IAMJ

INTERNATIONAL
AYURVEDIC
MEDICAL JOURNAL

Research Article | ISSN: 2320 5091 | Impact Factor: 5.344

ANTIBACTERIAL STUDY OF SHEETAMSHU RASA AND ITS MODIFIED METHODS

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https://doi.org/10.46607/iamj0508102020

(Published online: October 2020)

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Article Received:09/09/2020 - **Peer Reviewed:**28/09/2020 - **Accepted for Publication:**03/10/2020

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ABSTRACT

Knowledge of microorganisms was well stablished in *Ayurveda* in terms of *krimi* since Vedic period and have told that the organisms were invisible, scattered and present everywhere in which some are harmful or harmless to human beings. In *Samhita* clear cut description of invisible *krimi* is found and also said that they are causes various diseases like *Jawar, Kushtha* considered as contagious disease by *Accharya Sushruta*. Number of *Ayurvedic* formulation possess *krimighna* (antimicrobial) properties. Therefore, need of study to evaluate antimicrobial property of *Ayurvedic* formulations due to resistance of antibiotic which is challenge for modern medical science. *Sheetamshu Rasa* (SR) is one of the unique herbomineral formulation explained in text *Basavarajiyam* under *Sannipataja Jwara Rogadhikara* and also it has many indications. Four samples of SR were prepared according to textual reference and slight modification in levigation media & ratio of ingredients. Antimicrobial study was done in different concentrations in DMSO, against three bacteria *Klebsiella pneumonia, Pseudomonas aeruginosa & Staphylococcus aureus* producing fever as a symptom by Agar well diffusion method and Ciprofloxacin (5 µg/ml) as antibacterial standards. Result shows that SR samples had antibacterial activity moderate to high. SR1 and SR2 showed the best result in higher concentration than the other two samples (ZI= 11mm and AI= 0.92) on *Klebsiela pneumonia*. *Trikatu* and levigation media have no special significance in antibacterial activity.

Keywords: Antimicrobial, Herbomineral, *Krimi*, Microorganism, *Sheetamshu Rasa*.

INTRODUCTION

From the *Vedic* period the Indian physicians were well aware regarding the presence of the microorganism and this knowledge was passed through the generations and described in *Upanishads puranas* and *Samhitas*. They have described about both visible & invisible *Krimis*. A clear-cut description of *Adrista* (Invisible) *Krimis* is found in *Charaka* and *Sushruta Samhita* regarding *Raktaja Krimi* due to their minuteness and cause the disease like *kustha*. Also, in *kustha Roga* has described the presence of *Krimis* in every *Kustha* along with the three *Doshas*, later in the same chapter *Sushruta* has described the mode of the transmission of contagious diseases and listed *Kustha*, *Jawar* as a contagious disease. Most of the diseases produced by the *Krimi* find in the chapters of *Jwara* and *Kustha* in our ancient *Samhitas*. It is the greatness of *Ayurveda* that when there were no scientific tools available, in those days itself, they identified and described that the *Krimi* is a causative factor of diseases[1], which may be correlated to the micro-organism in present era. Theory of bacterial causation is established by them with the identification of factors responsible for the transmission of these *Krimi* from person to person[2]. It is important to note that Some *Ayurvedic* drug possess *Krimighna* property may considered as antibiotic by the modern scholar. That is why the Antimicrobial studies of many Ayurvedic formulations are being done. *Sheetamshu Rasa* (SR) is one of the unique formulations explained in text *Basavarajiyam* (15th century A.D.) under *Sannipatja Jwara Rogadhikara* and also it has many indications like *kushta, kamala, kasa, Visarp, Mandal, Shosha, Danta, karna Roga*, etc."*Sannipaatadi Sarvaroganam Sheetamshu Rasa*", this sentence suggests that it is indicated in all type of diseases.[3]

Aim & Objective: The aim of present study was to evaluate the antibacterial property of *Sheetamshu Rasa* against selected bacteria which produces disease having fever as a symptom, by Agar well diffusion method.

Materials & Methods:

Ingredients of SR samples: Four samples of SR are prepared using *Shuddha Hartala, Shuddha Manahshila & Trikatu Churna* in different ratio and different liquid preparation of *Nimba* (*Azadirachta indica*) as *Bhavana Dravya* with seven times *Bhavana*.

Table 1: Ratio of ingredients and *Bhavana Dravya* in different Samples of SR

Samples	*SuddhaHartala*	*SuddhaManahshila*	*Trikatu*	*Bhavana drava*	Reference
SR1	1 part	1 part	2 part	*Nimba twak kwath*	*Basavarajiyam*
SR2	1 part	1 part	2 part	*Nimba twak Hima*	Modified Method
SR3	1 part	1 part	2 part	*Nimba patra kwath*	Modified Method
SR4	1 part	1 part	6 part	*Nimba twak kwath*	Modified Method

Method of Preparation: The powder of all ingredients in specified ratio was taken into in a *Khalva* and *Mardana* (grind) was done till all gets properly mixed. For *Bhavana, kwatha/ Hima* were added in that quantity which was sufficient to soak the powder well and ground to a soft mass. Then this mixture was triturated well till whole liquid had dried up. After that, next *Bhavana* of fresh *kwatha/ Hima* were given.

Testing Bacteria:[4]

Table 2: Detail of Selected Bacteria.

Sr. No	Species	Gram -ve/+ve	MTCC No.
1.	*Klebsiella pneumonia*	-ve	3384
2.	*Pseudomonas aeureginosa*	-ve	7925
3.	*Staphyllococcus aureus*	+ve	737

Method of Antimicrobial Study:

Study Conduct At: S.R Lab, Pratap Nagar, Jaipur (Raj.).

Preparation of inoculums: Prepared the inoculums. Harvest the bacterial and fungal cultures, sterile peptone saline used, washed the surface growth, collected it in suitable glassware, and adding sufficient sterile peptone saline to obtain a microbial count of about 1 × 108 colony-forming units (CFU) per ml. Determined the number of CFU per ml in each suspension, using the conditions of media and microbial recovery incubation times 72 hours to confirm the initial CFU per ml. This value serves to calibrate the size of inoculums used in the test. The bacterial and yeast suspensions were used within 24 hours of harvest, but the fungal preparation was stored under refrigeration for up to 7 days.

Preparation of Media: For weighing media used Calibrated Balance, the glass wares and utensil are dehydrogenated in oven at 250^0C for 60 min, weighed media carefully and dissolved in distilled water, then shacked well and heated on hot plate for complete dissolve. Checked the water level of autoclave if necessary, adjust level with DMW. Loaded all prepared Media, carefully close the lid of autoclave, check power supply & run the autoclave for sterilization. After reach the temp. of 121^0C hold 15 min on this temp then off supply and release steam slowly. Before testing switch on the U.V light of Biosafety Cabinet, Pass Box & Biosafety Cabinet room for 30 mins. Opened the lid of autoclave taken all the media on SS trays and sent to pass Box. Enter in air lock and then secondary change room & change the dress and wear sterilize full dress, enter on Biosafety Cabinet Room off the UV Light of Biosafety Cabinet and switch on white light with airflow. Sterilized hands & work bench with IPA 70%.

Test Procedure: In vitro antibacterial activity of 4 different sample of *SR* was carried out by using the Agar well diffusion method. This classic method yields a zone of inhibition in mm result for the amount of antibacterial that is needed to inhibit growth of specific microorganisms. For each sample two different concentration solution 100 & 200mg/ml were prepared by dissolving in DMSO. For the determination of zone of inhibition (ZOI), bacterial strain was taken and as a standard antibiotic and control DMSO for comparison of the results. The dilution (100 & 200 mg/ml) of formulation in DMSO and Ciprofloxacin (5 µg/ml) as antibacterial as positive reference standards /antibiotics were prepared in double distilled water. Diameter of well was 6 mm and volume applied in each well 100 µl. DMSO did not show any activity against test organism and Ciprofloxacin 5 ppm as Standard or Positive control. Muller Hinton agar plates for bacteria were seeded with liquid culture of bacterial strains and allowed to stay at 37°C for 24 hours. The zones of growth inhibition around the wells were measured after 18 to 24 hours of incubation at 37°C for bacterial. The sensitivity of the microorganism species to formulation were determined by measuring the sizes of inhibitory zones (including the diameter of well) on the agar surface with comparison to the standard antibiotic zones.

Results:

Table 3: Showing result of Zone of Inhibition in mm (Values are mean of triplicate) of SR-1.

S. N.	Microbes	Zone of Inhibition		
		Standard (Ciprofloxacin 5ppm)	Test in DMSO	
			100 mg/ml	200 mg/ml
1.	*Klebsiella pneumonia*	12	8	11
2.	*Pseudomonas aeureginosa*	15	9	11
3.	*Staphyllococcus aureus*	12	6	10

Table 4: Showing result of Zone of Inhibition (Values are mean of triplicate) of SR-2.

S. N.	Microbes	Zone of Inhibition		
		Standard (Ciprofloxacin 5ppm)	Test in DMSO	
			100 mg/ml	200 mg/ml
1.	*Klebsiella pneumonia*	12	11	11
2.	*Pseudomonas aeureginosa*	15	10	12
3.	*Staphyllococcus aureus*	12	8	10

Table 5: Showing result of Zone of Inhibition in mm (Values are mean of triplicate) of SR-3.

S. N.	Microbes	Zone of Inhibition		
		Standard (Ciprofloxacin 5ppm)	Test in DMSO	
			100 mg/ml	200 mg/ml
1.	*Klebsiella pneumonia*	12	6	8
2.	*Pseudomonas aeureginosa*	15	8	13
3.	*Staphyllococcus aureus*	12	6	9

Table 6: Showing result of Zone of Inhibition in mm (Values are mean of triplicate) of SR-4.

S. No.	Microbes	Zone of Inhibition		
		Standard (Ciprofloxacin 5ppm)	Test in DMSO	
			100 mg/ml	200 mg/ml
1.	*Klebsiella pneumonia*	12	6	6
2.	*Pseudomonas aeureginosa*	15	6	9
3.	*Staphyllococcus aureus*	12	6	7

Measurement of Activity Index:

$$\text{Activity Index} = \frac{\text{Zone of Inhibition of sample}}{\text{Zone of Inhibition of standard}}$$

Fig.1

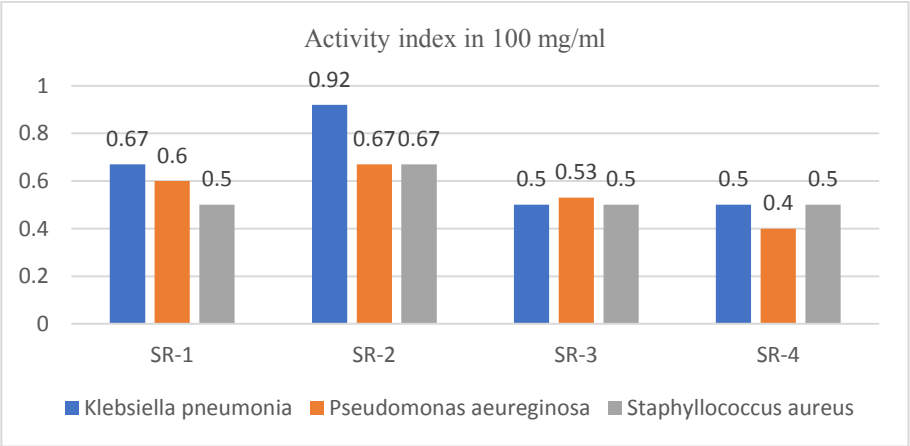
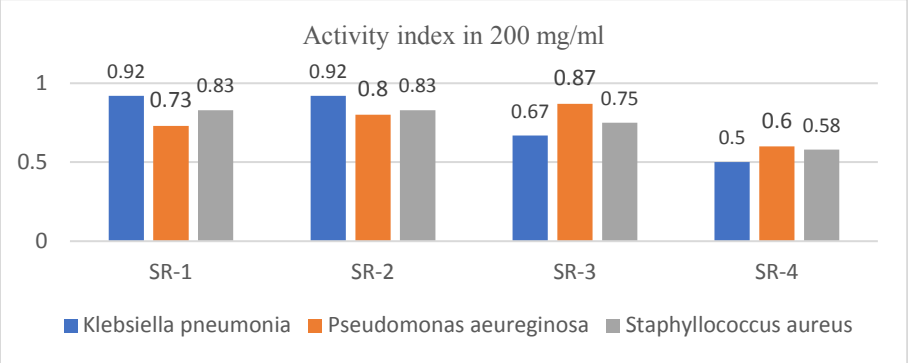


Fig.2



DISCUSSION

In our *Rasa* classics *Guna* (action) of mineral has been explained clearly. *Shuddha Manahshila* has many properties in which *Bhutanashak* or *Bhutaupdravanashini* properties mentioned by many *Rasa* Acharyas (R.R.S-3/71, A.P -2/218 and R.T- 11/116). *Shuddha Hartala* also has *Jwaraghna, Kushthaghna, Bhutanashak* etc. properties (R.T 11/60-71), so we may consider the word *Bhutanashak* as antimicrobial activity in present era. As per Database of Medicinal Plant *Pippali, Shunthi, Maricha* (collectively known as *Trikatu*) have also antibacterial properties. Bark and leaves of *Nimba* also have antibacterial action (API, part-1 vol-2, pg- 134). Therefore, it is clear from above discussion that all ingredients which were used in the preparation of *Sheetamshu Rasa* have antimicrobial properties. For established Antibacterial susceptibility of SR against three pathogenic bacteria viz *Klebsiella pneumonia, Pseudomonas aeruginosa* and *Staphylococcus aureus* which are responsible for fever as symptom were done in DMSO solution with comparison to 5ppm Ciprofloxacin and the method was adopting as Well diffusion method where Muller Hilton agar plates were seeded with indicated bacterial strains. Each sample of *Sheetamshu Rasa* were dissolved in DMSO at two different concentration 100 mg/ml & 200 mg/ml. Antibacterial activity was discussed below with comparison between standard reference of Ciprofloxacin (5 ppm) and Samples as follows-

***Klebsiella pneumonia*:** In comparison between four samples, SR1 showed the best result at 200 mg/ml concentration and SR2 showed same result at both concentrations i.e. 11mm Zone of Inhibition. SR4 shows least results in both concentrations. It was also proved by in the parameter of Activity index.

***Pseudomonas aeruginosa*:** Activity index showed the good result in higher concentration in SR1, SR2 and SR3 whereas least result in SR4 in both the concentration. It was also observed that among the first three samples SR3 showed best result.

Staphylococcus aureus: Both SR1 and SR2 had best result in higher concentration in comparison to others two samples according to Activity Index. SR4 showed least results in both concentrations. According to above discussion SR4 had lest antibacterial activity but it was containing more quantity of *trikatu* with compare to other samples. Therefore, it was showed that *trikatu* have no extra significance in antibacterial activity. It was also observed that there was no special significance of the levigation media.

CONCLUSION

All most all concentration had antibacterial activity moderate to high except SR4 against Pseudomonas aeruginosa in 100mg/ml concentration. Among all bacteria, SR1 and SR2 showed the best result in higher concentration than the other two samples (ZI= 11mm and AI= 0.92) on *Klebsiela pneumonia*. According to activity index higher concentrated samples had more effective than lower concentration. *Trikatu* and levigation media have no special significance in antibacterial activity.

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Source of Support: Nil
Conflict of Interest: None Declared

How to cite this URL: Parween Bano et al: Antibacterial Study of Sheetamshu Rasa And Its Modified Methods. International Ayurvedic Medical Journal {online} 2020 {cited October, 2020} Available from: http://www.iamj.in/posts/images/upload/4600_4605.pdf