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Antifungal Properties of Garlic and the Azole Group of Drugs against *Candida albicans* – A Comparative Study

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Candidiasis or thrush is a fungal infection (mycosis) of *Candida* species, *Candida albicans* being the most common. The dimorphic fungus *Candida albicans* is a commensal of the human oral, gastrointestinal, vaginal, cutaneous, and mucosal surfaces. In immunocompetent as well as immunocompromised individuals,*C. albicans* causes cutaneous or subcutaneous infections such as vaginitis or oral thrush or infections of the nails and skin. Catheters and other medical implants and devices serve as an excellent substrate for biofilm formation and can act as a source of nosocomial infections. Fluconazole and Itraconazole are well-established drugs of choice for both localized and systemic *C. albicans* infections. However, prolonged use of these drugs could lead to the development of drug resistant strains of *C .albicans*. The present study deals with understanding the antimycotic effect of both garlic and fluconazole on *Candida albicans*. Both aqueous and organic extracts of garlic are compared for their antimycotic activity along with Fluconazole. The use of natural antimycotics in synergism with Azole drugs in the treatment of *Candida* infections has also been investigated in order to make the treatment of *Candida* infections more cost effective and reduce the possibility of pathogenic *Candida albicans* strains from becoming drug resistant to the conventional anti mycotic drugs.

Key words: Antimycotic, *Candida albicans*, Candidiasis, Synergism, Garlic.

Candida albicans, a commensal fungus of the oral cavity and gastrointestinal tract in humans, represents one of the major causes of mucosal and systemic infection, which can be life threatening if not treated. In recent years, fungal infections have increased in immunocompromised hosts as a consequence of HIV infection, aggressive therapies for cancer, autoimmune diseases and organ or tissue transplantation. Commonly used antifungal drugs inhibit membrane component sterol biosynthesis and directly interact with the cell membrane or target cell wall biosynthesis.(Casalinuovo et.al., 2004)

Advanced medical equipments and surgery has also led to the increased spread of commensal *Candida* to tissues as pathogens. Medical devices such as catheter, dental implants, artificial joints, pacemakers and others have provided the opportunity to form biofilms, a stage more resistant to drugs and capable of greater invasion to tissues. These devices are easily colonized by *Candida* cells from mucosal surfaces and also blood stream, and frequently spread from one tissue site to another. Prolonged antibiotic therapy provides more available nutrients and space for *Candida* to multiply as other commensal microbial flora is diminished.

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The treatment regimes used to manage Candida infections vary and depend on the anatomical location of the infection foci, on the immune status of the patient and the risk factors of the patient associated with the infection. However, Echinocandins, Extended spectrum azoles and Amphotericin B are the drugs of choice. Fluconazole is considered as the first choice of drug in nonnetropenic patients with Candidemia or suspected invasive Candidiasis. (Jose A Hidalgo 2014)

There are a number of natural antifungals that are extremely effective. Allium sativum, commonly known as garlic is cited to be the most effective. Allicin, one of the active principles of freshly crushed garlic homogenates, has a variety of antimicrobial activities. Allicin in its pure form was found to exhibit antibacterial activity against a wide range of Gram-negative and Gram-positive bacteria, including multidrug-resistant enterotoxicogenic strains of *Escherichia coli*; antifungal activity, particularly against *Candida albicans*; antiparasitic activity, including some major human intestinal protozoan parasites such as *Entamoeba histolytica* and *Giardia lamblia*; and antiviral activity.(Ankri S, Mirelman D 1999) Recent studies have shown that ajoene, the factor responsible for anticoagulation in garlic, was superior to allicin in antifungal activity against *Candida albicans* and *Aspergillus niger*. (S Yoshida 1987) . *Candida* species possess a wide arsenal of glycoproteins located at the exterior side of the cell wall, many of which play a determining role in these steps. (Bennett et.al., 2010)

The aqueous extract of Garlic showed anti fungal activity against Trichophyton rubrum, a fungal skin pathogen isolated from infected patients. The extract was found to be heat labile at 60^0C (J. Karunyal Samuel et al 2000). Garlic Oil and Allyl Alcohol extracted from Alliin in garlic cause growth inhibition in yeast. Studies were carried out on Candida *utilis* ATCC 42416. Garlic oil showed fungistatic activity while Allyl alcohol showed fungicidal activity. (Chung I et al 2007)

MATERIALS AND METHODS

Two isolates of *Candida albicans* were used

Lab strain from the Culture Collection of Microiology department of S.I.E.S college of Arts, Science and Commerce, Sion (west), Mumbai

Hospital strain collected from the Microbiology department of Lokmanya Tilak Municipal Hospital, Mumbai, as it was a pathogenic isolate exhibiting a high MIC against both Fluconazole and Itraconazole. The pathogenicity of the lab strain and the hospital strain was confirmed by the germ tube test.

Preparation of samples

Fluconazole

Two tablets of Fluconazole (each of 150mg) were dissolved in 30ml of sterile MYE broth to get the original stock of 10mg/ml. Further dilutions were carried out to bring the stock at 100µg/ml.

Itraconazole

Content of one Itraconazole capsule (each of 100mg) was dissolved in 25ml of sterile MYE broth to get the original stock of 2mg/ml. Further dilutions were carried out to bring the stock at 1000µg/ml.

Garlic

Fresh Garlic paste (60gm) was dissolved in 20ml of sterile MYE broth to get the original stock of 3gm/ml

Extraction of active component from Garlic

Aqueous extract of garlic was prepared by pressing cloves of garlic through a garlic press into muslin cloth and then squeezed to extract garlic juice in deionised water.

The active component of Garlic was extracted in Alcohol and Acetone using 10 gm of garlic paste. After extraction, it was evaporated overnight and then checked for antimycotic activity.

Antimycotic activity of Garlic

To check whether inhibition of *Candida albicans* could be brought about by Garlic, Ditch plate method was performed. The technique is used to determine the antimicrobial spectrum of an insoluble drug. 3 gm of the freshly prepared stock was added to 7 ml of sterile molten and cooled MYE agar and poured in a ditch measuring 2cm by 7cm cut out on a sterile MYE agar plate. The 24 hour old test strains (OD 0.1 at 530nm) were streaked perpendicular to the ditch . The plate was incubated for 24 hours at 37^0C and the result was recorded .

Malt yeast extract agar contains polysaccharides which are used as energy source. It makes the medium acidic as well. Peptone serves as a nitrogen source. Thus, MYE medium was selected for the further studies.

MIC by Disc-Diffusion Method

Minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation. Minimum inhibitory concentrations are important inorder to confirm the dose response of microorganisms to an antimicrobial agent. A lower MIC is an indication of a more potent antimicrobial agent.

The agar diffusion test, or the Kirby-Bauer disk-diffusion method, is a means of measuring the effect of an antimicrobial agent against bacteria grown in culture. The plate test using the Kirby Bauer disk diffusion method was used for performing MIC and was preferred over the tube test as the drugs tested showed turbidity by themselves, thus masking the growth of the test organism.

A range of 0.1mg to 10.0mg was used. Whatman paper discs were loaded with 5micro litres of the different concentrations of the drug ,dried at room temperature and used for study. Overnight broth cultures of the Lab strain and Hospital strain of *C.albicans* (OD 0.1 at 530nm) were swabbed on MYE agar plates and the drug loaded discs were placed on the plates , one in each quadrant.The plates were incubated overnight at 37°C.

In Vitro Synergism: In vitro Synergistic activity using MIC concentrations of Fluconazole and Aqueous extract of garlic was performed. Aqueous extract of garlic was used because it gave the best results with respect to inhibition of *Candida albicans*. Sterile Whatman Filter paper strips dipped in the MIC concentrations of the test drugs were placed perpendicular to each other on a sterile MYE Agar plate swabbed with an over night culture of *Candida albicans*. The plates were incubated ay 37°C for 24 hours.

The inhibition around each strip and at the junction of both the strips was measured. Synergism was calculated as follows: h= "a^2 +b^2. If z >h, then the action of the two drugs tested is synergistic, where a = the inhibition around the strip loaded with drug A; b= the inhibition around the strip loaded with drug B and z= the inhibition at the junction of the two drugs.

RESULTS AND DISCUSSION

Antimycotic Activity of garlic

No growth on the ditch indicates that both the strains are inhibited by Garlic . Fig 1 shows the inhibition of both the strains under study on the ditch and around it too. Fig 2 shows the inhibition of the hospital strain on the ditch and around it too.

Ditch plate method showed the inhibition of both Lab and Hospital strains of C.albicans by garlic. Various Natural Antifungal agents have demonstrated inhibition against C.albicans. Origanum oil and carvacrol are studied for their antifungal activity. Using *Candida albicans* in broth cultures and a micro-dilution method, comparative efficacies of origanum oil, carvacrol, nystatin and amphotericin B were examined *in vitro*. Origanum oil at 0.25 mg/ml was found to completely inhibit the growth of *C. albicans*. (ManoharV et.al., 2001) Allyl alcohol and garlic extract also showed inhibition against C.albicans because of an oxidative stress produced on Candida albicans. (Chung I et al 2007) The results obtained in this study confirm the fact that some garlic components possess significant anticandidal properties. Studies have reported that Allyl alochol, a metabolic breakdown product of allicin, plays a major role in the inhibition of growth of *C. Albicans* (Katey et.al. 2005).

Candida albicans strain type	Growth on the ditch	Inhibition around the ditch in mm
Lab Strain	No growth	4mm on either side of the ditch
Hospital Strain	No growth	7mm on either side of the ditch

Minimum Inhibition Concentration of Fluconazole

MIC of Fluconazole against the lab strain showed an MIC of 300 mcg/ml. However, the hospital strain showed the maximum resistance, it showed an MIC of 5 mg/ml

Concentration: 0.1 to 1.0mg/ml

Concentration(µg/ml)	Lab strain	Hospital strain
100	+	+
200	+	+
300	–	+
400	–	+
500	–	+
600	–	+
700	–	+
800	–	+
900	–	+
1000	–	+
Concentration: 1-10mg/ml		
1	–	+
2	–	+
3	–	+
4	–	+
5	–	–
6	–	–
7	–	–
8	–	–
9	–	–
10	–	–

Key: + indicates growth, no inhibition.
-Indicates no growth, hence inhibition.

Minimum Inhibition Concentration of Garlic

Concentration(gm/ml)	Lab strain	Hospital strain
0.5	+	+
1.0	–	+
1.5	–	+
2.0	–	–
2.5	–	–
3.0	–	–

Key: + indicates growth, no inhibition.
-Indicates no growth, hence inhibition.

The Lab strain showed a MIC of 1.0 gm / ml with garlic as against a MIC of 0.3mg/ml with Fluconazole. The Hospital strain on the other hand showed a MIC of 2.0 gm/ml with garlic and a MIC of 5mg/ml with Fluconazole. This could be due to the fact that the active components in the garlic paste are not taken in the concentrated form for the study.

Headaches, dizziness, diarrhoea, vomiting,nausea, heartburn are some of the side effects of Fluconazole reported. (www.medicinenet.com) As the resistance towards the Azole drugs is increasing , as can be seen from the results of MIC obtained with the hospital strain of C.*albicans*,the dose to be given to patients increases. Every drug has its own side-effects so increasing the dose for the patients leads to adverse effects making the drug therapy ineffective. Thus, Natural Antifungal agents may be recommended for avoiding the deleterious side effects and also attaining efficient treatment.

The minimum inhibition concentration (MIC) of garlic extract reported was 0.78, 1.56, and 3.125 mg/ ml for *Candida tropicalis, C. glabrata*, and *C. albicans* respectively. (Jafari et.al)

Extraction of the active component of Garlic

Alcoholic, Acetone and Aqueous extraction of Garlic was carried out and the extract was used to check the inhibition of Lab and Hospital strain of *Candida albicans*

In the present study, it was found that Aqueous extract showed the strongest inhibitory activity followed by Acetone and then Alcoholic extracts of garlic. The strains tested were the Lab strain and the hospital strain.

Studies were carried out to check the inhibition of fungi (*A. niger and C. albicans*) with the component Ajoene derived from garlic. Garlic has been shown to inhibit the growth of variety of organisms, not only bacteria but also fungi and viruses. It is believed that the activity of garlic is

Strains	Aqueous garlic extract Zone of inhibition in mm	Acetone garlic extract Zone of inhibition in mm	Alcoholic garlic extract Zone of inhibition in mm
Lab strain	7mm	5mm	5mm
Hospital strain	5mm	4mm	3mm

Drug Tested	Zone of Inhibition
Drug A Fluconazole	3mm
Drug B Aq Garlic extract	5mm
Junction of two drugs	6.5mm

Fig. 1

Fig. 2.

Fig. 3. The lab strain shows no inhibition at 100mcg/ml, a hazy zone of inhibition at 200mcg/ml and a proper zone of inhibition from 300 mcg/ml onwards

Fig. 4. The hospital strain shows no inhibition of growth till 3mg/ml, there is an insignificant zone of inhibition at 4mg/ml and proper zone of inhibition at 5mg/ml

Fig 5. Shows the inhibition of the lab strain with 1.0gm of garlic

due to Allicin, the major component of garlic. There are also various other components in garlic which will help in the inhibition of organisms. (Yoshida et.al., 1987)

Invitro Synergism

The results obtained are suggestive of a possible synergy between the aqueous extract of garlic and Fluconazole.

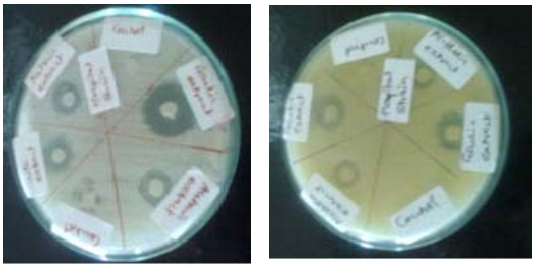
The present study shows synergy between the aqueous extract ogf Garlic and Fluconazole.

The results obtained thus suggest that Garlic and Fluconazole both can be used in synergy

Fig. 6. Shows the inhibition of the hospital strain with 2 gm/ml of garlic

Acetone extract Alcoholic extract

Fig. 7. Shows the zones of inhibition obtained using the various extracts using the lab strain



Acetone extract Alcoholic extract

Fig. 8. Shows the zones of inhibition obtained using the various extracts against the hospital strain

with each other. Khodavandi et al (2010) , used allicin, an allyl sulfur derivative of garlic, to demonstrate both its intrinsic antifungal activity and its synergy with the azoles, in the treatment of Candida spp. in vitro. In their study, the MIC(50) and MIC(90) of allicin alone against six *Candida* spp. ranged from 0.05 to 25 microg/ml. However, when allicin was used in combination with fluconazole or ketoconazole, the MICs were decreased in some isolates. The results demonstrated the existing synergistic effect between allicin and azoles in some of the Candida spp. such as *C. albicans, C. glabrata* and *C. tropicalis*, but synergy was not demonstrated in the majority of Candida spp. tested . Khodavandi et al (2010) also suggest that In vivo testing needs to be performed to support these findings.

CONCLUSION

In conclusion, it can be stated that Garlic does have a potent anti candidal activity, with the aqueous extract showing the most activity as compared to the acetone and alcoholic extracts. The hospital strain used in the study was found to tolerate higher concentrations of the azole drug, Fluconazole, the drug of choice in treating Candida infections, hence suggesting the possible drug resistance. The regular lab strain however was inhibited at lower concentrations of Fluconazole. Natural antimycotic substances like Garlic can be used along with the Azole drugs in order to reduce the dose of the Azole drugs. This would also reduce the possibility of the Candida strain from becoming drug resistant.

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