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Research Article

EVALUATION OF ANTIBACTERIAL ACTIVITY OF *HINGULESHWAR RASA*

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ABSTRACT

In this era infectious diseases are posing problems for human beings. In order to avoid various infections, production and use of antibiotics is on rise. The widespread misuse of antimicrobials is responsible for emerging microbial resistance. Numbers of Ayurvedic classical preparations were being used in cases of infections, and they were found to be effective clinically. Therefore, to make our treatment scientifically more validated, we can assess the antimicrobial activity of such preparations in vitro (i.e., culture and sensitivity tests). The development of bacterial resistance to known antibiotics and adverse effect to presently available antibiotics has necessitated the search for new antibacterial agents in different systems of medicine. So *Hinguleshwar rasa,* a known traditional medicine, has been selected for this study. The antibacterial activity of the *Hinguleshwar rasa* was tested against pathogenic bacteria strain Streptococcus pyogenes, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, and Salmonella typhi. In order to study antibacterial action of *Hinguleshwar rasa* in vitro well diffusion method. During this study *Hinguleshwar rasa* was trailed with bacteria at different concentrations. To correlate the result, control solutions were prepared by streptomycin. Experimental groups were compared with control groups and observations were noted.

INTRODUCTION

Now a day's infectious diseases are posing problem for humanity. In order to avoid different infections, production and use of antibiotics is on rise, which derived from the microbial sources in synthetic manner.

However all synthetic antimicrobial agent are local irritants and are responsible for hypersensitivity reactions. Secondly the global misuse of antimicrobials is responsible for emerging microbial resistance. The adverse effect to presently available antibiotics and development of bacterial resistance has necessitated the search for new antibacterial agents in different systems of medicine.

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Thus the idea of less intrusive alternative is alluring so to overcome the problem like limited shelf life and adverse effect etc, the mixture of traditional antibiotics are currently underway to look for natural origin.

Number of Ayurvedic classical preparations is being used in cases of infections, and they are found to be effective clinically. Therefore, to make our treatment scientifically more validated, we can assess the antimicrobial activity of such preparations Invitro (i.e., culture and sensitivity Tests). So *Hinguleshwar rasa* a known traditional medicine has been selected for this study.

Hinguleshwar Rasa is one of the celebrated and most popular drug compound mentioned in Ayurvedic texts and used for the management of *Ajirna* (indigestion), *Antrik Jwara* (enteric fever), *Amavata* (rheumatoid arthritis), *Yakrit Pliha vikriti* (hepato-splenomegaly), *Rajyakshma* (pthysis), *Udar roga* (abdominal disorders), *Pakshaghatha* (paralysis) and generally in all types of *Jwara* (fevers).

Different formulae of *Hinguleshwar* Rasa[1] are available in the classical *Rasashastra* texts. For present study Formula of *Hinguleshwar* rasa described in A.F.I., Volume-2, page no. 297, Rasa yoga- 16:18) (Bhaishajya Ratnawali, Chap. 5, 483) have been selected. It contains - *Hingula* (Cinnabar), *Pippli* (*Piper longum*), *Vatsanabha* (Purified *Aconitum ferox*) in equal proportion.

AIMS AND OBJECTIVES

To evaluate the anti-bacterial activity of *Hinguleshwar* Rasa against common pathogenic bacteria.

MATERIALS AND METHOD

For present study samples of *Hinguleshwar* rasa was taken and three different concentration solutions 50, 100, 125 (1mg/ml) were prepared of sample with solvent Dimethyl sulfoxide (DMSO). To validate the result control solution was also prepared by streptomycin in same concentration in same solution[3].

Chemicals

All chemicals used for the preparation of nutrient media and for present study were of Analytical grade.

Glass wares and Polywares

All the glassware was of sterilizable type and polywares were of disposable type.

Micro-organisms

Micro-organisms selected for the present research work are those which cause general infections along with fever[7]. The pathogenic strains of different species of bacteria used for study were maintained on the following media as mentioned in table given below-

Table I: The pathogenic strains of different species of bacteria[2]

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

The antibacterial Study was done at "Chemind Diagnosis and biosolution", Jaipur.

Culture Media

Like all other living forms, micro-organisms need suitable nutrients and favorable environments for growth. A simple way to obtain bacteria is to grow them in a test tube/ or a small flask in broth medium.

As per directed by IMTECH, different growth media's used for the micro-organisms.

Nutrient Agar

Beef extract- 1.0gm
Yeast extract- 2.0gm
Peptone- 5.0gm
NaCl- 5.0gm
Agar- 15.0gm
Distilled water- 1.0 L

Nutrient Broth

Peptic digests of animal tissue- 5.0 g/L
Sodium chloride- 5.0 g/L
Beef Extract- 1.5 g/L
Yeast Extract-1.5 g/L

Blood agar

Protease peptone- 15.0gm
Liver extract- 2.5gm
Yeast extract- 5.0gm
NaCl- 5.0gm
Agar- 15.0gm
Distilled water- 1.0L

Agar

Agar is a complex, long chain, polysaccharide derived from certain marine algae has several useful properties. When added to a solution it melts at 100°C forming a slightly viscous liquid that solidifies at 42°C. After solidification the agar will not melt unless the temperature is again raised to 100°C. This is a useful property. Some other useful properties of agar include its resistance to microbial degradation and its translucence for easy viewing of colonies embedded in the agar.

If a solid medium is necessary, agar is usually added as the solidifying agent. For plates or slants, 2.0% concentration of agar is needed.

Preparation of Media

With regards to this experiment, firstly Nutrient broth (13gms/1000ml of distilled water) was dissolved in distilled water in a conical flask, then Nutrient Agar (28gms/1000ml of distilled water) was added and dissolved as well in a conical flask having Nutrient broth. In another flask Blood Agar Base (21.25gm/500ml distilled water) was dissolved distilled water.

Both flasks were then plugged with cotton balls and autoclaved for complete sterilization of the solutions.

On cooling, media containing Agar solidify at about 42°C. So, after autoclaving, both the flasks were cooled to 45 to 47°C. Then, sterile human blood (7%) was added in a flask containing Blood agar base aseptically.

Preparation of Media Plates

- Sterilization of culture media was done by autoclaving at the pressure of 15 lbs for 20 minutes then media was taken out, kept on bench for a while.
- The media was then poured into glass petridishes, in laminar flow cabinet.
- Petridish – 90mm diameter. Lid has shallow rim and is larger in diameter. Base is smaller and deeper, base section should be labelled with details of medium, date, etc.
- About 30ml. of media to be poured into each petridish, if too little agar is poured there, it may not be enough to cover the dish or the agar plate will dry up easily. If too much agar is poured, the cover dish will come in contact with the nutrient agar, leaving no room for microbial growth. Either way the plates are rendered useless.
- The plates were left in isolation until the agar solidified. Then the plates were kept at room temperature overnight for observation of contamination.
- If contamination was found, the plates were discarded. If no contamination found, these plates were wrapped in a foil and kept in cold room at 4°C for further use.
- The media and media plates were prepared as per requirement and used for Antibacterial evaluation.

Study of the Evaluation of Antimicrobial

This experiment was carried out on solid media. On solid media it was done by "Well diffusion method".

Well Diffusion Method

In this method 100ml of test bacterial subculture was prepared in sterile broth medium. For this in an eppendrof tube, took 100ml sterile broth medium and few colonies of microbial culture left inside tube.

After that prepared medium was spread on media plates. It was allowed to dry for 30 minutes and then 2 holes (each 0.3cm diameter) was made at suitable distance in each media plates by using a sterile borer. Total 15 media plates were prepared for study.

In each media plate one hole was filled by sample drug 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 0.3cm diameter well.

The plates were incubated at 37°C for 24 hours and after then diameter of the inhibition zone was measured. [3]

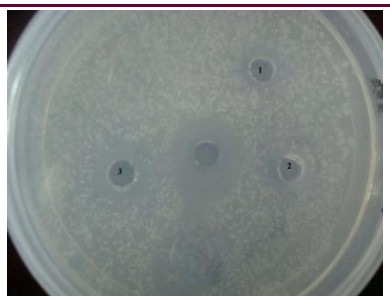
OBSERVATIONS

Table II: Showing Antibacterial activity of *Hinguleshwar Rasa* on different bacterial Strains

S. No.	Name of Bacteria	Zone of Inhibition (cm.) in Different Concentrations (mg/ml)		
		50	100	125
1.	Streptococcus pyogenes	0.65	0.92	1.1
2.	Staphylococcus aureus	0.3	0.86	1.05
3.	Pseudomonas aeruginosa	0.65	0.81	1.4
4.	Escherichia coli	0.72	0.8	1.01
5.	Salmonella typhi	0.63	0.9	1.2

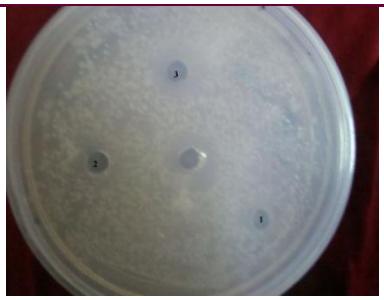
Table III: Showing Antibacterial activity of Straptomtcin on different bacterial Strains

S. No.	Name of Bacteria	Zone of Inhibition (cm.) in Different Concentrations(mg/ml)		
		50	100	125
1.	Streptococcus pyogenes	0.7	1.0	1.4
2.	Staphylococcus aureus	0.7	1.0	1.28
3.	Pseudomonas aeruginosa	0.75	0.98	1.3
4.	Escherichia coli	0.9	1.25	1.4
5.	Salmonella typhi	0.8	1.15	1.3



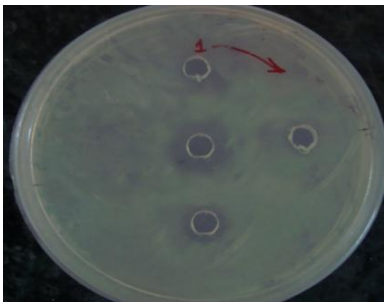
E Coli (50mg/ml)

E Coli (100mg/ml)

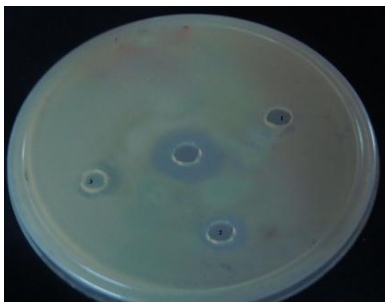


E Coli (125mg/ml)

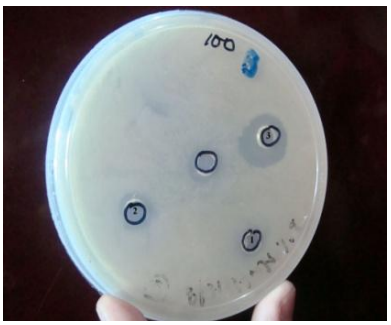
P. aeruginosa (50mg/ml)



P. aeruginosa (100mg/ml)

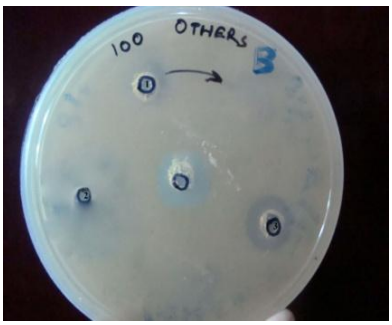


P. aeruginosa (125mg/ml)

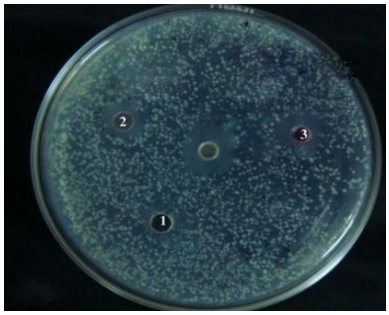


Staphylo.aureus (50mg/ml)

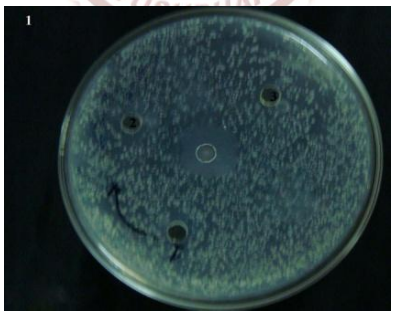
Staphylo.aureus (100mg/ml)



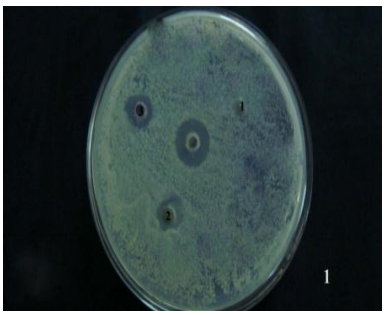
Staphylo.aureus (125mg/ml)



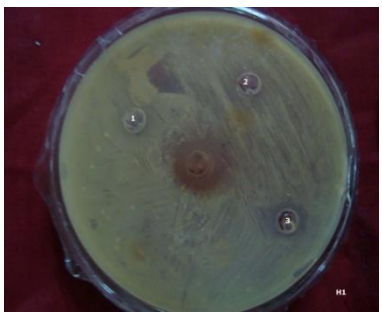
Strepto.pyogenes (50mg/ml)



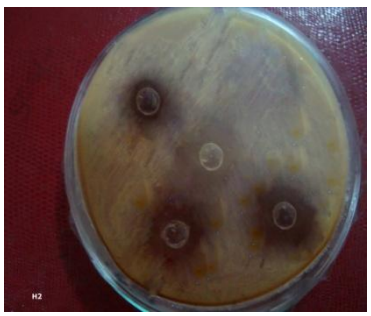
Strepto.pyogenes (100mg/ml)



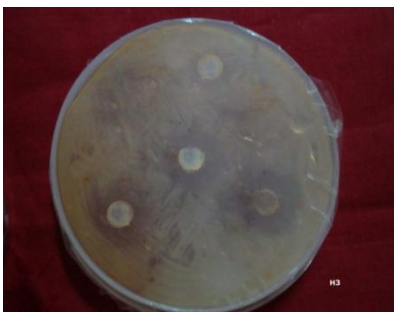
Strepto.pyogenes (125mg/ml)



Sal. Typhi (50mg/ml)



Sal. Typhi (100mg/ml)



Sal. Typhi (125mg/ml)

Inhibition Zone Observed In different pathogens
Due To Different Concentration of *Hinguleshwara Rasa* in DMSO Solution (Cup Plate Method)

RESULTS

The results were summarized according to table No.III which are given below

Table IV: 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. < Standard I.Z.	Highly sensitive (H.S.)

After comparing to standard solution following observations were obtained

- Streptococcus pyogenes was highly sensitive to 12.5% Concentration solution and 10.0% Concentration and 5.0% concentration of *Hinguleshwar rasa*.
- Staphylococcus aureus was highly sensitive to 10.0% Concentration and moderate sensitive to 12.5% Concentration solution and insensitive to 5.0% concentration of *Hinguleshwar rasa*.
- Pseudomonas aeruginosa was very highly sensitive to 12.5% concentration of *Hinguleshwar rasa* and highly sensitive to 5.0% and 10.0% concentration of *Hinguleshwar rasa*.
- Salmonella typhi was highly sensitive to all concentration of *Hinguleshwar rasa*.
- E.coli was moderately sensitive to 12.5% Concentration of *Hinguleshwar rasa* and less sensitive to
- 5.0% and 10.0% concentration of *Hinguleshwar rasa*.

Thus, by this view *Hinguleshwar rasa* was highly effective against salmonella typhi and streptococcus pyogenes and pseudomonas aeruginosa.

DISCUSSION

In the present study, it has been observed that *Hinguleshwar Rasa* inhibits different microbes. The nature of this antimicrobial activity cannot be categorized in a fixed format. It is clear that various concentration solutions has its own typical characteristics and differentiated action. But the exact clarification of this behavior will be available only after detailed analysis with sophisticated equipments and techniques.

CONCLUSION

The highly encouraging results obtained from antimicrobial study of *Hinguleshwar Rasa* are purely based on *in vitro* experimental methods.

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