

COMPARATIVE ANTI MICROBIAL STUDY OF SHUDDHA KASISA AND KASISA BHASMA

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ABSTRACT

In Rasa shastra, minerals are categorized as Maharasa, Uparasa and Sadharana rasa based on different criteria. Kasisa, one among the uparasa¹ is being therapeutically used since centuries. Kasisa Bhasma has Ushna virya, Kashaya amla rasa properties. It act as netrya, vishaghna, Kapha Vata nashaka, Vranaghna, Svitraghna, Kshayaghna, Kesharanjaka. It is also Kandughna, Pandughna, Krimighna, Rakta sanjanana, Raja pravartaka, Balya, Jwaraghna, Pleehanashana² So an attempt was made to comparatively analyze the antimicrobial properties of shuddha Kasisa and Kasisa Bhasma by in Vitro study. Qualitatively prepared shuddha kasisa and kasisa bhasma was evaluated against staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Candida albicans and compared with standard drugs like ampicillin, gentamycin and amopteracin. In the present study, sensitivity testing was done by disc diffusion technique pattern.

Key words: Shuddha kasisa(SK), Kasisa bhasma(KB), Ampicillin, Gentamycin, Amopteracin.

INTRODUCTION

The antimicrobial activities of any therapeutic agent are understood by the degree of growth inhibition of microorganisms it produces as well as its bactericidal property. Usually different microbial species or even 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. In present the study, the antibiotic, antibacterial and antifungal effect of ferrous sulphate (Kasisa) is evaluated. Shodhana of Kasisa was performed according to Rasa Tarangini 21/230 (Bhavana). Marana of Kasisa was done as per the method explained in Rasatarangini 21/259.

Antimicrobial study

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The antimicrobial activity of a drug is generally expressed as its inhibitory action on the growth of the bacterium in nutrient broth or nutrient agar.

For the purpose of 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 inhibition of growth.
4. The activity of test drug should be observed and determined by the growth response of microorganisms.

Procedure

Bacterial strain used

- **Gram negative Strain** - Escherichia coli(NCIM 2574) and Pseudomonas aeruginosa (NCIM 2036)
- **Gram positive Strain** - Staphylococcus aureus (NCIM 2079)
- **Fungal Strain** – Candida albicans
- **Media used**- Mueller Hinton agar, Mueller Hinton broth, Sabouraud dextrose broth

and Sabouraud dextrose agar.

Standard drug disc – Gentamycin (10 µg/disc) for Gram negative, Ampicillin (10 µg/disc) for Gram positive, Amphotericin B (20 µg/disc) for fungi

PROCEDURE

Preparation of Inocula³

For preparation of inoculum, growth from the agar slant was scrapped by adding 3 ml of sterile saline solution. This saline cell suspension was then spread evenly on large sterile Petri plates containing solidified Muller Hinton agar (for bacteria) and

Sabouraud dextrose agar (for fungi) using a sterile glass spreader. These plates were incubated in bacteriological incubator at 37⁰C for 24 hours and at 28⁰C for 48 hours for bacteria and fungi respectively. After profuse growth of the organism in the Petridish, it was scrapped using sterile spatula and adding small portion of sterile saline. This suspension was transferred to a sterile 100ml conical flask. The final volume of the suspension was made upto 50ml with sterile saline.

Standardization of Inocula

For the determination of MIC, the inoculum density was adjusted to contain 5 x 10⁶ CFU/ml which has turbidity equal to 0.5 McFarland standard. For this, 0.5 McFarland standard was prepared by adding 0.05ml of 0.048M BaCl₂ (1.17% w/v BaCl₂.2H₂O) to 9.95ml of 0.18M H₂SO₄ (1% w/v) with constant stirring. The standard was transferred to a glass screw capped bottle. Absorbance of the McFarland standard was checked at 625nm (absorbance at 625nm should range between 0.08- 0.13).

Preparation of drug Dilution

Each drug was suspended in sterile water with the help of 1% tween 80 at the concentration of 1mg/ ml. Sterile water with 1% tween 80 was also prepared to use a blank for the drug.

Disk Diffusion Assay

Disk diffusion assay of drug was performed in 40 mm Petri plates to observe growth inhibition of test organism in term of zone of diameter (mm). Mueller Hintonagar (3 in No.) and Sabouraud dextrose agar (1 in No.) medium was prepared and sterilized. Molten agar media were poured into sterile Petri plates (4 in No.) and left for Solidification. Each plate was neatly labeled with all the details. Each plate was divided into four parts which were labeled as ST, SK, KB and

BL. Standard cultures (100 µl of each) of test organism were transferred to the respective solidified agar aseptically. Transferred cultures were uniformly distributed all over the surface of agar medium using L spreader. Respected standard was kept in the center of the respective part of the plate. Three sterile discs were transferred into the

remaining parts of the plate. Each sterile disc was loaded with the 20 µl of respective drug or blank. First, Petri plates were kept in fridge for 30 min. for drug diffusion and then transferred into the respective incubator. Zone of inhibition were measured using antibiotic zone reader after 24 h for bacteria and 48 h for fungi.

Table No 1 Report of antimicrobial assay of Shuddha Kasisa

Name of tested organism	Zone Diameter (mm) of Growth Inhibition		
	Test drug	Blank	Standard drug
E.coli	12	9	21
P. aeruginosa	10	8	32
S. aureus	-	-	24
C. albicans	-	-	8

Table No 2 Report of antimicrobial assay of Kasisa Bhasma

Name of tested organism	Zone Diameter (mm) of Growth Inhibition		
	Test drug	Blank	Standard drug
E.coli	10	9	21
P.aeruginosa	8	8	32
S.aureus	-	-	24
C.albicans	-	-	8

Results

Shuddha Kasisa was found to be partially active against the used species of Gram negative bacteria at the tested concentration, while it was found to be inactive against the Gram positive and the fungal strain.

DISCUSSION

Comparative evaluation of Shuddha Kasisa and Kasisa Bhasma for antimicrobial potential was the main aim of the study. Based on the claim of Krimighna property of Kasisa Bhasma and external use of Shuddha Kasisa for wound healing, the antimicrobial study was planned. Disc diffusion method was followed. As a standard protocol Gram Negative Strain - Escherichia coli (NCIM 2574) and Pseudomonas aeruginosa (NCIM 2036),

Gram positive Strain – Staphylococcus aureus (NCIM2079) and **Fungal Strain** – Candida albicans were selected. Standard drugs used were Gentamycin (10 µg/disc) for Gram negative, Ampicillin (10 µg/disc) for Gram positive and Amphotericin B (20 µg/disc) for fungi. The products were found to be partially active against the used species of Gram negative at tested concentration while found to be inactive against the Gram positive and the fungal strain. Shuddha Kasisa was found to be slightly better in antimicrobial activity as the zone of inhibition was slightly higher than that of Kasisa Bhasma (for E coli 12mm against 10 mm of Kasisa Bhasma), which may be due to better solubility and highly acidic pH of Shuddha Kasisa. However, the zone of inhibition was far low when compared to the

standard drugs. However, using the media in which the products are more soluble may be useful. Multiple strains of microorganisms may have to be tried. Mode of action

of Kasisa Bhasma and Shuddha Kasisa may be different than that of antimicrobial agents. Evaluation of antioxidant profile and wound healing activity may be more useful.

CONCLUSION

The products were found to be partially active against the used Gram negative at tested concentration while found to be inactive against Gram positive and fungal strain. Shuddha Kasisa was found to be slightly better in activity as the zone of inhibition was slightly higher. than that of Kasisa Bhasma (for E coli 12mm against 10 mm of Kasisa Bhasma) which may be because of the better solubility and highly acidic pH of Shuddha Kasisa.

Figures:

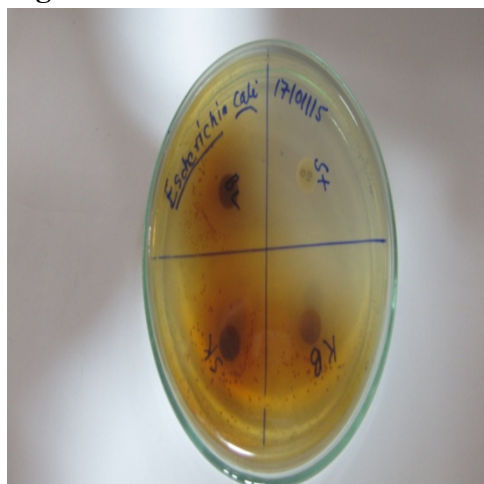


Figure1: Escherichia coli



Figure2: Pseudomonas aeruginosa

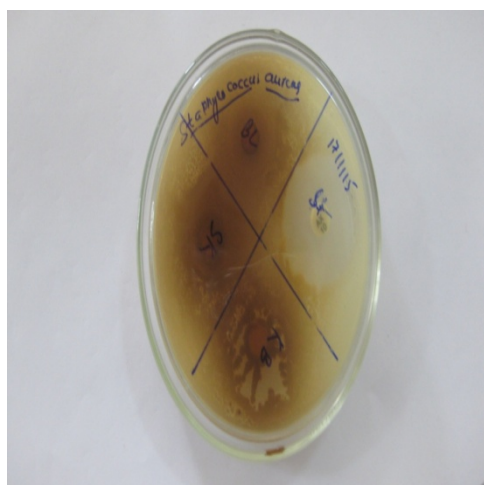


Figure3: Staphylococcus aureus



Figure 4: Candida albicans

REFERANCE

1. Shri Vagbhata, Rasarathna samuchchaya, with Hindi Commentary of Ras Prabha, Translate by Indra Dev Tripathi, Chaukhamba Sanskrit Sansthan, Varanasi, edition 2009, 3rd Chapter, page no.26. Pp418.
2. Shri Sadananda Sharma, Rasa Tarangini by Pandith Kashinath Shastri, Motilal Banarasidas, New Delhi, edition 2000, 21th Chapter,page no.564,Pp772.
3. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-First Informational Supplement. CLSI document M100S21 (ISBN 1-56238-742-1). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania19087 USA, 2011.

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