

## Pharmaceutical Standardization

# Evaluation of antimicrobial activity of *Rasaka Bhasma*

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### Abstract

*Rasaka*, one among the *Maharasas*, has been indicated in various diseases like *Netrarogas*, *Prameha*, etc. Lately, the use of *Rasaka bhasma* has been declined as an identification of *Rasaka*. The present study aims to prepare *Rasaka bhasma* from two different samples of *Rasaka* and undertake comparative antimicrobial activity study against Gram-positive and Gram-negative organisms by agar disk diffusion method.

**Key words:** Agar disk diffusion method, antimicrobial activity, gram negative, gram positive, *Rasaka*.

## Introduction

Antimicrobial activities of any therapeutic agent are understood by its degree of growth inhibition of microorganisms as well as bacterial property.

Usually different microbial species and strains have different degrees of susceptibility to therapeutic agents. The susceptibility of microorganisms can change with time even during therapy with a specific drug. Thus, it is essential for the physician to know the sensitivity of the pathogen before treatment.

### Antibacterial study

The antibacterial activity of a drug is generally expressed as its inhibiting effect toward the growth of the bacterium in nutrient broth or nutrient agar.

For this study, the following conditions are required.

1. The substance or test drug must be in contact with the test organisms.
2. Conditions must be favorable for the growth of microorganisms in the absence of antimicrobial substances.
3. There must be a means of estimating the amount of growth and thereby percentage of growth of inhibition.
4. The activity of test drug should be observed and determined by the growth response of microorganisms.

### Aims and objectives

The aim of present study was to evaluate the antibacterial property of *Rasaka bhasma* by agar disk diffusion method.

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## Materials and Methods

### Diffusion method/Agar plate method/Cup plate method

Cup plate method is one of the official methods in IP, where the test samples diffuse from the cup through an agar layer in a Petri dish or plate to such an extent that the growth of added microorganisms is restricted entirely to a circular area or zone around the cavity containing the solution of an antibiotic substance.<sup>[1]</sup>

The antimicrobial activity is expressed as zone diameter in millimeters, which is measured by a scale.

### Organisms

Gram-positive organisms used in the study were *Staphylococcus aureus*, *Streptococcus pyogenes* and *Streptococcus pneumoniae*. Gram-negative organism used was *Klebsilla pneumoniae*.

### Standards used in the study

Oflaxcin 5 µg (Himedia labs, Mumbai, India.) was used for Gram-positive and Gram-negative organisms.

### Preparation of test/stock solution

Suspension of *Rasaka bhasma* A was prepared with the following:

*Rasaka bhasma* sample A: 100 mg

Tween 80: 1 g

Distilled water: 10 ml

Thus, the final concentration of the test solution obtained was 10 mg/ml.

Suspension of *Rasaka bhasma* B was prepared with the following:

*Rasaka bhasma* sample B: 100 mg

Tween 80: 1 g

Distilled water: 10 ml

Thus, the final concentration of the test solution obtained was 10 mg/ml.

## Materials used

- Nutrient agar
- Sterile petri dishes
- Sterile micropipette
- Sterile cotton swab
- Sterile cork borer
- Sterile test tubes containing the solutions of the test compounds of desired concentrations.

## Preparation of nutrient agar

Definite volumes of peptone (0.6%), yeast extract (0.15%) and di-potassium dihydrogen phosphate (0.36%) were dissolved in distilled water and the pH was adjusted to 7.2. This solution was sterilized by autoclaving at 15 psi for 20 minutes.

## Preparation of peptone water

Definite volumes of peptones (0.6%), yeast extract (0.3%) and beef extract (0.13%) were dissolved in distilled water and the pH adjusted to 7.2 and sterilized by autoclaving.

## Preparation of subculture

One day prior to the testing, inoculations of the above bacterial cultures were made in the nutrient agar and incubated at 37°C for 18–24 hours.

## Preparation of base layer medium

Base layer medium was prepared with Muller-Hinton agar (Micro-master lab., Maharashtra, India).

Its formula per liter is as follows:

Beef infusion: 300.0 g

Casein acid hydrolysate: 17.5 g

Starch: 1.5 g

Agar: 17.0 g

Final pH (at 25°C): 7.3 ± 0.2.

## Directions

The medium was prepared by dissolving 38 g of medium in 1 l of distilled water. The solution was heated to boiling to dissolve the medium completely and sterilized by autoclaving at 120°C, 15 lbs pressure for 15 minutes. Then it was mixed well and poured into sterile Petri dishes using aseptic conditions.

## Sterilization

Sterilization of the medium, tubes, borer, etc. was done by autoclaving at 15 lbs/inch<sup>2</sup> for 20 minutes. The glass wares like syringes, Petri dishes, pipettes and empty test tubes were sterilized by dry heat in an oven at a temperature of 160°C for 1 hour.

## Preparation of agar plates

The Petri dishes which measured around 32 cm diameter and 2 cm thickness were selected after sterilizing by dry heat in an oven. Base layer was obtained by pouring around 20–30 ml of Muller Hinton Agar solution to obtain a thickness of 4 mm. It was then kept for solidification.

The overnight grown subculture was taken in definite volumes of peptone water and incubated at 37°C at least for 2–4 hours prior to plating. After incubation with the help of cotton swab, the organisms were streaked on Petri dish containing base layer medium.<sup>[2]</sup>

## Experimental procedure

- The sterile borer was used to prepare six cups of 8 mm diameter, in the medium of each Petri dish.
- At the center, one more cup was made for standard drug; its zone of inhibition was measured to compare with the zone of inhibition of the test drug.
- At the right side of the Petri dish, three cups were marked as A, where *Rasaka bhasma* sample A (prepared using ZnCO<sub>3</sub>) was introduced with the help of a micropipette at concentrations of 10, 25, and 50 µl, respectively.
- Left side of the Petri dish had three cups marked B in which suspension prepared out of *Rasaka bhasma* sample B (prepared using ZnO) was introduced with a micropipette at concentrations of 10, 25, and 50 µl, respectively.
- At the center of the Petri dish, control drug, i.e., Ofloxacin 5 µg was placed.
- All the plates were kept at room temperature for effective diffusion of the test drug and standard.
- Later, they were incubated at 37 ± 1°C for 24 hours.
- The presence of definite zones around the cup of any size indicated antibacterial activity.
- The diameter of the zone of inhibition was measured and recorded.

## Results and Observation

- The drug concentration in each microliter of test solution was as follows:  
10 µl: 100 µg  
25 µl: 250 µg  
50 µl: 500 µg
- The zone of inhibition was measured by a scale and the measurements are tabulated [Table 1].

**Table 1: Zone of inhibition measured in mm**

Drug		Zone of inhibition (mm)			
		<i>Staphylococcus aureus</i>	<i>Streptococcus pyogenes</i>	<i>Streptococcus pneumoniae</i>	<i>Klebsiella pneumoniae</i>
Sample A <sub>2</sub>	10 µl	17	20	17	12
	25 µl	20	25	20	15
	50 µl	24	29	23	16
Sample B <sub>2</sub>	10 µl	15	19	15	10
	25 µl	19	20	17	12
	50 µl	21	24	20	14
Standard Ofloxacin	5 µl	28	30	25	18

## Discussion

Both the samples were studied for their antimicrobial activity on *Sta. aureus*, *Str. pyogenes*, *Str. pneumoniae* and *K. pneumoniae*.

The sample contains zinc salts which is an inorganic material not soluble in any organic solvents to prepare its solution. Hence, the suspension was prepared by using "Tween 80" as a surfactant.

The samples were assessed by agar method, by comparing zone of inhibition between test drug against control drug. It was found that the drug showed better antimicrobial result against *Streptococcus* compared to other organisms. Between the two samples, *bhasma* prepared out of  $ZnCO_3$  showed better results than that prepared with  $ZnO$ .

## Conclusion

- Antimicrobial result of *Rasaka bhasma* prepared out of  $ZnCO_3$  has shown a better result than *Rasaka bhasma* prepared out of  $ZnO$ .
- *Bhasma* prepared out of  $ZnCO_3$  has shown better antimicrobial results over *Str. pyogenes* compared to other organisms at the concentration of 50  $\mu$ l.

## Reference

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## हिंदी सारांश

### रसक भस्म के जीवाणुप्रतिबंधक प्रभाव का विश्लेषण

शुभा एच. एस., आर.एस.हिरेमठ

रसक जो महारसों में एक है, अनेक व्याधियों में उल्लेखित है उदा. प्रमेह, नेत्र रोग आदि। आज कल इसका उपयोग कम हो रहा है। यह अध्ययन उचित रसक भस्म निर्माण और उसका जन्तुघ्न क्रिया का परीक्षण करने के हेतु से लिखा गया है।