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A Comparative Antimicrobial Study of Mritunjaya Rasa Prepared by two Different Method

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ABSTRACT

Number of Ayurvedic classical preparations was 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).

"***MRITYUNJAYA RASA"*** is one of the celebrated and most popular drug compounds mentioned for the management of *Jwara*. But, nowadays, it is routinely prescribed for curative measures in certain diseases especially Indigestion, Enteric fever, *Amavata, Yakrit pliha vikriti, Rajyakshma, Udar roga, Pakshaghata* and generally in all types of fevers.

Therefore, to make our treatment scientifically more validated, we can assess the antimicrobial activity of both preparations M1 & M2 In vitro (i.e. culture and sensitivity Tests). So *MRITUNJAYA RASA* is a very well-known traditional medicine has been selected for this study.

In this study we prepared *MRITUNJAYA RASA* by two different methods

1. Sample M2 With *Bhavana of Aadraka Swarasa* with reference of *Yoga Ratnakar*
2. Sample M1 With *Bhavana of Nimbu Swarasa* with reference of Ayurved *Sara Sangrah*

The difference between above formulations were that in sample M2 *bhavana* given by *adaraka swarasa* and in sample M1 *bhavana* given by *nimbu swarasa* was used in the place of *adaraka swarasa*. This was because, the preparation of sample M2 as per *yog ratanakar* and sample M1 As per Ayurveda *sara Sangrah*.

The aim of this study was to assess the comparative antimicrobial activity of different samples.

The above said formulations were subjected to antimicrobial investigations to determine their quality, standards, and anti-microbial effects on 5 pathogenic bacterial strain Streptococcus pyogenes, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, and Salmonella typhi. These pathogens are very common for fever.

In order to study antibacterial action of *MRITUNJAYA RASA* in vitro well diffusion method. During this study *MRITUNJAYA RASA* was trailed with bacteria at different concentrations. To correlate the result control solution was prepared by streptomycin. The experimental group was compared with control group and observation were noted.

The encouraging results obtained from anti-microbial study of both samples of *MRITUNJAYA RASA*. *MRITUNJAYA RASA*1 & *MRITUNJAYA RASA*2 was highly significant for some pathogen with the different concentration & moderately significant for some pathogen with the different concentration & no significant for some pathogen with the different concentration. So, an attempt was made to find a safe and effective ayurvedic medicine.

Key words – Preparation of *MRITUNJAYA RASA*1 & 2, Antimicrobial study of *MRITUNJAYA RASA*1 & 2 etc.

सारांश

मत्युंजय रस 1 & 2 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 मत्युंजय रस 1 & 2 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

Infectious diseases are posing problems for human beings in this modern era. 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 agents are local irritants & are responsible for hypersensitivity reactions. The 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 currently 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.

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 proven, we can assess the antimicrobial activity of such preparations In vitro (i.e. culture & sensitivity Tests). "*MRITYUNJAYA RASA*" is one of the celebrated and most popular drug compound mentioned for the management of *Jwara*.

But, nowadays, it is routinely prescribed for curative measures in certain diseases especially Indigestion, Enteric fever, *Amavata, Yakrit pliha vikriti, Rajyakshma, Udar roga, Pakshaghata* and generally in all types of fevers.

Many pharmaceutical companies prepare *MRITYUNJAYA RASA* by different references of Rasashastra Classics including special reference of A.F.I. They have different only in *bhawana dravya*. So, it is necessary to investigate their comparative efficacy.

Therefore, to make our treatment scientifically more validated, we can assess the antimicrobial activity of both preparations M1 & M2 In vitro (i.e. culture and sensitivity Tests). So *MRITUNJAYA RASA* is a very well-known traditional medicine and has been selected for this study.

Different formulas of *MRITUNJAYA RASA*[1] are available in the classical Rasashastra texts. For present study Formula of *MRITUNJAYA RASA*2 described in yoga *ratanakar* (U.K.A.12/20-25) P.222, and *MRITUNJAYA RASA*1 was described in ayurveda *sara sangrah* have been selected.

The Sample M2 was prepared with *Shuddha Hingula Churna, Shuddha Tankana Churna, Shuddha Gandhaka Churna, Shuddha Vatsanabha Churna, Maricha Churna, Pippali Churna.*

The above mixture was triturated with *Ardraka swaras* and made into pill form. The Sample M1 was prepared with *Shuddha Hingula Churna, Shuddha Tankana Churna, Shuddha Gandhaka Churna, Shuddha Vatsanabha Churna, Maricha Churna, Pippali Churna*.

The above mixture was triturated with *Nimba swaras* and made into pill form.

***MRITUNJAYA RASA*1 (M1)** It contains –

Table no. I

Sr. No.	Ingredients	Form	Quantity taken (in gm)
1	*Shuddha Hingula*	*Churna*	Twice
2	*Shuddha Tankana*	*Churna*	Equal
3	*Shuddha Gandhaka*	*Churna*	Equal
4	*Shuddha Vatsanabha*	*Churna*	Equal
5	*Maricha*	*Churna*	Equal
6	*Pippali*	*Churna*	Equal

Bhavana followed by *mardana* in a *kharal* with lemon juice. [1]

MRITUNJAYA RASA2 **(M2)** It contains –

Table no. II

Sr. No.	Ingredients	Form	Quantity taken (in gm)
1	*Shuddha Hingula*	*Churna*	Twice
2	*Shuddha Tankana*	*Churna*	Equal
3	*Shuddha Gandhaka*	*Churna*	Equal
4	*Shuddha Vatsanabha*	*Churna*	Equal
5	*Maricha*	*Churna*	Equal
6	*Pippali*	*Churna*	Equal

Bhavana followed by *mardana* in a *kharal* with *Adaraka* juice. [1]

Aims & Objectives

To Evaluate the Anti-bacterial activity of ***MRITUNJAYA RASA*1** & ***MRITUNJAYA RASA*2** against common pathogenic bacteria.

MATERIALS AND METHOD

For present study samples of ***MRITUNJAYA RASA*1** & ***MRITUNJAYA RASA*2** were taken and three different concentration solutions 50, 100, 125 (1mg/ml) were prepared of sample with solvent Dimethyl sulfoxide (DMSO). To tally the result control solution was also prepared by streptomycin in same concentration in same solution. [3]

Chemicals:

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

Glass wares and Polymer wares:

All the glassware was of sterilizable type and polymer wares were of disposable type.

Micro-organisms:

Micro-organisms selected for the this research work are those which cause general infections along with fever[5]. The pathogenic strains of different species of bacteria that are used for study were maintained on the following media as mentioned in table given below –

Table: III

S.No.	Species	MTCC No.	Media Used
1.	Streptococcus pyogenes [8]	1928	Blood Agar
2.	Staphylococcus aureus [7]	3160	Nutrient Agar
3.	Escherichia coli [10]	1652	Nutrient Agar
4.	Pseudomonas aeruginosa [9]	647	Nutrient Agar
5.	Salmonella typhi [11]	734	Nutrient Agar

The antibacterial analysis 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 mediums 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, 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 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 petri dishes, in laminar flow cabinet.
- Petri dish - 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 petri dish, 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 agar is poured, the cover dish will meet the nutrient agar, leaving no room for microbial growth. The plates are left useless either way.
- The plates were left undisturbed 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 not contaminated, these plates were wrapped in foil and kept in cold room at 4^0C for further use.
- The media and media plates were prepared from time to time as per requirement and used for Antibacterial evaluation.

Evaluation of antimicrobial analysis:
This was carried out on solid media. On solid media it was done by “Well diffusion method”.

Well diffusion method:
In this method 100µl 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 thin prepared medium was spread on media plates. It was allowed to dry for 30 minutes and then 2 holes

(each 0.3cm diameter) were made in each media plate by using a sterile borer in suitable distance. A total of 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 placed 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. [7]

Observations

Showing Antibacterial activity of ***MRITUNJAYA RASA*1** on different bacterial Strains:-

Table No. IV

S. No.	Name of Bacteria	Zone of Inhibition (cm.) in Different Concentrations (mg/ml)		
		50	100	125
1.	Streptococcus pyogenes	0.36	0.61	1.2
2.	Staphylococcus aureus	0.00	0.36	0.58
3.	Pseudomonas aeruginosa	0.31	0.59	0.91
4.	Escherichia coli	0.40	0.64	0.78
5.	Salmonella typhi	0.00	0.45	0.66

Showing Antibacterial activity of ***MRITUNJAYA RASA*2** on different bacterial Strains:-

Table No. V

S. No.	Name of Bacteria	Zone of Inhibition (cm.) in Different Concentrations (mg/ml)		
		50	100	125
1.	Streptococcus pyogenes	0.00	0.39	0.52
2.	Staphylococcus aureus	0.32	0.49	0.85
3.	Pseudomonas aeruginosa	0.32	0.46	0.82
4.	Escherichia coli	0.41	0.49	0.82
5.	Salmonella typhi	0.33	0.54	0.85

Table No. VI

Showing Antibacterial activity of Streptomycin on different bacterial Strains

S. No.	Name of Bacteria	Zone of Inhibition (cm.) in Different Concentrations (mg/ml)		
		50	100	125
1.	Streptococcus pyogenes	0.40	0.70	1.10
2.	Staphylococcus aureus	0.40	0.70	0.98
3.	Pseudomonas aeruginosa	0.45	0.68	1.00
4.	Escherichia coli	0.60	0.95	1.10
5.	Salmonella typhi	0.50	0.85	1.00

RESULTS

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

Table No. VII
Showing the relation between zones 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 extremely sensitive to 12.5% Concentration of sample M1, M2 and 10.0% Concentration of sample M1, M2, 5.0% Concentration of sample M1.
- E.coli was lightly sensitive to both Concentration of sample M1 & M2.
- Staphylococcus aureus was moderately sensitive to both Concentration of sample M1 & M2. except 5.0% Concentration of sample M1.
- Pseudomonas aeruginosa was moderately sensitive to both Concentration of sample M1 & M2.
- Salmonella Typhi was moderately sensitive to both Concentration of sample M1 & M2 except 5.0% Concentration of sample M1.
- No sensitivity was recorded at 5.0% Concentration of sample M2, against Streptococcus pyogenes; 5.0% Concentration of sample M1 while comparing against Salmonella Typhi & Staphylococcus aureus.
- Both samples of *MRITYUNJAYA RASA* have high sensitivity against Streptococcus pyogenes.

DISCUSSION

In the present study, it has been observed that sample **M2** which was prepared as per the Y.R. specifications found to be more effective than M1.

- Results which are given by all concentration of the sample M1 are comparatively better than sample M2 against streptococcus pyogenes and pseudomonas aeruginosa.
- Results which are given by 5%, 10% & 12.5% concentration of sample M2 is comparatively better against sample M1 against Salmonella Typhi.
- Result which is given by 5%, 10% & 12.5% concentration of sample M2 is also comparatively better against sample M1 Against staphylococcus aures.
- Results which is given by 5% & 12.5% concentration of sample M2 is also comparatively better against sample M1 Against Ecoli.

Variation in the results of antibacterial activity of sample M1, M2 could be attributed due to *bhavana dravya*.

M1 & M2 inhibits different microbes. The nature of these antimicrobial activities cannot be categorized in a predefined format. However, it is clear that different concentration solutions has its individual typical characteristics and differentiated action. But the exact clarification of these behaviors will be available only after detailed analysis with sophisticated equipment and techniques.

CONCLUSION

Upon comparing the effects of two samples of *MRITUNJAYA RASA* it was found that sample M2 which was prepared as per the Y.R. specifications found to be more effective against sample M1.

Upon comparing the effects of two samples of *MRITUNJYA RASA* it was found that sample M2 which was prepared by Y.R. specifications was found to be more effective against Ecoli, Staphylococcus aureus and Salmonella Typhi.

Upon comparing the effects of two samples of *MRITUNJYA RASA* it was found that sample M1 was found to be more effective against streptococcus pyogenes and pseudomonas aeruginosa.

The encouraging results obtained from antimicrobial study of *MRITUNJAYA RASA*1 & *MRITUNJAYA RASA*2 are purely based on in vitro experimental methods.

The encouraging results obtained from antimicrobial study of ***MRITUNJAYA RASA*1 & *MRITUNJAYA RASA*2** are purely based on in vitro experimental methods.

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