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Study of the Effect of Fluconazole, Alcohol Extract Curcumenind Nano Curcumenon *Candida Albicans* Isolated From Cancer Patients

DoaaKhudair Abbas, Fatima Abdul-Hussein Mejbel

[1,2]University of Kufa-Faculty of Science-Department of Biology, Iraq
d56273297@gmail.com

Abstract
Candidosis or candidiasis is the most common fungal infection in the oral cavity and is caused by *Candida species*. The most common species in infected and healthy mouths is *Candida albicans.*
During the period from September to November 2020, a total of 150 clinical samples were collected from Middle Euphrates Cancer Center in AL Najaf Governorate, which included swabs from the mouth. The Cultivation and identification methods of *Candida albicans* isolates were based on the morphological, cultural and biochemical characteristics, beside to the confirmative systems such as chromagar medium. And determine virulence factors (lipase , phospholipase and exopolysaccrid production)tube and in microtiter plate). Study the effect of fluconazole, alternative material (alcoholic extract curcumin&nanocurcumin) on growth *C. albicans* and their virulence factors. This research seems to be, Fluconazole and nanocurcumin are affected on culture and virulence factors of *Candida albicans* while alcoholic extract curcumin are affected on virulence factors only.
Key words: alcoholic extract curcumin, Fluconazole, *Candida ablicans.*

Introduction:-

Candidosis or candidiasis is the most common fungal infection in the oral cavity and is caused by *Candida species* . The most common species in infected and healthy mouths is *Candida albicans*. Oral *candidiasis*, as one of the most common opportunistic fungal infections, can appear in individuals with immunodeficiency, diabetes, and other predisposing conditions . (JasminkaTalapko*etal*, 2020). Virulence factors in *C. albicans* such as hydrolytic enzymes, lipases, phospholipases, and exopolysaccrid production that allow successful colonization and infection of the host under suitable predisposing conditions . The colonizing oral population can also serve as a reservoir for dissemination leading to potentially lethal infections. .(Morad H.O.J.*atel*,2018) There are currently five classes of antifungal agents used in the treatment of fungal infection. Curcumin is a kind of medicinal plant, a member of the ginger family,Zingiberaceae. As the herbal medicine has been globally demanded in recent years and due to the anti-viral, anti-inflammatory, and antifungal properties of curcuminCurcumin nanoparticles exhibit increased solubility and bioavailability, Several studies proved that curcumin nanoparticles act as a therapeutic agent against a wide spectrum of fungal disease (Dr. L.H.Hiranandani.*atel* ,2021)

Materials and Methods:-

Specimen's collection and IdentificationThe samples were then transported to the laboratoryFor culture on Sabouraud's dextrose agar and Chrom agar.Then incubated at 37C° for 24-48 hr. for visible growthof *Candida spp*. colonies for diagnosis and study (Sayyada*et al*., 2010)

Preparation of alcoholic extract of curcuminaccording to
(K. Diba1 *,et al.,2011)*

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A concentration stock solution was used in the experiment. ofanti fungal drugs and (alcoholic extract curcumin , nanocurcumen and Fluconazole) was prepared using DMSO 2%. For experimental purpose used80 &60 mg/ml concentration from all

Effects of Fluconazole on growth of candida albicans by well diffusion

An amount of 50 µl was taken by micropipette from Fluconazole solution into each well in petri dish and incubated at 37C° for 48 hours then inhibition zones were recorded.

Effects of (alcoholic extract curcumin ,nanocurcumen) on growth of candida albicans by well diffusion&micro titer test:

An amount of 50 µl was taken by micropipette from (alcoholic extract andnano solution) into each well in petri dish and incubated at 37C° for 48 hours then inhibition zones were recorded.The microbroth dilution technique was used in a 24-well microtitre plate (MTP) as per the Clinical and Laboratory Standards Institute [CLSI] recommendations. In 1 ml ofsabroud broth, around 1% of an overnight C. albicans culture was allowed to develop in the presence and absence of (nanocurcumen, extract ofcurcumen). As a blank, wells containing 1 mL of ordinary sabroud broth were used. For 16 hours, the plate was incubated at 37°C. A multi-mode plate reader was used to measure the growth absorbance at OD600 nm after incubation(ELIZA , United States) (Baillie and Julia Douglas, 1998).

E**ffects on Phospholipase Production**

For qualitative analysis of phospholipase production, 1 l of inoculum was placed in the center of an SDA plate containing 1 percent peptone, 3 percent glucose, 5.73 percent NaCl, 0.055 percent CaCl2, and 10% egg yolk emulsion in the absence andpresence of (curcumen, nanocurcumen), and the plates were incubated at 37°C for 72 hours (Ribeiro et al., 2010). The precipitation zone's diameter around the colony was measured. (Baillie and Julia Douglas, 1998)

Effects on lipase Production:

l of inoculum was inserted in the center of the Rhan medium for qualitative investigation of lipase synthesis. The plates were then incubated at 37°C for 4 days in the absence and presence of curcumen,nanocurcumen (Alexpandi*et al*., 2019)

Effects on Exopolysaccharides Production of C. albicans: The influence of alternate materials on the synthesis of exopolysaccharides in Candida albicans(Viszwapriya et al., 2016) . The assay was performed with andwithoutalter nativematerialsalcoholicextractofcurcumen and nanoparticalsofcurcumen at and 80 µg\ml of nanocurcumen and alcoholic extract for

16h at 37 C. After incubation, planktonic and biofilm cells were extracted and resuspended in 200 ml of 0.9 percent NaCl by centrifugation at 12,000 rpm for 10 minutes. A 5 percent phenol solution was added to the cell suspension in an equal amount. The reaction mixture was then added five volumes of H2SO4 and incubated for one hour in the dark at room temperature. The supernatant was collected after incubation and the absorbance was measured at OD490 nm.

Results and Discussion

Morphological identification

Identification Candida albicans on sabrouaddextroseagar

All of the samples were cultivated on Sabouraud dextrose agar (SDA), and the colonies of Candida spp. ranged in color from cream to yellowish, matured quickly in 24-48 hours, and had a smooth, glistening, or dry texture depending on the species. These findings were accepted (Bhavan et al., 2010). The colonies of C. albicans on SDA, in particular, appeared white to creamy in color, with round borders, soft and smooth edges, a curved top, and a yeast odor. as show in Fig. 1.

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Identification of Candida albicans on Chrom agar medium:-

Chrom agar is a selective medium for the isolation of yeast that simultaneously provides direct differentiation and identification of several Candida spp. (Sayyada et al., 2010). This study has showed that using Chrom agar *Candida* which is considered a differential agar the colonies appear *C. albicans* characterized by leaf-green.

Fig. 1: Showing growth of Candida spp. on SDA at 37°C for 48 Hours

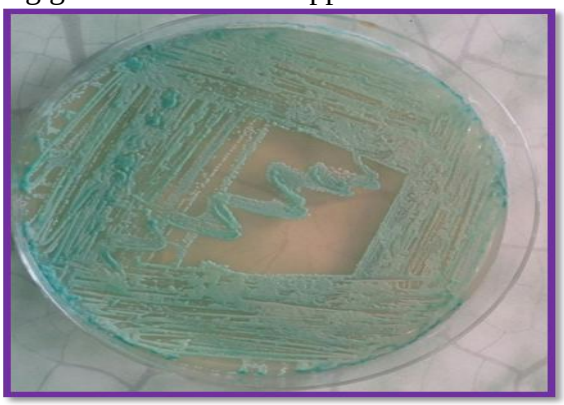

Fig. 2: Show colonies of C. albicans, on Chrom agar medium .**At 37C for 48 hours**

The result of these study show the effect of fluconazole on growth of *C. albicans* by usind well diffiusion method(figure 3) in the concentration (100µg/ml) for antifungal drug fluconazole was 1.4mm ,while at the concentration of (200 µg/ml) for fluconazole, showed inhibition zone on *C.albicans* 2.2mm . The primary target of azoles(fluconazol) may be the heme protein, which cocatalyzes cytochrome-P450-dependent 14a-demethylation of lanosterol in the last stage of ergosterolbiosynthesisfluconazole (last step) of ergosteroln‘s biosynthesis has principal role in a play of depletion of ergosterol and agglomeration of sterol precursors, resulting some alteration in the structure and function of cell membrane in *Candida* cells (Leonardi*et al*,.2013)

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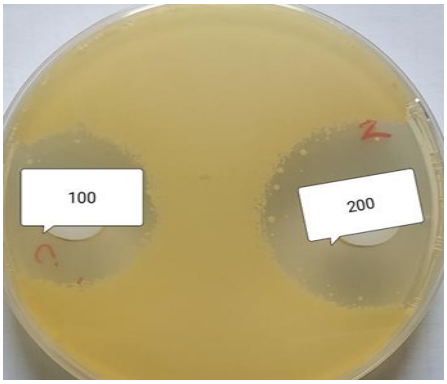

Figure 3:effect of fluconazole on *Candida albicans.*

Effects of (alcoholic extract curcumin ,nanocurcumen) on growth of *candida albicans* by well diffusion&micro titer test

the result in table (1) of well diffusion method revealed antifungal activity alcoholic extract curcumin and nanocurcumin 80 µg\ml and 60µg\mlfrom,nanocurcumen and alcoholic extract curcumen concentration give antifungal effect against *C. Albicans* The significant effect of nanocurcumen in two concentration (60,80) µg/ml were inhibtition zone on *C.albicans* (1,1.7)mm respectively.whiel the effect of alcoholic extract curcumin in two concentration (60,80) µg/ml on *c.albicans were No inhibiton zone*

Alternative materials	**Concentration**	***C.albicans***
Alcoholic extract of curcumen	**80 µg\ml** **60 µg\ml**	**No inhibiton zone**
Nano curcumen	**80 µg\ml** **60 µg\ml**	1.7mm 1mm

Table(4-2): effect of alcoholic extract curcumin and nanocurcumen on candida albicans

The result of thise test show effect of alcoholic extract curcumin and nanocurcumen on growth candida albicans inconcentration 80 µg\ml .The result that shown in the figure(4,19) explain the effect of alcoholic extract curcumin, and nanocurcumin in the growth of c. albicans that lead to reduce their growth of c.albicans amount of absorbance (OD600) explain the greatest impact on the growth of candida caused by nanocurcumin 1.02 OD and extract curcumin1.1 OD wen it well compare with control 1.7 OD

Nanocurcumin has more significant antimicrobial activities, compared to curcumin against the selected bacteria and fungal strains (e.g., penicilliumnotatum and aspergillusniger). This finding could be attributed to the smaller particle size and higher water solubility of nanocurcumin (without any modifications in its structure) (Javidi MA *at el* 2015)

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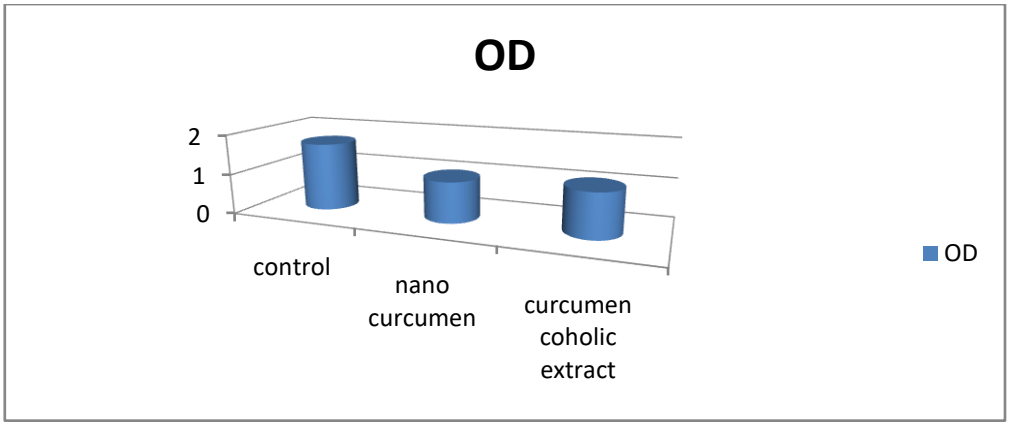

Figure (4): effects of (curcumen,nanocurcumen) on growthof *C. albicans* by microtiter plate

Effect on Phospholipase Production of *C. Albicans*

the result show activity of alcoholic extract curcumin and nanocurcumen 80 µg\ml to inhibtion phospholipase production. The positive result of these test it not apear of phospholipase precipitate around the colony in egg yolk agar plates show figure (5) .The phospholipase production in *C. albicans*wasqualitatively as well as quantitatively estimated containing appropriate substrates in the absence and presence of alternative material.nanoparticals it give high effect to inhibiton than alcoholic extract.

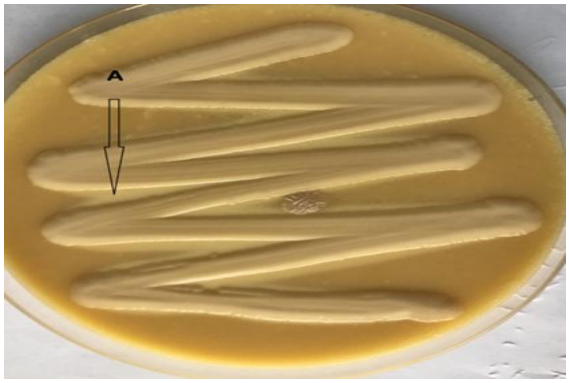

Figure (5): A- Phospholipase Enzyme Production for C.albicans.(untreated) B- Non Phospholipase Enzyme production for Other C.albicans(treated).

Effect on lipase production of *C. Albicans*:-

The result of this test show the ability of alcoholic extract curcumin and nanocurcumen 80 µg\ml toinhbition lipase production in *Candida albicans* . Positive result not appear as white precipitate around the colony the on Rhan media for 4 days at 30 C show figure (6).˚ when treated the media with nanoparticals and alcoholic extract.

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Figure (6): non lipase production in treated Rhan media

Effect on onexopolysaccharides Production of C. Albicans

The results of the study showed the effect alcoholic extract curcumin and nanocurcumen 80 µg\ml toinhibtion in reduce the exopolysaccharides production in *c.albicans* . Were the amount of exopolysaccharides was 90 µg\ml in c.albicans treated with coholic extract curcumen while 150 µg\mlinnanocurcumen was more great effect in inhibition exopolysaccharidesformation.The determined of exopolysaccharides Among many colorimetric methods for carbohydrate analysis, the phenol-sulfuric acid method is the easiest and most reliable method. It has been used for measuring neutral sugars in oligosaccharides, proteoglycans, glycoproteins, and glycolipids. This method is used widely because of its sensitivity and simplicity(Durgadevi et al. 2019)..

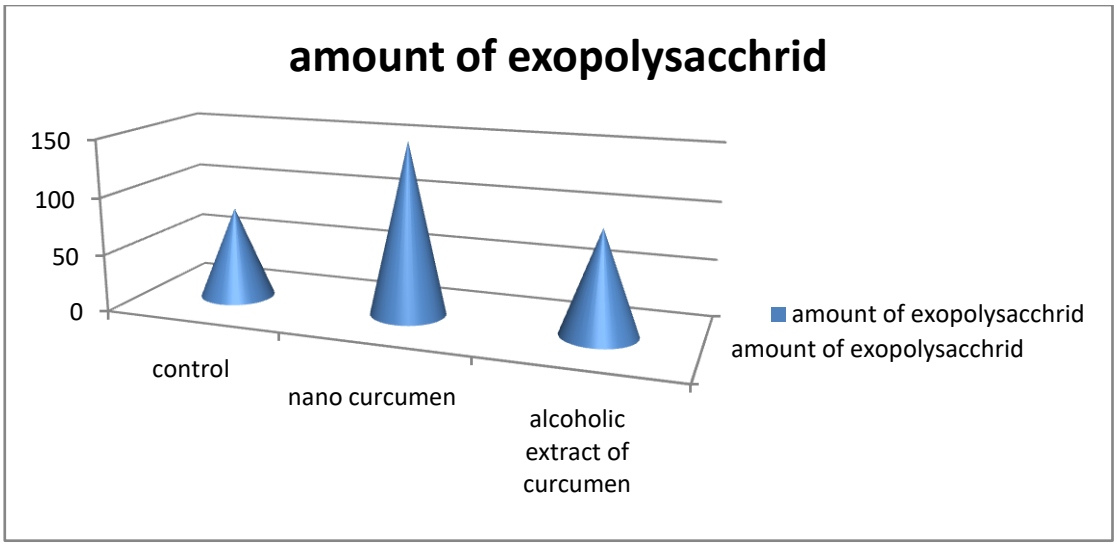

Figure (7): effects of alcoholic extract curcumin and nanocurcumen 80 µg\ml toinhibtion on exopolysaccharides production of C. albicans

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