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An Insight into the traditional formulation Mathiiushna Rasayanam as per the Siddha text Yuuki vaithiya kaaviyum in the prevention of Infectious diseases

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Abstract

Aim: The aim of the study is to examine the sample drug Mathiiushna Rasayanam through Microbial Limit Testing(Microbial Contamination Test, Specific Pathogen Testand the screening of antimicrobial activity by agar well diffusion method).

Methodology: Mathiiushna Rasayanam was prepared as per Siddha Materia Medica procedures.Extract of test sample Mathiiushna Rasayanam was prepared. Microbial Limit Testing-Total bacterial counting , total fungal counting and the screening of antimicrobial activity by agar well diffusion method were done.

Result: Thus the present study shows that Mathiiushna Rasayanam is free from microbial contamination. Both Gram positive and Gram negative bacteria were found to be high sensitive to Mathiiushna Rasayanam when compared to the standard drug Gentamycin (Broad spectrum).

Conclusion: It is concluded that the test drug Mathiiushna Rasayanam can be prescribed as the medicine for infectious disease due to pathogenic micro organism namely, *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Pseudomonas aeuroginosa* and *Staphylococcus aureus* infections. This study also reveals that the Siddha literature evidence which was mentioned thousands years before the advent of modern science by Siddhars in Yuukivaithiyakaaviyum becomes true to this modern era.

Keywords: Siddha, Mathiiushna Rasayanam**,** Microbiological examination.

Introduction

The Siddha medicine is one of the oldest medical systems known to mankind. The word Siddha comes from the word Siddhi which means an object to be attained perfection or heavenly bliss. Siddha is focused to “Ashtamahasiddhi” that is the eight supernatural powers. Those who attained or achieved the “Ashtamahasiddhi” powers are known as Siddhars. Siddha medicine is claimed to revitalize and rejuvenate dysfunctional organs. The Siddha medicine given by practitioners includes leaves, flowers, fruit and various roots in a mixed basis.

Infectious disease, also known as transmissible disease or communicable disease is illness resulting from an pathogenic microbial infection. Dating back to Ancient Siddha literature, the Siddha formulations were found to be highly effective in prevention of the infectious diseases.

Objective:

The objective of the present study is to examine the test drug Mathiiushna Rasayanam through Microbial Limit Testing- Total bacterial counting, total fungal counting and the screening of antimicrobial activity by agar well diffusion method

Materials and Methods

The ingredients of the test drug Mathiiushna Rasayanam were identified, collected, purified as per the Siddha text book of Yuuki vaithiya kaaviyum and made into fine rasayanam form.

Table: Information about the traditional Siddha formulation - Mathiiushna Rasayanam

S.No	Vernacular name	Botanical name	Medicinal used part
1	Parangi pattai	*Smilax china*	Bark
2	Kadukkaai	*Terminalia chebula*	Seed outer coat
3	Nellikaai	*Phyllanthus emblica*	Seed
4	Thandrikaai	*Terminalia bellarica*	Seed outer coat
5	Ellam	*Elletaria cardamomum*	Seed
6	Chukku	*Zingiber officinale*	Rhizome
7	Melagu	*Piper nigrum*	Seed
8	Thippili	*Piper longum*	Unriped fruit
9	Cheivium	*Piper nigrum* (root)	Root
10	Lavangapattai	*Cinnamomum verum*	Bark
11	Seeragam	*Cumium cyminum*	Seed
12	Saathipatri	*Taxus buccata*	Leaves
13	Thippili moolam	*Piper longum* (root)	Root
14	Kanduparangi	*Clerodendrum divaricatum*	Leaves
15	Vaaivilangam	*Embelia ribes*	Seed
16	Kiraambu	*Syzygium aromaticum*	Flower bud
17	Vetpaalai	*Wrightia tinctoria*	Leaves
18	Arathai	*Alpinia officinarum*	Rhizome
19	Koraikilangu	*Cyperus rotundus*	Leaves
20	Nannari	*Hemidesmus indicus*	Leaves
20	Santhanam	*Santalum album*	Wood
21	Kostam	*Costus speciosus*	Leaves
22	Saatamanjil	*Nardostachys jatamansi*	Leaves
23	Takolam	*Illicium verum*	Flower
24	Omam	*Trachyspermum ammi*	Seed
25	Kaatu milagu	*Capsicum frutescens*	Seed
26	Mullai ver	*Jasminum sambac*	Root
27	Athimaduram	*Glycyrrhiza glabra*	Leaves

Evaluation of Total Aerobic Bacterial Count

Preparation of Sample for Experimental Work

Weighed 10 gm of the homogenized test drug sample Mathiiushna Rasayanam aseptically and dissolved in 10 ml of sterile water and made upto 100ml with the sterile water. The insoluble drug product was suspended in 100 ml of buffered sodium chloride-peptone solution (pH 7.0).

Serial dilution of Sample

A serial dilution is the dilution of a sample; in 10-fold dilutions. From the sample, 1 ml of the sample was added to 9 ml of sterile water and mixed it well. This dilution was denoted as 10^{-1} dilution. From this dilution, one ml was taken from that mixture is added to 9 ml and designated as 10-^{2} dilution. The same procedure was repeated upto 10^{-4}.

Isolation of Total Viable Aerobic Microbial Count

Isolation of Bacteria by Plate Count Method

In this test, the bacteria in sample was made to grow as colonies by inoculating a known volume of sample into a solidifiable nutrient medium (Casein Soybean Digest agar or Nutrient agar medium) in petridish. The agar plate was prepared by mixing growth medium with agar and then sterilized by autoclaving. Once the agar was cooled to 45° C, approximately 15 to 20 ml of medium was poured into a sterile petri dish under aseptic condition and left to solidify for 15 minutes. After solidification ,each plate was smear with 0.1 ml of sample from the dilution of 10-^{1} and 10^{-2}.After inoculations, all the plates were incubated at 37° C for 24 hours. After incubation , the bacterial colonies were developed as visible to the naked eye and the number of colonies on the plate was counted using Quebec Colony Counter .Plates with an average of from 30 to 300 colonies of the target bacterium were selected for colony count .Because of the statistical problems, plates with lower than 30 colonies greater than 300 colonies were rejected.

Composition of Nutrient Agar Media

Peptone	: 5.0 gm
Sodium chloride	: 5.0 gm
Beef extract	: 1.5 gm
Yeast extract	: 1.5 gm
Agar	: 15.0 gm
Distilled water	: 1000 ml
pH (at 25° C)	: 7.4

Isolation of Fungi

From each of the above prepared samples,0.1 ml of sample was transferred to Sabouraud Dextrose agar (SDA) prepared with Chloramphenicol. The plates were then incubated for 5 days at room temperature (20 to 25° C). After incubation, the fungal colonies were observed and calculated.

Composition of SDA

Dextrose	: 40 gm
Peptone	: 10 gm
Agar	: 15 gm
Distilled water	: 1000 ml

Evaluation of Antimicrobial Activity of test drug Mathiiushna Rasayanam

Antimicrobial activity was performed by agar well diffusion method on agar.

Preparation of test drug Mathiiushna Rasayanam extracts solutions for the experiment

The dried drugs were weighed and dissolved in sterile distilled water to prepare appropriate dilution to get required concentrations. They were kept under refrigerated condition unless they were used for the experiment.

Procedure for the Agar Well Diffusion Test

The antibacterial screening of the drugs were carried out by determining the zone of inhibition using agar well diffusion method. All the drug extracts were tested against four pathogenic bacterial strains of gram positive and gram negative organism by agar well diffusion method.

Bacterial Inoculums Preparation

Inoculums of *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris* and *Bacillus substilis* were prepared in nutrient broth medium and kept for incubation at 37° C for 8 hours.

Agar well-diffusion method

This method was followed to determine the antimicrobial activity. Muller-Hinton Agar media plates were swabbed (sterile cotton swabs) with 8 hour old-broth culture of respective bacteria. After inoculation, wells with the size of 10 mm diameter and about 2 cm a part were made in each of these plates using sterile cork borer. Stock solution of each drug extract was prepared at a concentration of 1 mg/ml in water. About 100 microlitre of different concentrations of drug solvent extracts were added into the wells and allowed to diffuse at room temperature for 2 hours. The plates were incubated at 37° C for 24 hours. After incubation, the diameter of the zone (mm) was measured.

Composition of Muller Hinton Agar Media

Beef Extract	:02.00 gm
Acid Hydrolysate of Casein	:17.50 gm
Starch	:01.50 gm
Agar	:17.00 gm

Evaluation of Specified Micro organisms

Isolation and identification of *Escherichia coli*

One ml of the prepared sample was added in a sterile screw-capped container containing 50 ml of nutrient broth and mixed well. Then, it was allowed to stand for 1 hour and mixed again. After one hour, the screw caps of the bottle was loosened and incubated at 37° C for 18 to 24 hours.

Primary Test

From the above prepared enrichment culture, 1.0 ml was taken and transferred aseptically into a tube containing 5 ml of Mac-Conkey broth. Inoculated tubes were incubated in a water –bath at 36° C for 48 hours.

Secondary Test

From the primary test, 1.0 ml of the enrichment culture was taken and transferred aseptically into 5 ml of peptone water .It was then incubated in a water –bath at 43.5° to 44.5° C for 24 hours and observed the tubes for acid and gas. Then, the culture was subjected to biochemical tests of IMViC and the results were observed and correlated.

Isolation and identification of Salmonella sp.

One ml of the prepared sample was added in a sterile screw-capped container containing 100 ml of nutrient broth and mixed well. Then, it was allowed to stand for 1 hour and mixed well again. After one hour, the screw caps of the bottle was loosened and incubated at 37° C for 18 to 24 hours.

Primary Test

From the above prepared enrichment culture,1.0 ml was taken and transferred aseptically into a tube containing 10 ml of Selenite F broth. Inoculated tubes were incubated in a water –bath at 36°to 38° C for 48 hours. After incubation the culture was sub cultured on two of the agar media namely Bismuth sulphate agar and Deoxy cholate citrate agar and incubated the plates at 36° to 38° C for 18 to 24 hours. After incubation, colonies were observed on the medium and confirmed the genus Salmonella based on guidelines.

Secondary Test

The suspected colonies of the primary test were subcultured on the slant of triple sugar-iron agar in test tube and in urea broth. Both media were incubated at 37° C for 24 hours. After incubation, the results were observed according to the development of colour change and acid or gas in media. The presence of Salmonella was confirmed by agglutination tests

Isolation and Identification of *Pseudomonas aeruginosa*

From the above prepared enrichment culture ,1.0 ml was taken and transferred aseptically into 100 ml of fluid soyabean-casein digest medium and mixed well. The inoculated tubes were incubated at 37° C for 24 hours. After incubation, the growth of bacteria was checked. From this, a loop full of culture was streaked on the surface of Cetrimide agar medium and Pseudomonas Isolation Agar medium and incubated at 37° C for 24 hours. After incubation, the colonies from the agar surface of these two media were checked for detection of fluorescein and pyocyanin.

Isolation and identification of *Staphylococcus aureus*

From the above prepared enrichment culture, a loopfull of culture was taken and transferred aseptically on Mannitol salt agar and incubated at 37° C for 24 hours. After incubation the colonies were subjected to confirmation by hemeagglutination test.

Results and Discussion

The Results of the microbiological analysis for microbial contamination of the test drug Mathiiushna Rasayanam was given in the Table 1. The total viable aerobic bacterial counts on Nutrient agar plate was 73 X 10^2 CFU/g and the fungal count on SDA agar plates was 1X10^2 CFU/g. This results were found to comply with the specification limit for total bacterial count and the total fungal count (Protocol for testing Ayurveda, Siddha and Unani medicines).

Microbial Limit Tests

Table 1: Results of Microbial Contamination Test

S.no	Test Particulars	Colony Counts (CFU/g)	Limits Value (CFU/g)
1	Total Viable Aerobic Bacterial Count	73X10^2	1X10^5
2	Total Viable Fungal Count	1X10^2	1X10^3

The analytical screening of sample Mathiiushna Rasayanam showed in Table 2 that the product is free from specific pathogens like *Escherichia coli*, *Salmonella*, *Pseudomonas aeuroginosa* and *Staphylococcus aureus*.

Table 2 : Results of Specific Pathogen Test

S.No	Test for Specific Pathogen	Colony Counts (CFU/g)	Limits Value (CFU/g)
1	*Salmonella* sp.	No growth	-
2	*Staphylococcus aureus*	No growth	-
3	*Escherichia coli*	No growth	-
4	*Pseudomonas aeruginosa*	No growth	-

Microbial contamination usually occurs because of improper drying or storage of the plant material which eventually results in degradation of the plant constituents. Microbial contamination can also render plants material toxic, either by transforming the chemicals in the plant material or through the production of toxic compounds by the microbes. Therefore, microbial quality tests should be applied to starting plant materials intermediate and finished products where necessary. During the quality analysis, precautions must be taken to ensure that the conditions do not adversely affect any micro organisms that are to be measured.

Thus the present study proves that Mathiiushna Rasayanam is free from microbial contamination and also highlighted the safety of the same.

The information obtained from microbial screening tests will be useful in finding out the quality of the drug.

The good antibacterial activity of polyhebal formulation Mathiiushna Rasayanam implies that the anti microbial compounds present in herbal medicines are possibly controlling the microbial activity .Herbal medicines showed varying degrees of *in vitro* anti bacterial activity against test bacteria.

Both Gram positive and Gram negative bacteria, *Escherichia coli, Klebsiella pneumonia, Salmonella typhi*, *Pseudomonas aeuroginosa* and *Staphylococcus aureus* were found to be high sensitive to herbal medicine when compared to the standard drug Gentamycin (Broad spectrum) (Table 3).

The polyhebal formulation Mathiiushna Rasayanam, exhibited broad spectrum activity against bacterial pathogens at 100 mg/ml concentration of the drug.

Table 3: Antimicrobial Activities of test drug Mathiiushna Rasayanam by Agar Well Diffusion Method

S.No	Test Pathogens	Result	Zone of inhibition(mm) at 30ul	
			Positive Control (Gentamycin)	Size of Inhibition
1	*Escherichia coli*	Sensitive	20 mm	18 mm
2	*Klebsiella pneumonia*	Sensitive	22 mm	17 mm
3	*Staphylococcus aureus*	Sensitive	19 mm	13 mm
4	*Pseudomonas aeuroginosa*	Sensitive	21 mm	11 mm
5	*Salmonella typhi*	Sensitive	22 mm	17 mm

Conclusion

From these results, it is accomplished that this study would lead to the establishment of several important compounds that have to be used to formulate new, different and more potent antimicrobial drugs of Siddha medicinal origin. However, further studies are required to screen the biologically active compounds and to evaluate the efficacy of this compounds against pathogenic micro organisms associated with various human diseases.

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