Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Issue 4, 2012

Research Article

BIO-CONTROL OF CLINICAL FUNGAL ISOLATES ASSOCIATED WITH FUNGAL KERATITIS USING MEDICINAL PLANT EXTRACT

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Received: 07 Jun 2012, Revised and Accepted: 13 July 2012

ABSTRACT

Most common causes of corneal ulcer is mycotic infestation, it damage cornea and iris. On the culture incidence of *Penicillium* sp. is high followed by *Fusarium* sp., *Microsporium* sp., *Aspergillus* sp. Plants are reservoir of biological active compounds to combat various pathogens, aqueous and methanolic extracts of four plants *Thuja occidentalis,* Catharanthus roseus, Withania sominifera, Lawsonia *inermis* were tested for their antifungal activity against fungal clinical isolates from fungal keratitis as *Fusarium* sp., *Microsporium* sp., *Aspergillus* sp. and *Penicillium* sp. Maximum inhibition was recorded by methanolic extracts in comparison to aqueous extracts. Methanolic extract of *Thuja occidentalis* (10% v/v) were found to be significantly inhibiting all clinical isolates as *Fusarium* sp. (89.05%), *Microsporium* sp. (72.22%), *Aspergillus* sp. (90.59%) and *Penicillium* sp. (90.0%) whereas, 10% methanolic extract of *Lawsonia inermis* showed mycelial inhibition of *Microsporium* sp. (81.33%) and *Fusarium* sp. (93.08%). Percent inhibition by W*ithania sominifera* extract significantly reduces *Fusarium* sp (85.45%) and *Microsporium* sp. (89.6%) by10% *Catharanthus roseus* extract. It was recorded that *Aspergillus flavus* was only inhibited by methanolic extracts of *Thuja occidentalis.* Aqueous extracts could not significantly control the incidence of the test fungi but only 10% aqueous extract of *Withania sominifera* control *Fusarium* sp. (83.69%).

Keywords: Bio-control, Clinical fungal isolates, Fungal keratitis, Medicinal plant

INTRODUCTION

Fungal keratitis was first described by Leber in 1879. Fungal keratitis is a suppurative, ulcerative, and sight-threatening infection of the cornea that sometimes leads to loss of the eye. It represents one of the major causes of infectious keratitis in tropical areas of the world. Corneal ulcer is an open sore on the cornea, the thin clear structure overlying the iris, which is the colored part of eye. Damage may occur as a result of injury, a foreign body in the eye, or excessive or inappropriate wearing of contact lenses. The infections that can result in ulceration of the cornea may be caused by viruses, bacteria, fungi or acanthamoeba. Fungal keratitis is caused mainly due to infection by *Candida* sp., *Fusarium* sp., *Aspergillus* sp., *Penicillium* sp., *Cephalosporium* sp. and mycosis fungoides species[5]. Most of the chemical formulations used against fungal keratitis contain azole, trienes and flucocytosine. Medicinal plants synthesize a vast array of secondary metabolites that are important for human life[7]. For medicinal purposes, antimicrobial activity of substances derived from plant extracts have been recognized for many years.

The present study aims at evaluating the antifungal activity of plant extract (aqueous and methanolic) of medicinal plant against the clinically isolated fungi from fungal keratitis.

MATERIALS & METHODS

Test Plant

Four wild medicinal plants extracts of Catharanthus roseus, Thuja occidentalis, Withania sominifera and Lawsonia *inermis* were used. Fresh leaves were collected washed thoroughly 2-3 times with running tap water and once with sterile distilled water, air dried at room temperature on a sterile blotter and used for preparation of extracts[9].

Solvent extraction

The dried powdered leaves were subjected to two types of extraction (methanolic and aqueous). Plant extract were prepared by 15 grams fine powder of leaves was filled in the thimble and extracted successively with water and methanol for 48 hours at 55^{0}C (methanolic) and 85oC (aqueous). All the solvent extracts were concentrated using rotary flash evaporator under reduced pressure. The extracts were preserved in airtight brown bottle until further use 10,6. A series of concentration of plants extract were prepared as 2.5%, 5%, 7.5%, 10% were taken to find the significant control of the clinically isolated fungi by the food poisoning method12,13.

Test fungi

Fungal cultures were collected from Dr. B. Lal Clinical Laboratory, Jaipur. Samples were collected from mycotic ulcer and all the samples were subjected to culture on Sabouraud Dextrose Agar medium (SDA). Four species of clinically isolated fungi from fungal keratitis as *Fusarium* sp., *Microsporium* sp., *Aspergillus* sp. and *Penicillium* sp. were used as test fungi for antifungal activity assay.

Antifungal activity assay by poisoned food technique

Solvent extract

One gram of each of the solvent extract was dissolved in 10ml of respective solvents, which served as the mother solvent extracts. Sabouraud Dextrose Agar medium (SDA) with different concentration of each of the solvent extracts viz., 2.5%, 5.0%, 7.5% and 10% were prepared. SDA medium amended with the same concentrations of these respective solvents served as control. Five mm mycelial discs from the margins of seven day old cultures of test fungi were placed in the center of SDA medium. The plates were incubated at 25±1^{o} C for seven days and ten replicates were maintained for each treatment. The percent inhibition of mycelial growth was determined[12,13]. The data were subjected to statistical analysis by ANOVA and Tukey's HSD.

$$\text{Percent inhibition} = \frac{\text{Mycellial growth (C)} - \text{Mycellial growth (T)}}{\text{Mycellial growth (C)}} \times 100$$

RESULTS

The clinical samples (50) suspected for mycotic keratitis were collected from Dr. B. Lal clinical Laboratory. The relative percent occurrence (RPO) of *Fusarium* sp. (22), is high followed by *Aspergillus* sp. (12), *Microsporium* sp. (4), *Trichophyton* sp. (4), *Penicillium* sp. (2) (Fig. 1).

Antifungal activity assay by poisoned food technique

Among the four different plant extract (Aqueous and methanolic) against clinically isolated fungi from the mycotic keratitis maximum inhibition was recorded by methanolic extracts in comparison to aqueous extracts. Maximum inhibition of the test fungi was recorded at 10% concentration (v/v) out of 2.5%, 5.0%,

7.5% and 10.0% with respect to their control. Methanolic extract of *Thuja occidentalis* (10% v/v) were found to be significantly inhibiting all clinical isolates as *Fusarium* sp. (89.05%), *Microsporium* sp. (72.22%), *Aspergillus* sp. (90.59%) and *Penicillium* sp. (90.0%) whereas, 10% methanolic extract of *Lawsonia inermis* showed inhibition of *Microsporium* sp. (81.33%) and *Fusarium* sp. (93.08%). Percent inhibition by W*ithania sominifera* extract significantly reduces *Fusarium* sp (85.45%) and *Microsporium* sp. (89.6%) by10% *Catharanthus roseus* extract. It was recorded that *Aspergillus flavus* was only inhibited by methanolic extracts of *Thuja occidentalis.* Aqueous extracts could not significantly control the incidence of the test fungi but only 10% aqueous extract of W*ithania sominifera* control *Fusarium* sp. (83.69%). (Table 1, Fig. 2).

Fig. 1: Showing relative percent occurrence (RPO) of fungal clinical isolates

Table 1: Analysis of antifungal properties of *Thuja occidentalis, Catharanthus roseus, Withania sominifera, Lawsonia inermis* plant extracts (Aqueous & Methanolic) against clinical isolates (fungus)

Clinical isolate (fungus)	Plant extract	Conc. (v/v)	Percent Inhibition (%)			
			Thuja occidentalis	***Catharanthus roseus***	***Withania sominifera***	***Lawsonia inermis***
***Fusarium* sp.**	Aqueous	2.5%	40.30±0.1	24.60±0.0	44.62±0.2	32.31±0.1
		5%	54.15±0.0	38.77±0.1	52.62±0.0	61.85±0.1
		7.5%	69.53±0.2	41.85±0.1	63.08±0.0	55.38±0.0
		10%	61.54±0.1	42.77±0.0	**83.69±0.1**	44.62±0.0
	Methanolic	2.5%	11.79±0.0	31.20±0.1	29.09±0.2	7.07±0.1
		5%	57.05±0.1	30.00±0.0	31.27±0.1	52.31±0.1
		7.5%	62.95±0.0	38.80±0.0	43.27±0.0	71.08±0.1
		10%	**89.05±0.0**	74.80±0.1	**85.45±0.0**	**93.08±0.1**
***Microsporium* sp.**	Aqueous	2.5%	16.00±0.2	63.69±0.3	44.00±0.1	30.77±0.1
		5%	38.00±0.2	51.08±0.1	24.60±0.0	38.77±0.0
		7.5%	59.60±0.1	58.15±0.2	36.62±0.2	41.23±0.0
		10%	96.20±0.0	61.54±0.0	53.23±0.2	48.92±0.1
	Methanolic	2.5%	14.00±0.0	54.80±0.0	10.40±0.0	30.22±0.0
		5%	44.78±0.1	50.00±0.0	39.20±0.0	64.44±0.0
		7.5%	66.26±0.0	62.40±0.1	62.80±0.1	65.33±0.2
		10%	**72.22±0.1**	**89.60±0.1**	66.80±0.0	**81.33±0.2**
***Aspergillus* sp.**	Aqueous	2.5%	I	I	I	I
		5%	I	I	I	I
		7.5%	I	I	I	I
		10%	I	I	I	I
	Methanolic	2.5%	I	I	I	I
		5%	I	32.94±0.2	I	I
		7.5%	51.76±0.0	58.82±0.0	I	42.35±0.1
		10%	**90.29±0.1**	**83.52±0.1**	74.00±0.0	63.53±0.0
***Penicillium* sp.**	Aqueous	2.5%	I	I	I	I
		5%	I	I	I	I
		7.5%	I	I	I	I
		10%	I	I	I	I
	Methanolic	2.5%	I	I	I	I
		5%	I	I	I	I
		7.5%	38.75±0.1	48.75±0.0	I	I
		10%	**90.00±0.0**	56.25±0.2	68.75±0.1	I

Note: I- Insignificant

Values are the mean of five replicates, ±standard error.

1. The means followed by the same letter (s) are not significantly different at P 0.05 when subjected to Tukey's HSD.
2. Pattern of percentage inhibition increase is not uniform for all the microorganisms
3. Diameter of cork borer is 5 mm

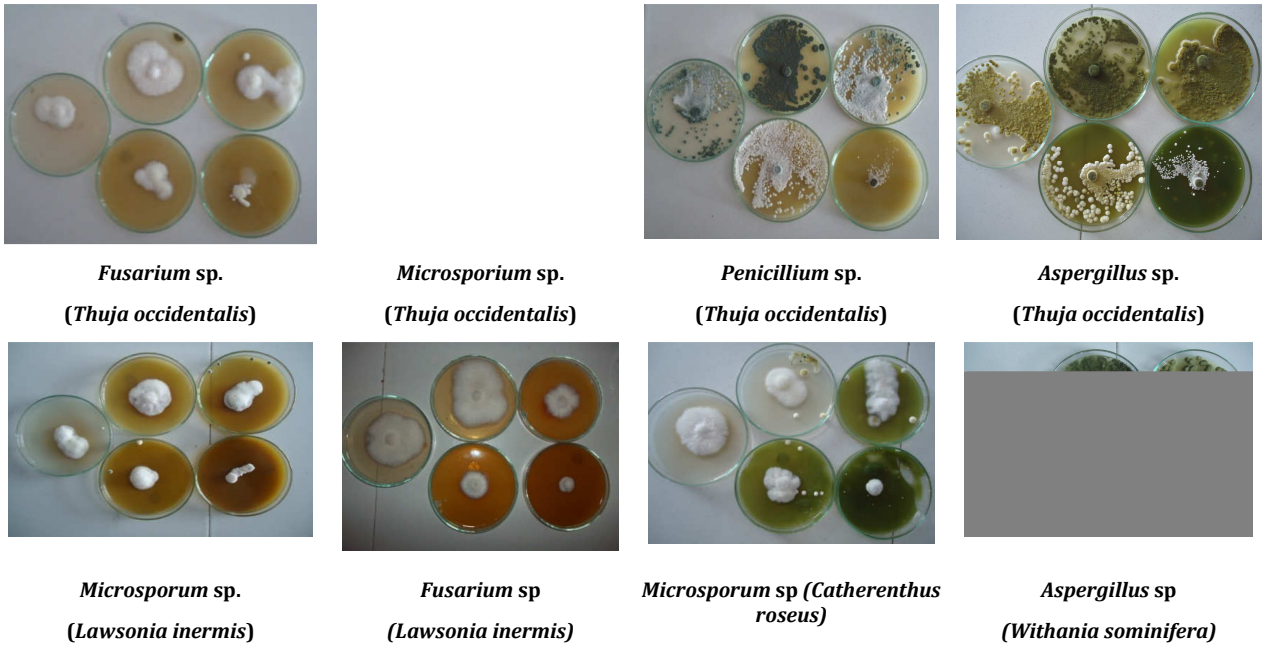

Fig. 2: Antifungal activity of methanolic extract control, 2.5%, 5.0%, 7.5%, 10.0% (clockwise from left) of *Thuja occidentalis, Catharanthus roseus, Withania sominifera, Lawsonia inermis* against clinically isolated *Fusarium* sp., *Microsporium* sp., *Penicillium* sp. and *Aspergillus* sp.

DISCUSSION

Development of synthetic products to control plant diseases has become difficult because of strict requirements of their efficacy, selectivity, toxicology and general impact on the environment. Consequently, there is an increasing interest in evaluating other mechanisms of control including the effects of plant metabolites on plant pathogens[10]. Several higher plants and their constituents have shown success in plant disease control and proved to be harmless and non phytotoxic unlike chemical fungicides[13]. Indiscriminate use of chemical not only hazardous to living beings but adversely affects the microbial population present in the ecosystem[1].

Alternative to this effect, to control plant diseases, plant products are gaining prominence as fungicides and bactericides[8]. Antifungal compounds from higher plants are advantageous over synthetic fungicides due to their easily biodegradable nature[13]. During the study of 50 case study the relative percent occurrence (RPO) of *Fusarium* sp. (22), is high followed by *Aspergillus* sp. (12), *Microsporium* sp. (4), *Trichophyton* sp. (4), *Penicillium* sp. (2) (Fig. 1) whereas, Two (28.6%) fungi (*Aspergillus fumigatus* and *Fusarium* spp.) were isolated from 31.8% patients with fungal keratitis were males and 28.6% were females[14].

In the present investigation, four wild medicinal plants extract (aqueous and methanolic) were analyzed for their antifungal properties. Various concentration of the wild medicinal plant extract were used 2.5%, 5%, 7.5%, 10% with respect to their control to evaluate MIC. Maximum inhibition was recorded by methanolic extracts in comparison to aqueous extracts. Methanolic extract of *Thuja occidentalis* (10% v/v) were found to be significantly inhibiting all clinical isolates as *Fusarium* sp. (89.05%), *Microsporium* sp. (72.22%), *Aspergillus* sp. (90.59%) and *Penicillium* sp. (90.0%) whereas, 10% methanolic extract of *Lawsonia inermis* showed inhibition of *Microsporium* sp. (81.33%) and *Fusarium* sp. (93.08%). Percent inhibition by W*ithania sominifera* extract significantly reduces *Fusarium* sp (85.45%) and *Microsporium* sp. (89.6%) by10% *Catharanthus roseus* extract. It was recorded that *Aspergillus flavus* was only inhibited by methanolic extracts of *Thuja occidentalis.* Aqueous extracts could not significantly control the incidence of the test fungi but only 10% aqueous extract of W*ithania sominifera* control *Fusarium* sp. (83.69%) (Table-2, Fig.-2). Plants are reservoir of biological active compounds to combat various pathogens. Certain plant extracts contained alkaloids flavonoids, saponins, steroids and tannins. Saponin was found in all extracts.

The mode of action of extracts was determined on cell wall and enzyme production of fungi. *Lawsonia inermis* inhibited the production of catalase in *Aspergillus niger* and *Fusarium oxysporum*.. The active compounds were proteinaceous in nature or proteins and they are effective against plant pathogens[5]. The water base extraction technique was the most effective in inducing antifungal properties of lime whiles ethanol base extraction technique was the best for ginger. Garlic on the other hand exhibited a good antimicrobial (antifungal) property in both ethanol and water extraction media. The study thus confirms the antifungal properties of these medicinal plants and suggests the type of extraction to yield the best results[4]. The methanol extract of *Sida cordifolia* exhibited significant antifungal activity against *F. verticillioides*[5]. Qualitative phytochemical tests, thin layer chromatography and TLC-bioautography of certain active extracts demonstrated the presence of common phytocompounds in the plant extracts including phenols, tannins and flavonoids as major active constituents[7]. Antifungal activity of the leaves of *Calotropis procera* was carried against *Aspergillus fumigatus, Candida albicans, Penicillium chrysogenum, Aspergillus flavus* antifungal activity against *Aspergillus niger*. The finding shows that this leaf extracts having the potential to inhibit the growth of bacteria and fungi[15]. *Cassia* spp of caesalpiniodeae were used against fungal phytopathogens (*Aspergillus niger*, *Rhizoctonia solani*, *Macrophomina phaseolina*, *Fusarium oxysporum* and *Colletotricum falcatum).* Among all the extracts tested, methanol extracts of *C. laevigata* and *C. fistula* leaf significantly inhibit the growth of *F. oxysporium* and *A. niger* on agar[16]. The differential antimycoses activities of chloroform, methanol and ethyl acetate extracts of *Calotropis spp* were evaluated against dermatophytes and *Aspergillus flavus* using agar-well diffusion assay. Chloroform extracts showed highest activity, methanol extracts showed moderate activity and ethyl acetate extract showed least activity[17].

Leaf extract (methanolic) of *Thuja occidentalis* serves as a potent plant against clinically isolated fungi from mycotic keratitis and further work is necessary to isolate the bioactive compound which is an eco friendly approach.

ACKNOWLEDGEMENT

The authors are thankful Dr. Siddharth Kothari, Cornea consultant, Opthalmology M.N.A.M.S., D.M.B., M.B.B.S. for providing the samples from the case study and Dr. B. Lal Clinical Laboratory, Jaipur for providing the test pathogens.

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