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PHARMACOLOGICAL EVALUATION OF POLYHERBAL EXTRACTS OF *Amorphophallus paeoniifolius, Citrus sinensis* AND *Plumbago zeylantia* FOR ANTI FUNGAL ACTIVITY

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ABSTRACT
Fungal infections may take the form of superficial infections involving the skin or mucous membranes or systemic involving various internal organs. Deep infections are systemically treated with drug therapy. Among the fungi that cause systemic infections are Aspergillus spp., Nocardia asteroides, Histoplasma capsulatum and Coccidiodes immitis. Among ancient civilizations, India has been known to be rich repository of medicinal plants. Recently, WHO estimated that 80 percent of people worldwide rely on herbal medicines for some aspect of their primary health care needs. Treatment with medicinal plants is considered very safe and there is no or minimal side effects. Medicinal plants are considered as rich sources of ingredients which can be used in drug development. The Ayurvedic literature Sarangdhar Samhita' highlighted the concept of polyherbalism to achieve greater therapeutic efficacy. The active phytochemical constituents of individual plants are insufficient to achieve the desirable therapeutic effects. When combining the multiple herbs in a particular ratio, it will give a better therapeutic effect and reduce the toxicity. This research mainly focuses on important of the polyherbalism and its clinical significance.

KEY WORDS
Polyherbals, Fungal infections, Amorphophallus Paeoniifolius, citrus sinensis, Plumbago Zeylantia.

INTRODUCTION
Infectious diseases represent a critical problem to health, and they are one of the main causes of morbidity and mortality worldwide (World Health Organization 1998). Despite the significant progress in human medicine, infectious diseases caused by microorganisms such as fungi are still a major threat to public health. The impact is even more in developing countries due to the unavailability of medicine and the emergence of widespread drug resistance.

One of the major public health problems is Dermatophytosis. Dermatophytes include three types of fungus such as Microsporum, Trichophyton and Epidermophyton which commonly causes skin diseases in animals and humans. Dermatophytosis is an infectious disease of skin, hair and nails which attack on the keratinized tissue.

The rich content of antifungal substances in plants are being used biopesticide since up to the beginning of human civilization. Antifungal effects of plant and plant products emerge clearly every day. Antifungal substances which are obtained from plants have no side effect against environment thus, giving a significant advantage. There are many synthetic and natural product-based drugs available for treating fungal infections, but they are not consistently effective. Furthermore, the development of resistance in fungi against most of the drugs has now been reported for several years. The use of amphotericin B, known as the "gold standard", is limited because of its infusion-related problems and nephrotoxicity. In addition, the low efficacy, and side-effects and resistance associated with the existing drugs, highlight the advent of safe, novel, and effective antifungal drugs. Plants produce a great deal of secondary metabolites, many of them with antifungal activity. Similarly, traditional medicine has made use of many different plant extracts for treatment of fungal infection and many of these have been tested for in vitro antifungal activity. Based on the knowledge that plants develop their own defense against fungal pathogens, they appear as an interesting source for antifungal compounds.

MATERIAL AND METHODS

Amorphophallus paeoniifolius (*Dennst*.) *Nicolson* (Araceae) is a commonly available tuber in South India, widely used in folk medicine for treatment of acute rheumatism, tumors, lung swelling, asthma, vomiting, and abdominal pain. So far, no attempts have been made to evaluate the chemical composition and medicinal properties of *A. paeoniifolius.* Hence, the present study was performed to investigate the antifungal potential of ethanol extract of *A. paeoniifolius* using different *in vitro* models.

Citrus sinensis L. is an evergreen tree belonging to the family Rutaceae family. It is native to China but is widely cultivated throughout the world. It currently ranks as the most commonly cultivated and commercialized specie of citrus. Citrus fruits, including their by-products, have been reported to possess high medicinal value in addition to their economic values. The fresh fruits are consumed on a large scale and large quantities are processed to produce juice. The wastes produced, after consumption and juice extraction, such as the peels, pulps and seeds are a potential source of valuable byproducts. The oil obtained from the seeds, flowers, fruits and rinds of different species of Citrus also find wide applications in the toiletry, confectionary, and perfumery industry. The oils extracted from the peels and seeds of C. sinensis have also been reported to possess different type of activities ranging from insecticidal activity, to antimicrobial activity against a wide range of microbial organisms, hence this study was designed to evaluate and validate the activity of C. sinensis seed oil through an *in vitro* antifungal assay.

Plumbago zeylanica L. is a multipurpose medicinal herb of family Plumbaginaceae. A native of South Asia, the species is distributed throughout most of the tropics and subtropics. In India P. zeylanica commands an important place among medicinal herbs in India since ancient times. Ayurveda, the Indian indigenous system of medicine dating back to the Vedic ages (1500-8000 BC), has described chitraka as tumor-negating and anti-dyspepsic. Plumbagin has shown antibacterial activity against both gram-positive (e.g. Staphylococcus, Streptococcus, Pneumonococcus sp.) and gram-negative (e.g. Salmonella, Neisseria) bacteria. It is also active against certain yeasts and fungi (Candida, Trichophyton, Epidermophyton and Microsporum spp.) and protozoa (Leishmania).

Preparation of extracts

Air dried coarsely powdered plant materials of (*Amorphophallus Paeoniifolius, Citrus Sinensis* and *Plumbago Zeylantia*) were extracted with ethanol (95%) using soxhlet apparatus for 4-5 hrs. All the extracts were concentrated at low pressure by rotary flash evaporator and finally air-dried.

Microbial cultures and Growth Stains

The plant extracts were assayed for antifungal activity against the fungal strain Bacillus subtilis (Gram +ve) and Escheritia coli (Gram –ve).

Potato Dextrose Agar (PDA) Medium (pH 6.7)

Potato - 250g
Dextrose - 15g
Agar - 18g
Distilled water - 1000ml

Effect of Plant Extracts on Mycelium Growth

The poisoned food method was used in the preliminary screening of aqueous extracts for their antifungal properties evaluation. First, the mycelia growths were evaluated in 60 mm Petri dishes filled with PDA solid medium amended with 10% and 20% aqueous extracts of each plant. Next, the center of each Petri dish was inoculated with 5 mm diameter disc of fungal mycelium, taken from pure culture (7 days old). Then, all inoculated dishes were incubated at 25∘ C for 6 days. After that, the radial mycelial growth was measured 6 days after inoculation. For each treatment, three replicates were maintained.

Finally, the antifungal activity of each extract was calculated in terms of inhibition percentage of mycelia growth by using the following formula:

% inhibition = $(dc - dt)\ / dc \times 100$

where dc is the average increase in mycelia growth in control and dt is the average increase in mycelia growth in treated.

RESULTS

Table 1: Effects of 10% and 20% concentrations of aqueous extracts of different plants on growth of *Asperigillus* and *C albicans* for mycelia growth index.

Treatment	Concentration	Mycelia growth index (mm/hr)		Mean
		Asperigillus	*C albicans*	
Control	0%	0.64 ± 0.015	0.70 ± 0.028	0.67
Amorphophallus Paeoniifolius	10%	0.58 ± 0.027	0.61 ± 0.012	0.60
Amorphophallus Paeoniifolius	20%	0.51 ± 0.052	0.47 ± 0.024	0.54
Citrus Sinensis	10%	0.60 ± 0.041	0.63 ± 0.054	0.61
Citrus Sinensis	20%	0.56 ± 0.072	0.61 ± 0.038	0.58
Plumbago Zeylantia	10%	0.62 ± 0.036	0.68 ± 0.018	0.65
Plumbago Zeylantia	20%	0.57 ± 0.023	0.62 ± 0.056	0.59
Polyherbal Extract	10%	0.43 ± 0.072	0.54 ± 0.012	0.48
Polyherbal Extract	20%	0.41 ± 0.052	0.53 ± 0.024	0.47

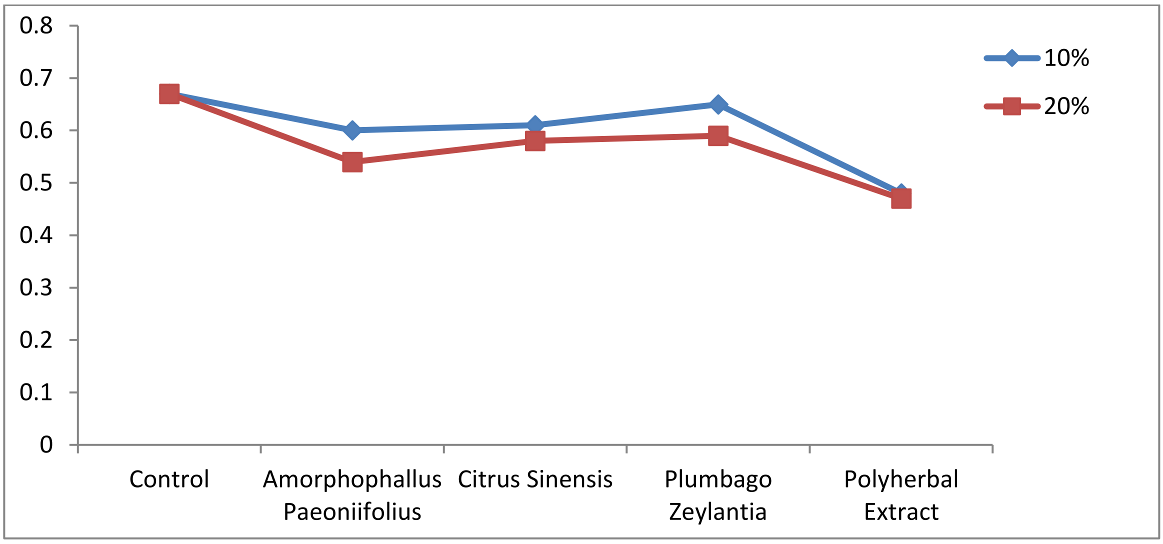

Figure 1: Mycelia growth index of *Asperigillus* and *C albicans* on various treatments

DISCUSSION

This study showed the treatment with various extracts on *Asperigillus* and *C albicans* results in anti-fungal activity when compared with control. The extracts of *Amorphophallus Paeoniifolius, Citrus Sinensis* and *Plumbago Zeylantia* showed anti-fungal activity with very much little variance and when the combination of extract (polyherbal extract) was given, results in gradual enhancement in anti-fungal activity. This shows the synergistic activity achieved by polyherbal extracts.

CONCLUSION

The Polyherbal Extracts of *Amorphophallus Paeoniifolius, Citrus Sinensis* and *Plumbago Zeylantia* for Anti-Fungal Activity was evaluated and was found to be very potent regarding anti-fungal activity when they were given in combination.

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