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ANTIFUNGAL ACTIVITY OF *NIGELLA SATIVA AND LAWSONIA INERMIS* AND ITS COMPARATIVE STUDY

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ABSTRACT

Human fungal infections are uncommon in healthy persons. However in immunodeficient persons a no. of mild or non pathogenic fungi can cause fatal infection. Aspergillus can infect lungs, inner ear sinuses. Infection caused by it called as aspergillosis. Candida is normal flora but can infect immune compromised host. It is leading cause of nosocomial infection. It's infection is called as candidosis. Cryptococcus cause systemic infection like meningitis. Constant exposure to fungal spores of it in atmosphere can also induce respiratory allergies. Commonly it is called as cryptococcosis. Fungal infection that is mycoses are still difficult to treat by drug treatment. Being eukaryotes fungi are not amenable to treat with antibiotics. In present investigation the antifungal activity of Nigella sativa and Lawsonia inermis were tested on different strains of fungi such as Candida, Aspergillus and Cryptococcus. The antifungal activity is tested by disc diffusion method. The activity of both is compared and it is found that aqueous extract of Lawsonia inermis has better activity than Nigella sativa.

KEYWORDS:- Antifungal, Disc diffusion method, Nosocomial, Aspergillosis, Candidosis.

INTRODUCTION

Nigella sativa is an annual flowering plant belonging to family Ranunculaceae. It has pungent bitter taste and smell. It's seed is used as spice. The seed is known as kallonji. It is used in cough, asthama, diabetes, eye problems, kidney stones, hypertension, heart complaints, antibacterial, antifungal [1,8] Lawsonia inermis is commonly known as heena belonging to family Lythraceae. It is also flowering plant. Dye prepared from plant is widely used to dye skin, hair, fabrics. It has good antimicrobial antifungal activity. [2]

Fungi are opportunistic organisms and they cause systemic fungal infection. Such fungi cause infections in persons whose defense is impaired due to some diseases condition or immunosuppressive therapy. Many drugs are now available but still there is need of safer and broad spectrum fungicidal drug which has minimum toxic effects.

Hence antifungal screening of various extract of Nigella sativa and Lawsonia inermis are tested by disc diffusion method.[3]

MATERIAL AND METHOD

Chemicals

Methanol , Dextrose, Peptone, Agar,(Rajesh chemicals) Distilled water, Amphotericin B, Barium chloride dehydrate, Sulphuric acid.

Fungal strains

The fungal strains for the study were obtained from Govt. Medical college, (Microbiology and bacteriology department). The fungal strains used in the study are Candida albicance, Aspergillus flavus, Cryptococcus fumigates.

Collection of plant material

Plant material that is Nigella sativa seeds was collected from Velankar Aushadhalaya, Sangli. Leaves of Lawsonia inermis was collected from residential area (Trimurty colony, Sangli). Both the plant material are authenticated by Dr. Datar. Kalpana R. (Botany Department of Willingdon college, Sangli.)

Extract Preparation

1. Alcoholic extract of Nigella sativa – 100 gram of finely powdered seeds are extracted by maceration in 200ml ethanol and kept for 6 days. After 6 days extract was filtered through Whatman filter paper and evaporated till dried.[4]

2. Aqueous extract of Lawsonia inermis – 25 grams powder of dried leaves are soaked for 12 hrs in 250ml distilled water. After that it is filtered through Whatman filter paper and evaporated till dryness.[2]

3 Alcoholic extract of Lawsonia inermis – 25 grams powder of dried leaves are soaked for 12 hrs in 250ml ethanol. After that it is filtered through Whatman filter paper and evaporated till dryness.[2]

Assesment of Antifungal activity

1. Preparation of Inocula – From fungal cultured slants, several colonies were transferred to 5ml of sterile distilled water. It is mixed for some seconds to ensure homogenicity and further diluted to match the turbidity with 0.5 McFarland standard solution (Corresponding to 1-5 × 10^6 CFU/ml).[5,6]

2. Preparation of samples – i) Nigella sativa alcoholic sample solutions were prepared at 50mg/ml, 75mg/ml, 100mg/ml concentrations in alcohol . ii) Lawsonia inermis (alcoholic and aqueous extract) sample solutions were prepared at 50mg/ml, 100mg/ml, 150mg/ml concentrations in distilled water. iii) Amphotericin B is taken as standard at 50mg/ml concentration.

3. Disc diffusion method [6]-

i) Sabouraud Dextrose agar is prepared as fungal media and sterilized.[7]

ii) All glasswares, filter disc, petriplates, extract dilutions were sterilized in autoclave.

iii) In aseptic technique, using sterile swab a bacterial lawn is made on sterile petri plates from microbial inoculum suspension. Swab is made in one direction by rotating plate at 90º

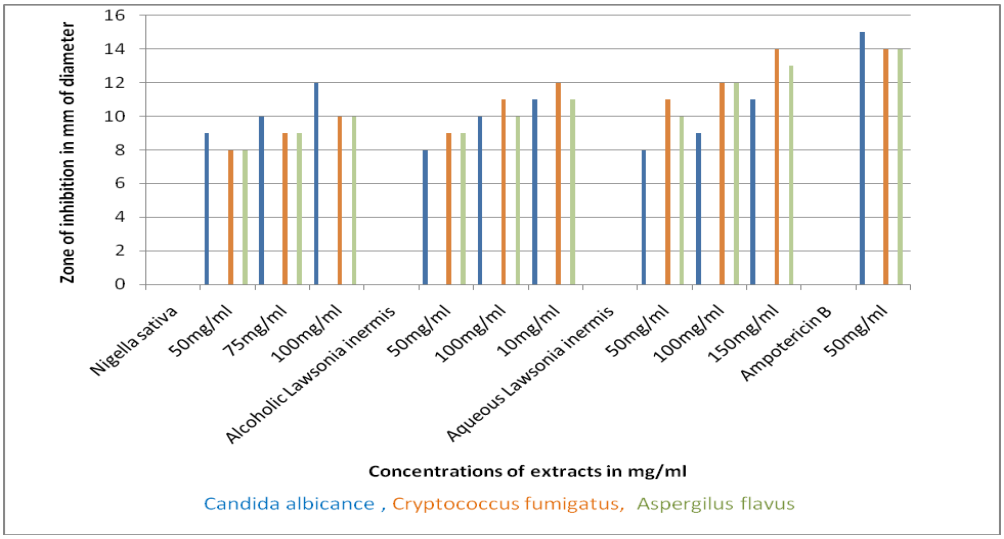
iv) Sterile filter discs of 6mm diameter were impregnated with about 0.1ml/disc of each extract dilution solution and placed on agar plate in aseptic condition.

v) Plates are incubated at 28ºc-30ºc for 2 days. Alcohol, Sterile distilled water are kept as control. After 2 days zone of inhibition was measured. In case of alcoholic dilutions of Nigella sativa the zone of inhibition of alcohol is substracted from it.

Figure:- Effect of alcoholic extract of Nigella sativa on Candida albicance.

Observation Table: Zone of inhibition for three fungi in mm of diameter.

Sr. No.	Extract	Zone of Inhibition in mm of diameter		
	Concentration	Candida albicance	Cryptococcus fumigatus	Aspergillus flavus
1.	**Nigella sativa**			
	50mg/ml	09	08	08
	75mg/ml	10	09	09
	100mg/ml	12	10	10
2.	**Alcoholic Lawsonia inermis**			
	50mg/ml	08	09	09
	100mg/ml	10	11	10
	150mg/ml	11	12	11
3.	**Aqueous Lawsonia inermis**			
	50mg/ml	08	11	10
	100mg/ml	09	12	12
	150mg/ml	11	14	13
4.	Amphotericin B (50mg/ml)	15	14	14



Graphical Representation – Effect of different concentrations of plant extract on fungi

RESULT AND DISCUSSION

From disc diffusion method it is observed that both the plant extracts show significant inhibition of fungi. Among them Nigella sativa shows better activity against Candida albicance than Lawsonia inermis.

Nigella sativa has more activity against Candida albicance than Cryptoccus fumigates and Aspergilus flavus. Alcoholic extract of Lawsonia inermis show more inhibition for

Cryptococcus than rest of two. While Aqueous extract of it shows relative similar activity on Cryptococcus fumigates and Aspergilus flavus .

Agueous extract of Lawsonia inermis has better activity than it's alcoholic extract. It show high inhibition zone against Cryptococcus fumigates and Aspergillus flavus.

CONCLUSION

From the results obtained it can be concluded that extracts of both species exhibit potent inhibition of almost these three fungal strains. These extract show same potency as that of standard Amphotericin B. From study it is concluded that these plant species can be a good pharmacophore source of in future.

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