IAMJ

Research Article *International Ayurvedic Medical Journal* *ISSN:2320 5091*

ANTIMICROBIAL STUDY OF MRITYUNJAYA RASA

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ABSTRACT

The idea of less intrusive alternative to the antibiotics is alluring due to the adverse effect, sensitivity and development of resistance against microbes. This provokes the researchers to investigate the antimicrobial effects of formulations mentioned in traditional system of medicine. *Mrityunjaya Rasa* is one of the Ayurvedic preparation vividly used for treating fever. Antimicrobial activity of *Mrityunjaya Rasa* was carried out against various strains of bacteria. Anti microbial activity of *Mrityunjaya Rasa* was investigated against both gram positive and gram negative bacteria in in vitro disc and minimum inhibitory concentration methods. The result evidenced the antimicrobial potential of *Mrityunjaya Rasa*.

Keywords: *Mrityunjaya Rasa*.

INTRODUCTION

Ancient Ayurvedic physicians knew the existence of microbes; they agreed that they are contagious "*upsargaja*". But, it didn't bother them as the primary causal factor in the onset for disease, unlike conventional physician. Now a days "*upsargaja vyadhi*" (infectious diseases) makes trouble for human being. The conventional remedy "antibiotics" all synthetic antimicrobial agents are local irritants, responsible for hypersensitivity and repeated use may develop resistance against so antibiotics from microbial sources. These drawbacks to presently available antibiotics has necessitated the search for new antibacterial agents.

Number of Ayurvedic classical preparations was being used in cases of infections, and they were found to be effective clinically. So far, little is known about the antibacterial properties of these medicines and the scientific basis of its application in treating infectious bacterial diseases. Therefore, to make our treatment scientifically more validated, we can assess the antimicrobial activity of one of such preparation Mrityunjaya Rasa such preparations in vitro (i.e. culture and sensitivity Tests).

MATERIAL AND METHODS

Preparation and Extraction of Mrityunjaya Rasa

Three samples of the trial drug *Mrityunjaya Rasa* were prepared using different *bhawana dravya*

M_3 → With *Bhavana* of *Jala* (*A.F.I.* Vol.-I, 20/30g) [2] M_2 → With *Bhavana* of *Aadraka Swarasa (Y. R.)* [4] M_1 → With *Bhavana* of *Nimbu Swarasa (A.S.S.)* [1]

Ingredients

Shuddha Hingula Churna- 40 gms
Shuddha Vatsanabha Churna-20 gms
Maricha Churna-20 gms
Pippali Churna- 20 gms
Shuddha Tankana- 20 gms
Shuddha Gandhaka- 20 gms
Total Wt.-140 gms

Method of Preparation

The powdered *Shuddha Vatsanabha*[3] and *Shuddha Tankana* were taken first in a mortar and *Mardana* was

carried out. This process of grinding *Vatsanabha* with ***Tankana*** is also termed as "*Marana* of *Vatsanabha*". After that, Shuddha Hingula was added into the mixture and grinded well till it gets properly mixed. Then, other ingredients were properly mixed and grinded one by one.

For *Bhavana, Swarasa*/water was added in that quantity which was sufficient to soak the powder well and ground to a soft mass. Then this mixture was triturated well with the help of mortar and pestle till whole *swarasa* had dried up. After that, next *Bhavana* of fresh *swarasa* /water was given.

For present anti microbial study three different sample of *Mrityunjaya rasa* (M1, M_2, M_3) was taken. From each sample of *Mrityunjaya rasa* three different concentration solution 5%, 10%, 12.5% (1gm sample was mixed in 100 ml DMSO FOR 1% Solution) was prepared with solvent Dimethyl sulfoxide (DMSO).

Bacterial cultures

Micro-organisms were selected that causes general infections along with fever. The standard pathogenic bacteria cultures were procured from IMTECH, Chandigarh, India and used in the present study (Table I). The bacterial cultures were rejuvenated in Mueller- Hinton broth (Hi-media laboratories, Mumbai, India) at 37 ^{0}C for 18h and then stocked at 4 ^{0}C in Mueller- Hinton Agar. The antimicrobial Study was done at "Chemind Diagnosis and biosolution", Jaipur.

Culture Media

Different growth media's used for the micro-organisms, as directed by IMTECH. Nutrient Agar, Nutrient Broth and Blood agar were used.

Table No. I showing bacterial strain with their MTCC No.

S.No.	Species	MTCC No.	Staining	Media Used (Himedia Lab. Pvt. Ltd.)
1.	*Streptococcus pyogenes*	1928	Gram-positive	Blood Agar
2.	*Staphylococcus aureus*	3160	Gram-positive	Nutrient Agar
3.	*Escherichia coli*	1652	Gram-negative	Nutrient Agar
4.	*Pseudomonas aeruginosa*	647	Gram-negative	Nutrient Agar
5.	*Salmonella typhi*	734	Gram-negative	Nutrient Agar

Preparation of Media

Nutrient broth (13gms/1000ml of distilled water) was dissolved in distilled water in a conical flask; Nutrient Agar (28gms/1000ml of distilled water) was also added and dissolved in a conical flask having Nutrient broth. In another flask containing distilled water, Blood Agar Base (21.25 gm/500 ml distilled water) was dissolved. On cooling, media containing Agar solidify at about 42ºC. So, after autoclaving, both the flasks were cooled to 45 to 47ºC. Then, sterile human blood (7%) was added in a flask containing Blood agar base aseptically.

Preparation of Media Plates

- Sterlization of culture media was done by autoclaving at 15 lbs pressure for 20 minutes. The media poured into glass petridishes, in laminar flow cabinet.
- Petridish - diameter = 90 mm. Lid is larger in diameter and has shallow rim. Base is smaller and deeper, base section should be labelled with details of

medium, date, etc.

- About 30 ml. of media to be poured into each petridish (diameter=90 mm, labelled with details of medium, date etc). The plates were left undisturbed until the agar solidified. Then the plates were kept overnight at room temperature for observation of contamination.
- The contaminated plates were discarded. Rest of the plates were wrapped in a foil and kept in cold room at 4^0C for further use.

Well diffusion method

100 µl of test bacterial subculture was prepared in sterile broth medium (few colonies of microbial culture were left in 100µl sterile broth medium side in an eppendrof tube).

Prepared medium was spread on media plates. It was allowed to dry for 30 minutes and then 4 hole (each 3 mm diameter) was made in each media plates by using a sterile borer at equal distance. Total 15 media plates (3 x 5) were prepared for study. In each media plate 3 holes was filled by three different samples (same concentration solution) and one hole was filled by same concentration solution of ***streptomycin*** **(standard or control)**. The samples and the control (0.1ml) were places in 3-mm diameter well. The plates were incubated at 37°C for 24 hours and diameter of the inhibition zone was measured by scientific scale.

RESULTS/ OBSERVATIONS

Pictures - Antimicrobial Activity of Mrityunjaya Rasa on different Bacterial strain

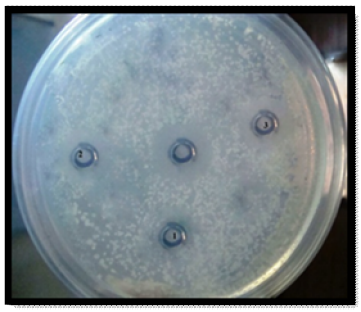


Fig.1 E.Coli-1

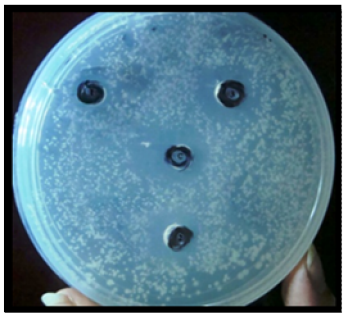


Fig.2 E.Coli-2

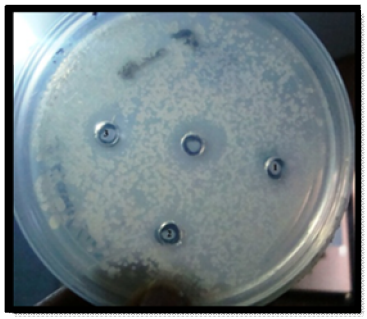
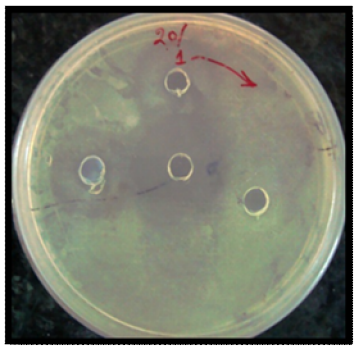
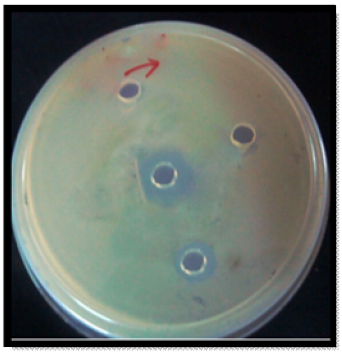


Fig.3 E.Coli-3





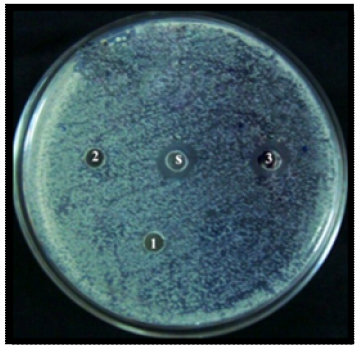


Fig. 4 S.aureus-1

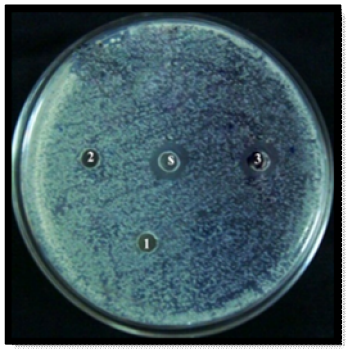


Fig.5 S.aureus-2

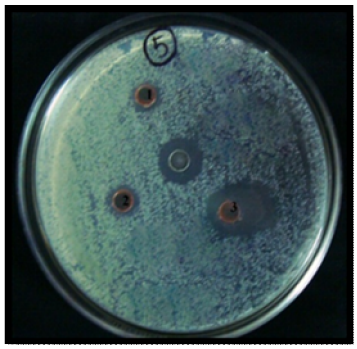


Fig.6 S.aureus-3

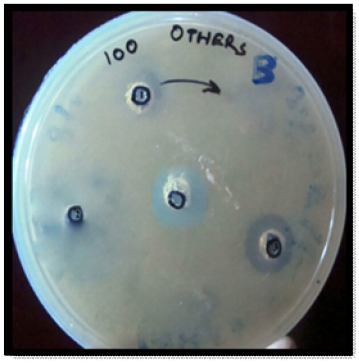


Fig.7 P.aeruginosa-1

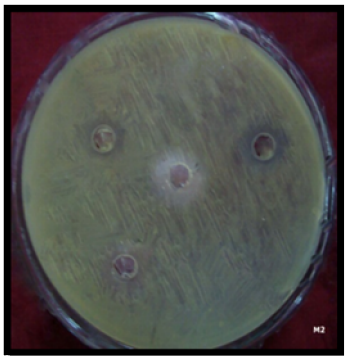


Fig.8 P.aeruginosa-2

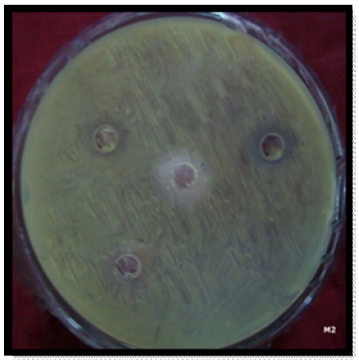


Fig.9 P.aeruginosa -3

Fig.10 S. pyogenes-1

Fig.11 S.typhi -1

Table No. II showing Zone if Inhibition. Antibacterial activity

Bacteria culture	Samples	Zone of Inhibition (cm) @		
		50 mg/ml	100 mg/ml	125 mg/ml
Escherichia coli	M_1	0.4	0.65	0.79
	M_2	0.42	0.5	0.8
	M_3	0	0.42	0.76
	Streptomycin	0.6	0.95	1.1
Streptococcus pyogenes	M_1	0.38	0.6	1.1
	M_2	0	0.38	1.51
	M_3	0.45	0.63	0.85
	Streptomycin	0.4	0.7	1.1
Staphylococcus aureus	M_1	0	0.37	0.57
	M_2	0.32	0.48	0.85
	M_3	0	0.45	0.8
	Streptomycin	0.4	0.7	0.98
Pseudomonas aeruginosa	M_1	0.32	0.6	0.9
	M_2	0.35	0.47	0.8
	M_3	0	0.35	0.7
	Streptomycin	0.45	0.68	1
Salmonella Typhi	M_1	0	0.45	0.65
	M_2	0.34	0.55	0.85
	M_3	0.40	0.65	0.85
	Streptomycin	0.5	0.85	1

Table No.III Showing the relation between Zone of Inhibition drug sensitivity.

S.No.	Inhibition Zone (I.Z.)	Drug Sensitivity
1.	No Inhibition Zone	Insensitive (I.S.)
2.	Drug I.Z. << Standard I.Z.	Moderate sensitive (M.S.)
3.	Drug I.Z. $\leq$ Standard I.Z.	Highly sensitive (H.S.)

DISCUSSION AND CONCLUSION

Streptococcus pyogenes was highly sensitive to 12.5% Concentration of sample M_1, M_2 & 5.0% Concentration of sample M_3. It was moderately sensitive to 12.5% Concentration of sample M_3 and 10.0% Concentration of sample M_1, M_2, M_3, 5.0% Concentration of sample M_1, M_3. E.coli was moderately sensitive to all Concentration of sample M_1, M_2, M_3 except 5.0% Concentration of sample M_3. *Staphylococcus aureus* was moderately sensitive to all Concentration of sample M_1, M_2, M_3 except 5.0% Concentration of sample M_1, M_3. *Pseudomonas aeruginosa* was moderately sensitive to all Concentration of sample M_1, M_2, M_3 except 5.0% Concentration of sample M_3. *Salmonella Typhi* was moderately sensitive to all Concentration of sample M_1, M_2, M_3 except 5.0% Concentration of sample M_1.

No sensitivity was observed at 5.0% Concentration of sample M_3 against *Pseudomonas aeruginosa*, *E. coli* & *Staphylococcus aureus*; 5.0% Concentration of sample M_2, against *Streptococcus pyogenes*; 5.0% Concentration of sample M_1 against *Salmonella Typhi* & *Staphylococcus aureus*.

Formulation M_2^4 which was prepared as per the Yoga Ratnakara specifications found to be highly effective than other formulations.

All sample of *Mrityunjaya Rasa* have high sensitivity against *Streptococcus pyogenes*.

Bhavana dravya also contribute their share in the antibacterial efficacy of the prepared medicine M_2 sample where ginger juice has been used as *bhavana dravya* showed better results than the other two samples.

REFERENCES

1. Ayurveda Sar Samgraha, 17th edition 1993, published by Shri Baidyanath Ayurveda Bhavana Ltd. Nagpur.
2. Ayurvedic Formulary of India, Part I & II. Published by Govt. of India, Ministry of Health and Family welfare.
3. Rasa Tarangini of Shri Sadananda Sharma with Prasadini Sanskrit Commentary by Shri Haridatta Shastri and Rasavigyana hindi commentary by Pt. Dharmananda Shastri. Edited by Kashinath Shastri (reprint 1994), Published by Motilal Banarsidas, New Dehli.
4. Yoga Ratnakara of Mayurapad Bhikshu, 6th edition. Edited by Shri Brahmashankar Shastri with Vidyotini Hindi commentary by Vaidya Laxmipati Shastri. Published by Chaukhamba Sanskrit Bhavan, Varanasi.

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