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Physicochemical Evaluation and Standardization of *Siddha* Formulation *Ratthi Naagara Rasa Mezhugu* by Modern Techniques

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ABSTRACT

Siddha medicine has own idiosyncratic way to treat various diseases. The *Siddha* system has different formulation in herbal, mineral, poly-herbal, herbo-mineral. *Ratthi Naagara Rasa Mezhugu* is a herbo-mineral formulation mentioned in the Siddha literature which indicated for Rheumatoid arthritis, penile cancer, vaginal cancer. The present study indicates the quality of the drug Ratthinaagara rasa mezhugu identified by physicochemical analysis, phytochemical analysis and organoleptic characters and other analytical techniques was based on the PLIM guidelines. High-performance thin-layer chromatography finger printing was done through CAMAG software. Physicochemical analysis of this drug was done such as Loss on drying at 105ºC was 15.2 ± 0.7211%, Total Ash value was 0.47±0.1323%, Acid insoluble Ash valve was 0.036±0.015%, Water soluble Extractive value was 4.9±0.5292%, Alcohol soluble extractive value was 11.37±1.097% and PH value was 4. The phytochemical analysis results in the presence of alkaloid, steroid, phenols. The drug RRM was free from microbial contamination; the heavy metal analysis and pesticide residues below quantification limit. The results indicate the drug is of standard quality and can be used as reference standard in laying pharmacopoeia standard.

***Keywords*:** *Siddh*a, Physicochemical analysis, Standardization, *RatthiNaagara Rasa Mezhugu*, PLIM guidelines.

1. INTRODUCTION

Siddha system of medicine was formed by *Siddhar's*. The *Siddh*a medicine is split into two groups, internal medicine and external medicine are available in *Siddha* literature. One such form of *mezhugu* is internal medicine, prepared by way of grinding well the ingredients with certain juices or extracts till a soft waxy consistency is attained.[1] The present of study deals with standardization of Siddha herbo mineral formulation of *Ratthinaagara Rasa Mezhugu (RRM)* drug is treating Rheumatoid arthritis, penile cancer, vaginal cancer. Still now there is no proper documentary evidence available on standardization and investigation aspect of this formulation. This prompted through the systematic standardization of *Ratthinaagara Rasa Mezhugu* by physicochemical analysis, phytochemical analysis and HPTLC finger print, organoleptic characters and other analytical techniques was based on the PLIM guidelines.

2. MATERIALS AND METHODS

Selection of the test drugs [2]

The test drug "*Ratthinaagara Rasa Mezhugu*" is one of the Herbo mineral formulation for Rheumatoid arthritis which indicated in the *Siddha* literature "*Anubooga Vaithiya Navaneedam*" written by Abdulla sahib, part 5 Page No148,149. Table-1

Ingredients of the drug Ratthinaagara Rasa Mezhugu:

Table-1 Ingredients of *Ratthinaagara rasa mezhugu*

Name of the drugs	Botanical/chemical name	Quantity
Purified Rasam	Mercury	35 grams
Purified Ganthagam	Sulphur	35 grams
Purified serankottai	Semecarpus anacardium	30 nos
Citramanakkennai	Castor oil	8.75 grams
Pasunei	Ghee	35 grams
Panaivellam	Palm jaggery	70 grams

Identification and Authentication of Drugs:

All raw materials were identified and authenticated by Botanist and *Gunapadam* experts, Government *Siddha* Medical College, Arumbakkam, Chennai.A specimen sample of each raw material Mercury, Sulphur, Semecarpus anacardium, Castor oil, Ghee, Palm jaggeryhas been labeled as 1021-1026/PGG/321912107/ GSMC-GH/2019-2022 respectively and were kept in the PG *Gunapadam* department for future reference.

Purification of raw drugs

Take 35 grams of mercury triturated with brick powder and turmeric powder for 60 minutes respectively, and washed with water. Then the mercury was boiled with 1 litre of juice of *kuppaimeni (Acalypha indica)* until the juice was evaporated completely and taken.[3] Sulphur was placed in an iron spoon. A small quantity of melted cow's butter was taken and this mixture was immersed in inclined position in cow's milk. This procedure is repeated for 7 times to get purified form of sulphur. Each time, use fresh milk.[4] The tip of Nuts of *Serankottai* was carefully cut by avoiding contact with the exudation of oil and the pieces were soaked in the solution of cow dung for three days. Then it was boiled for three hours, cleaned with water and dried.[5]

Preparation of *Ratthi Naagara Rasa Mezhugu*

First castor oil was added in a vessel and heated. The purified sulphur was powdered and mixed with the heated castor oil. After sulphur was melted, semecarpus seeds are cut into pieces and put into the oil. After the seed turned red, floating over the oil, it was known as *Ratthinaagara thylam*. Mercury and *Ratthinaagara Rasa thylam* was ground well, Until *Rasam*is fully perished. To this Palm jaggery and ghee was added and grounded to attain *mezhugu* form. These are stored in air tight container and labelled as *RRM*

Organoleptic characters

State, Nature, odour, touch, flow property, appearance of the drug was noted.
These following studies were done at Noble Research Solution, Perambur at Chennai.

2.1 Physicochemical Evaluation [6-7]

Solubility Test:

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

Loss on Drying:

An accurately weighed 2gm of RRM formulation was taken in a tarred evaporating dish. The crude drug was heated 105ºc for 5 hours in an oven till a constant weight and then weighed. The percentage moisture content of the sample was calculated with reference to the shade dried material.

Determination of Total Ash:

RRM was accurately weighed 2g in silica dish and incinerated in furnace at a temperature 400 ºC until it turns white in colour which indicates absence of carbon. Percentage of total ash will be calculated with reference to the weight of air-dried drug.

Determination of Acid Insoluble Ash:

The ash obtained by Total ash test was boiled with 25ml of hydrochloric acid for 6 minutes and filtered using ash less filter paper. Then the Insoluble matter retained on filter paper was washed with hot water and filter paper was burnt to a constant weight in a muffle furnace. The percentage of acid insoluble ash was calculated with reference to the weight of air-dried ash.

Determination of Water Soluble Extractive:

5gms of RRM was macerated with 100ml of chloroform water in a closed flask for twenty-four hours, shaken frequently for six hours and it was allowed to stand for eighteen hours. The solution was filtered rapidly with taking precautions to prevent loss of solvent. 25 ml of the filtrate was evaporated to dryness in a tarred flat bottom shallow dish, further dried at 105ºC to constant weight and weighed. The percentage of water soluble extractive was calculated with reference to the air dried drugs.

Determination of Alcohol Soluble Extractive:

RRM was macerated with 100 ml of Alcohol in a closed flask for twenty-four hours, shaken frequently for six hours and it was allowed to stand for eighteen hours. The solution was filtered rapidly with taking precautions to prevent loss of solvent. 25 ml of the filtrate was evaporated to dryness in a tarred flat bottom shallow dish, further dried at 105ºC to constant weight and weighed. The percentage of alcohol soluble extractive was calculated with reference to the air dried drugs.

pH value:

About 5 gram of RRM will be dissolved in 25 ml of distilled water and filtered the resultant solution is allowed to stand for 30 minutes and then subjected to PH evaluation.

2.2 Phytochemical Evaluation[8]

Test for alkaloids:

Mayer's Test:

To the test sample, 2ml 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 is indicated by the formation of yellow color.

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 color showed the presence of tannins.

Test for glycosides

Borntrager's Test

Test drug is hydrolysed with concentrated hydrochloric acid for 2 hours on a water bath, filtered and the hydrolysate is subjected to the following tests. To 2 ml of filtered hydrolysate, 3 ml of chloroform is added and shaken, chloroform layer is separated and 10% ammonia solution is added to it. Pink colour indicates presence of glycosides.

Test for flavonoids:

To the test sample about 5 ml of dilute ammonia solution were been added followed by addition of few drops of conc. Sulfuric acid. Appearance of yellow color 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, 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.

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

A.Aanthocyanin:

To the test sample, 1 ml of 2N sodium hydroxide was added and heated for 5 min at 100^{o}C. Formation of bluish green colour indicates the presence of anthocyanin.

B.Betacyanin

To the test sample, 2mi of HCl was added and heated for 5mins at 100^{o}C.Formation of pink colour indicates the presence of betacyanin.

Test for Carbohydrates

Benedict's test

To the test sample about 0.5 ml of Benedict's reagent is added. The mixture is 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.

2.3 Biochemical Analysis of Acid and Basic Radicals [9]

Table-2. Analytical Investigation On Test For Acid Radicals

S.No	Test For Specific Acid Radical	Indication/Observation
1.	**Test for Carbonates** To 1 ml of the test solution about 1 ml of concentration (conc.) HCL was added.	Formation of brisk effervescence indicates the presence of carbonates
2.	**Test For Chlorides** To 2 ml of test solution, about 1 ml of silver nitrate solution was added.	Appearance of White precipitate indicates the presence of chlorides.
3.	**Test For Sulfates** To 1 ml of the test sample add diluted H2SO4 till effervescence ceases followed by this about 1 ml of barium chloride solution was added.	Appearance of white precipitate indicates the presence of sulfates.
4.	**Test For Sulfides** To 1 ml of the test sample about 2 ml of HCL was added with slight warming the mixture	Formation of colorless gas with the smell of rotten egg indicates the presence of sulfides.
5.	**Test For Phosphates** To 2 ml of test solution treated with 2 ml of ammonium molybdate solution followed by addition of 2ml of concentrated nitric acid	Formation of yellow precipitate Indicates the presence of phosphates
6.	**Test For Fluoride And Oxalate** To 2 ml of the test solution about 2 ml of dil acetic acid and 2ml of calcium chloride solution was added	Formation of white precipitate Indicates the presence of Fluoride/ Oxalate
7.	**Test For Borates** 2ml of the test solution was added with sulphuric acid and 95% alcohol followed by exposure to flame	Appearance of green flame Indicates the presence of Borates
8.	**Test For Nitrates** 0.5 ml of test solution heated with copper turning followed by addition of sulphuric acid	Appearance of reddish brown gas Indicates the presence of Nitrates

Table-3. Analytical Investigation on Test For Basic Radicals

S. No	Test For Specific Basic Radical	Indication/Observation
1.	**Test For Lead** 1 ml of the test solution added with 2 ml of potassium chromate solution	Formation of yellow precipitate indicates the presence of lead
2.	**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 indicates the presence of Arsenic
3.	**Test For Mercury** 1 ml of the test solution added with 2 ml of 10% (2N) sodium hydroxide (NaOH) solution	Formation of yellow precipitate indicates the presence of mercury.
4.	**Test For Copper** 1 ml of the test solution added with 1 ml of Ammonium hydroxide (NH4OH) solution	Formation of blue precipitate indicates the presence of copper.
5.	**Test For Ferric** To 1 ml of test solution, about 2 ml of potassium ferrocyanide was added	Formation of blue precipitate indicates the presence of ferric
6.	**Test For Ferrous** To 1 ml of test solution, about 1 ml of potassium ferric cyanide solution was added.	Formation of blue precipitate indicates the presence of ferrous.
7.	**Test For Zinc** 1 ml of the test solution added with 2 ml of sodium hydroxide (NaOH) drop wise until indication appears.	Formation of white precipitate indicates the presence of Zinc
8.	**Test for Silver** 1 ml of the test solution was added with 1 ml of conc. HCL followed by appearance of curdy white precipitate. Boil the precipitate with water. It does not dissolve. Add NH4OH solution in it and add 1 ml dilute HNO3	Formation of curdy white precipitate indicates the presence of silver.
9.	**Test For Magnesium** 1 ml of the test solution added with 2 ml of sodium hydroxide (NaOH) drop wise until indication appears.	Formation of white precipitate indicates the presence of Magnesium

2.3 Thin layer chromatography-TLC Analysis [10]

Test sample was subjected to thin layer chromatography (TLC) as per conventional one dimensional ascending method using silica gel 60E254, 7X6 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 liter 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 254mm and light long-wave UV light 365 nm.

2.4 High Performance Thin Layer Chromatography Analysis [11]

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 analyzed. 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.

2.5 Heavy Metal Analysis ByAutomic Absorption Spectrometry (AAS)[12]

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 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 HCl

Cd & Pb- 100 ppm sample in 1mol/L HNO3

2.6 Sterility Test by Pour Plate Method[13]

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 37o C for 24-48 hours and further extended for 72 hrs for fungal growth observation. Grown colonies of organism was then counted and calculated for CFU.

Observation

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

2.7 Test for Specific Pathogen[14]

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 37oC for 24 - 72h for observation. Presence of specific pathogen identified by their characteristic colour with respect to pattern of colony formation in each differential media.Table-4

Table-4 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

Observation

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

2.8 Analysis Of Pesticide Residue Pesticides Organochlorine, Organophosphorus, Organo Carbamates, Pyrethroids.[15-16]

Methodology

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 40^{o}c 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 Will Be Dissolved Using Toluene And Filtered Through Membrane Filter.

2.9 Aflatoxin Assay By Thin Layer Chromatography (TLC) (B1,B2,G1,G2) [17]

Standard

Aflatoxin B1, Aflatoxin B2, AflatoxinG1, AflatoxinG2

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. RESULT AND DISCUSSION

3.1 Organoleptic Characters

The drug *Ratthi Naagara Rasa Mezhugu* a dark blackish in colour with characteristic odour, no greasy, semi solid, and no free flowing(figure1). The organoleptic characters of RRM are tabulated in Table No: 5

Table-5 Organoleptic characters

S. No	Specification	character
1.	State	Semi solid
2.	odour	characteristic
3.	Taste	Sweet
4.	Touch	Non greasy
5.	Flow property	Non free flowing
6.	Appearance	Dark blackish

3.2 Physicochemical Parameters

The results were tabulated in table-6.

Table-6 Result of Physicochemical Evaluation Of RRM

S. No	parameter	Mean (n=3)SD
1.	Loss on drying at 105ºC %	15.2 ± 0.7211%
2.	Total Ash	0.47±0.1323%
3.	Acid insoluble Ash	0.036±0.015%,
4.	Water soluble Extractive	4.9±0.5292%,
5.	Alcohol soluble Extractive	11.37±1.097%
6.	pH	4

Solubility Profile

The resultwere tabulated in Table-7.

Table-7 solubility profile

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

3.3 Qualitative Phytochemical Analysis of RRM

The results were tabulated in Table-8.

Table-8: Preliminary Phytochemical Analysis of RRM

S. No	Name of the Test	observation
1.	Test for Alkaloids (Mayer's test)	present
2.	Test for Steroids	present
3.	Test for Phenols (Lead acetate test)	present
4.	Test for Tannin	present
5.	Test for Proteins (Biuret test)	present
6.	Test for Saponins	present
7.	Test for sugar (Benedict's test)	present
8.	Test for Betacyanin	present

Figure 1: prepared drug

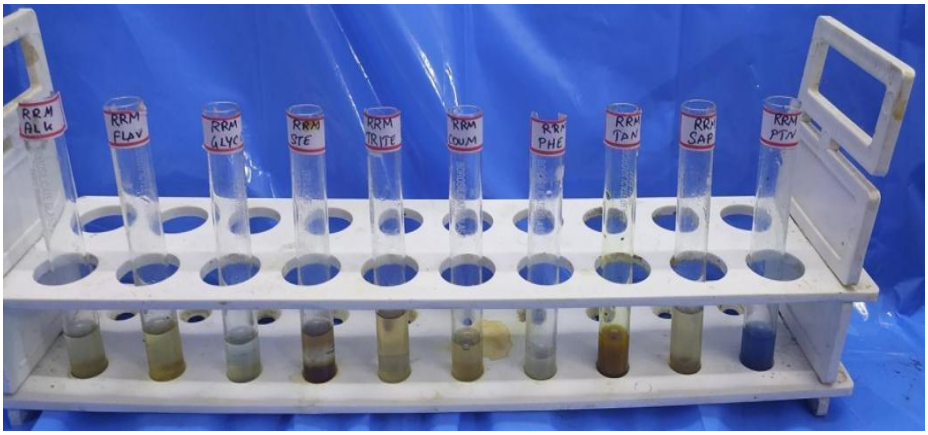

Figure No: 2 Qualitative Phytochemical Investigation

3.4 Biochemical Analysis Of RRM

The result was tabulated IN Table-9

Table-9: Test for Acid Radicals

Specific Radical	Test Report
Test for chloride	Positive – Indicates present
Test for nitrates	Positive – Indicates present

3.5 HPTLC Analysis of RRM

HPTLC finger printing analysis of the sample reveals the presence of six prominent peaks corresponds to presence of six versatile phytocomponents present with in it. Rf value of the peaks ranges from 0.03 to 0.74.

TLC Visualization of REC at 3363D-Chromatogram

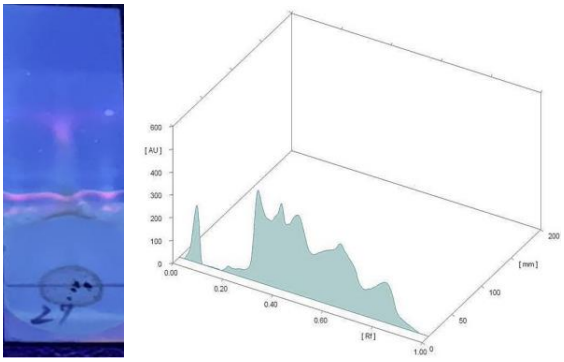

Figure No: 3 HPTLC finger printing of sample RRM

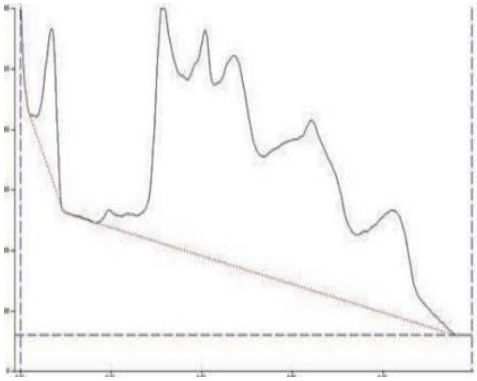

Figure No: 4

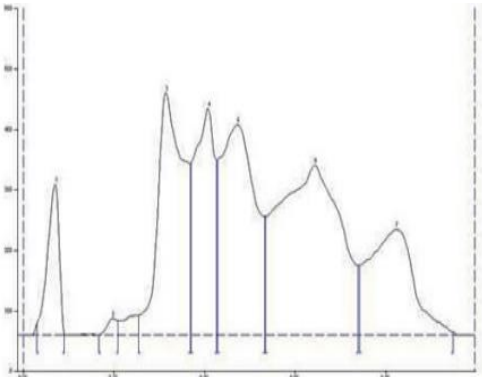

Figure No: 5

Table-10: Peak Table

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.03	19.0	0.07	250.1	13.45	0.09	1.9	2966.5	5.02
2	0.17	0.2	0.20	27.9	1.50	0.21	23.7	293.9	0.50
3	0.26	33.1	0.32	400.7	21.56	0.37	283.9	10474.5	17.72
4	0.37	285.6	0.41	375.4	20.20	0.43	289.5	7464.1	12.63
5	0.43	290.5	0.47	348.0	18.72	0.53	196.5	12071.9	20.42
6	0.54	196.6	0.65	281.6	15.15	0.74	115.3	17809.5	30.13
7	0.74	115.7	0.83	174.9	9.41	0.95	5.9	8029.6	13.58

3.6 Heavy metal Analysis by AAS

Result of the present investigation have clearly shown that the sample *RRM* has no traces of heavy metals such as Lead, Arsenic, cadmium and Mercury.

Table-11: Heavy Metals Analysis Report

Name of the Heavy Metal	Absorption Max	Result Analysis	Maximun 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

3.7 Microbial Contamination by Pour Plate Method

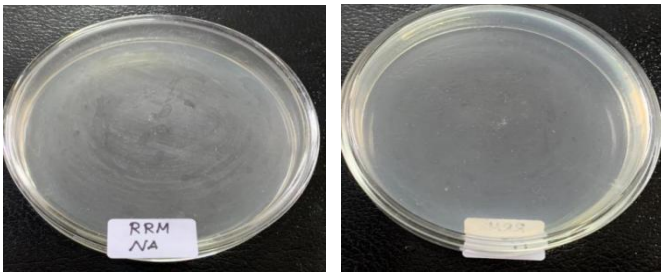

Figure No: 6

Observation

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

Result

No growth / colonies were observed in any of the plates inoculates with the test sample shown in the table No: 12

Table-12: Sterility Report

Test	Result	Specification	As per AYUSH/ WHO
Total Bacterial count Total Fungal count	Absent Absent	NMT 105CFU/G NMT 103CFU/G	As per AYUSH specification

3. 8Test for specific pathogen

Result

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

Table-13: specific pathogen Report

Organism	Specification	Result	Method
Coli Salmonella Staphylococcus Aureus Pseudomonas Aeruginosa	Absent Absent Absent Absent	Absent Absent Absent Absent	As per AYUSH specification

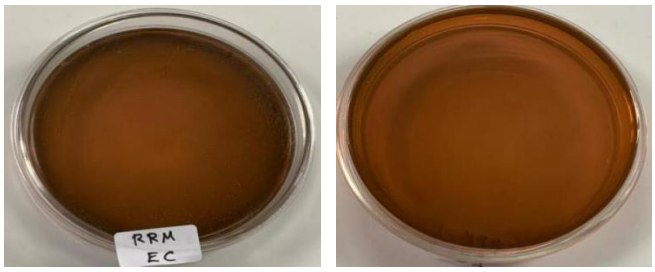

Figure No: 7 Culture Plate with E-Coli Specific Medium

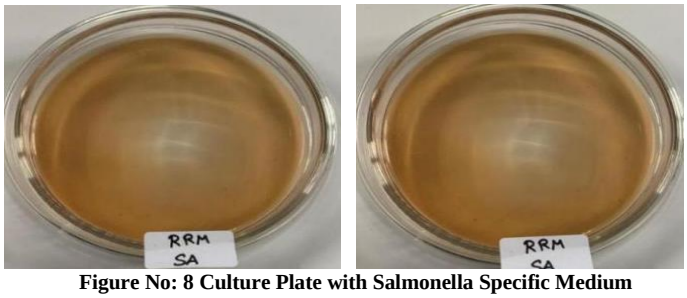

Figure No: 8 Culture Plate with Salmonella Specific Medium

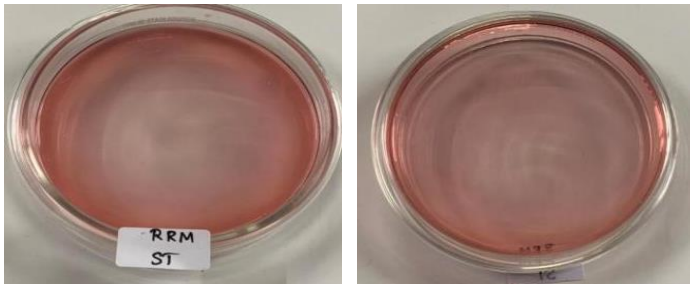

Figure No: 9 Culture Plate with Staphylococcus Aureus Specific Medium

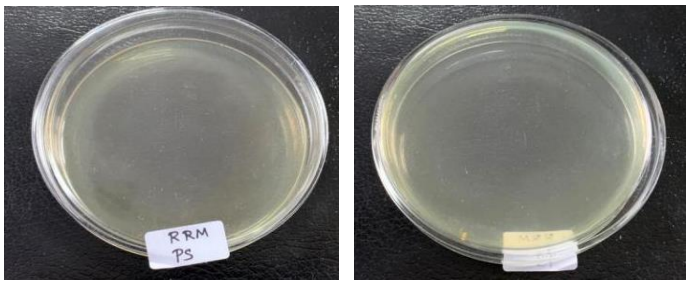

Figure No: 10 Culture Plate with Pseudomonas Aeruginosa Specific Medium

3.9 Analysis Of Pesticide Residue Organochlorine, Organophosphorus, Organo Carbamates, Pyrethroids

The result showed that there were no traces of pesticides residues such as organo chlorine, organo phosphorus, organo carbamates and pyrethroids in the sample *RRM* provided for analysis.

Table-14: Pesticide Residue Report

Pesticide Residue	**Sample**	**AYUSH Limit (mg/kg)**
Organo Chlorine Pesticides		
Alpha BHC	BQL	0.1mg/kg
Beta	BQL	0.1mg/kg
Gamma	BQL	0.1mg/kg
Delta	BQL	0.1mg/kg
DDT	BQL	1mg/kg
Endosulfan	BQL	3mg/kg
II.organo phosphorus pesticides		
Malathion	BQL	1mg/kg
Chlorpyriphos	BQL	0.2mg/kg
Dichlorovos	BQL	1mg/kg
III. Organo carbamates		
Carbofuran	BQL	0.1mg/kg
IV. pyrethroid		
Cypermethrin	BQL	1mg/kg

BQL - Below Quantification Limit

3.10 Aflatoxin Assay by AAC (B1,B2,G1,G2)

The result shown that were no spots were being identified in the test sample loaded on TLC plates when compare to the standard which indicates that the sample were free from Aflatoxin B1, B2,G1,G2.

Table No 15: Aflatoxin Report

Aflatoxin	**Sample RRM**	**Ayush specification Limit**
B1	Not detected -Absent	0.5ppm
B2	Not detected -Absent	0.1ppm
G1	Not detected -Absent	0.5ppm
G2	Not detected -Absent	0.1ppm

DISCUSSION

Organoleptic property of the *Ratthi Naagara Rasa Mezhugu* justifies the genuineness of the raw drugs and finished formulation with respect to its Darkblackish, characteristic odour, sweet taste, semi solid, non free flowing and non greasy by identity. Loss on drying value of *RRM* is 15.2%. This indicates the stability and shelf life of the drugs. The total ash value of *RRM* is 0.47%. This indicates the

amount of minerals and earthly present in the drug and purity of the drugs. The Acid insoluble ash value of the *RRM* is 0.036% which determines the superior quality and it gives the information about the siliceous matter. The pH of *RRM* is 4 and it is acidic. In oral administration the acidic nature of the drug enhances rapid absorption in the stomach. So *RRM* is suitable for oral administration. Solubility is the basic requirement for the absorption of the drug from Gastro intestinal tract. Thecommon causes of low oral bioavailability are attributed to poor solubility and low permeability. RRM is soluble in major solvents like Water and DMSO, thereby it proves its efficiency of solubility increasing in bio-availability in the stomach indirectly.[18] Plants alkaloid are one of the largest group of nature product, represent a highly diverse group of chemical entities. Alkaloid containing anti-inflammatory, analgesic and immuno regulative activities.[19] Plants steroid are unique class of chemical compound that are found through the plant kingdom. It contains anti-inflammatory activity with lesser side effect.[20] Dietary phenolic compounds in RA because of their action exhibit to modulate pro-oxidant and pro-inflammatory pathways reducing the onset of arthritic disease progression. These natural phenolic compounds can tune both the action and the production of inflammatory mediators either directly or indirectly by tuning the action of other molecules involved in RA pathology.[21] Tannin are water soluble phenol derivatives naturally synthesized and accumulated by higher plants as secondary metabolism. It contains anti-microbial, anti-oxidant, anti-inflammatory properties.[22] The protein GBP5 appears to play a key role in suppressing inflammation in rheumatoid arthritis.[23] Saponins contains anti-inflammatory, antiallergic, immuno-modulating activities.[24] Chloride help to maintain the water balance and pH balance they activate of salivary amylase. Chloride provides the acid medium for the activation of the gastric enzymes and digestion in the stomach.[25] Nitrates can help keep vessel dilated so that oxygen, vital components, and nutrients And drug that prevent or treat blood disorder can help keep it flowing through.[26] HPTLC finger printing 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 0.03 to 0.74. Further the peak 6 occupies the major percentage of area of 30.13 which denotes the abundant existence of such compound. Result of the present investigation have clearly show that the RRM has no traces of heavy metals such as Arsenic and cadmium. This reveals the safety of the drug. Further the result shows the present of mercury and lead can be below the permissible limit. Hence the safety of the *RRM* is ensured. It was observed that there were no growth / colonies in any of the plates inoculated with the *RRM* which confirms that the product is free from presence of viable micro-organisms. It was observed that there were no growth / colonies in any of the plates inoculated with the test sample *RRM* which confirms that there are no viable aerobic microorganisms present in the sample. The results showed there were no traces of pesticides residues such as Organo chlorine, Organo phosphorus and Pyrethroids in the *RRM*. Analysis of Pesticide residue is a parameter for quality control of drug. This result suggests that RRM have good quality. The results shown that there were no spots were been observed in the *Ratthi Naagara Rasa Mezhugu* loaded TLC plates when compare to the standard, which gives that the sample was free from Aflatoxin B1, Aflatoxin B2, Aflatoxin G1, Aflatoxin G2.

CONCLUSION

Standardization of *Ratthinaagara rasa mezhugu (RRM)* has been carried out according PLIM guidelines and standardized procedure. The obtained result of standardization of *Siddha* herbal mineral formulation RRM by different parameters such organoleptic characters,

physicochemical parameters, phyto-chemical analysis, Biochemical analysis, TLC visualization of drug at 366nm, HPTLC finger printing analysis and Heavy metal will be useful a tool for authentication and analysis their safety and quality of herbal drug. These standardization parameters could be considered reference standard of this drug for quality assessment in future.

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