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Screening of Antifungal Activity of Ornamental Flower Extracts against Dermatophytic Fungi

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Abstract: In India, tones of *Calendula officinalis* and *Chrysanthemum maximum L*. flowers are used for worshipping in temples and homes. *Calendula officinalis* and *Chrysanthemum* flower extracts have known to have medicinal value. Chemical studies have underlined the presence of various classes of compounds, the main being triterpenoids, flavonoids, alkaloid, coumarines, quinones, volatile oil, carotenoids and amino acids. In the present study dry and fresh extracts of *Calendula officinalis* and *Chrysanthemum maximum L* showed different levels of inhibitory effects on four human and animal dermatophytic fungi. Chloroform extract of Chrysanthemum showed higher inhibition of the pathogenic fungi as compared to ethanolic extract.

Keywords: *Calendula officinalis , Chrysanthemum maximum L, dermatophytic fungus*

I. INTRODUCTION

Pathogenic fungi are fungi that cause disease in humans or other organisms. Although fungi are eukaryotic organisms many pathogenic fungi are also microorganisms[1]. Among the animal pathogenic fungi the most extensively studied have been those that cause disease in humans[2]. Dermatophytes (Ringworm fungi) are anamorphic hyphomycetous, keratinophilic fungi that parasitize keratinized tissue (hair, skin, nails) of humans and animals and cause dermatophytoses (dermatomycoses). These fungi occur widely in soil and other keratin containing substrata[3]. The dermatophyte have the ability to invade keratinized tissue (skin, hair, and nails) but are usually restricted to the nonliving cornfield layer of the epidermis because of their inability to penetrate viable tissue. India, being a tropical country, is rich in medicinal plant diversity [4]. Invasion of dermatohytes elicits a host response ranging from mild to severe which causes the development of cell-mediated immunity and an inflammatory response which is associated with clinical cure[5]. This has led to a search for therapeutic alternatives, particularly among medicinal plants and compounds isolated from them used for their empirically antifungal properties. One possible approach is to screen local medicinal plants in search of suitable antifungal chemotherapeutic substances[6], [8]. The molecules present in plants are secondary metabolites that have led to the development of pharmacologically active extracts[7]. In the present study waste flowers of *Calendula officinalis Linn*. (Asteraceae) and *Chrysanthemum maximum L* have been used. These flowers are used as ornamental flowers to be made into garlands for worshipping of Gods. The Calendula flowers contains triterpenoid esters (an anti-inflammatory chemical) and the carotenoids, flavoxanthin and auroxanthin (antioxidants) [9]. The Chrysanthemum flower contains significant amounts of flavonoids (up to 30%, w/w) and hydroxycinnamoylquinic acids that are considered to be the biologically active components[11]. Thus *Calendula officinalis* and *Chrysanthemum maximum L* are known to have medicinal importance due to pharmacological activity of its flowering part. [12] The aim of the this work was to study in vitro antifungal activity of the *C. officinalis* and *Chrysanthemum maximum L*. flower extract by disc diffusion method, against the human and animal pathogenic fungi.

II. MATERIALS AND METHODS

The flowers of Calendula and Chrysanthemum were collected from temples dried in oven for overnight so as to completely remove moisture. 3 different concentration i.e. 20%, 50% and 80% of fresh as well as dry flower extract were prepared in chloroform and ethanol. These extracts were then filtered through membrane filter in laminar air flow chamber and were used for testing the antifungal properties against the dermatophytes. . Four fungal strains were collected from humans and pets : 1. *Malassezia pachydermatis* (Fungus 2) which is a *yeast* found on the skin and ears of dogs. Though a normal inhabitant of these regions, an abnormal overgrowth of the yeast can cause *dermatitis*, or inflammation of the skin. 2. *Sporothrix schenckii* (Fungus 3) is a fungus that has the potential to infect the skin, respiratory system, bones and sometimes the brain, causing a diseased state called sporotrichosis. 3. *Trichophyton* (Fungus 4) is the most common cause of athlete's foot, fungal infection of nail, jock itch, and ringworm worldwide.4. *Malassezia furfures or Dandruff* (Fungus 5) causes dandruff in humans and leads to itiching and disturbance.

Antifungal activity of the extracts was observed by well diffusion method. 100 ul of dry and fresh flower extracts were inoculated with the pathogenic fungal strain. The plates were incubated for 24 hours and then the zone of inhibition (in mm) was observed and documented.

To detect the presence of alkaloids in the flowers. Detection of alkaloids was done through Dragondroff's method then subjected to quantitative estimation by UV-Spectrophotometer. This method is based on the reaction between alkaloid and bromocresol green (BCG).[15]

III. RESULTS AND DISCUSSION

The present study involved the use of ornamental flower extracts of Calendula and Chrysanthemum. The extracts were made in ethanol and chloroform and both dry and fresh flower material was used. Dermatophytic fungi were isolated from humans and animals and were characterized based on their morphological and microscopic structure. The Fungi which were isolated were identified to be

1. *Malassezia pachydermatis* (Fungus 2) 2. *Sporothrix schenckii* (Fungus 3) 3. *Trichophyton* (Fungus 4) 4. *Malassezia furfures or Dandruff* (Fungus 5)

The antifungal activity of the flower extracts was studied by well diffusion where in 50% and 80% of the flower extract was made and used in the wells and in vitro testing against the fungal strains was achieved.

Table 1: Table showing Zone of Inhibition of ethanolic extracts (in mm) against fungal pathogens. (D(E)- Dry and ethanolic extract, F(E) – Fresh and ethanolic extract)

Fungal Sample		D(E) Calendula	D(E) Crysantham um	F(E) Calendula	F(E) Crysantham um
		Zone of inhibition in mm	Zone of inhibition in mm	Zone of inhibition in mm	Zone of inhibition in mm
Fungal 2	**50 %**	27	33	21	17
	80 %	34	45	24	26
Fungal 3	**50 %**	33	25	27	20
	80 %	40	32	34	23
Fungal 4	**50 %**	19	35	38	31
	80 %	23	48	41	37
Fungal 5	**50 %**	13	34	45	38
	80 %	24	43	51	45

The ethanolic extracts of both the dried and fresh flower extracts gave inhibition of all the four fungal strains. In case of Fungus 3 calendula gave better inhibition (Table 1, Fig 1,2)but all the other 3 fungi were inhibited more than 40 percentage by Chrysanthemum extracts.

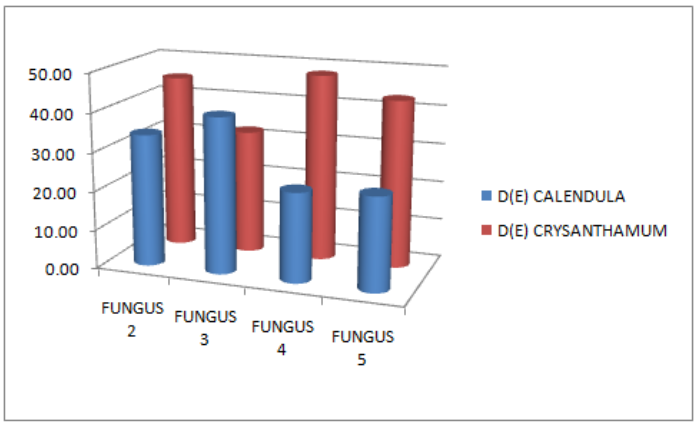

Figure 1. Comparative effects of dry ethanolic extract (80%) of C*alendula* and C*rysanthamum* on different fungi.

The chloroform extracts of both the dried and fresh flower extracts gave higher level of inhibition of all the four fungal strains as compared to ethanolic extracts. This suggests that the extraction of all the antifungal molecules is better when chloroform is used. In case of Fungus 2 and 3 calendula shows better inhibition with the dry flower extract (Table 2, Fig 1) while Fungus 3 and 4 were inhibited to more than 50% by chloroform extract of fresh flowers. (Table 2, Fig 2)

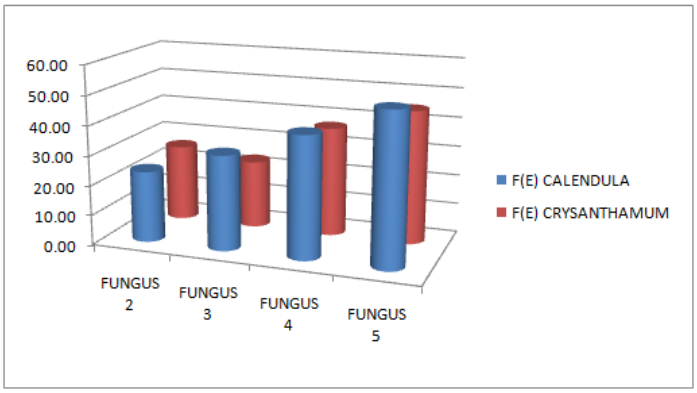

Figure 2. Comparative analysis of effect of fresh ethanolic extract (80%) of *calendula* and C*rysanthamum* on different fungi.

Table 2: Table showing Zone of Inhibition of chloroform extracts (in mm) against fungal pathogens. (D(C)- Dry and chloroform extract, F(C) – Fresh and chloroform extract)

Fungal Sample		D(C) Calendula	D(C) Crysantham um	F(C) Calendula	F(C) Crysantham um
		Zone of inhibition in mm	Zone of inhibition in mm	Zone of inhibition in mm	Zone of inhibition in mm
Fungal 2	**50 %**	45	15	47	44
	80 %	56	23	53	51
Fungal 3	**50 %**	46	22	19	21
	80 %	53	31	24	28
Fungal 4	**50 %**	41	50	25	51
	80 %	49	52	37	57
Fungal 5	**50 %**	35	21	33	46
	80 %	47	27	49	51

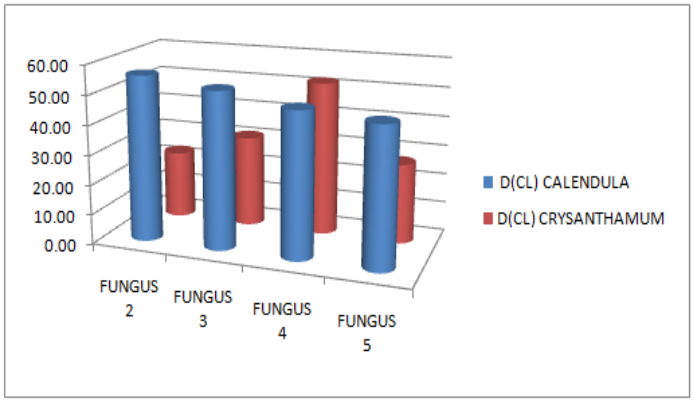

Figure 3. Comparative analysis of effect of dry chloroform extract (80%) of C*alendula* and C*rysanthamum* on different fungi

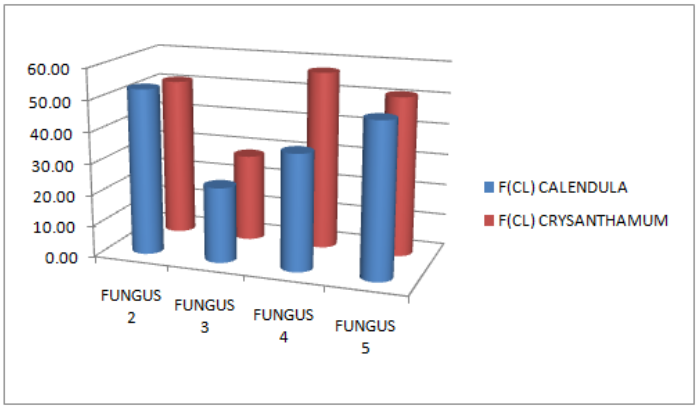

Figure 4. Comparative analysis of effect of fresh chloroform extract (80%) of *Calendula* and *Crysanthamum* on different fungi

The results of the present study clearly show that the waste flowers can be used as medicinal extracts to make skin ointmentsor solutions against skin fungal diseases in both humans and animals. Chrysanthemum gives better inhibitory activity in case of *Trichophyton* and *Malassezia furfur*.

Dermatophytes are a common label for a group of three types of fungus that commonly causes skin disease in animals and humans. Dermatophytes cause infections of the skin, hair and nails due to their ability to obtain nutrients from keratinized material. The organisms colonize the keratin tissues and inflammation is caused by host response to metabolic by-products. They are usually restricted to the nonliving cornfield layer of the epidermis because of their inability to penetrate viable tissue of an immune competent host. Invasion does elicit a host response ranging from mild to severe. The development of cell-mediated immunity correlated with delayed hypersensitivity and an inflammatory response is associated with clinical cure. The spread of multidrug-resistant strains of fungus and the reduced number of drugs available makes it necessary to discover new classes of antifungal and compounds that inhibit these resistant mechanisms. This has led to a search for therapeutic alternatives, particularly among medicinal plants and compounds isolated from them used for their empirically antifungal properties. Thus the results of the present study can be used to create antifungal solutions or creams for medicinal purposes.

CONCLUSION

The results revealed that the chloroform extracts of both dry and fresh flower gives good inhibition on the four dermatophytic fungi as compared to ethanolic extract. It is also clear that chloroform extracts of Crysanthamum showed better inhibition of *Trichophyton* and *Malassezia furfur* while the fresh ethanolic of calendula inhibits fungi *Sporothrix*.

References

[1] San-Blas G, Calderone RA. Pathogenic fungi: insights in molecular biology: Horizon Scientific Press; 2008.
[2] Hibbett DS, Binder M, Bischoff JF, Blackwell M, Cannon PF, Eriksson OE, et al. A higher-level phylogenetic classification of the Fungi. Mycological research. 2007;111:509-47.
[3] Aneja KR, Joshi R, Sharma C, Surain P, Aneja A. Biodiversity of dermatophytes: an overview.
[4] Singh I, Kumaravadivel N, Gnanam R, Vellaikumar S. RP-HPLC analysis for camptothecin content in Nothapodytes nimmoniana, an endangered medicinal plant. J Med Plants Res. 2010;4:255-9.
[5] Shimamura T, Kubota N, Shibuya K. Animal model of dermatophytosis. BioMed Research International. 2012;2012.
[6] Aqil F, Ahmad I. Broad-spectrum antibacterial and antifungal properties of certain traditionally used Indian medicinal plants. World journal of microbiology and biotechnology. 2003;19:653-7.
[7] Gurib-Fakim A. Medicinal plants: traditions of yesterday and drugs of tomorrow. Molecular aspects of Medicine. 2006;27:1-93.
[8] Muley B, Khadabadi S, Banarase N. Phytochemical constituents and pharmacological activities of Calendula officinalis Linn (Asteraceae): a review. Tropical Journal of Pharmaceutical Research. 2009;8.
[9] Zaman S. medicinal plant, QoQnus publication. Tehran, İran Country. 2003:45-90.
[10] Chu Q, Fu L, Guan Y, Ye J. Determination and differentiation of Flos Chrysanthemum based on characteristic electrochemical profiles by capillary electrophoresis with electrochemical detection. Journal of agricultural and food chemistry. 2004;52:7828-33.
[11] Harborne JB, Baxter H. The handbook of natural flavonoids. Volume 1 and Volume 2: John Wiley & Sons; 1999.
[12] Dwivedi S, Khatri P, Kumar V. Calendula Officinalis.
[13] Mahesh B, Satish S. Antimicrobial activity of some important medicinal plant against plant and human pathogens. World journal of agricultural sciences. 2008;4:839-43.
[14] Hussain AI, Anwar F, Sherazi STH, Przybylski R. Chemical composition, antioxidant and antimicrobial activities of basil (Ocimum basilicum) essential oils depends on seasonal variations. Food Chemistry. 2008;108:986-95.
[15] Ajanal M, Gundkalle MB, Nayak SU. Estimation of total alkaloid in Chitrakadivati by UV-Spectrophotometer. Ancient science of life. 2012;31:198.