

## ANTI-MICROBIAL ACTIVITY OF BRAHMA RASA- IN VITRO STUDY

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**ABSTRACT**

Antibiotics are certainly considered one among our most essential weapons in combating bacterial and fungal infections and have significantly benefited the health. Associated high-satisfactory of human life since their introduction. However, during the last few decades, these health benefits are under risk due to much less impact of antibiotics towards certain illness not, only due to the fact a lot of them produce toxic reactions, but additionally due to emergence of drug resistant bacteria. so it is important to analyze newer drugs having anti-microbial activity. Some formulations in Ayurveda containing both herbal and mineral act as Krimighnata by herbal drugs, rapid and accurate in their action by their mineral combination. Brahma Rasa is one of such various Khalviya Rasayana which is indicated in Mandala Kushta is compared to psoriasis can be caused by streptococcal infections. The present study was achieved to validate the anti-microbial action of Brahma Rasa by In-Vitro method.

**KEYWORDS:** Antibiotics, Agar-well diffusion method, Brahma Rasa, Krimighnata, MIC Method, Rasa-Sindoora.

**INTRODUCTION**

Now a day the Antimicrobial resistance (AMR) has emerged as a major global threat, the inappropriate use of Antibiotics has resulted in more and more bacterial strains developing resistance over the last few decades with fatal consequences. According to World Health Organization (WHO) estimates, around 25,000 people die each year from AMR infections in Europe alone. On prolonged usage of antibiotics having side effects like Gynaecomastia, Nephro-toxicity, etc. hence there is a need for establishment of new antimicrobial compound with lesser resistance.

Some of formulations in Ayurveda containing both herbals and minerals act as Krimighnata by herbal drugs, fast and accurate in their action by their mineral combination. There are huge numbers of formulations in Ayurvedic Classics which act as a Antimicrobial but they are yet to explore to replace the active compounds of Allied Sciences. Among four major Rasayana Kalpanas, Khalviya Rasayana is the one among them and is the Primary and essential Pharmaceutical Technique as these are used as an ingredient of other three Rasayanas. The speciality of this lies in binding different varieties of drugs into a single molecular form and thereby minimizing dose and more efficacious in their action.

Brahma Rasa is one among the Khalviya Rasayana which is explained in Rasendra Chintamani, which consists of Rasa Sindoora, Shodhita Gandhaka, Chitrakamula Churna, Bakuchi Churna, Palashabeeja Churna, Guda and Madhu. It will act as a Kaphavatashamaka, Kushtaghna and Jantughna. Indicated in Mandala and Prasupta Kushta. Shuddha Gandhaka is having Krimighna Karma and Palasha beej also act as Krimighna. Brahma Rasa as indicated in Mandala Kushta is compared to Psoriasis can be caused by Streptococcal Infections.

**Aim of the Study**

To evaluate the antimicrobial activity of Brahma Rasa.

**MATERIALS AND METHODS****Test Drug**

The test drug was prepared from Rasashastra and Bhaishajya Kalpana Department Rasashala of N.K.Jabshetty Ayurvedic Medical College, Bidar.

**Source of Chemical and Reagents**

All the Chemical Reagents and other requirements of experimental study used from stock of Skanda life sciences Private limited, R&D Centre, Sri Shaila

Bhramara Complex, Sy.No 47, No.10-12, Chandana Layout, Srigandakaval, Nagarabhavi, Bengaluru.

### Test Organisms

Staphylococcus aureus, Staphylococcus epidermis, Staphylococcus pyogenes, Escherichia coli, Pseudomonas aeruginosa, Candida albicans, Trichophyton rubrum.

### Test Compound as Standard

Ciprofloxacin (0.1mg/ml)  
Itracanzole

### Sample Details

Brahma Rasa.

### Test compound

Sample (100mg/ml)  
S-Standard: Ciprofloxacin (0.1mg/ml) and Itraconazole (1mg/ml)  
Control: Water

### Procedure

#### a) Well Diffusion Method<sup>[1]</sup>

##### Inoculum

S.aureus, S. epidermis, S. pyogenes, E.coli and P. aeruginosa cell suspension were prepared and grown on Peptone broth. C.albicans and T.rubrum cell suspension were grown on potato dextrose broth. Cultures were incubated for 24hrs at 37°C for bacteria and 27°C for fungi. The cell suspensions of all the cultures were adjusted to 1-2x 10<sup>6</sup> cells/ml.

#### Determination of Antimicrobial activity

- S.aureus, S.epidermis, S.pyogenes, E.coli, P.aeruginosa were inoculated on Soya bean Casein Digested agar plates. C.albicans and T.rubrum were inoculated on Potato dextrose agar plates. (90 mm).
- Test compounds: Sample (25µl), Standard Ciprofloxacin (25µl) for S.aureus, E.feacalis, K.pneumoniae, E.coli and P.mirabilis were added to the 5mm well on agar plates.
- The treated plates with S.aureus, S.epidermis, S.pyogenes, K.pneumoniae, E.coli, P. aeruginosa,

C.albicans and T.rubrum were incubated in aerobic chamber at 37°C for 24hrs. The treated plates were observed for zone of inhibition around the wells.

#### b) Determination of Minimum inhibitory Concentration (MIC)<sup>[2]</sup>

##### Sample preparation

2mg/ml stock solutions will be prepared by using Water. From that stock solution dilutions will be made up to 1mg/mL by using broth for the initial screening.

##### Inoculum

Cell suspension prepared from bacterial cultures grown on Peptone water broth and cells were adjusted to 1-2 x 10<sup>8</sup> cells/ml.

#### Drug concentrations: drug concentration prepared

- Ciprofloxacin (8µg/ml): 8, 4, 2, 1, 0.5, 0.25 and 0.125 µg/ml in Peptone water broth.
- Ciprofloxacin (100µg/ml): 100, 25, 12.5, 6.25, 3.125 and 1.56 µg/ml in potato dextrose broth.
- Test compounds (Sample): 1, 0.5, 0.25, 0.125, 0.0625, 0.0315, 0.015mg/ml in 90µl broth.
- Control: Peptone water broth and potato dextrose broth was inoculated with test culture and without test compounds.

#### CLSI broth micro dilution method

- Mix 90µl test samples / standard of different test concentration with 10µl inoculum (1- 2x10<sup>8</sup>) in 96 well plates.
- Control: Mix 90µl Peptone water broth and potato dextrose broth without drug with 10µl inoculum in 96 well plates.
- After incubation for 24 h at 37 °C, resazurin (0.015 %) was added to all wells (30 µl per well), and further incubated for 2–4 h for the observation of colour change. On completion of the incubation, columns with no colour change (blue Resazurin colour remained unchanged) were scored as above the MIC value. iv. Metabolism of Resazurin by active bacterial cells leads to reduction of Resazurin (Purple-blue) to resorufin (pink-colorless) Pink color.

Table 1: Ingredients of Brahma Rasa.<sup>[3]</sup>

Ingredients	Quantity
Rasa Sindoor	150 gm
Shuddha Gandhaka	150 gm
Chitraka Mula Churna	150 gm
Bakuchi Churna	150 gm
Palasha Beeja Churna	150 gm
Guda	375 gm
Madhu	150 gm

### Procedure

- Rasa Sindoor was taken in Khalva Yantra and Shuddha Gandhaka churna, Chitraka mula churna,

Bakuchi churna and Palasha beeja churna was added and trituration was done.

- Trituration was done to get a homogenous mixture.

- To the homogenous mixture Guda and Madhu was added and triturated well.
- Later vati were prepared out of the homogenous mixture.
- After it was collected and stored in a clean air tight container.

**Table No 2: Properties of Brahma Rasa Ingredients.**

SLNO	Ingredients	Latin Name	Rasa Panchaka
1	Rasa Sindoor <sup>4</sup>		Katu Rasa, Laghu Guna, Ushna Virya, Katu Vipaka
2	Shuddha Gandhaka <sup>5</sup>	Sulphur	Tikta, Katu, Kashaya Rasa, Laghu, Snigdha Guna, Ushna Virya, Katu Vipaka
3	Chitraka Mula Churna <sup>6</sup>	Plumbago zeylanica	Katu Rasa, Laghu, Ruksha, Tikshna Guna, Ushna Virya, Katu Vipaka
4	Bakuchi Churna <sup>7</sup>	Psoralea corylifolia	Katu, Tikta Rasa, Laghu, Ruksha Guna, Ushna Virya, Katu Vipaka
5	Palashabeeja Churna <sup>8</sup>	Butea monosperma	Katu, Tikta, Kashaya Rasa, Laghu, Snigdha Guna, Ushna Virya, Katu Vipaka
6	Guda <sup>9</sup>	Saccharum officinarum	Guru, Snigdha Guna
7	Madhu <sup>10</sup>	Honey	Madhura Rasa, Kashaya Anurasa, Laghu, Ruksha Guna

**Antimicrobial Study**

The Study was done at Skanda Life Sciences Private Limited, R & D Centre, Sri Shaila Bramara Complex, Sy.No 47, No.10-12, Chandana Layout, Srigandakaval, Nagarabhavi, Bengaluru.

**OBSERVATION AND RESULTS****Evaluation of antimicrobial activity by Well Diffusion Method**

The inhibitory effect of the sample against *S.aureus*, *S.epidermis*, *S.pyogenes*, *E.coli*, *P.aeruginosa*, *C.albicans* and *T.rubrum* is as shown in the table below.

**Table No- 3: Inhibitory activity of test compounds against test organisms.**

Test Organisms	Test Compounds	Conc. per well( $\mu$ g/ml)	Zone of inhibition (mm)	Figure reference number
<i>S.aureus</i>	Control	-	-	Figure 1.1
	Ciprofloxacin (Standard)	2.5	25	
	(Sample) 25 $\mu$ l	2500	8	
<i>S.epidermis</i>	Control	-	-	Figure 1.2
	Ciprofloxacin (Standard)	2.5	26	
	(Sample) 25 $\mu$ l	2500	10	
<i>S.pyogenes</i>	Control	-	-	Figure 1.3
	Ciprofloxacin (Standard)	2.5	25	
	(Sample) 25 $\mu$ l	2500	12	
<i>E.coli</i>	Control	-	-	Figure 1.4
	Ciprofloxacin (Standard)	2.5	16	
	(Sample) 25 $\mu$ l	2500	18	
<i>P. aeruginosa</i>	Control	-	-	Figure 1.5
	Ciprofloxacin (Standard)	2.5	22	
	(Sample) 25 $\mu$ l	2500	-	
<i>C.albicans</i>	Control	-	-	Figure 1.6
	Itracanzole (Standard)	25	30	
	(Sample) 25 $\mu$ l	2500	13	
<i>T.rubrum</i>	Control	-	-	Figure 1.7
	Itracanzole (Standard)	25	14	
	(Sample) 25 $\mu$ l	2500	10	

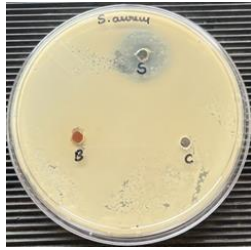


Figure 1.1: Inhibitory activity of test sample against *S.aureus* S-Standard (Ciprofloxacin); C -Control (distilled water).

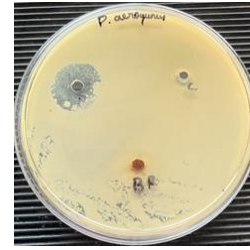


Figure 1.5: Inhibitory activity of test sample against *P. aeruginosa* S-Standard (Ciprofloxacin); C -Control (distilled water).

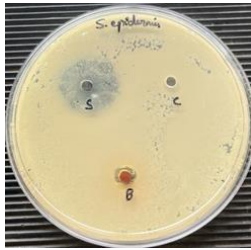


Figure 1.2: Inhibitory activity of test sample against *S. epidermis* S-Standard (Ciprofloxacin); C -Control (distilled water)

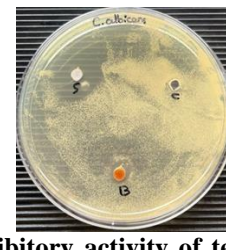


Figure 1.6: Inhibitory activity of test sample against *C.albicans* S-Standard (Itracanazole); C-Control (distilled water).



Figure 1.3: Inhibitory activity of test sample against *S. pyogenes* S-Standard (Ciprofloxacin); C -Control (distilled water)

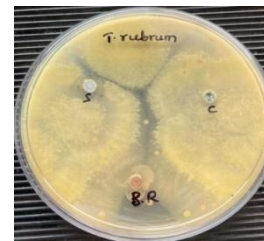


Figure 1.7: Inhibitory activity of test sample against *T.rubrum* S-Standard (Itracanazole); C -Control (distilled water).

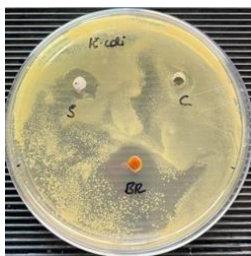
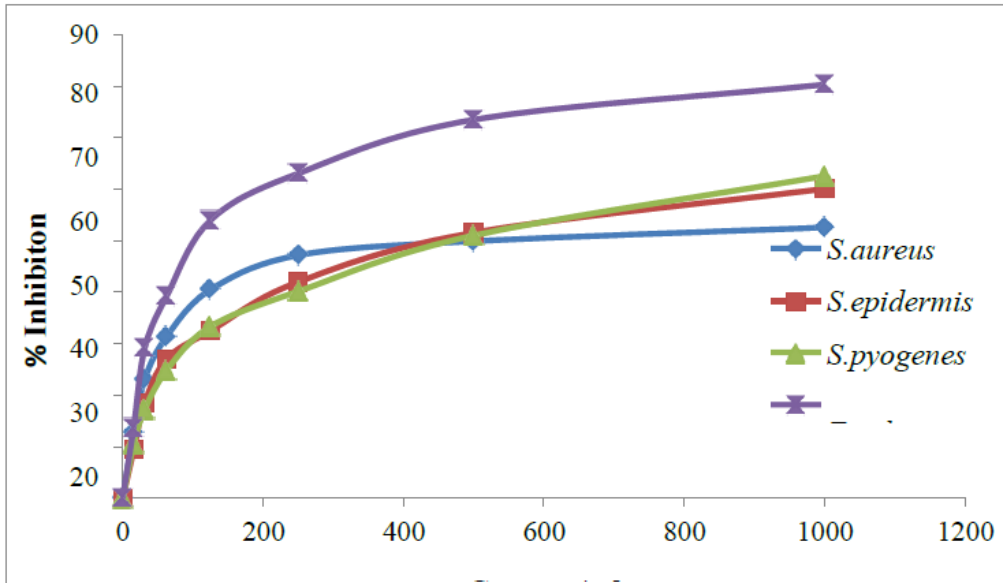


Figure 1.4: Inhibitory activity of test sample against *E.coli* S-Standard (Ciprofloxacin); C -Control (distilled water).

Table No - 4: Inhibitory activity of test sample against test organisms.

Test sample Conc .µg/ml	<i>S.aureus</i>		<i>S.epidermis</i>		<i>S.pyogenes</i>		<i>E.coli</i>		<i>P.aeruginosa</i>	
	OD@ 590nm	% Inhibition	OD@ 590nm	% Inhibition	OD@ 590nm	% Inhibition	OD@ 590nm	% Inhibition	OD@ 590nm	% Inhibition
0	0.615	0.00	0.606	0.00	0.633	0.00	0.584	0.00	0.616	0.00
15.62	0.537	12.74	0.549	9.41	0.566	10.56	0.503	13.74	0.579	6.02
31.25	0.473	23.10	0.494	18.46	0.524	17.19	0.414	29.08	0.547	11.23
62.5	0.422	31.39	0.442	27.03	0.476	24.84	0.355	39.20	0.502	18.54
125	0.366	40.50	0.409	32.48	0.422	33.34	0.269	53.96	0.464	24.72
250	0.325	47.17	0.351	41.97	0.379	40.17	0.216	63.04	0.427	30.63

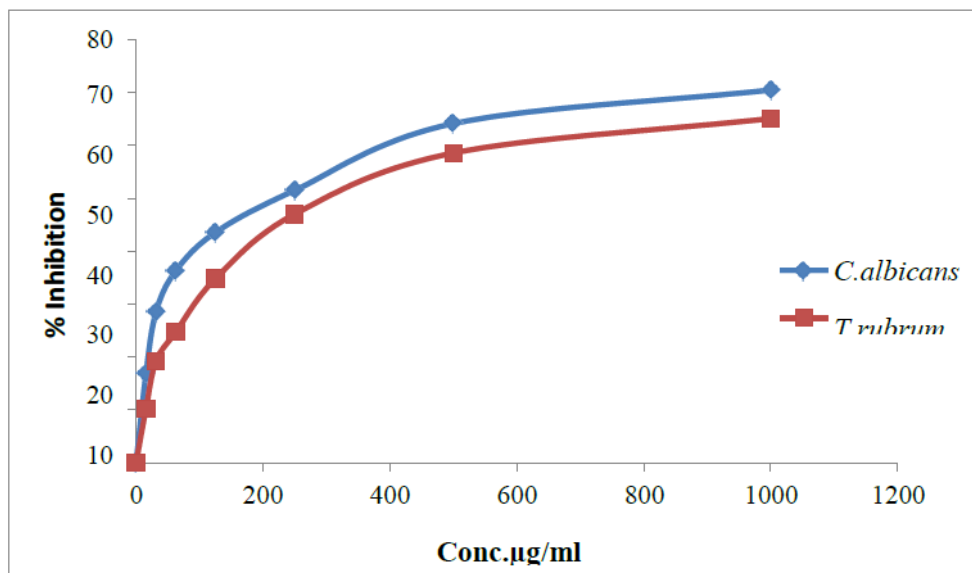
500	0.308	49.90	0.294	51.54	0.310	51.01	0.155	73.47	0.378	38.70
1000	0.292	52.59	0.242	60.07	0.237	62.56	0.115	80.31	0.326	47.14
<b>MIC</b>	1000		500		500		125		NA	



Graph - 1: Inhibitory activity of test sample against test organisms.

Table No - 5: Inhibitory activity of test sample against test organisms.

Test sample Conc. µg/ml	<i>C. albicans</i>		<i>T. rubrum</i>	
	OD@ 590nm	% Inhibition	OD@ 590nm	% Inhibition
0	0.632	0	0.615	0.00
15.62	0.525	16.88	0.552	10.24
31.25	0.452	28.46	0.497	19.19
62.5	0.403	36.26	0.463	24.72
125	0.357	43.52	0.401	34.80
250	0.306	51.48	0.326	46.99
500	0.227	64.09	0.255	58.54
1000	0.187	70.44	0.215	65.04
<b>MIC</b>	250		500	



Graph - 2: Inhibitory activity of test sample against test organisms.

## RESULT

The Study shows that Brahma Rasa has inhibitory action against *S.aureus*, *S.epidermis*, *S.pyogenes*, *E.coli*, *C.albicans* and *T.rubrum* except *P.aeruginosa*.

## DISCUSSION

Parada is said to be Shadrasatmaka, saptadhatuvaradhaka, panchabhatatmaka, tridosha shamaka and having Madhura Vipaka, Ushna Virya, Rasayana and Krimighna Property.

Gandhaka which is having Katu Rasa, Ushna Virya and Madhura, Katu Vipaka with Kandugna, Krimighna, Vishahara and Rasayana Property. Sulphur is reviewed to be having target specific organ action and antibiotic properties.

Rasa Sindoor possess Katu Rasa, Katu Vipaka, Ushna Guna, Tridosha shamaka with Kushtaghna, Pramehaghna, Yogavahi and Balya Property.

The co-drugs of Brahma Rasa like Chitraka mula, Bakuchi & Palasha beeja are having Katu Rasa, Katu Vipaka, Ushna Guna & Kapha-Vata Shamaka, Krimighna Property.

Bakuchi is having Kushtaghna Property. All drugs are having Deepaka and Pachaka property which helps to combat the Ama condition.

In the Present Study of Well Diffusion method it was observed that Inhibitory Activity of Test sample against *S. aureus*, *S.epidermidis*, *S.pyogenes*, *E.coli*, *C.albicans* and *T.rubrum* shows 8mm, 10mm, 12mm, 18mm, 13mm, 10mm Zone of Inhibition respectively. Sample has not shown any Inhibition against *P.aeruginosa*.

whereas in MIC Method the Test Sample has shown above 50% inhibition against *S.aureus*, *S.epidermidis*, *S.pyogenes*, *E.coli*, *C.albicans*, *T.rubrum* in Microbroth dilution technique with MIC Concentration 1000, 500, 500, 125, 250 & 500 µg/ml respectively. Test Sample with 1000 µg/ml MIC Concentration has shown 52.59%, 60.07 %, 62.56 %, 80.31 %, 70.44 %, 65.04% inhibitory activity against test organisms.

## CONCLUSION

Brahma Rasa is explained by Acharya Dundukanath in Rasendra Chintamani which is a herbomineral compound having kaphavatashamaka, Jantughna and kushtaghna property. In the Anti-microbial study that was conducted got satisfied result in inhibiting the growth of microbes, Brahma Rasa has shown inhibition against *S.aureus*, *S.epidermidis*, *S.pyogenes*, *E.coli*, *C.albicans* and *T.rubrum*.

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