Journal of Developing Drugs
ISSN: 2329-6631

Salih, J Dev Drugs 2016, 5:3
DOI: 10.4172/2329-6631.1000165

Research Article Open Access

Synergistic Effects of Plant Extracts and Antifungal Drugs on *C. albicans*

Kamal A Salih*

Faculty of Science and Science Education, Department of Biology, University of Sulaimani, Iraq

***Corresponding author:** Salih KA, Faculty of Science and Science Education, Department of Biology, University of Sulaimani, Iraq, Tel: 07709893454; E-mail: hunar.h96@gmail.com

Received date: Nov 03, 2016; **Accepted date:** Nov 21, 2016; **Published date:** Nov 25, 2016

Abstract

Background: 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 we can use plant extracts to produce valuable protection against infection.

Objective: In this study, we used agar well diffusion method to investigate the effect of antifungal drug of plant extracts on *Candida albicans*.

Methods: *Candida albicans* was isolated from oral cavity of Sulaimani Emergency hospital patients. The isolates consisted *C. albicans* (n=15). The microorganisms were divided into four groups in a factorial design: control, FGE with blackseed, 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 (BL 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*.

Conclusion: The result suggests that FGE can improve the antibiotic sensitivity and BI don't effect on *C. albicans*.

Keywords: Plant extract; Fluconazole; *Candida albicans;* Black seed; Fresh garlic extract; Antifungal

Introduction

Fungi are eukaryotic organism that includes unicellular (like yeast) or very complex multicellular (like filamentous) microorganism, don't have chlorophyll A (mean non-photosynthetic-heterotrophic). 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 infection (like *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 also digestive system [1]. 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 [2].

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 medically antifungal treatment can use plant like natural antifungal which may have greater effect than it, mostly focused on garlic, black seed and both together.

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 [3].

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 wellbeing 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 favouring that might mend large portions diseases.

Too many years, 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 [4].

Fluconazole has more effect on *Candida albicans* or extract plant like garlic and black seed, synergistic of FGE and BI have more effect together or use separately on Candida.

We have focused on extract plant especially FGE and BI, effect on *Candida albicans*, and have focused on Fluconazole for affecting on *Candida albicans* and with the extract plant like FGE more affecting or less affecting on Candida, comparing the data with other papers [5].

Aims

The aim of the current project was to investigate the effect of Fluconazole on *Candida albicans*, throughout using fresh garlic extract and black seed.

Objective

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 [6].

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 minutes. The supernatant was then filtered by a clean gauze. Later, the weight of the precipitate along with the unfiltered garlic fibres were weighed and subtracted from the original weight of the peeled garlic bulb. Thus, the final concentration of Fresh Aqueous Garlic Extract 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

10% 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 [7].

Agar-well diffusion test

After preparing the agar media, agar wells were generated using a glass puncturing tool. The diameter of each well was 5 mm. The wells were created after spreading *C. albicans* on the agar media. After the incubation period, the diameter of the wells were 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 and observe 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" is used, but to compare "antifungal and plant extract" main effective t. test is used. P values of 0.05 were significant.

Results

The antifungal defenselessness of *C. albicans*, FGE with BI and FGE without BI and BI without FGE and F is summarized to Figure 1. The ranking is 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 an 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 (0.996 mm) compared fluconazole (0.833 mm), respectively (Figure 1).

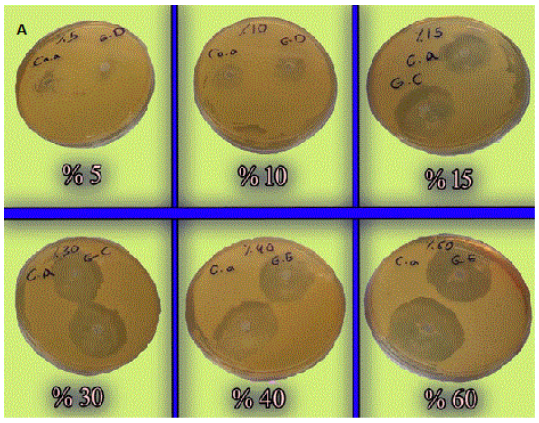

Figure 1A: Show the activity of FGE without black seed, FGE with black seed, Black seed without FGE and Fluconazole on *C. albicans*.

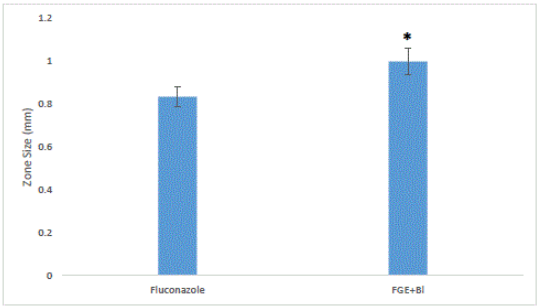

Figure 1B: The analysis of antibiotic activity of fluconazole, FGE with Black seed, FGE without black seed, black seed without FGE against *candida albicans*. *P. value<0.05 indicates a significant difference, and used bonferroni of post-hoc test. The chart shows descriptive statistic and one way anova, and shows FGE significant to fluconazole and FGE significant to FGE with black seeds and fluconazole no significant FGE with black seed.

Discussion

In this experiment, we discovered 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 allicin, 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. 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.

Black seeds effect on *C. albicans* is zero, which means it does not produce an inhibition zone this is because the components which contain it does not effect of *candida albicans* or not target for *Candida albicans*.

By comparing to other papers like we got few zones than that paper because we used different way to extract plant, and bzhy santare bioscience 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 *Candida albicans* and can inhibit growth of it by working in cytochrome and ergosterole of *C. albicans*, fluconazole has common side effects on humans like (headache, nausea, rash, diarrhea, stomach ache) and has rare side effects like; it may cause some unpredictable side effects including (dizziness, baldness). *Candida albicans* can make resistant to antibiotic and by mixing FGE and Fluconazole we get more yield of zone mean fluconazole with FGE have more effect on Candida than without FGE (Figure 2).

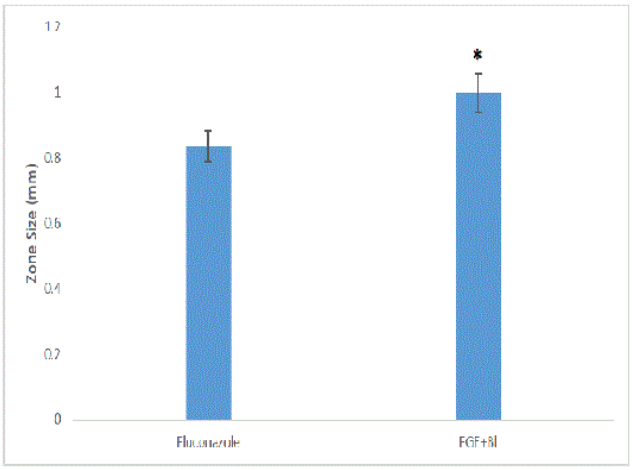

Figure 2: Show the activity of fluconazole and FGE with Black seed on *C. albicans*. The analysis of antibiotic activity of fluconazole and FGE with Black seed against *Candida albicans*. *P. value<0.05 indicates a significant difference from respective antibiotic and FGE with Black seed and used t. test.

We use different type for extracting plant like for black seeds we use ethanol after drying BI and for garlic we used juice's garlic, because that we get those data and have many ways to extract from plant and may better than the way we have used like maceration, percolation, soxhlet extraction and many ways which get different results for same sample.

In this experiment we may have done some errors in the steps, like; for FGE (choosing old or new of garlic, the way and quality of filtration for garlic, contamination of garlic by other microorganism, do pure culture for garlic or not), 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 dating of expire of fluconazole), and should all steps do by aseptic technique.

Conclusion

The result shows that FGE has effects on *Candida albicans*, black seeds don't have effects on *C. albicans*, synergistic of BI with FGE have less effect than FGE but more effect than fluconazole.

References

1. Li G, Ma X, Deng L, Zhao X, Wei Y, et al. (2015) Fresh Garlic Extract Enhances the Antimicrobial Activities of Antibiotics on Resistant Strains in vitro. Jundishapur Journal of Microbiology 8: e14814.
2. Khodavandi A, Alizadeh F, Harmal NS, Sidik SM, Othman F, et al. (2011) Comparison between efficacy of allicin and fluconazole against Candida albicans in vitro and in a systemic candidiasis mouse model. FEMS microbiology letters 315: 87-93.
3. Shuford JA, Steckelberg JM, Patel R (2005) Effects of fresh garlic extract on Candida albicans biofilms. Antimicrobial agents and chemotherapy 49: 473-473.

4. Tsao SM, Yin MC (2000) Enhanced inhibitory effect from interaction of curcumin with amphotericin B or fluconazole against Candida species. Journal of Food and Drug Analysis 8: 208-212.
5. Nadaf NH, Gawade SS, Muniv AS, Waghmare SR, Jadhav DB, et al. (2015) Exploring anti-yeast activity of Nigella sativa seed extracts. Industrial Crops and Products 77: 624-630.
6. Hassawi D, Kharma A (2006) Antimicrobial activity of some medicinal plants against Candida albicans. J Biol Sci 6: 109-114.
7. Ankri S, Mirelman D (1999) Antimicrobial properties of allicin from garlic. Microbes and infection 1: 125-129.