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Effect of sliver nanoparticles solution on *Candida glabrata* isolated from skin infection

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ABSTRACT

In recent years, a rapid increase in the fungal infections particularly infections by *Candida* species which contaminated by environmental sources, including soil, air, feeds and skin . Nowadays ,the researchers search in their studies for new antimicrobial agents which have the unique physical and chemical properties In this experiment , AgNPs solution was synthesized by chemical reduction, characterized and tested as the antifungal effects of sliver nanoparticles (Ag-Nps) on *Candida glabrata* . Sliver nanoparticles revealed unique physical and chemical properties that suitable to understand to possess a broad spectrum of antimicrobial activities. Sliver nanoparticles (AgNPs) characterized by UV- Visible spectrphotometry by exhibiting the typical surface plasmon absorption maxima at 400 nm. The morphology of the sliver nanoparticles was measured by Scanning Electron Microscope (SEM). Investigating Antimicrobial activity of nanoparticles were evaluated by using Minimum Inhibitory Concentration (MIC) technique. The experiment results showed that the lowest MIC and MBC of Ag-NPs to *C.glabrata* was 30 ppm, 300ppm, respectively. The obtained results suggested that Ag-NPs exhibit fungistatic and fungicidal effect towards *C.glabrata* . The present study indicates (AgNps) has considerable antifungal activity .

INTRODUCTION

Severe fungal infections have significantly to be increased in recent years , due to multiple drug resistant microbes . (Goffeau , 2008). Despite aggressive treatment with new or more established antifungal agents , these fungal infections causes of morbidity and mortality especially in immunocompromised patients (Pfaller and Diekema ,2007)

Nanotechnology has emerged up as a new promising technology for synthesis of nanoparticles during the past few decades . (Kim *et al*.,2007; Marambio- jones and Hoek , 2010).

Sliver ions are considered as the bioactive agent , used for clinical applications from different formulations: sliver salts, sliver chelates, sliver oxide, metallic sliver and sliver particles (Morones *et al*.,2005; Rai *et al* ., 2012; Dos *et al*., 2014).

Silver nanoparticles have antimicrobial activity against bacteria (Sharma *et al*., 2009; Lazar, 2011; Rai *et al*., 2012; Taraszkicz *et al*., 2013), Fungi (Kim *et al*., 2008), viruses (Rogers *et al*., 2008) and anti- inflammatory activity .(Nadworny *et al*.,2008).

Ag- Nps have been studied in a various field of biological, medical, and pharmaceutical applications. (Gauger *et al*., 2003; Li *et al*., 2006; Tian *et al*. 2007; Rai *et al*., 2009) therefore, from this present study, it was investigated the prepared of the sliver nano particles were evaluated for antifungal activity against *Candida glabrata*

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MATERIALS AND METHODS

Microorganism:

The fungal strain isolated from clinical specimens and characterized on SDA agar and biochemical tests .

Synthesis of sliver nanoparticles:

AgNPs prepared from a volume of 10 ml sliver nitrate solution with 4 x 10^{-4} mol / l was added drop wise from a glass burette to a flate – bottom flask (immersed in ice bath) containing 10 ml of 1x 10^{-2} mol /l sodium borohydride . The reaction protected from light . After the addition of sliver nitrate solution was finished , the mixture was than vigorously stirred during 15min .

Scanning Electron microscope examination:

The SEM analysis was performed on a FEI comp – Q nanta 450 instrument at Pharmacy college – Babylon. Scanning electron microscopy was done to characterize the morphology of the sliver nanoparticles **.**

Characterization of sliver nanoparticles:

After showing the color changing, the sample was subjected to UV – visible spectrophotometery

Antimicrobial activity of sliver nanoparicles :Measurement of Minimum Inhibitory Concentration (MIC):

Inoculation suspension of the *Candida glabrata* was prepared by picking colonies from Sabraudous agar for 24 h at $35C^0$ and using sterile saline (0.85%)v/v Nacl to determine tubidity equivalent to 0.5 McFarland standard ($1x10^8$) colony forming unites /ml. Different concentration of Ag- NPs (10, 20, 30, 40, 50, 100, 150, 250 – 300, 400 ppm) was added in LB medium. The fungal culture was incubated at 35 C^0 . Broth microdilution method was followed for measurement of MIC values. The test tubes were incubated at $37C^0$. The MIC values were estimated the lowest concentration in the test tube that showed no turbidity after incubation. The minimum fungicidal concentration was evaluated through sub culturing from each test tube showing no apparent growth .

RESULTS AND DISCUSSION

UV- visible analysis:

UV- visible spectroscopy is one of the most widely and sensitive techniques for structural characterization of sliver nanoparticles synthesis. In present study, Ag NPs solution was synthesized by chemical reduction method and characterized UV- vis spectra of the AgNPs solution showed absorption band at around 413 nm, which indicates the presence of spherical AgNPs (Fig 1).

SEM analysis:

The morphological details of the AgNPs were second examined by SEM measurement. Figure (2) shows a reperserntive SEM of Ag Nps It can been seen that the inner diameter of the NTs is approximately 43.12nm with the wall thicknessof about 6.0 nm.

Antimicrobial studies:

In the present investigation, the antimicrobial activity of sliver nanoparticles of (Ag Nps) was evaluated against *Candida glabrata* using broth dilution technique with different concentration. Table (1) showed the antimicrobial properties of Ag-NPs against *C. glabrata*. The Minimum inhibitory concentration of Ag- NPs against *C. glabrata* was found 30 ppm . While the Minimum fungicidal count of Ag-Nps against *C. glabrata* was found 300 ppm.

The obtained result of the antifungal activity (MIC) reveal the growth of yeast is inhibited at concentration as low as 30 ppm in microdilution method.

Antifungal activity of sliver nanoparticles has been investigated in several researchers whom demonstrated that MIC values reported a wide extent of variation due to no standard protocol for evaluation of antifungal activity of nanoparticles and using different methods. In addition, the differences in MICs of the SN probably result from differences in the strains tested. Also MIC values are different due to the nature of the particles used especially in size. (Morones *et al*., 2005; Panacek *et al*., 2010; Kim *et al*., 2009; Monteriro *et al*., 2011; Nasrollahi *et al*., 2011; Amanda *et al* ., 2014).

According to our result there are significant differences between the different concentrations of sliver nanoparticles on the inhibition. fewer fungal growth in concentration 100 ppm and for concentration above 300 ppm, 100% of growth inhibition was observed. (Fig 3, Fig 4).

Lok *et al*. (2007) suggested that antimicrobial activity of sliver nanoparticles is dependent on the ability to cross the microorganism cell walls

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Fungicidal behavior of nanoparticles is attributed to the presence of electronic effects that are brought as a result of changes in local electronic structures of the surfaces due to smaller sizes. These effects are considered to be contributing towards enhancement of reactivity of sliver nanoparticles surfaces. It has been suggested that DNA loses its replication ability once the microorganisms are treated with sliver ions. (Morones *et al*., 2005; Shrivastava *et al*., 2007).

Sliver nanoparticles destabilize plasma membrane potential and depletion of the levels of intracellular adenosine triphosphate (ATP) by targeting bacterial membrane and finally the formation the formation of pores and subsequent cell death. (Kim *et al*., 2008; Kim *et al*., 2009). Therefore Sliver and sliver – based compounds have been in use in the treatment of burns and chronic wound (Klasen,2000; Castellano *et al*., 2007). I t is economical to consolidate sliver in polymers, composites, fabrics and catheters for antimicrobial activity. (Zeng *et al*., 2007; Falletta *et al*., 2008); Roe *et al*., 2008). Other workers Hwang *et al* (2012) demonstrated in their research to production of reactive oxygen species (ROS) , DNA fragmentation resulting in cell death. (Bawskar *et al*., 2015).

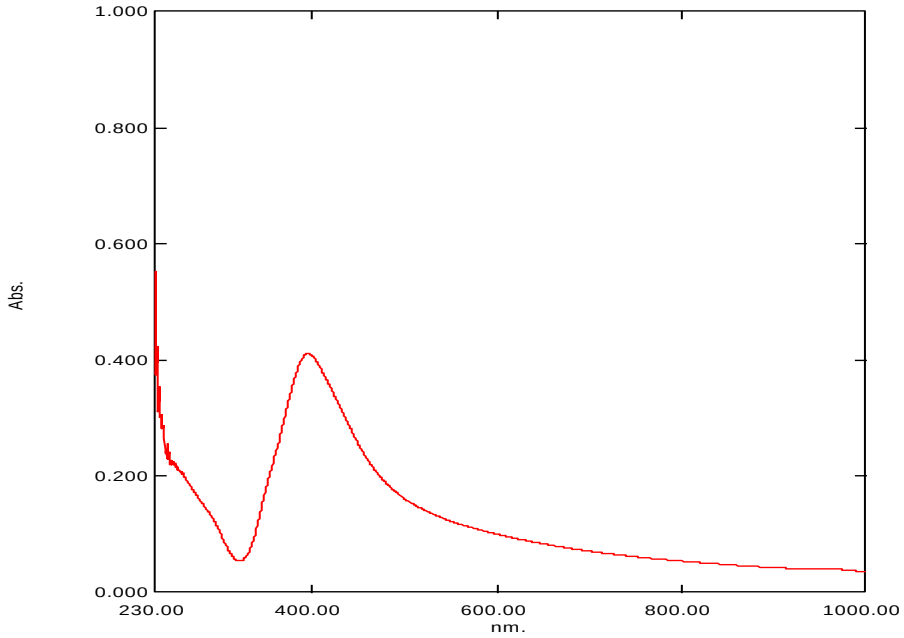

Fig. 1: Uv – Vis spectra . The maximum absorbance was at 400 nm

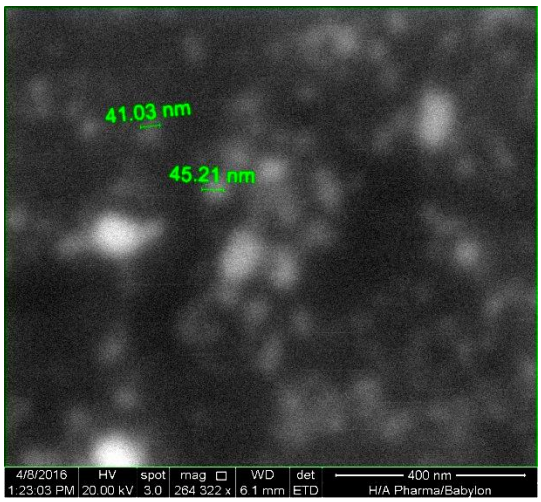

Fig. 2: SEM image of AgNps solution.

Table1: Determination of MIC and MBC for nanoparticles

Isolate	Con(ppm)	Visible growth In Tubes	MIC (ppm)	MFC (ppm)
C. glabrata	10 20 30 40 50 100 150 250 300 400	Turbidity Turbidity NoTurbidity NoTurbidity NoTurbidity NoTurbidity NoTurbidity NoTurbidity NoTurbidity NoTurbidity	30	300

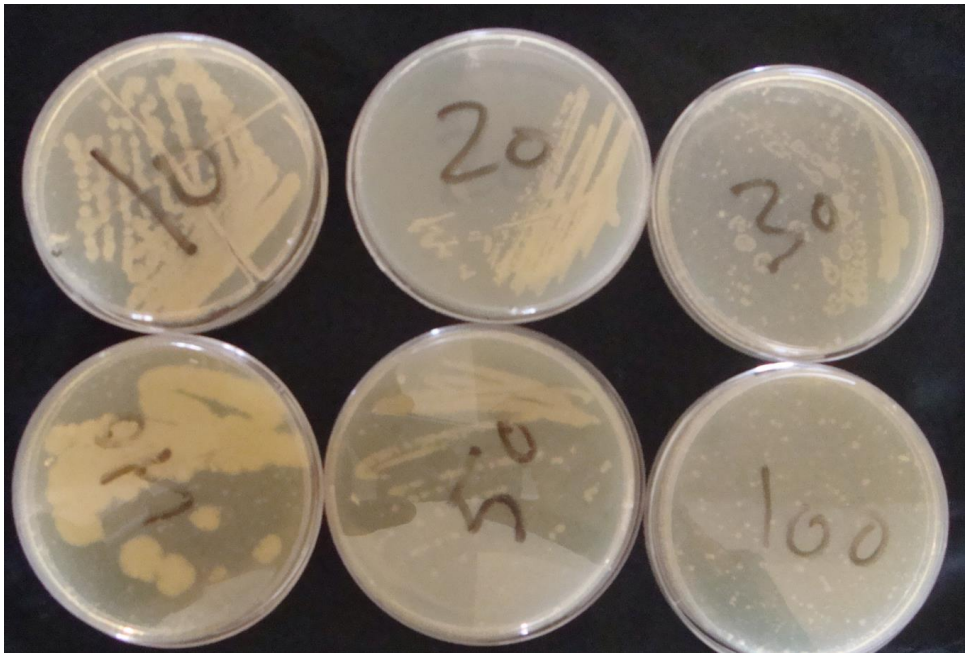

Fig. 3: *C. glabrata* growth in conc (10, 20, 30 , 40 , 50 , 100 ppm .)

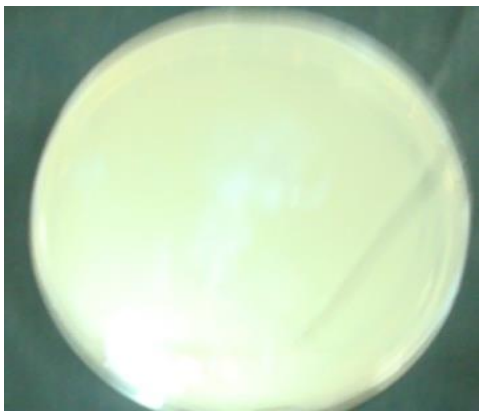

Fig. 4: *C. glabrata* growth in conc (300ppm).

Conclusions:

Results in present study indicate that Ag Nps had good antifungal activity which obtained by reduction of sliver nitrate, as described was effective and confirmed by different characterization methods.

MIC result have demonstrated that synthesized AgNps show significant antifungal effect as evidenced at low concentration 30 ppm.

Sliver nanoparticls with size range of 41.03 – 45.21nm have antifungal activity , which make them suitable for many practical applications.

It was conclude that synthesized AgNPs showed the ability to inhibit the growth of *C. glabrata*. Therefore, it was stated that AgNPs has considerable antifungal activity which served for further studies for clinical applications and exhibit as a fungicidal agent which suggested for further studies in genotoxic and cytotoxic effects.

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