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FUNGICIDAL ACTIVITY OF GOLD NANOPARTICLES (AuNP) FORMED USING CITRATE SYNTHESIS METHOD AGAINST *Candida albicans*

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ABSTRACT

This study synthesized gold nanoparticles using the citrate synthesis method and tested it for its fungicidal activity against *Candida albicans,* the causative fungus for Candidiasis. Three concentrations of 750, 500 and 250 ug/ml of synthesized gold nanoparticles (AuNP) were subjected to paper disc assay using corn meal agar against *C. albicans*. Synthesized AuNPs at 750 ug/ml concentration recorded the most effective antifungal activity against *Candida albicans* that even surpassed the inhibitory effects of the positive control, ketoconazole. The prevalent antifungal activity of the gold nanoparticles (AuNP) using the citrate synthesis method showed promising utilization as fungicidal mediator. It is confirmed that AuNPs synthesized using sodium citrate account for high antifungal effectiveness and displays a great potential in the development of drugs against *C. albicans* that are resistant to common antifungal drugs and causes treatment failures in candidiasis.

Keywords: Gold nanoparticles, citrate synthesis, fungicidal activity, *Candida albicans*

INTRODUCTION

In recent years, nanotechnology has made an impact and has generated much interest and applications in the field of drug delivery and pharmacology due to its unique properties [1]. Nanoparticlesare now immensely used to enhance treatment of diseases due to their reduced dimensions and consequentially, its efficiency in drug delivery processes owing to their extremely small size and large relative surface area. In applications as an antimicrobial agent, these properties lead to their increased contact with bacteria or fungi significantly improving their bactericidal and fungicidal activity.

With this growing interest and recognition of its importance, several researches have now focused on its synthesis using several methods including chemical, sonochemical, or photochemical paths [2]. The most common chemical technique is precipitation of gold nanoparticles in aqueous solution from a dissolved gold precursor by a reducing agent and use of a stabilizing agent to prevent agglomeration of the particles. One of the most common stabilizing agents is sodium citrate [3]. Because of its simple procedure, the classical citrate method remains one of the most reliable pathways of mono dispersed gold colloids synthesis [4].

Gold in its substance form has long been considered an inert, noble metal with some therapeutic and medicinal value. AuNPs are also thought to be relatively non-cytotoxic [5]. In biology, AuNPs are used for the development of biosensors, DNA labels [6,7] and for the development of medicines [8]. Synthesized gold nanoparticles (AuNPs) in the classical citrate synthesis method accentuate an extensive range of applications in the field of medicine for preeminent treatment of diseases [3].

The increasing concerns on antibiotic resistance [10] has also brought about the use of metallic nanoparticles for the control of bacteria [11] as well as fungi to prevent the development of resistant strains. Although antifungal drug resistance does not seem to be as much of a problem as resistance to antibacterial agents in bacteria, one long-term concern is that the number of fundamentally different types of antifungal agents that are available for treatment remains extremely limited [12]. Among the resistant fungi is *Candida albicans,* a natural fauna on humans that cause virulence when mucosal barriers are at stake. It displays an acquired mechanism for antifungal resistance and readily adapts to changes in the environment and new

hosts during the course of its association with the host [13].

Antifungal drug resistance long-term concern is that the number of fundamentally different types of antifungal agents that are available for treatment remains extremely limited. Therefore, there is an inevitable and vital need for drugs with novel antimicrobial mechanisms without developing resistance [12].

MATERIALS AND METHODS

Preparation of materials through Aqua regia

Aqua regia was used to produce chloroauric acid for refining the highest quality of gold. The use of aqua regia method is a standard over the traditional chromic acid bath for cleaning NMR tubes leaving no traces of paramagnetic chromium can remain to spoil spectra [14].

Mixture of nitric acid and hydrochloric acid in a molar ratio of 1:4 were used to clean all the laboratory glassware for the synthesis of AuNPs. It was washed with the aqua regia solution and rinsed thrice with distilled Mili-Q water. The noble metals gold,platinum and other metallic particles were dissolved in the process.

Citrate synthesis of Gold Chloride

A 10^{-3} M concentration of gold chloride was prepared with sterilized double distilled Milli-Q water. Trisodium citrate was gradually mixed with 10^{-3} M gold chloride for the synthesis of gold AuNPs. The vessels were incubated under dark conditions at room temperature with constant stirring of magnetic stirrer and observations were recorded.

The resulting solution was kept for 60 minutes in that condition until it turned into pink red color indicating the formation of AuNPs and to ensure the stability of the nanoparticles. The change in color indicates the formation of AuNPs in the solution due to excitation of surface Plasmon vibration in the metal nanoparticles [15]. The AuNPs obtained from the solution were purified by centrifugation at 4000 rpm for 15 minutes followed by dispersion of the pellet thrice in deionized water to eliminate the water-soluble biomolecules and secondary metabolites.

UV-visible Spectrophotometry Analysis

Surface Plasmon resonance (SPR) peaks attained in UV-vis spectroscopy are one of the versatile techniques that confirm the formation of metal nanoparticles [16]. The UV–visible absorption spectra findings demonstrate a novel technique for the preparation of the AuNPs [14]. The formation of AuNPs was monitored by UV-visible spectroscopy using Thermo Scientific NanoDrop 2000 1-position

spectrometer by analyzing the excitation due to the applied electromagnetic field of Surface Plasmon Resonance (SPR) and absorption values of the wavelength were recorded.

Characterization of the synthesized gold nanoparticles

The surface morphology of synthesized AuNPs were predominantly spherical and has uneven shapes [9].

The size and morphology of the gold nanoparticles were analyzed using Scanning Electron Microscopy. The SEM grid was prepared by adding a small quantity of the sample into 10 ml ethanol and then ultrasonicated to ensure proper dispersion. A drop of this dispersed sample was then placed on a SEM grid coated with the carbon film [17].

Stability of AuNPs

The synthesized AuNPs were kept stationary over 3 months in dark condition for all concentrations and evaluated visually and under UV-vis spectrophotometer every week for probable colloid, aggregate or precipitate formation to ensure that the synthesized AuNPs were stable.

Analysis of Antifungal activity

Synthesized gold nanoparticles with three concentrations of 750, 500 and 250 ug/ml were used. Sterile paper discs (5 mm) were soaked and air dried on sterile petri plates with synthesized gold nanoparticles under a Biosafety laminar flow. Prepared media on petri plates of Corn Meal agar, a selective medium for the cultivation of *C. albicans,* swabbed with fungal culture were used. Air dried disc with three concentrations were individually seeded on plates in three concentrations. Ketoconazole served as positive control while sterile distilled water served as negative control. Plates were incubated at 37°C for 3-5 days.

RESULTS AND DISCUSSION

Visual Observation and UV vis spectrophotometry analysis

The resulting reddish pink color in the mixture was noticeable upon gradual addition of trisodium citrate to $AuCl_3$ that starts the reduction of the gold ions. Change of color in the mixture was attributed to the Surface Plasmon Resonance (SPR) arising due to the collective oscillation of induced free conduction electrons in gold nanoparticles [16].

Absorption values monitored by UV-visible spectroscopy recorded 0.312 at a wavelength of 524 nm clearly indicated the formation of AuNPs in the citrate synthesis method. Gold nanoparticles formation in the citrate synthesis method were confirmed using UV-vis spectrophotometer.

Synthesized gold nanoparticles yielded nanoparticles with different shapes such as nanorods, nanodiscs, cubes, prisms and polyhedral nanoparticles with an average size of 300 nm.

Antifungal Activity of AuNPs

Synthesized gold nanoparticles exhibited excellent fungicidal activity at the concentration of 750 ug/ml against *Candida albicans*.

The fungicidal activity of the synthesized gold nanoparticles using sodium citrate at 750ug/ml showed a zone of inhibition of 18.59 mm, which is higher than that of the positive control (ketoconazole) with 17.01 mm zone of inhibition. This indicates that the synthesized AuNPs is more efficient in controlling *C. albicans* than the positive control, making a clear impact that the drug delivery of the gold nanoparticles is more efficient. Other concentrations also exhibited significant antifungal activities against *C. albicans.*

This result may be due to a more efficient delivery that contributed to the inhibitory effect through different cellular mechanisms including binding to cytoplasmic membrane causing cell membrane destruction, forming depths on the cell surface and modifying cell wall permeability and lastly the inhibition of major cellular functions such as respiration, DNA replication, and cell division, resulting in loss of cell integrity and viability [15, 18].

The effect of the gold nanoparticles is spontaneous and irreversible signifying that there may be cellular sites targeted by these gold nanoparticles [16]. It is also probable that the gold nanoparticles may be directly interacting with Glucan and Chitin, which serves as the primary reason for their antifungal activity. Regulation of intercellular pH (pHi) is fundamental prerequisite for growth of *Candida* and activation of plasma membrane ATPase is involved in maintenance of pHi [14]. The gold nanoparticles is supposed to act both at the membrane and cytoplasmic level.

Further it may be assumed that there may be some morphology-specific interaction between the gold nanoparticles and the plasma membrane proteins that resulted in the higher antifungal activity of the gold nanoparticles. It is also suggested that the smaller gold nanoparticles might have diffused easily through the cell membrane to the inside of the cell. Gold being a lenient acid might have strongly interacted with the soft bases like Sulphur containing proteins in the membrane or phosphorus containing bases in the DNA, thus retarding their normal functions such as synthesis, repair and replication leading to cell death [22].

Other reports on metallic nanoparticles include the antifungal activity of silver nanoparticles in the size range of 7–20 nm synthesized by an exclusive bio stabilization process against *A. niger* and *C. albicans* [10] as well as the significant antifungal activity of modified denture base acrylic combined with silver nanoparticles against *C. albicans* strain [22].

SEM analysis shows that trisodium citrate is an efficient reducing agent in producing gold nanoparticles [20]. UV–visible absorption spectroscopy analysis of the gold nanoparticles showed surface plasmon resonance (SPR) peak located at 524 nm with 0.312 absorbance value. However, reduction produced mixture of nanoparticles of different morphologies containing cubes, hexagons and other polyhedral structures with an average size of 300 nm.

Table 1: Zones of inhibition (in mm) of synthesized Gold nano particles using sodium citrate

Treatments	Zone of Inhibition (mm)
750 ug/ml	18.59[e]
500 ug/ml	15.09[c]
250 ug/ml	11.98[b]
+ Control (ketoconazole)	17.01[d]
- Control	0[a]

***Superscripts of different letters indicate significant difference among the treatments**

CONCLUSION

The prevalent antifungal activity of the synthesized gold nanoparticles using the citrate synthesis method can lead to its promising utilization as fungicidal agent in the field of nano biotechnology.

AuNPs synthesized using sodium citrate are capable of rendering high antifungal efficacy and hence, has a great potential in the preparation of drugs that can pave the way to the development of techniques on preparing nanomedicines for fungal-related diseases but thorough investigations on the toxicity and mechanism of action should be studied.

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