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Biosynthesis Nickel Nanoparticle by Microorganism and Their Biological Activity

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ABSTRACT

Biosynthesis of metal nanoparticle using fungi Claviceps paspali by varying composition of media, pH and temperature. The synthesized nanoparticle was characterized by UV-Visible spectroscopy, Scanning Electron Microscope (SEM) and Fourier Transform Infrared (FT-IR) Spectroscopy. Antimicrobial property of the nanoparticle was carried out against tested strains. Nanoparticle was also tested for their antidiabetic and anti-angiogenic properties. This work revealed that metal nanoparticle can be easily produced by fungi and may be a feasible, low-cost, environmentally friendly method for generating stable nanoparticles.

Keywords: *Claviceps paspali*, Nanoparticle, Biological activity

INTRODUCTION

Green synthesis method is proved beneficial over other methods which are implemented for the synthesis of nanoparticles. Green synthesis methods are eco-friendly approach and compatible for pharmaceutical and other biomedical applications, as the toxic chemicals are not used in these methods [1]. This method does not require high pressure, temperature. Synthesis of various nanoparticles by microorganisms is used more than the other techniques due to its ecofriendly nature. The use of environmentally beneficial materials like plant leaf extract, fungi, bacteria and enzymes for the synthesis of zinc nanoparticles proposes abundant benefits of eco friendliness and compatibility for pharmaceutical and many other biomedical applications [2]. The chemotherapy drugs can also be delivered to cancer cells with applying heat to the cells. For example, gold nanorods to which DNA strands are attached. The DNA strands act as a scaffold which holds together the nanorods and the chemotherapy drug. When the infrared light illuminates the cancer cells, the infrared light absorbs by the gold nanorods which turns into heat. The produced heat releases the chemotherapy drug and destroys the cancer cells. Nanoparticles are also developed to defeat the viruses. The nanoparticles delivers an enzyme that prevents the reproduction of viruses in the patient's bloodstream instead of destroy the viruses. Some nanoparticles are developed which can be delivered across the brain barrier to tackle neurologic disorders. Nanoparticles are also used in the early detection of the cancer tumors. When the nanoparticles attach to cancer tumors, the nanoparticles release the biomarkers molecules known as the peptides. In this technique, each nanoparticle carries several peptides of a very high concentration of these markers which will occur at very early stages of cancer and also allowing the early detection of the disease.

Ergot fungus *Claviceps purpurea* is able to infect rye and other grains, and has caused several epidemics, particularly during the Middle Ages, due to consumption of rye products contaminated with *C. purpurea sclerotia* [3,4]. The resulting disease is called ergotism [5]. Patients show various symptoms depending on the amount and kind of alkaloids they consume. Painful spasms, paresthesia, diarrhea, nausea and vomiting, headache or psychosis are typical convulsive symptoms and gangrenous symptoms are observed especially for fingers and toes [6]. The development of a high-yielding strain of *Claviceps paspali* capable of producing compounds, when grown in submerged culture on a synthetic medium provides the suitable system for biosynthetic studies [7].

Different indole-diterpenoids were detected in the *C. paspali* [8] from high-resolution fourier transform Orbitrap mass spectrometry. The indole-diterpene profile of the extract from the ergotized Bermuda grass was similar to that of *C. paspali sclerotia* [9]. Isolation and identification of tremorgenic metabolites from sclerotia of *C. paspali* was reported [10]. In this work, an attempt has been made to synthesize metal nanoparticle using *C. paspali*. The shape of the synthesized particle was analyzed by Scanning Electron Microscope (SEM). The prepared particle was elementally analyzed by UV-Visible and Fourier Transform Infrared (FT-IR). Finally, the metal nanoparticle was tested for their biological activity.

MATERIALS AND METHODS

$NiSO_4$ required for metal nanoparticles synthesis, was obtained from Sigma-Aldrich. Other chemicals required for our research purpose were purchased from Merck. The culture *C. paspali* was obtained NCL, Pune (NCIM: 1013). It was subcultured from time to time to maintain its viability during the study period. Microorganisms *Streptococcus oralis* (MTTC 389) and *Streptococcus mitis* (MTTC 2696) were procured from Department of Microbiology, NCL, Pune. Pathogens are obtained from JSS Hospital, Mysuru.

Fertilized eggs were purchased from the Indian veterinary research Institute, Bangalore. All reagents were of analytical grade. P-nitrophenol α-D-glucopyranoside (pNPG), sodium azide and α- glucosidase type I (EC 3.2.20, lyophilized powder from (*Saccharomyces cerevisiae*), Potato dextrose broth were purchased from Hi-Media.

Synthesis of nickel nanoparticles

Nickel nanoparticle was synthesized using *C. paspali* by treating Nickel Sulfate ($NiSO_4$) of 1 mM solution independently to cell free supernatant. After 7 days, the cell free culture was centrifuged at 7500 rpm for 20 min at 30°C nickel nanoparticle was recovered independently. The experiment was also performed in different culture media, pH and at different temperature as it is done for the production of nanoparticle.

Characterization of nanoparticle

JSM-6480 LV SEM instrument was employed to study the morphology of synthesized nanoparticles which was performed at an accelerating voltage of 20 kV. Product samples were subjected to UV-Visible NIR spectroscopic study (Agilent, CARY 60) in the range of 190-1100 nm. Number of cycle was set to 1 per 1 second and lamp change value was set at 326 nm. The interaction of extract and nanoparticle was analyzed with FT-IR spectrophotometer (Agilent FT-IR ATR Cary 630) in the range of 7000-350 cm^{-1}.

Biological activity

Antimicrobial activity

Antibacterial activity of the metal nanoparticle was tested *in vitro* for their activity against gram positive and gram negative bacterial strains, *S. oralis, S. mitis, Staphylococcus aureus, Proteus, Klebsiella, Pseudomonas, Salmonella paratyphi and Salmonella typhi* which causes diseases [11]. Microbes were grown in Nutrient Broth (NB, Merck) medium at 37°C for 24 h. The bacterial number in the final inoculums was adjusted to 10^6 CFU/ml. A bacterial lawn was prepared by pouring 0.1 ml of bacterial suspension onto each plate of Nutrient Agar Medium (NA, Merck), spread by a sterile cotton swab, and allowed to remain in contact for 1 min. Metal nanoparticle of various concentrations (5, 10, 20 µg and 40 µg) was prepared in order to impregnate the paper discs. The sterile filter paper discs containing different metal NP concentrations (2 mm diameter) were then placed on the bacterial lawn. The Petri dishes were subsequently incubated at 37°C for 24 h and the inhibition zone around each disc was measured in cm.

Antifungal activity

The antifungal assay was performed by food poisoning method [12]. Potato Dextrose Agar (PDA) medium was used for the study. The plates containing medium with different concentrations (100 and 200 µg/ml) of metal nanoparticle was inoculated at the centre with 5 mm inoculum disc of pathogenic fungus and incubated at 25°C for 7 days. After 7 days the growth is measured using ruler and expressed in % inhibition.

Antidiabetic activity

α-amylase inhibition activity

Pancreatic α-amylase inhibition of nickel was studied *in vitro* with calcium chloride at 40 mg/l as calcium is an essential cofactor for α-amylase [13]. Aliquots of a α-amylase solution of 20 µl and metal nanoparticle solution of different concentrations were mixed in a 2 ml Eppendorf tube and incubated at 37°C for 15 min to allow interactions between α-amylase and metal nanoparticle. The reaction was initiated by adding 60 µl of starch solution into the mixture and lasted for another 5 min at 37°C. Afterwards, an aliquot of 100 µl of 3,5-Dinitrosalicylic acid (DNS) reagent solution was added followed by heating in boiling water for 15 min to develop color. The reaction was then stopped by cooling down in ice water. The absorbance of the reaction mixture was read at 540 nm using a spectrophotometer.

α-glucosidase assay

The α-glucosidase inhibitory activity was determined according to the procedure [14]. In short, the α-glucosidase inhibition assay was performed in 0.1 M sodium phosphate buffer (SPB, pH 7.5) containing 0.02% sodium azide and solutions of α-glucosidase (2.0 U/ml) and pNPG (10 mM) were prepared in this buffer. Solution of nickel of different concentrations, 1.3 ml of buffer Sodium Phosphate Buffer (SPB), and 200 µl α-glucosidase solutions were added to each test tube. The samples were shaken for 30 min and then incubated at room temperature for 10 min. 1500 µl of the pNPG solution were added to initiate the reaction. Enzyme inhibition was determined by measuring the absorbance of p-nitrophenol at 405 nm using a spectrophotometer. The maximum enzyme activity measured without addition of inhibitor was used as a control experiment. Percentage enzyme inhibition was calculated.

Anti-angiogenic activity

Chorioallantoic Membrane (CAM) assay was performed according to the method [15]. The fertilized eggs were divided into different treatment groups, which included control, the vehicle treated group and metal nanoparticle treated groups with minimum of six eggs in each group. The fertilized eggs were incubated for 6 days at 37°C in a humidified and sterile atmosphere. Window was made on the egg shell to assess the developmental stage of the embryo and was resealed and incubation was continued. On day 8 the windows were opened and the compound/vehicle were loaded on the Whatman paper discs separately, air dried and inverted over the CAM and the windows were closed. The window was resealed and the embryo was allowed to develop further. The windows were opened and observed on day 9 and inspected for changes in the micro vessel density in the area around the paper discs and photographed.

RESULTS AND DISCUSSION

PDB proved to be a suitable media for the production of microorganism, which in turn produces more amount of reducing metabolite, responsible for reducing metal complexes into respective metal. The color change observed for the extracellular sample was further confirmed by UV-Vis spectral analysis as part of primary confirmation. An absorption peak was observed at 370 nm and is an indication of formation of nickel nanoparticle.

The Table 1 shows yield, effect of pH and temperature, pH plays a major role in the production of nanoparticle. We subject the fungi to different pH, such as acidic pH of 3, the optimal pH and the alkaline pH of 11 fungi produced maximum production of the entire nanoparticle at pH 11 in PDB media. The production of Ni was found to be insignificant with the value of 49.3 mg/mM. *C. paspali* produces amount of Ni nanoparticle at 40°C, the yield is of 49.7 mg/mM.

Table 1: Yield, effect of pH and effect of temperature on the production of nanoparticles

Metal	*Claviceps paspali*		
	Yield in mg/mM	**PDB (pH-11, mg/mM)**	**Temperature at 40°C**
Nickel	52.0	49.3	49.7

The characteristic absorption peak at around 595 cm^{-1} is due to the formation of metal nanoparticle (Figure 1). So an absorption peak around 580 cm^{-1} confirms the formation of metal nanoparticle by the microorganisms. It also confirms the reduction of metal ions by the used fungi by the observed peaks are 675 cm^{-1}, 1162 cm^{-1}, 1551 cm^{-1}, 1654 cm^{-1} and 3362 cm^{-1}. The stretch for metal nanoparticle was found to be around 515 cm^{-1}.

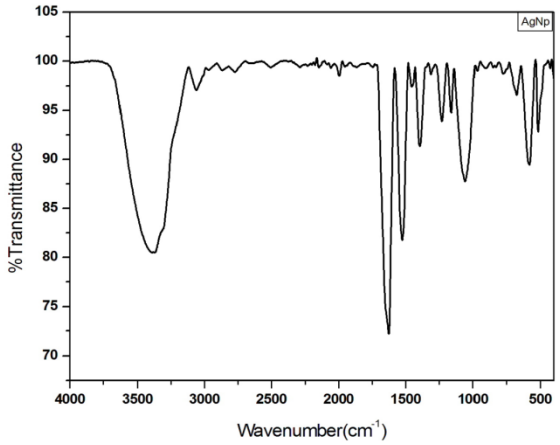

Figure 1: FT-IR spectra of nickel nanoparticle

The surface morphology of synthesized nanoparticle is studied using SEM technique, representative SEM micrographs of Ni is shown in Figure 2. The nickel nanoparticle was formed on the surface derived biological materials as indicated in figure. SEM reveals that the synthesized NiNP was powdery in form and they look almost spherical in shape. This SEM micrograph confirms the formation of metal NPs.

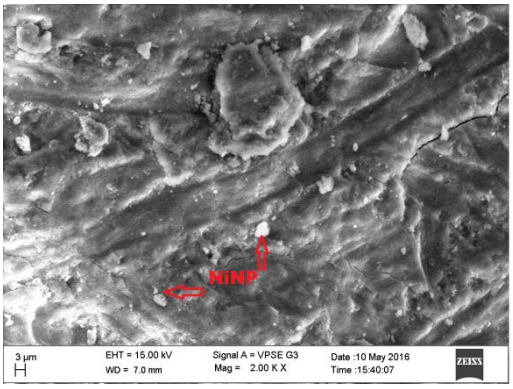

Figure 2: SEM micrographs of nickel nanoparticle

Biological activity

Antibacterial activity

Eight different Gram-positive and Gram-negative bacteria were subjected for the antibacterial activity by metal nanoparticle. Table 2 reveals that nickel nanoparticle showing antibacterial activity, the activity is not significant.

Table 2: Minimum Inhibitory Concentration (MIC) values of different metal nanoparticles

Minimum inhibitory concentration in µg								
Nanoparticle	***Salmonella typhi***	***Salmonella paratyphi***	***Proteus***	***Klebsiella***	***Pseudomonas***	***Staphylococcus aureus***	***Streptococcus oralis***	***Streptococcus mitis***
Ni	5.9	6.4	5.3	7.6	6.1	4.5	8	5.4

Antifungal activity

The synthesized metal nanoparticle was tested against *Pencillium*, *F. oxysporium* and *Helminthosporium* (Table 3). Nanoparticle showed varying degree of percentage inhibition. NiNP showed good antifungal activity against tested strain *Helminthosporium*. From the results, it is evident that the nanoparticle is inactive against *Pencillium*.

Table 3: Antifungal activity of different metal nanoparticle

Nanoparticle	*Pencillium*		*Fusarium oxysporium*		*Helminthosporium*	
	100	200	100	200	100	200
Ni	-	-	30	70	70	70

Antidiabetic activity

The Ni nanoparticle showed inhibition of alpha amylase with IC_{50} values of 58.2 µg/ml. The Ni nanoparticle showed alpha glucosidase inhibition with IC_{50} values of 64.8 µg/ml (Table 4).

Table 4: IC_{50} value of alpha amylase and alpha glucosidase inhibition

Alpha amylase		Alpha Glucosidase	
Compound	**IC_{50} µg/ml**	**Compound**	**IC_{50} µg/ml**
Acarbose	95.8	**Milglitol**	95.7
Ni	58.2	Ni	64.8

Antiangiogenic assay

The investigation of anti-angiogenic activity of metal nanoparticles in CAM assay showed (Figure 3) significant reduction of proliferation of capillaries around the zone of application of the discs loaded with the nanoparticle as compared to the control site where only the vehicle, 0.1% Polyethylene Glycol (PEG) were applied. These results indicate that the metal nanoparticle is potent antiangiogenic molecules *in vivo*. The angioinhibitory activity of the metal nanoparticle exhibiting significant positive results in the CAM assay model of developing embryos. The data shown represents the result using a minimum of six eggs in each group.

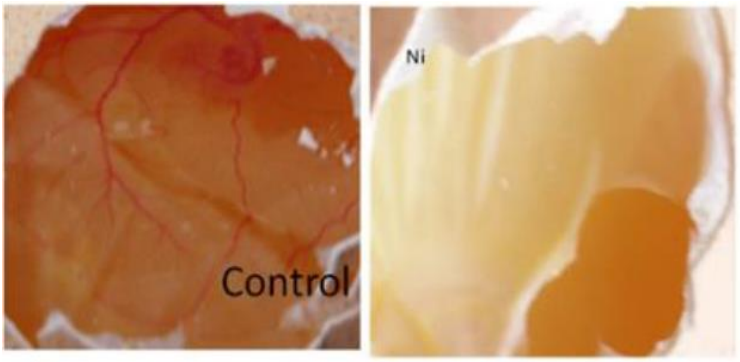

Figure 3: Chick Chorioallantoic Membrane (CAM) assay model of NiNP

Claviceps paspali was selected for the study and was found to have the ability to form metal nanoparticle as observed by change in color of the reaction. The yield of nanoparticle using the supernatant was investigated primarily through the observation of color change of the experimental samples. There is no growth of fungi in the acidic pH in any of the media and no formation of nanoparticle, only PDB media supported the growth of microorganisms, which in turn produces the secondary metabolites and proteins which could reduce the metal complexes to respective metals. Temperature is a key factor which decides the growth of organisms and its metabolism. A color change from pale yellow to brown occurred for supernatant within 24 h of incubation in the presence of light, nickel particle was confirmed by light yellow to light brown. The positive result as observed by the formation of nanoparticle was maintained throughout the 72 hrs period of observation. The metal nanoparticles comprise distinguishing color in colloidal solution due to its miniature dimension. Stabilization of the nanoparticles occurs by some capping agents which are confirmed by the sharp peaks. The color change observed for the extracellular samples were further confirmed by UV-Visible spectral analysis as part of primary confirmation.

FT-IR measurements of nanoparticle which was carried out to identify the possible biomolecules responsible for capping and efficient stabilization of the metal nanoparticle synthesized by the fungus. The FT-IR analysis confirmed the presence of carbonyl groups from the amino acid residues and proteins has the stronger capability to bind the metal designating the proteins which can probably from the metal nanoparticle, i.e. capping of metal nanoparticles which suggests the biological molecules has the capability to perform dual functions of formation and stabilization of metal nanoparticles. The image obtained by the SEM also showed, nanoparticle was spherical in shape.

In antimicrobial activity, nickel found to be a relatively better compound in killing the microorganisms. Metal nanoparticle showed better inhibition in antidiabetic activity. The investigation of anti-angiogenic activity of metal nanoparticle in CAM assay showed significant reduction of proliferation of capillaries around the zone of application of the discs loaded with the nanoparticle. The development of biogenic methods for metal nanoparticle synthesis is of considerable importance to expand their biotechnological applications, as these methods provide clean, nontoxic nanoscaled particles.

CONCLUSION

Metal nanoparticle was successfully synthesized by using biological green synthesis method using fungi. A FT-IR measurement was carried out to identify the possible biomolecules responsible for capping and efficient stabilization of the metal nanoparticle synthesized by the microbes. The SEM analysis confirms the formation of Ni nanoparticle. Antimicrobial property reveals that it shows good activity. It was found to have good antidiabetic property. It showed potent antiangiogenic activity. With the recent progress and the ongoing efforts in improving particle synthesis efficiency and exploring their biomedical applications, it is hopeful that the achievement of these approaches on a large scale and their viable applications.

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REFERENCES

[1] B. Willner, B. Basnar, *FEBS J*., **2007**, 274, 302.
[2] M.A. Willard, L.K. Kurihara, E.E. Carpenter, S. Calvin, *Int. Mat. Rev*., **2004**, 49, 125.
[3] P.L. Schiff, *Am. J. Pharm. Educ*., **2006**, 70, 1.
[4] W.P. Gary, W.K. Elmer, JB Lippincott Company, **2016**, 184, 200.
[5] D. Jakubczyk, J.Z. Cheng, S.E.O. Connor, *Nat. Prod. Rep*., **2014**, 31, 1328.
[6] C. Wallwey, S.M. Li, *Nat. Prod. Rep*., **2011**, 28, 496.
[7] F. Arcamone, E.B. Chain, A. Ferretti, A. Minghetti, P. Pennella, A. Tonolo, L. Vero, *Proc. R. Soc. B.*, **1961**, 155, 26.
[8] S. Uhlig, W. Egge-Jacobsen, T. Vralstad, C.O. Miles, *Rapid Commun. Mass Spectrom.*, **2014**, 28, 1621.
[9] S. Uhlig, C.J. Both, T. Vralstad, E. Rolen, C.O. Miles, *J. Agric. Food Chem.*, **2009**, 57, 11112.
[10] R.J. Cole, J.W. Dorner, J.A. Lansden, R.H. Cox, C. Pape, B. Cunfer, S.S. Nicholson, D.M. Bedell, *J. Agric. Food Chem.*, **1977**, 25, 1197.
[11] W. Salem, D.R. Leitner, F.G. Zingl, G. Schratter, *Int. J. Med. Microbiol*., **2015**, 305, 85.
[12] N. Khandelwal, A. Singh, D. Jain, M.K. Upadhyay, H.N. Verma, *Digest J. Nanomater. Biostruct*., **2010**, 5, 483.
[13] C. Morris, S.L. Fichtel, A.J. Taylor, *Chem. Percept*., **2011**, 4, 116.
[14] I. Schmidt, S. Thomas, P. Kain, B. Risse, *J. Neurosci*., **2012**, 32, 7466.
[15] P. Nowak-Sliwinska, T. Segura, M.L. Iruela-Arispe, *Angiogenesis.*, **2014**, 17, 779.