Int J Ayu Pharm Chem
ISSN 2350-0204
www.ijapc.com

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Comparative Anti-microbial Activity of *Rasapushpa* Prepared by Classical and Conventional Methods

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ABSTRACT

Introduction: The increasing prevalence of antibiotic-resistant pathogens has intensified the search for the safe and effective anti-microbial agents. *Rasapushpa*, a traditional Ayurvedic formulation with *Bhutavishapaha* (anti-microbial) properties, is indicated for microbial diseases like syphilis (*Phiranga*) and cholera (*Visuchika*) has not been widely explored till date. The research seeks to validate its traditional claims and explore its potential as an alternative or complement to conventional anti-microbial therapies in modern healthcare. **Aim:** To evaluate anti-microbial activity (*in vitro*) of *Rasapushpa* prepared by both methods. **Materials and methods:** This comparative experimental *in- vitro* study was conducted at the Microcare Laboratory & Tuberculosis Research Centre, Surat, using two samples of *Rasapushpa*: RPVY (classical method) and RPEMF (conventional method) was assessed using the Minimum Inhibitory Concentration (MIC) method by using different concentrations such as 5,25,50, 100 and 250 mg/mm to determine their efficacy against various pathogens like Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Vibrio cholerae, Candida albicans, Aspergillus niger and Mucor rouxii. **Result:** The anti-microbial study of *Rasapushpa*, prepared by both classical (RPVY) and conventional (RPEMF) methods, demonstrated its effectiveness against a range of microorganisms, including Vibrio cholerae, supporting its traditional use in treating *Visuchika* (Cholera). **Conclusion:** The anti-microbial study demonstrated that both RPVY and RPEMF are effective against a range of pathogens, including Vibrio cholerae and Mucor species, supporting their traditional use in treating *Visuchika* (Cholera) and fungal infections.

Key Words *Calomel, Mercurous Chloride, Syphilis, Cholera, Ayurvedic Mercurial Preparation*

Received 04th March 2025 Accepted 04th June 2025 Published 10th July 2025

INTRODUCTION

Man is closely influenced by the activities of microorganisms. They are part of our lives in more ways than most of us understand. They have shaped our present environment and their activities will greatly influence our future. Microorganisms should not be considered separate from human beings, but profound beneficial influence as a part of our life. They are

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employed in the manufacture of dairy products, certain foods, min processing of certain medicines and therapeutic agents, in manufacture of certain chemicals and in numerous other ways.

Despite the established useful functions and potentially valuable activities of microorganism, these microscopic dorms of life may be best known as agents of food spoilage and causal agents of human beings viz. Acquired immune deficiency syndrome [AIDS], herpes, influenza, jaundice, tuberculosis, typhoid, dermatomycoses, dysentery, malaria etc. in human beings. Animals (infected with brucellosis, tularaemia etc.) and plants infected with mildews, rusts, smuts, cankers, leaf spots, etc.) have also been known to be victims of microbial pathogens. So far as is known, all primitive and civilized societies have experienced diseases caused by microbes, frequently with disastrous results. Moreover, microorganisms have played profound roles in warfare, religion and the migration of populations. Control of microbial population is necessary to prevent transmission of disease, infection, decomposition, contamination and spoilage caused by them, man's personal comforts and convenience depend to a large extent on the control of microbial population.

In recent years, the quest for effective anti-microbial agents has gained considerable momentum. The emergence of bacterial resistance and the adverse effects associated with current antibiotics have highlighted the need for the discovery of new antibacterial agents across various medical systems. As a result, there is growing interest in exploring less invasive alternatives to address issues such as side effects and limited shelf life. Research is now focused on combining traditional antibiotics with natural substances to find more effective solutions. So, it is expected to contribute to the broader understanding of Ayurvedic formulations and their role in modern therapeutic practices.

Rasapushpa is a *Nirgandha, Saagni* and *Kanthastha* type of *Kupipakwa Rasayana* and is a sublimated mercurial preparation (Mercurous Chloride- Hg_2Cl_2). It is firstly mentioned by Rasatarangini with its synonyms, three preparation methods, therapeutic actions, indications and dosage guidelines. *Rasapushpa* contains *Shuddha Parada* (purified mercury), *Shuddha Kasisa* (purified green vitriol) and *Saindhava* (rock salt)[1]. *Rasapushpa* possesses *Bhutavishapaha* (Anti-microbial) property and *Rasapushpa* has been traditionally used to treat a wide range of conditions, including *Hikka, Jalodara, Krimi* and also indicated in the diseases like *Phiranga* (Syphilis) and *Visuchika* (Cholera) which are caused by microbes[2]. Emerging in the medieval period, it reflects the integration of alchemical principles with Ayurvedic practices, aiming to enhance the therapeutic efficacy of mercury-based compounds. Thus, *Rasapushpa* has emerged as a subject of interest due to its reputed therapeutic benefits.

This study aims to address this gap by investigating the anti-microbial properties of *Rasapushpa* through *in-vitro* experimentation. By evaluating its effects against a spectrum of

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pathogenic microorganisms, here is an attempt to provide empirical evidence supporting its traditional claims and to explore its potential as a viable alternative or complement to conventional anti-microbial therapies.

Hence, an attempt was made to screen the anti-microbial potential of *Rasapushpa* prepared by classical (*Valukayantra*- RPVY) and conventional method (Electric muffle furnace- RPEMF) in the control prevention of microbial infection against Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Vibrio cholerae, Candida albicans, Aspergillus niger and Mucor rouxii.

OBJECTIVES

- To evaluate anti-microbial activity (*in-vitro*) of *Rasapushpa* prepared by two methods.

MATERIALS AND METHODS

The objective of this study was to evaluate the *in-vitro* antimicrobial activity of *Rasapushpa* prepared by two different methods. A comparative experimental *in-vitro* study was conducted at the Microcare Laboratory & Tuberculosis Research Centre, Surat. The test substance, *Rasapushpa*, was prepared using two methods: RPVY- which was prepared by the classical *Valukayantra* method and RPEMF- which was prepared using the conventional electric muffle furnace method. The antimicrobial activity of both samples was assessed using the Minimum Inhibitory Concentration (MIC) method by using different concentrations such as 5,25,50, 100 and 250 mg/mm to determine their efficacy against various pathogens**.**

Study Design

1. All the trial drugs and standard drugs used for study

Table 1 Grouping of trial drugs with standard control and group code

Group no.	Drugs	Group code
1.	*Rasapushpa* prepared by classical *Valukayantra* method	RPVY
2.	*Rasapushpa* prepared by conventional method (electric muffle furnace)	RPEMF
3.	Ampicillin	Ampicillin
4.	Chloramphenicol	Chloramphenicol
5.	Ciprofloxacin	Ciprofloxacin
6.	Norfloxacin	Norfloxacin
7.	Nystatin	Nystatin

Vehicle: Dimethyl sulfoxide (DMSO) was used as a diluent/vehicle to get the desired concentration of drugs to test upon standard bacterial strains. Mueller Hinton Broth was used as a nutrient medium to grow and dilute the drug suspension for the test. The inoculum size for test strain was adjusted to 10^8 Cfu (Colony Forming Unit) per millilitre by comparing the turbidity.

2. All standard strains used for study

Following common standards strains were used for screening of antibacterial and antifungal activities. The strains were procured from Institute of Microbial Technology, Chandigarh.

Table 2 Common standards strains used for study

Sr.no.	Microbial culture	Microbial strains

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1.	Gram-negative	Escherichia coli MTCC-442
2.	Gram-negative	Klebsiella pneumoniae MTCC-109
3.	Gram-negative	Vibrio cholrae MTCC-906
4.	Gram-positive	Staphylococcus aureus MTCC-96
5.	Fungus	Candida albicans MTCC-227
6.	Fungus	Aspergillus niger MTCC-282
7.	Fungus	Mucor rouxii MTCC 386

3. All Necessary Controls

- Drug Control
- Vehicle Control
- Agar Control
- Organism Control
- Known Antibacterial Drugs Control

Evalution techniques

➢ In evaluating antimicrobial activity, it is essential to ensure intimate contact between the test organism and the substance being tested, while providing the necessary conditions for microbial growth. Uniform conditions must be maintained throughout the study and an aseptic environment should be strictly followed. Various methods are employed to assess antimicrobial effectiveness, including the turbidimetric method, agar streak dilution method, serial dilution method and agar diffusion method.

➢ Among these, the agar diffusion method is commonly used, with techniques such as the agar cup method, agar ditch method and paper disc method. In our study, we have employed the agar cup method to evaluate antimicrobial activity. This non-automated *in vitro* test involves the formation of a zone of inhibition, measured in millimetres, to determine the concentration of antimicrobial agents required to inhibit bacterial growth. The test is performed on petri plates, providing reliable results for bacterial susceptibility.

Study method: Agar cup diffusion method

➢ Liquefy a suitable medium for the assay conditions and inoculate it at an appropriate temperature (e.g., 48° to 50°C for vegetative forms) with a known quantity of microorganism suspension sensitive to the antibiotic under examination, ensuring clearly defined zones of inhibition at the antibiotic concentrations used. Immediately pour the inoculated medium into Petri dishes or large rectangular dishes to form a uniform layer 2 mm to 5 mm thick, or use two layers, with only the upper layer inoculated. Store the dishes to prevent appreciable growth or death of microorganisms before use, ensuring the surface of the medium is dry. Prepare solutions of the reference substance and the antibiotic in known concentrations and presumed equal activity using the solvent and buffer solution. Apply equal volumes of these solutions to the medium surface, such as in cavities prepared in the agar.

RESULTS

Table 3 Antibacterial activities of trial drugs and standard drugs against E. Coli

ZONE OF INHIBITION [ZOI] [micrograms/mm]						
Sr. no.	**Code no.**	**E. Coli MTCC 443**				
		5	**25**	**50**	**100**	**250**
1.	RPVY	-	11	13	17	21
2.	RPEMF	-	12	15	16	20
3.	Ampicillin	14	15	16	19	20
4.	Chloramphenicol	14	17	23	23	

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5.	Ciprofloxacin	20	23	28	28	28
6.	Norfloxacin	22	25	26	27	29

Table 4 Antibacterial activities of trial drugs and standard drugs against Kl. Pneumoniae

ZONE OF INHIBITION [ZOI] [micrograms/mm]						
Sr. no.	**Code no.**	**Kl. Pneumoniae MTCC 109**				
		5	**25**	**50**	**100**	**250**
1.	RPVY	-	9	11	14	19
2.	RPEMF	-	10	13	15	18
3.	Ampicillin	10	13	14	16	18
4.	Chloramphenicol	12	14	19	20	21
5.	Ciprofloxacin	17	19	21	22	22
6.	Norfloxacin	19	22	25	26	28

Table 5 Antibacterial activities of trial drugs and standard drugs against V. Cholerae

ZONE OF INHIBITION [ZOI] [micrograms/mm]						
Sr. no.	**Code no.**	**V. Cholerae MTCC 906**				
		5	**25**	**50**	**100**	**250**
1.	RPVY	-	10	12	14	18
2.	RPEMF	-	11	14	18	20
3.	Ampicillin	11	14	16	18	19
4.	Chloramphenicol	10	13	19	20	20
5.	Ciprofloxacin	16	19	21	21	22
6.	Norfloxacin	18	19	20	21	21

Table 6 Antibacterial activities of trial drugs and standard drugs against S. Aureus

ZONE OF INHIBITION [ZOI] [micrograms/mm]						
Sr. no.	**Code no.**	**S. Aureus MTCC 96**				
		5	**25**	**50**	**100**	**250**
1.	RPVY	-	10	14	18	21
2.	RPEMF	-	10	15	19	20
3.	Ampicillin	10	13	14	16	18
4.	Chloramphenicol	12	14	19	20	21
5.	Ciprofloxacin	17	19	21	22	22
6.	Norfloxacin	19	22	25	26	28

Table 7 Antifungal activities of trial drugs and standard drug against C. Albicans

ZONE OF INHIBITION [ZOI] [micrograms/mm]						
Sr. no.	**Code no.**	**C. Albicans MTCC 227**				
		5	**25**	**50**	**100**	**250**
1.	RPVY	-	12	14	17	19
2.	RPEMF	-	10	12	13	18
3.	Nystatin	18	19	24	29	29

Table 8 Antifungal activities of trial drugs and standard drug against A. niger

ZONE OF INHIBITION [ZOI] [micrograms/mm]						
Sr. no.	**Code no.**	**A. Niger MTCC 282**				
		5	**25**	**50**	**100**	**250**
1.	RPVY	-	11	13	15	18
2.	RPEMF	-	9	12	15	17
3.	Nystatin	18	19	24	29	29

Table 9 Antifungal activities of trial drugs and standard drug against Mucor Rouxii

ZONE OF INHIBITION [ZOI] [micrograms/mm]						
Sr. no.	**Code no.**	**Mucor Rouxii MTCC 386**				
		5	**25**	**50**	**100**	**250**
1.	RPVY	-	10	12	15	17
2.	RPEMF	-	10	13	16	18
3.	Nystatin	18	19	24	29	29

DISCUSSION

This study evaluates the antimicrobial potential of *Rasapushpa*, known for its *Bhutavishapaha* (anti-microbial) properties, using the Minimum Inhibitory Concentration (MIC) method. *Rasapushpa*, prepared by classical and conventional methods, showed significant inhibition against pathogens including Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Vibrio cholerae, Candida albicans, Aspergillus niger and Mucor rouxii. These results align with Ayurvedic references, particularly in treating cholera (Vibrio cholerae) and Mucormycosis (Mucor rouxii). The findings

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highlight potential of *Rasapushpa* as a therapeutic option in combating antimicrobial resistance (AMR), especially in resource-limited areas, with comparable efficacy to standard drugs.

Table 10 Comparative Zone of Inhibition of trial drugs against various pathogens

Sr. No.	Species	Concentration	ZOI [micrograms/mm]	
			RPVY	RPEMF
1.	**E. Coli MTCC 443**	25	11	12
		50	13	15
		100	17	16
		250	21	20
2.	**Kl. Pneumoniae MTCC 109**	25	9	10
		50	11	13
		100	14	15
		250	19	18
3.	**V. Cholerae MTCC 906**	25	10	11
		50	12	14
		100	14	18
		250	18	20
4.	**S. Aureus MTCC 96**	25	10	10
		50	14	15
		100	18	19
		250	21	20
5.	**C. Albicans MTCC 227**	25	12	10
		50	14	12
		100	17	13
		250	19	18
6.	**A. Niger MTCC 282**	25	11	9
		50	13	12
		100	15	15
		250	18	17
7.	**Mucor Rouxii MTCC 386**	25	10	10
		50	12	13
		100	15	16
		250	17	18

CONCLUSION

Anti-microbial study of RPVY and RPEMF samples showed their effectiveness against all microorganisms and both samples were the same effective as reference drugs against Vibrio cholerae supporting the textual reference of *Rasapushpa* indicated in *Visuchika* (Cholera). Both samples were also proven effective against Mucor species, especially in the context of rising Mucormycosis cases among COVID-19 patients. Its efficacy suggests it could serve as an alternative treatment, addressing both fungal infections and the challenge of anti-microbial resistance.

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