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Evaluation of Antibacterial Activity of Mrityunjaya Rasa

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ABSTRACT

Now a day's infectious diseases are posing problem for human beings. In order to avoid different infections, production and use of antibiotics is on rise. The widespread misuse of antimicrobials is responsible for emerging microbial resistance.

The development of bacterial resistance and adverse effect to presently available antibiotics has necessitated the search for new antibacterial agents in different systems of medicine. So *Mrityunjaya rasa* a known traditional medicine has been selected for this study.

The antibacterial activity of the *Mrityunjaya rasa* was tested against pathogenic bacterial strain Streptococcus pyogenes, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, and Salmonella typhi.

In order to study antibacterial action of *Mrityunjaya rasa* in vitro well diffusion method. During this study *Mrityunjaya rasa* was trailed with bacterial at different concentration. To correlate the result control solution were prepared by streptomycin. Experimental group were compared with control group and observation were noted.

Key wards – Mrityunjaya rasa, Antibacterial study, Bacteria etc.

सारांश

मृत्युन्जय रस 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 वैल डिफ्युजन मेथड को काम मे लिया गया।इस अध्ययन हेतु मृत्युन्जय रस 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।

INTRODUCTION

Now a day's infectious diseases are posing problem for human beings. 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 & are responsible for hypersensitivity reactions. Second important thing is this; the widespread misuse of antimicrobials is responsible for emerging microbial resistance.

The development of bacterial resistance and adverse effect to presently available antibiotics has necessitated the search for new antibacterial agents in different systems of medicine.

Thus the idea of less intrusive alternative is alluring so to overcome the problem like adverse effect and limited shelf life 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 *In vitro* (i.e. culture and sensitivity Tests). So *Mrityunjaya rasa* a known traditional medicine has been selected for this study.

Mrityunjaya 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-spleenomegaly), *Rajyakshma* (Pthysis), *Udar roga* (Abdominal disorders), *Pakshaghatha* (paralysis) and generally in all types of *Jwara* (fevers).

Different formulae of Mrityunjaya Rasa are available in the classical Rasashastra texts. For present study Formula of Mrityunjaya rasa described in Yogaratnakar, have been selected. It contains -- **Hingula** (Cinnabar), **Marich** (Piper nigrum), **Pippli** (Piper longum), **Tankana** (Borax), **Vatsanabha** (Purified Aconitum ferox), and **Gandhaka** (Sulphur) in equal proportion except **Hingula**. **Hingula** is double in quantity and ***Adraka Swarasa*** for trituration.

Aims and Objectives:
To Evaluate the Anti-bacterial activity of **Mrityunjaya Rasa** against common pathogenic bacteria.

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

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. 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

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.

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

Nutrient Agar

Beef extract	-	1.0 gm.
Yeast extract	-	2.0 gm.
Peptone	-	5.0 gm.
NaCl	-	5.0 gm.
Agar	-	15.0 gm.
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	-	15.0 gm.
Liver extract	-	2.5 gm.
Yeast extract	-	5.0 gm.
NaCl	-	5.0 gm.
Agar	-	15.0 gm.
Distilled water	-	1.0 L.

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 100^0C forming a slightly viscous liquid that solidifies at 42^0C. After solidification the agar will not melt unless the temperature is again raised to 100^0C. 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
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. In another flask containing distilled water, Blood Agar Base (21.25 gm/500 ml distilled water) was dissolved.

Both flasks were then plugged with cotton and autoclaved for complete sterilization. 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

- Sterilization of culture media was done by autoclaving at 15 lbs pressure for 20 minutes then media was taken out, kept on bench for a while.
- 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 labeled with details of medium, date, etc.

- About 30 ml. of media to be poured into each petridish, if too little agar is poured there may not be enough to cover the dish or the agar plate will dry up easily. If too much is poured, the cover dish will come in contact with the nutrient agar, leaving no room for microbial growth. The plates are rendered useless either way.
- The plates were left undisturbed until the agar solidified. Then the plates were kept at room temperature for overnight for observation of contamination.
- If contamination was there, the plates were discarded. If not contaminated, these plates were wrapped in a foil and kept in cold room at 4^0C for further use.
- The media and media plates were prepared time to time as per requirement and used for Antibacterial evaluation.

Evaluation of antimicrobial study

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

Well diffusion method

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

After than 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 in each media plates by using a sterile borer in suitable distance. 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.

OBSERVATIONS

Table No. I Showing Antibacterial activity of Mrityunjaya 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	0.38	1.51
2.	Staphylococcus aureus	0.32	0.48	0.85
3.	Pseudomonas aeruginosa	0.35	0.47	0.8
4.	Escherichia coli	0.42	0.5	0.8
5.	Salmonella typhi	0.34	0.55	0.85

Table No. II 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	0.4	0.7	1.1
2.	Staphylococcus aureus	0.4	0.7	0.98
3.	Pseudomonas aeruginosa	0.45	0.68	1
4.	Escherichia coli	0.6	0.95	1.1
5.	Salmonella typhi	0.5	0.85	1

RESULTS

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

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. ≤ 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 of Mrityunjaya rasa and moderately sensitive to 10.0% Concentration.
- E. coli, Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella Typhi was moderately sensitive to all Concentration of of Mrityunjaya rasa.
- No sensitivity was observed at 5.0% Concentration of Mrityunjaya rasa against Streptococcus pyogenes.

Thus by this view all Mrityunjaya rasa was highly effective against streptococcus pyogenes and less effective for other microbes.

DISCUSSION

In the present study, it has been observed that Mritunjaya 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 encouraging results obtained from antimicrobial study of Mrityunjaya Rasa are purely based on *in vitro* experimental methods.

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