Original Article

The Effecting of Ovalbumin on the Candida Albicans in Harsh Environment

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Abstract

We studied the effect of ovalbumin on *Candida albicans* by agar well difussion method, OVA showed Anifungul activity against all studied cultured. *Candida albicans* is a, commensal yeast, fungus of the human oral, gastric, vaginal mucosal surfaces, and skin. *C. albicans* may become an opportunistic, pathogen and lead to life systemic, infections, severe mucocutaneous illnesses, and/or antibiotic-, induced dysbiosis, immunodeficiency, and/or medical procedures that disrupt protective host defense mechanisms and/or damage the integrity of the mucocutaneous barrier.

Keywords: *Candida Albicans*, Ovalbumin, Simulated Body Fluid.

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INTRODUCTION

Ovalbumin which accounts up at least, 54% of the protein, content in egg whites,, is thought to be, their major allergen., With 385 amino, acids, approximately a,quarter of which are charged, it is a glycoprotein, with a molecular weight of around 42.7 kDa, Ovalbumin has antimicrobial properties that can inhibit several types of bacteria and yeast cells, including *C.albicans* (Arzumanya V.G. *et al*., 2019). Egg white, protein's antimicrobial, antifungal, and, antioxidant properties are increasingly finding new applications (Geng F *et al*., 2012), which has helped in the creation of health, and pharmaceutical-related products. also, OVA is primarily responsible for most of its functional properties. Furthermore, albumin also inhibited the activity of certain hydrophobic toxins produced by bacteria, fungi, and insects. This led to the discovery of a vital biological, mechanism by which albumin, may maintain host organisms against the effects of toxins (Croguennec T *et al*., 2000).

Blood is a crucial and special habitat during the development of systemic candidiasis, housing a variety of stimuli and hazards for the fungus. Multiple studies have shown how *C.albicans* interacts with blood-borne immune cells, immunological mediators, such complement, proteins, and coagulation, factors (Duhring. S *et al*., 2015). Additionally, human serum has the ability to control *C.albican's* pathogenicity (Ding. X *et al*., 2014).

For instance, *C. albican's* invasive phenotype but the serum harm the fungus causes to host cells (Samaranayake YH., 2013). It was discovered that albumin controls the pathogenicity of *C. albicans* by hydrophobically interacting with the peptide toxin candidalysin.

Only a small number of the more than 150 species in, the genus *Candida* are, known to infect humans. It was discovered that *Candida albicans* was, the most common species, (50-60 percent). (Alhussein., 2019).

The yeast *C.albicans*, is one of the most, prevalent fungi in the human microbiome and frequently responsible for mucosal and systemic, infections. Most people's guts, mouths, or vaginal tracts are colonized by *C. albicans* in good health as a non-harmful commensal but may be become opportunistic pathogen in immunocompromised patients (Witchley. JN *et al*., 2019).

However, many as 400,000 systemic fungi illnesses have species of *Candida*. Nearly 70% of fungal diseases worldwide are caused by the species *Candida albicans*, which is the most frequent cause of mucosal infections, and systemic, infections. For many years, it has been the main contributor of invasive infections that can be fatal. Even with therapy, the death rate is about 40%, particularly in hospital settings (Chen, H *et al*., 2020).

1.1 Candida albicans, morphology

The morphological forms of *C. albicans* include hyphae, pseudohyphae, and blastospores. Asexual division of blastospores occurs by budding The Clinical Signs and Symptoms of *Candida albicans* infections can range from mucocutaneous illnesses that are only superficial to invasive infections that affect many organs (Walker, G.M. *et al*., 2017).

1.2 Oral candidiasis

Oral candidiasis (OC), often known as "thrush," is an, infection of the tongue, and other oral mucosal regions and is distinguished by fungal proliferation and penetration of superficial tissues, their symptoms might not always show or may be White or yellowish lesions on your, tongue, tonsils, gums, or lips, as well as bumps on your, inner cheeks, also the discomfort or burning in the mouth and the, feeling of cotton in, your mouth; cracked, dry skin at the edges of your mouth, too a bad, taste in your tongue or a lack of flavor, difficulties swallowing(Hellstein, J.W., 2019).

1.3 Candida albicans, in Gastroenterology

Each person has a different gut flora, which in healthy individuals is largely consistent. Its content varies among individuals based on food or hygiene practices, age, and other factors throughout the digestive system (Jeziorek M., 2019).

1.4 Candida albicans in Dermatology

C.albicans often causes superficial, skin infections, but "deep" mycoses, in which *Candida* also affects the dermis and subcutaneous tissue, are uncommon.as well as, Acute and, chronic paronychia, onychomycosis may also be brought on by *candida*. The condition known as paronychia, commonly known as whitlow, is an infection of the skin, around a nail. Another cause diaper dermatitis. Additionally, invasive candidiasis occur in various organs such as bone, brain, heart, spleen, liver, kidney, aye and lung (Leggit, J.C., 2013).

METHOD AND MATERIALS

Ovalbumin poweder and Simulated body fluid from sigma aldrige and sabauroud dextros agar medium.

2.1 Isolation of C.albicans

C.albicans isolated from Saliva and sputum specimens for patient among both sexual and different ages by diagnostic this isolates via cultured on Sabauroud Dextrose agar with chloramphenicol then the plates incubated at 37ºC for 24-48 hours. also, Examined under light microscope using germ tube test which made by adding the suspected colonies into small tube contain 0.5ml of human serum, Then Incubated the tubes at 37°C for 2 to 4 hours, Transfer a drop of the serum to a slide for examination after air dried, heat fixed and complet a staining to gram stain.

Too, we diagnostic of all samples using VITEK2YSTcard in VITEK 2 system Biomeriux (France).

2.2 Preparation of antifungul solution

Put some concentrations of OVA on the 4ml of simulated body fluid in beaker and well mixed to noticed the change of a color from clear to a pale yellow. Where three different concentrations of ovalbumin solution were prepared, as follows: (5,10,15 mg/ml) respectively, as 20, 40,60 mg were wighted per 4ml of solution.

2.3 Agar well diffusion test

C. albicans suspention were done via compared with 0.5 × 108 colony forming unit/ml (macFarland standered solution) where cultured through spreader on SDA. After that, made the wells via tips size 10mm in sabouraud dextrose agar which were previously inoculated with yeasts. Then, add the solutions into a hole at a volume (20 ul).therefore, agar plates were incubated in the proper conditions overnight under 37 °C, The fungal inoculum being examined is inhibited from growing when the antimicrobial drug diffuses in the agar medium and make zone inhibition (Valgas C., *et al* 2007).

RESULT AND DISCUSSION

3.1 Isolation and identification of Candida spp

3.1.1 Characteristic of culture

All sample were cultivated on Sabouraud Dextrose agar with Chloramphenicol as a selective media, As shown in figure (3-1) white to cream, round, curved, soft and smooth with a characteristic a yeast odor.

Figure (3-1): C.albicans colonies was cultured on SDA

3.1.2 Gram stain

Microscopic examination for all candida isolates after gram staining were done to confirmatory of *candida albicans*, untle appeared gram positive, spherical to oval with present pseudohypha and budding yeast cells as well as in figure(3-2).

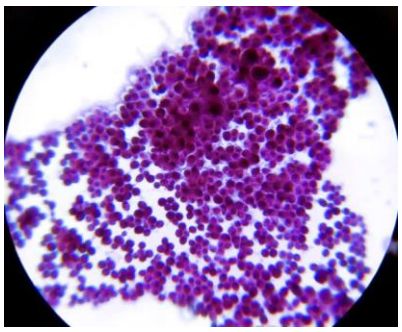


Figure (3-2): C.albicans under the light microscope

3.1.3: Germ tube formation test

Candida albicans isolates were apeared positive results with the production long tube like extentions that protruded from the yeast cells which was the germ tube, at the location of attachment to the yeast cells seen at figure (3-3).

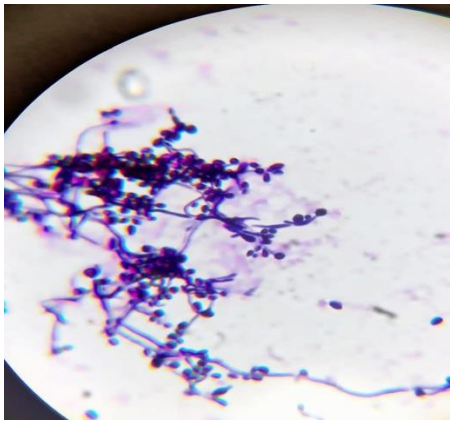


Figure (3-3): Illustrated germ tube formation in the C.albicans

3.2 Production of colloidal suspension

The changes of coloration solution were noticed from a clear to a pale yellow as in figure (3-4).

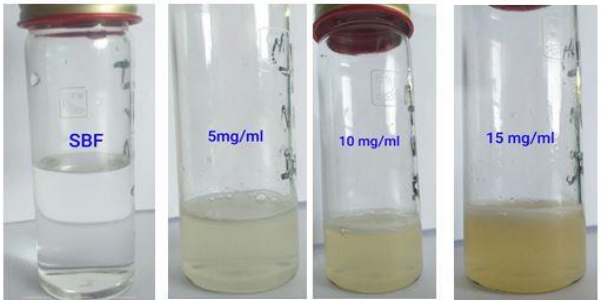
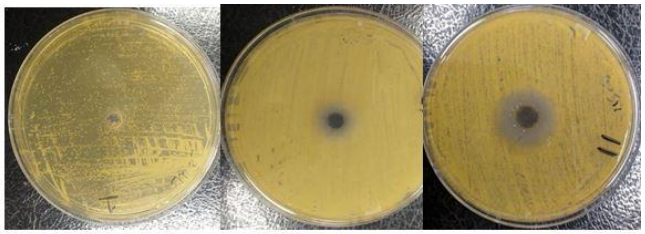


Figure (3-4): It exhibits the differences in color between the various concentrations of the antifungal ovalbumin solution.

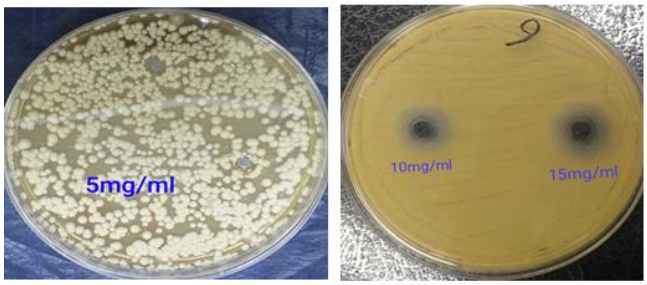
3.3 Well difussion assay

The results of an overnight incubation at 37°C revealed that the concentration of the solution affected its inhibitory effect against the fungal isolates. With an average inhibition diameter approximately between of (18-21mm) for all isolates seen in the below to the solution with a concentration of 15 mg/ml had the maximum inhibitory ability for fungul isolates. Compared to the second concentration, which provided a lower level of inhibition with an average diameter of (14-16mm) for all isolates also shown in below The isolates did not exhibit any inhibitory action at the final dosage of 5 mg/ml. Consequently, the results indicate that the inhibitory activity rises, so does inhibition zone. Our research therefore shows that albumin inhibits *C. albicans* development. This particular yeast was chosen due to it having the rare capacity of all microbial pathogens to infect almost all bodily tissues and organs.



(a) (b) (c)

Figure (3-5): illustrated antifungul activity against the C.albicans isolates in concentrations (a) 5mg/ml where observed no inhibitory effect (b) 10mg/ml shown the diameter of zone inhibtion 14mm (c) 15mg/ml seen the diameter of zone inhibtion 21mm



(a) (b)

Figure (3-6): illustrated antifungul activity against the C.albicans isolates in concentrations (a) 5mg/ml where observed no inhibitory effect (b) 10mg/ml and15mg/ml where shown the diameter of zone inhibtion 16mm and 19mm respectively.

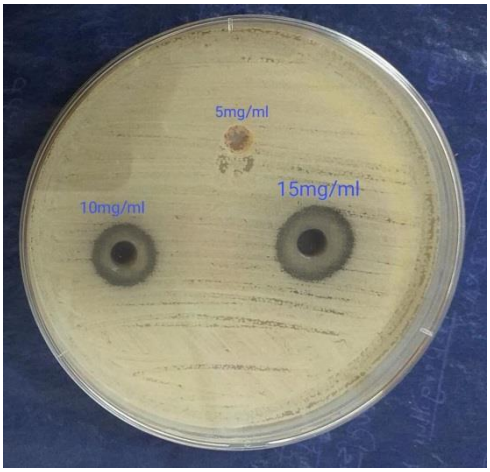


Figure (3-7): illustrated antifungul activity against the C.albicans isolate in concentrations 5mg/ml where observed no inhibitory effect, 10mg/ ml and 15mg/ml shown the diameter of zone inhibtion were 14mm and 18mm respectively.

Table (3-1): It displays the fungus inhibition results for various ovalbumin solution concentrations

The Average Of diameters in mm	Zone inhibition in mm of *C.albicans* 3	Zone inhibition in mm of *C.albicans* 2	Zone inhibition in mm of *C.albicans* 1	Concentrations of Ovalbumin solution
0	0	0	0	5mg/ml
14.6	14	16	14	10mg/ml
19.3	18	19	21	15mg/ml

According to the study's findings, smaller concentrations of this antifungal were unaffected, while rising concentrations caused an increase in the inhibitory activity.this study is simeller with another study (Arzumanyan V.G., 2019) which revealed in the high concentrations the celles of yeast distroyed with formation of dibris vesicles while at lower concentrations not effect on this yeasts, also the (Austermeier, S., 2021) shown that the albumin which modulates *C.albicans* pathogenicity

CONCLUSION

Ova was dissolved in liquid and used in a harsh environment as a simulated body fluid because it is similar to blood plasma because it contains the same ions and salts and is also close to the conditions of the body. We concluded that OVA has antifungal activity on *C.albicans* at certain concentrations, but the inhibitory capacity increases as the concentration rises. *C. albicans* are grown on Sabauroud dextrose agar in a suitable environment at least 37°C for 24 hours.

REFERENCES

Abd Alhussein, H. (2019). Candidiasis (Doctoral dissertation, Ministry of Higher Education).

Arzumanyan, V. G., Ozhovan, I. M., & Svitich, O. A. (2019). Antimicrobial effect of albumin on bacteria and yeast cells. Bulletin of Experimental Biology and Medicine, 167(6), 763-766.

Austermeier, S., Pekmezović, M., Porschitz, P., Lee, S., Kichik, N., Moyes, D. L.,... & Gresnigt, M. S. (2021). Albumin neutralizes hydrophobic toxins and modulates Candida albicans pathogenicity. MBio, 12(3), e00531-21.

Chen, H., Zhou, X., Ren, B., & Cheng, L. (2020). The regulation of hyphae growth in Candida albicans. Virulence 11 (1): 337–348. [CrossRef] [PubMed]

Croguennec, T., Nau, F., Pezennec, S., & Brule, G. (2000). Simple rapid procedure for preparation of large quantities of ovalbumin. Journal of Agricultural and Food Chemistry, 48(10), 4883-4889.

Dühring, S., Germerodt, S., Skerka, C., Zipfel, P. F., Dandekar, T., & Schuster, S. (2015). Host-pathogen interactions between the human innate immune system and Candida albicans—understanding and modeling defense and evasion strategies. Frontiers in microbiology, 6, 625.

Ding, X., Liu, Z., Su, J., & Yan, D. (2014). Human serum inhibits adhesion and biofilm formation in Candida albicans. BMC microbiology, 14(1), 1-9.

Geng, F., Huang, Q., Wu, X., Ren, G., Shan, Y., Jin, G., & Ma, M. (2012). Co-purification of chicken egg white proteins using polyethylene glycol precipitation and anion-exchange chromatography. Separation and purification technology, 96, 75-80.

Hellstein, J. W., & Marek, C. L. (2019). Candidiasis: red and white manifestations in the oral cavity. Head and neck Pathology, 13(1), 25-32. [CrossRef] [PubMed]

Jeziorek, M., Frej-Madrzak, M., & Choroszy-Król, I. (2019). The influence of diet on gastrointestinal Candida spp. colonization and the susceptibility of Candida spp. to antifungal drugs. Roczniki Państwowego Zakładu Higieny, 70(2).

Leggit, J. C. (2017). Acute and chronic paronychia. American Family Physician, 96(1), 44-51.

Samaranayake, Y. H., Cheung, B. P., Yau, J. Y., Yeung, S. K., & Samaranayake, L. P. (2013). Human serum promotes Candida albicans biofilm growth and virulence gene expression on silicone biomaterial. PLoS One, 8(5), e62902.

Valgas, C., Souza, S. M. D., Smânia, E. F., & Smânia Jr, A. (2007). Screening methods to determine antibacterial activity of natural products. Brazilian journal of microbiology, 38, 369-380.

Walker, G. M., & White, N. A. (2017). Introduction to fungal physiology. Fungi: biology and applications, 1-35.

Witchley, J. N., Penumetcha, P., Abon, N. V., Woolford, C. A., Mitchell, A. P., & Noble, S. M. (2019). Candida albicans morphogenesis programs control the balance between gut commensalism and invasive infection. Cell host & microbe, 25(3), 432-443.