AYUSHDHARA
ISSN: 2393-9583 (P)/ 2393-9591 (O)
An International Journal of Research in AYUSH and Allied Systems

Research Article

A COMPARATIVE ANTIMICROBIAL STUDY OF *DEEPIKA RASA* PREPARED BY TWO DIFFERENT METHODS

Deepika Upadhyay[1]*, K.Shankar Rao[2]

*[1]Lecturer, Dept. of Ras shastra & B.K., MJF Ayurved College, Chomu, Rajasthan.

[2] Dean and Professor, Dept. of Ras shastra & B.K., Vijyashree Ayurvedic Medical College and Hospital, Jabalur, Madhya Pradesh .

Article info

Article History:
Received: 08-08-2022
Revised: 28-08-2022
Accepted: 15-09-2022

KEYWORDS:
Deepika rasa 1 & 2, Antimicrobial study, *Rasa Ratan Samuccaya.*

ABSTRACT

The Indian classic of Rasashastra have contribute numerous *Rasayoga* for various disease. In modern era for the development of Ayurvedic medicine with the proof scientifically and use of new search antibiotics to be increase, *Deepika rasa* is not popular traditional medicine so an attempt has been made to develop a safe & less expensive antimicrobial drug. *Deepika rasa* formulations are selected for the present study from *Rasa Ratan Samuccaya* (U.K.A12). *Deepika rasa* was indicated as "*Sarva Jwarhar Vinashanam*". *Deepika rasa* is one among *Kharaleeya Kalpana.* In this study we prepared *Deepika rasa* by two different methods: 1. *Deepika rasa* formulation- Rasa Ratan Samuccaya *(U.K.A.)* [D1] 2. *Deepika rasa* formulation- Modified method [D2]. The difference between above formulations was that *Rasa Sindoora* was used in the place of *Parad* and *Gandhak* in the formula two of *Deepika rasa.* This was because of the preparation *Rasa sindoora* was more potent as compared to *Kajjali* of first formula. The above said formulations were subjected to antimicrobial investigations to determine their quality, standards and anti-microbial effects on 5 pathogenic bacteria 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 *Deepika rasa* in vitro well diffusion method. During this study *Deepika 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 observations were noted. The encouraging results obtained from anti-microbial study of both sample of *Deepika rasa. Deepika rasa* 1 & *Deepika rasa* 2 was highly significant for some pathogen with the different concentration and moderately significant for some pathogen with the different concentration and no significant for some pathogen with the different concentration. So an attempt was made to find a safe and effective Ayurvedic medicine.

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.

Access this article online

Quick Response Code

https://doi.org/10.47070/ayushdhara.v9iSuppl1.1038

However all synthetic antimicrobial agent are local irritants and 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.

Deepika rasa is an organo-mineral preparation, used for '*Sarva Jvar Vinashanam'* and for many other diseases.[1] This product is not available in the market due to lake of standards, but at the same time it is being manufacturing by physicians in their day to day practice of life. Therefore, to make our treatment scientifically more validated, we can assess the antimicrobial activity of both preparations D1 & D2 *In vitro* (i.e., culture and sensitivity Tests). So *Deepika rasa* is not very known traditional medicine has been selected for this study.

Different formulae of *Deepika rasa* [1] are available in the classical Rasashastra texts. For present study formula of *Deepika rasa 1* described in *Rasa Ratan Samuccaya* (U.K.A.12/20-25)P.222, and *Deepika rasa* 2 was modified method have been selected.

The formula of *Deepika rasa* 1 (D1) in which the Mercury, Sulphur, *Naga bhasma, Pippali, Chitraka*, etc. drugs are given *Bhavana* with herbal juices and made into pill form.

The formula of *Deepika rasa* (D2) has prepared from *Rasa Sindoora, Naga bhasma, Pippali, Chitraka, Saindhav lavana* and *Souvarchal lavana* and given *Bhavana* with herbal juices and made into pill form.

Table 1: Formula of *Deepika rasa* 1 (D1)

S. No.	Ingredients	Form	Quantity taken (in gm)
1	*Shudhha Parada*	*Churna*	Equal
2	*Shudhha Gandhaka*	*Churna*	Equal
3	*Naga bhasma*	*Churna*	Equal
4	*Pippali*	*Churna*	Equal
5	*Chitraka*	*Churna*	Equal
6	*Saindhav lavana*	*Churna*	Equal
7	*Souvarchal lavana*	*Churna*	Equal

Bhavana followed by *Mardana* in a *Kharal* with lemon juice, cow's urine, goat urine, juice of *Meghnad* and juice of *Grita kumari*. [1]

Table 2:: Formula of *Deepika rasa (D2)*

S. No.	Ingredients	Form	Quantity taken (in gm)
1	*Rasa Sindoora*	*Churna*	Equal
2	*Naga bhasma*	*Churna*	Equal
3	*Pippali*	*Churna*	Equal
4	*Chitraka*	*Churna*	Equal
5	*Saindhav lavana*	*Churna*	Equal
6	*Souvarchal lavana*	*Churna*	Equal

Bhavana followed by *Mardana* in a *Kharal* with lemon juice, cow's urine, goat urine, juice of *Meghnad* and juice of *Grita kumari*.[1]

AIMS AND OBJECTIVES

To evaluate the anti-bacterial activity of *Deepika Rasa* 1 & *Deepika Rasa* 2 against common pathogenic bacteria.

MATERIALS AND METHOD

For present study samples of *Deepika rasa 1* & *Deepika rasa 2* 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. [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 sterilize 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 3: The pathogenic strains of different species of bacteria [2]

S.No.	Species	MTCC No.	Media Used (Himedia Lab Pvt. Ltd.)
1.	Streptococcus pyogenes [3]	1928	Blood Agar
2.	Staphylococcus aureus [2]	3160	Nutrient Agar
3.	Escherichia coli [5]	1652	Nutrient Agar
4.	Pseudomonas aeruginosa [4]	647	Nutrient Agar
5.	Salmonella typhi [6]	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 favourable 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°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

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 (21.25gm/500ml distilled water) was dissolved.

Both flasks were then plugged with cotton and autoclaved for complete sterilization. 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 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 labelled with details of medium, date, etc.
- About 30ml 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°C 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 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 that the 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. [3]

OBSERVATIONS

Table 4: Showing Antibacterial activity of *Deepika Rasa 1* on different bacterial Strains

S. No.	Name of Bacteria	Zone of Inhibition (cm.) in Different Concentrations (mg/ml)		
		50	100	125
1.	Streptococcus pyogenes	0.38	0.6	1.1
2.	Staphylococcus aureus	0	0.37	0.57
3.	Pseudomonas aeruginosa	0.32	0.6	0.9
4.	Escherichia coli	0.3	0.65	0.79
5.	Salmonella typhi	0	0.45	0.65

Table 5: Showing Antibacterial activity of *Deepika Rasa 2* 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	0.51
2.	Staphylococcus aureus	0.32	0.48	0.85
3.	Pseudomonas aeruginosa	0.35	0.47	0.8
4.	Escherichia coli	0.42	0.50	0.80
5.	Salmonella typhi	0.34	0.55	0.85

Table 6: 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 7: 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:-

- E.coli was moderately sensitive to all Concentration of sample D1 & D2.
- Streptococcus pyogenes was highly sensitive to 12.5% Concentration solution of sample D1. It was moderately sensitive to 12.5% Concentration of sample D1 & D2.

- Staphylococcus aureus was moderately sensitive to all Concentration of sample D1 & D2 except 5% Concentration of sample D1.
- Pseudomonas aeruginosa was moderately sensitive to all Concentration of sample D1 & D2.
- Salmonella Typhi was moderately sensitive to all concentration of D1 & D2 except 5% concentration of sample D1
- No sensitivity was observed at 5% Concentration of sample D1 against Salmonella typhi and staphylococcus aureus; 5% Concentration of sample D2 against Streptococcus pyogenes.

Variation in the results of antimicrobial activity of sample D1 & D2 could be attributed due to *Kajjali* & *Rasa Sindoor* respectively in sample D1 & D2

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

DISCUSSION

In the present study, it has been observed that sample D1 which was prepared as per the R.R.S. specifications found to be highly effective against Streptococcus pyogenes.

Result which is given by all concentration of sample D2 is comparatively better then sample D1 against Salmonella Typhi and Staphylococcus aureus.

Result which is given by 10%, 12.5% Concentration of sample D1 is comparatively better then sample D2 against Pseudomonas aeruginosa.

Result which is given by 5%, 12.5% Concentration of sample D2 is comparatively better then sample D1 against E.coli.

D1 & D2 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 behaviour will be available only after detailed analysis with sophisticated equipments and techniques.

CONCLUSION

On comparing the effects of two sample of *Deepika rasa* it was found that sample D1 which was prepared as per the R.R.S. specifications found to be highly effective against Streptococcus pyogenes.

On comparing the effects of two sample of *Deepika rasa* it was found that sample D2 which was prepared by *Rasa sindoor* (modified method) was found to be more effective against Salmonella Typhi and Staphylococcus aureus.

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

REFERENCES

1. Rasa Ratna Samucchya of Acharya Vagbhata with Vigyanbodhini Hindi commentary by Prof. D.A. Kulkarni, Reprint 1998. Published by Meherchand Laxman Das Publication, New Delhi. (U.K.A. 12/20-25) P.222
2. Short Textbook of Medical Microbiology by Shri Satish Gupte, 6th edition 1995 Published by Jaypee Brothers Medical Publishers (P.) Ltd, New Delhi., Cha.21, P. 181-188
3. Short Textbook of Medical Microbiology by Shri Satish Gupte, 6th edition 1995 Published by Jaypee Brothers Medical Publishers (P.) Ltd, New Delhi, Cha.22, P.192-197
4. Short Textbook of Medical Microbiology by Shri Satish Gupte, 6th edition 1995 Published by Jaypee Brothers Medical Publishers (P.) Ltd, New Delhi, P.202-205
5. Short Textbook of Medical Microbiology by Shri Satish Gupte, 6th edition 1995 Published by Jaypee Brothers Medical Publishers (P.) Ltd. New Delhi, P.256
6. Short Textbook of Medical Microbiology by Shri Satish Gupte, 6th edition 1995 Published by Jaypee Brothers Medical Publishers (P.) Ltd, New Delhi, P.268-271
7. Textbook of Microbiology by R.Ananthanarayana and C.K. Jayanam Paniker, 6th edition (reprint 2002), Published by Orient Longman pvt. Ltd.

Cite this article as:
Deepika upadhyay, K.Shankar Rao. A Comparative Antimicrobial Study of Deepika Rasa Prepared by Two Different Methods. AYUSHDHARA, 2022;9(Suppl 1):90-96.
https://doi.org/10.47070/ayushdhara.v9iSuppl1.1038
Source of support: Nil, Conflict of interest: None Declared

***Address for correspondence**
Dr Deepika upadhyay
Lecturer,
Dept. of Ras shastra & B.K.,
MJF Ayurved College, Chomu,
Rajasthan.
Email:
drdeepikaupadhyay@gmail.com
Mob. No.7000539495

Preparation of *Deepika rasa*


KAJJALI
KUPI
RASA SINDOOR SET-UP
AGNI
RESULT NECK
RASA SINDOOR
DEEPIKA RASA 1
DEEPIKARASA 2
DEEPIKA RASA 1 VATI SAMPLE
DEEPIKARASA 2 VATI SAMPLE