 varagan (36 grams)

Purification of the drug

All the drugs mentioned here were purified as per the Siddha literature.

Method of preparation

The required quantity of the purified drugs was taken and grinded into fine powder and sieved by vasthrakayam procedure and pulverised in kalvam by adding warm water. Made it as a sundaikaai sized pills when the required consistency obtained.

Dosage: 1 tab (35 mg) – od with hot water.
Indication: Valippu noi
Duration: 45 days

Physicochemical analysis

Table 1: Physicochemical analysis of Jathikaai Mathirai

State	Solid
Nature	Hard Solid
Odor	Strong characteristic
Touch	Rigid
Flow Property	Non free flowing
Appearance	Brown

Percentage Loss on Drying

10 gram of test drug was accurately weighed in evaporating dish. The sample was dried at 105ºC for 5 hours and then weighed.

Percentage loss in drying = Loss of weight of Sample / Wt. of the Sample x 100

Determination of Total Ash

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

Total Ash = Weight of Ash/ Wt. of the crude drug taken X 100

Determination of Acid Insoluble Ash

About 0.5 gram of the ash obtained by total ash test was boiled with 25 ml of dilute hydrochloric acid for 6 minutes. Then the insoluble matter was collected in crucible and washed with hot water and ignited to constant weight. Percentage of acid insoluble ash was calculated with reference to the weight of air-dried ash.

Acid insoluble Ash = Weight of Ash/ Wt. of the crude drug taken X 100

Determination of Alcohol Soluble Extractive

About 5 gram of 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. Filtered rapidly, took precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish, and dried at 105ºC, to constant weight and weigh. Calculated the percentage of alcohol-soluble extractive with reference to the air-dried drug.

Alcohol soluble extract = Weight of extract/ Wt. of the sample taken X 100

Determination of Water-Soluble Extractive

About 5 gram of the test sample was macerated with 100 ml of chloroform water in a closed flask for twenty-four hours, shaking frequently during six hours and allowed it to stand and for eighteen hours. Filtered rapidly, took precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish, and dried at 105ºC, to constant weight and weigh. Calculated the percentage of water-soluble extractive with reference to the air-dried drug.

Water soluble extract= Weight of extract/ Wt. of the sample taken X 100

Phytochemical analysis

Test for alkaloids
To the 5 gram of test sample, 2 ml of Mayer's reagent was added, a dull white precipitate revealed the presence of alkaloids.

Test for coumarins
The test sample were mixed with 1 ml of 10% sodium hydroxide. The presence of coumarins is indicated by the formation of yellow colour.

Test for saponins
To the test sample, 5 ml of water was added, and the tube was shaken vigorously. Copious lather formation indicated the presence of Saponins.

Test for tannins
To the test sample, ferric chloride was added, formation of a dark blue or greenish black colour showed the presence of tannins.

Test for glycosides- Bontrager's Test
Test drug was hydrolysed with concentrated hydrochloric acid for 2 hours on a water bath, filtered and the hydrolysate were subjected to the following tests. To 2 ml of filtered hydrolysate, 3 ml of chloroform was added and shaken, chloroform layer was separated, and 10% ammonia solution was added to it. Pink colour was not present, it indicated absence of glycosides.

Test for flavonoids
To the test sample about 5 ml of dilute ammonia solution were added followed by addition of few drops of conc. Sulfuric acid. No appearance of yellow colour indicated the absence of Flavonoids.

Test for phenols - Lead acetate test
To the test sample, 3 ml of 10% lead acetate solution was added. A bulky white precipitate indicated the presence of phenolic compounds.

Test for steroids
To the test sample, 2 ml of chloroform was added with few drops of conc. Sulphuric acid (3 ml) and shaken well. The upper layer in the test tube was turned into red and sulphuric acid layer showed yellow with green fluorescence. It showed the presence of steroids.

Test for Triterpenoids - Liebermann–Burchard test
To the chloroform solution, few drops of acetic anhydride was added then mixed well. 1 ml concentrated sulphuric acid was added from the sides of the test tube, no appearance of red ring indicated the absence of triterpenoids.

Test for Cyanins
To the test sample, 1 ml of 2 N sodium hydroxide was added and heated for 5 min at 100°C. No formation of bluish green colour indicated absence of anthocyanin.

Test for Carbohydrates - Benedict's test
To the test sample about 0.5 ml of Benedict's reagent was added. The mixture was heated on a boiled water bath for 2 minutes. A characteristic-coloured precipitate indicated the presence of sugar.

Test for Proteins - Biuret Test
To extracts 1% solution of copper sulphate was added followed by 5% solution of sodium hydroxide, no formation of violet purple colour indicated absence of proteins.

TLC Analysis

Test sample was subjected to thin layer chromatography (TLC) as per conventional one-dimensional ascending method using silica gel 60F254, 7 X 6 cm (Merck) were cut with ordinary household scissors. Plate markings were made with soft pencil. Micro pipette was used to spot the sample for TLC applied sample volume 10-micro litre 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 254 nm and light long-wave UV light 365 nm.

High Performance Thin Layer Chromatography Analysis

HPTLC method was 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) was 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 provided chromatographic fingerprint of phytochemicals which was suitable for confirming the identity and purity of phytotherapeutics.

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 analysed. After elution, plates were taken out of the chamber and dried[6].

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

Heavy metal analysis by AAS

Standard
Hg, As, Pb and Cd – Sigma

Methodology
Atomic Absorption Spectrometry (AAS) was 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 of heavy metals such as mercury, arsenic, lead and cadmium concentrations in the test item.

Sample Digestion
Test sample was digested with 1 mol/L HCl for determination of arsenic and mercury. Similarly, for the determination of lead and cadmium the sample were digested with 1 mol/L of HNO_3.

Standard reparation
As & Hg- 100 ppm sample in 1 mol/L HCl
Cd & Pb- 100 ppm sample in 1 mol/L HNO_3

Aflatoxin Assay

Standard
Aflatoxin B1, Aflatoxin B2, Aflatoxin G1, Aflatoxin G2

Solvent
Standard samples 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 each of aflatoxin B2 and aflatoxin G2.

Test solution
Concentration 1 µg per ml

Procedure
Standard aflatoxin 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 Allowed the spots to dried and developed 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 had moved not less than 15 cm from the origin. Removed the plate from the developed chamber, marked the solvent from and allowed the plate to air-dry. Locate the spots on the plate by examination under UV light at 365 nm[7].

Test for specific pathogen

Methodology
Test sample was directly inoculated into the specific pathogen medium (EMB, DCC, Mannitol, Cetrimide) by pour plate method. The plates were incubated at 37°C for 24 – 72 h for observation. Presence of specific pathogen identified by their characteristic colour with respected to pattern of colony formation in each differential media.

Table 2: List of Specific Pathogens

Organism	Abbreviation
E-coli	*EC*
Salmonella	*SA*
Staphylococcus aureus	*ST*
Pseudomonas aeruginosa	*PS*

Sterility test by Pour plate method

Objective
The pour plate techniques were adopted to determine the sterility of the product. Contaminated / unsterile sample (formulation) when met the nutrition rich medium it promoted 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 were referred to as Colony Forming Units (CFUs).

Methodology

Test sample was inoculated in sterile petri dish to which about 15 mL of molten agar 45ºC 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 37ºC for 24-48 hours and further extended for 72 hours for fungal growth observation. Grown colonies of organism was then counted and calculated for CFU.

Pesticide residue analysis

Extraction

Test sample were extracted with 100 ml 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 had almost completely evaporated. To the residue added a few millilitres of toluene and heated again until the acetone was completely removed. Resultant residue was dissolved using toluene and filtered through membrane filter[8,9].

RESULT AND DISCUSSION

Out of 32 types of internal medicines described in Siddha Literatures, Mathirai was a type of Internal Medicine that can be correlated with tablet preparation. Usually, tablet form of medicine was easy to administer even to the children. But nowadays, in the commercial world, there was a possibility for adulteration of the ingredients and hence there was necessity for standardization of the formulation. Standardization gives information on chemical, biological, physio-chemical profile of Jathikaai Mathirai. In the present study, the organoleptic characters, Physico-chemical character as per AYUSH guidelines[9,10] were carried out and are compared with AYUSH standards. Phytochemical quantification of the Mathirai is also studied using HPTLC technique[11].

Organoleptic characters

Table 3: Organoleptic characters of Jathikaai Mathirai

State	Solid
Nature	Hard Solid
Odor	Strong characteristic
Touch	Rigid
Flow Property	Non free flowing
Appearance	Brown

Physiochemical analysis

Table 4: Physiochemical analysis of Jathikaai Mathirai

Parameter	Mean (n = 3) SD
Loss on Drying at 105 °C (%)	0.65 ± 0.11
Total Ash (%)	8.7 ± 0.43
Acid insoluble Ash (%)	0.18 ± 0.03
Water soluble Extractive (%)	22.1 ± 0.64
Alcohol Soluble Extractive (%)	16.5 ± 0.95

Loss on drying indicated the total volatile content and moisture content of the drug. The loss on drying at 105ºC of Jathikaai Mathirai was 0.65 ± 0.11.

Phytochemical analysis

Table 5: Phytochemical analysis of Jathikaai Mathirai

Test	Observation
Alkaloids	+
Flavonoids	-
Glycosides	-
Steroids	+
Triterpenoids	-
Coumarin	+
Phenol	+
Tannin	+
Protein	-
Saponins	+
Sugar	+
Anthocyanin	-
Betacyanin	+

+ -> Indicates Positive and - -> Indicates Negate

Result analysis of HPTLC

Figure 1: TLC Histogram of the Sample Jathikaai Mathirai

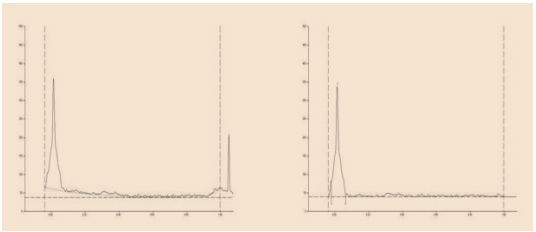

Figure 2: HPTLC Chromatogram of Jathikaai Mathirai

Table 6: R_f value of Jathikaai Mathirai

Peak	Start R_f	Start Height	Max. R_f	Max. Height	Max. %	End R_f	End Height	Area	Area %
1	-0.02	42.3	0.02	299.3	100.0	0.07	8.1	5650.3	100.00

The result of HPTLC finger printing of the drug at UV 366 nm revealed that the peak at R_f 0.02 constitutes 100% of the total area of the separated peaks, which denoted the abundant existence of the drug at minimum peak level itself. It indicated that the drug contains phytochemicals.

Heavy Metal Analysis

Table 7: Heavy Metal analysis of Jathikaai Mathirai

Name of the heavy metal	Absorption Max - A max	Result Analysis	Maximum Limit
Mercury	253.7 nm	BDL	1 ppm
Lead	217.0 nm	0.18	10 ppm
Arsenic	193.7 nm	BDL	3 ppm
Cadmium	228.8 nm	BDL	0.3 ppm

BDL- Below Detection Limit

The present investigation had clearly showed that the sample had no traces of heavy metals such as Mercury, Arsenic and Cadmium. Whereas the sample showed the presence of heavy metal Lead at 0.18 ppm which was less than the recommended limit.

Aflatoxin

Table 8: Aflatoxin analysis in Jathikaai Mathirai

Aflatoxin	Sample JM	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

It showed that there were no spots had been identified in the test sample loaded on TLC plates when compared to the standard which indicated that the sample were free from Aflatoxin B1, Aflatoxin B2, Aflatoxin G1, Aflatoxin G2.

Specific pathogen

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

Table 9: Specific pathogen analysis in Jathikaai Mathirai

Organism	Specification	Result	Method
E-coli	Absent	Absent	As per AYUSH specification
Salmonella	Absent	Absent	
Staphylococcus aureus	Absent	Absent	
Pseudomonas aeruginosa	Absent	Absent	

No growth / colonies were observed in any of the plates inoculated with the test sample

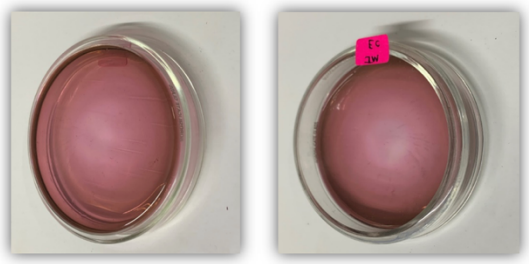

Figure 3: Culture plate with *E-coli* (EC) specific medium

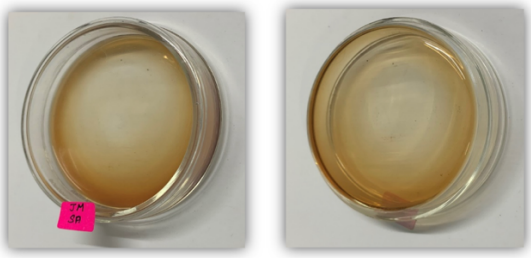

Figure 4: Culture plate with *Salmonella* (SA) specific medium

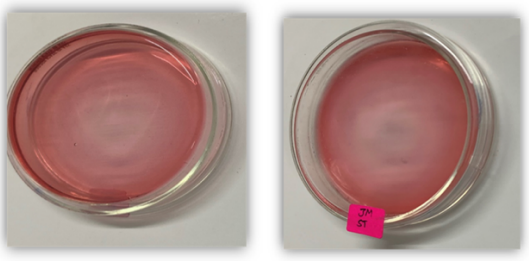

Figure 5: Culture plate with *Staphylococcus aureus* (ST) specific medium

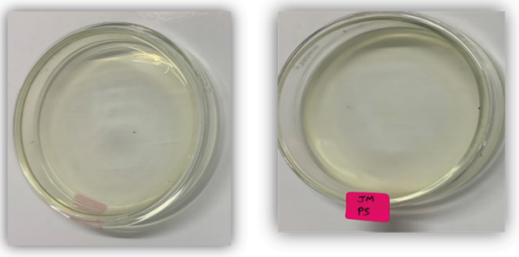

Figure 6: Culture plate with *Pseudomonas aeruginosa* (PS) specific medium

Sterility Test by Pour Plate Method

No growth / colonies were observed in any of the plates inoculated with the test sample.

Table 10: Sterility test report

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	

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

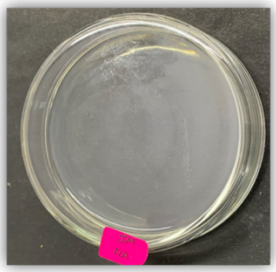

Figure 7: Sterility test by pour plate method

Table 11: Pesticide Residue Analysis of Jathikaai Mathirai

Pesticide residue	Sample JM	AYUSH Limit (mg/kg)
I. Organo Chlorine Pesticides		
Alpha BHC	BQL	0.1 mg/kg
Beta BHC	BQL	0.1 mg/kg
Gamma BHC	BQL	0.1 mg/kg
Delta BHC	BQL	0.1 mg/kg
DDT	BQL	1 mg/kg
Endosulfan	BQL	3 mg/kg
II. Organo phosphorus pesticides		
Malathion	BQL	1 mg/kg
Chlorpyriphos	BQL	0.2 mg/kg
Dichlorvos	BQL	1 mg/kg
III. Organo carbamates		
Carbofuran	BQL	0.1 mg/kg
IV. Pyrethroid		
Cypermethrin	BQL	1 mg/kg

BQL- Below Quantification Limit

It showed that there was no trace of pesticide residues such as Organo chlorine, Organo phosphorus, Organo carbamate and pyrethroids in the sample Jathikaai Mathirai. It further showed the above-mentioned residues were not detected in the sample Jathikaai Mathirai provided for analysis.

The present study of standardisation of Jathikaai Mathirai in respect to aflatoxin assay, sterility test for microbial contamination, test for specific pathogen and pesticide residue analysis revealed the absence of microorganisms, aflatoxins and pesticides in the Mathirai preparation and ensures the safe usage of the formulation. Safety studies of herbal drugs and other products used in traditional medicines have become mandatory in order to ensure their quality and risk-free therapeutic application.

CONCLUSION

From this current study, preclinical standardisation of Jathikaai Mathirai indicated for Valippu Noi which was mentioned in Siddha texts, showed that the drug was hard solid in nature with brown colour. The phytochemical analysis showed the presence of bioactive phytoconstituents such as steroids, alkaloids, coumarins, saponins, tannins, phenols, glycosides and carbohydrates. Heavy metal analysis revealed that there was no traces of heavy metals such as arsenic, cadmium and mercury whereas the level of lead are within the prescribed limit there by ensures its safe usage. In addition to these, the study revealed that the Mathirai is sterile and free of bacteria, fungi, and specific pathogens like *E-coli, Salmonella*, *Staphylococcus aureus, Pseudomonas aeruginosa* and pesticide residues. As a result, Jathikaai Mathirai, Siddha Mathirai formulation was subjected to many studies to validate its efficacy and safety through the defined standardization procedure, and it was recommended to take the formulation to the next level of investigation like pharmacological studies and clinical trials.

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Cite this article as:

K. Rajeswari *et al*. Physicochemical Standardisation of siddha tablet preparation Jathikaai Mathirai. Int. J. Res. Ayurveda Pharm. 2021;12(4):87-93 http://dx.doi.org/10.7897/2277-4343.1204112

Source of support: Nil, Conflict of interest: None Declared