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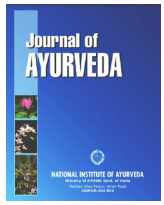

PHARMACOLOGICAL STUDY

A Comparative - In Vitro Study of Hinguleshwara Rasa Against Different Pathogenic Microbes

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ABSTRACT

Though there is little concept of antibiotics in Ayurveda, many Ayurvedic drugs show significant results in infectious diseases, these results are comparable with modern antibiotics. So it is presumed that the Ayurvedic Rasaushadhi has also possesses antimicrobial property. Hinguleshwara Rasa is widely used as anti-pyretic agent. Hence, it is considered as it posses antimicrobial activity against pyrexia (Jwara) causing organism.

In present study, three different samples of Hinguleshwara Rasa (first sample was prepared according to Ayurvedic text (A.F.I.) & the other two samples according to modified method) were tested for antimicrobial activity against common five pathogenic microbes causing pyrexia (jwara) by culture and sensitivity method (Cup Plate Method).

The results were encouraging, all three sample of Hinguleshwara Rasa exhibits good antimicrobial activity against microbes. But as compared to each other sample H1 (AFI) was more effective than sample H2 & H3 (modified methods).

Keywords : *Hinguleshwara Rasa, Kajjali, Rasa Sindoora & Antimicrobial.*

Introduction

Now a day's infectious disease makes a trouble for human being. In order to avoid different infections there are lots of antibiotics which derived from the microbial sources in synthetic manner. However all synthetic antimicrobial agent are local irritants & are responsible for hypersensitivity reactions. Second important thing is this that antibiotics from microbial sources have become ineffective & the infectious organism develops resistance against them.

Antimicrobial sensitivity test is mainly necessary when usually effective agents fail to reduce the desired effects in the treatment and control of infectious diseases which are caused by pathogens that are drug resistance. Sensitivity testing is helpful in selecting effective antimicrobial drugs.

Antimicrobial activity is a process by which response of an organism to a drug or crude extract can be evaluated as its inhibiting effect towards the growth of bacterium in nutrient broth or nutrient agar. To evaluate the efficacy of these agents for their antimicrobial activity different scientific procedures are established.

Number of Ayurvedic preparations were being used in cases of infections, and they were found to be effective clinically. Therefore, to make the treatment

scientifically more validation, there is need to assess the antimicrobial activity of such preparations in vitro (i.e. culture and sensitivity Tests).

Materials and method

For present study three different sample of Hinguleshwara Rasa (H1, H2 and H3) were prepared in N.I.A. laboratory, which were codes as: -

- H1 - Hinguleshwara Rasa prepared as per the specification mentioned in

  A.F.I. vol. - 2, page no. – 297, rasa yoga- 16:18.

The other two Samples were prepared on the basis of modified methods.

- H2 - Hinguleshwara Rasa prepared by using Hingula (HgS) in place of

  Kajjali (HgS).

- H3 - Hinguleshwara Rasa prepared by using Rasa Sindoor (HgS) in place of Hingula/Kajjali (HgS).

To study of antibacterial property of Hinguleshwara Rasa-

Three different concentration solutions 5%, 10%, 12.5% of each sample of Hinguleshwara Rasa was prepared with solvent DMSO (Di Methyl Sulfoxide). The method employed was 'Hot extraction method' recommended by W.H.O. (Quality Control Methods for Medicinal Plant Materials).The Antibacterial Study was done at "Chemind Diagnosis and biosolution", Jaipur.

Microbial strains

The antimicrobial activity of three different sample of Hinguleshwara Rasa was tested against different five species of common pathogenic bacteria. The strains of different microbes were procured from 'Institute of Microbial Technology' (IMTECH), and 'SMS medical college, Jaipur' as mentioned below table No. 1.

Table No.I. Showing Pathogens used for the study along with method & source.

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

Microbiological techniques adopted

For the study, standard techniques were used and were taken from "Indian Pharmacopeia.

- **Preparation of Media and Media Plates**

In this regard, first of all 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 also added and dissolved in a conical flask having Nutrient broth. Flasks were then plugged with cotton and autoclaved for complete sterilization. After autoclave, media was immediately poured in sterile Petri dishes aseptically in a Laminar flow cabinet. The Agar, which is added in a broth medium, hardens as it cools. After solidifying of Agar plates, they were kept in incubator at 37°C for overnight for checking any contamination.

- **Cup Plate Method / Cylinder Plate Method**

It is also called Well Diffusion Method. In this method, sectors were marked on the media plate for different samples and one for Standard. A 24 hr. test bacterial subculture was prepared in sterile broth medium and then 100 -l of it was spread on the plate with the help

of spreader. It was allowed to dry at room temperature for 30 min. After than 4 well (holes each 3 mm diameter) was made in each media plates by using a sterile borer in suitable 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 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 after then diameter of the inhibition zone was measured.

Observation & Results

The antibacterial activity of three different formulations of Hinguleshwara Rasa, in different concentration as mentioned earlier were evaluated against a number of pathogenic bacterial strains and zone of inhibition was observed in DMSO solution. The zone of Inhibition and results of drug sensitivity compared from standard (Streptomycin) was mentioned as below.

Table no-II 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.	Less sensitive (L.S.)
3.	Drug I.Z. << Standard I.Z.	Moderate sensitive (M.S.)
3.	Drug I.Z. ≤ Standard I.Z.	Highly sensitive (H.S.)
4.	Drug I.Z. > Standard I.Z.	Very Highly sensitive (V.H.S.)

The results are summarized in the form of tabular form as below -

Table no-III showing Antibacterial activity of three Samples of Hinguleshwara Rasa (in different concentrations) on Staphylococcus aureus MTCC no. 3160

S. No.	Drug conc. mg/ml	Inhibition zone in different Sample (In cm)			
		H_1	H_2	H_3	Standard (Streptomycin)
1.	50	0.3	0.3	0.65	0.7
2.	100	0.86	0.62	0.9	1.0
3.	125	1.05	0.76	1.17	1.28

Staphylococcus aureus was highly sensitive against the sample H3 compared to Standard in all concentration. But sample H1 & H2 was moderate sensitive in con. 100 & 125 mg/ml.

Table no-IV showing Antibacterial activity of three Samples of Hinguleshwara Rasa (in different concentrations) on Staphylococcus pyogenes MTCC no. 1928

S. No.	Drug conc. mg/ml	Inhibition zone in different Sample(In cm)			
		H_1	H_2	H_3	Standard (Streptomycin)
1.	50	0.65	0.67	0.3	0.7
2.	100	0.92	0.75	0.85	1.0
3.	125	1.1	0.83	1.01	1.4

Staphylococcus pyogenes was highly sensitive against the sample H1 compared to Standard in all concentration. But sample H2 & H3 was moderate sensitive in con. 100 & 125 mg/ml.

Table no-V showing Antibacterial activity of three Samples of Hinguleshwara Rasa (in different concentrations) on Pseudomonas aeruginosa MTCC no. 0647

S. No.	Drug conc. mg/ml	Inhibition zone in different Sample(In cm)			
		H_1	H_2	H_3	Standard (Streptomycin)
1.	50	0.65	0.62	0.3	0.75
2.	100	0.81	0.78	0.7	0.98
3.	125	1.4	1.1	0.85	1.3

Pseudomonas aeruginosa was Very highly sensitive against the sample H1 compared to Standard at the con. 125 mg / ml, But sample H2 was highly sensitive & sample H3 was moderate sensitive.

Table no- VI showing Antibacterial activity three Samples of Hinguleshwara Rasa (in different concentrations) on Escherichia coli. MTCC no. 1652

S. No.	Drug conc. mg/ml	Inhibition zone in different Sample (In cm)			
		H_1	H_2	H_3	Standard (Streptomycin)
1.	50	0.72	0.81	0.3	0.9
2.	100	0.8	1.05	0.75	1.25
3.	125	1.01	1.3	0.9	1.4

Escherichia coli were highly sensitive against the sample H2 compared to Standard in all concentration but sample H1 & H3 was moderate sensitive.

Table no- VII showing Antibacterial activity three Samples of Hinguleshwara Rasa (in different concentrations) on Salmonella typhi MTCC no.734

S. No.	Drug conc. mg/ml	Inhibition zone in different Sample (In cm)			
		H_1	H_2	H_3	Standard (Streptomycin)
1.	50	0.63	0.7	0.7	0.8
2.	100	0.9	0.98	1.0	1.15
3.	125	1.2	1.15	1.1	1.3

Salmonella typhi was highly sensitive against the all sample compared to Standard in all concentration.

Discussion & Conclusion

From the present study, the antimicrobial activity of three different samples of Hinguleshwara Rasa has got good antimicrobial property against all selected microbes. But H1 sample (according to Ayurvedic text) was more effective from other two samples H2 & H3 (according modified method). So it is presumed to conclude that these encouraging results obtained are purely based on invitro experimental methods. For establishment of authentic conclusion, in vivo efficacy on well diagnosed patients should also be carried out. And this study supports the therapeutic potential of said drugs as they have inhibited the growth of micro-organisms responsible for different diseases. However, proper understanding of formulation and its mechanism action appropriate models and parameters on pharmacological studies are necessary.

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सारांश:

पी.हनसमग्अत ते की जीवाणु प्रतिरोधक क्षमता की परीक्षा पाँच रोगोत्पादक जीवाणुओ पर की गई जो इस प्रकार है– स्टेफाइलोकोकस ओरियस , ई कोलाई , स्ट्रेप्टोकोकस पायोजिनस ,स्युडोमोनास ऐरुजिनोसा एवम् साल्मोनेला टाईफी ।

जीवाणु प्रतिरोधक क्षमता की परीक्षा के लिये ाच च्सम3 उम4क को काम मे लिया गया।इस अध्ययन हेतु पी.हनसमग्. अत ते की अलग –अलग सान्द्रता वाले विलियनो को तैयार कर उनका अध्ययन उपरोक्त लिखित जीवाणुओ पर किया गया । अध्ययन से प्राप्त परिणाम की तुलना समान सान्द्रता वाले मानक विलियन जो की स्ट्रेप्टोमाईसिन से तैयार किया गया था से की गयी। अध्ययन से प्राप्त तुलनात्मक परिणाम का विश्लेषण किया गया।