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Antifungal activity of polyherbal Siddha formulation- Seemai Agathi ointment

[1] Ganesh T*, [1] Sivakumar L, [2] Ramasamy MS and [2] Chitra Jayaram.

[1] R&D Division, SKM Siddha and Ayurveda Company (India) Ltd, Erode, Tamil Nadu, India.

[2] AU-KBC Research centre, ISM & Natural products laboratory, MIT Campus, Anna University, Chennai, Tamil Nadu, India.

***Corresponding Author:** E-Mail: ganeshmpharma@gmail.com

ABSTRACT

This study was carried out with an objective to investigate the antifungal potential of Seemai Agathi ointment- a polyherbal Siddha formulation. The aim of the study is to evaluate the potential of antimicrobial activity against medically important fungal strains and to determine the zone of inhibition of Seemai Agathi ointment. The antifungal activity of Seemai Agathi (250, 500, 750, 1000 µg/ml) were tested against six fungal strains- *Aspergillus niger, Aspergillus fumigatus, Cryptococcus laurentii, Candida albicans, Fusarium oxysporum* and *Microsporum gypseum*. Zone of inhibition of the polyherbal formulation were compared with that of the standard drug fluconazole for antifungal activity. The results showed that the formulation exhibits significant inhibition of the fungal growth against the tested organisms. So this study confirms the ethno medical use of this formulation in Siddha medicine for treating various fungal infections.

Keywords: Siddha formulation, Antifungal activity, *Cassia alata,* Ayush medicine.

1. INTRODUCTION

Skin is the most sensitive organ in the human body. Infection of the skin is caused by various pathogens such as bacteria, fungi and virus. Among the pathogens, fungi are the most causative organisms causing skin infections. Fungal infections represent an important paradigm in immunology, as they can result from either a lack of recognition by the immune system or over activation of the inflammatory response [1]. An antifungal agent is a drug that selectively eliminates fungal pathogens from a host with minimal toxicity to the host. Examples of antifungal agents include Amphotericin, nystatin, pimaricin, Fluconazole, Itraconazole and ketoconazole. Fluconazole is now routinely used to treat candidemia in non-neutropenic hosts, and is gaining acceptance for use in cryptococcosis and selected forms of coccidioidomycosis [2]. However, plant based medicines are of interest in this context because they comprise safer or more effective substitutes for synthetically produced antimicrobial agents [3].

Seemai Agathi also called as Vandukolli is the best medicinal plant against bacteria, fungi, virus and parasite. The botanical name of Seemai Agathi is *Cassia alata* which belongs to the family Caesalpiniaceae. This medicinal plant has got several uses including skin infections caused by bacteria, fungi etc. The ointment which was prepared by grinding the leaves with coconut oil or gingelly oil was applied externally over the affected areas and it is very good remedy against skin infection mainly fungal infections [4].

Table - 1: Composition of the formulation- Seemai Agathi ointment

Siddha name	Scientific name	Quantity
Karun Seeragam	*Nigella sativa*	20 %
Kattu Seeragam	*Vernonia anthelmintica*	20 %
Neeradimuthu	*Hydnocarpus pentandra*	20 %
Kandhagam	Elemental sulphur	20 %
Karboga Arishi	*Psoraela corylifolia*	20 %

Processed in:
Cassia alata, Citrus aurantifolia, Aristolochia bracteata, Lawsonia inermis, Apis mellifera, Cocos nucifera.

2. MATERIALS AND METHODS

2.1. MATERIALS

2.1.1 Collection of drug and test organisms

Seemai Agathi ointment is manufactured by SKM Siddha and Ayurveda Company (India) Limited, Erode, Tamil Nadu. The product is

obtained from the SKM Siddha and Ayurveda Company (India) Ltd, Erode (Batch No: OAA13008 Mfg Date: August 2013, Batch No: OAA13011, OAA13012 Mfg date: October 2013). The test organisms (fungal pathogens) such as *Candida albicans (MTCC 183), Aspergillus niger (MTCC 281), Aspergillus fumigatus (MTCC 8877), Cryptococcus laurentii (MTCC 3954), Microsporum gypseum (MTCC 4524)* and *Fusarium oxysporum (MTCC 7677)* were purchased from MTCC, Chandigarh, India.

2.1.2. Media requirements

Sabauroud's Dextrose agar, Nutrient medium, well maker, DMSO, micropipette, Conical flasks, petri dishes, test tubes, beakers, sterilized tips, Bunsen burner, loop, etc.,

2.2. METHODS

2.2.1. Culturing of organisms

The medium Sabauroud's dextrose agar and nutrient broth were prepared and sterilized at 121˚C for 20 minutes using the autoclave. The glass wares used in this study were also sterilized before use. The fungal pathogens *Candida albicans (MTCC 183), Aspergillus niger (MTCC 281), Aspergillus fumigatus (MTCC 8877), Cryptococcus laurentii (MTCC 3954), Microsporum gypseum (MTCC 4524), Fusarium oxysporum (MTCC 7677)* were subcultured in the nutrient medium.

2.2.2. Extraction of drugs

The topical ointment was dissolved in water and prepares a stock solution of 10 mg/ml and different concentrations of the ointment were prepared by serial dilution technique. The concentrations of Seemai Agathi were 250µg/ml, 500µg/ml, 750µg/ml and 1000µg/ml.

2.2.3. Screening for antifungal activity

The sterilized Sabauroud's Dextrose agar was poured into the petri plates aseptically and allowed to solidify at room temperature.The antibacterial agent Tetracycline (500mg) was added to the agar medium and mixed well before pouring to the petri plates. The antifungal activity was done by agar well diffusion method as follows. Once the medium had solidified, four wells, each 5 mm in diameter, were cut out of the agar and each fungal pathogen *Candida albicans (MTCC183), Aspergillus niger (MTCC 281), Aspergillus fumigatus (MTCC 8877) , Cryptococcus laurentii (MTCC 3954), Microsporum gypseum (MTCC 4524)* and *Fusarium oxysporum (MTCC 7677)* was swabbed into each plate. 50 µl of the Seemai Agathi were placed into each well at different concentrations (250µg/ml, 500µg/ml, 750µg/ml and 1000µg/ml). Fluconazole (FLC) (standard antifungal agent) was used as a positive control. The plates were kept in incubator to observe the zone of inhibition. The zone of inhibition was measured from the agar well to the end of the zone (mm). The minimal inhibitory concentration of the ointment was also determined [5-8]. Triplicates were maintained.

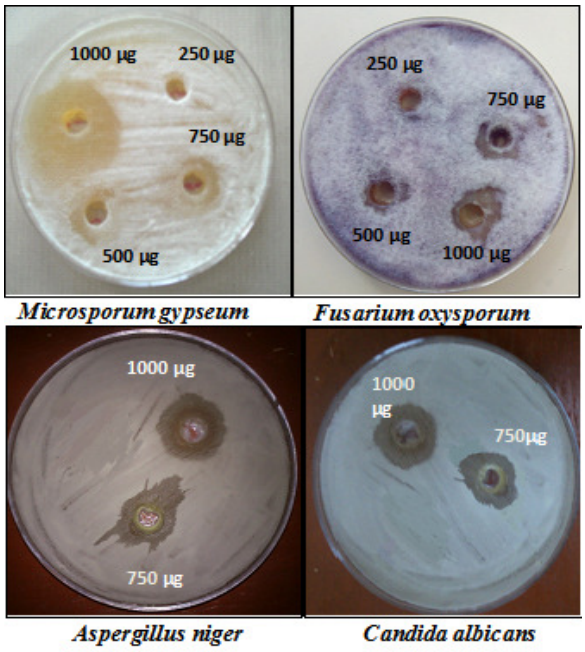

Figure - 1: Antifungal activity of Seemai Agathi ointment.

3. RESULTS AND DISCUSSION

The dose dependent antifungal activity of Seemai Agathi ointment was observed. The broad spectral antifungal activity was observed for all the fungal pathogens. The results were shown in the tables 2 and 3. The minimum inhibitory concentration was observed as 20 µg/ml against *Aspergillus fumigatus*. The important medicinal plant in this formulation is found to be *Cassia alata*[9-11].

Table - 2: Antifungal activity of Seemai Agathi ointment

Name of the fungal pathogens	Seemai Agathi Diameter of zone of inhibition (mm)			
	Concentration (µg/mL)			
	250	500	750	1000
Aspergillus niger	16	22	23	25
Aspergillus fumigatus	14	21	22	23
Cryptococcus laurentii	8	12	14	17
Candida albicans	14	16	18	21
Fusarium oxysporum	6	8	11	14
Microsporum gypseum	9	12	14	16
Fluconazole (500 µg/ml)	18	22	23	26

Table - 3: Minimal inhibitory concentration of Seemai Agathi ointment

Name of the fungal pathogens	Seemai Agathi (MIC*) in µg/mL
Candida albicans	25
Aspergillus niger	25
Aspergillus fumigatus	20
Cryptococcus laurentii	75
Microsporum gypseum	75
Fusarium oxysporum	75

4. CONCLUSION

In summary, the polyherbal Siddha formulation-Seemai Agathi ointment shows significant activity against the clinically important fungal strains. The results were compared with standard antifungal drug. Many investigations are being carried out throughout the world to discover plant products to inhibit the clinically important fungal pathogens. The World Health Organization (WHO) estimates that plant extracts or their active constituents are used as folk medicine in traditional therapies of 80% of the world's population. Hence, plant based medicines that inhibit their growth without harming the host represent potential therapeutic agent. Each and every country has their own indigenous system of medicine and many of the formulations were not validated for their purported claims and it is the need of the hour to scientifically prove the claimed effects to spread those systems to gain acceptance at the global level.

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