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**Abstract:**

*Rasoushadhi* (Metallic medicine) being most efficacious drug in Ayurveda is known for its minute dosage and quick action. *Rasasindura* is a organo-metallic compound widely utilized by clinicians in infectious diseases but lack of preclinical data has been impediment for its wide acceptance. Hence study was planned to assess *in vitro* antimicrobial potential of *Shadguna Rasasindura* (SRS). Four gram-ve, four gram+ve and three fungal strains were studied for Minimum Inhibitory Concentration (MIC) and Zone of Inhibition (ZOI) against standard drugs and test drugs. Test drug SRS showed moderate zone of inhibition when compared to standard drugs in all the bacterial and fungal strains taken for study, however test drug was not active at 5mg concentration in all the experiments. MIC was observed at either 250 or 500 mg concentration but not in lower.

**Introduction:**

Rasashastra a mercurial science, converts several minerals and metals to bio assailable drug. These are used after subjecting to proper purificatory procedures which are elaborately dealt in classics of Rasashastra [1]. As a matter of fact, any drug or formulation, even if it is *Visha* (toxic) and intense, they can act as very useful medicament, where even a simple drug or formulation can become dangerous if not used properly [2]. These formulations especially *Bhasmas*, are having minute dosage and quick acting. *Rasasindura* is a compound formulation of mercury and sulphur prepared by subjecting to specialized pharmaceutical process known as *Kupipaka* [3]. Studies carried out on *Rasasindura* have emphasized on its pharmaceutical standardization, Mercury sulfide (crystals ranging from 25-50nm) associated with several organic macro molecules and trace elements in different amounts [4]. *Drosophila melanogaster* did not show any heavy metal toxicity [5]. *Shadguna Rasa Sindura* (SRS) has reported to be efficacious in various kinds of infectious diseases like *Jwara* (Fever), *Kushta* (Skin Disease), *Dushtavruna* (Infected wound) and *Mootra vikara* (Renal Disease). On this quotation the role of micro-organisms can be linked to the following diseases which indicate that *Rasasindura* (SRS) acts against microbes. Hence the present study has been carried out for its anti-microbial activity.

**Materials and methods:**

Present study has been carried out to assess *in vitro* antimicrobial potential of *Shadaguna Rasa Sindura* (SRS).

**Parameter:**

Zone of Inhibition (ZOI), Minimum Inhibitory Concentration (MIC).

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### Anti-Bacterial study:

Four Gram-positive (*S.aureus* and *S. Pyogenes*, *B. Megatarium*, *B. Subtilis*)

Four Gram-negative (*E. coli* and *P. Aeruginosa*, *K Pneumoniae*, *P. Morganni*)

### Standards:

Ampicilline, Chloramphenicol, Ciprofloxacin, Norfloxacin and these four with gentamycin were taken as standard for Minimum Inhibitory Concentration (MIC)

### Anti-Fungal study:

*A.Niger*-MTCC282, *A.Clavatus*-MTCC1323 and *C. Albicans*-MTCC227

**Standards:** Griseofulvin and Nystatin

### Methodology:

These were investigated by the agar disk diffusion method. Each purified extracts were dissolved in Dimethyl Sulfoxide (DMSO) sterilized by filtration using sintered glass filter and stored at 4°C. For the determination of ZOI, pure Gram positive, Gram negative and fungal strains were taken. The sets of five dilutions (5, 25, 50, 100 and 250 µg/mL) of both extracts and standard drugs were prepared in double distilled water using nutrient agar tubes. Muller Hinton sterilized agar plates were seeded with indicator bacterial strains (10<sup>8</sup>cfu) and allowed to kept at 37°C for 3 h and same experiments were carried out for standard using ampicilline, Chloramphenicol, Ciprofloxacin and Norfloxacin used for antibacterial activity. The zones of growth inhibition around the disks were measured after 18 to 24 h of incubation at 37°C for bacteria and 48 h to 96 h for fungi at 28°C. At the end of the incubation period, the medias were observed for ZOI (including the diameter of disk) and values <8 mm were considered as not active against microorganisms [6]. ZOI were measured in millimetre using verniercallipers. The results are as per shown below.

MIC of drug was determined by Broth dilution method. It is one of the non automated *in vitro* bacterial susceptibility tests. This classic method yields a quantitative result for the amount of antimicrobial agents that is needed to inhibit growth of specific microorganisms. It is carried out in tubes [7].

### Observation & Results:

Table 1-4 shows ZOI of test drug compared with standard drugs in gram +ve bacteria in various dose levels. ZOI of test drug on gram ve bacteria

comparison with standard drugs has been depicted in Table 5-8. MIC of test drugs compared with standards has been shown in Table 9. Effect of trial drugs compared to standard drugs on fungal strains at various concentrations (ZOI) has been given in Table 10-12 where as MIC of the same is tabulated in Table 13.

### Discussion:

The result of antibacterial activity of different concentration of *Shadaguna Rasasindura* (SRS) with reference to Zone of Inhibition (ZOI) and Minimum Inhibitory Concentration (MIC) has been tabulated in Table 1-8. The reference antibiotic Norfloxacin showed the highest antibacterial activity against all tested microorganism. It was observed that SRS sample with concentration of 5 µg showed no antibacterial activity against any tested microorganism. *S.aureus* MTCC96 was found most sensitive to SRS sample at concentration of 250 µg with zone of 19 mm which is equivalent to the activity given by reference antibiotic ampicillin at given concentration. *E. coli* MTCC 443 was found more susceptible at concentration of 25 µg with 13 mm zone followed by *B. subtilis* MTCC 441 with 12 mm zone. But at higher concentration of 250 µg SRS showed good results against *B. subtilis* MTCC 441 which was more than reference antibiotic Ampicillin. *P. Aeruginosa* MTCC 424 was found least susceptible to SRS at all given concentrations. *P. Morganni* MTCC 8426 showed no activity at lower concentrations of SRS but showed good results with increased concentrations. MIC of strains tested has been tabulated in Table 9. MIC of SRS was found much higher compared to most of the reference antibiotics, but showed good results when compared to reference antibiotic ampicillin indicating good antibacterial activity. SRS was found least effective against *K.Pneumoniae* with MIC of 500µg/ml.

The antifungal activity of different concentration of SRS with reference to ZOI and MIC has been shown in Table 10-12. SRS showed no antifungal activity at lower concentration of 5µg/ml, however in higher dosage mild to moderate activity was observed. *A.clavitus* gave with ZOI 15 mm at concentration of 250µg/ml which was more as compared to other two tested organisms. ZOI of SRS was found much lower compared to most of the

**Table 1: Effect of trial drugs on *S. aureus* MTCC96 at various concentrations (ZOI)**

Concentrations	SRS	Ampicillin	Chloramphenicol	Ciprofloxacin	Norfloxacin
5	-	10	12	17	19
25	08	13	14	19	22
50	11	14	19	21	25
100	16	16	20	22	26
250	19	18	21	22	28

**Table 2: Effect of trial drugs on *S. Pyogens* MTCC442 at various concentrations (ZOI)**

Concentrations	SRS	Ampicillin	Chloramphenicol	Ciprofloxacin	Norfloxacin
5	-	11	10	16	18
25	10	14	13	19	19
50	15	16	19	21	20
100	17	18	20	21	21
250	21	19	20	22	21

**Table3: Effect of trial drugs on *B. Megatarium* MTCC6305 at various concentrations (ZOI)**

Concentrations	SRS	Ampicillin	Chloramphenicol	Ciprofloxacin	Norfloxacin
5	-	10	12	19	17
25	08	15	14	22	19
50	10	16	19	25	21
100	14	17	20	26	22
250	17	19	21	28	22

**Table 4: Effect of trial drugs on *B. Subtilis* MTCC 441 at various concentrations(ZOI)**

Concentrations	SRS	Ampicillin	Chloramphenicol	Ciprofloxacin	Norfloxacin
5	-	10	10	18	16
25	12	13	13	19	29
50	15	16	19	20	21
100	18	18	20	21	21
250	22	20	20	21	22

**Table 5: Effect of trial drugs on *P. Morganni* MTCC 8426 at various concentrations (ZOI)**

Concentrations	SRS	Ampicillin	Chloramphenicol	Ciprofloxacin	Norfloxacin
5	-	14	14	20	18
25	11	15	17	23	19
50	13	15	18	24	21
100	17	18	19	26	23
250	20	20	21	27	23

**Table 6: Effect of trial drugs on *K. Pneumonie* 109 at various concentrations (ZOI)**

Concentrations	SRS	Ampicillin	Chloramphenicol	Ciprofloxacin	Norfloxacin
5	-	14	14	20	22
25	11	15	17	23	25
50	14	16	23	28	26
100	18	19	23	28	27
250	20	20	23	28	29

**Table7: Effect of trial drugs on *P. Aeruginosa* MTCC 424 at various concentrations (ZOI):**

Concentrations	SRS	Ampicillin	Chloramphenicol	Ciprofloxacin	Norfloxacin
5	-	14	14	20	18
25	09	15	17	23	19
50	12	15	18	24	21
100	15	18	19	26	23
250	17	20	21	27	23

**Table 8: Effect of trial drugs on *E. Coli* MTCC 443 at various concentrations (Zone of inhibition):**

Concentrations	SRS	Ampicillin	Chloramphenicol	Ciprofloxacin	Norfloxacin
5	-	14	14	20	22
25	13	15	17	23	25
50	15	16	23	28	26
100	18	19	23	28	27
250	20	20	23	28	29

**Table 9: Effect of MIC of 4gram positive and 4gram negative bacilli on SRS and standard antibiotics:**

STANDARD STRAIN	MIC [microgram/ml]					
	SRS	Gentamycin	Ampicillin	Chloramphenicol	Ciprofloxacin	Norfloxacin
<i>E.coli</i> MTCC 443	250	1	100	50	25	10
<i>Kl.pneumoniae</i> MTCC 109	500	1	125	50	50	50
<i>P.Aeruginosa</i> MTCC 424	250	1	--	50	50	50
<i>P.Morgani</i> MTCC 8426	250	0.5	125	50	50	50
<i>S.Aureus</i> MTCC 96	250	0.5	250	50	50	10
<i>S.pyogenus</i> MTCC 442	500	0.5	100	50	50	10
<i>B.Subtilis</i> MTCC441	250	0.5	100	50	25	10
<i>B.Megateruim</i> MTCC 6305	250	1	50	50	25	10

**Table 10: Effect of trial drugs on *A.Niger* MTCC 282 at various concentrations (ZOI):**

Concentrations	SRS	Greseofulvin	Nystatin
5	-	19	18
25	10	23	19
50	11	25	24
100	12	25	29
250	14	28	29

**Table 11: Effect of trial drugs on *A.Clavatus* MTCC 1323 at various concentrations (ZOI):**

Concentrations	SRS	Greseofulvin	Nystatin
5	-	19	18
25	10	21	20
50	11	26	25
100	12	26	27
250	14	28	29

**Table 12: Effect of trial drugs on *C.Albicans* MTCC 227 at various concentrations (ZOI):**

Concentrations	SRS	Greseofulvin	Nystatin
5	-	18	18
25	10	21	21
50	12	22	24
100	13	22	25
250	14	24	26

**Table 13: Effect of MIC of fungal strains on SRS and standard antibiotics:**

STANDARD STRAIN	MIC [microgram/ml]		
	SRS	Greseofulvin	Nystatin
<i>A.Niger</i> MTCC 282	500	100	100
<i>A.Clavatus</i> MTCC 1323	>1000	100	100
<i>C.Albicans</i> MTCC 227	1000	500	100

reference antibiotics. Minimum Inhibitory concentration of SRS against *A.niger* was found to be 500µg/ml, however MIC of SRS was found to be more than 1000µg/ml for other two tested fungal strains. (Table 13)

*Rasaushadhies* are organo-metallic complex compositions may not work until they undergo a series of biotransformation after digestion and absorption in gastrointestinal tract. Hence, the effective compositions of a drug working in vivo may differ and it is difficult to achieve same results in experiment study in vitro.

#### **Conclusions:-**

*Rasasindura* possesses moderate antibacterial and antifungal activity but is less in compared to standard drugs in Disc diffusion method. Minimum inhibitory concentration of SRS for bacterial strains ranges from 250 to 500µg. However further studies with different solvents with higher solubility and different methods of screening could be crucial to prove its efficacy.

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