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Antibacterial and Antifungal activity of Arseno-Mercurial formulation *Sameerapannaga Rasa*: An in-vitro study

Research Article

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Abstract

Background: *Sameerapannaga Rasa* (SPR) is a herbomineral formulation categorized as a *Kupipakwa Rasayana*, one of the four types of *Rasayanas* in *Rasashastra*. Materials & Methods: After proper *shodhan* of the individual ingredients, the SPR was prepared by the *kupipakwa* method and the anti-microbial activity was assessed through standard and internationally accepted methods like MIC (Minimum inhibitory concentration) & MBC (Minimum bactericidal concentration) methods. Results and Discussion: The minimum inhibitory concentration of *Sameerapannaga rasa* showed very good inhibitory activity against all the tested organisms. SPR demonstrated strong antimicrobial activity against *S. aureus, K. pneumoniae, C. albicans, S. sanguis,* and *S. pneumoniae* at low concentrations (0.9 mg). *Sameerapannaga rasa* exhibited antimicrobial effects against *S. epidermidis* (31.25 mg), *P. aeruginosa* (125 mg), *P. mirabilis* (62.5 mg), *E. coli*, and *C. krusei* (7.8 mg). Minimum bactericidal concentration of *Sameerapannaga rasa* showed very significant inhibitory activity against all the tested organisms. SPR demonstrated strong antibacterial and antifungal activity against *E. coli, S. pneumoniae*, and *C. albicans* at low concentrations (0.9 mg). It also showed activity against *S. aureus* (15.6 mg), *K. pneumoniae* (1.96 mg), *S. sanguis* (7.8 mg), *C. krusei* (62.5 mg), *P. aeruginosa* and *P. mirabilis* (125 mg), and *S. epidermidis* (250 mg). Conclusion: The results of both Minimum inhibitory concentration and Minimum bactericidal concentration methods provided scientific evidence of the potential antimicrobial activity of SPR, therefore, *Sameerapannaga rasa* could be considered as a potential alternative to the contemporary treatment.

Keywords: Antimicrobial activity, *Sameerapannaga Rasa, Rasa-aushadhi,* Herbomineral Drug, *Vata-pannaga Rasa*.

Introduction

The National Health Portal of India reported in 2019 that there were 41,996,260 cases and 3,740 deaths from respiratory infections in India in 2018. Acute respiratory infections (ARI) accounted for 69% of the total cases of communicable diseases, and this scenario is before the era of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). After the coronavirus disease 2019 (COVID-19) pandemic, the total number of infected numbers rose to millions and cases are still getting added (01). The other main issues are facing by the medical practitioners is antimicrobial resistance. With 700000 people losing battle to antimicrobial resistance per year and another 10 million people projected to die from it by 2050. Antimicrobial resistance alone is killing more people than cancer and road accidents combined together (02).

In ayurveda, *Rasashastra* is the branch which deals with herbometallic and herbomineral preparations. In *Rasa chikitsa*, *chaturvidha rasayanas* (*Kharaliya rasayana, Parparti rasayana, Kupipakwa rasayana* and *pottali rasayana*) are four pillars for *vyadhiharanartha* which inturn helps in *Dehasiddhi*. Among them, *Kupipakwa rasayana* is one of such preparation categorized under the *chaturvidha rasayana*. *Kupipakwa rasayana* are highly potent because the time taken for *murchana* and *jarana* of *parada* with other drugs is relatively high when compared to three *rasayanas*. This quality enables them to treat a wide range of diseases, from acute to chronic and ensures quicker action, which is an inherent property of *rasaushadhis*.

Sameerapannaga rasa (SPR) is a *kupipakwa rasayana* prepared by trituration of *Parada* (Mercury-Hg), *Gandhaka* (Sulphur-S), *Haratala* (Orpiment-As_2S_3), *Manashila* (Realgar-As_2S_2), and *Gauripashana* (White Arsenic-As_2O_3) in equal quantity with *bhavna dravya* of *Tulsi swarasa* (juice of *Ocimum sanctum* Linn.). It is a unique herbo-mineral preparation in which the resultant medicine is obtained *ubhayastha* from the bottle (*Kupi*) i.e., at neck and bottom of the *kanchakupi*.

SPR is indicated in *sannipataja jwara, kaphaja unmada, sandhibandha (sandhivata) and kapha-amaya vikara (kasa, swasa, jwara, rajayakshama* etc.) and it has *vata-kapha hara* properties (03). The classical

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indications given for the formulation are more near to the diseases of infectious origin. Despite its traditional therapeutic utility, the evidence of antimicrobial activity pertaining to SPR is not available. Therefore, this research work was undertaken to validate SPR as a potential antimicrobial agent.

Objective of the study is To evaluate the antimicrobial activity of *Sameerapannaga Rasa* against test organisms at different concentration.

Materials and Methods

i. Pharmaceutical preparation of the formulation.
ii. Solubility testing of formulation
iii. Antimicrobial Susceptibility Testing

Preparation of *Sameerapannaga rasa*

The Pharmaceutical study was carried out in the Dept. of *Rasashastra & Bhaishajya Kalpana.* At First, the *shodhana* of the individual ingredients is done i.e, *Parada, Gandhaka, Haratal, Manahshila & Gauripashana* as per text. It is followed by *Kajjali* preparation from *shodhita Parada* and *Gandhaka*. After that, remaining ingredients were added & triturated with the *bhavana dravya* of *Tulasi swarasa* for three days to obtain the SPR *kajjali* and subjected to *kramagni kupipaka.* The final product was obtained at the base of *kanchkupi* (03).

Table 1: Ingredients of Sameerapannaga rasa

S.N.	COMPONENTS	QUANTITY
1	*Parada* (Hg)	1 Part (80grams)
2	*Gandhaka* (S)	1 Part (80grams)
3	*Hartala* (As_2O_3)	1 Part (80grams)
4	*Manahshila* (As_2S_2)	1 Part (80grams)
5	*Gauripashana* (As_2O_3)	1 Part (80grams)
6	*Tulasi swarasa* (*Ocimum sanctum* Linn.)	Quantity sufficient
7	Final product obtained (SPR)	352grams

Solubility of *Sameerapannaga Rasa*

Prepared SPR was subjected for solubility with different solvents, like Ethanol, Methanol, Ether, and Dimethyl Sulfoxide by using a cyclometer. But it was not soluble in any of these solvents except in DMSO (Dimethyl sulfoxide) to some extent. Hence, DMSO was selected for the solubility of the SPR. So, 01 gm of SPR dissolved in 1 ml of DMSO solution is taken for the further study.

Figure 1: Solubility of SPR in different solvents

Methods of Antimicrobial Study

The antimicrobial study was carried out by method following Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) against the 08 selected bacterial culture stains and 02 fungi were conducted at Microbiology Lab, Arihant Super Specialty Hospital, Belagavi, Karnataka.

a) **Gram-positive Bacteria-** *Streptococcus pneumoniae, Staphylococcus aureus, staphylococcus sanguis* and *Staphylococcus epidermidis.*

b) **Gram-negative Bacteria-** *Klebsiella pneumoniae, Pseudomonas aeruginosa, Protius species* and *Esch. Coli.*

c) ***Fungus:*** *Candida albicanse* and *Candida krusei.*

Minimum Inhibitory Concentration (MIC): Microdilution Method (04) (05).

96 well micro-titer plates were taken and Brain heart infusion broth (HI media) of 100 microliter volume was added in all 12 wells in a row.

Test drug: A 100-microliter volume of test drug sample was taken and mixed properly in 1_{st} well. After mixing, the same 100 microliter volume was taken from the first well and transferred to the next well. This serial dilution was done till the 10_{th} well.

Micro-organism Concentrations: $01\times10_6$ per ml CFU of micro-organism was taken and 10-10 microliter volumes of micro-organisms were added in 01 to 10 well and 12_{th} well respectively and mixed properly.

Incubation: Now this microtiter plate was placed in an incubator for 24 hours at 37 Celsius temperatures.

Observations: After 24 hours, the MIC plates were inoculated with 0.01% of Resazurin sodium (Dye) and incubated for the next 4 hours. The active bacterial metabolism changes the dye colour irreversibly to pink which shows the presence of viable (active) microbes and the blue colour represents only compounds/drugs concentration that kills microorganisms. The last well in a row with the lowest conc. of drug, containing blue colour is considered as the MIC value of drug.

Positive control: The 11^{th} well which contains broth and the organism showing pink colour is known as positive control which reflects the purity and proper growth of the organism taken for study.

Negative control: The 12^{th} well which contains broth only shows a blue color known as negative control which suggests the broth used in the study is sterile completely.

Minimum Bactericidal Concentration (MBC):

Broth media: Different broth used were

a. Blood Agar: *S. pneumoniae* and *S. sanguis.*
b. Sabouraud Agar: *C. albicans* and *C. krusei.*
c. Brain heart infusion Agar: used for remaining micro-organism.

These agar media were taken in a sterilized petri dish. After solidifying it was divided into four quadrants. From each well of the MIC plates 10-10 microliter inoculum was taken and placed over respected marked concentrations of quadrants. These plates were incubated for 24 hours at 37 Celsius and inspected for growth of organisms in each quadrant.

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The minimum concentration which did not show any growth of the organism was considered as Minimum bactericidal concentration for that particular micro-organism (05).

The prepared SPR was tested from the concentration range 500 mg to 0.9 mg towards selected micro-organisms. Test results of MIC and MBC are mentioned in Tables No. 02 and 03.

Observations and Results

Minimum Inhibitory Concentration (MIC):

Figure 2: MIC observations of Microbes

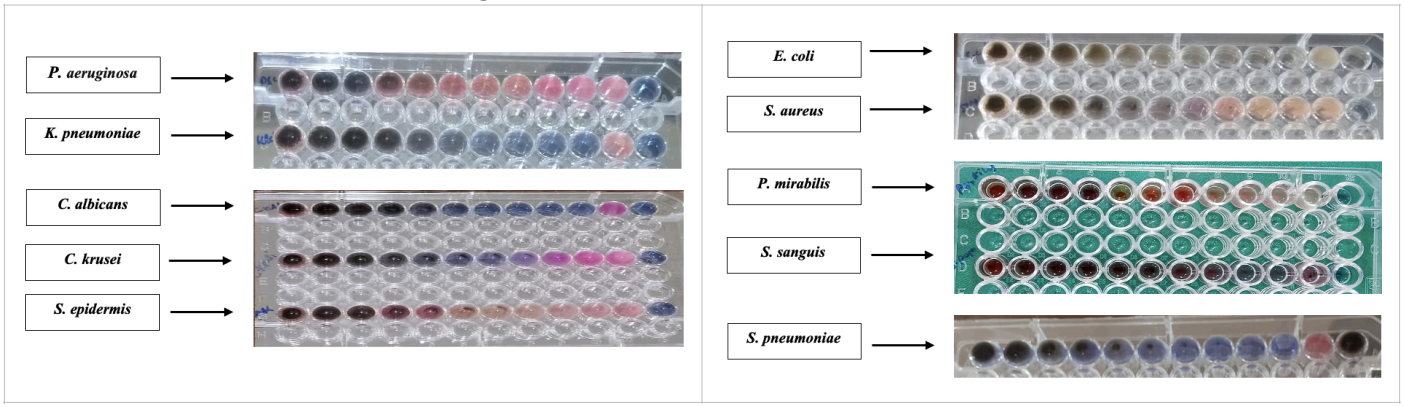


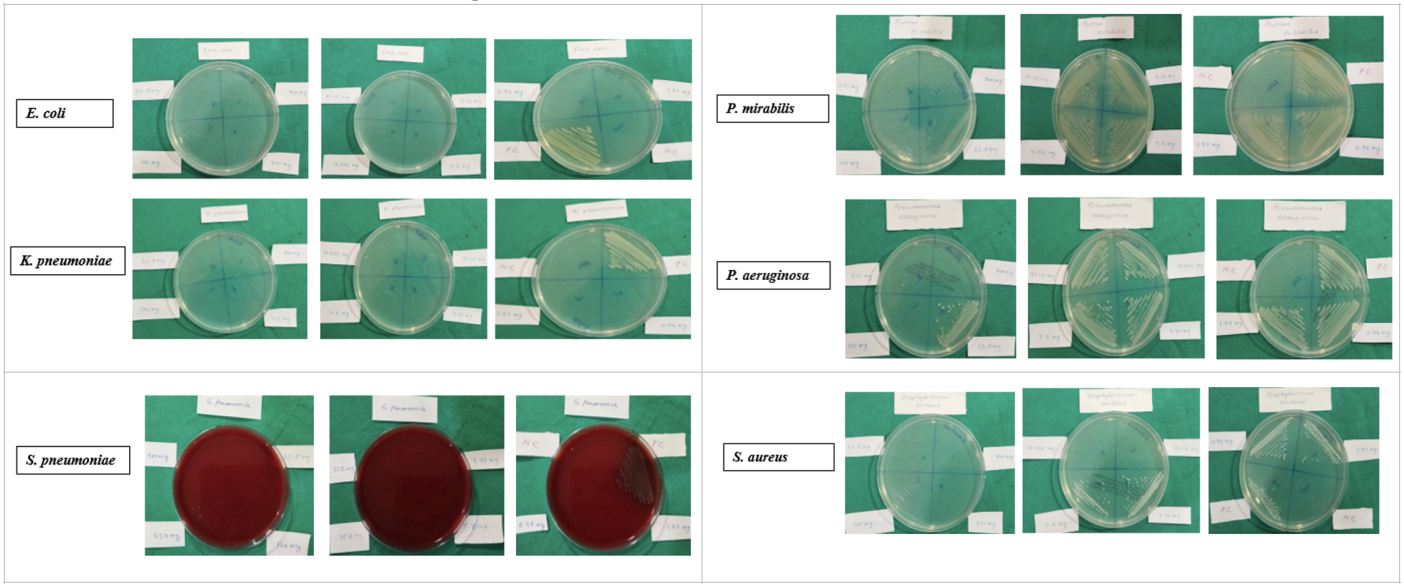
Table 2: MIC Antimicrobial results

Bacteria & Fungus	Concentration of Drug (*Sameerapannaga rasa*)										No Drug	
	500mg	250mg	125mg	62.50mg	31.25mg	15.60mg	7.80mg	3.90mg	1.96mg	0.90mg	PC	NC
S. aureus	S	S	S	S	S	S	S	S	S	S	G	NG
E. coli	S	S	S	S	S	S	S	R	R	R	G	NG
K. pneumoniae	S	S	S	S	S	S	S	S	S	S	G	NG
P. aeruginosa	S	S	S	R	R	R	R	R	R	R	G	NG
S. epidermis	S	S	S	S	S	R	R	R	R	R	G	NG
S. sanguis	S	S	S	S	S	S	S	S	S	S	G	NG
P. mirabilis	S	S	S	S	R	R	R	R	R	R	G	NG
S. pneumoniae	S	S	S	S	S	S	S	S	S	S	G	NG
C. albicans	S	S	S	S	S	S	S	S	S	S	G	NG
C. krusei	S	S	S	S	S	S	S	R	R	R	G	NG

S- Sensitive, R- Resistant, G- Growth, NG- No growth, PC- Positive Control, NC- Negative Control

Minimum Bactericidal Concentration (MBC):

Figure 3: MBC Observations of Microbes



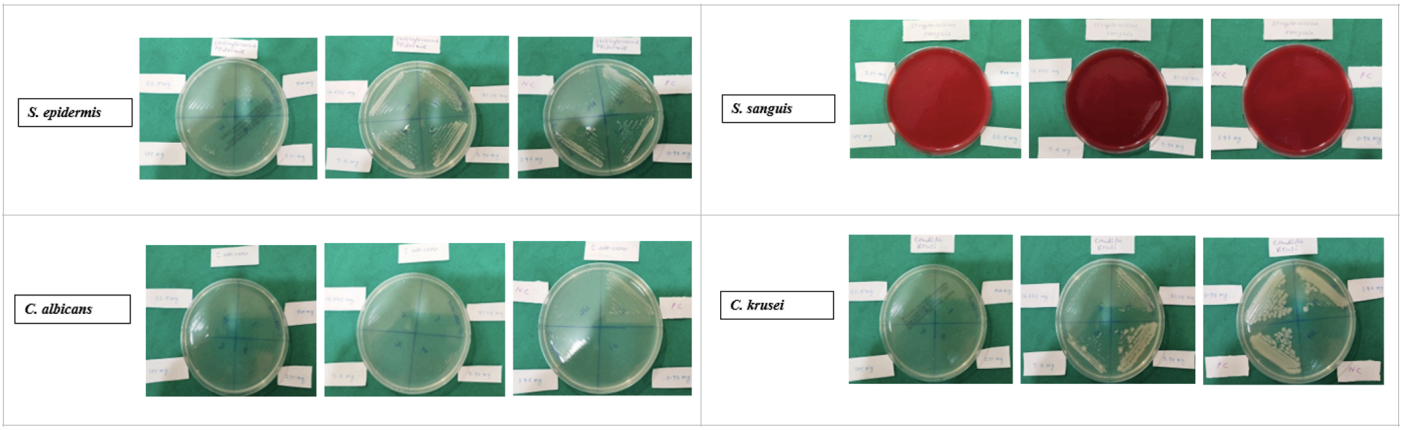


Table 3: MBC Antimicrobial results

Bacteria & Fungus	Concentration of Drug (*Sameerapannaga rasa*)										No Drug	
	500mg	250mg	125mg	62.50mg	31.25mg	15.60mg	7.80mg	3.90mg	1.96mg	0.90mg	PC	NC
S. aureus	S	S	S	S	S	S	R	R	R	R	G	NG
E. coli	S	S	S	S	S	S	S	S	S	S	G	NG
K. pneumoniae	S	S	S	S	S	S	S	S	S	R	G	NG
P. aeruginosa	S	S	S	R	R	R	R	R	R	R	G	NG
S. epidermis	S	S	R	R	R	R	R	R	R	R	G	NG
S. sanguis	S	S	S	S	S	S	S	R	R	R	G	NG
P. mirabilis	S	S	S	R	R	R	R	R	R	R	G	NG
S. pneumoniae	S	S	S	S	S	S	S	S	S	S	G	NG
C. albicans	S	S	S	S	S	S	S	S	S	S	G	NG
C. krusei	S	S	S	S	R	R	R	R	R	R	G	NG

S- Sensitive, R- Resistant, G- Growth, NG- No growth, PC- Positive Control, NC- Negative Control

Discussion

The prevalence of respiratory diseases in India which has increased further after the pandemic covid -19 and with the introduction of antibiotic resistant, there is a need for introducing newer possibilities for treating these infections. SPR containing total six ingredients in which five are minerals and one herbal ingredient *Tulasi*. *Haratal* (Orpiment/As_2S_3), *Manahshila* (Realgar/ As_2S_2) and *Gauripasana* (White Arsenic/As_2O_3) are comprising of Arsenic and known for its antimicrobial activity. Paul P.N. et.al. has concluded that arsenic-containing compounds are known to have antibacterial, antiviral, antiparasitic & anticancer activity (06).

Similarly, *Tulasi* (*Ocimum sanctum* Linn.) which is used as the *bhavana dravya* (Trituration) for the preparation of SPR been used for respiratory disorders such as dyspnoea (*Shwasha*), cough (*Kasa*), hiccups (*Hikka*) and pleural effusion (*Parshovshola*) (07)(08)(09). In a study, Broncho–protective effectiveness of aqueous extract of *Tulsi* (*Ocimum sanctum* Linn.) has been proven (10).

A randomized double blind clinical study was conducted on 52 patients were treated with SPR at a dose of 30 mg twice a day for 04 weeks along with *nagavallidala* (betel leaf). The results reveal that the SPR has a significant action in cases bronchial asthma and it could suppress total leukocyte count, eosinophil count, Erythrocyte sedimentation rate (ESR) and can improve Peak expiratory flow rate (PEFR) along with providing symptomatic relief. Analysis of the data generated during the study shows that; all the groups of SPR have been highly significant in treating the condition. The study reveals that SPR can be used as an effective drug in bronchial asthma (11). Above mentioned studies & research collectively supports its effectiveness in *kaphaja vyadhi* as well as antimicrobial activity.

Minimum inhibitory concentration and Minimum bactericidal concentration are the standard and internationally accepted methods for antimicrobial study. They can be used for many concentrations/ dilutions simultaneously compared to other antimicrobial methods. There is no bias of diffusion of the testing drug.

The **Minimum inhibitory concentration** of SPR showed very good inhibitory activity against all the test organisms. In this method, SPR showed excellent activity against *S. aureus, K. pneumoniae, C. albicans, S. sanguis,* and *S. pneumoniae* even at lowest concentrations (0.9 mg). *Sameerapannaga rasa* has shown antimicrobial activity against *S. epidermis* at lower conc. 31.25 mg, *P. aeruginosa* at conc. 125 mg, *P. mirabilis* at conc. 62.5 mg, *E. coli* and *C. krusei* at conc. 07.8 mg.

The **Minimum bactericidal concentration** of SPR showed very significant inhibitory activity against all the test organisms. In this method, SPR showed excellent antibacterial and antifungal activity against *E. coli, S. pneumoniae*, and *C. albicans* even at lowest concentrations (0.9 mg). SPR has shown antimicrobial activity against *S. aureus* at lower conc. 15.6 mg, *K.*

pneumoniae at 01.96 mg, *S. sanguis* at conc. 07.8 mg, *C. krusei* at conc. 62.5 mg, *P. aeruginosa* and *P. mirabilis* at conc. 125 mg and *S. epidermis* at lower conc. 250 mg.

Conclusion

Sameerapannaga rasa has shown effective antimicrobial activity in almost all concentrations against both bacterial (Gram-positive and Gram-negative) stains and fungus.

The results of both Minimum inhibitory concentration and Minimum bactericidal concentration methods provided scientific evidence of the potential antimicrobial activity of *Sameerapannaga Rasa*. Hence, *Sameerapannaga rasa* could be considered as a potential alternative to the contemporary treatment.

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