Research Article

www.ijrap.net (ISSN:2229–3566)

STANDARDIZATION OF PULIYAARAI NEI: A SIDDHA POLY HERBAL FORMULATION

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Received on: 10/08/20 Accepted on: 07/10/20

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DOI: 10.7897/2277-4343.1105172

ABSTRACT

Siddha is one of the Indian systems of medicine which is widely practiced in Tamil Nadu, India. Siddha insists to lead a healthy life both physically and mentally. Siddhars have classified 4448 disease, based on the three humoral pathologies. Among them *Ratha Moolam* (Bleeding piles) is classified under *Eruvaimulainoi*. *Puliyaarai Nei* has indication for *Ratha Moolam* (Bleeding piles) as per siddha text Aathmaratchamirthamennumvaithiyasaarasangiragam (siddha vaithiyathinaathinool). The drug is prepared as per the method mentioned in the Siddha literature. Drug standardization of herbal formulations is essential for their acceptance in this scientific medical world. In this article Physicochemical, Phytochemical, biochemical analysis and other standardization methods of one such polyherbal medicated ghee *Puliyaarai nei* are discussed. The organoleptic characteristics, pH, Iodine number, Saponification value, Peroxidase value, Acid value were studied. The Qualitative study was done in biochemistry and the results are as follows, puliyaarai nei showed the Presence of carbonate, aluminium, iron, zinc, calcium, magnesium, ammonium, mercury, starch, arsenic and alkaloid. Preliminary phytochemical screening was done for the presence of flavonoids, steroids and betacyanin. HPTLC finger printing, Heavy metal analysis, Pesticide residues, Microbial contamination were studied as per the Pharmacopoeial laboratory standards of Indian medicine. In results, it was found that the specified drug was containing various phytochemicals and is free from microbial contamination, and pesticide residues. The heavy metals such as Arsenic, Mercury, Cadmium and Lead are not detected.

Keywords: Siddha, Standardization, Puliyaarai Nei, Polyherbal, *Ratha Moolam*.

INTRODUCTION

Saint Agasthiyar identified 4448 diseases and one among them is *Eruvai Mulainoi*. Saint Yugi classified *moola noi* into 21 types. The signs and symptoms of *Ratha Moolam* such as pain around the umbilicus, splashing of blood during defecation, weakness in the limbs, giddiness can be correlated with Bleeding Haemorrhoids mentioned in Modern science. Siddha system of medicine paves a way in the treatment of *Moolam* at its early and later stages as well. If the early stage is not being taken care of it may lead to complications and need to be corrected with surgery. "*Anilapithathondhamalathumoolamvaraathu*" (*Anilam-vaatham, pitha-pitham*). As per saint Theriyar derangement of *Vatham* and *Pitha* humor due to lifestyle changes diet and deeds resulting *Moolam*. The main *Vatha* humor and *Pitha* humor get affected cause in *Moola noi* (Haemorrhoids). In *Moola noi*, increased *keelvaikanal* stimulates *Vatha* humor these totally stimulate *Pitha* humor, constipation develops due to effects of *keevaikanal.* So the symptoms like loss of appetite, emaciation, mental depression, decreased body fluids and blood volume are developed in treatment aspect in order to suppress *Vatha* humor, cooling agents were prescribed to suppress *keelvaikanal.*[1-2]

A siddha formulation "*Puliyaarai Nei*" has been specifically indicated for *Moola noi*. Most of the ingredients of these formulations possess Styptic, Anti -inflammatory, Anthelmintic and Laxative activities. Hence, here comes the importance of standardization of drugs to exhibit conformation of its identity and determination of its purity, safety, potency and efficacy for safer practice. The Standardization of any drug is done by stepwise quality control methods as prescribed by Pharmacopeial laboratory standards of Indian medicine. Siddha pharmacopeia has given preparations in various forms as *Chooranam* (powder), *Maathirai* (tablet), *Manapagu* (syrup) and *Nei* (medicated ghee) for the treatment of different diseases. Here the herbal medicated ghee, which is lipid-based formulation, has the ability to cross blood-brain barrier thereby having beneficial effects on the brain. Also, drugs prepared with ghee are easily digested and quickly absorbed. The lipid solubilized drugs are quickly distributed in the intra and extracellular spaces in our body.

Puliyaarai Nei is one such Siddha polyherbal medicated ghee chosen from the text Aathmaratchamirthamennumvaithiyasaarasangiragam (siddha vaithiyathinaathinool) is indicated for *Ratha Moolam.* Till date, no standards are available for Puliyaarai nei. Hence, the current study has been carried out to assess its Physico-chemical, Phyto-chemical and other standardization parameters as a part of their scientific validation.

MATERIAL AND METHODS

Identification of raw drugs

The herbal ingredients were authenticated from Botanist, National Institute of Siddha, Tambaram sanatorium, Chennai.

Table 1: Ingredients of Puliyaarai nei

S. No.	Drug Name	Measures
1	Puliyaaraiilai (*Oxalis corniculata* Linn.)	20 Palam (700 gm)
2	Thanneervittan Kizhangu (*Asparagus racemosus* Linn.)	30 Palam (1050 gm)
3	Ponmusuttai Ver (*Sida acuta* Linn.)	30 Palam (1050 gm)
4	Nannari Ver (*Hemidesmus indicus* Linn.)	30 Palam (1050 gm)
5	Kariveppilai (*Murraya koenigii* Linn.)	30 Palam (1050 gm)
6	*Koraikizhangu* (*Cyprus rotundus* Linn.)	30 Palam (1050 gm)
7	Vilamitcham Ver (*Plectranthus vettiveroides* Linn.)	30 Palam (1050 gm)
8	Vilwa Ver (*Aegle marmelos* Linn.)	30 Palam (1050 gm)
9	Cow's Ghee	1 Padi (1.34 lts)
10	Thalisa-Paththiri (*Abies spectabilis* Linn.)	1 Palam (35 gm)
11	Thippili (*Piper longum* Linn.)	1 Palam (35 gm)
12	Chiru-Nagappu (*Mesua nagassarium* Linn.)	Palam (35 gm)
13	Chukku (*Zingiber officinale* Linn.)	1 Palam (35 gm)
14	Vetpalai Arisi (*Wrightia tinctoria* Linn.)	½ Palam (18 gm)
15	Karkadaka Singi (*Rhus succedanea* Linn.)	½ Palam (18 gm)
16	Seeragam (*Cuminum cyminum* Linn.)	½ Palam (18 gm)
17	Sanna Lavangapattai (*Cinnamomum verum* Linn.)	½ Palam (18 gm)
18	Narukku Moolam (Root of *Piper longum* Linn.)	½ Palam (18 gm)
19	Lavangapathiri (*Cinnamomum tamala* Linn.)	½ Palam (18 gm)
20	Elam (*Elettaria cardamomum* Linn.)	½ Palam (18 gm)
21	Karunjeeragam (*Nigella sativa* Linn.)	½ Palam (18 gm)
22	Sathikkai (*Myristica fragrans* Linn.)	1 Varagan (4 gm)
23	Chathi- Pathiri (Leaf of *Myristica fragrans* Linn.)	1 Varagan (4 gm)
24	Kirambu (*Syzygium aromaticum* Linn.)	1 Varagan (4 gm)
25	Athimathuram (*Glycyrrhiza glabra* Linn.)	1 Palam (35 gm)
26	Naatusarkarai	5 Palam (175 gm)
27	kargandu	5 Palam (175 gm)[3]

Method of Purification

Purification of raw drugs was done as per the methods given in Siddha text *Sigichaarathnadeepam*.[4]

Method of Drug Preparation

- Step 1: Above drugs no 1-8 are coarsely powdered and taken in a wide mouth vessel.
- Step 2: 2 Padi (2.6 lit) of water is added to the above mixture and decoction is prepared by boiling the content till reduced to 1/8th of its quantity.
- Step 3: To the prepared decoction add cow's ghee 1 Padi (1.3 lit).
- Step 4: Drugs no 10-13 each 1 Palam (35 gm), drugs no 14-21 each ½ Palam (17.5 gm), drugs no 22 – 24 each 1 Varagan (4.2 gm), are finely powdered and mixed with the decoction and boiled to a thick consistency.
- Step 5: Finally drugs 25 and 26 each 5 Palam (175 gm), drug no 27 1 Palam (35 gm) is finely powdered and added.

Figure 1: Physicochemical evaluation

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 50^{O}C 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.

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 Wijs 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.

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.

Determination of Viscosity value

Viscosity determination was 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 was 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.

Determination of Refractive Index

Determination of RI was carried out using Refractometer.

Determination of Weight per ml

Weight per ml was determined using the comparative weight calibration method. In which the weight of 1 ml of the base of the formulation was calculated and then weight of 1 ml of finished formulation were calculated. The difference between weight variations of the base with respect to finished formulation calculated as an index of weight per ml.

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)

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 0.5 ml 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 0.01 M 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 0.01 M sodium thiosulphate in the blank determination must not exceed 0.1 ml.

$$\text{Peroxide value} = 10\ (a - b)/w$$

Phytochemical analysis

Test for alkaloids

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

Test for Coumarins

To the test sample, 1 ml of 10% sodium hydroxide was added. The presence of coumarins was 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 indicates 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 was 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 indicates presence 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. Appearance of yellow colour indicates the presence 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 turns into red and sulphuric acid layer showed yellow with green fluorescence. It showed the presence of steroids.

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, appearance of red ring indicates the presence of triterpenoids.

Test for Cyanins

Anthocyanin

To the test sample, 1 ml of 2N sodium hydroxide was added and heated for 5 min at 100°C. Formation of bluish green colour indicates the presence 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 boiling water bath for 2 minutes. A characteristic coloured precipitate indicates the presence of sugar.

Proteins (Biuret Test)

To extracts 1% solution of copper sulphate was added followed by 5% solution of sodium hydroxide, formation of violet purple colour indicates the presence of proteins.

Biochemical analysis

Preparation of extract

10 gm of Puliyaarai Nei was measured accurately and placed in 250 ml of clean beaker and added with 250 ml of distilled water. Then it was boiled well for 10 minutes. Then it was cooled and filtered in a 100 ml volumetric flask and made up to 100 ml with distilled water.

Experiment	Observation	Inference
Appearance of sample	Dark brown in colour	
Solubility Little of the sample was shaken well and mixed with distilled water.	Insoluble	Absence of Nitrate
Action of heat: A small amount of the sample was taken in a dry test tube and heated gently at first and then strong.	No brown fumes No white fumes evolved.	Absence of Nitrate Absence of Carbonate
Flame test: A small amount of sample was made into paste with con.HCL in a watch class and introduced into nonluminous part of the Bunsen flame.	No bluish green flame appeared.	Absence of Copper
Ash test: A filter paper was soaked into a mixture of sample and cobalt nitrate solution introduced into the Bunsen flame and ignited.	No Yellow coloured flame appeared	Absence of **Sodium**

Test for acid radicals

Test for Sulphate: 2 ml of above prepared extract was taken in the test tube to this added2 ml of 4% ammonium oxalate Solution.	No Cloudy Appearance present	Absence of Sulphate
Test for Chloride: 2 ml of the above prepared solution was added with dil. HNO_3 till the effervescence ceases. Then 2 ml of Silver nitrate solution was added.	No cloudy appearance present	Absence of Chloride
Test for Phosphate: 2 ml of the extract was treated with 2 ml of Ammonium molybdate Solution and 2 ml of Con. HNO_3.	Absence of cloudy yellow appearance	Absence of Phosphate
Test for Carbonate: 2 ml of the extract was treated with 2 ml of Magnesium sulphate Solution.	Cloudy appearance present	Presence of Carbonate
Test for Nitrate: 1 drop of the substance was heated with Copper tunics and concentrated H_2SO_4 and viewed the test tube vertically down.	No characteristic changes of formed	Absence of Nitrate
Test for Sulphide: 1 ml of substance was treated with 2 ml of Con. HCL.	No rotten egg smelling gas evolved	Absence of Sulphide
Test for Fluoride and Oxalate: 2 ml of the extract was added with 2 ml of dis. Acetic acid and 2 ml Calcium chloride solution and heated.	Cloudy appearance present	Presence of fluoride and Oxalate
Test for Nitrite: 3drops of the extract was placed on the filter paper on that 2 drops of Acetic acid and 2 drops of Benzidine solution is placed.	No characteristic changes observed	Absence of Nitrite
Test of Borate: 2 pinches of the substances were made into paste by sulphuric acid alcohol (95%) and introduce into blue Flame.	Bluish yellow coloured flame not appeared.	Absence of Borate

Test for basic radicals

Test for Lead: 2 ml of extract was added with 2 ml of Potassium iodide solution.	Yellow coloured precipitate was not obtained.	Absence of Lead.
Test for Copper: One pinch of substance was made into paste with Con. HCl in a watch glass and introduced into the non- luminous part of the flame.	No blue coloured flame appeared	Absence of copper
Test for Aluminum: To the 2 ml of the extract Sodium hydroxide was added in drops to excess.	No characteristic changes observed	Presence of **Aluminium**
Test for Iron: To the 2 ml of extract add 2 ml of ammonium Thiocynate solution. To the 2 ml of extract add 2 ml ammonium Thiocynate solution and 2 ml of con HNO_3 is added.	Mild red colour appear Blood red colour appear	Presence of **Iron**

Test for Zinc: To 2ml of the extract sodium hydroxide solution was added in drops to excess.	White precipitate was appeared.	Presence of Zinc
Test for Calcium: 2 ml of the extract was added with 2 ml of 4% ammonium oxalate solution.	cloudy appearance present	Presence of Calcium.
Test for Magnesium: To 2 ml of extract sodium hydroxide solution is added in drops to excess.	White precipitate was not appeared.	Presence of Magnesium
Test for Ammonium: To 2 ml of extract few ml of Nessler's reagent and excess of sodium hydroxide solution are added.	Brown colour appeared	Presence of ammonium

Test for Potassium: 1 ml of substance was treated with 2 ml of sodium and then treated with 2 ml of cobalt nitrate in 30% glacial acetic acid.	No yellowish precipitate was obtained.	Absence of Potassium
Test for Sodium: 2 pinches of the substance was made into paste by using HCL and introduced into the blue flame of Bunsen burner.	No yellow colour flame appeared.	Absence of Sodium
Test for Mercury: 2 ml of the extract was treated with 2 ml of sodium hydroxide solution.	Yellowish precipitate was obtained.	Presence of Mercury
Test for Arsenic: 2 ml of the extract was treated with 2 ml of sodium hydroxide solution.	Brownish red precipitate was obtained.	Presence of Arsenic

Miscellaneous

Test for Starch: 2 ml of the extract was treated with weak iodine solution.	No blue colour developed.	Presence of **Starch.**
Test for reducing sugar: 5 ml of Benedict's qualitative solution was taken in a test tube and allowed to boil for two minutes and added 8 to 10 drops of the extract and again boil it for 2 minutes, the colour is noted.	No brick red colour developed.	Absence of Reducing sugar.
Test for alkaloids: 2 ml of extract was treated with 2 ml of picric acid.	Yellow colour developed.	Presence of **Alkaloid**
Test for Tannic acid: 2 ml of extract was treated with 2 ml of ferric chloride solution.	Black colour Precipitate is not appeared.	Absence of Tannic acid.
Test for Unsaturated compounds: To the 2 ml of extract 2 ml of potassium permanganate solution was added.	Potassium permanganate is not de-coloured.	Absence of Unsaturated compounds
Test for Amino acids: 2 drops of the extract were placed on a filter paper and dried well.	No violet colour developed.	Absence of Amino acids
Test for type of compound: 2ml of the extract is treated 2ml of ferric chloridesolution.	No green colour developed. No red colour developed. No violet colour developed. No blue colour developed.	Absence of Oxyquinole epinephrine and pyrocatechol. Anti pyrine, Aliphatic amino acid and meconic acid absent. Apomorphine, Salicylate and Resorcinol are absent. Morphine, Phenol cresol and Hydro quinine are absent.

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 were 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 were 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 provides chromatographic fingerprint of phytochemicals which was suitable for confirming the identity and purity of phyto-therapeutics.

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 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 Atomic Absorption Spectrometry 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 the 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

Sterility test by pour plate method

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 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

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 add a few milliliters of toluene and heat again until the acetone was completely removed. Resultant residue would be dissolved using toluene and filtered through membrane filter[5-10].

Table 2: Test Result Analysis of the Sample PN

Pesticide Residue	Sample PN	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

RESULTS AND DISCUSSION

Table 3: Organoleptic characters

State	Dense viscous liquid
Nature	Dense
Odor	Mild characteristic
Touch	greasy
Flow Property	Non free flowing
Appearance	Dark greenish

Table 4: Physicochemical evaluation report

S. No.	Parameter	Puliyaarai Nei - PN
1	Specific Gravity	0.7997
2	Viscosity at 50ºC (Pa s)	91.27
3	Refractive index	1.86
5	Iodine value (mg I2/g)	70.48
6	Saponification Value (mg of KOH to saponify 1 gm of fat)	265.25
7	Weight per ml	0.411
8	Acid Value mg KOH/g	1.57
9	Peroxidase Value mEq/kg	5.72

Phytochemical Analysis Report

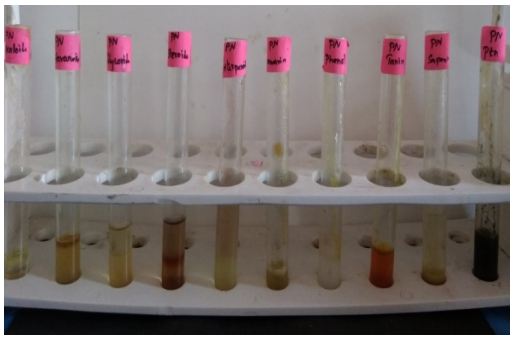

Figure 2: Qualitative Phytochemical Investigation

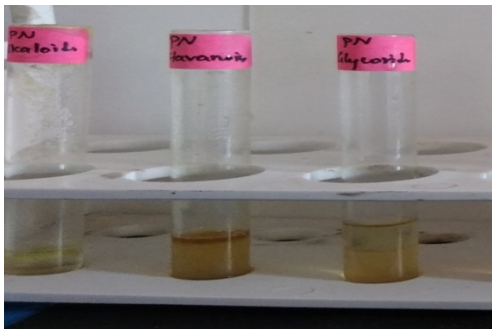

Figure 3: Test for Alkaloids, Flavonoids, Glycosides

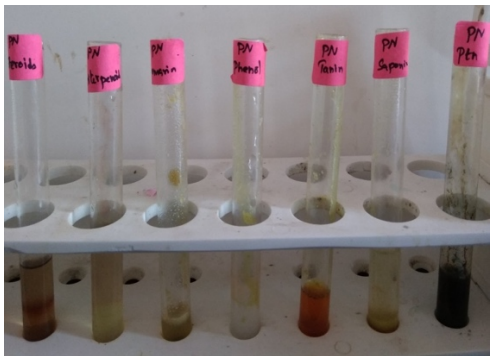

Figure 4: Test for Steroids, Triterpenoids, Coumarin, Phenol, Tannins, Saponin, Proteins

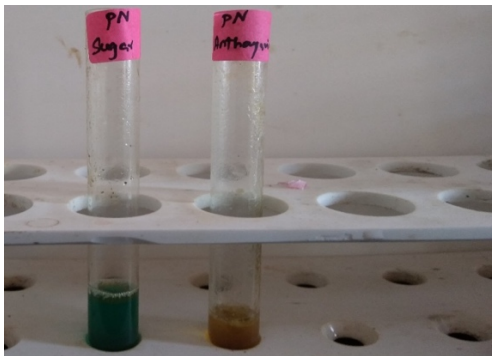

Figure 5: Anthocyanin and carbohydrates

Table 5: Biochemical Analysis Report

S. No.	Presence	Absence
1.	Carbonate	Nitrate
2.	Aluminium	
3.	Iron	Copper
4.	Zinc	Sulphate
5.	Calcium	Chloride
6.	Magnesium	Phosphate
7.	Ammonium	Sulphide
8.	Mercury	Nitrite
9.	Starch	Lead
10.	Arsenic	Reducing sugar
11.	Starch	Tannic acid
12.	Alkaloid	Amino acids
13.		Type of compound

TLC Visualization of PN - TLC plate visualization at 366 nm

HPTLC finger printing of Sample PN

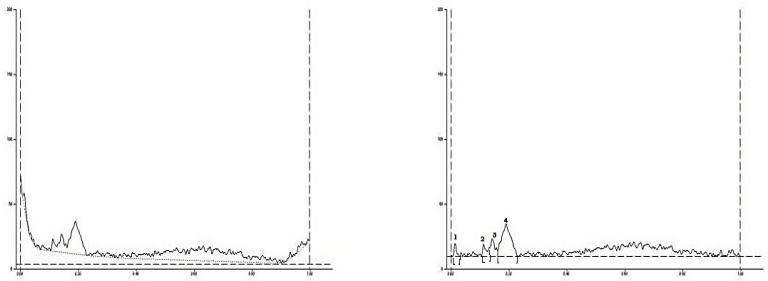

Figure 6: TLC and HPTLC analysis report

Table 6: HPTLC Peaks

Peak	Start Rf	Start height	Max Rf	Max Height	Max %	End Rf	End Height	Area	% Area
1	0.05	0.0	0.01	13.1	1.52	0.08	0.0	193.1	6.21
2	0.10	1.1	0.11	18.4	1.56	0.14	0.3	242.8	3.44
3	0.12	2.8	0.14	12.4	1.95	0.17	0.2	281.0	3.32
4	0.16	4.3	0.19	25.2	94.97	0.23	0.5	609.4	87.03

HPTLC fingerprinting analysis of the sample reveals the presence of four prominent peaks corresponds to presence of four versatile phytocomponents present within it. Rf value of the peaks ranges from 0.05 to 0.16. Further the peak 4 occupies the major percentage of area of 87.03 which denotes the abundant existence of such compound.

Table 7: Heavy metal analysis test report

Name of the Heavy Metal	Absorption Max λmax	Result Analysis	Maximum Limit
Mercury	253.7 nm	BDL	1 ppm
Lead	217.0 nm	0.49	10 ppm
Arsenic	193.7 nm	BDL	3 ppm
Cadmium	228.8 nm	BDL	0.3 ppm

BDL – Below Detection Limit

Results of the present investigation have clearly shown that the sample had no traces of heavy metals such as Mercury, Arsenic and Cadmium; whereas the sample shows the presence of heavy metal Lead at 0.49 ppm which may be less than the recommended limit.

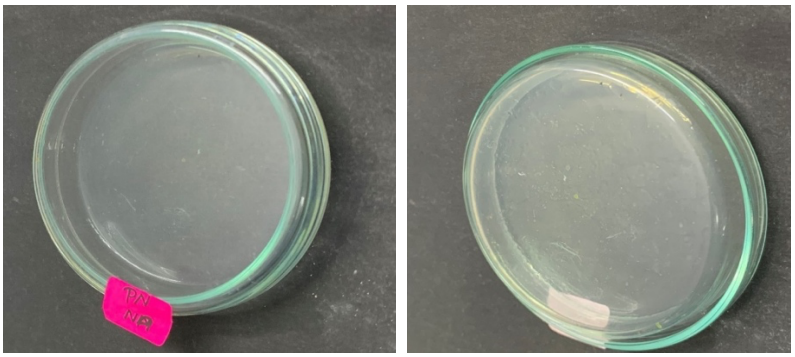

Figure 7: Sterility Test Report

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

Table 8: Sterility test

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	

Pesticide residue test report

The results showed that there were no traces of pesticides residues such as Organochlorine, Organophosphorus, Organ carbamates and pyrethroids in the sample provided for analysis.

CONCLUSION

In the current scenario drug standardization is vital for even centuries old traditional Siddha formulations for its global acclimatization. Evaluation of parameters such as peroxide value, saponification value, iodine value, HPTLC studies were determined, which signifies standard parameters to ensure the purity and quality of the drug.

The Physicochemical analysis for *Puliyaarai Nei*, the results showed that specific gravity -0.7997, Viscosity at 50°C (Pa s) -91.27, Refractive index -.86, Iodine value (mg I2/g) -70.48, Saponification Value (mg of KOH to saponify 1 gm of fat) -265.25, Weight per ml- 0.411, Acid Value mg KOH/g -1.57, Peroxidase Value mEq/kg - 5.72 respectively. The phytochemical findings of the study confirm the presence flavonoids, steroids and betacyanin has Anti -inflammatory, anti-radical and anti-oxidant activity. The Qualitative biochemical study was done and the results are as follows, *Puliyaarai Nei* showed the Presence of carbonate, aluminium, iron, zinc, calcium, magnesium, ammonium, mercury, starch, arsenic and alkaloid which are essential to fulfil the therapeutic need. HPTLC fingerprinting analysis of the sample reveals the presence of four prominent peaks corresponds to presence of four versatile phytocomponents present within it. Rf value of the peaks ranges from 0.05 to 0.16. Further the peak 4 occupies the major percentage of area of 87.03 which denotes the abundant existence of such compound. Pesticides residues study of *Puliyaarai Nei*, the results showed that there were no traces of pesticides residues such as Organochlorine, Organo phosphorus, Organo carbamates and pyrethroids in the sample provided for analysis. Microbial contamination study the results showed that no growth / colonies were observed in any of the plates inoculates with the test sample.

From the above study puliyaarai neiwas prepared as per Classical textual Standard operative procedure and subjected to various studies to reveal its potency and effectiveness against the disease mentioned in Aathmaratchamirthamennumvaithiyasaarasangiragam (siddha vaithiyathinaathinool). This present study suggests puliyaarai neihas remarkable medicinal value in the treatment.

ACKNOWLEDGEMENT

My sincere thanks to the Tamil Nadu Dr. M. G. R. Medical University, National Institute of Siddha for providing the necessary facilities to carry out this research work and to the Dr. D. Aravind, M.D(s), M.Sc [Medicinal plants], Assistant Professor, for the authentication of crude herbal drugs and my sincere thanks to Noble Research Institute for standardization of drug puliyaarai nei.

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Cite this article as:

S. Priyadarshini *et al*. Standardization of Puliyaarai Nei: A Siddha Poly Herbal Formulation. Int. J. Res. Ayurveda Pharm. 2020;11(5):218-227 http://dx.doi.org/10.7897/2277-4343.1105172

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