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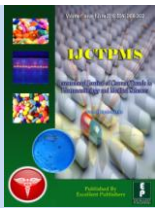

Original Research Article

Synergistic Effects of Plant Extracts and Antifungal Drugs on *Candida albicans*

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Abstract

Fungi have surrounded us and have affected our life, when changed or getting out from control it should be treated and when change to multi drug resistant it is difficult to treat by antibiotics, so plant extracts can be used to produce valuable protection against infection. In this study, agar-well diffusion method was used to investigate the effect of antifungal drug of plant extracts on *Candida albicans. C. albicans* was isolated from oral cavity of Sulaimani Emergency hospital patients. The isolates consisted of *C. albicans* (n=15). The microorganisms were divided into four groups in a factorial design: control, FGE with black seed, FGE without black seed, black seed without FGE and antibiotic (fluconazole). The minimum inhibitory concentration of FGE was calculated by using a gradient of concentrations and observing their inhibitory effects on *C. albicans*. Results: fresh garlic extract (FGE extract) displayed evident inhibition against Candida albicans by producing more inhibition zone (p<0.05), black seed extract (BI extract) showed no inhibitory effect on *C. albicans* at 10% concentration. However, synergism of BI with FGE have shown significant effects on it (p<0.05) and fluconazole had little effect on *Candida albicans*. The results of the present study suggest that FGE can improve the antibiotic sensitivity and BI had no effect on *C. albicans*.

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Keywords

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Introduction

Fungi are eukaryotic organism that include unicellular (like yeast) or very complex multicellular (like filamentous) microorganisms, do not have chlorophyll A (mean non-photosynthetic heterotrophic). They have rigid cell walls which contain chitin-chitosan, and they have relationships with animals, some of them symbiotic, commensals or parasite in/on animals which cause infections (e.g.: *Candida albicans*), *Candida* is a type of yeast that is the reason for a number of undesirable symptoms, this yeast may be only those gut flora, an assembly of microorganisms that live in mouth and also in digestive system (Chaffin, 2008). The point when the *C. albicans* populace begin getting crazy for control eventually periods for stress alternately after a course from claiming antibiotics, this equalization is lost, the states for *Candida* have the ability to extend quickly until they control an expansive bit about your gut what's more infiltrating through under those circulation's system also discharging its poisonous repercussions all around for muscles, too. For a long time, human try to produce antifungal (An antifungal executor may be a medication regardless that specifically dispenses with contagious

pathogens starting with a host with insignificant poisonous quality of the host) to kill parasite fungi, for example antifungal fluconazole.

Fluconazole (F) will be used to treat contagious infections, including yeast infections of the vagina, mouth, throat (tube heading adrift from mouth of the stomach), abdomen (the area above the waist), lungs, blood, and furthermore other organs. Fluconazole may be likewise utilized will treat meningitis (infection of the membranes coating this cerebrum and spine) initiated by parasite. Fluconazole will be over a class from claiming antifungals known as triazoles. It meets expectations by abating this development from claiming growths that result in spoiling. Other medical antifungal treatment can use plant like natural antifungal which may have greater effect than it, mostly focused on garlic, black seed and both together (Hassawi and Kharma, 2006; Khodavandi et al., 2011).

Fresh garlic extract (FGE) has been utilized as both sustenance and drug for a long time. It has been found that garlic helps prevent a number of infections. Some advanced investigations affirm that garlic has definite anti-microbial properties Also may be powerful against totally range bacteria, growths also infections. For addition, the antimicrobial exercises from claiming garlic need aid interfaced of the vicinity of a few bioactive mixes (Ankri and Mirelman, 1999; Tsao and Yin, 2000; Li et al., 2015; Shuford et al., 2005).

Too many years, black seeds (BI) have been utilized to their recuperating and also medicinal properties. Recently, specialists and researchers need aid to start to remember those stunning well being profits of black seeds. Black seeds are the seeds of a plant called '*Nigella sativa*' that initially originate starting in Egypt. Numerous nations in Middle East used black seeds 'the seeds from claiming favoring that might mend large portions diseases'. The black seeds have been utilized concerning illustration an incredible antibacterial, anti-inflammatory, antifungal, antiviral, cell reinforcement and antispasmodic. Black seeds might additionally make an incredible cure to icy, furthermore would consume eventually those figure fast at made over little dosages (Nadaf et al., 2015).

Fluconazole has more effect on *C. albicans* or extract plant like garlic and black seed, synergistic of FGE and BI have more effect together or use separately on *Candida.* The present study has been focused on plant extract especially FGE and BI, and their effect on *C. albicans*, and have focused on Fluconazole for affecting on *C. albicans* and with the extract plant like FGE more affecting or less affecting on *Candida*, comparing the data with other papers.

The aim of the current study was to investigate the effect of fluconazole on *C. albicans*, throughout using fresh garlic extract and black seed. To reach our aim, we want to experiment the effect of plant extract and antifungal to *C. albicans* by using agar well diffusion.

Materials and methods

Isolation of *C. albicans*

Candida albicans was isolated from oral cavity of Sulaimani Emergency hospital patients. After taking a swab from their throat, the swab was subsequently used to grow the fungus on Sabaroud Dextrose Agar plates. The plates were then brought back to the lab and incubated at 37°C for 48 hrs. Another culture of *C. albicans* was kindly provided by Dr. Haider Mussa from advanced microbiology lab. A subculture was then prepared for later use.

Preparation of fresh garlic extract (FGE)

Fresh garlic was peeled off, and 100 g was weighed and mixed up with 200 ml distilled water then it was crushed using a juicer. The resulting paste was then centrifuged at 3000 rpm for 30 mins. The supernatant was then filtered by clean gauze. Later on, the weight of the precipitate along with the unfiltered garlic fibers were weighed and subtracted from the original weight of the peeled garlic bulb. Thus the final concentration of Fresh Aqueous Garlic Extract (FGE) was calculated. In this experiment, we obtained 60% FGE (w/v) and used it as a stock solution to prepare other concentrations like 40, 30, 20, 10, 5, 3, 1 and 0.5% (w/v).

Preparation of black seed extract

Ten percent black seed oil extract was prepared the same as the above procedure. However, there were some changes in the preparation process, the solvent used here was Ethanol, and this was to obtain oil extract from the black seeds. The mixture was then subjected to air drying in an incubator at 37°C for 72 hrs. Then, the dried product was used to prepare 10% Black seed extract in distilled water.

Agar well diffusion test

After preparing the agar media, agar wells were generated using a glass puncturing tool. The diameter of each well was 5mm. The wells were created after spreading *C. albicans* on the agar media. After the incubation period, the diameter of the wells was subtracted from the diameter of the clear zones generated as a result of the extracted products.

Calculation of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration of FGE was calculated by using a gradient of concentrations for observing their inhibitory effects on *C. albicans*. It was noted that FGE at 10% completely inhibits the fungal growth. However, concentrations below 10% only partially inhibited the fungal growth. Thus, 10% FGE was the MIC. Due to time and resource availability constraints, we could not perform the same procedure for Black seeds and we hypothesized that 10% of black seed would show some inhibitory effects on *C. albicans* similar to FGE.

Statistical analysis

In this experiment, data analysis has been used for each action at least four times using Microsoft excel 2013. To compare the groups, One Way ANOVA was used, but to compare antifungal and plant extract main effective *t*-test was used. *p*-values of 0.05 were considered to be significant.

Results and discussion

The antifungal defenselessness of *C. albicans*, FGE with BI and FGE without BI and BI without FGE and F is summarized to Fig. 1. The ranking observed were FGE > FGE with BI > F > BI and control. The information has been indicated that FGE without BI could prompt an increasing in the size of the inhibition zones against *C. albicans* compared to FGE with black seeds, but FGE with black seeds might prompt a increasing in the size of the inhibition zone against *C. albicans* compared to fluconazole and black seeds without FGE does not produce an inhibition zone ($p<0.05$). Those factorial dissection show to extraordinary positive connection effect ($p<0.05$) (data is not shown). The fungicide activity of black seeds increase by FGE, and the synergism black seeds with FGE produce more zone (0.996mm) compared fluconazole (0.833mm), respectively (Fig. 1).

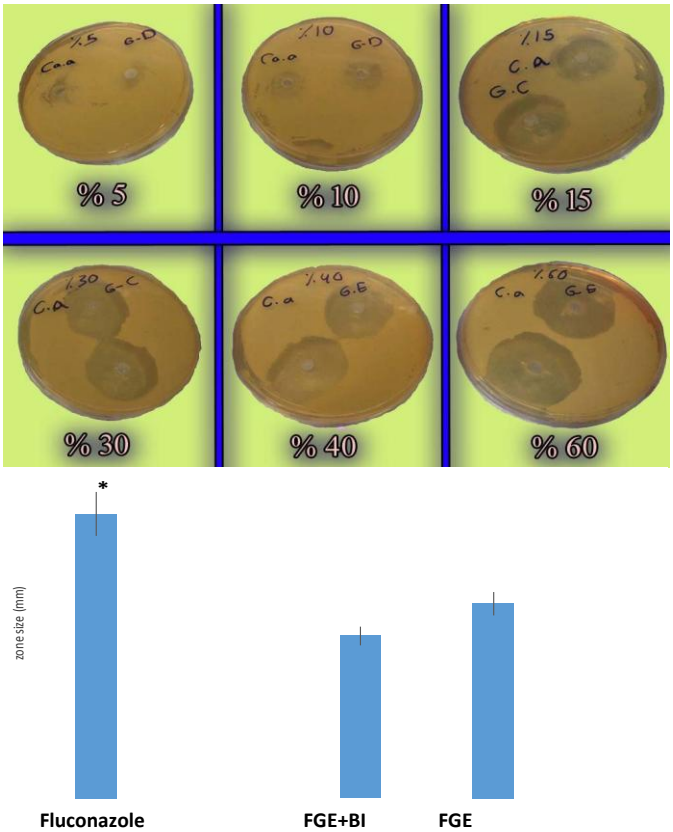

Fig. 1: Activity of FGE on *C. albicans*. The analysis of antibiotic activity of fluconazole, FGE with Black seed, FGE without black seed, black seed without FGE against *C. albicans*. *p-value <0.05 indicates a significant difference, and used Bonferroni of Post-hoc test. The chart shows descriptive statistic and One Way ANOVA, and shows FGE significant to fluconazole and FGE significant to FGE with black seeds and fluconazole no significant FGE with black seed.

In this experiment, it was found that FGE shows restraint properties against *C. albicans*. A standout amongst the way exacerbating garlic will be ajoene, and turned out antifungal that has been indicated to make successful against a number contagious strains.

Ajoene will be structured from a compound named allicin also a chemical named allinase. At these two common mixes come into contact (by chopping this garlic, pulverizing it or by other means), they manifestation an antibacterial agonization named, which after that combines to structure ajoene. However, allicin is rapidly oxidized, unstable and volatile, mean allicin rapidly breakdown after raw garlic is cracked. So it means garlic extract has more activity than an equal amount of allicin (Ankri and Mirelman, 1999; Khodavandi et al., 2011). In spite of the fact that this has

turned out antifungal properties, those correct system toward which this happens is not reasonable. Concerning illustration with different antifungals, researchers suspect that it works by disrupting the cells walls of the *C. albicans* yeast cells.

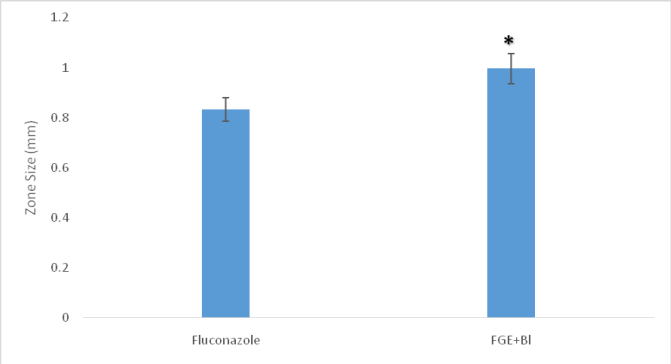

Fig. 2: Activity of fluconazole and FGE with black seed on *C. albicans.* The analysis of antibiotic activity of fluconazole and FGE with Black seed against *C. albicans*. **p*-value <0.05 indicates a significant difference from respective antibiotic and FGE with Black seed by *t*-test

Black seeds' effect on *C. albicans* was zero, which means it did not produce an inhibition zone, because the components contained in it did not affect *C. albicans* or not target for *C. albicans*. By comparing to other papers, Li et al. (2015), zones were obtained than that paper because we used different way to extract plant, and we use agar well method. However, they use disk diffusion method to investigate the antimicrobial activities of fresh garlic extract, in that paper just FGE was used but we used FGE and black seeds for plant extraction, we didn't mix fluconazole with FGE, however, we mix FGE with black seeds and compared to fluconazole.

Fluconazole has few effect on *C. albicans* and can inhibit growth of it by working in cytochrome and ergosterols of *C. albicans*, fluconazole has common side effects on humans (headache, nausea, rash, diarrhea, stomach ache, etc.) and has rare side effects like; it may cause some unpredictable side effects including (dizziness, baldness). *C. albicans* can make resistant to antibiotic and by mixing FGE and fluconazole more inhibition zone means fluconazole with FGE have more effect on *Candida* than without FGE. For extracting plant materials-black seeds ethanol was used after drying BI and for garlic juice's garlic was used, because of that different data was obtained. In this experiment errors might have occurred in the steps, for FGE (choosing old or new of garlic, the way and quality of filtration for garlic, contamination of garlic by other microorganism, for black seeds (choosing old or new of black seed and should not damage, the calculating of percentage of ethanol when extracted), for fluconazole (the date of expiry of fluconazole), and whether all steps were done by aseptic technique.

In conclusion, the result shows that FGE has effects on *C. albicans*, black seeds did not have effects on *C. albicans*, synergistic of BI with FGE had less effect than FGE but more effect than fluconazole.

Conflict of interest statement

Author declares that he has no conflict of interest.

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