

Original Research Article

<https://doi.org/10.20546/ijcmas.2017.604.036>Antifungal Activity of Biogenic Platinum Nanoparticles: An *in vitro* Study

Kapil Dev Sharma*

Research Scholar, Shri JTT University, Jhunjhunu, Rajasthan 333001, India

*Corresponding author

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The aim of this study was to screen biogenic platinum nanoparticles for antifungal properties by a combination of *in vitro* methods. During this study, marine actinobacteria (*Streptomyces* sp. mediated biogenic platinum nanoparticles were screened for their antifungal activity against *Aspergillus flavus*, *A. niger*, *Penicillium* sp., *Candida albicans* and *C. tropicalis*. Biologically synthesized platinum nanoparticles exhibited broad spectrum antifungal activity against test organisms. Particles exhibited high activity against yeast *viz.*, *Candida albicans* (16.66±1.52) and *C. tropicalis* (18.33±1.52), while moderate antifungal activity against mold *viz.*, *A. flavus* (11.00±1.00), *A. niger* (09.33±1.52), *Penicillium* sp. (06.00±1.00). These particles exhibited antifungal activity with minimum inhibitory concentration ranging from 10-40µg/ml.

Introduction

Fungi are very important group of microorganisms with maximum number of representatives. Fungi provide several direct or indirect benefits to mankind such as decomposition of organic material, bioremediations of toxic materials, production of antibiotics and other industrial products etc (Rani *et al.*, 2014; Kawaguchi *et al.*, 2013; Sunna *et al.*, 1997). In contrast to the benefits, fungi can also leads a variety of diseases in plants, humans and animals. Fungal infection such as, Aspergillosis, Candidiasis and Mucormycosis etc. pose a significant negative impact on human health, most of the fungi produces slow and long term infection in human body and is quite difficult to eliminate. Development of drug resistance in pathogenic strains is an important virulence factor of fungi, therefore there is a constant need to develop newer products with antifungal

potential (Vanden *et al.*, 1997). Nanotechnology is a science that deals with the synthesis, development, manipulation and applications of tiny molecules that measure 100 nanometers or less. Due to their very small size, they carry unique properties and can be utilize for the development of new products. Most recently, there is a global interest among the scientists to develop, improve, employ and use nanoparticles for solving various problems encountered by mankind. In recent past, a variety of metallic and non-metallic nanoparticles are extensively used in various fields such as pharmaceuticals, optical devices, catalyst, bioremediation, electronic and sensor technology (Praetorius *et al.*, 2007; Karthik *et al.*, 2013; Anderson *et al.*, 2006; Jiang *et al.*, 2005; Agarwal *et al.*, 2014; Sharpe *et al.*, 2014).

Various nanoparticles *viz.* silver, gold, zinc oxide, platinum are being excessively used in several industries such as food, pharmaceutical and bioelectronics. Among them platinum nanoparticle is one of the rare and very useful variety of nanoparticle. In recent past, platinum nanoparticles have been reported to exhibit great antioxidant properties. Platinum nanoparticles are also being used for coating materials, and for the development of nanofibers and polymer membranes.

These particles are also reported to have great applications in the development of fuel cells and hydrogen storage materials (Wen *et al.*, 2008; Li *et al.*, 2007). In recent past nanoparticles have been reported to exhibit significant antimicrobial activity (Ahmed *et al.*, 2016; Elhusseiny *et al.*, 2013), however the reports on antifungal activity remains limited. Therefore, this study is designed to screen the platinum nanoparticles for their properties to inhibit the growth of fungi.

Materials and Methods

Platinum nanoparticles were previously biologically synthesized using marine actinobacteria *Streptomyces* sp. isolated from soil sediments samples collected from coastal areas of Chennai, India, while Chloroplatinic acid hexahydrate was used as substrate. These nanoparticles were characterized using UV-visible spectrophotometer, FTIR Spectroscopy, XRD and TEM analysis (Data not shown).

Antifungal activity of the platinum nanoparticles

Test organisms

Aspergillus flavus, *A. niger*, *Penicillium* sp., *Candida albicans* and *C. tropicalis* were used for studying the antifungal activity of

biogenic platinum nanoparticles. All fungal cultures were maintained on potato dextrose agar medium and stored at 4°C.

Antibiogram

All the clinical isolates of fungi were screened for their sensitivity towards standard antibiotics by disc diffusion method. Antibiotics included Miconazole (10 µg/disc), Clotrimazole (10 µg/disc), Ketoconazole (10 µg/disc), Amphotericin -B (20 µg/disc) and Fluconazole disc (10µg/disc). Drug sensitivity test was performed by disc diffusion method on Potato dextrose agar plates. Fungal isolates were inoculated in to potato dextrose broth for 8 hours. Isolates were seeded on potato dextrose agar plates by using sterilized cotton swabs. The standard antibiotic discs were placed on the agar surface using a sterilized forceps. Plates were incubated at 28°C for 48-72 hours. Plates were observed for zone of inhibition. The experiment was performed in triplicates (Perez *et al.*, 1990).

Antifungal activity

Antifungal activity of the biologically synthesized platinum nanoparticles was checked by agar well diffusion method on Potato dextrose agar plates. The fungal cultures were lawn cultured on potato dextrose agar plates by using sterilized cotton swabs. In each of these plates, three wells were cut out using a standard cork borer (7 mm diameter). Using a micropipette, 100 µl of Chloroplatinic acid hexahydrate solution (100µg/ml), 100µl of platinum nanoparticle (100µg/ml) and 100µl of distilled water was added to separate wells. Plates were incubated for 48-72 hours at 28°C. Anti-fungal activity was evaluated by measuring the zone of inhibition (Kumar *et al.*, 2010). Experiment was performed in triplicates.

Determination of relative percentage inhibition

The relative percentage inhibition of the biologically synthesized platinum nanoparticles with respect to positive control was calculated by using the following formula (Kumar *et al.*, 2010).

Relative percentage inhibition of the biologically synthesized platinum nanoparticles =

$$[100 \times (x-y)] / (z-y)$$

Where,

x: total area of inhibition of the biologically synthesized platinum nanoparticles

y: total area of inhibition of the solvent

z: total area of inhibition of the standard drug

The total area of the inhibition was calculated by using $\text{area} = \pi r^2$; where, r = radius of zone of inhibition.

Determination of minimum inhibitory concentration (MIC)

MIC of the biologically synthesized platinum nanoparticles was performed by modified agar well diffusion method. Sample was diluted in sterilized distilled water to make a concentration range from 1-1000 $\mu\text{g/ml}$. Test cultures were inoculated in PDB and seeded on PDA plates using sterilized cotton swabs. In each of these plates four wells were cut out using a standard cork borer (7 mm). Using a micropipette, 100 μl of each dilution was added in to wells. Plates were incubated at 28°C for 72 the results were recorded. The minimum concentration of each extract showing a clear zone of inhibition was considered to be MIC (Rios *et al.*, 1988; Okunji *et al.*, 1990).

Statistical analysis

The results of the antifungal activity of biologically synthesized platinum

nanoparticles are expressed as mean \pm standard deviation of the response of 3 replicates determinations per sample. Results were analyzed by using Microsoft Excel 2007.

Results and Discussion

Platinum is a very rare and one of the most costly metal available on earth. It's highly resistance to corrosion and is the least reactive metal therefore used widely for specific applications. Platinum is being widely used in nanotechnology and can be reduced to nanoparticles by various physical, chemical and biological processes. These nanoparticles can exhibit unique properties depending upon the method used for the production of particles and their size.

In recent past, platinum nanoparticles has been reported to exhibit several pharmaceutical properties such as antioxidant activity, anticancer activity (Borse *et al.*, 2015) and antimicrobial activity (Rajathi *et al.*, 2014).

In a study, pectin and sodium borohydride synthesized platinum nanoparticles exhibited significant antibacterial activity against *Escherichia coli* and *Aeromonas hydrophila*. In another study, phytofabricated platinum nanoparticles exhibit strong antibacterial activity toward *Vibrio cholera*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Salmonella typhi* and *E. coli*. During this study the biologically synthesized platinum nanoparticles were screened for their antifungal activity by using various *in vitro* methods.

Antibiogram study

Antibiogram studies performed against fungal isolates provide systemic information about the drug resistant pattern of the test cultures against commonly used antibiotics.

Table.1 Antibiogram study of fungal isolates

Test organisms	Antibiotics used				
	Miz	Ctz	Kt	Ap	Fu
<i>A. flavus</i>	S	R	S	S	S
<i>A. niger</i>	R	S	S	S	S
<i>Penicillium</i> sp.	R	S	S	S	S
<i>C. albicans</i>	S	I	I	I	S
<i>C. tropicalis</i>	S	S	S	S	S

S: Sensitive, I: Intermediate, R: Resistant, n.a.: not applied,
Miz: Miconazole (10 µg/disc), Ctz: Clotrimazole (10 µg/disc), Ketoconazole (10 µg/disc), Ap: Amphotericin-B (20 µg/disc), and Fu: Fluconazole disc (10µg/disc)

Table.2 Antifungal activity of biologically synthesized platinum nanoparticles

Test organism	Zone of inhibition (mm)		
	Pt NPs	PC	NC
<i>Aspergillus flavus</i>	11.00±1.00	17.33±2.08	0.0±0.0
<i>Aspergillus niger</i>	09.33±1.52	16.00±3.00	0.0±0.0
<i>Penicillium</i> sp.	06.00±1.00	15.66±1.52	0.0±0.0
<i>Candida albicans</i>	16.66±1.52	19.33±1.52	0.0±0.0
<i>Candida tropicalis</i>	18.33±1.52	23.00±1.73	0.0±0.0

Here, PC: positive control, NC: negative control
Values are expressed as mean ± standard deviation of the three replicates,
Zone of inhibition not include the diameter of the well.

Table.3 Relative percentage inhibitions of biologically synthesized platinum nanoparticles

Test organism	RPI (%)
<i>Aspergillus flavus</i>	40.27
<i>Aspergillus niger</i>	34.02
<i>Penicillium</i> sp.	14.66
<i>Candida albicans</i>	74.31
<i>Candida tropicalis</i>	63.53

RPI: Relative percentage inhibition

Table.4 Minimum inhibitory concentrations of biologically synthesized platinum nanoparticles

Test organism	MIC (µg/ml)
<i>Aspergillus flavus</i>	20
<i>Aspergillus niger</i>	40
<i>Penicillium</i> sp.	20
<i>Candida albicans</i>	10
<i>Candida tropicalis</i>	10

MIC: Minimum inhibitory concentration

During this study, *Aspergillus flavus*, *A. niger*, *Penicillium* sp., *Candida albicans* and *C. tropicalis* were subjected to antibiogram study against antifungal drugs such as miconazole,

clotrimazole, ketoconazole, amphotericin-B and fluconazole disc. Results represents that almost all the fungal cultures were sensitive towards the tested antibiotics, however *A. flavus* exhibited resistance towards clotrimazole, while *A. niger* and *Penicillium* sp. resist miconazole. Results of antibiogram study are summarized in Table 1.

Antifungal activity of biologically synthesize platinum nanoparticles

In this study, biologically synthesize platinum nanoparticles was screened for antifungal activity against *Aspergillus flavus*, *A. niger*, *Penicillium* sp., *Candida albicans* and *C. tropicalis*. Biologically synthesized platinum nanoparticles exhibited high antifungal activity, while highest antibacterial activity was shown against *C. tropicalis* (18.33 ± 1.52), followed by *C. albicans* (16.66 ± 1.52), *A. flavus* (11.00 ± 1.00), *A. niger* (09.33 ± 1.52) and *Penicillium* sp. (06.00 ± 1.00). Results of antifungal activity of biologically synthesize platinum nanoparticles are summarized in Table 2.

Relative percentage inhibition

During this study, antifungal activity of biologically synthesize platinum nanoparticles was compared with the antifungal activity of standard drugs for evaluating relative percentage inhibition. The biologically synthesize platinum nanoparticles exhibited maximum relative percentage inhibition against *C. albicans* (74.31%), followed by *C. tropicalis* (63.53%), *A. flavus* (40.27%), *A. niger* (34.02%) and *Penicillium* sp. (14.66%). Results of relative percentage inhibition are summarized in Table 3.

Minimum inhibitory concentration

Minimum inhibitory concentration values of the biologically synthesize platinum

nanoparticles against fungal strains were range between 10-40 $\mu\text{g/ml}$. The biologically synthesize platinum nanoparticles exhibited minimum inhibitory concentration against *C. albicans* (10 $\mu\text{g/ml}$), *C. tropicalis* (10 $\mu\text{g/ml}$), followed by *A. flavus* (20 $\mu\text{g/ml}$), *Penicillium* sp. (20 $\mu\text{g/ml}$) and *A. niger* (40 $\mu\text{g/ml}$). Results of MIC values of the biologically synthesize platinum nanoparticles are summarized in Table 4.

During this work, actinobacteria mediated biogenic platinum nanoparticles were evaluated for its antifungal property. These particles exhibited a significant antifungal activity against a broad range of fungi including *C. albicans*, followed by *C. tropicalis*, *A. flavus*, *A. niger* and *Penicillium* sp. It could be concluded that marine actinobacteria can effectively produce platinum nanoparticles with broad spectrum antifungal properties.

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