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In Vitro Antifungal Activity Of Selected Siddha Herbo-Mineral Preparations: Linga Chend Ooram, Kandha Rasavillai, Vajra Kandi And Rasa Chunnam

J Savarimuthu Michael[1]*, A Kalirajan[1], V Lakshmanan [1], C Padmalatha[2], R Venkataraman[3] and AJA Ranjit Singh[1]

[1]Department of Zoology, Sri Paramakalyani College, Alwarkurichi, M.S. University, Tirunelveli, Tamil Nadu, INDIA
[2]Department of Zoology, Rani Anna Govt. College for women, M.S. University, Tirunelveli, Tamil Nadu, INDIA
[3]PG and Research Department of Chemistry, Paramakalyani College, Alwarkurichi. M.S. University, Tirunelveli, Tamil Nadu, INDIA

ABSTRACT

Nowadays the usage of Siddha Herbo-mineral drugs has increased to fight with the acute and chronic diseases of human kind. Considering the indigenous uses of the Herbo-mineral drugs, the present study deals with the investigation of *in vitro* antifungal activity by disc diffusion method against different fungal strains (*Candida sps*, *Aspergillus niger*, *Trichophyton rubrum*, *Aspergillus flavus*, *Aspergillus fumigates*). It was observed that certain herbo-mineral preparations have significant activity against the fungal strains.

Key words: Herbo-mineral, antifungal activity, indigenous, disc diffusion method.

Corresponding author

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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, Chedooram 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*]. Minerals are combined with herbs that assist the assimilation and delivery of the ingredients to the human body [4, 5]. These herbo mineral medicine are prepared by repeated incineration of metals or their salts (preferably oxides) with medicinal herbs or their extracts so as to eliminate their harmful effects and are taken along with honey, milk, butter, or ghee (a preparation from milk) [6].

The siddha herbo-mineral preparations represent a rich source of antimicrobial agents [7]. There are a limited numbers of *in vitro* studies on herbo - mineral preparations. Therefore, there is a need to identify antifungal potential of herbo - mineral products based on diseases for which no medicine or only palliative therapy is available. Hence in the present study, the antifungal activities present in the selected siddha herbo-mineral preparations were studied.

MATERIALS AND METHODS

The selected Siddha medicines Linga Chendooram (LC), Kanda Rasavillai (KR), Vajra Kandi (VK) and Rasa Chunnam (RC) were prepared according to the standard Siddha procedures with the help of local Siddha practitioners [8].

Test Microorganism

For the antifungal screening five species of fungal isolates were selected. The following fungal strains were obtained from National Collection of Industrial Microorganism (NCIM), Pune, India.

S. No	TYPES OF FUNGI TESTED
1	*Candida sps.*
2	*Aspergillus niger*
3	*Trichophyton rubrum*
4	*Aspergillus flavus*
5	*Aspergillus fumigatus*

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Screening for antifungal activity

Antifungal activity was carried out by Kirby Bauer disc diffusion method [9] against *Candida sps. Aspergillus niger, Trichophyton rubrum*, *Aspergillus flavus*, *Aspergillus fumigatus.* The microorganisms were inoculated on Potato dextrose broth (Hi-media, Bombay) during 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 Potato dextrose agar with 4% glucose. The sterile discs were impregnated in the seeded agar. The sterile disc were loaded with selected Siddha herbo-mineral preparations at a concentration 40µl and 80µl for LC & RC, and 20 µl, 40v for LC and 10 µl, 20 µl for VK. Fluconozole (100U/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 a mean diameter.

RESULTS AND DISCUSSION

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 [11]. The same mineral and mercury processed with different herbs acts on different organs in the human body. These Siddha herbo mineral preparations have great potential as anti microbial agent against many enteric pathogens [8]. The specialty of Siddha drug is its adaptogenecity. The same drug can be prescribed successfully for various diseases simply by changing the vehicle accordingly.

According to the antifungal activity of herbo – mineral preparations, it is observed that the Rasa Chunnam and Kantha Rasavillai have moderate activity against the fungal strains (Fig:1,2,3) *Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Candida sps and Trichophyton rubrum* at the concentration of 40 µl & 80 µl for RC and 20 µl & 40 µl for KR. And the Vajra Kandi has good activity against *Aspergillus fumigatus* & *Candida sps* at the concentration of 10 µl & 20 µl. “Kanda Rasavillai (KR), Vajra Kandi (VK)” are potentially controlling the fungal pathogen even in lesser concentration. But the Linga Chendooram has no activity against the five fungal strains (Table: 1).

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.

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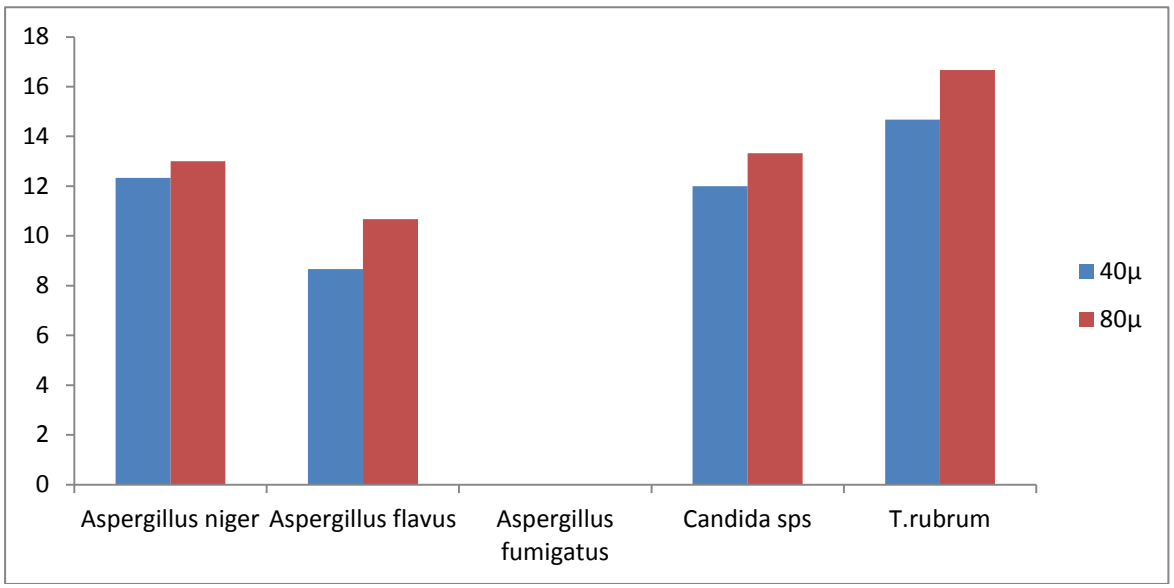

Fig: 1 Antifungal activities of Rasa Chunnam against various fungal strains

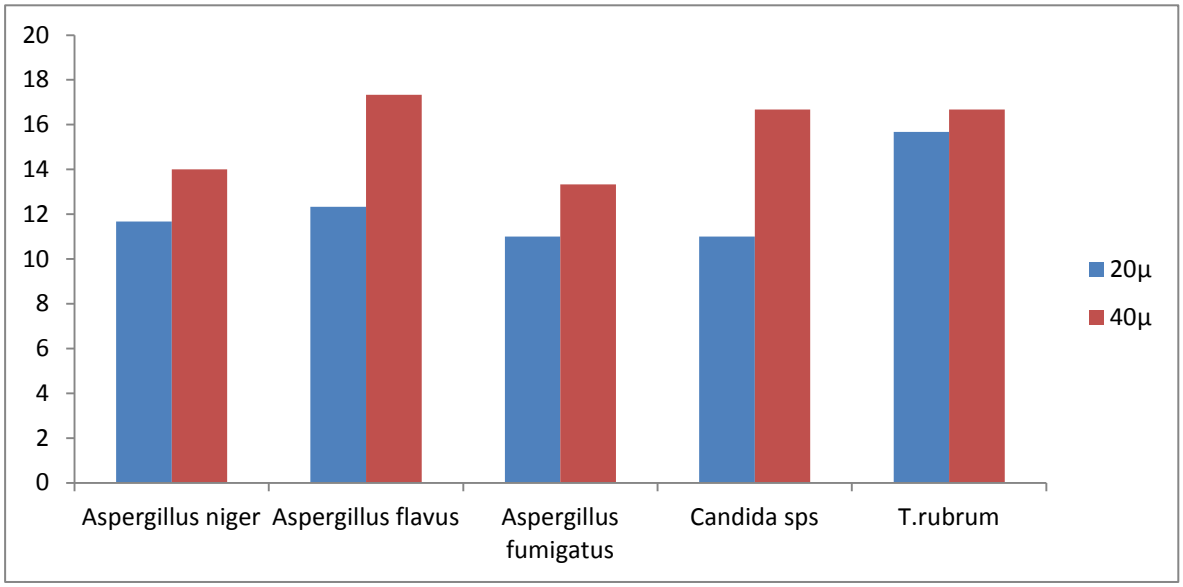

Fig: 2 Antifungal activities of Kandha Rasavillai against various fungal strains

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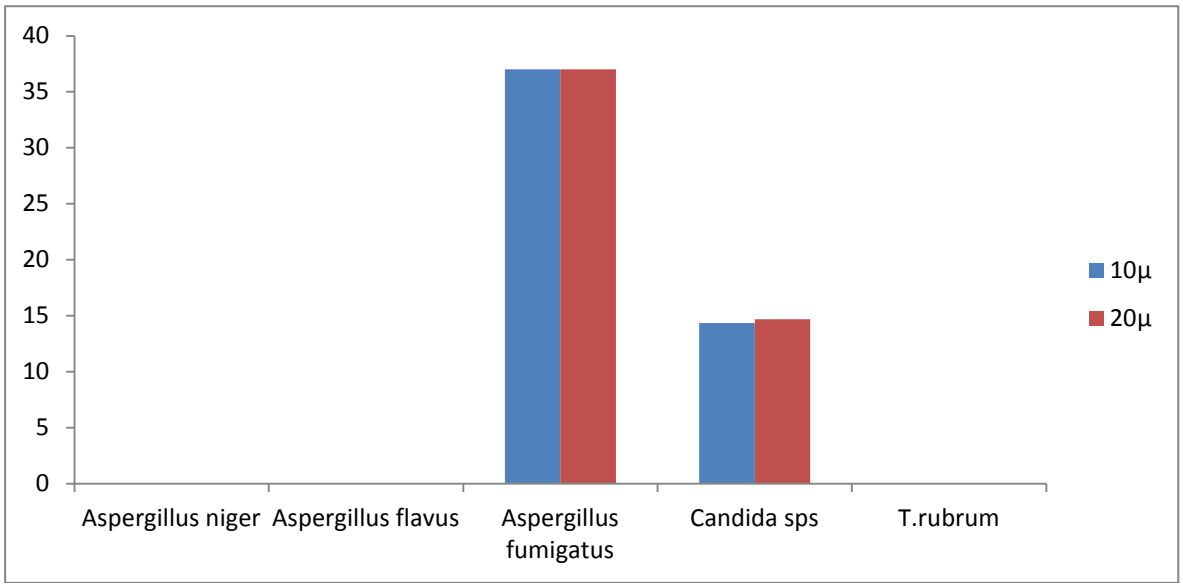

Fig: 3 Antifungal activities of Vajra Kandi against various fungal strains

Table: 1. Antifungal Potential of selected Siddha herbo-mineral against various fungal pathogens

Pathogenic Organism	Control		LC		RC		KR		VK	
	-ve	+ve	40µ	80µ	40µ	80µ	20µ	40µ	10µ	20µ
Aspergillus niger	-	-	-	-	12.33±0.33	13.00±0.58	11.67±0.33	14.00±0.58	-	-
Aspergillus flavus	-	-	-	-	8.67±0.88	10.67±0.33	12.33±0.88	17.33±0.33	-	-
Aspergillus 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

CONCLUSION

From the above study, it was observed that the Herbo-mineral Drugs such as Kanda Rasavillai (KR), Vajra Kandi (VK) and Rasa Chunnam (RC) was found with significant antifungal activity. The Herbo-mineral medicine, Linga Chendooram (LC) has no significant antifungal activity against selected five fungal strains. It is concluded that these three Siddha preparations can be used to control or prevent the fungal infection. Modern techniques are necessary to standardize and bring out high quality herbal products owing to their complex nature.

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