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Antimicrobial attributes of *Streptomyces tuius* mediated gold nanoparticles and coated materials

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Extracellular gold nanoparticles mediated by *Streptomyces tuius* DBZ39 were employed for their distinct characteristic properties and antimicrobial activities as a major objective of the present investigation. Defined size and distinct gold nanoparticles, obtained from biological sources in general and microbial sources in particular reveal spectacular biomedical applications. Scanning and Transmission Electron Micrographs, revealed the characteristic properties of extracellular gold nanoparticles in a controlled size of 24nm. X-ray diffraction analysis exhibits crystal phases of gold nanoparticles. Different materials including cotton, plastic and glass were used for coating with nanosolution and used to detect the antimicrobial activity against bacterial and fungal pathogens. An antimicrobial property of gold nanoparticles employing coated materials is a significant attribute. The antibacterial activity of gold nanoparticles was encouraging against gram negative bacterial pathogens *E. coli* and *Pseudomonas aeruginosa*, which can be explored further for their efficacy. The results suggest exploration of gold nanoparticles as a therapeutic agent in medical and pharmaceutical field.

Keywords: Streptomyces, Gold nanoparticles, Antibacterial, Antifungal, Goldnano coated materials

INTRODUCTION

Microbial nanotechnology is the science which deals with the use of microorganisms for the synthesis of nanoparticles. The interaction of microorganisms with certain minerals leads to the formation of nanosized materials. Thus, the employment of several microorganisms in the synthesis of nanoparticles is relatively a recent phenomenon and is the attractive field of research. Many microorganisms both unicellular and multicellular, which may be prokaryotes or eukaryotes are known to produce nanoparticles, either intra or extracellularly [Ahmad et al 2003b] with properties similar to chemically synthesized

materials, while exercising strict control over size, shape and composition [Alt et al., 2004]. Several bacteria namely, *E. coli*, *Pseudomonas*, *Lactobacillus*, Fungi- *Fusarium*, *Aspergillus*, *Penicillium fellutanum* and Yeast- *Saccharomyces* sp are known to produce different nanoparticles [Bater et al., 2005; Mazhari et al., 2014; Mazhari et al., 2017]. Among actinomycetes, *Rhodococcus* sp. [Bruins et al., 2009], *Thermomonospora* sp. and *Streptomyces* sp. [Duran et al., 2007] were reported to produce nanoparticles. Even though many biotechnological applications, such as remediation of toxic metals employ microorganisms, such as bacteria [Fayaz et al.,

2010], fungi [Jain and Pradeep, 2004] actinomycetes [Alt et al., 2004] and yeast [Jeong et al., 2005]. It is only recently that materials scientists have proposed microorganisms as possible eco-friendly nanofactories, for synthesis of nanoparticles [Kathiresan et al., 2009]. At the same time the biologically synthesizing gold nanoparticles has many applications includes catalysis in chemical reactions [Kim et al., 2008] biolabelling, antimicrobial agent, electrical batteries [Kora et al., 2009] and optical receptors [Krolikowska et al., 2003]. Recently gold nanoparticles [Kumar et al., 2003] exhibiting antimicrobial activity have been synthesized. Antibacterial activity of the gold containing materials can be used, for example, in medicine to reduce infections in burn treatment and arthroplasty [Lee et al., 2003] as well as to prevent bacteria colonization on prostheses, catheters, vascular grafts, dental materials and sunscreen lotions [Li et al., 2006], stainless steel materials and human skin [Mandal et al., 2006]. Gold containing materials can be employed to eliminate microorganisms on textile fabrics [Mann et al., 1996] or they can be used for water treatment [Tripathi et al., 2010; Stephen et al., 1999]. Antifungal activity [Mehra et al., 1999], anti inflammatory [Murali et al., 2003], and antibacterial activity capable of killing about 650 types of diseases causing microorganisms [Mann et al., 1996].

Although, a lot of information is available on the synthesis of metallic nanoparticles, literature on the antimicrobial activity of extracellular gold nanoparticles is little and the exploration of actinomycetes for the synthesis of nanoparticles is very meager. In view of this the present work is undertaken to explore the antimicrobial activity of gold nanoparticles

MATERIALS AND METHODS

Synthesis of gold nanoparticles by *Streptomyces tuius* DBZ39

An isolate of *Streptomyces tuius* DBZ39 obtained from limestone quarry soil earlier [Mazhari et al., 2014] was employed for the synthesis of extracellular gold nanoparticles, as per the standard protocol prescribed by [Murali et al., 2003]. The gold nanoparticles synthesized in the solution were visually confirmed by the development of deep purple color and UV-visible absorption spectrum in the range of 500-550nm. The gold nanoparticles produced were characterized by Transmission and Scanning

electron microscopy [Bater et al., 2003] for their size, shape and dispersion and EDAX for elemental confirmation as per the standard protocols.

Antimicrobial activity

The antimicrobial activity of the extract containing gold nanoparticles was carried out by following well diffusion and disc diffusion methods [Navazi et al., 2010]. The antimicrobial activity, mainly antibacterial and antifungal activity of gold nanoparticles was carried out by employing the procedure as follows [Peto et al., 2002].

A) Antibacterial and Antifungal activity of Gold nanosolution

The antibacterial activity [Raffi et al., 2008] of the solution of gold nanoparticles was carried out by well diffusion method against both Gram positive and Gram negative multidrug resistant pathogens such as *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Proteus vulgaris* was carried out.

The antifungal activity of gold nanoparticle solution was also carried out by well diffusion method as explained earlier. Fungal pathogens namely *Aspergillus niger*, *Fusarium oxysporum*, *Candida albicans* and *Cladosporium* sp.

C) Antibacterial and Antifungal activity of gold nano coated substances

Antibacterial activities of gold nano coated substances were also carried out by following the procedure similar to the guide lines of antibacterial activity as mentioned earlier. *Staphylococcus aureus* and *Pseudomonas aeruginosa* were the two pathogens employed in the study.

Adequate size of different materials such as cotton cloth, filter paper, cotton, polythene pieces and glass pieces were coated with gold nanoparticles by immersing them into 10 ml nanosilver solution for about 2 min. The antibacterial activity of coated and uncoated antibiotic discs (Vancomycin-30mg, Norfloxacin-10mg, Imipenin-10mg, Chloramphenicol-30mg, Polymixin b-30mg, Cefazidime-30mg, Amoxicillin-30mg) with gold nano solution was also assessed [SAdhasivan et al., 2010; Shahverdi et al., 2007; Stephen et al., 1999; Taylor et al., 2005].

Antifungal activity of gold nano coated substances as mentioned earlier was also carried out against *Fusarium oxysporum* [Tripathi et al., 2010].

RESULTS AND DISCUSSION

The interactions between metals and microorganisms has been well documented. Among microorganisms, actinomycetes are considered as best nanofactories as they possess dual characteristics of bacteria and fungi. Several

actinomycetes are known to produce various bioactive molecules such as enzymes, antibiotics and other variety of secondary metabolites. Recently, they are regarded as producers of bionanomaterials.



Figure1: Antibacterial activity of gold nanoparticles synthesized by *S. tuius* on *S. aureus*



Figure 2: Antibacterial activity of Gold nanoparticles synthesized by *S. tuius* on *E. coli*



Figure 3: Antibacterial activity of gold nanoparticles synthesized by *S. tuius* on *S. typhi*



Figure 4: Antibacterial activity of gold nanoparticles synthesized by *S. tuius* on *Pseudomonas aeruginosa*



Figure 5: Antibacterial activity of gold nanoparticles synthesized by *S. tuius* on *Klebsiella pneumoniae*



Figure 6: Antibacterial activity of gold nanoparticles synthesized by *S. tuius* on *Proteus vulgaris*



Figure7: Antifungal activity of gold nanoparticles synthesized by *S. tuius* on *Aspergillus niger*



Figure 8: Antifungal activity of gold nanoparticles synthesized by *S. tuius* on *Fusarium* sp



Figure 9 : Antifungal activity of gold nanoparticles synthesized by *S. tuius* on *Cladosporium* sp.



Figure 10: Antifungal activity of gold nanoparticles synthesized by *S. tuius* on *Candida albicans*

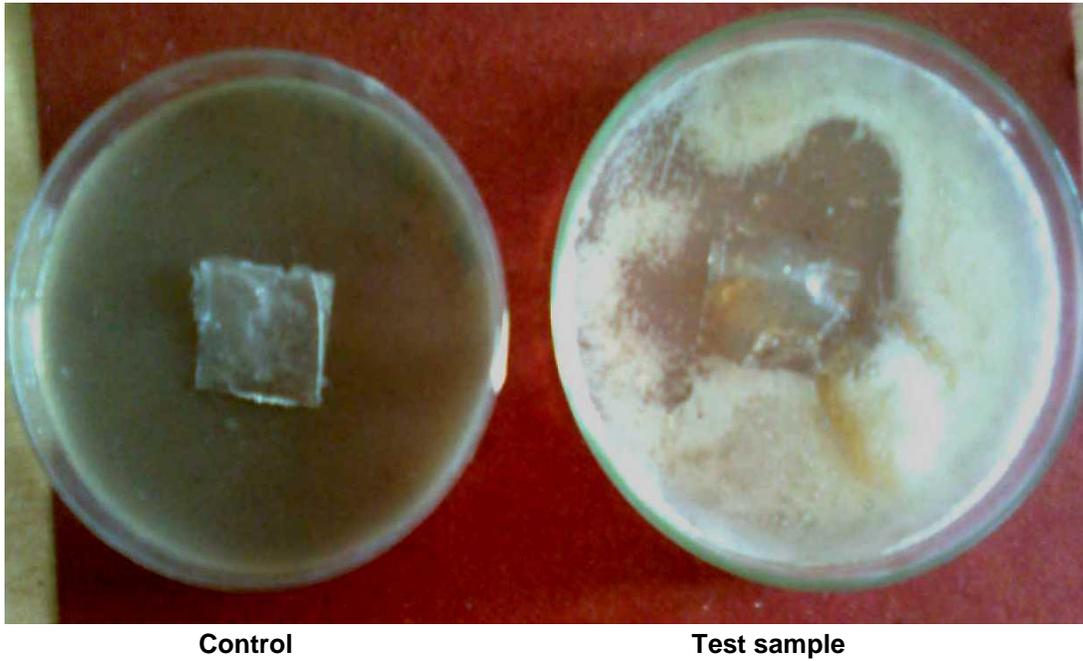


Figure 11: Antibacterial activity of gold nanoparticles coated plastic against *S.aureus*

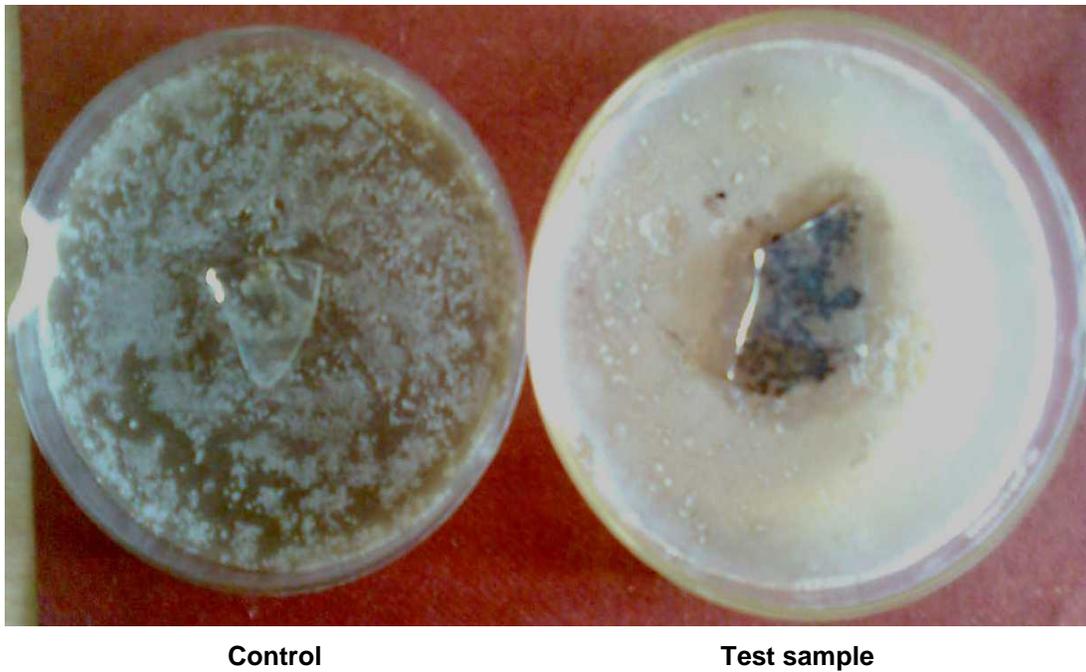


Figure 12: Antibacterial activity of gold nanoparticles coated glass piece against *S. aureus*



Control

Test sample

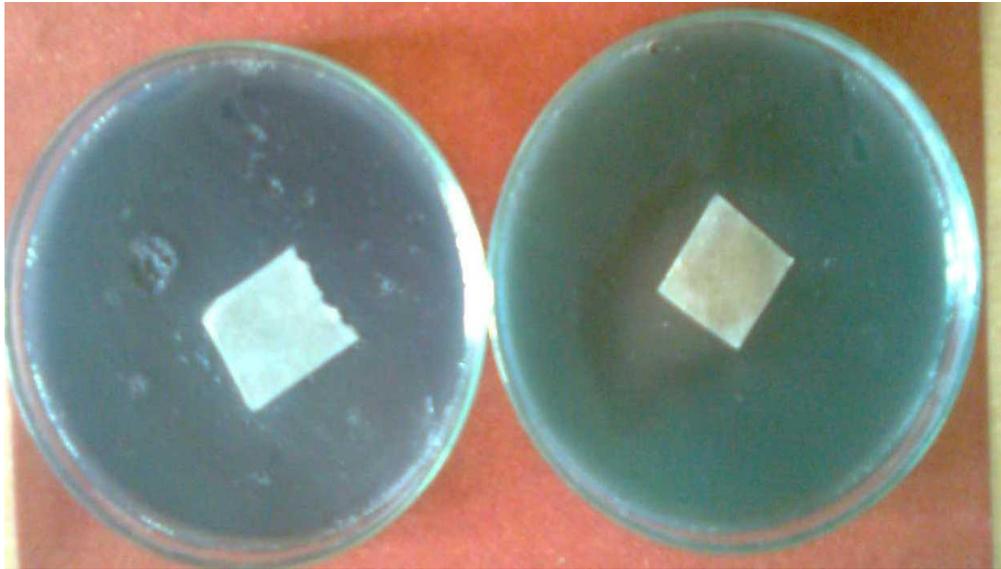
Figure 13: Antibacterial activity of gold nanoparticles coated cotton against *S. aureus*



Control

Test sample

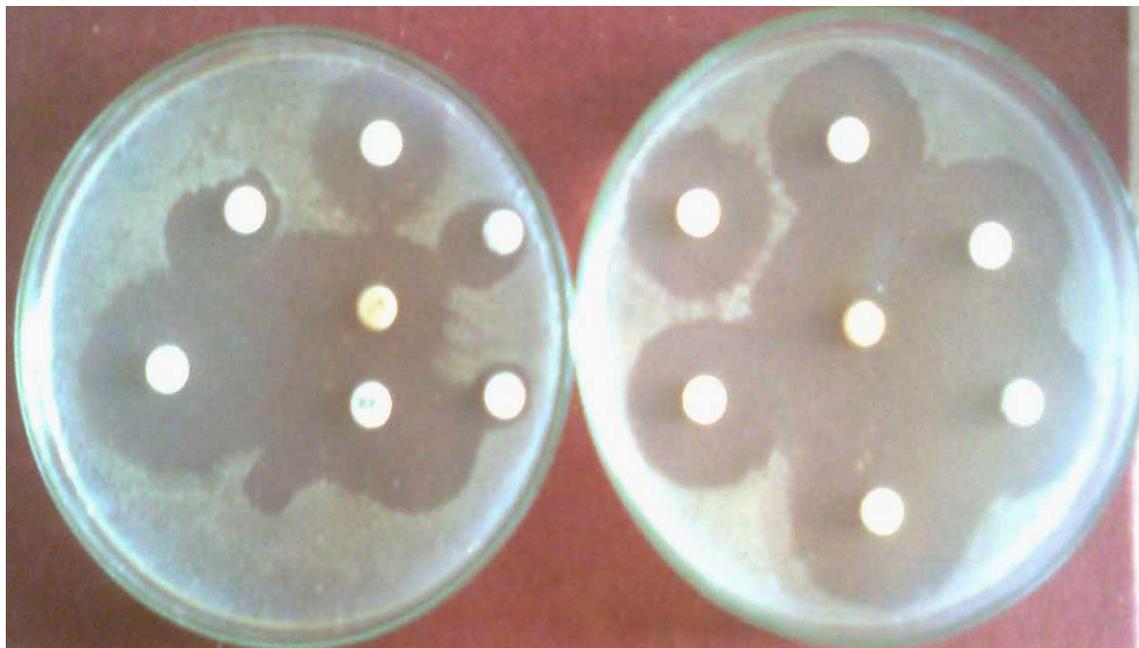
Figure14: Antibacterial activity of gold nanoparticles coated cloth against *S. aureus*



Control

Test sample

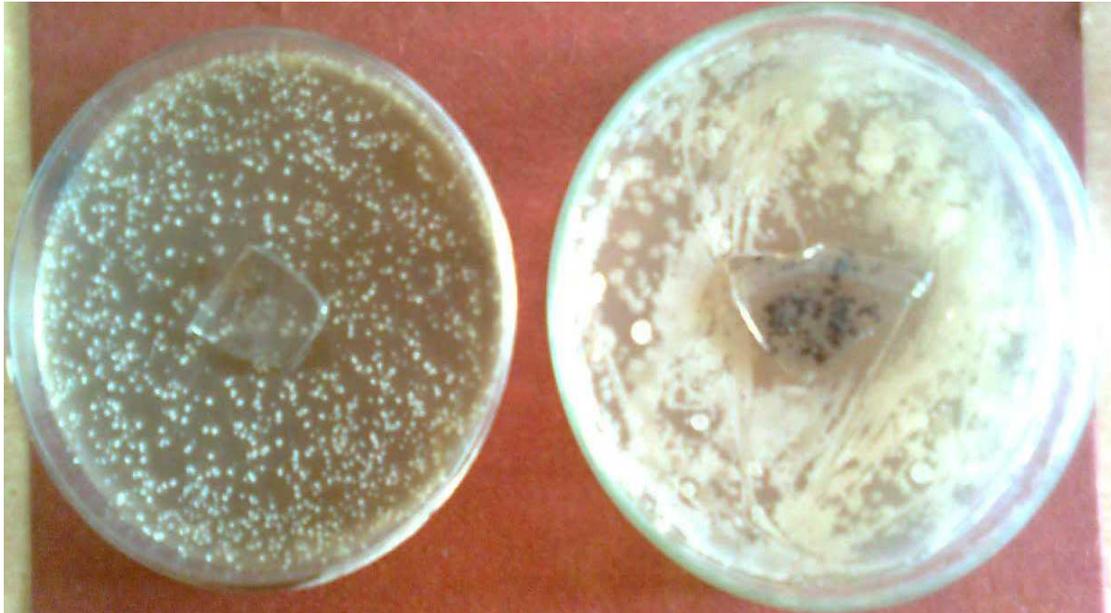
Figure 15: Antibacterial activity of gold nanoparticles coated filter paper against *S. aureus*



Control

Test sample

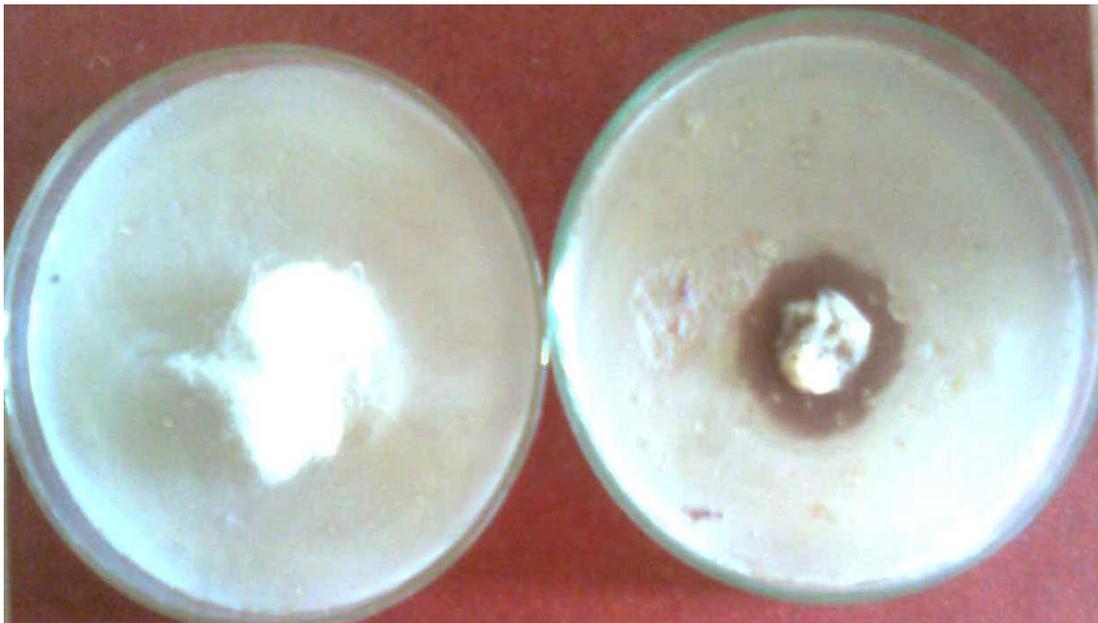
Figure 16: Antibacterial activity of gold nanoparticles coated antibiotics against *S.aureus*



Control

Test sample

Figure 17: Antifungal activity of gold nanoparticles coated glass piece against *Fusarium sp*



Control

Test sample

Figure 18: Antifungal activity of gold nanoparticles coated cotton against *Fusarium sp*.



Control

Test sample

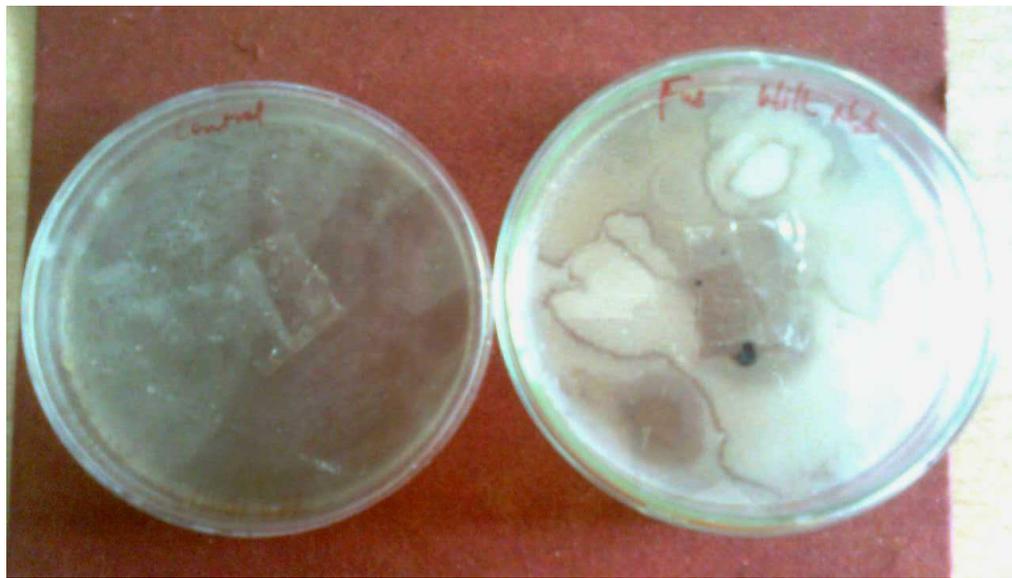
Figure 19: Antifungal activity of gold nanoparticles coated cotton cloth against *Fusarium sp.*



Control

Test sample

Figure 20: Antifungal activity of gold nanoparticles coated filter paper against *Fusarium sp.*



Control

Test sample

Fig. 21: Antifungal activity of gold nanoparticles coated plastic against *Fusarium* sp.

The genus *Streptomyces* being one of the major antibiotic producers among the actinomycetes is a wide and major known organism.

Gold nanoparticles have gained much popularity recently, owing to the broad spectrum of antimicrobial activity, wound dressings and contraceptive devices. Thus, gold nanoparticles are becoming more and more widespread use in medicine and related applications. The antimicrobial activity of gold nanoparticles synthesized by *Streptomyces tuius* DBZ39 was carried out against few Gram positive, Gram negative bacterial pathogens and few fungal pathogens. The antibacterial activities of gold nanoparticles are as presented in Figure 1 to 6. The antibacterial activity of gold nanoparticles against both Gram positive and Gram negative bacterial pathogens is considerably great when compared to the zone of inhibition with the standard prescribed synthetic antibiotics. However, from among the tested bacterial pathogens *S. aureus* (Fig. 1) and *E. coli* (Fig. 2) were more affected with the gold nanoparticles to a greater extent, when compared to other bacterial pathogens. *Proteus vulgaris* (Fig.6) and *Klebsiella pneumonia* (Fig. 5) were least affected. *Salmonella typhi* (Fig.3) and *Pseudomonas aeruginosa* (Fig.4) which are known to be the potential human pathogens were also affected by the gold nanoparticles. These observations

required to be explored further for the use of gold nanoparticles as antibacterial therapeutic agents specifically.

The antifungal activities of gold nanoparticles synthesized by *Streptomyces tuius* DBZ39 are as presented in Figure 7 to 10 against *Aspergillus niger*, *Fusarium* sps., *Cladosporium* sps., and *Candida albicans* respectively. The efficacy of gold nanoparticles is reported to be very high against *Fusarium* sps. (Fig. 8) and *Candida albicans* (Fig. 10) in comparison with the other two fungal pathogens.

The surface application of the solution of gold nanoparticles as antimicrobial agents against bacterial and fungal infections is not an uncommon practice. In view of this, various inert materials were employed with the coating of gold nanoparticles to study the antimicrobial activity. The antibacterial activity of *S. aureus* employing gold nano coated plastic piece (Fig. 11), glass piece (Fig. 12), cotton piece (Fig. 13), cloth piece (Fig. 14) and filter paper piece (Fig. 15) were observed and compared with the antibacterial activity of gold nano coated antibiotic discs (Fig. 16). Results clearly reveals that, none of the gold nano coated materials were efficient enough when composed to gold nano coated antibiotics. However, the coated materials did exhibit the antibacterial properties showing the zone of inhibition when compared with the control. Similarly, the antifungal activity using *Fusarium*

sps., with different gold nano coated materials was also carried out and the results are as presented in Figure 17 to 21. However, the observations were almost similar with that of the antibacterial properties.

CONCLUSION

Exploration of antimicrobial activity of gold nanoparticles proved to be quite potential. Antibacterial attributes of gold nanoparticles were significant when compared with synthetic antibiotics. *S.aureus* and *E.coli* showed greatest sensitivity to gold nanoparticles whereas *S.typhi* and *Pseudomonas aeruginosa* showed resistivity to gold nanoparticles. *Cladosporium* sps. and *Aspergillus niger* were resistant to gold nanoparticles compared to *Fusarium* and *Candida albicans*. Gold nanoparticles coated inert materials including plastic, filter paper, cotton and glass were not efficient enough to show the antimicrobial activity compared to gold nanocoated antibiotics. Therefore these observations can be considered for the exploration of gold coated antibiotics as a powerful weapon for topical and therapeutic agents in the field of medical and pharmaceutical sciences..

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

All authors are equally contributed in performing the experimental part and participated in writing the manuscript and submission.

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