

**ANTIMICROBIAL ACTIVITY OF KRIMIGHNA RASA - AN IN VITRO STUDY****Dr. Shabeen Ali<sup>1\*</sup>, Dr. Bandeppa Sangolge<sup>2</sup> and Dr. Pavitra G.<sup>3</sup>**

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**ABSTRACT**

In Ayurveda, various formulations are mentioned for the management of Krimi. Krimighna rasa is a herbomineral formulation which is the combination of both Kharliya and Kupipakwa rasayana mentioned in Rasendra Sara Sangraha in context of Krimi Roga Adhikara. The main ingredients of Krimighna rasa are Rasasindura (Parada and Gandhaka), Palash Beej Churna, Nimba Beej Churna, Vidangachurna and indicated in Krimirogas. Krimighna rasa has properties like Kandughna, Kustaghna, Krimighna. Hence here in the present study an attempt will be made to get more scientific knowledge based on classical and modern parameters about Krimighna rasa will be updated. And Pharmaceutical- Analytical and Antimicrobial effects of Krimighna rasa will be evaluated.

**KEYWORDS:** Krimighna rasa, Rasasindura, Krimighna.

**INTRODUCTION**

Adoption of unhealthy lifestyles, specifically reduced physical exercise and increased mental stress has resulted in drastic increase in the incidence and prevalence of diseases. Globally, infectious diseases are the leading cause of deaths. With changing of environment and global setup, increasing population, poverty, malnutrition, unauthorised use of antibiotics or prolonged use of immunosuppressant drugs or increased prevalence of drug resistance microbes there is emergence of viral disease which indicate that threat of tackling emerging infections also prevails.

In recent era most microbial infections have gained resistance to antibiotic drugs, due to this multi gained drug resistance microbial strains, there is a need to search potential antimicrobial agents. As antimicrobial agents are essentially important in reducing the global burden of infectious diseases. There is a need to combat the above issue. Since ancient time's plants, mineral origin drugs and various formulations have impact on human health. In Ayurvedic classics Antimicrobial activity can be correlated with Krimighna karma. There are 20 types of Krimis are mentioned in Ayurveda.<sup>[2]</sup> Krimi refers both macro as well as microorganisms, which can be correlated to bacteria, fungi, virus or parasites.

In Ayurveda, various formulations are mentioned for the management of Krimi. Among these, Krimighna rasa<sup>3</sup> is a Herbomineral formulation which is the combination of both Kharliya and Kupipakwa rasayana mentioned in rasendra sara sangraha in context of Krimi roga. Where Kharaliya rasayana and Kupipakwa rasayana induces organic quality in the final product for better therapeutic efficacy and least toxic effect, target specific action and for acceleration of drug activity inside the body. The ingredients of Krimighna rasa are Rasasindura (Parada and Gandhaka)<sup>4</sup>, Palash Beej Churna, Nimba Beej Churna, Vidangachurna and indicated in Krimirogas. Krimighna rasa has properties like Kandughna, Kustaghna, Krimighna and Katu, Tikta rasa.

**AIMS AND OBJECTIVES**

1. To Prepare Krimighna Rasa as per classics
2. To study Physico- chemical aspect and instrumental analysis of Krimighna Rasa.
3. To evaluate the Antimicrobial effect of Krimighna Rasa by In vitro method.

**MATERIALS AND METHOD****Test drug**

The test drug was prepared from RasaShastra and Bhaishajya kalpana Department Rasashala of N.K.Jabshetty Ayurvedic Medical college & P.G Centre, Bidar.

**Sources of Chemical and Reagents**

All the chemical reagents and other requirements of experimental study used from stock of Skanda Life Science Private Limited, R & D Centre, Sri Shaila bramara Complex, Sy. No 47, No 10-12, Chandana layout, Srigandakaval, Nagarbhavi, Bengaluru.

**Test organisms**

Staphylococcus aureus, Staphylococcus epidermis, Shigella, Klebsiella Pneumoniae, Candida albicans, Aspergillus fumigatus.

**Test compound as standard**

1. Ciprofloxacin (0.1mg/ml)
2. Itraconazole(1mg/mg)

**Sample details**

Krimighna Rasa.

**Test compound**

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

**Procedure****a) well diffusion method**

**Inoculum**  
S.aureus, S. epidermis, Shigella, K.pneumoniae, cell suspension were prepared and grown on peptone broth. C.albicans and A. fumigatus culture suspension were grown on Potato dextrose broth, bacterial cultures were incubated for 24hrs at 37°C. Fungi cultures were incubated for 24-48hrs at 27°C. The cell suspensions of all the cultures were adjusted to 1-2x 10<sup>6</sup> cells/ml.

**Determination of Antimicrobial activity**

- i. S.aureus, S. epidermis, S.flexneri and K.pneumoniae were inoculated on Soya bean Casein Digest agar plates. (90 mm).
- ii. C.albicans and A. fumigatus were inoculated on Potato dextrose agar plates.
- iii. Test compounds: Sample (25µl), Standard Ciprofloxacin (25µl), Itraconazole (25µl) for S.aureus, S. epidermis, S.flexneri, K.pneumoniae, C.albicans and A. fumigatus were added to the 5mm well on agar plates.
- iv. The treated plates with S.aureus, S. epidermis, S.flexneri and K. pneumoniae, were incubated in aerobic chamber at 37°C for 24hrs. C.albicans and A. fumigatus, were incubated in aerobic chamber at 27°C for 24-48hrs. The treated plates were observed for zone of inhibition around the wells.

**b) Determination of Minimum inhibitory Concentration(MIC)****Sample preparation**

2mg/ml stock solutions will be prepared by using Water. From that stock solution dilutions will be made up to 1 mg/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**

- I. Ciprofloxacin (8µg/ml): 8, 4, 2, 1, 0.5, 0.25 and 0.125 µg/ml in Peptone water broth.
- II. Itraconazole (100µg/ml): 100, 50, 25, 12.5, 6.25, 3.125 and 1.56 µg/ml in potato dextrose broth.
- III. Test compounds (Sample): 1, 0.5, 0.25, 0.125, 0.0625, 0.0315, 0.015mg/ml in 90µl broth.
- IV. Control: Peptone water broth and potato dextrose broth was inoculated with test culture and without test compounds.

**b) CLSI broth microdilution method.**

- i. Mix 90µl test samples / standard of different test concentration with 10µl inoculum (1-2x10<sup>8</sup>) in 96 well plates.
- ii. Control: Mix 90µl Peptone water broth without drug with 10µl inoculum in 96 well plates.
- iii. 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 colourless) Pink colour.

**Table 1: Krimighna Rasa requires the following ingredients.**

Ingredient	Quantity
Rasasindoor <sup>[5]</sup>	30gms
Palash beeja <sup>[6]</sup>	30gms
Nimba beeja <sup>[7]</sup>	30gms
Vidanga <sup>[8]</sup>	30gms

30 grams of each i.e rasasindura, nimba beeja, vidanga, palasha beeja churna is taken in a khalvayantra in equal quantity. Trituration was done and all the drugs were mixed properly. A total of 120 grams of the compound drug i.e Krimighna rasa was obtained. And preserved in airtight containers.

**Table 2: Properties of Krimighna Rasa ingredients.**

SR. NO	INGREDIENT	RASA	GUNA	VEERYA	VIPAKA	KARMA
1.	Parada <sup>[11]</sup>	Shadrasatmak	Snigdha	Ushna	madhur	Yogavahi, rasayana, lohasiddhikara
2.	Gandhaka	Madhur	-	Ushana	Katu	Kustaghna, krimigha raagnideepaka,
3.	Vidanga	Katu	Laghu	Ushana	Katu	Kustaghna, krimighna, Truptighna
4.	Palasha	Kashaya, katu	snigdha	Ushana	Katu	Vrana, kriminasak,
5.	Nimba	Tikta, kashaya	Laghu	Sheeta	Katu	Krimighna, kandughna, Kustaghna, jwarahara, kasa, swasahara.

**Antimicrobial Study**

The study was done at Skanda Life Science Private Limited, R & D Centre, Sri Shaila bramaraComplex, Sy.

No 47, No 10-12, Chandana layout, Srigandadakaval, Nagarbhavi, Bengaluru.

**Observation and results****Evaluation of antimicrobial activity by well Diffusion Method.****Table 3: Inhibitory activity of Krimighna Rasa against test organisms.**

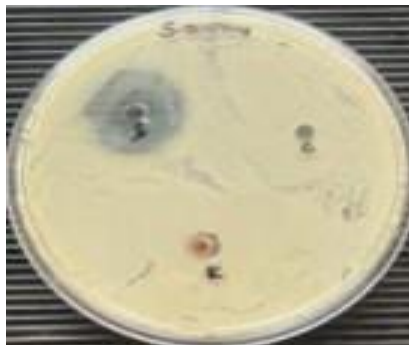
Test Organisms	Test Compounds	Conc. per well( $\mu$ g/ml)	Zone of inhibition (mm)	Figure reference number
S.aureus	Control	-	-	Figure 1.1
	Ciprofloxacin (Standard)	2.5	26	
	(Sample) 25 $\mu$ l	2500	10	
S. epidermis	Control	-	-	Figure 1.2
	Ciprofloxacin (Standard)	2.5	24	
	(Sample) 25 $\mu$ l	2500	09	
S.flexneri	Control	-	-	Figure 1.3
	Ciprofloxacin (Standard)	2.5	25	
	(Sample) 25 $\mu$ l	2500	11	
K.pneumoniae	Control	-	-	Figure 1.4
	Ciprofloxacin (Standard)	2.5	22	
	(Sample) 25 $\mu$ l	2500	14	
C.albicans	Control	-	-	Figure 1.5
	Ciprofloxacin (Standard)	2.5	24	
	(Sample) 25 $\mu$ l	2500	13	
A. fumigatus	Control	-	-	Figure 1.6
	Ciprofloxacin (Standard)	2.5	15	
	(Sample) 25 $\mu$ l	2500	10	

**Table 4: Inhibitory activity of sample against test organisms *S.aureus*, *S.epidermis*, *S.flexneri*, and *K.pneumoniae*.**

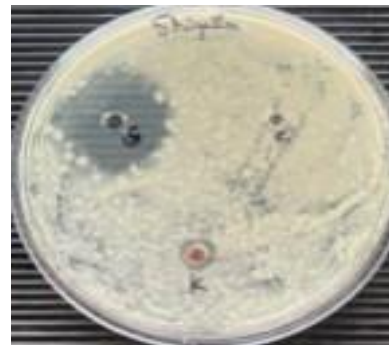
Test samples	<i>S.aureus</i>		<i>S.epidermis</i>		<i>S.flexneri</i>		<i>K. pneumoniae</i>	
	OD@ 590nm	% Inhibition	OD@ 590nm	% Inhibition	OD@ 590nm	% Inhibition	OD@ 590nm	% Inhibition
0	0.708	0.00	0.682	0.00	0.709	0.00	0.704	0.00
15.62	0.623	12.07	0.582	14.66	0.664	6.35	0.633	10.10
31.25	0.560	20.89	0.501	26.54	0.591	16.64	0.589	16.28
62.5	0.460	35.04	0.442	35.19	0.459	35.26	0.496	29.47
125	0.435	38.58	0.401	41.20	0.391	44.85	0.435	38.16
250	0.362	48.89	0.355	47.95	0.331	53.31	0.339	51.80
500	0.292	58.78	0.309	54.69	0.268	62.20	0.209	70.36
1000	0.231	67.39	0.221	67.60	0.106	85.05	0.109	84.47
MIC	500		500		250		250	

**Table 5: Inhibitory activity of Sample against *C.albicans* and *A.fumigatus*.**

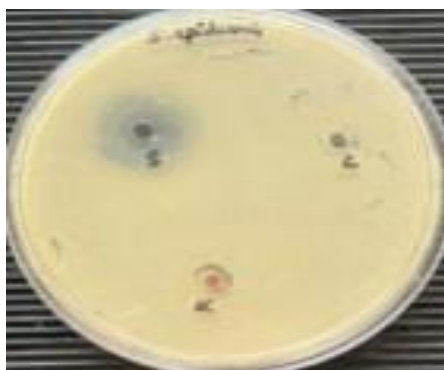
Itra(Std) Conc.µg/ml	<i>C.albicans</i>		<i>A. fumigatus</i>	
	OD@ 590nm	% Inhibition	OD@ 590nm	% Inhibition
0	0.699	0.00	0.704	0.00
15.6	0.639	8.67	0.633	10.10
31.25	0.570	18.44	0.589	16.28
62.5	0.527	24.70	0.496	29.47
125	0.427	38.90	0.435	38.16
250	0.340	51.37	0.392	44.25
500	0.268	61.67	0.309	56.15
1000	0.121	82.69	0.239	66.00
MIC	250		500	



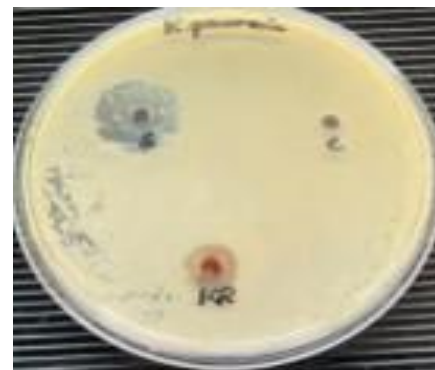
**Figure 1.1: Inhibitory activity of test sample against *S.aureus* S-Standard (Ciprofloxacin); C -Control (distilled water).**



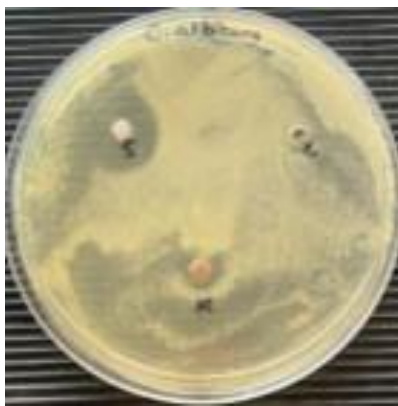
**Figure 1.3: Inhibitory activity of test sample against *S.flexneri* S-Standard (Ciprofloxacin); C -Control (distilled water).**



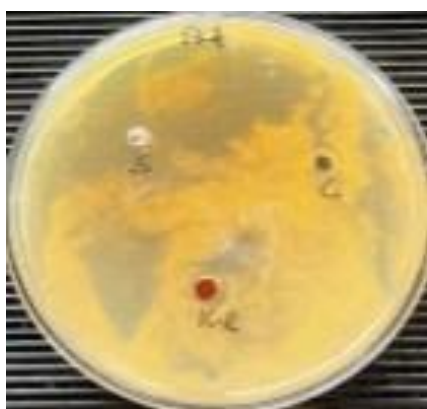
**Figure 1.2: Inhibitory activity of test sample against *S.epidermis* S-Standard (Ciprofloxacin); C -Control (distilled water).**



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



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



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

## RESULT

The study shows that Krimighna rasa shows inhibitory activity against *S.aureus*, *S. epidermis*, *Shigella*, *K.pneumoniae*, *C.albicans* and *A. fumigatus*.

## DISCUSSION

Krimighna rasa is a Herbomineral formulation which is the combination of both Kharliya and Kupipakwa rasayana mentioned in Rasendra Sara Sangraha in context of Krimi roga. The ingredients as per literature were brought to shodhana to avoid toxic and adverse effects. The above formulation is prepared in powdered form.

Krimighna rasa possesses ushna snigdha katu tikta kashaya rasa and katu vipaka, properties. They are mainly Krimighna, Kustaghna, Kandughna and Rasayana in action, acting as antimicrobial in activity.

Parada It is good appetiser, rejuvenator and aphrodisiac. It is Sadrasatmak, yogavahi, Rasayana Shuddha Gandhaka has been used for Deepan, Pachana, Amanashan, Rasayana and to destroy the skin disorders, Krimi roga, Swasa, Arthritis due to its Katu, Ushna properties.

Rasasindura It is Shada Rasatmak, Guru, Snigdha, Madhura Vipaka, Ushna Virya, Balya, Aayushya, Yogavahi, Vrushya, Sarva Roga Hara etc.

Nimba beeja has tikta, Kashya Guna Laghu, Katu, Vipaka, Sheeta veerya properties and is used as Vrana, Twak dosha, Krimi, Kustha.

Palasha is used in Vatadivarga and it is indicated in Krimi, Arshas, Prameha, Kushta, gulma and Udara Roga.

Vidanga is with Katu Kashaya rasa, Ushna virya and Katu vipaka. These qualities are favourable for destroying bacteria or stopping its further growth. It contains Embelin as an active constituent so it shows its activity like Antibacterial, Antifertility, Antiprotozoal, Antifungal, Pneumonia, Analgesic, Anti-inflammatory, Antioxidant, Anthelmintic, Antidiabetic etc.

In the present study of well diffusion method by performing Antimicrobial sensitivity test of bacteria, it was observed that *S.aureus*, *S. epidermidis*, *Shigella*, *K.pneumoniae*, *C.albicans* and *A. fumigatus* to final solution was found to be 10,09,11,14,13,10 respectively to that of ciprofloxacin. Whereas MIC of Krimighna rasa has shown inhibitory activity against *S.aureus*, *S. epidermis*, *Shigella*, *K.pneumoniae*, *C.albicans* and *A. fumigatus* in Micro broth dilution technique showing an inhibition of above 50% at MIC Concentration 500, 500, 250, 250, 250 and 500µg/ml. And 67.39% (*S.aureus*), 67.60% (*S.epidermis*), 85.05% (*Shigella*), 84.47% (*K.pneumoniae*) and 82.69% (*C.albicans*), 66.00% (*A. fumigatus*) of inhibition at 1000µg/ml.

## CONCLUSION

Krimighna rasa is a herbomineral formulation which is the combination of both kharliya and kupipakwa rasayana, where kharaliya rasayana induces organic quality in the final product for better therapeutic efficacy and least toxic effect and target specific action. In the antimicrobial study conducted Krimighna rasa was found to be effective against the microorganisms *S.aureus*, *S. epidermis*, *Shigella*, *K.pneumoniae*, *C.albicans* and *A. fumigatus* by virtue of property of ingredients.

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