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ANTIMICROBIAL ACTIVITY OF SELECTED NANO PREPARATIONS OF TRADITIONAL MEDICINE AGAINST HUMAN PATHOGENS

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ABSTRACT

Siddha medicine is one of the most ancient medical systems of India. Siddha is the mother medicine of ancient Tamils/Dravidians of peninsular South India. This system has enormous pharmacopoeia containing vegetable, animal and mineral products. Although the Siddha herbo-mineral preparations represent a rich source of antimicrobial agents. The selected Siddha herbo-mineral preparations were tested for antibacterial and antifungal activities. For antimicrobial assay five strains of bacteria viz. *E. coli, S. typhi, S. aureus*, *K. pneumonia* and *V. cholerae* were used, For antibacterial assay four concentrations of the Siddha drug (for LC, KR, VK and RC - 5 µl, 10 µl, 15 µL and 20 µl were used, Of the five bacteria tested, the growth of all the bacteria were well inhibited by the Siddha drugs. The disc diffusion assay indicated a dose dependent effect of the Siddha drugs to inhibit the growth of bacteria. From the study, it was observed that the Herbo – mineral Drugs such as *Kantha rasavillai* (KR), *Vajera kandi* (VK) and *Rasa chunnam* (RC) was found to have antifungal activity. But the Herbo-mineral medicine, *Linga chendhuram* (LC) has no antifungal activity against the selected five fungal strains. So It is concluded that these three Siddha preparations KR, VK, and RC can be used to control or prevent the fungal infections. Modern techniques are necessary to standardize and bring out high quality herbal products owing to their complex nature.

Keywords: *Antibacterial, Antifungal, Nano medicine, Traditional medicine and pathogen.*

I. INTRODUCTION

Siddha medicine is one of the most ancient medical systems of India. Siddha is the mother medicine of ancient Tamils/Dravidians of peninsular South India. The Siddha system of medicine, which has been prevalent in the ancient Tamil land, is the foremost of all other medical systems in the world. Its origin goes back to B.C 10,000 to B.C 4000 [1,2]. The uniqueness of Siddha system is evident by its continuous service to the humanity for more than 5000 years in combating diseases and also in maintaining its physical, mental and moral health while many of its contemporaries had become extinct long ago.

Siddha Nagarjuna has been considered to be the father of Indian alchemy and Rasa Sastra is one of the disciplines in which Parpam, Chendhuram and Chunnam were first described as intriguing formulations of metals and minerals such as gold, silver, copper, iron, zinc, mercury, and so forth, apparently associated with organic macromolecules derived from the herbal juices by alchemic processes making these biologically assimilable [3]. This system has enormous pharmacopoeia containing vegetable, animal and mineral products. All Siddhars are well versed in using mineral drugs. Silver, gold, zinc, copper and other metals which are well known to have anti-microbial effect in modern medicines had been used as wonderful life saving drugs against infectious diseases for over thousands of years without any adverse effects. Role of these herbo-mineral preparations for curing skin diseases such as psoriasis, eczema, alopecia, diabetic ulcer, warts, vitiligo and leprosy are well studied [4]. Subash Chandren *et al*., [5] had evaluated the antibacterial activity of the Siddha drug Seethabethi sanjeevi. The chloroform extracts of Seethabethi sanjeevi had shown the maximum inhibitory action against *E.coli* and *Shigella* sp. In aqueous extract Seethabethi sanjeevi had shown the highest action against *Citrobacter* sp. The acetone extract of medicine had showed the peak sensitivity against *E.coli* and *Micrococcus* sp. and peak inhibitory action against *Klebsiella* sp.

Thomas *et al.*, [6] studied the antibacterial activity of the Siddha medicine Thalaga Parpam‘ and Thurusu Chendhuram‘. The drug Thalaga parpam showed inhibitory action against one organism, *Streptococcus pneumoniae* with 18 diameter zone of inhibition. The next drug, Thurusu chendhuram was effective to inhibit the growth of *E.coli, Klebsiella sp., Staphylococcus aureus, Pseudomonas aeruginosa* and *Candida albicans* whereas the maximum zone of inhibition was against *Proteus* and *Streptococcus pneumoniae*. This drug had been used to Gunmam, Peruvayiru (Acidities) and diseases due to the derangement of Vatha, Pitha and Kapha. Tambekar and Dahikar [7] had reported the antibacterial potential of some Ayurvedic preparations such as *Mandura bhasma, Tamra bhasma, Lauha bhasma* and *Kashis bhasma* against enteric bacterial pathogens such as *Escherichia coli, Staphylococcus aureus, Enterobacter aerogenes, Pseudomonas aeruginosa, Bacillus subtilis, Klebsiella pneumoniae, Salmonella typhi, Staphylococcus epidermidis, Salmonella typhimurium* and *Proteus vulgaris.* Although the Siddha herbo-mineral preparations represent a rich source of antimicrobial agents [8] there is a limited number of *in vitro* antimicrobial studies on herbo-mineral nano preparations. Although the Siddha herbo-mineral preparations represent a rich source of antimicrobial agents.

II. MATERIALS AND METHOD

2.1. Antimicrobial assay (Kirby Bauer -Disc diffusion method)

The antimicrobial activity of the selected Siddha herbo-mineral preparations were determined by an agar-diffusion method using discs. The details of microbes used for the testing is given below.

2.2 Test microorganisms for antimicrobial studies

For the antimicrobial screening five species of bacterial isolates (*Escherichia coli, Salmonella typhi, Vibrio cholera, Staphylococcus aureus* and *Klebsiella pneumoniae*) and five species of fungal isolates (*Candida* sp.,

Aspergillus niger, *Trichoderma rubrum*, *Aspergillus flavus* and *Aspergillus fumigatus*) were used. The bacterial and fungal strains were obtained from National Collection of Industrial Microorganism (NCIM), Pune, India. The bacterial cultures used for antimicrobial testing were maintained on nutrient agar slant and the fungal strains were maintained on Sabouraud dextrose agar slant at 4^0C. The fresh bacterial cultures were obtained by growing the test organisms at 37^0C, for 24h while fungi were grown at 28^0C for 48h.

2.3. Antibacterial assay

Antibacterial activity was determined against certified strains of *Escherichia coli*, *Salmonella typhi*, *Vibrio cholerae*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, using a modification of agar diffusion assay method [9]. Discs of 6 mm diameters were used. Microorganisms were inoculated on nutrient broth (Himedia, Bombay) for 24h at 37^0C. The inoculate absorbance was established between 0.08 and 0.10 AU (equivalent to 0.5 McFarland 10^8 CFU/ml) adding sterile nutrient broth, before incorporating bacteria (λ=625 nm). Bacterial strains were seeded on Muller-Hinton agar. The sterile discs were impregnated in the seeded agar. From the stock solution, different concentrations of Siddha drug (ie) LC, KR and VK (200mg/ml) and for RC (1g/5ml). were taken. The sterile disc was loaded with the selected Siddha herbo-mineral preparations at concentrations of 5µl, 10µl 15µl and 20µl for LC, KR, VK and RC. A disc of Streptomycin (20 µg/disc) was used as a positive control. The plates were incubated at 37^0C for 24 h. All the assays were carried out in triplicate. The diameter of the growth inhibition zone was measured with standard zone reader scale (Himedia, Bombay) and recorded the mean diameter.

2.4. Antifungal assay

Antifungal activity was carried out by Kirby Bauer disc diffusion method against *Candida sp.*, *Aspergillus niger*, *Trichoderma rubrum*, *Aspergillus flavus*, *Aspergillus fumigatus.* The microorganisms were inoculated on Potato dextrose broth (Hi-media, Bombay) for 24h at 25^0C. The inoculate absorbance was established between 0.08 and 0.10 AU (equivalent to 0.5 McFarland 10^8 CFU/ml) adding sterile Potato dextrose broth, before incorporating fungi (λ=530 nm). Fungal strains were seeded on Potato dextrose agar with 4% glucose. The sterile discs were impregnated in the seeded. From the stock concentration of Siddha drug LC and RC (1g/5ml) and for KR and VK (200mg/5ml) were taken. The sterile discs were loaded with selected Siddha herbo-mineral preparations at a concentration of 40µl and 80µl for LC and RC, and 20 µl, 40 µl for KR and 10 µl, 20 µl for VK. Fluconozol (100µg/disc) antifungal agent was used as positive control. The plates were incubated at 25^0C for 48h. All the assays were carried out in triplicate. The diameter (mm) of the growth inhibition zone was measured with standard zone reader scale (Himedia, Bombay) and recorded the mean diameter.

III. RESULTS AND DISCUSSION

3.1. Antibacterial activity

Metallic herbal preparations offer advantages over plant drugs by virtue of their stability over a period, lower dosage, easy storability, and sustained availability as it contain minerals and metals as integral part of the formulations [10]. The metals and minerals are mixed with herbs because they are considered non-living and by

treating them with herbs they are converted to a living state thereby becoming bio-compatible. The same mineral and mercury processed with different herbs acts on different organs in the human body [7].

The selected Siddha herbo-mineral preparations were tested for antibacterial and antifungal activities. For antimicrobial assay five strains of bacteria viz. *E. coli, S. typhi, S. aureus and K. pneumonia* and *V. cholerae* were used, For antibacterial assay four concentrations of the Siddha drug (for LC, KR, VK and RC - 5 µl, 10 µl, 15 µL and 20 µl were used, Of the five bacteria tested, the growth of all the bacteria were well inhibited by the Siddha drugs. The disc diffusion assay indicated a dose dependent effect of the Siddha drugs to inhibit the growth of bacteria (Table 1). Due to the antibacterial activity of herbo-mineral preparations, *Linga chendhuram* used to treat fevers, skin diseases and also venereal diseases. It is given along with honey in a dose of 50 – 100mg/day. *Linga chendhuram* is derived from cinnabar. It has been widely used in clinical therapy [11]. It was observed that Linga Chendhuram was found to have a strong antibacterial activity against *E.coli, S.aureus, K. pneumoniae* at 20 µl concentrations and moderate anti-bacterial activity against *S. typhi* and *V. cholerae* at 10 µl and 15 µl concentrations (Fig 1).

Kantharasa villai is useful in skin diseases, cancer, gastric and arthritis. These herbo mineral drugs are prepared from purified mercury, cinnabar, white arsenic, camphor, magnet and other plant material. It was found that the *Kantha rasavillai* had a strong anti-bacterial activity against *S.aureus, Vibiro cholorae* and *K.pneumoniae* at 5 µl, 10 µl, 15 µl, 20 µl concentrations and mild antibacterial activity against *E.coli*, and *S.typhi* at 10 µl and 20 µl concentrations (Fig 2). *Vajera kandi* is a mineral based Siddha preparation. In this preparation cinnabar, calomel and Hydrargrum per chloride corrosive sublimate are purified and used. These herbomineral preparations are used to treat fevers, body pain, arthritis and for wound healing. Vajera kandi was found to have a strong antibacterial activity against *K.pnumoniae, S.typhi, and S.aureus* at 10 µl, 15 µl, 20 µl concentrations and moderate activity against *V.cholera* and *E.coli* (Fig 3).

Rasa chunnam is used to enhance immunity. It is prepared from purified mercury, egg shell, Alam and nitric acid. *Rasa chunnam* was found to show moderate antibacterial activity against S. *aureus, K. pneumoniae, V. cholerae and S. typhi* at 5 µl, 10 µl, 15 µl concentrations and moderate activity against *E.coli* at 20 µl concentrations (Fig 4).The present study clearly indicates that the selected Siddha drugs contain a good anti-bacterial potency against bacteria.

3.2. Antifungal activity

From the antifungal activity of herbo – mineral preparations, it was observed that the *Kantha rasavillai* and *Rasa chunnam* had moderate activity against the fungal strains (Fig:5) *Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Candida* sp *and Trichophyton rubrum* at the concentration of 40 µl and 80 µl for RC and 20 µl & 40 µl for KR. And the Vajera kandi had good activity against *Aspergillus fumigatus* and *Candida* sp at the concentration of 10 µl and 20 µl. *Kantha rasavillai* (KR), *Vajera kandi* (VK) (Fig: 5) were potentially controlling the fungal pathogen even in lesser concentration. But the *Linga chendhuram* had no activity against the five fungal strains (Table: 2) (Fig 5).

IV. CONCLUSION

The findings of the present study suggest that, Siddha herbo mineral preparations have great potential as anti-microbial agent against many enteric pathogens. The speciality of Siddha drugs is its adaptogenecity. The same drug can be prescribed successfully for various diseases simply by changing the vehicle accordingly. Thus these herbo mineral preparations can be used to control or prevent the enteric bacterial infection. Further research is deserved to identify the compounds responsible for the observed antibacterial activity. From the study, it was observed that the Herbo – mineral Drugs such as *Kantha rasavillai* (KR), *Vajera kandi* (VK) and *Rasa chunnam* (RC) was found to have antifungal activity. But the Herbo-mineral medicine, *Linga chendhuram* (LC) has no antifungal activity against the selected five fungal strains. So It is concluded that these three Siddha preparations KR, VK, and RC can be used to control or prevent the fungal infections. Modern techniques are necessary to standardize and bring out high quality herbal products owing to their complex nature.

The alternative systems of medicine such as Siddha, Unani and Ayurveda have always come to the rescue of modern medicine, whenever there was dearth for new drugs. The literature of Siddha medicine reveals several such drugs that are available for the treatment of fungal infections. However they do not have scientific data to substantiate their use as antifungal agents, though, they have been in use for centuries by the practitioners of Siddha systems of medicines [12]. The main purpose of this study is to provide scientific validation of the Siddha drugs tested for their antifungal activity. Further studies with these formulations *in vivo* may reveal interesting facts about these medicines. In these days of growing antibiotic resistance in bacteria, the identified herbo-mineral preparation in the present study will provide valuable drug to drug resistant bacteria in the cellular system.

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Table 1 Antibacterial Potential of selected Siddha herbo-mineral preparations against various enteric bacterial pathogens.

Source	**Conc.**	**Pathogenic microorganism**				
		E.coli	*S.typhi*	*S.aureus*	*K.pneumoniae*	*V.cholerae*
LC	5 µl	-	-	-	-	-
	10 µl	7.67± 0.33	7.67± 0.33	18.67±0.33	13.67±0.33	7.67±0.33
	15 µl	8.00± 0.00	7.67±0.33	19.67± 0.33	18.67±0.33	8.00±0.00
	20 µl	9.67± 0.33	7.67±0.33	25.67±0.33	21.67±0.33	7.67±0.33
KR	5 µl	6.33± 0.33	9.67± 0.33	10.67±0.33	15.67±0.33	11.67±0.33
	10 µl	7.67±0.33	13.67±0.33	14.67±0.33	19.67±0.33	15.67±0.33
	15 µl	11.33±0.33	15.67±0.33	19.67±0.33	23.67±0.33	18.67±0.33
	20 µl	12.67±0.33	16.67±0.33	20.67±0.33	21.33±0.33	21.67±0.33
VK	5 µl	7.67±0.33	9.67±0.33	14.67±0.33	19.67±0.33	11.67±0.33
	10 µl	14.33±0.33	18.00±0.00	20.00±0.00	29.67±0.33	18.67±0.33
	15 µl	14.67±0.33	19.67±0.33	23.67±0.33	30.00±0.00	20.33±0.33
	20 µl	15.67±0.33	22.67±0.33	26.00±0.00	34.67±0.33	19.67±0.33
RC	5 µl	7.67±0.33	8.00±0.00	9.67±0.33	7.67±0.33	8.00±0.00
	10 µl	8.67±0.33	8.33±0.33	9.00±0.33	8.67±0.33	9.67±0.33
	15 µl	9.00±0.00	9.67±0.33	9.33±0.00	9.67±0.33	9.67±0.33
	20 µl	9.67±0.33	10.67±0.33	10.67±0.33	10.67±0.33	10.67±0.33
Standard (20µl)		19.67± 1.20	22.67±0.88	28.33±0.33	10.67±0.33	27.67±0.33

Stock solution: For LC, KR, VK – 200 mg / 5 ml & For RC – 1 g / 5 ml

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Table 2 Antifungal Potential of selected Siddha herbo-mineral against various fungal pathogens.

Pathogenic Organism	Control/ Fluconozole	LC		RC		KR		VK	
	+ve	40µl	80µl	40µl	80µl	40µl	80µl	40µl	80µl
A. niger	-	-	-	12.33± 0.33	13.00± 0.58	11.67 ±0.33	14.00 ±0.58	-	-
A. flavus	-	-	-	8.67±0 .88	10.67± 0.33	12.33 ±0.88	17.33 ±0.33	-	-
A.fumigatus	-	-	-	-	-	11.00 ±0.58	13.33 ±0.88	37.00± 1.53	37.00± 1.73
Candida sps	29.00±0.58	-	-	12.00± 0.58	13.33± 0.67	11.00 ±0.58	16.67 ±0.33	14.33± 0.33	14.67± 0.33
T. rubrum	-	-	-	14.67± 0.33	16.67± 0.33	15.67 ±0.33	16.67 ±0.33	-	-

Stock solution: For LC & RC - 1g / 5 ml & For KR & VK - 200 mg / 5 ml, Positive control: Fluconozole

Figure 1 Antibacterial activity of Siddha drug –*Linga Chendhuram*

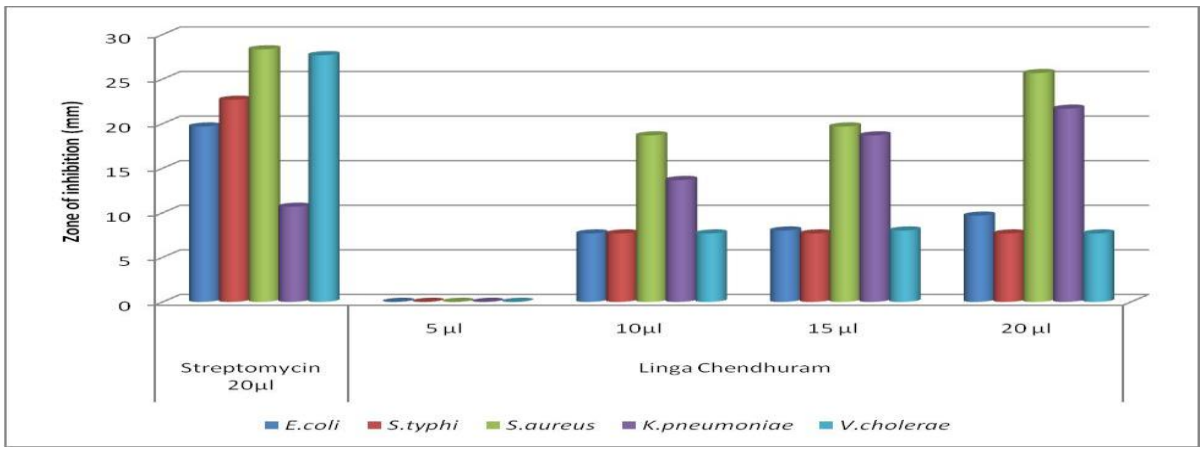

Figure 2 Antibacterial activity of Siddha drug – *Kantha Rasavillai*

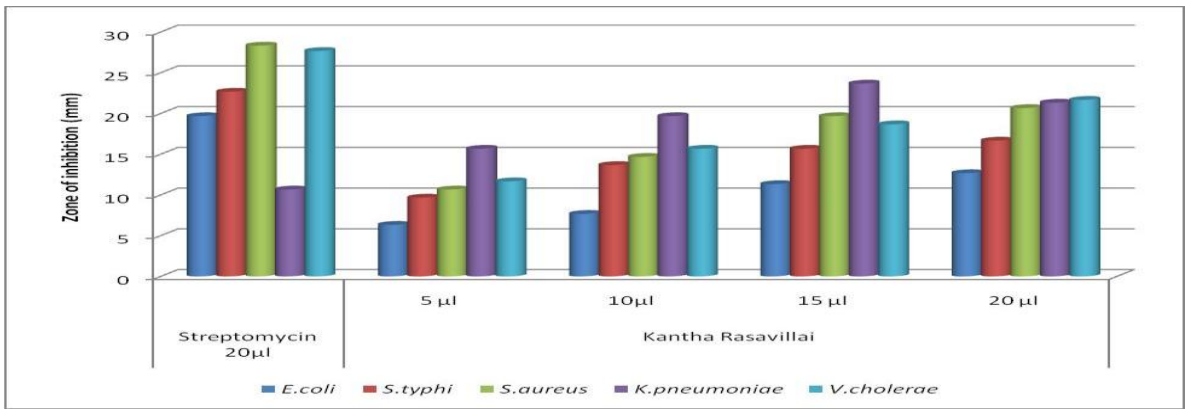

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Figure 3 Antibacterial activity of Siddha drug – *Vajera Kandi*

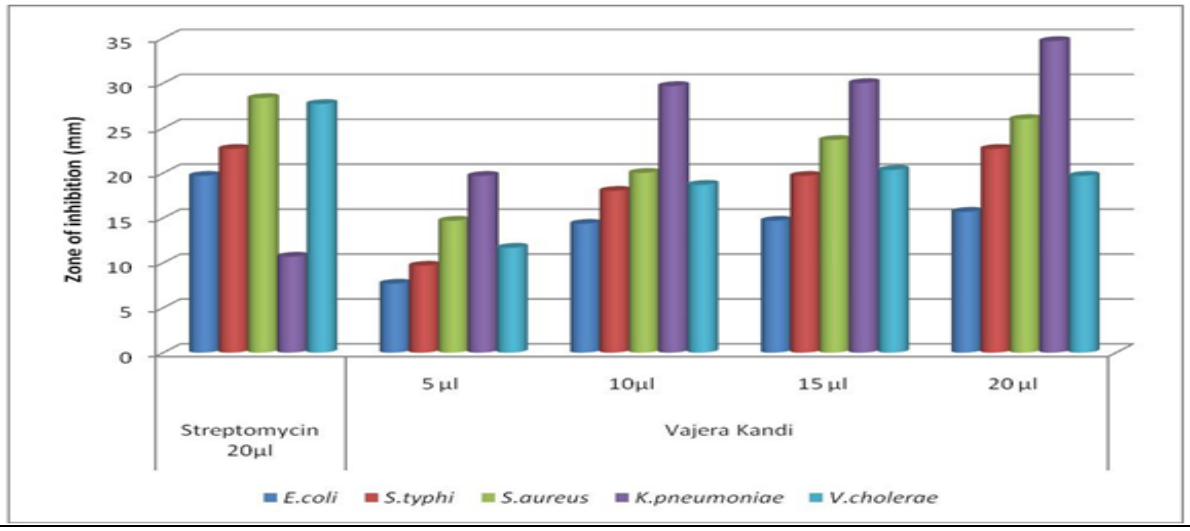

Figure 4 Antibacterial activity of Siddha drug – *Rasa Chunnam*

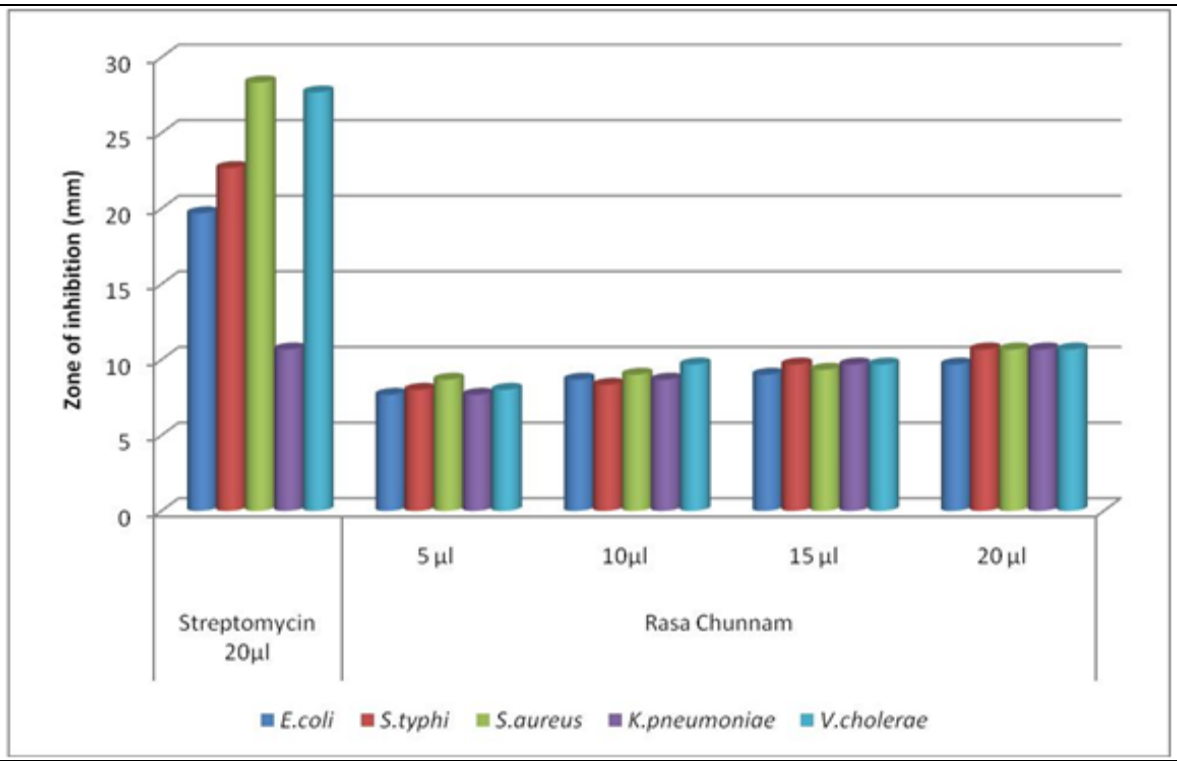

Figure 5 Antifungal activities of Siddha medicines RC, KR, and VK against various fungal strains.

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