

Physicochemical characterization, Phytochemical analysis and Anti-microbial activity of *Elathy mathirai*

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Abstract

Elathy Mathirai is a Siddha herbomineral formulation indicated to cure *sori*(itching), *sirangu*(Scabies), *suram*(fever), *vaayu*, *maandham*(gastro intestinal disorder) . The aim of the study is to standardize *Elathy Mathirai* to ensure its quality, purity and safety through its physicochemical, chromatographic, phytochemical, heavy metal, pesticide residue and aflatoxins analysis and the secondary objective is to evaluate its antimicrobial property. This study determines the physicochemical parameters that are loss on drying, total ash, acid insoluble ash, water soluble extractive, alcohol soluble extractive and pH as 5.667% , 5%, 0.014 % , 14.93 % , 8.3% and 5.6 respectively denotes purity and quality of the drug. From the study results it is ensured that the test drug *Elathy mathirai* is free from pesticide toxicity and aflatoxins. Qualitative preliminary phytochemical analysis shows the presence of alkaloids, Flavanoids,steroids, triterpenoids, saponins, coumarins, phenol, tannin, sugar and betacyanin. HPTLC finger printing analysis reveals the presence of seven prominent peaks Rf value ranges from 0.04 to 0.39 corresponds to the presence of seven components present within it. It was observed from the results of In vitro anti microbial assay that the formulation EM reveals significant level of activity against bacteria *Klebsiella pneumonia* and observed moderate activity against *Staphylococcal aureus*, *Pseudomonas aeruginosa* and possess consistent anti fungal activity against *Candida albicans* and there is no significant effect on *Aspergillus niger*. The obtained results shows that the EM complies with the regulatory standard and also possess significant anti microbial property against the tested microbes.

Keywords: Standardisation; Herbomineral; Siddha Medicine; Anti microbial

1. Introduction

In Siddha medicine, numerous drugs are mentioned in various literatures that have therapeutic value in nature, but because of a lack of standardization, they are not widely accepted. Standardization is most important to ensure the quality, safety, efficacy and to bring Siddha Medicine into the current medical system on a broad basis and to facilitate natural drug discovery. In Siddha literature Siddha Medicine *Elathy Mathirai* indicated to cure *Sori*(Itching), *sirangu*(Scabies), *suram*(Fever), *Maandham*(gastrointestinal disorder). This study aims to standardise the Herbomineral drug *ELATHY MATHIRAI* as a first step towards scientific approval and also to evaluate its antimicrobial effects against some skin causing micro organisms.

2. Materials and Methods

The test drug *Elathy mathirai* is a herbomineral formulation mentioned in the literature of *Kannusamiyam parambarai vaithiyam* indicated for itching, scabies, fever, vaayu, maandham.

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As per the reference literature Elathy Mathirai is prepared with the ingredients of

- Elam (*Elettaria cardamomum*)
- Kirambu (*Syzygium aromaticum*)
- Nervaalam(*Croton tiglium*)
- Pooram(*Hydragyrum sub chloride*)[Calomel]

After the proper purification of the mentioned drugs, they were grounded and mixed together in mortar. By adding sufficient amount of water it has been grinded and rolled into approximately 45 mg tablets. After the tablets were dried, it was stored in an air tight container.

2.1. Collection of Raw drugs

All the drugs were bought from authenticated drug store in Palayamkottai, Tamil Nadu.

2.2. Authentication of Raw drug

The raw drugs were identified and authenticated by the experts in Pg Gunapadam Department, Government Siddha Medical College, Palayamkottai.

2.2.1. Organoleptic characters

Nature, Colour, Odour, Appearance were noted.

2.2.2. Physico-chemical Evaluation

Percentage of Loss on Drying

Test drug was accurately weighed in evaporating dish. The sample was dried at 105°C for 5 hours and then weighed.

$$\text{Percentage loss on drying} = \text{Loss of weight of sample} / \text{Weight of the sample} \times 100$$

2.2.3. Determination of Total ash

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

$$\text{Total Ash} = \text{Weight of Ash} / \text{Weight of the crude drug taken} \times 100$$

2.2.4. Determination of Acid Insoluble Ash

The ash obtained by total ash test will be boiled with 25 ml of dilute hydrochloric acid for 6mins. Then the insoluble matter is collected in crucible and will be washed with hot water and ignited to constant weight. Percentage of acid insoluble ash will be calculated with reference to the weight of air-dried ash.

$$\text{Acid insoluble ash} = \text{weight of ash} / \text{weight of the crude drug taken} \times 100$$

2.2.5. Determination of Alcohol Soluble Extractive

Test sample was macerated with 100 ml of Alcohol in a closed flask for twenty-four hours, shaking frequently during six hours and allowing it to stand for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish, and dry at 105°C, to constant weight and weigh. Calculate the percentage of alcohol-soluble extractive with reference to the air-dried drug.

$$\text{Alcohol soluble extract} = \text{Weight of extract} / \text{weight of the sample taken} \times 100$$

2.2.6. Determination of Water Soluble Extractive

Test sample was macerated with 100 ml of chloroform water in a closed flask for twenty-four hours, shaking frequently during six hours and allowing it to stand and for eighteen hours. Filter rapidly, taking precautions against loss of solvent,

evaporate 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish, and dry at 105°C, to constant weight and weigh. Calculate the percentage of water-soluble extractive with reference to the air-dried drug.

2.2.7. Determination of pH

Required quantity of test sample was admixed with distilled water and the subjected to screening using pH meter.

About 5gm of test sample was dissolved in 25ml of distilled water and filtered the resultant solution is allowed to stand for 30 minutes and then subjected to pH evaluation.

Solubility Test

In a dry test tube one gram of sample was taken and to it 2 ml of the solvent added and shaken well for about a minute and the results are observed. The test done in solvents Chloroform, Ethanol, Water, Ethylacetate, Hexane and Dimethyl sulfoxide(DSMO).The results are observed individually.

Uniformity of weight

20 tablets/Native formulations previously selected at random were weighed. The average weight was determined. Tablets were weighed individually and the percentage of deviation of its weight from the average weight was determined for each tablet.The deviation if individual weight from the average weight should not exceed the prescribed pharmacopoeial limits.

2.3. High Performance Thin Layer Chromatography

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.

2.4. 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.

2.4.1. 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 R_f values weretabulated.

2.5. Phytochemical analysis

2.5.1. 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.

2.5.2. 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.

2.5.3. 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.

2.5.4. 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.

2.5.5. 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.

2.5.6. Test for flavonoids

Alkaline reagent test

Two to three drops of sodium hydroxide were added to 2 mL of extract. Initially, a deep yellow colour appeared but it gradually became colourless by adding few drops of dilute HCl, indicating that flavonoids were present.

2.5.7. 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.

2.5.8. 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.

2.5.9. 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.

Betacyanin

To the 5ml of aqueous extract of EM 2ml of HCl was added and heated for 5 mins at 100C. Formation of pink colour indicates the presence of Betacyanin.

2.5.10. 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.

2.5.11. 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.

2.6. Heavy metal analysis by atomic absorption spectrometry(AAS)

2.6.1. 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 preparation

As & Hg- 100 ppm sample in 1mol/L HCl

Cd &Pb- 100 ppm sample in 1mol/L HNO₃

2.6.2. Sterility test by pour plate method

Methodology

Test sample was inoculated in sterile petri dish to which about 15 mL of molten agar 45°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° 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.

Test for Specific Pathogen

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.

Pesticide residue

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°C until the solvent has almost completely evaporated. To the residue add a few milliliters of toluene and heat again until the acetone is completely removed. Resultant residue will be dissolved using toluene and filtered through membrane filter

Aflatoxin assay by Thin layer Chromatography

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

3. Results and discussion

3.1. Organoleptic characters

Organoleptic characters reveal that EM is hardsolid with smooth surface, brownish in colour, Mild characteristic in odour.

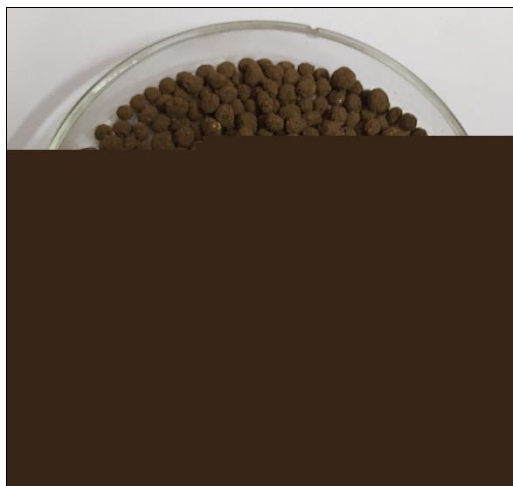


Figure 1 *Elathy Mathirai*

Table 1 Organoleptic characters

State	Solid
Nature	Hard Solid with smooth surface
Odour	Mild Characteristic
Touch	Rigid
Flow Property	Free flowing
Appearance	Brownish

3.2. Physico-chemical parameters

The analysis of physicochemical parameter determines that the percentage of loss on drying, total ash, acid insoluble ash, water soluble extractive, alcohol soluble extractive and pH as 5.667%, 5%,0.014%,14.93%, 8.3% and 5.6 respectively.The observed results of physicochemical analysis were tabulated

Table 2 Physicochemical characters

S.No	Parameter	Mean (n=3) SD
1.	<i>Loss on Drying at 105 °C (%)</i>	5.667 ± 0.73
2.	<i>Total Ash (%)</i>	5 ± 0.81
3.	<i>Acid insoluble Ash (%)</i>	0.014 ± 0.004
4.	<i>Water soluble Extractive (%)</i>	14.93 ± 1.81
5.	<i>Alcohol Soluble Extractive (%)</i>	8.3 ± 0.95
6.	<i>pH</i>	5.6

3.3. Uniformity of weight

Based on the evaluation, the uniformity of weight was determined by using EM in order to make sure that the dosage units are consistent. From the result obtained, it was observed that the tablets used has an average weight of 0.0378gm which is under the category of 'Less than 80 mg' as per pharmacopeia limit.

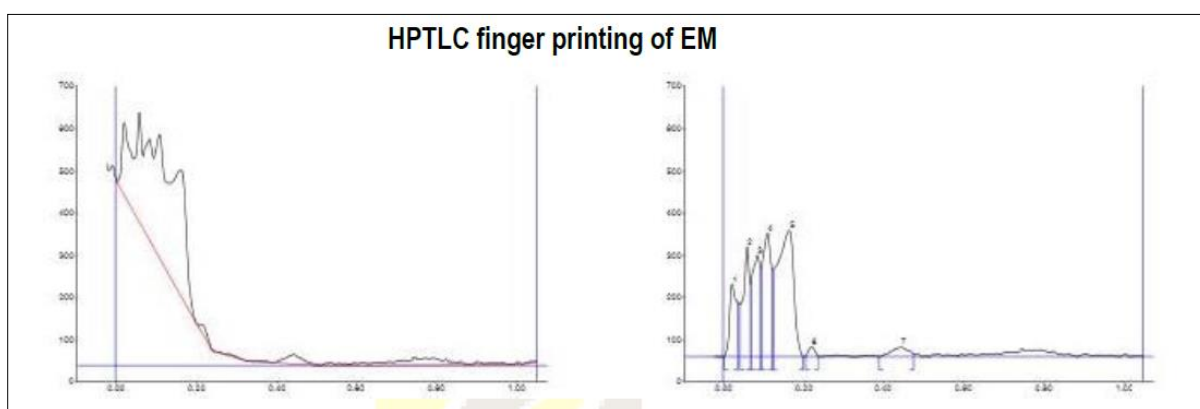
Table 3 Percentage Deviation on uniformity of Weight of the sample EM

Average Weight of Tablet/urundai	Number of Tablets/urundai	Deviation (%)	Pharmacopoeial Category	Limit
0.0378gm	10	0 - 3 %	Less than 80 mg	± 10% for Minimum 18 Tabs ± 20% for Maximum 02 Tabs
	0	Above 3 %		
	10	Negative deviation value -0.15 to -2.86		

Based on the trial it was observed that the start of disintegration time of the tablets was found to be 36 ± 8.78 mins. Further tablet took average of 131.5 ± 22.4 mins for completion of 100% disintegration.

3.3.1. HPTLC

TLC Visualization of EM at 366 nm 3D – Chromatogram

**Figure 2** HPTLC finger printing of EM**Table 4** HPTLC peak value

Peak	Start Rf	Start height	Max Rf	Max height	Max%	End Rf	End height	Area	Area%
1	0.00	1.2	0.02	171.8	13.12	0.04	130.2	1801.2	9.50
2	0.04	124.0	0.06	259.9	19.85	0.07	171.3	2526.5	13.33
3	0.07	178.9	0.08	240.0	18.33	0.09	210.3	2811.3	14.83
4	0.09	212.8	0.11	292.5	22.34	0.12	105.5	3742.0	19.74
5	0.12	205.8	0.16	298.4	22.79	0.20	1.1	7277.2	38.38
6	0.20	1.2	0.22	23.7	1.81	0.23	1.4	221.3	1.17
7	0.39	0.0	0.45	23.2	1.77	0.48	7.5	580.1	3.06

HPTLC fingerprinting analysis of the sample reveals the presence of seven prominent peaks corresponds to the presence of nine components present within it. Rf value of the peaks ranges from 0.04 to 0.39.

3.4. Phytochemical analysis

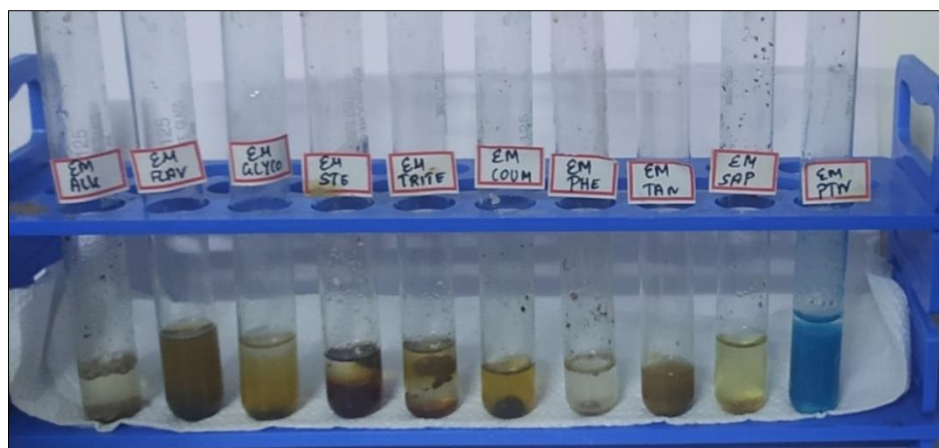


Figure 3 Phytochemical Analysis

Table 4 Phytochemical Analysis

S.No	Result	Observation
1.	Alkaloids	+
2.	Flavonoids	+
3.	Glycosides	-
4.	Steroids	+
5.	Triterpenoids	+
6.	Coumarin	+
7.	Phenol	+
8.	Tannin	+
9.	Proteins	-
10.	Saponins	+
11.	Sugar	+
12.	Anthocyanin	-
13.	Betacyanin	+

3.5. Heavy metal analysis by AAS

Table 5 Heavy metal analysis table

Name of the heavy metal	Absorption Max	Result analysis	Maximum limit
Lead	217.0 nm	0.717	10 ppm
Arsenic	193.7 nm	BDL	3 ppm
Cadmium	228.8 nm	BDL	0.3 ppm
Mercury	253.7 nm	0.179	1 ppm

Result shows the sample has no traces of Arsenic and cadmium whereas the lead and mercury at 0.717 and 0.179 ppm as list in the table.

3.6. Sterility test by pour plate method

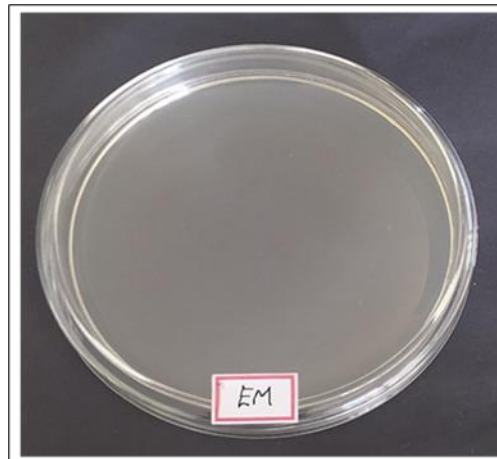


Figure 4 Sterility test plate

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

Table 6 Sterility test result

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

3.7. Test for specific pathogens

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

Table 7 Specific pathogens

Organism	Specification	Result	Method
<i>E-coli</i>	Absent	Absent	As per AYUSH specification
<i>Salmonella</i>	Absent	Absent	
<i>Staphylococcus Aureus</i>	Absent	Absent	
<i>Pseudomonas Aeruginosa</i>	Absent	Absent	

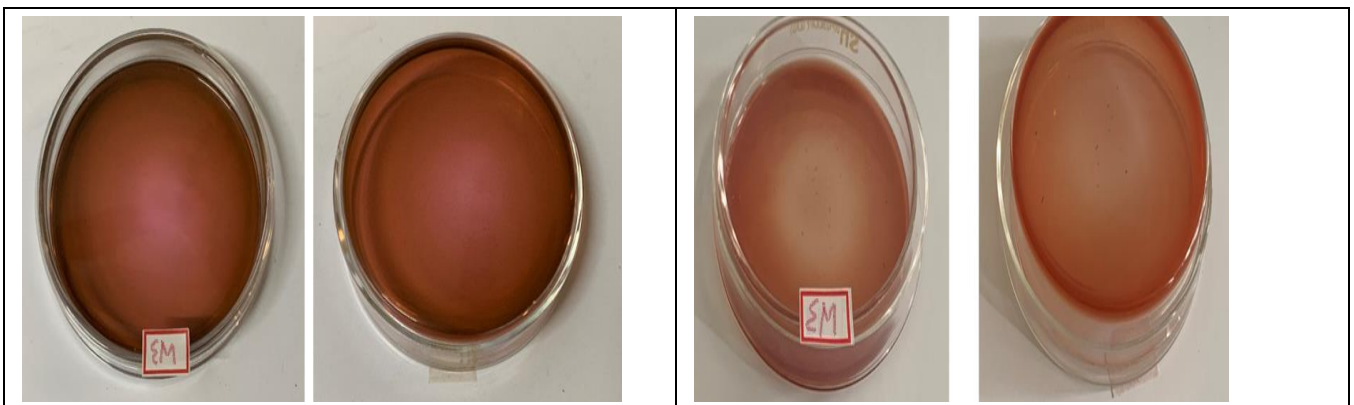


Figure 5 Culture plate with *E-coli* (EC) specific medium

Figure 6 Culture plate *Salmonella* specific Medium

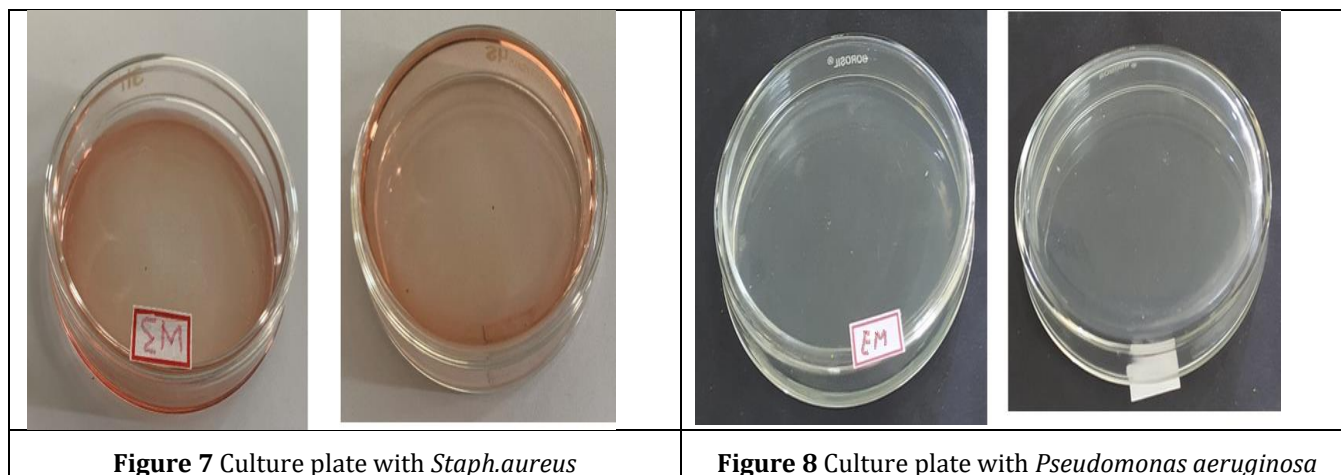


Figure 7 Culture plate with *Staph.aureus*

Figure 8 Culture plate with *Pseudomonas aeruginosa*

3.8. Pesticide residue

The result shows that there were no traces of pesticides residues such as Organochlorine, Organophosphorous, Organocarbamates and pyrethroids in the sample provided for analysis.

3.9. Aflatoxin assay by TLC

Table 8 Aflatoxin result

Aflatoxin	Sample EM	AYUSH Specification limit
B1	Not detected - absent	0.5 ppm (0.5 mg /kg)
B2	Not detected - absent	0.1 ppm (0.1 mg/kg)
G1	Not detected - absent	0.5 ppm (0.5 mg /kg)
G2	Not detected -absent	0.1 ppm (0.1 mg /kg)

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

3.10. Antimicrobial activity

3.10.1. Determination of Minimum inhibitory concentration (MIC) of the siddha formulation ElathyMathirai (EM) using Resazurin Microtitre Assay

Procedure

Test was carried out in a 96 well Plates under aseptic conditions. A sterile 96 well plate was labelled. Volume of sample in DMSO comprises of 1000 µg was pipetted into the first well of the plate and transferred to subsequent wells by half of its weight until 8th Well. To all other wells 50 µl of nutrient broth was added and serially diluted it. To each well 10 µl of resazurin indicator solution was added. 10 µl of bacterial/ fungal suspension was added to each well.Each plate was wrapped loosely with cling film to ensure that bacteria did not become dehydrated. The plate was incubated at 37 °C for 24-48 h. The colour change was then assessed visually. Any colour changes from purple to pink or colourless were recorded as positive. The lowest concentration at which colour change occurred was taken as the MIC value.

Table 9 Anti-Bacterial Activity- EM Growth of inhibition Chart for the Sample and Standard Drug

S.No	Sample/ Microorganisms	Growth of inhibition										DMSO	Culture
		W-1 1000 µg	W-2 500 µg	W-3 250 µg	W-4 125 µg	W-5 62.5 µg	W-6 31.2 µg	W-7 15.6 µg	W-8 7.8 µg	STD Chloramphenicol (10µg)			
1	<i>Staphylococcus aureus</i> Gram +ve	-	-	+	+	+	+	+	+	-	+	+	
2	<i>Pseudomonas aeruginosa</i> Gram -ve	-	-	+	+	+	+	+	+	-	+	+	
3	<i>Klebsiella pneumonia-KP</i> Gram-ve	-	-	-	+	+	+	+	+	-	+	+	

Table 10 Anti-Fungal ActivityGrowth of inhibition Chart for the Sample and Standard Drug

S. No	Sample/ Microorganisms	Growth of inhibition										DMSO	Culture
		W-1 1000 µg	W-2 500 µg	W-3 250 µg	W-4 125 µg	W-5 62.5 µg	W-6 31.2 µg	W-7 15.6 µg	W-8 7.8 µg	STD Fluconazole (20µg)			
1.	<i>Candida albicans</i>	-	-	-	+	+	+	+	+	-	+	+	
2.	<i>Aspergillus niger</i>	+	+	+	+	+	+	+	+	-	+	+	

(+) No anti-microbial activity for the sample in that particular well; (-) Good anti-microbial activity for the sample in that particular well

3.11. Observation

It was observed from the results of the present investigation that the sample reveals convincing anti-microbial activity among all tested organisms except *Aspergillus niger*. It was observed that the sample reveals moderate activity against the pathogen *Staphylococcus aureus* (MIC 500µg) and *Pseudomonas aeruginosa*(MIC 500µg)and significant level of activity against *Klebsiella pneumonia*(MIC 250µg). Similarly the sample demonstrate consistent anti-fungal activity against *Candida albicans* with the MIC value of 250 µg. There is no significant activity against *Aspergillus niger*.

4. Discussion

The drug EM was a hard solid brownish in colour with mild characteristic odour. Organoleptic properties colour, appearance, odour indicates the genuinity, purity and quality of the test drug EM. The loss on drying value indicates the moisture content of this medicine evaluated as 5.667 + 0.73. The total ash value of the test drug EM was 5 ± 0.81%. The Acid insoluble ash value was 0.014 ± 0.004% which indicates that the drug contains only negligible amount of siliceous matter. Ash value is one of the most significant measurable factors for the quality control of herbal drug. A high ash value represents presence of more inorganic residues such as phosphates, carbonates and silicates. The water soluble extractive value and alcohol soluble extractive value were determined as 14.93 ± 1.81 % and 8.3± 0.95 % respectively. Extractive values are useful in the determination of amount of the phytoconstituents present in herbal drug and helpful in estimating the chemical proportions soluble in a particular solvent. The pH value is calculated as 5.6 which indicates that the drug is weekly acidic. Solubility property impacts bioavailability and pharmacological efficacy of a drug. EM is soluble in water, ethanol, dimethyl sulfoxide and insoluble in chloroform and ethyl acetate. Drugs that poorly soluble in water often need enhanced doses to attain therapeutic plasma concentration after intake. Qualitative Preliminary Phytochemicals test result reveals the presence of alkaloids, flavonoids, steroids, triterpenoids, coumarin, phenol, tannin, saponin, sugar and betacyanin in the test drug EM. Phytochemicals are chemical compounds present in plants and act as pharmacologically active substance which useful in treating human diseases as medicinal ingredients and

nutrients. These phytochemicals are responsible for potential therapeutic properties of EM. HPTLC finger printing analysis of the sample EM reveals the presence of seven prominent peaks corresponds to the presence of nine components present with Rf value of peak ranges from 0.04 to 0.39. Heavy metal analysis shows that the test drug EM has no traces of Arsenic, Cadmium whereas the lead and mercury at 0.717ppm and 0.179 ppm which is under the limit. The term heavy metal represents to a metallic chemical elements that has a relatively high density and poisonous even at minute concentration. Some metals like mercury, lead, arsenic and cadmium cause poisonous and carcinogenic effects when administrated even at low concentration. No growth/ colonies were observed in any of the plates inoculated with the test sample EM indicates that the test drug is sterile and free from bacterial and fungal contamination. EM has pesticide in below quantity limit which means there is no contamination of pesticides. Pesticides are chemical compounds used to control or eradicate pest. According to their chemical structure, they are classified into Organo Chlorine, Organo Phosphate, Organocarbamates and Pyrethroids. When these pesticides are used on herbal plants during agricultural practices will remain in plants that become a significant source of contamination of herbal medicines. The results shown that there were no spots were being identified in the test sample loaded on TLC plates when compare to the standard which indicates that the sample were not contaminated from Aflatoxin B1, Aflatoxin B2, Aflatoxin G1, Aflatoxin G2 .Aflatoxins are toxic fungal secondary metabolites. As aflatoxins are highly toxic, carcinogenic, mutagenic, teratogenic and hepatotoxic, it is important to determine the aflatoxins in herbal medicines. It was observed from the results of In vitro anti microbial assay that the formulation EM reveals significant level of activity against bacteria *Klebsiella pneumonia* and observed moderate activity against *Staphylococcal aureus* and *Pseudomonas aeruginosa* and possess consistent anti fungal activity against *Candida albicans* and there is no significant effect on *Aspergillus niger*.

5. Conclusion

Based on the data gathered from the current study findings, The Siddha herbomineral formulation EM complies with the regulatory standard and also possess significant anti-microbial property against the tested microbes which could be due to the presence of significant biologically active phytocomponents and it will work therapeutically in treating diseases. In future these data can be used as references for the standardization of the drug EM. This medicine shows inhibitory activity against *Candida albicans*. So it may treat *Candidiasis*. It also shows the inhibition against *Klebsiella pneumonia*. So it may treat the UTI infections caused by this organism. Thus In vivo and clinical studies have to be done in future to prove its efficacy.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest.

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