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# Screening of Antifungal Activity of Some Essential Oils and Antifungals on Clinical Fungal Isolates from Diabetic Patients

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**ABSTRACT**

Diabetic patients are highly susceptible to various kinds of skin infections or mycoses. As compared to healthy population, diabetics seem to suffer from certain types of mycoses more frequently. With the view of this fact 28 skin samples were collected from diabetic patients in the Railway hospital and Netaji Subhash Chandra Bose Medical College, Jabalpur. Out of these 13 samples were found to be positive for fungal infection. The clinical isolates were subjected to *in vitro* susceptibility against antifungals, herbs and essential oils. Essentials oils have been shown to possess antifungal as well as antioxidant properties. Amp B and ketoconazole were found to be the effective synthetic antifungals. Clove oil and garlic proved to be the potential natural antifungals.

**Keywords:** Antifungals, essential oils, *in vitro* susceptibility and clinical isolates

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## INTRODUCTION

Diabetic patients are more susceptible to skin infections [1]. Although the overall incidence of skin mycoses in diabetics is not higher as compared with healthy population. Diabetics suffer from certain types of mycoses more frequently. Approx 30% of patient with diabetes have disease related dermatologic problems [13]. Fungal infection occur as opportunistic or secondary infection in compromised patient or susceptible to infection [5]. The cutaneous signs of diabetes are the manifestations of multiple factors. Skin of diabetic patient's increases the capillary fragility; blood vessels show decreased circulation and abnormal carbohydrate metabolism etc.

Fungal infections may be classified according to the site of infection, route of acquisition and type of virulence. Superficial fungal infections of the skins are some of the most common dermatologic conditions seen in clinical practice. Superficial infection or mycoses are limited to the stratum corneum and essentially elicit no inflammation. Superficial fungal infections of the skin are some of the most common dermatologic condition seen in clinical practice.

There are many natural products which have been used for the treatment of mycoses. The antimicrobial properties of volatile aromatic oils from medicinal as well as other edible plants have been recognized as antibiotic. Some experiments have documented the antimicrobial properties of spices, herbs & their components [11]. Clove oils had shown antibacterial and antifungal activity [3, 17]. Itraconazole, Fluconazole, Amphotericin-B have been reported to have activity against *Candida sp*., some dermatophytes and many agents of systemic mycoses [6]. Fluconazole , a triazole has been reported to be active against *Candida* species, few dermatophytes and many agents of systemic mycoses [21]. Ketoconazole has been found to be effective against dermatophytes, yeasts, some agents of systemic mycoses and superficial cutaneous fungal infection.

## MATERIALS AND METHOD

### Sample Collection

The location of the infection site considerably helps in the diagnosis of a mycotic infection. The collections of the sample for the study of mycotic infection in diabetic patients were done at the dermatological ward of the central Railway hospital & Netaji Subhash Chandra Bose Medical College, Jabalpur.

### Fungal Isolation

The infected skin was treated with 10% KOH to destroy tissue elements and then examined microscopically. Wet mount method was used to identification of fungal isolates. Fungal pathogens were isolated on Sabouraud Dextrose Agar (SDA) with chloramphenicol at 26°C-28°C.

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**In vitro susceptibility method**

These fungal isolates were subjected to disc diffusion test (antifungal & herb sensitivity).the antifungals used for in vitro sensitivity were Ketoconazole, nystatin, fluconazole, clotrimazole, Itraconazole, & Amphotericin-B. the herbs used for in vitro antifungal sensitivity against antifungals were garlic, turmeric, eucalyptus, neem and tulsi .

**Thermotolerance test**

Isolates were inoculated in SDA slants in 6- sets. The 6 sets of fungal isolates along with control were incubated at different temperatures i.e. at 26ºC, 30ºC, 35ºC, 40ºC, 45ºC, 50ºC for 3-4 days. After incubation fungal growth in all the sets was observed. All the tests were performed in triplicates, along with a control and a standard.

## RESULTS AND DISCUSSION

The spread of drug resistance pathogens is one of the most serious threats to successful treatment of microbial diseases [8]. The mortality rate of acute mycotic infection is also very high even in non-immuno-compromised patients. [14] If the cause of the infection can be brought under control, the infection will disappear after an antifungal treatment. The fungal pathogenicity obtained during the study can be helpful for further studies concerned with superficial mycoses.

Out of the 28 skin samples collected 13 samples were found to be positive for fungal infection. The common fungi isolated were *Aspergillus brevipes ,Aspergillus fumigatus. Pythium, Alternaria sp., Fusarium, Rhizopus, Candida sp., Penicillium nigricans Helminthosporium and Absidia sp.*

The results of the thermo tolerance test are expressed in the table no. 1. From the table it is evident that *Aspergillus brevipes, Aspergillus fumigatus and Candida* can grow at all the temperature and therefore can be considered as potentially pathogenic. The effect of various antifungals, essential oil and herbal extracts were studied by Disc diffusion method (Kirby Bauer method) (2).The susceptibility of clinical isolates against various natural and synthetic antifungals are expressed in table no. 2, 3 and 4.From table no. 2 it is evident that clove oil exhibits antifungal property. The clinical isolates were found to be resistant to mustard oil. *Pythium sp*. was also found to be sensitive to mustard oil. From table no.3 it is evident that garlic exhibits antifungal property as almost all clinical isolates were sensitive to it. The clinical isolates were resistant to eucalyptus and tulsi except for *Alternaria sp*. and *Fusarium sp*. Amongst all the clinical isolates only *Aspergillus brevipes* and *Alternaria sp*. were sensitive to turmeric and neem. The results of in vitro susceptibility test of clinical fungal isolates are expressed in table no. 4 .From the table it is clear that the clinical isolates were sensitive to Amphotericin B followed by Clotrimazole, Ketoconazole, itraconazole and nystatin.*Helminthosporium sp* was found to be resistant to all antifungals used in the study.

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**Table 1: Results of thermotolerance test of clinical fungal isolates**

| Fungal Isolates | 25°C | 30°C | 35°C | 40°C | 45°C | 50°C |
|---|---|---|---|---|---|---|
| *A. brevipes* | ++ | ++ | ++ | ++ | ++ | + |
| *A. fumigatus* | ++ | ++ | ++ | ++ | ++ | + |
| *Rhizopus sp.* | ++ | ++ | ++ | ± | - | - |
| *Alternaria sp.* | + | ± | ± | - | - | - |
| *Candida sp.* | ++ | ++ | ++ | + | ± | + |
| *Pythium sp.* | + | + | + | + | + | - |
| *Absidia sp.* | ++ | ++ | ++ | ++ | ++ | - |
| *P. nigricans* | ++ | ++ | ++ | + | + | - |
| *Fusarium sp.* | + | + | + | - | - | - |
| *Helminthosporium sp.* | ± | ± | ± | - | - | - |

- : No growth
+ : Growth
++ : Excess growth
± : Partial growth

**Table 2: Results of in vitro susceptibility test of clinical isolates against various oils expressed as zone of inhibition in cms.**

| No. of Isolates | Clove | Eucalyptus | Mustard Oil |
|---|---|---|---|
| *Aspergillus brevipes* | 2 cm. | - | - |
| *A.fumigatus* | 4.6 cm | - | - |
| *Rhizopus sp.* | 4 cm | - | - |
| *Alternaria sp.* | 3.2 cm | - | - |
| *Candida sp.* | 2 cm | - | - |
| *Pythium sp.* | 5.4 cm | - | 1.2 cm |
| *Absidia sp.* | - | - | - |
| *P. nigricans* | 3.2 | - | - |
| *Fusarium sp.* | 4 cm | - | - |
| *Helminthosporium sp.* | 3 cm | - | - |

**Table 3: Results of in vitro susceptibility test of clinical isolates against various herbal extracts by disc diffusion method expressed as zone of inhibition in cms.**

| No. of Isolates | Neem | Turmeric | Eucalyptus | Garlic | Tulsi |
|---|---|---|---|---|---|
| *Aspergillus brevipes* | 2 | 2 | - | 1 | - |
| *A.fumigatus* | - | - | - | 0.4 | - |
| *Aspergillus sp.* | - | 0.2 | - | 0.4 | - |
| *Rhizopus sp.* | - | - | - | 0.3 | - |
| *Alternaria sp.* | 0.8 | 1 | 2 | 1.3 | 1.4 |
| *Candida sp.* | - | - | - | 1.2 | - |
| *Pythium sp.* | - | - | - | 2.4 | - |
| *Absidia sp.* | 0.4 | - | - | - | - |
| *P. nigricans* | - | - | - | 0.8 | - |
| *Fusarium sp.* | 0.4 | 0.2 | 0.2 | 2 | 0.4 |
| *Helminthosporium sp.* | - | - | - | 0.7 | - |

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**Table 4: Results of in vitro susceptibility test of clinical isolates against various antifungals by disc diffusion method expressed as zone of inhibition in cms.**

| Isolates | Ketoconazole | Itraconazole | AMP-B | Nystatin | Clotrimazole | Fluconazole |
|---|---|---|---|---|---|---|
| *A. brevipes* | 2 | 0.2 | 2.1 | 1.6 | 1.3 | 1.4 |
| *A.fumigatus* | 1.7 | 0.8 | 0.8 | 1.5 | 1.5 | 0.8 |
| *Aspergillus sp.* | 1.4 | 1.4 | 1.5 | 0.6 | 1.5 | 1.8 |
| *Rhizopus sp.* | - | - | - | 1.0 | 1.2 | - |
| *Alternaria sp.* | 1.2 | 1.5 | 1.2 | - | - | - |
| *Candida sp.* | 0.7 | 0.5 | 1.8 | - | 1.1 | - |
| *Pythium sp.* | 0.9 | 0.3 | - | - | 2.0 | 2.4 |
| *Absidia sp.* | 0.6 | 1.2 | 1.7 | - | 1.6 | - |
| *P. nigrican s* | 1.2 | 1.5 | 1.2 | - | - | - |
| *Fusarium sp.* | - | 1 | 0.4 | 1.5 | 1.0 | - |
| *Helminthosporium sp.* | - | - | - | - | - | - |

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