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Synthesis of Gold Nanoparticles from *Candida Dubliniensis* for use as an Anti-Bacterial Agent against some Bacteria-Caused Vaginal Infections

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Abstract

This work in our paper aimed to discuss the synthesis in extracellular of gold nanoparticles (AuNPs) by utilizing the supernatant of the yeast Candida dubliniensis, which was recluse from the oral mucosa of patients wearing removable dentures and identified through molecular assays. Various techniques were used to characterize the AuNPs. The medium's color shifted (light yellow-dark violet), and a sucking peak was detected at wavelength 550 nm utilizing ultraviolet (UV)-visible spectroscopy. Scanning electron microscopy (SEM) uncovered that the AuNPs were spherical, with sizes ranging from 58.07 to 72.15 nm. Additionally, Fourier Transform Infrared (FT-IR) spectroscopy was utilized to distinguish active functional groups. Gold nanoparticles have been evaluated for antibacterial activity against human pathogenic bacteria isolated from Vaginitis infections, including Staphylococcus aureus, Staphylococcus haemolyticus, Escherichia coli, Klebsiella sp., and Micrococcus sp. On Muller Hinton agar plates, the activity of anti-bacterial was assessed utilizing the agar well diffusion technique with concentrations ranging from 31.25 to 500 µg of AuNPs. The produced gold nanoparticles demonstrated variable growth inhibition activity (1-23 mm) inhibitory zones against the assessed harmful bacteria.

Keyword: Sabouraud dextrose agar, Candida dubliniensis, Gold nanoparticles AuNP.

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Introduction

Recently, nanotechnology topics have received great attention from researchers. The rapid growth and development of research has increased the use of nanoparticles (NPs). Gold nanoparticles (AuNPs) are among the most common particles. Nanoparticles are significant and have found widespread usage in both medical and non-medical applications. (Zhan et al, 2022) It is a suitable material due to its unique properties of inertness, biocompatibility, and low toxicity. When reduced oxidation of gold Au+1 (gilding) or silver Au under these conditions can be produced on a large scale. On the other hand, under various conditions by adding a reducing agent through different physical, chemical and biological mechanisms, Au+3 (earthy) can be transformed into Au0 (Hammami & Alabdallah 2021).

Endogenous and exogenous production of gold nanoparticles can be met by microbes, although extracellular handling is simple in terms of isolation. Exceptional diagnostic and therapeutic applications have demonstrated this, including biosensor applications, the ability to target tumors (anticancer activity) to treat cancer, and precise drug administration. Nanocomposites have been used to treat damaged tissue.

Antibiotic resistance is a growing global concern, with drug-resistant bacteria frequently arising as a result of antibiotic usage in hospitals (Opaland Calandra 2009). As a result, treating device-associated infections is getting more complicated and costly. Long-term antibiotic therapy has led to the evolution of resistant bacteria. Gold nanoparticles are electrostatically adsorbed to bacteria. It has a significant interaction with lysine on bacterial membranes, namely Gram-positive bacteria. (Li *et al* 2021).

Materials and methods

Biosynthesis of Gold Nanoparticles

The biosynthesis of AuNPs was performed utilizing the supernatant of the yeast *Candida dubliniensis*. The sample was isolated from the oral cavity of a patient wearing dentures using a sterile cotton swab. On a (SDA) plate, the isolate was cultured and at 37°C is been incubated for 48 h. Candida dubliniensis was identified through molecular testing. Additionally, a sample of the culture was placed in Erlenmeyer flasks with a 250 ml capacity, each 100 ml of Sabouraud dextrose broth. These flasks were incubated at 37°C in a shaking incubator at 150 rpm for one to two days.

Preparation and Purification of AuNPs

In a flask with a volume of 250 mL, 100 mL of Candida dubliniensis supernatant was combined with HAuCl4 to reach a final concentration of 1 mm for the synthesis of gold nanoparticles (AuNPs). The solution was then stored overnight at 37°C. A control flask containing only the supernatant, without the addition of HAuCl4, was maintained for comparison. The formation of AuNPs was indicated by a color change and increased turbidity in the culture after incubation. The resulting AuNP solution was centrifuged at 6000 rpm for 25 m. Following centrifugation, the supernatant was discarded, and the pellet was washed three times with deionized water. The pellet at the bottom of the tube was then dried at 40°C. The dried powder was carefully and currency collected to be kept at 4°C for other tests in same work field (Sarvamangala *et al*., 2013).

Characterization of Biosynthesis of Gold Nanoparticles

Several methods were used to characterize AuNPs biosynthesized using *Candida dubliniensis* which are Spectrophotometer (UV-VIS). A scanning electron microscope (SEM) was utilized to describe the morphology and size of AuNPs, recording the absorbance spectra of the NPs in the range (300-800) nm, and energy-dispersive X-ray spectroscopy (X-RD) was utilize for analysis structure of the nanoparticles. samples AuNPs, and performs FT-IR spectroscopy to identify the functional sets introduce in the organic and inorganic compound.

Evaluation of Anti-Bacterial Activity of Mycosynthesized AuNPs Using the Diffusion Method

Using a well diffusion test, the antibacterial potential of gold nanoparticles dissolved in dimethyl

sulfoxide (DMSO), as described (Pérez et al. 1990), was analyzed and evaluated. Five strains of bacteria were tested in this study for pathogenic strains associated with vaginitis. The previously used bacterial strains obtained from the central laboratory at the College of Veterinary Medicine were diagnosed and worked on, which included three Gram-positive bacteria (Staphylococcus aureus, Staphylococcus hemolyticus, and Micrococci) and two Gram-negative bacteria (Escherichia coli and Klebsiella). Bacterial culture, at 37°C for 18 h Bacteria were cultured in nutrient broth. In addition, the cell suspensions were then adjusted to approximately 10^8 CFU/mL, which corresponds to the 0.5 McFarland criterion (Balouiri et al., 2016; Sharma et al., 2016). Bacteria tested included Enterococcus macrophages, Klebsiella, Staphylococcus haemolyticus, and Staphylococcus aureus.

Overnight cultures appeared punctate on sterile Mueller Hinton agar (MHA) plates. Wells, each 6 mm in diameter, were created in agar plates utilizing a sterile stainless steel cork drill. These wells were filled with a solution of gold nanoparticles at five different concentrations (500, 250, 125, 62.5, and 31.25 µg/ml). At 37°C for 24 h, the plates were inoculated, and the zones of inhibition around the wells were carefully observed and measured.

Result and discussion

Candida. dubliniensis was used to obtain gold nanoparticles from gold tetrachloride. When compared to the control sample, the color of the solution changed from light yellow to purple 24 hours after the reaction began (Fig. 1). Our results are similar to those of Salem & Fouda, (2021), who mentioned the color of a solution changes to purple (AuNPs), implying the formation of nanostructured materials. This was observed as an indicator for the synthesis of gold nanoparticles. The color of medium is changing may be due to the reduction of gold ions to AuNPs. It shows the formation of metal nanoparticles that lead to a color change in the solution due to the reduction of metal ions (Rónavári *et al*., 2021).

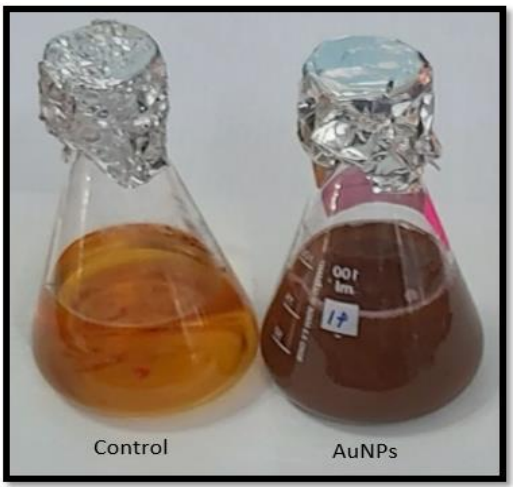


Figure 1. Biosynthesis of gold nanoparticles: **Control:** conical flasks containing the culture supernatant of the *Candida. dubliniensis* **AuNPs:** purple in color synthesized AuNPs solution

To detect gold nanoparticles, we established optical measurements where ultraviolet (UV) visible light with a wavelength of (350-800) nanometers was used for this purpose. As illustrated in Figure 2, the results indicated an sucking top-peak at wavelengh550 nm, which falls within the distinctive range for AuNPs. where the absorption maximum was. These results are consistent with the study (Niranjan Dhanasekar et al., 2015) which found that the maximum absorption peak wavelength was within the range.

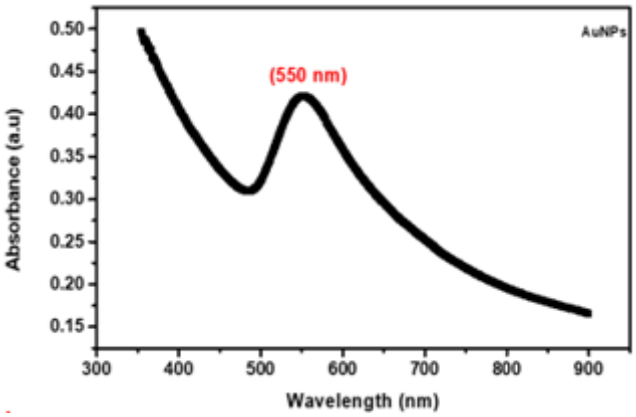


Figure 2. The UV-Vis spectrum of AuNPs synthesized using the supernatant of Candida dubliniensis.

The dimensions and form of gold nanoparticles were measured using an SEM. The study found that the gold nanoparticles biosynthesized were pseudo-spherical spherical and dispersed in size ranging in size from ((58.07-72.15) nm. See Fig.3.

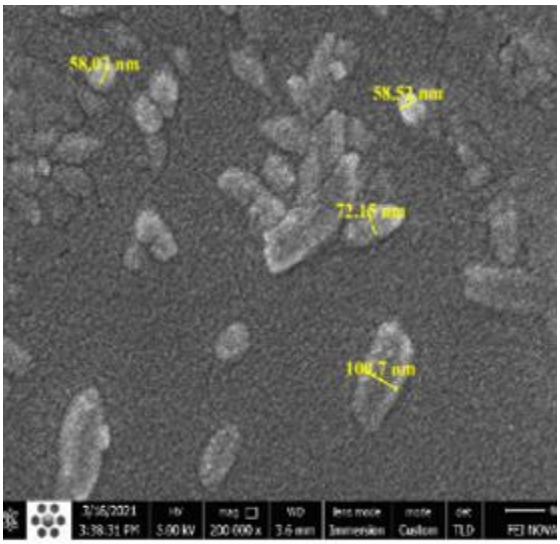


Figure 3. SEM image of synthesized AuNPs of *Candida. dubliniensis*

For determining whether a substance is crystalline or amorphous. X-ray diffraction is a contemporary method that analyzes materials at various radiation angles. In our study, XRD was used to validate the crystal structure of gold NP extracts. The figure proves that there is a distinct and clear diffraction line in the XRD pattern at low angles that fall within the range (10°-80°). Bragg reflections at 2θ angles for C. Dublinesises at 31.39o, 38.24o, 64.73o, and 73.50o. Planes 111, 200, 220, and 311 are represented by the peaks, respectively (Fig.4). This pattern verified the face-centered cubic (FCC) arrangement of the gold AuNPs. In Candida extract, XRD data confirmed the validity of the crystal structure of gold, and the results of this study can be matched and compared with the results of many existing medical literature studies (Clarence et al., 2020).

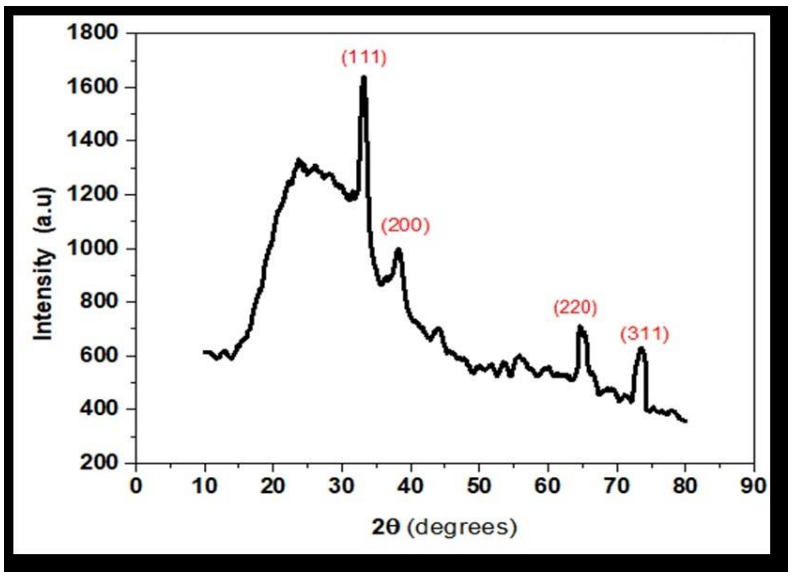


Figure 4: XRD spectrum of lyophilized cell supernatant with AuNPs from isolates of *Candida dubliniensis*

The FTIR spectrum serves as a fingerprint for functional groups found in organic and inorganic materials. The principle of this spectrum is that electromagnetic energy is absorbed by particles in the infrared region of this spectrum, and this leads to vibration in subatomic particles. These vibrations are measured in specific and clear locations, based on a pre-determined wave number, and absorption occurs. Also the ranges of wavelength are operated in (4000-400) cm-1. The shape of nanoparticles is determined by the band position, by the intensity of the peaks obtained, the size in the FT-IR spectrum is determined (Devi et al., 2019).

The primary functional sets identified in the FT-IR analysis of biosynthesized AuNPs. The FT-IR spectra reveal distinct bond stretches at various peaks: 3419.17 for the N-H stretch, 2361.41 for the C-N stretch, and 1631.48 cm^{-1} for the N-H bend (see Fig. 5).

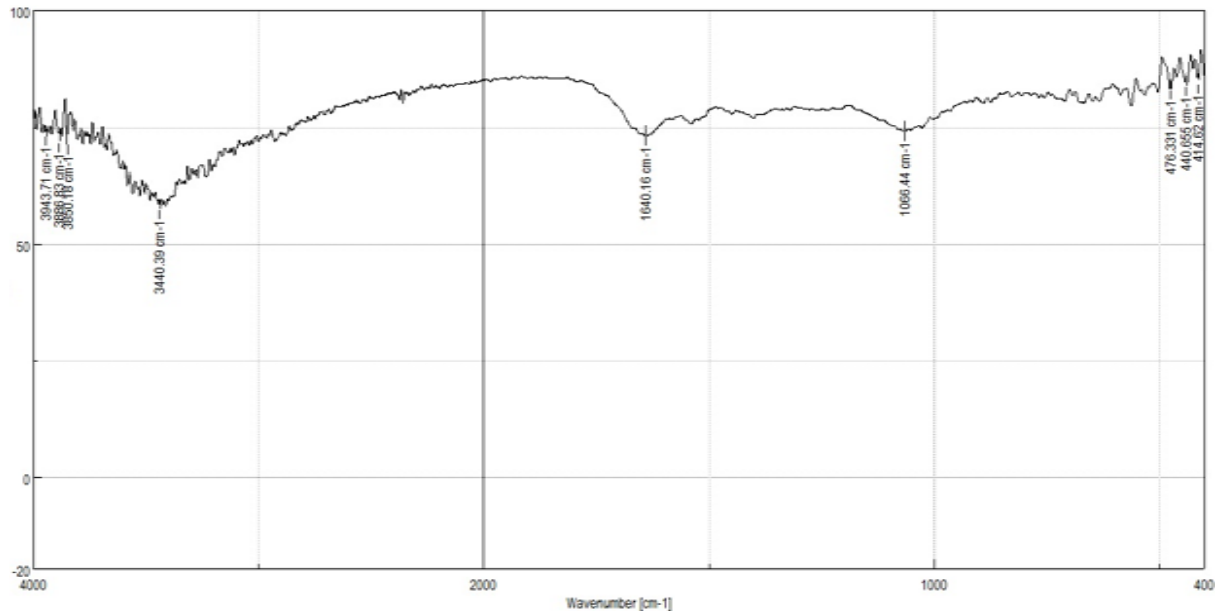


Figure 5. FTIR spectrum of AuNPs extracted from isolates of *Candida. Dubliniensis.*

Biological activity of AuNPs

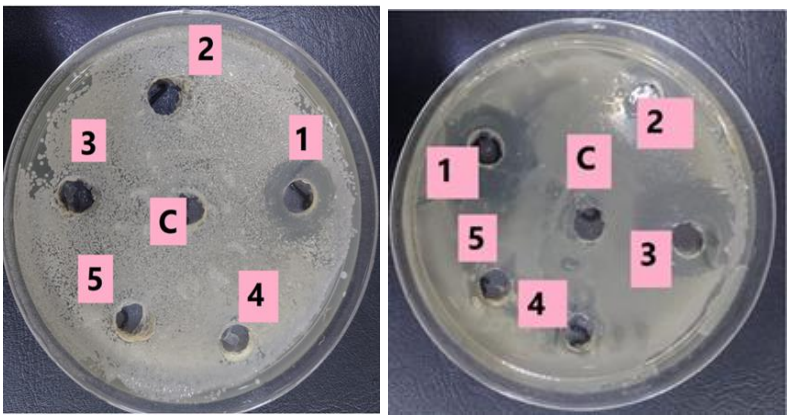
Different concentrations of the compound's- AuNPs (500, 250, 125,62.5and 31.25 ug/ml) were used to test its biological activity. The agar well spread method was utilized to evaluate the anti-microbial

susceptibility of the samples (Saeed B.M.S. *et. al*., 2020). The results showed that anti-bacterial efficacy increases with an increase in AuNps concentration Table (1) and Figure (1). Recently, the antibacterial activities of AuNPs have seen a promising and evolving emergence. Although controversial, through several experiments individual AuNPs and conjugates containing AuNPs have been verified for their ability to inhibit some microbial growth (Tian, E. K. et al., 2021).

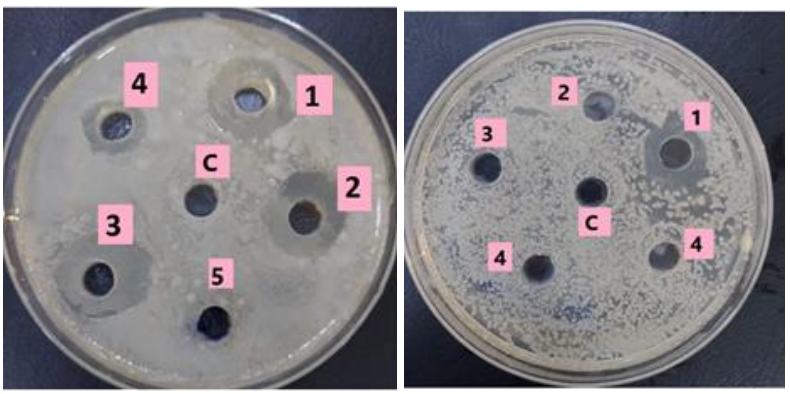
Our results showed the maximum inhibition (23) mm was recorded against *Staphylococcus haemolyticus*. at a concentration of 500µl followed by S. *aureus* (22) mm. The minimum inhibition (18) mm was recorded towards *Escherichia coli* at the same concentration while there was no effect on *Klebsiella spp* Table (1).

Table (1) Inhibition zones in diameter (mm) of gold nanoparticles against bacterial pathogenic

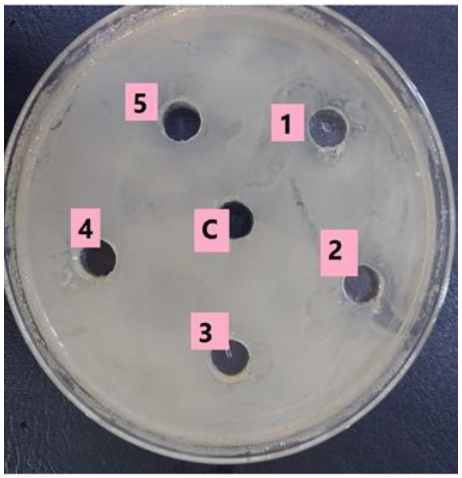
Bacteria of isolate	*Inhibition zone (mm)*				
	500 µg	250 µg	125 µg	65.5 µg	32.25 µg
Staphylococcus haemolyticus	23	22	21	18	10
Staphylococcus aureus	22	18	10	8	2
Escherichia coli	12	8	2	0	0
spp. Micrococcus	18	16	0	0	0
Klebsiella spp.	0	0	0	0	0



Staphylococcus aureus Micrococcus spp.



Staphylococcus haemolytioccus Escherichia coli



Klebsiella spp.

Figure(1) Inhibition zone in (mm) by anticandidal effect at different concentrations of AuNPs (µg/ml) biosynthesized by *C. dubliniensis.* (1=500 ug/ml 2=250 ug/ml 3=125 ug/ml 4=62.5 ug/ml 5=31.25 ug/ml.

Antibacterial Susceptibility Test

The 4 types of antibiotics were tested against (5) pathogenic bacteria isolated from vaginal infections, and this was done according to method of the disk diffusion. In the inhibition zone results showed variation between the different isolates, see the Table (2) antibacterial resistance profile of bacterial isolated the *Escherichia coli, and Klebsiella spp.* Showed a high resistance to Amoxicillin.while *Staphylococcus haemolyticus* recorded a high inhibition zone against Ciprofloxacin (38) mm. Our paper's findings for this study are consistent with a study by Yalew et al. (2022) which showed resistance to amoxicillin. Mahmoud and Al-Hadan (2022) demonstrated extreme sensitivity to gentamicin when using Staphylococcus aureus isolates, high resistance to azithromycin, as well as moderate resistance to ciprofloxacin.

Table (2) Inhibition zones in diameter (mm) of some antibacterial agents bacterial pathogenic.

Bacteria pathogenic	*Inhibition zone (mm)*			
	Ciprofloxacin 5mcg	*Amoxicillin 10mcg*	*Gentamicin 12mcg*	*Tetracycline 30mcg*
Staphylococcus haemolyticus	27	30	32	25
Staphylococcus aureus	38	36	36	35
Escherichia coli	30	0	37	36
spp. Micrococcus	34	18	35	36
Klebsiella spp.	20	0	37	20

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