Antifungal Activity of Silver Nanoparticles Using *Penicillium Chrysogenum* Extract Against The Formation of Biofilm for *Candida Glabrata*

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Abstract

The results showed that 71 isolates of *Candida* spp were isolated from patients with leukemia both women and men, Isolate 59 from *C. glabrata* while the number of isolates of *C.albicans*, *C.tropicalis*, *C.krusei* and *C.kefyer* were 6,3,2,1 respectively. The size of the nanoparticle was measured using AFM, The highest peak was 455 nm due to the presence of surface plasmons and another 243 nm wavelength, SEM showed the presence of particle of different sizes and distributed regularly and small silver nanoparticle. Effect of synergistic silver nanoparticle and antifungal agent (fuconazole) on the biofilm of *Candida glabrata*, capable of *C.gabrata* on adhesion of epithelial cells in the absence of silver nanoparticle and fluconazole, no adhesion between epithelial and yeast cells when adding silver nanoparticles, Decrease in surface adhesion between biofilm of the yeast and the epithelial cell when adding fuconazole, When collecting silver nanoparticles with fluconazole and adding it to epithelial cells exposed to *C.glabrata*, It led to the inability of the yeast to adhere to epithelial cells and then died . All experiments showed the least significant differences at 0.001 level.

Key Words: *Penicillium chrysogenum, silver nanoparticle, Candida glabrata, antifungal.*

Introduction

Candida yeast is transformed from a saprophytic organism into a pathogen due to Candida's factors such as adhesion, protease production, phospholipids, hemolysin proteins, biofilm and germ tube formation, Pathogenesis also depend on the host's immune system(1). One of the factors causing an increase in candidiasis is the chronic illness of people such as diabetes, weak immune system, malignant tumors, pregnancy and excessive use of antibiotics, which are factors for the emergence of infection(2). *C.glabrata* is a mono-chromosome group (haploid) that has no dimorphic form and severe opportunism in the genitourinary system and in the bloodstream Candidemia is particularly prevalent in older people and infected with HIV(3). *C.glabrata* is common in 15-20% of infections and many of its isolates are resistant to fungal antibiotics such as Amphotericin B and Fluconazol(4). (5) indicated that yeast has the ability to form a biofilm which is environmentally important and helps them to survive as human pathogens by allowing them to escape host immunity mechanisms, resist antifungal and compete with other microorganisms, The formation of the biofilm is a key factor in species survival.

Penicillium chrysogenum is common in temperate and subtropical regions and is found in food products such as citrus and grains(6). (7) noted that *P.chrysogenum* was widely used in the industry and in the treatment of certain plant wastes and the production of enzymes such as Polyamine Oxidae and Phospho-gluconate dehydrogenase. It also has a high potential for production of Penicillin antibody and the first commercially produced penicillin.

(8) noted that fungi contains some distinctive advantages when used as biosynthesis for the production of nanoparticle compared to bacteria by producing larger

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amounts of Mechanism of action of silver nanoparticles (AgNPs) against yeast by targeting the biofilms of *Candida glabrata*, Analysis of the active electron microscopy revealed that the interaction between nano-Ag and *C. glabrata* cells during AgNPs exposure leads to changes in membranes which can be observed as holes on the surface of the membrane and lead to cell death[9]. The technique of silver nanoparticles led to the movement of the drug into the tissues of the body, which was previously unreachable and was based on several factors including pH, temperature, solubility in the medicine, absorption of the surface-related drug and the spread of the drug through the matrix of nanoparticles[10].

Materials and Method

Collections of Samples: Collected130 clinical samples taken from patients of leukemia from the City of Medicine/ Leukemia Department in Baghdad City

Isolation of yeast: Placing 100 microliters of blood on the sabroud dextrose agar (SDA)[11].

Identification of *Candida*: For the purpose of diagnosing *Candida* was studied, Characterstions of Morphological[12], Purification of Colonies[13].

Virulence of factor test: The following experiments were performed, germ tube test (14). Biofilm formation test[15], Candida Chromgenic agar[16], the Vitek2 Compact System.

Identification of *Penicillium chrysogenum*: The fungus of *Penicillium chrysogenum* according[17].

Biomass of *Penicillium chrysogenum*: For the fungal biomass by[18].

Preparation of silver nanoparticles in *Penicillium chrysogenum*: The silver nanoparticles were composed by observing in kind the color change of the yeast from the transparent color to the dark brown color[19].

Characterization of nanoparticle using different microscopes:

A.Atomic Force Microscope: Use this microscope to find out the size of nanoparticles and monitor the bio-processing of nanoparticles and know the particle size[20].

B. UV-ViS Spectrophoto meter: The UV spectrometer is used to monitor the biotransformation of silver ions by means of UV spectroscopy of the reaction[21].

C. Scanning Electron Microscope: Use this microscope to determine the size and shape of the nanoparticles and to know the structural[20].

Studying the synergistic effect of nanoparticle and antifungal for the biofilm agent of the *Candida glabrata*: This technique was used to test epithelial cells of the mouth on adhesion the biofilm to *Candida glabrata* [22] as follows:

A- First treatment: Take 0.5 ml of sediment containing epithelial cells (control treatment).

B-Second treatment: Take 0.5 mL of the sediment containing the epithelial cells and add 0.5 ml of *Candida glabrata.*

C- Third treatment: Take 0.5 ml of sediment containing epithelial cells and add 0.5 ml *Candida glabrata* and 50 microliters of silver nanoparticles composed with *Penicisllium chrysogenum.*

D- Treatment 4: Take 0.5 ml of sediment containing epithelial cells and add 0.5 ml *Candida glabrata* and 100 mg of antifungal Fuconazole.

E- Treatment 5: Take 0.5 ml of sediment containing epithelial cells and add 0.5 ml *Candida glabrata* and add 100 mg of Fuconazole and add 50 microliters of silver nanoparticles with *Penicillium chrysogenum.*

Statistical analysis: Statistical Analysis System SAS (2012)[23].

Results and Discussion

Distribution of infected patients with candidiasis: The results showed that 71 isolates of *Candida* spp were isolated from patients with leukemia, both women and men, with 34 clinical cases of women. The 50-65 age group recorded 17 cases of 50% and 9 cases of the 20-30 years and 26.5%. The age group between 40-50 years recorded 5 cases and 14.7%. Finally, the age group of 17 years had the lowest rates of 8.8% and three clinical cases as in Table (1).

Table (1): Shows the distribution of women by age group Candidiasis patients due to leukemia.

Age group	No. of infected of women\34	%Percentage
50-65 years	17	50
40-50 years	9	26.5
20-30 years	5	14.7
Less than 17 years	3	8.8
Total	34	100%
Chi-Square (χ^2) P-value	---	13.594 ** 0.0036

** (P<0.01).

In the case of men, there were 37 clinical and positive cases of yeast *Candida* spp. The results showed positive results for yeast for leukemia patients in the age group 50-65 years in 14 cases and 37.9%, followed by age group 40-50 years and 11 cases and 29.7% The age group between 20-30 years, which was 7 cases and 18.9%. Finally, the lowest age group of 17 years recorded the lowest rates of 13.5% and 5 clinical cases table (2).

Table (2): shows the distribution of men by age group. Candidiasis patients due to leukemia

Age group	No. of infected of Men \37	%Percentage
50-65 years	14	37.9%
40-50 years	11	29.7
20-30 years	7	18.9
Less than 17 years	5	13.5
Total	37	100%
Chi-Square (χ^2) P-value	---	5.270 * 0.0530

* (P<0.05).

This is consistent with[24] which showed that Candidiasis is the fourth most common type of infection of the bloodstream and causes candidasis for patients in hospital. The increase in infection in these age groups is due to the availability of appropriate conditions such as immunodeficiency, long-term use of antibiotics and malignant tumors.

Identification of *Candida* spp.: Table (4), 71 isolates were obtained from clinical samples of women and men with leukemia, 59 *C. glabrata* from 71 isolates and 83.1%, while the number of isolates of *C.albicans*, *C.tropicalis*, *C.krusei* and *C.kefyer* were 6,3,2,1 respectively. These results were consistent with[25], indicating that *C.glabrata* was the second most common cause and 24% of Candida in the United States of America. In 2004 *Candida glabrata* was the main cause of Candidiemia, and mortality rates for *Candida glabrata* patients were detected. 50% in cancer patients, and 100% in bone marrow patients.

Table (4): shows the distribution of *Candida* isolated from patients with leukemia from women and men.

Candida spp	No. of Isolates of Leukemia/Women and Men	Percentage%
C.glabrata	59	83.1
C.albicans	6	8.5
C.tropicalis	3	4.2
C.krusei	2	2.8
C.kefyer	1	1.4
Total	71	100
Chi-Square (χ^2) P-value	---	177.662 ** 0.0001

** (P<0.01).**4.Biosynthesis of silver nanoparticles**

Combining the biomass of *Penicllium chrysogenum* with the silver nitrate solution results in color difference. This indicates the formation of silver nanoparticle by the presence of surface plasmon, which is consistent with[26]. The difference in color is due to the difference in the electron density of the nanoparticles that are different nano-size.

Characterization of silver nanoparticle.

Atomic Force Microscope (AFM) Results: The average square root value is equal to Root Mean Square = 2.12nm. The surface roughness of the membrane is

average Roughness = 1.54nm. This value is a proof of surface roughness, The particle size was found to be 18.83 nm. [27]showed that the nanoparticle were modified by *Fusarium graminaerum* in different sizes and measured using AFM and began with a diameter of 1 nm.

UV spectrophotometer results:

The highest peak was 455 nm and another 243 nm wavelength and the highest peak due to the presence of surface plasmons either the second peak may indicate the presence of tyrosine and tryptophan residues found in the protein released from the yeast. This is explained[28] suggests that the reduction of silver nitrate to silver nanoparticles can be easily by using the UV spectrometer because silver nanoparticles can absorb light in the visible area due to Plasmon surface.

Scanning Electron Microscope (SEM) Results:

Fig. (1) showed the presence of spherical particle of different sizes and distributed regularly and small silver nanoparticles, this supports[29]. That the surface of the plasmon reached a peak of 420 nanometers and that silver nanoparticles have a spherical shape.

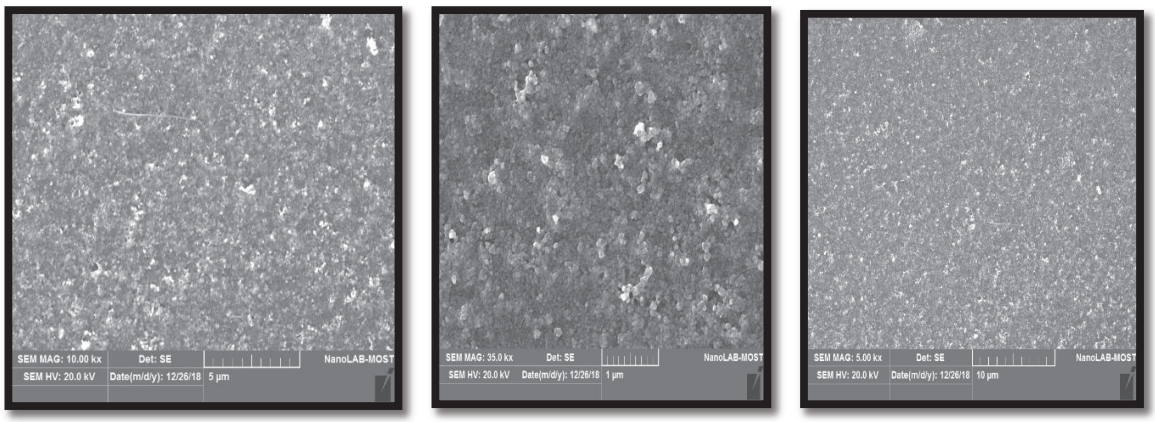

Figure (1) SEM for prepared silver nanoparticles.

Effect of synergistic silver nanoparticle of *Penicillium chrysogenum* and antifungal agent on the biofilm of *Candida glabrata*: Results showed the effect coefficients the synergy of silver nanoparticle particles For *Penicillium Chrysogenum* and antifungal agent against the *Candida glabrata* has different effects. Figure (5-A) shows the normal shape of the epithelial cells that represent the control treatment, between Figure (5-B) capable of *C.gabrata* on adhesion of epithelial cells in the absence of silver nitrate and antifungal. Figure (5-C) showed no adhesion between epithelial and yeast cells when adding silver nanoparticles with *Penicillium chrysogenum*. This indicates the inability of *C.glabrata* to adhere to the presence of silver nanoparticles. The exposure of epithelial cells with *C.glabrata* and antifungal fuconazole showed a decrease in surface adhesion between biofilm of the yeast and the epithelial cell as shown in Figure (5-D). When collecting silver nanoparticles with fluconazole and adding it to epithelial cells exposed to *C.glabrata*, Yeast inability was observed on adhesion with epithelial cell and then its death due to the presence of synergistic nanoparticle with fluconazole showed stable and strong antifungal activity as in Fig. (5-E). The results showed that silver nanoparticle have properties antifungal.It can also provide synergy with antifungal when evaluating the synergistic effect of silver nanoparticles and the fluconazole against the adhesion cells formed for the biofilm of *C.glabrata*, This is consistent with the study of silver nanoparticle antifungal such as floconzole against *Candida albicans* and a strong synergistic effect between silver nanoparticles and antifungal[30]. [31]showed that silver nanoparticles are associated with important cellular structures of proteins and DNA and cause cellular damage to yeast. [32]explain The association of the silver atoms with the thiol group (SH) in the enzymes, which change the composition and function of the enzymes in the cell membrane, which makes the adhesion ineffective.

Figure (2) A- Normal epithelial cells, B- Epithelial cells with *C.glabrata*, C- Epithelial cells with *C.glabrata* when adding silver nanoparticles, D- Epithelial cells with *C. glabrata* when adding Fluconazole, E- Epithelial cells with *C. glabrata* when adding silver nanoparticles with Fluconazole.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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