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EXAMINING KALMIA LATIFOLIA'S ANTIFUNGAL PROPERTIES IN THE CONTEXT OF HOMOEOPATHIC MEDICINE: AN *IN VITRO* STUDY

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ABSTRACT

According to the World Health Organization (WHO), the prevalence rate of superficial mycotic infection worldwide has been found to be 20-25%. Medicinal components from plants play an important role in conventional as well as western medicine in treating fungal diseases. Plant derived compounds as potent drugs have been a part of human evolution and healthcare for thousands of years. Main aim of this study is to analyze the zone of inhibition produced by homeopathic medicine in various potencies in the culture media proving its antifungal activity. The medicine Kalmia Latifolia 30C, 200C and 100 is used for the study. Fungi *Aspergillus Niger, Aspergillus Flavus* and *Candida Albicans* were purchased from Microbial Type Culture Collection and Gene Bank (MTCC) Chandigarh. The bacterial strains were maintained on Nutrient Agar (NA). Antibiotic susceptibility tests were determined by agar disc diffusion (Kirby-Bauer) method. Kalmia 200C shows maximum effectiveness against the fungal strains.

KEYWORDS: Antifungal, Antibiotic, Conventional, Kalmia Latifolia, Potencies.

INTRODUCTION

According to the World Health Organization (WHO), the prevalence rate of superficial mycotic infection worldwide has been found to be 20-25%. Its prevalence varies in different countries [1]. Fungal strains selected in this study were Aspergillus, Candida Albicans and Penicillin. Herbal medicines have been playing a vital role in treatment and cure for various fungal diseases in traditional methods such as Ayurveda, Unani, Homoeopathy and Siddha. Medicinal components from plants play an important role in conventional as well as western medicine. Plant derived compounds as potent drugs have been a part of human evolution and healthcare for thousands of years [2].

Our study demonstrates the homeopathic medicine Kalmia Latifolia in 30C, 200C and 1M had inhibitory action against Aspergillus Niger, Aspergillus Flavus and Candida Albicans. Further detailed analysis of various different potencies of homeopathic medicines is a subject testing on the principles of homeopathy viz., "Similia Similibus Curentur" in identifying cellular level and exact molecular targets and mechanism of action of these ultra-high diluted medicines [3].

Candida Albicans is a fungal commensal of human skin and mucosal surfaces that can transit into an invasive fungal pathogen within immune-compromised individuals. C. Albicans infection results in over 400000 cases of invasive disease worldwide and systemic infection leads to high mortality rate annually [4]. Like many opportunistic fungi, a key virulence factor of C. Albicans has the ability to undergo a reversible morphological switch from a unicellular (yeast) to a filamentous (hyphal or pseudo hyphal) growth form. This switch, resulting in changes of both cell shape and cell physiology, is thought to allow fungal pathogens to adapt in different environmental conditions and has been correlated with pathogenicity traits [4].

However, the management of Candida infections faces a number of problems including limited number of effective antifungal agents, toxicity and the high cost of antifungal agents. Besides these things, the indiscriminate and prolonged use of antifungal drugs has led to therapeutic failures associated with an emergence of multi drug resistance to pathogenic organisms. Therefore, there is a need for the development of alternative therapies where solutions for the optimal treatment of fungal infections could be found. Medicines from plant origin could be a possible solution since herbal drugs have been used as a traditional treatment for numerous diseases. However, detailed studies of antifungal activity of homeopathic medicines against C. Albicans are limited. Therefore, the homeopathic medicines used in the present study were selected based on their clinical indications and through literature search [4]. Fungal keratitis is a leading cause of ocular morbidity throughout the world, particularly in warm climates and developing countries. It is estimated that corneal ulceration and ocular trauma result in 1.5 to 2 million new cases of corneal blindness annually. In certain parts of the world, such as South India, nearly half of these corneal ulcers may be secondary to fungal infection [5]. Epidemiologic studies in India have identified filamentous fungi, Fusarium and Aspergillus, as the two most common causes of fungal keratitis [5].

MATERIALS AND METHODS

Following medicine Kalmia Latifolia 30C, 200C, 1M were collected from Schawabe India Pvt Ltd.

Fungal Strains

Standard fungal strains of the following organism used in this study were *Aspergillus Flavus, Aspergillus Niger* and *Candida Albicans.*

Preservation of Fungal Strains

The fungal test strain of *Aspergillus Niger, Aspergillus Flavus* and *C. Albicans* were freeze-dried culture was aseptically opened in microbiology lab and the suspension was made as per protocol. 0.4 ml sterilized water was taken in a micro centrifuge tube, and freeze-dried culture was transferred into it and mixed well. The mixture was allowed to stand for 20 min before transferring it on solid media. Petri plates containing sabouraud dextrose agar (SDA) medium and incubated for 24–48 h at 35°C to give white round colonies against a yellowish background. Approximately, 1-mm colonies were picked up and suspended in 5 ml of sterile SDA and kept as broth culture/stock culture. Microorganisms were repeatedly subculture using streaking methods and maintained to obtain pure isolation on the SDA for further drug sensitivity assay [4]. Optical density of the Fungal strains was measured after 24 hrs., of incubation at 28ºC (pH, 5.5) using Spectrophotometer at 600 nm. (14) Cultural inoculum was standardized for all experiments as OD 0.511A at 600nm [3].

Preparation of Disc for Antifungal Assay

Antibiotic susceptibility tests were determined by agar disc diffusion (Kirby-Bauer) method. Fungal strains Aspergillus Flavus and Candida Albicans were swabbed using sterile cotton swabs on SDA agar plate. Up to 80 µl of 1 mg/ml concentration of the extract was introduced in the sterile discs (10 mm) using sterile pipettes. The standard drug Fluconazole 150 mcg concentration was introduced in the sterile disc (10 mm) for positive control and empty sterile disc was used for negative control. The disc was then placed on the surface of SDA medium and the compound was allowed to diffuse for 5 minutes and the plates were kept for incubation at 22°C for 48 hours. At the end of incubation, inhibition zones were examined around the disc and measured with a transparent ruler in millimeters. [6]

RESULTS AND DISCUSSION

The Marked anti-fungal was observed with Kalmia Latifolia. The growth of A. Niger was inhibited and showed maximum zone of inhibition up to 7mm by Kalmia 100 and upto 9mm by Kalmia 200C. No inhibition was observed in Kalmia 30C as compared to control. The growth of A. Flavus was inhibited and showed maximum zone of inhibition upto 9mm by Kalmia 1M upto 10mm by Kalmia 200C and upto 12mm by Kalmia 30C.As compared to control the antifungal activity of Kalmia against C. Albicans was profound. The growth was inhibited with an inhibition zone upto 10mm by Kalmia 1M, 9mm by Kalmia 200C and 10mm by Kalmia 30C as compared to control. Other studies also prove the antifungal activity of Kalmia against A. Niger. The marked antifungal activity was observed of Zingiber Officinale, the growth of A. Niger was inhibited and showed maximum zone of inhibition up to 15.4 ± 2.88 mm. [7]

Table 1: Antifungal Activity of Kalmia Latifolia against Various Pathogens

Fungi	***Kalmia 1M***	***Kalmia 200C***	***Kalmia 30C***	***+ve control***	***+ve control***
Aspergillus Niger	7mm	9mm	NZ	26mm	NZ
Aspergillus Flavus	9mm	10mm	12mm	30mm	NZ
C. Albicans	10mm	9mm	10mm	30mm	NZ

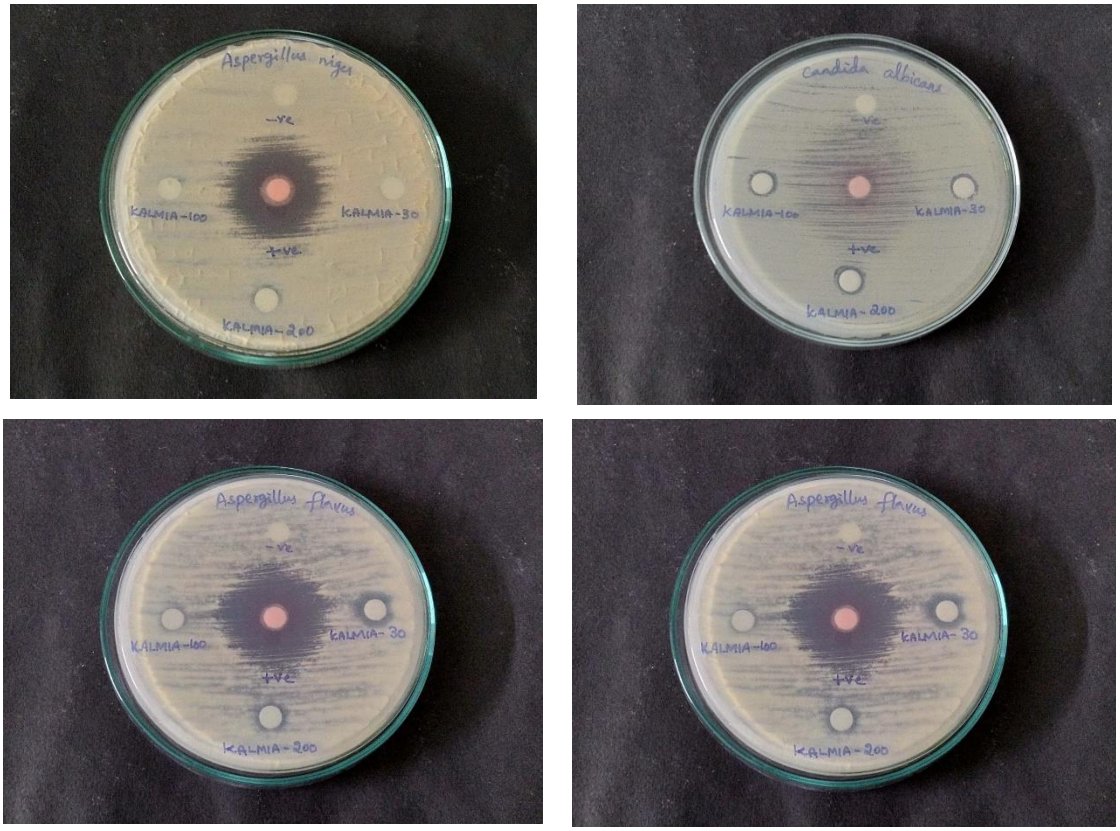

Fig 1: Antifungal Activity of Kalmia Latifolia against Various Pathogens

CONCLUSION

The findings of study concluded that potencies of Kalmia Latifolia can effectively inhibit the growth of A. Niger A. Flavus and C. Albicans in vitro. This study paves the way for development of homeopathic antifungal treatments. However, further investigations are required to get more information about the mechanistic approach, their mode of action and in vivo evaluation. The other homeopathic medicines that have effective action against fungal strains are Sepia Officinalis, Syzygium Jambolanum, Calcarea carbonica, Petroleum, Benzoic acid, Apis Mellifica, Mezerium, Sulphur, Graphites, Silicea, Iodium, Phosphorus, Zincum Metallicum. Hence we able conclude

that Homeopathic medicines have the antioxidant property thus we can use homeopathic medicines against free radicals.

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