

ANTI-MICROBIAL ACTIVITY OF RATHI NAGARA RASA

MEZHUGU

Dr. Veerasivaraman M.^{1*}, Dr. Brunda S.², Dr. Anbarasan B.³ and Dr. Madhavan R.⁴

¹PG Scholar, Dept. of Nanju Maruthuvam, National Institute of Siddha, Chennai.

²PG Scholar, PG Dept. of Maruthuvam, Govt. Siddha Medical College, Chennai.

³Siddha Consultant, Dr Jay's Ayush Hospital, Chennai.

⁴Lecturer, Dept. of Nanju Maruthuvam, National Institute of Siddha, Chennai.

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***Corresponding Author**

Dr. Veerasivaraman M.

PG Scholar, Dept. of Nanju
Maruthuvam, National
Institute of Siddha, Chennai.

ABSTRACT

The Siddha system of medicine describes various treatments to cure the disease by the siddhars in the struggle to preserve human health for healthy human life. In the present study antimicrobial activity and microbial load of Siddha herbo mineral drug, Rathi nagara rasa mezhugu (RNM) were studied. Anti-microbial activity against gram positive and gram negative microorganisms was studied by using the cup plate method. The antimicrobial screening shows that the drug RNM exhibited bigger zone of inhibition of about 8-22 mm against candida albicans followed by 9-20 mm against pseudomonas

aeruginosa, 8-19mm against bacillus cereus, 7-10 mm against staphylococcus aureus, 7-9 mm against Escherichia coli at different concentrations in volume of 80 µl/well. Microbial load to evaluate the total bacterial count, total fungal count, *E.coli*, *Salmonella spp*, *Staphylococcus aureus*, *Enterobacter* was done by using pour plate method. The result of the study further proves the bacterial and fungal count are within permissible limits as per WHO 2007, and absence of pathogens such as *E.coli*, *Enterobacter*, *Salmonella Spp* and *Staphylococcus aureus*.

KEYWORDS: Siddha, Rathinagara rasa mezhugu, antimicrobial activity, microbial load.

INTRODUCTION

Infectious diseases remain key agents of the debilitating poverty afflicting so much of the world today. Each year these diseases kill almost nine million people, many of them children under five, and they also cause enormous burdens through life-long disability. Stepping up

research into their causes and how to effectively treat them and prevent them from spreading could have an enormous impact on efforts to lift people out of poverty and to build a better world for future generations.^[1]

In recent years Siddha system has been gained much importance as a result of its increased use in the successful treatment of infectious diseases. Throughout history, medicinal plant-based remedies have been used with varying degrees of success for the management of infectious diseases.

The scientist Paracelsus quoted that all substances are poisons; there is none, which is not a poison. The right dose differentiates a poison from a remedy. Likewise, the liability of inimical effects while administering the metals and minerals containing traditional medicines is to be explored for its safeness. In Siddha system of medicine, the raw materials like plant, mineral, and animal resources are acquired from the natural surroundings. They have been used extensively for many centuries after thorough evaluation of the drug by traditional way. Siddha system emphasizes the dose regimen and pertinent vehicle for every medicine intake. Metals have been used as therapeutics since time immemorial. Siddha medicine has vast range of mercurial usage in therapeutic application for treating acute and chronic diseases.^[2]

In the present study *Rathi nagara rasa mezhugu*, a classical Siddha formulation mentioned in Siddha Literature *Anubogavaidhaya* naveenedham,^[3] is indicated for Linga puttru (Penile cancer), Algul puttru (Cervix cancer), Araiyaappu (Adenitis), Kandamaalai (Cervical adenitis), Karunkuttam, Senkuttam (Leprosy), Megaranam (Syphilis), Kaalkai mudakku (Rheumatoid Arthritis).to evaluate the antimicrobial and microbial load study.

MATERIALS AND METHODS

Collection of the drugs

All the raw materials were obtained from Country drug shop, Ramaswamychetti, Parrys, Chennai.

Identification and Authentication

All the raw drugs were identified and authenticated by the experts of Gunapadam (Pharmacology) at National institute of Siddha, Tambaram sanatorium, Chennai. The specimen samples of the identified raw drugs were preserved in the laboratory of P. G Gunapadam for future references.

Ingredients of Rathi nagara rasa mezhugu

- Sitramanakkennai (Castor oil)
- Purified Gandhakam (Sulphur)
- Purified Serankottai (Semecarpus Anacardium)
- Purified Vaalairasam (Mercury)
- Pasunei (Ghee)
- Panaivellam (Palm jaggery)

Preparation**Step 1**

First castor oil is taken in a vessel and heated. Then Purified sulphur is powdered and mixed with the heating castor oil. When the sulphur melts, Semecarpus seeds are cut into two pieces and put in the oil. Then thailam is taken when semecarpus seeds turns red and floats. The thailam is called *Rathi nagara thailam*.

Step 2

Mercury and Rathi nagara thailam is mixed and grinded. When mercury is grinded well, ghee and palm jaggery is added and grinded to get *Rathi nagara rasa mezhugu*.

Therapeutic Uses

- Linga puttru (Penial cancer),
- Algul puttru (Cervix cancer),
- Araiyaappu (Adenitis),
- Kandamaalai (Cervical adenitis),
- Karunkuttam, Senkuttam (Leprosy),
- Megaranam (Syphilis),
- Kaalkai mudakku (Rheumatoid Arthritis).

Antimicrobial activity

The cup plate antibacterial susceptibility test method was followed. Muller Hinton agar plates were prepared and Swabbed with different isolates of log phase cultures of above organisms. The plates were allowed to stand for few minutes. Wells were made over the plates at an equidistant position. Wells were loaded with 50µl of the drug at the concentration of 250mg/ml Dimethylsulphoxide (DMSO) was used as the solvent. Control well was also included using the solvent. The ampicillin 10 mcg disc used as standard for comparison. All

the plates were kept at 37°C for 18-24 hrs. The zone of inhibition was measured using the verniercaliper.^[4,5,6]

Microbial load

Determination of Microbial load

Total bacterial count Strains for evaluation E.coli, Salmonella typhimurium, Staphylococcus aureus, Enterobacter. By using pour plate method.

Microbial count

Test drug was prepared at the concentration of 10- 100 µg/ ml and dissolved in 100 ml of peptone water. Using the standard loop which carried 0.01 ml of the extract solution was inoculated in nutrient agar, blood agar and MacConkey agar plates. The plates were incubated at 37° C overnight and the colonies were counted. The organisms were identified using biochemical tests such as Indole test, Triple sugar iron (TSI), Urease, Citrate, Mannitol Motility and phenyl pyruvic acid.

Total Bacterial Count

About 1ml of the test solution contains test drug at fixed concentration as diluted with minimum of 1/ 10th dilution ratio was poured on to each petri dish (Petri dishes 9-10 cm in diameter) aseptically, add to each dish 15- 20 ml of sterilized agar medium, previously melted and kept below 45°C, and mix. Growth of bacteria was detected using soybean- casein digest agar medium. After the agar solidifies, plates were been incubated at least for 2 days at 30-35°C. The number of colonies was counted and the values were expressed as CFU.

Total Fungal Count

About 1ml of the test solution contains test drug at fixed concentration as diluted with minimum of 1/ 10th dilution ratio was poured on to each petri dish (Petri dishes 9-10 cm in diameter) aseptically, add to each dish 15- 20 ml of sterilized agar medium, previously melted and kept below 45° C, and mix. Growth of fungi was detected using potato- dextrose agar, plates were been incubated at least for 2- 3 days at 20- C. The number of colonies was counted and the values were expressed as CFU.^[7]

RESULTS**Effect on RNM on Antimicrobial activity**

The test drug RNM exhibited significant zone of inhibition in four organisms out of eight selected for microbial screening in which the maximum inhibition zone diameter (IZD) of about 8-22 mm against candida albicans followed by this 9-20 mm of zone against pseudomonas aeruginosa, 8-19mm of zone against bacillus cereus, 7-10 mm against staphylococcus aureus, 7-9 mm of zone against Escherichia coli at different concentration in volume of 80 µl/well The ampicillin 10 mcg disc used as standard shown inhibition against *Candida albicans*, *Staphylococcus aureus*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Escherichia coli*. All IZD corresponding to test organisms are tabulated in Table 1 and represented in figure 1-6. No significant IZD was observed in the screening against the following organisms namely *Klebsiella pneumoniae*, *Proteus vulgaris*, *Salmonella typhimurium*.

Table 1: Anti-microbial activity of Rathi Nagara Rasa Mezhu.

MIC - Chloroform extract of the sample (80µl/ well)												
Zone of inhibition in mm												
S. No.	Organism	1	2	3	4	5	6	7	8	9	10	Std Amp (10mcg /disc)
1.	<i>Escherichia coli</i> NCIM 2931	9	8	7	-	-	-	-	-	-	-	+
2.	<i>Klebsiella pneumoniae</i> NCIM 2957	-	-	-	-	-	-	-	-	-	-	-
3.	<i>Proteus vulgaris</i> NCIM 2027	-	-	-	-	-	-	-	-	-	-	-
4.	<i>Pseudomonas aeruginosa</i> NCIM 2945	20	19	18	17	14	13	11	9	-	-	+
5.	<i>Bacillus cereus</i> NCIM 2458	19	17	14	13	11	8	-	-	-	-	+
6.	<i>Staphylococcus aureus</i> NCIM 5021	10	8	7	-	-	-	-	-	-	-	+
7.	<i>Salmonella typhimurium</i> NCIM 2501	-	-	-	-	-	-	-	-	-	-	-
8.	<i>Candida albicans</i> NCIM 3471	22	21	19	18	17	16	12	9	8	-	+

Drug concentration: 1. 125mg/ml; 2. 62.5mg/ml; 3. 31.25mg/ml; 4. 15.6mg/ml; 5. 7.8mg/ml; 6. 3.9mg/ml; 7. 1.95mg/ml; 8. 0.97mg/ml; 9. 0.48mg/ml; 10. 0.24mg/ml.

Antimicrobial activity

Chloroform extract of the sample

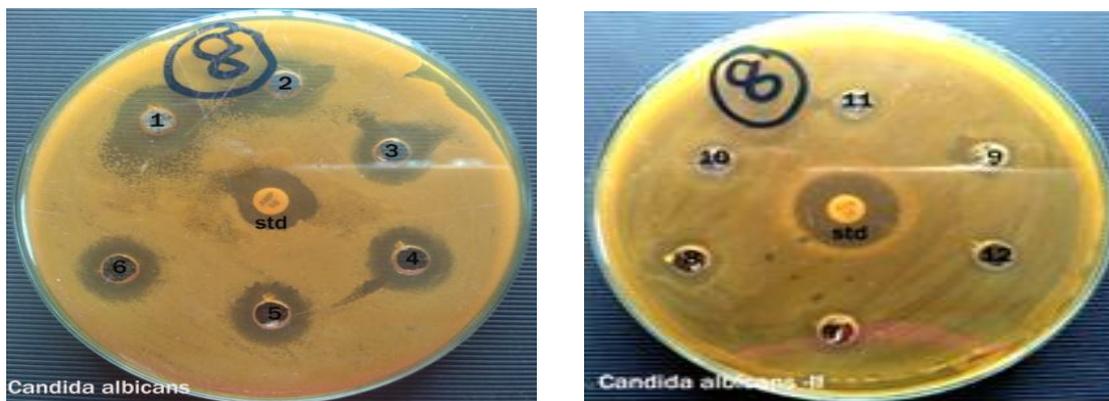


Fig. 1,2: Anti-microbial activity of RNM against *Candida albicans-I*, *Candida albicans-II*.

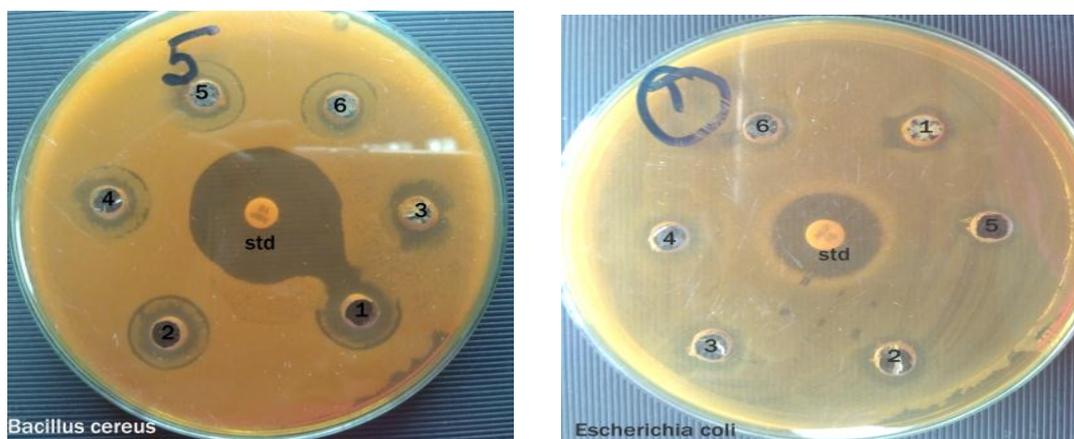


Fig. 3: Anti-microbial activity of RNM against *Bacillus cereus* and *Escherichia coli*.



Fig. 4: Anti-microbial activity of RNM against *Staphylococcus aureus*.



Fig. 5,6: Anti-microbial activity of RNM against *Pseudomonas aeruginosa-I*, *P. aeruginosa-II*.

Analysis of Microbial load of RNM

Microbial contamination signifies more dreadful diseases in humans and it aggravates the co-infection. The result of microbial analysis of study drug RATHI NAGARA RASA MEZHUGU reveals the absence of bacterial and fungal strains. The results of the study further proves the absence of pathogens such as *E.coli*, *Enterobacter*, *Salmonella typhimurium* and *Staphylococcus aureus*. The results were tabulated in Table 02.

Table 2: Microbial load of Rathi Nagara Rasa Mezhugu.

S. No.	Parameters	Reference Limits as per WHO (2007) ^[8]	Results	Remarks
1	Total Bacterial Count (TBC)	10 ⁵ CFU/gm	Less than 1 cfu/gram	Within permissible limits
2	Total Fungal Count (TFC)	10 ³ CFU/gm	Less than 1 cfu/gram	
3	Enterobacteriaceae	10 ³	Absent	
4	<i>Escherichia coli</i>	10	Absent	
5	<i>Salmonella Spp</i>	Absent	Absent	
6	<i>Staphylococcus aureus</i>	Absent	Absent	

CONCLUSION

The antimicrobial and Microbial load study was done for the drug Rathinagara Rasamezhugu. Anti microbial screening results showed that the drug RNM exhibited maximum zone of inhibition of about 8-22 mm against *Candida albicans* followed by 9-20 mm against *Pseudomonas aeruginosa*, 8-19 mm against *Bacillus cereus*, 7-10 mm against *Staphylococcus aureus*, 7-9 mm against *Escherichia coli* at different concentrations in volume of 80 µl/well. The result of the study further proves the bacterial and fungal count are within permissible limits as per WHO 2007, and pathogens such as *E.coli*, *Enterobacter*, *Salmonella Spp* and

Staphylococcus aureus are absent in the drug. Further studies are required for the evaluation of its therapeutic efficacy and safety.

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CONFLICT OF INTEREST

Nil.

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