International Journal of Health Sciences and Research
Vol.12; Issue: 5; May 2022
Website: www.ijhsr.org
Original Research Article *ISSN: 2249-9571*

Standardization and Physicochemical Evaluation of the Drug *Raja Elathi Chooranam* - A *Siddha* Polyherbal Formulation

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DOI: https://doi.org/10.52403/ijhsr.20220523

ABSTRACT

Siddha drugs are natural products obtained from herbal, herbo-mineral, mineral and animal kingdom. Most of the *Siddha* medicines are effective but they have not been standardized yet. So, there be in need to progress a standardization technique. *Raja Elathi Chooranam* is a poly-herbal formulation mentioned in the classical *Siddha* literature for the medication of Menorrhagia. The aim of this study to estimate quality of *Raja Elathi Chooranam* by conducting physicochemical analysis, phytochemical analysis and other analytical techniques. This study was done based on Pharmacopoeial laboratory for Indian Medicine guidelines. High performance thin layer chromatography was done through CAMAG software. The organoleptic character of the sample drug justifies the solid and its aromatic and fine powder nature confirms that the formulation is of good quality. The results obtained after phytochemical analysis of test drug showed 2.427 ± 0.4839 % of Loss on drying at 105°C, 9.633 ± 0.7506% of Total Ash, 0.054 ± 0.01652 % of Acid insoluble ash etc. The pH value of the drug 6.5 which is indicates it is weakly acidic. The results obtained after preliminary phytochemical analysis confirmed the presence of alkaloids, flavonoids, steroids, triterpenoids, coumarin, phenols, tannins, saponins and sugar. Biochemical analysis of the test drug reveals the presence of Carbonates, Chlorides, Sulphates and Phosphates. The test drug is devoid of microbial contamination, heavy metals and pesticide residues were below quantification limit. From the result we infer that the drug is of standard quality and shall be used as reference in pharmacopoeia standards.

***Keywords*:** Standardization, *Raja Elathi Chooranam*, Poly herbal, Siddha, Physicochemical analysis, phytochemical analysis.

1. INTRODUCTION

The *Siddha* system of Medicine is a complete comprehensive medical system that has been practised in India for 2000 years and above. Herbal medicine uses whole plants, seeds, berries, roots, leaves, bark and flowers for medicinal purposes.[1] *Siddha* system offers effective treatment

options for various common illness, helps to improve the quality of life by better management of lifestyle disorders and ailment of various systems of the body. The subject of Herbal drug standardization is wide and deep.

Standardization is avital factor for herbal formulation to evaluate the quality, purity and efficacy of the drugs based on the active principles.[2] As per World Health Organization (WHO), more than 80% of global population uses plants or their products as the primary source of medicinal agents.[3]

Chooranam is referred to the powder prepared by a single or a combination of two or more medicinal plant ingredients.[4] It is simple and affordable. Thus, the present study deals with standardization of *Siddha* herbal formulation, *Raja Elathi Chooranam* a *Siddha* drug mentioned in the text *Koshayi Anuboga Vaithiya Navaneetham* Part-2 which is used to treat Menorrhagia, Gonorrhoea and Tuberculosis.[5] Till now there is no clear documentation available on standardization and phytochemical investigation aspect of this formulation. This is proved through the systematic standardization of the test drug by physicochemical, phytochemical evaluation and HPTLC finger printing aspects according to PLIM guidelines.

2. MATERIALS AND METHODS

Selection of the trial drug

For this present study, the Poly-herbal formulation "*Raja Elathi Chooranam*" a compound drug preparation for *Perumbadu* (Menorrhagia) has been chosen from classical *Siddha* literature – "*Koshayi Anuboga Vaithiya Brahmma Ragasiyam*" -Part 2, Publisher: R.C. Mohan, Publication: *Thamarai noolagam*, Page no: 101-102.[5]

Table No1: Ingredients with Botanical name of *Raja Elathi Chooranam*

S.no	Ingredients	Botanical Name	Quantity
1.	Cardamom	*Elettaria cardamomum*	268.8gms
2.	Dried ginger	*Zingiber officinalis*	134.4gms
3.	Indian arrow root	*Maranta arundinacea*	67.2gms
4.	Ceylon ironwood	*Mesua nagassarium*	33.6gms
5.	Many spiked flacortia	*Abies spectabilis*	16.8gms
6.	Pepper	*Piper nigrum*	8.4gms
7.	Cloves	*Syzygium aromaticum*	4.2gms
8.	Sugar	*Saccharum officinarum*	420gms

Collection of the drug materials

The raw drugs *Elettaria cardamomum*, *Zingiber officinalis*, *Maranta arundinacea*, *Mesua nagassarium*, *Abies spectabilis*, *Piper nigrum*, *Syzygium aromaticum* and *Saccharum officinarum* were bought from authenticated country drug store in Chennai.

Identification and authentication of the drugs

All the raw materials were identified and authenticated by the Botanist, Government Siddha Medical College, *Arumbakkam* and Chennai. The specimen sample of each raw materials are *Elettaria cardamomum*, *Zingiber officinalis*, *Maranta arundinacea*, *Mesua nagassarium*, *Abies spectabilis*, *Piper nigrum*, *Syzygium aromaticum* and *Saccharum officinarum* has been labelled as 1027 to 1034/PGG/321912107/GSMC-GH/2019-2022 respectively and were kept in the PG *Gunapadam* department, Government Siddha Medical College, Chennai-106 for future reference.

Purification of the Drugs[6]

All the raw drugs specified here were purified as per Siddha literature. All the impurities like sand and dust were removed from *Elettaria cardamomum*, *Mesua nagassarium* and *Abies spectabilis* and were roasted. Dried *Zingiber officinalis* was soaked in lime water for 3 hours. It was washed and dried. Then the outer layer was removed. Water was added to *Maranta arundinacea* powder and dissolved. The supernatant water was filtered. This was repeated for 7 times. The resulting powder was dried. *Piper nigrum* was soaked in butter milk for one hour 15 minutes and then dried. *Syzygium aromaticum* was fried in low flame. The dust of *Saccharum officinarum* was removed and sugar was powdered well.

Preparation of the drug

All the ingredients were finely powdered separately by pounding in an Iron mortar and sieved through a mesh. Then the powdered sugar was added to the *Chooranam.* Then the finely powdered *Chooranam* was kept in an air tight container and labelled as *REC*.

Dosage:0.8 to 1gram
Adjuvant: Water
Indications: It cures menorrhagia, wounds, gonorrhoea and tuberculosis.

Organoleptic characters

State, nature, odour, touch, flow property, appearance of the drug was noted.

These following studies were done at Noble Research Solutions, Peramburat Chennai.

2.1. Physicochemical Evaluation [7-9]

Percentage Loss on Drying

Test drug was accurately weighed 2gm of *REC* formulation was taken in a tarred in evaporating dish. The sample was dried at 105^{o}C for 5 hours and then weighed.

Determination of Total Ash

Test drug *REC* was accurately weighed 2gram in silica dish and incinerated at the furnace at 400 ºC until it turns white which shows absence of carbon. Percentage of total ash was calculated with reference to the weight of air-dried drug.

Determination of Acid Insoluble Ash

The ash obtained by total ash test was boiled with 25 ml of dilute hydrochloric acid for 6mins. 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.

Determination of Alcohol Soluble Extractive

Test sample *REC* 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 tarred 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 *REC* 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 tarred flat bottomed shallow dish and dry at 105ºC, to constant weight and weight. Calculate the percentage of water-soluble extractive with reference to the air-dried drug.

Determination of pH

About 5g of test sample *REC* was dissolved in 25ml of distilled water and filtered. The resultant solution was allowed to stand for 30mins and then subjected to pH evaluation.

Particle Size Determination by Microscopic Method

Methodology

Particle size determination was carried out by optical microscopic method. In which the sample were dissolved in the sterile distilled water (app 1/100th dilution). Diluted sample were mounted on the slide and fixed with stage of appropriate location. Light microscopic image was drawn with scale micro-meter to arrive at the average particle size. Minimum 30 observations were made to ascertain the mean average particle size of the sample.

Solubility test

A pinch of sample was taken in a dry test tube and to it 2ml of the solvent was added and shaken well for about a minute and the results were observed. The test was done for solvents like Chloroform, Ethanol, Water, Ethyl Acetate, Hexane, Dimethyl sulphide (DMSO) and the results were observed individually.

2.2 Phytochemical Analysis[10]

Test for Alkaloids:

Mayer's Test

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

Test for Coumarins:

To 5ml test sample *REC*, 1 ml of 10% sodium hydroxide was added. The presence of coumarins was showed by the formation of yellow colour.

Test for Saponins

To 5ml test sample *REC*, 5 ml of water was added and the tube was shaken vigorously. Copious lather formation shows the presence of saponins.

Test for Tannins

To 5ml test sample *REC*, Ferric chloride was added, formation of a dark blue or greenish black colour showed the presence of tannins.

Test for Glycosides

Borntrager's Test

Test drug *REC* was hydrolyzed with concentrated hydrochloric acid for 2 hours on a water bath, filtered and the hydro-lysate was subjected to the following tests. To 2 ml of filtered hydro-lysate, 3 ml of chloroform is added and shaken, chloroform layer is separated and 10% ammonia solution is added to it. Pink colour shows presence of glycosides.

Test for Flavonoids

To 5ml test sample *REC* about 5 ml of dilute ammonia solution was added followed by addition of few drops of conc. Sulphuric acid. Appearance of yellow colour shows the presence of Flavonoids.

Test for Phenols:

Lead Acetate test

To 5ml test sample *REC*; 3 ml of 10% lead acetate solution was added. A bulky white precipitate showed the presence of phenolic compounds.

Test for Steroids

To 5ml test sample *REC*, 2ml of chloroform was added with few drops of conc. Sulphuric acid (3ml), 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.

Test for Triterpenoids

Liebermann-Burchard test

To 5ml of test sample *REC* was mixed with chloroform solution and 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 shows the presence of triterpenoids.

Test for Cyanine's

Anthocyanin

To 5ml test sample *REC*, 1 ml of 2N sodium hydroxide was added and heated for 5 min at 100°C. Formation of bluish green colour shows the presence of anthocyanin.

Test for Carbohydrates

Benedict's test

To 5ml test sample *REC*, 0.5 ml of Benedict's reagent was added. The mixture is heated on a boiling water bath for 2 minutes. A characteristic coloured precipitate shows the presence of sugar.

Test for Proteins

Biuret Test

To 3ml of *REC* extracts, 1ml of 1% solution of copper sulphate was added followed by 5% solution of sodium hydroxide; formation of violet purple colour shows the presence of proteins.

2.3 Thin Layer Chromatography (TLC) Analysis[11]

Test sample was subjected to thin layer chromatography (TLC) as per conventional one dimensional ascending method using silica gel 60F254, 7X6 cm (Merck) were cut with ordinary household scissors. Plate markings were made with soft pencil. Micro pipette was used to spot the sample for TLC applied sample volume 10-micro litter 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 were observed using visible light Short-wave UV light 254nm and light long-wave UV light 365 nm.

High Performance Thin Layer Chromatography Analysis (HPTLC)[12]

HPTLC method is a modern sophisticated and automated separation technique derived from TLC. Pre-coated HPTLC graded plates and auto sampler was used to achieve precision, sensitive, significant separation both qualitatively and quantitatively. High performance thin layer chromatography (HPTLC) is a valuable quality assessment tool for the evaluation of botanical materials efficiently and cost effectively. HPTLC method offers high degree of selectivity, sensitivity and rapidity combined with single-step sample preparation. Thus this method can be conveniently adopted for routine quality control analysis. It provides chromatographic fingerprint of phytochemicals which is suitable for confirming the identity and purity of phytotherapeutics.

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.

Scanning

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

2.4 Biochemical analysis of Acid and Basic radicals [13]

Test for Acid Radicals

Test for Carbonates

To 1 ml of the *REC* solution about 1 ml of concentration (conc.) hydrochloric acid (HCL) was added. Formation of brisk effervescence shows the presence of carbonates.

Test for Chlorides

To 2 ml of *REC* solution, about 1 ml of silver nitrate solution was added. Appearance of White precipitate shows the presence of chlorides.

Test for Sulphates

To 1 ml of the test sample *REC* add diluted sulphuric acid(H_2SO_4) till effervescence ceases followed by this about 1 ml of barium chloride solution was added. Appearance of white precipitate shows the presence of sulphates.

Test for Sulphides

To 1 ml of the test sample *REC* about 2 ml of hydrochloric acid (HCL) was added with slight warming the mixture. Formation of colourless gas with the smell of rotten egg shows the presence of sulphides.

Test for Phosphates

To 2 ml of *REC* solution treated with 2 ml of ammonium molybdate solution followed by addition of 2ml of concentrated nitric acid. Formation of yellow precipitate shows the presence of phosphates.

Test for Fluoride and Oxalate

To 2 ml of the *REC* solution about 2 ml of dil. acetic acid and 2ml of calcium chloride solution was added. Formation of white precipitate shows the presence of Fluoride/ Oxalate.

Test for Borates

2ml of the *REC* solution was added with sulphuric acid (H_2SO_4) and 95% alcohol followed by exposure to flame. Appearance of green flame shows the presence of Borates.

Test for Nitrates

0.5 ml of *REC* solution heated with copper turning followed by addition of sulphuric acid. Appearance of reddish brown gas shows the presence of Nitrates.

Test for Basic Radicals

Test for Lead

1 ml of the test solution added with 2 ml of potassium chromate solution. Formation of yellow precipitate shows the presence of lead.

Test for Arsenic

1 ml of the test solution added with 2 ml of 10% (2N) sodium hydroxide (NaOH) solution. Formation of brownish red precipitate shows the presence of Arsenic.

Test for Mercury

1 ml of the *REC* solution added with 2 ml of 10% (2N) sodium hydroxide (NaOH) solution. Formation of yellow precipitate shows the presence of mercury.

Test for Copper

1 ml of the *REC* solution added with 1 ml of Ammonium hydroxide (NH4OH) solution. Formation of blue precipitate shows the presence of copper.

Test for Ferric

To 1 ml of *REC* solution, about 2 ml of potassium Ferro cyanide was added. Formation of blue precipitate shows the presence of ferric.

Test for Ferrous

To 1 ml of *REC* solution, about 1 ml of potassium ferric cyanide solution was added. Formation of blue precipitate shows the presence of ferrous.

Test for Zinc

1 ml of the *REC* solution added with 2 ml of sodium hydroxide (NaOH) drop wise until indication appears. Formation of white precipitate shows the presence of Zinc.

Test for Silver

1 ml of the *REC* solution was added with 1 ml of conc. hydrochloric acid (HCL) followed by appearance of curdy white precipitate. Boil the precipitate with water. It does not dissolve. Add Ammonium hydroxide (NH4OH) solution in it and add 1 ml dilute HNO3. Formation of curdy white precipitate shows the presence of silver.

Test for Magnesium

1 ml of the *REC* solution added with 2 ml of sodium hydroxide (NaOH) drop wise until indication appears. Formation of white precipitate shows the presence of Magnesium.

2.5 Heavy Metal Analysis byAtomic Absorption Spectrometry (AAS)[14]

Standard: Hg, As, Pb and Cd – Sigma

Methodology

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

Sample Digestion

Test sample was digested with 1mol/L hydrochloric acid (HCL) for determination of arsenic and mercury. Similarly, for the determination of lead and cadmium the sample were digested with 1mol/L of HNO3.

Standard reparation

As &Hg- 100 ppm sample in 1mol/L Hydrochloric acid (HCL)

Cd & Pb- 100 ppm sample in 1mol/L Nitric acid (HNO3)

2.6 Sterility Test by Pour Plate Method[15]

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.

2.7 Test for Specific Pathogen[16]

Methodology

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

Table No 2: Detail of Specific Medium and their abbreviation

Organism	Abbreviation	Medium
E-coli	EC	EMB Agar
Salmonella	SA	Deoxycholate agar
Staphylococcus aureus	ST	Mannitol salt agar
Pseudomonas aeruginosa	PS	Cetrimide Agar

2.8 Analysis of Pesticide Residue Organochlorine, Organophosphorus, Organocarbamates, Pyrethroids[17-18]

Extraction

Test sample were extracted with 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 millilitres of toluene and heat again until the acetone is completely removed. Resultant residue was dissolved using toluene and filtered through membrane filter.

2.9 Aflatoxin assay by Thin Layer Chromatography (TLC) (B1, B2, G1, G2)[19]

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.

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 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 the solvent from and allow the plate to air-dry. Locate the spots on the plate by examination under UV light at 365 nm.

3. RESULTS

Organoleptic characters

The inferences are illustrated in Figure 1and tabulated in Table 3.

Figure1: Prepared drug

Table No 3: Organoleptic characters

S.no	Specification	Character
1.	State	Solid
2.	Nature	Fine
3.	Odour	Aromatic
4.	Touch	Soft
5.	Flow Property	Non Free flowing
6.	Appearance	Pale Brownish

Physicochemical parameters

Results tabulated in table 4,5 and illustrated in Figure 2.

Table No 4: Results of Physicochemical evaluation of *Raja Elathi Chooranam*

S.no	Parameter	Mean (n=3) SD
1	Loss on Drying at 105 °C (%)	2.427 ± 0.4839
2	Total Ash (%)	9.633 ± 0.7506
3	Acid insoluble Ash (%)	0.054 ± 0.01652
4	Water soluble Extractive (%)	30.9 ± 1.153
5	Alcohol Soluble Extractive (%)	16 ± 2.138
6	pH	6.5
7	Particle size	16.71 ± 7.25

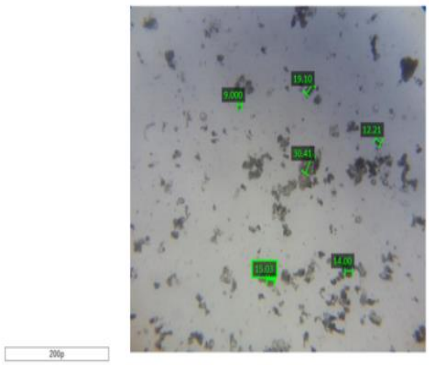

Figure 2: Microscopic Observation of Particle Size for the sample *REC*

Table No 5: Solubility Profile

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

Qualitative Phytochemical Analysis of *REC*

Results tabulated in Table 6 and illustrated in Figure 3.

Table No 6: Preliminary Phytochemical Analysis of *REC*

S.no	Name of the Test	Observation
1	Test for Alkaloids (Mayer's test)	Present
2	Test for Flavonoids	Present
3	Test for Steroids	Present
4	Test for Triterpenoids (Liebermann-Burchard test)	Present
5	Test for Phenols (Lead acetate test)	Present
6	Test for Tannin	Present
7	Test for Saponins	Present
8	Test for Sugar (Benedict's test)	Present

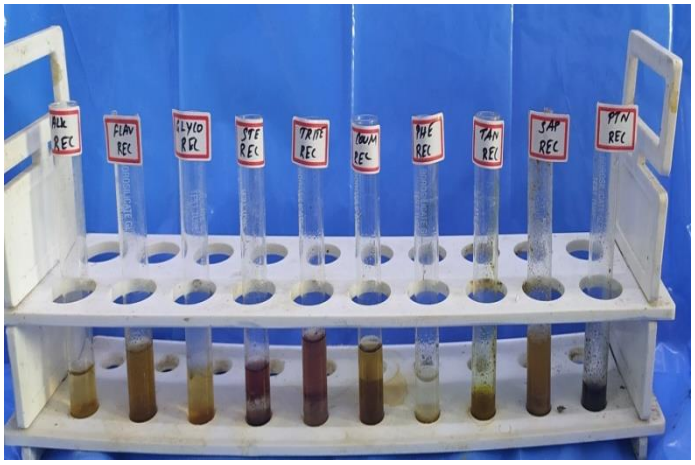

Figure 3: Qualitative Phytochemical Investigation

High Performance Thin Layer Chromatography Analysis (HPTLC) of *REC*

Results illustrated in Figure 4 and 5and tabulated in Table 7.

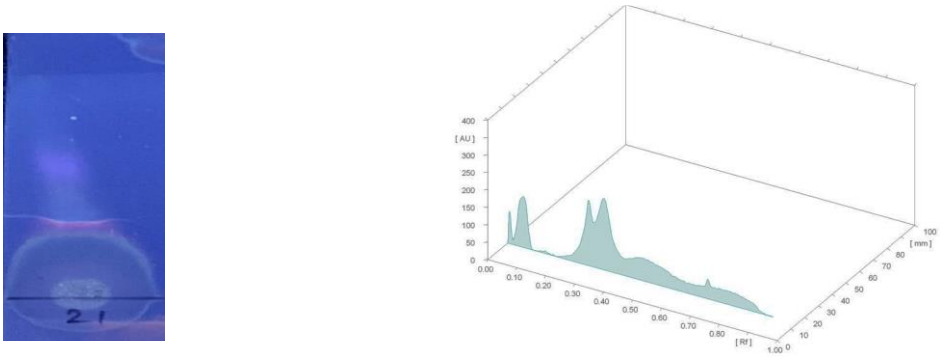

Figure 4: TLC Visualization of REC at 366 nm3D – Chromatogram

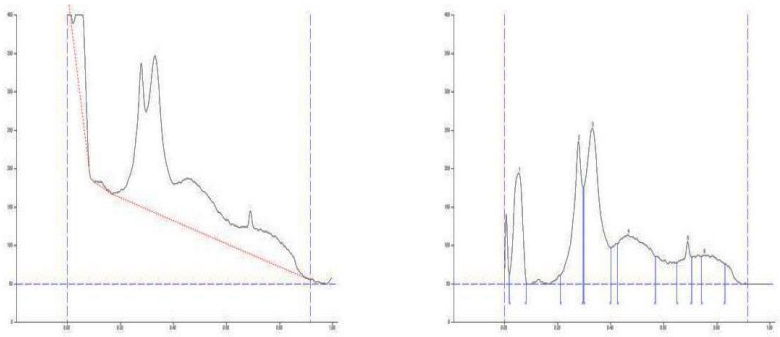

Figure 5: HPTLC finger printing of Sample *REC*

Table 7: Peak value

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.02	11.8	0.05	144.8	20.92	0.08	1.4	2716.3	14.13
2	0.21	11.5	0.28	186.3	26.91	0.30	126.6	3591.6	18.68
3	0.30	126.9	0.33	203.7	29.42	0.40	47.8	6359.7	33.07
4	0.43	53.2	0.47	64.1	9.26	0.57	36.4	3951.7	20.55
5	0.65	28.3	0.69	55.3	7.99	0.71	35.3	1067.6	5.55
6	0.74	36.6	0.75	38.1	5.50	0.83	26.6	1542.1	8.02

Biochemical analysis of *REC*

Results tabulated in Table 8 and 9.

Table No 8: Test for Acid Radicals

S.no	Specific Radical	Test Report
1	Test for carbonates	Positive
2	Test for chlorides	Positive
3	Test for sulphates	Positive
4	Test for phosphates	Positive

Table No 9: Test for Basic radicals

S.no	Specific Radical	Test Report
1	Test for Ferric	Positive
2	Test for Magnesium	Positive

Heavy Metal Analysis by Atomic Absorption Spectrometry (AAS)

Results tabulated in Table 10.

Table No 10: Heavy Metals Analysis report

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

BDL-Below Detection Limit

Microbial contamination test by pour plate method

Results illustrated in Figure 6 and tabulated in Table 11.

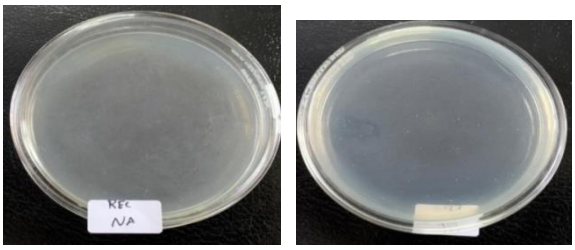

Figure 6: Microbial contamination test by pour plate method

Table No 11: Sterility 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	

Test for Specific Pathogen

No growth was observed in any of the plates inoculated with the test sample shown in Table 12 and illustrated in Figure 7, 8, 9 and 10.

Table No 12: Specific Pathogen Report

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

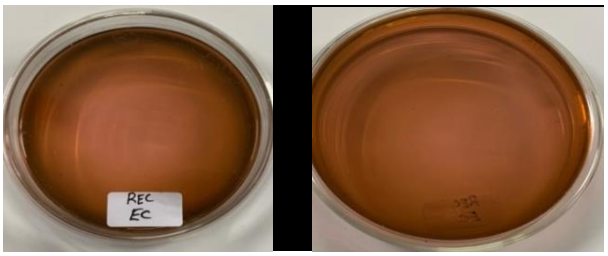

Figure 7: Culture plate with E-coli (EC) specific medium

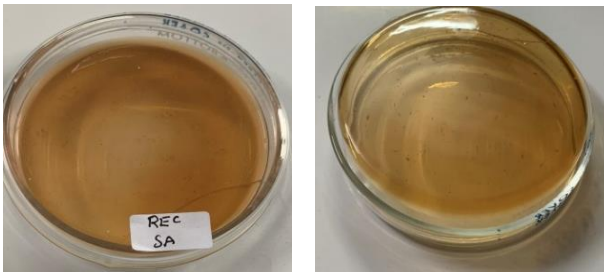

Figure 8: Culture plate with Salmonella (SA) specific medium

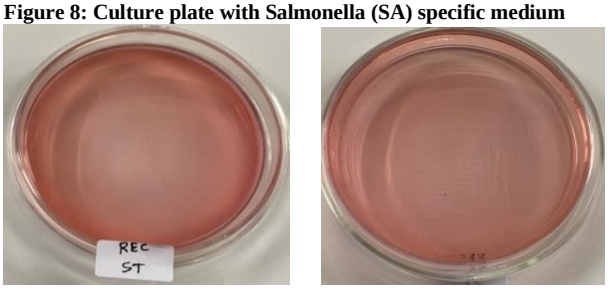

Figure 9: Culture plate with Staphylococcus Aureus (ST) specific medium

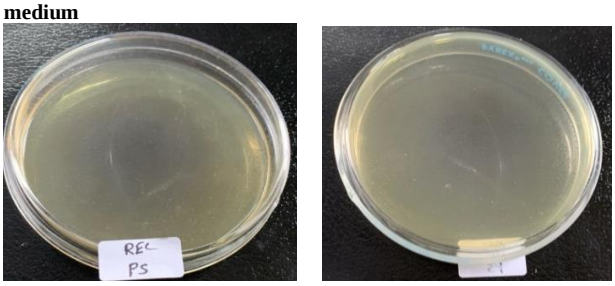

Figure 10: Culture plate with Pseudomonas Aeruginosa (PS) specific medium

Analysis of Pesticide residue Organochlorine, Organophosphorus, Organo carbamates, Pyrethroids

The results showed that there were no pesticide traces such as Organo chlorine, Organo phosphorus, Organo carbamates and Pyrethroids in the sample *REC* provided for analysis. Results tabulated in Table 13.

Table No 13: Pesticide residue report

Pesticide Residue	Sample REC	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
Endosulfan	BQL	3mg/kg
II. Organo Phosphorus Pesticides		
Malathion	BQL	1mg/kg
Chlorpyriphos	BQL	0.2 mg/kg
Dichlorovos	BQL	1mg/kg
III. Organo carbamates		
Carbofuran	BQL	0.1mg/kg
IV. Pyrethroid		
Cypermethrin	BQL	1mg/kg

BQL- Below Quantification Limit

Aflatoxin Assay by Thin Layer Chromatography (TLC) (B1, B2, G1, G2)

Results tabulated in Table 14.

Table No 14: Aflatoxin report

Aflatoxin	Sample REC	AYUSH Specification Limit
B1	Not Detected - Absent	0.5ppm
B2	Not Detected - Absent	0.1ppm
B3	Not Detected - Absent	0.5ppm
B4	Not Detected - Absent	0.1ppm

4. DISCUSSION

Standardization of the drugs is more essential to derive the efficacy, potency of the drug. The standardization of *Raja Elathi Chooranam* (*REC*) was achieved through various procedures like analysing the organoleptic characters, physicochemical characters and elements present in the drug. The physical parameters like state, nature, odour, taste, touch, flow Property and appearance revealed that it was solid, fine in nature with aromatic odor, sweet with pungent taste, soft to touch, non-free flowing and pale brownish in color. The fineness of the C*hooranam* represents easy absorption and better availability of the drug. So the drug *REC* is good in nature and safe to consume.

The results derived from the physicochemical evaluation divulge that Loss on drying value of *REC* was 2.427% which indicates low moisture content could increase the stability and shelf life of the drug which is suitable for medicine preparation. The Total Ash value of *REC* was 9.33% which indicates the purity of the drug. Acid insoluble ash value of *REC* was 0.054% which ensures the trial drug is not contaminated with siliceous material like sand, dust. The water soluble extractive value of *REC* was 30.9% which represents easy facilitation of diffusion and osmosis mechanism. The Alcohol soluble extractive value of *REC* was 16% which indicates that the test drug has good quality, purity and no adulteration. The pH value of *REC* was 6.5% which indicates that the drug is acidic in nature. In oral administration the acidic nature of the drug enhances rapid absorption in the stomach. Particle size analysis reveals the average particle size of the sample as 16.71 ± 7.25 µm further the sample has particle with the size range of lowest 9 µm to highest 30 µm. So the drug *REC* is easily absorbed and suitable for oral administration. *REC* is well soluble in major solvents like Water, Ethanol and DMSO. Thereby it proves its efficiency of solubility and increases the bio- availability in the stomach indirectly.

The result of the qualitative phytochemical analysis indicates that the formulation *REC* reveals the presence of alkaloids, flavonoids, steroids, triterpenoids, phenols, tannins, saponins and sugar.Alkaloids are used for bleeding disorders and eye diseases, and antiseptics, sedatives, stomatics, anti-inflammatory and analgesics.[20] Flavonoids strengthen the capillaries, connective tissue and function as antihistaminic, anti-inflammatory, anti-allergic effects, antioxidant, antithrombotic

and vasoprotective properties.[21]It can suppress endometrial prostaglandins and control heavy menstrual bleeding.[22]

Steroids are relevant to their powerful anti-inflammatory and immune-modulator properties. The anti-inflammatory properties of steroids have been ascribed to their inhibitory effects on the action of phospholipase A2, an enzyme critical to the production of inflammatory compounds.[23] Triterpenoids are studied for their antioxidant and anti-inflammatory and regulates apoptosis that attributes its therapeutic effects in numerous diseases. It is used for hyperglycaemia, liver fibrosis, wound healing, cerebral ischemia, dementia, metabolic syndrome and obesity.[24] Phenolic compounds are used as antioxidant, anti-inflammatory, antimicro-bial, antiviral, antitumor, antipyretic, and analgesic.[25] Caustic effect of phenol induces vascular thrombosis on endothelium.[26] Tannins are glucosidal organic compounds, which gives acidic reaction and are astringent. They are effective in medicine due to astringent nature. It increases blood clotting, decreases blood pressure and serum lipid level. [27] It helps in healing of wounds and inflammation of mucous membrane and inhibition of carcinogenesis.[28] Saponins cause bulging and rupture of erythrocytes causing release of haemoglobin. This has been one of the most investigated properties of saponins. Saponins to act as immunological adjuvants by enhancing the immune response to antigens have been recognized since 1940s. [29] The sugars are the carbohydrate which gives instant energy to the body is present in the test drug *REC.* A synergistic effect of all these compounds helps to increases the potency of the test drug *REC.*

The results of HPTLC finger printing analysis of the sample *REC* reveals the presence of 6 prominent peaks corresponds to presence of six versatile phyto-components present with in it. Rf value of the peaks ranges from 0.02 to 0.74 with percentage area of 5.55% to 33.07%. This method was used well to develop a chemical fingerprint for authentication and good confirmation of the presence of bioactive compounds in the sample *REC.* Hence, the result supports the ethno medical uses the drug *REC* to treat Menorrhagia.

The result of the Biochemical analysis of the test for Acid radicals reveals the presence of Carbonates, Chlorides, Sulphates, and Phosphates in the test drug *REC*. Carbonate is a salt of carbonic acid, along with calcium it is used as an antacid to relieve heart burn, acid indigestion and stomach upset. Chloride is the most important electrolyte in the blood and it is necessary for maintaining our body's homeostasis. It assists with fluid balance, delivery of oxygen to cells and acid-base balance. [30] Haemostatic action of ferric is due to the agglutination of blood proteins which occurs due to reaction of blood with ferric and Sulphate ions in acidic pH.[31] Approximately 60% of the Phosphates are absorbed in the intestine. Haemostatic mechanisms that regulate circulating phosphates and intestinal absorption depends on the diet.[32] The test for Basic radicals reveals the presence of Ferric and Magnesium in the test drug of *REC.* Magnesium is found in serum, red blood cells and also essential for immune system.[33] Presence of Iron in the drug has increased haemoglobin concentration in the blood.

Results of the Heavy metal analysis have clearly shown that the sample *REC* has no heavy metals traces such as Lead, Arsenic, Mercury and Cadmium. These results indicate that the trial drug is extremely safe as it contains heavy metals below detection limits. This reveals the safety of the drug. The result of the Sterility test shows no growth was seen in any of the plates inoculates with the test sample *REC.* The revealed that the drug *REC* is free from the viable microorganisms and the absence of total bacterial and fungal colonies which indicates that the drug *REC* have good quality and safer drug.

The result of the Specific pathogen was observed that there was no growth in any of the plates inoculated with the test sample *REC* which confirms that there are no viable aerobic microorganisms present in the sample. The results showed that there were no pesticide traces such as Organo chlorine, Organo phosphorus, Organo carbamates and Pyrethroids in the *REC* for analysis. This result suggests that *REC* have good quality.

The results shown no spots 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 G2. So the drug *REC* is non-toxic and there is no contamination and does not possess carcinogenic property. As a result, *Raja Elathi Chooranam* was proved for its safety over the defined standardization method.

5. CONCLUSION

Standardization of *Raja Elathi Chooranam* was done as per PLIM guidelines and standardized procedure. The obtained results of standardization of *Siddha* herbal formulation *REC* by different parameters such as organoleptic characters, physicochemical parameters, preliminary phytochemical analysis, TLC visualization of drug at 366nm, HPTLC finger printing analysis, Heavy metals and Biochemical analysis will be useful as tool for authentication and analysis their safety and quality of herbal drug. These standardization parameters could be considered as a reference standard of this drug for quality control assessment in future.

Acknowledgement: Authors are wish to thank the Principal and Faculties of post graduate department of *Gunapadam*, Government Siddha Medical College, Chennai, Tamil Nadu and India for their support, guidance and advice for this research work and also my sincere thanks to The Tamil Nadu Dr MGR University and Noble research solutions, Chennai.

Conflict of Interest: The authors declare that they have no conflict of interest.

Source of Funding: None

Ethical Approval: Approved

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How to cite this article: J. Manimekalai, P. Aswini, K. Nalina Saraswathi et.al. Standardization and physicochemical evaluation of the drug *raja elathi chooranam* – a siddha polyherbal formulation. *Int J Health Sci Res.* 2022; 12(5):213-227.
DOI: *https://doi.org/10.52403/ijhsr.20220523*

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