https://doi.org/10.48047/AFJBS.6.15.2024.4418-4437

AFJBS

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

Green synthesis, characterization, and evaluation of Silica nanoparticles from *Fusarium oxysporum*

Roshini Sri R[1], Divya A[1], Sumitha R[1]*

[1]Department of Biomedical Sciences, Faculty of Biomedical Sciences and Technology, Sri Ramachandra Institute of Higher Education and Research, Porur, Chennai-116.

Corresponding author: Dr R Sumitha Assistant Professor Department of Biomedical Sciences Sri Ramachandra Institute of Higher Education and Research (D.U.) Porur, Chennai 600116, India

Telephone : 044 -044 24768027/29 – Extn: 8296

E-mail address: sumithar@sriramachandra.edu.in

Volume 6, Issue 15, Sep 2024

Received: 15 July 2024

Accepted: 25 Aug 2024

Published: 05 Sep 2024

doi: 10.48047/AFJBS.6.15.2024.4418-4437

Abstract

Nanotechnology is a burgeoning field that plays a very prominent role in the biomedical applications. Among metallic nanoparticles, Silica nanoparticle has gained core attention in nanotechnology owing to its tunable size, shape, porosity, biocompatibility and unique properties. These silica nanoparticles are used in the field of diagnostics, theranostics and treatments. The conventional procedures used in nanoparticle production are extremely hazardous since they entail using hazardous substances, high temperatures, and expensive equipment. As a result, adopting environmentally friendly methods of nanoparticle fabrication has become critical. The green approach of nanoparticles synthesis uses biological entities, ambient parameters and easy downstream processing modalities. Urinary tract infection is a microbial infection that occurs in the urinary tract which is a major concern among women and children. One of the solutions in treating Urinary tract infections is antibiotics which in repeated use leads to antimicrobial resistance and unnecessary side effects. Other alternatives are still in the infancy stage, therefore preferring the use of nanoparticles will be able to overcome the disadvantages faced by antibiotics. The present research investigated the green synthesis of silica nanoparticles with the bioleaching of fungi *Fusarium oxysporum* from wilted banana pseudo stem and fly ash used in the cement industry. The silica nanoparticles were further characterized by Transmission Emission Microscopy (TEM) and Ultra Violet Visible spectroscopy (UV-Vis). Furthermore, antimicrobial activity of silica nanoparticles was evaluated against UTI causing pathogens Gram positive bacteria (*Enterococcus faecalis* and *Staphylococcus aureus*), Gram negative bacteria (Escherichia *coli* and *Klebsiella pneumoniae*) and fungal strains (*Aspergillus flavus* and *Candida albicans*)through Agar well diffusion test.The toxicity of these nanoparticles would also be a worry; from medical therapeutics to commercial sectors, these particles would need to be created more sustainably to avoid toxicity that might affect everyone's lives. In vitro toxicity evaluation of the biocompatibility of the nanoparticles with blood components are a necessary part of early preclinical development. The purpose of this study is to green synthesize silica nanoparticles from the fungal species *Fusarium oxysporum* and to characterize the bio transformed silica composites by XRD and FTIR to determine their size. This study also aims at performing the *in-vitro* toxicity evaluation by chromosomal aberration assay and hemolytic assay and asses the antimicrobial activity of the bio synthesized silica nanoparticle.

Key words: Silica nanoparticles, Fly ash, green synthesis, *Fusarium oxysporum*, bioleaching, and antimicrobial susceptibility

Introduction

Nanotechnology is a field of fabrication, production and application of nanoparticles in different fields of medicine [1]. The conventional methods of synthesis are Stöber, sol-gel, and flame techniques, which use high range parameters, toxic chemicals, expensive equipment, longer duration, which is hazardous, and are limited to a few sectors for applications. Green synthesis is the chosen method of obtaining nanoparticles due to its eco-friendliness, cost-effectiveness, manufacture of nanoparticles at optimal parameters without the use of harmful chemicals, and use of natural repositories.[2]. Sources of nanoparticles from natural repositories are microbes which are ubiquitous and easy to be cultured in laboratories at optimum pH and temperature. Micro-organisms are the nano factories that have both reductase and hydrolytic enzymes which act on the substrate and lead to the formation of nanoparticles. These enzymes play a prominent role as capping and reducing agents. The synthesis of nanoparticles with distinguishing morphology and unique properties entirely depends on the nano factory analog, duration of incubation, ambient conditions (pH, temperature, growth media used) and the type of source used [3,]. Another advantage of the green system is downstream processing which is facile and easier to acquire nanoparticles through green synthesis.

Among inorganic nanoparticles, Silica colloquially called as Silicon di oxide, is the chosen compound of interest to procure nanoparticles owing to its biocompatibility, good morphology, and purity. Silica nanoparticles are being used as a subsidiary agent for antimicrobial purposes [4,5]. Silica nanoparticles as nanocarriers require three intricate properties: porosity, size, and shape, therefore, providing a good pharmacokinetic and pharmacodynamic profile of the nanoparticle [6]. In addition to the above parameters like the balance between stability and degradability, biocompatibility and non-toxicity play an influential role in drug delivery systems of silica nanoparticles. Under various porous analogies, mesoporous silica nanoparticles are promising drug delivery choices owing to their tunable pore size and volume, functionalization, biocompatibility, and increased drug loading capacity [7,8]. These synthesized nanoparticles have gained attention because of the toxicity they impose on the environment during the manufacturing and disposal hence toxicity assessment is necessary. The smaller the size of the particle they are believed to be more harmful than larger nanoparticles because they can pass biological barriers more quickly [9]. Assessing the toxicity of nanoparticles is aimed at identifying the potential hazards that are useful for the safety evaluation of nanomedicines. The rapid emergence of drug resistance due to drug accumulations, drug efflux by efflux pumps and/or enzymatic degradation continues to outpace the development of new antibiotics in the treatment of infectious diseases. To increase access, nanocarrier-based targeted delivery could enable an unprecedented increase in intracellular drug transport and retention. Hence Silica nanoparticles (SiNPs) plays a vital role in drug delivery applications. The goal of this study is to green synthesize silica nanoparticles from the fungus *Fusarium oxysporum* and characterize the bio-converted silica composites using TEM, XRD, UV Vis, and FTIR to determine their size, chemical complexity, and structure, as well as to evaluate their toxicity and anti-microbial activity.

Materials and Method

Isolation and identification of *Fusarium oxysporum*

Fusarium wilt affected Pseudo stem of the banana tree was cut transversally and the sheath rich in mesophilic fungi was shredded by scalpel into the Potato Dextrose agar plates aseptically. The plates were incubated at 25°C for 72 hours. The morphological appearance of the fungal growth was viewed and was further confirmed by lactophenol cotton blue staining.

Synthesis of silica nanoparticles through bioleaching method

The isolated fungus *Fusarium oxysporum* was inoculated into Potato Dextrose Agar slants and was incubated for 4 days at 25°C. The mycelial fungi were inoculated into the 100 ml of Potato Dextrose Broth (200gms of infused potatoes, 20grms of dextrose was added to the 100 ml of sterile distilled water aseptically with a pH 5.1±0.2) in 250 ml conical flask at 25°C the culture was incubated with continuous shaking on a rotatory shaker at 200 rpm for 96 hours. After 4 days of fermentation, the mycelial growth was observed. The mycelial mass was then separated from the media by centrifuged at 3500rpm, 20°C for 20minutes. The pellet was retrieved and the supernatant was discarded. Sterile distilled water was added to the pellet, vortexed and was centrifuged at 3500rpm, 20°C for 20minutes. The step was repeated thrice under sterile conditions. The mycelial mass obtained was weighed and transferred to 500ml conical flasks containing 100ml of sterile distilled water. To the solution, about 10gms of fly-ash was added. The reactant mixture was adjusted to pH 7 which was then kept in a rotary shaker at 200rpm, 25°C for 24hrs in the dark. The resultant mixture was filtered and the filtrate obtained was kept in hot air oven for 2hrs at

60°C to obtain granular silica nanoparticles (10). The grinding of the granular silica nanoparticles procures a fine powder and was stored in an Eppendorf tube.

Characterization of synthesized silica nanoparticle

Transmission Electron Microscopy (TEM)

The morphology, size and the diameter of the bio transformed silica nanoparticles was analyzed using JEOL JEM-3010 at a voltage of 200kV. The results were obtained by adding pinch of nano silica powder to 1ml of Milli-Q water and was dissolved well. The solution obtained was micro pipetted into copper coated grid and the morphology was analyzed. The TEM analysis was carried out at IIT Guindy, Chennai.

Ultra violet visible spectroscopy (UV-Vis)

UV-Vis measurements for the synthesized silica nanoparticles were recorded in reflectance mode against the wavelength ranging from 190 to 800 nm. The measurements were performed at room temperature using UV 3092 UV Visible Spectrophotometer. About 1 gram of Barium Sulphate ($BaSO_4$) was weighed and 20 mg of the sample was taken in a mortar and pestle, grinded finely and transferred into the disc and the measurement was taken. The UV-Vis measurements was carried out at Anna University Chennai.

X-Ray Diffraction (XRD)

XRD patterns were recorded using a PHILIPS X'PERT PRO instrument equipped with a fast solid-state detector on a drop-coated sample on a glass substrate. The sample was scanned using X'celerator with a total number of 121 active channels. Iron-filtered Cu Ka radiation (l = 1.5406A˚) was used. XRD patterns were recorded in the 2h range of 20u–80u with a step size of 0.02u and a time of 5 seconds per step at 40 kV voltage and a current of 30 mA.The XRD pattern was carried out at Anna University Chennai.

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectroscopy measurements of bioleached silica nanoparticles powder was taken in a KBr pellet. Then the sample was recorded using the instrument Agilent Cary 630 FTIR spectrophotometer in the range of 650 to 4000 cm^{-1}.The FTIR spectroscopy measurements was carried out at Anna University Chennai.

In vitro toxicity evaluation

Hemolytic assay

The heparinized human blood was transferred to a 15ml centrifuge tube and centrifuged at 1000rpm for 10min. The supernatant was discarded and the pellet was suspended with equal volume of 1XPBS. The contents of the was centrifuged for 1000rpm for 10min this step was repeated until the white supernatant was obtained. The whole blood obtained out of which 100 µl of blood was added to 900 µl of PBS (1% RBC suspension). Varying concentration (20 µl, 40 µl, 60 µl, 80 µl and 100 µl) of bioleached silica nanoparticles along with positive control (100 µl of triton X) and negative control (1% RBC) were added labelled respectively to which 1ml of 1% RBC was suspended and incubated for 2hrs. The incubated Eppendorf were then centrifuged at 1000 rpm for 10 mins. 100µl of supernatant was transferred into a 96 well flat bottom plate and OD was measured at 545 nm [11]. The percentage of hemolysis due to exposure was calculated.

Chromosomal aberration assay

Chromosomal aberration assay was performed using human peripheral blood. 1ml of peripheral blood was added aseptically along with RPMI 1640 media, 20% of FBS, 400µL of Phytohemagglutinin (PHA) in a sterile culture flask. The culture initiation was done by exposing cultures to varying concentration of the bioleached silica nanoparticles (50, 100, 200 and 400 µg/ml) alongwith positive control of Ethyl Methane Sulphonate (EMS) exposed to blood culture, and negative control (unexposed blood culture). The culture flasks were incubated at 37°C for 48 hours with 5% CO_2. After 48 hours of exposure the samples were harvested, casted, stained and 100metaphases were scored and evaluated under each concentration including the positive and negative control [12].

Antimicrobial activity of silica nanoparticles

Agar well diffusion method

The antimicrobial activity of bioleached silica nanoparticles was evaluated against gram positive bacteria (*Enterococcus faecalis* and *Staphylococcus saprophyticus*), gram negative bacteria (*Escherichia coli* and *Klebsiella pneumonia*) and fungal strains (*Candida albicans* and *Aspergillus flavus*). The Mueller Hinton Agar and Potato Dextrose Agar plates were prepared for antibacterial and antifungal activity respectively. Lawn culture was performed for gram positive, gram negative and fungal strains aseptically. Wells of 7mm depth were cut in agar plates and aseptically loaded with various concentrations (20,40,60,80, and 100g/ml) of bioleached silica nanoparticles. Commercially available antibiotics Fluconazole and ciprofloxacin prepared were prepared used as positive control for fungal and bacterial strainsand negative control as distilled water were added to the wells respectively (13).

Results and Discussion

Isolation and Identification of *Fusarium oxysporum*

The wilted banana pseudo stem was collected from the farmland of Peravurani, Thanjavur district, Tamil Nadu, India, (10.2866° N, 79.2008° E). After the incubation period the fungi *Fusarium oxysporum*colony morphology was observed to be white orange spongy colonies as represented in Fig 1. The microscopic observation was done by the lactophenol cotton blue staining technique. The septate hyphae with microconidia were observed respectively. The isolated fungi were bioleached with the fly ash for the synthesis of Silica nanoparticles as shown in **Figure 1**.

Figure 1: Isolation, identification and Synthesis of Silica nanoparticles with *Fusarium oxysporum*

Characterization of synthesized silica nanoparticle

Transmission Electron Microscopy

The topographic structure and shape of the bioleached nanoparticleswas analyzed by Transmission Electron Microscopy JEOL-JEM 3010 at 200kV. The shape and size of SiNp revealed a quasi-spherical morphology with size range of 10 - 20 nm as shown in the **Figure 2** respectively. The agglomeration of the SiNp was observed during the analysis.

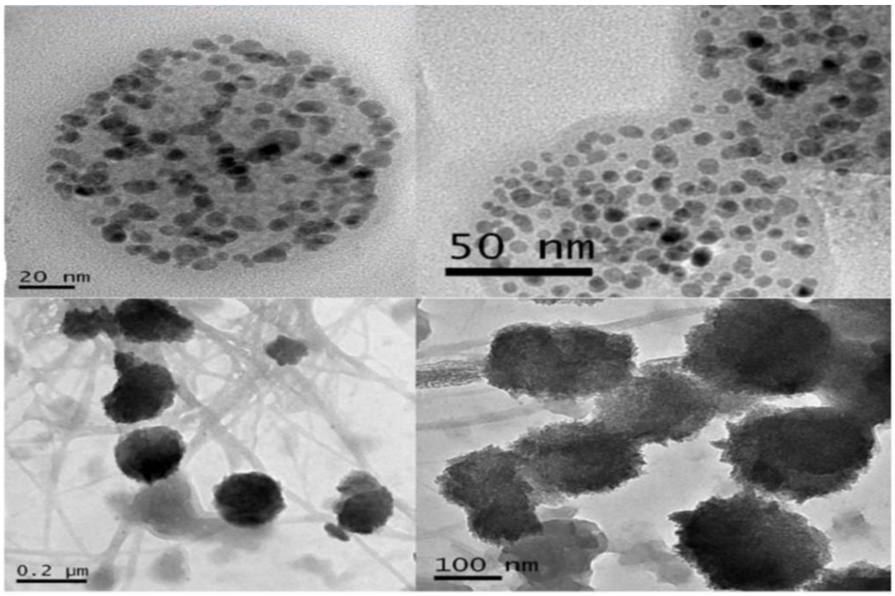

Figure 2: TEM analysis of bioleached silica nanoparticles

Ultraviolet-visible spectrophotometry

The synthesized silica nanoparticles exhibit a broad-spectrum in the UV range which was confirmed by the absorbance in the visible light region with UV–visible spectroscopy. A maximum intensity peak can be observed at ~216nm between the wavelength (200nm-300nm) which may be attributed to the signature of silica nanoparticles. The width of the peak is considerably large indicating the good crystallinity as represented in the **Figure 3**. A small hump seen in the range 300-400 nm could be due to the surface plasmon effect.

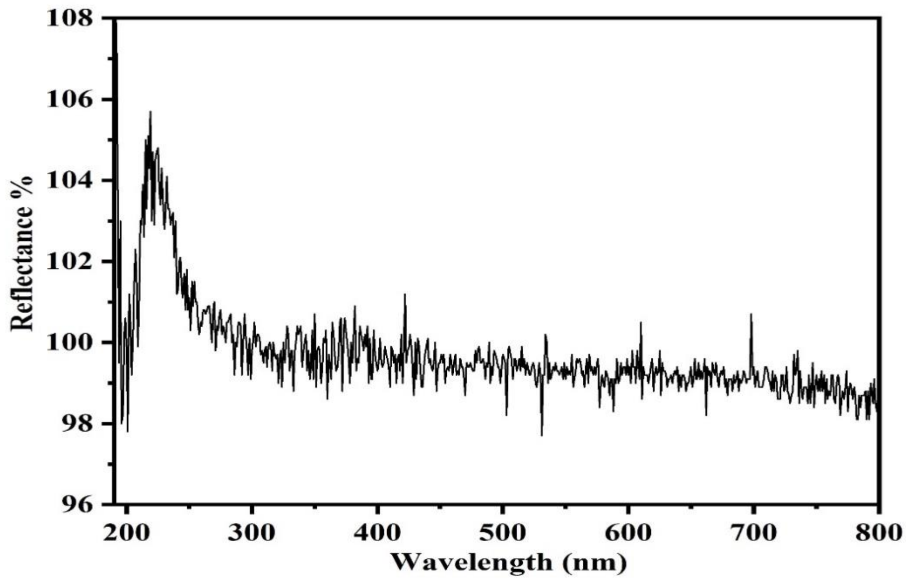

Figure 3: The UV-Vis analysis of bioleached silica nanoparticles

X-Ray Diffraction (XRD)

The bioleached silica nanoparticles were characterized by X-ray powder diffraction (XRD) to analyse the structure of silica nanoparticles. The examination of the crystal structure in XRD analysis is used to determine the crystalline phases contained in a material and hence give chemical composition information. The XRD pattern of bioleached silica nanoparticles showed peaks at {2192}, {1034}, {563}, {3000}, {422}, {048}, {051}, {514}, {057}, {622} and {631} in the 2θ range of 20°–80°C and agrees well with crystalline polymorph of silica as shown in the **Figure 4**. The X-ray diffraction analysis of Fly-ash powder showed different peaks corresponding to different components of Fly-ash (such as Al, Si, Fe, Mg and Ca). This confirmed the presence of silica nanoparticles.

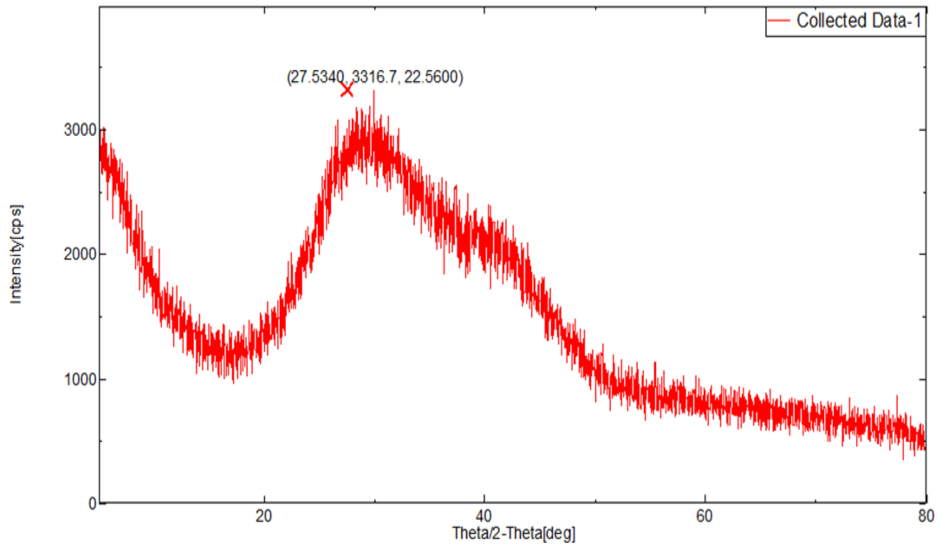

Figure 4: XRD graph of the bioleached silica nanoparticles

Fourier transform infrared (FTIR) spectroscopy:

The bioleached silica nanoparticles were characterized by Fourier transform infrared (FTIR) spectroscopy for the identification of organic, inorganic, and polymeric materials utilizing infrared light for scanning the nanoparticle sample. It enables the in-situ analysis of interfaces to investigate the surface adsorption of functional groups on the nanoparticle. The peak at 3450 cm-1 represents the Si-O-Si asymmetric stretching vibrations, 2080cm-1 indicates the and H-O-H bending, 1650 cm-1 represents the C=C stretching vibrations, 1420 cm-1 denotes the =CH bending vibrations, 1130 cm-1 represents the -C-O-C- vibrations, and 710cm-1 represents the stretching vibrations of -C-H bonds as shown in the **Figure 5**. This confirms that the synthesized nanoparticle is silica nanoparticles.

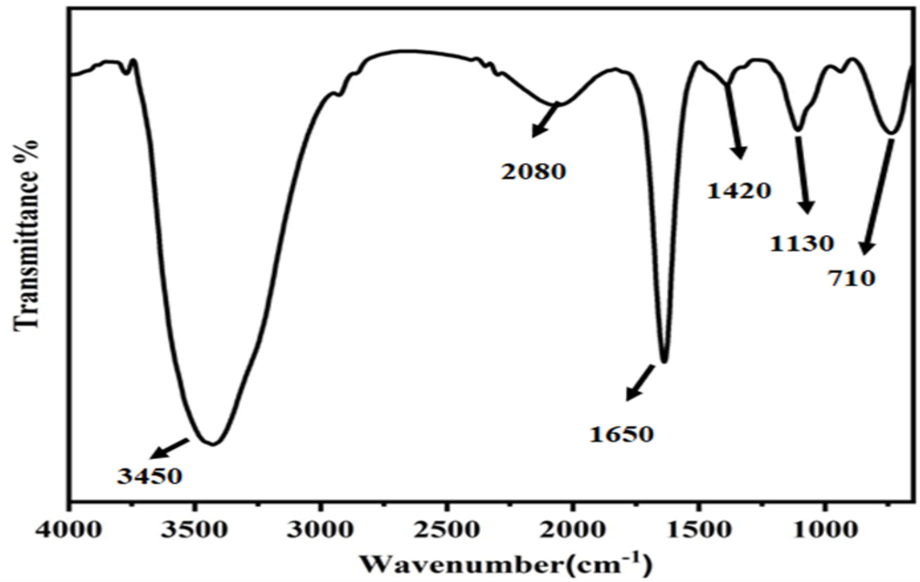

Figure 5: FTIR graph of the bioleached silica nanoparticles

In vitro toxicity evaluation

Hemolytic assay and Chromosomal Aberration assay

In vitro toxicity assessment is crucial for analyzing nanoparticles since it provides information on the nanoparticle's detrimental implications. In this study, the synthesized silica nanoparticle was tested against human peripheral blood employing hemolytic and chromosomal aberration assay. The hemolytic assay was performed with defined concentrations of bioleached silica nanoparticle (20, 40, 60, 80, 100 g/ml). The percentage of hemolysis for the defined concentration of the silica nanoparticles as shown in the **Table 1** indicates that percentage of hemolysis gradually increasing depending on the concentration on comparison with the positive control showing 100% of lysis as shown in the **Figure 6**. The results indicates that the Si NP is considered to be non-hemolytic.

Table1: Hemolytic Assay

Concentration of the Si Np	20µg/ml	40µg/ml	60µg/ml	80µg/ml	100µg/ml	Positive control	Negative control

Percentage of lysis	3.6%	3.78%	3.78%	4.85%	5.3%	100%	0

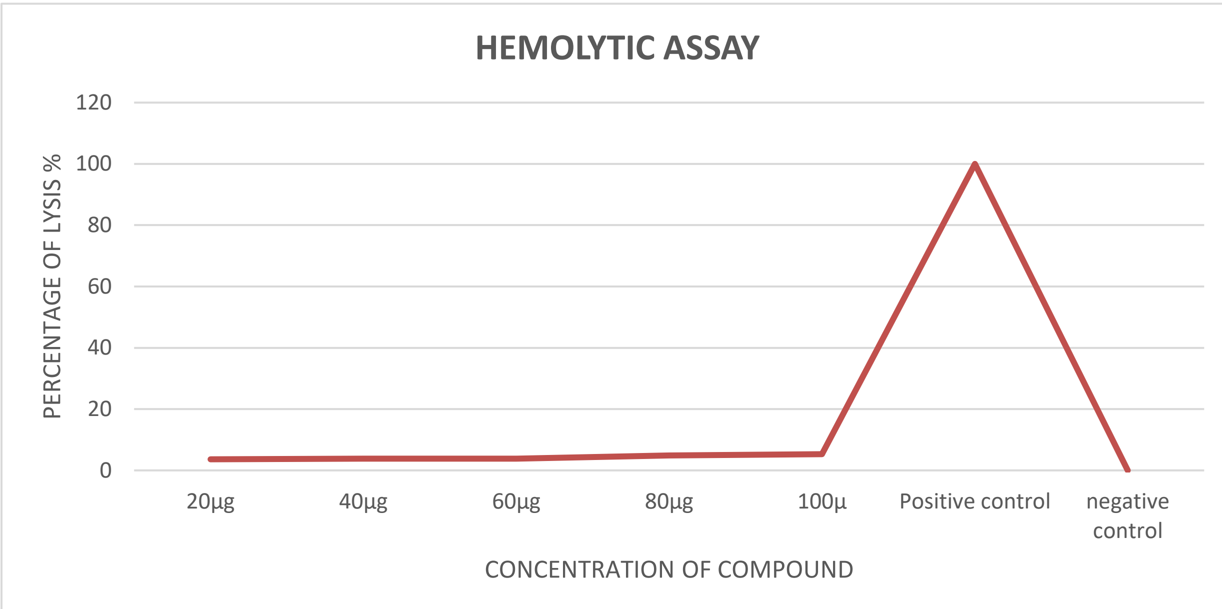

Figure 6: Hemolytic Assay

Chromosomal Aberration Assay

Chromosomal aberration assay was performed with defined concentrations (20,40,60,80, and100µg/ml) of the Si Np was exposed to the human peripheral blood. The chromosomes were isolated, casted, and stained, and 100 metaphases were scored and analyzed under each concentration as shown in **Table 2**. Microscopic examinations indicated no structural or numerical abnormalities in all of the stated concentrations as shown in the **Table 2**. 80 g/ml and 100 g/ml, showed no structural aberration but numerical deviation in 4 and 8 spreads were observed.The exposure of blood to the defined concentration of SiNp were determined to be non-genotoxic.

Table 2: Chromosomal aberration assay

S. No	Concentration of sample	The total number of	The total number of	Aberration per cell ± standard error

	(µg/ml)	metaphases scored	aberrations scored	
1	20µg	100	0	0.00
2	40µg	100	0	0.00
3	60µg	100	0	0.00
4	80µg	100	4	0.04±0.025
5	100µg	100	8	0.08±0.05
6	Positive Control	100	375	3.75 ± 0.5
7	Negative Control	100	0	0.00

Antimicrobial activity of silica nanoparticles

Well diffusion method

The microbial susceptibility testing by agar well diffusion method was performed against gram-positive bacteria, gram-negative bacterial, and fungal strains as shown in **Figure (7)**. The antibacterial activity was evaluated against gram-positive bacteria *Staphylococcus saprophyticus* strain and *Enterococcus faecalis* strain and gram-negative bacteria *Escherichia coli* strain and *Klebsiella pneumoniae* strain against the defined concentration of Si Np and the commercial antibiotic Ciprofloxacin used as the positive control. The antifungal activity was evaluated against two important strains *Candida albicans* strain and *Aspergillus flavus* (against the defined concentration of *largest zone of inhibition (*70.5±0.5*) at100*µg/ml of Silica nanoparticles was observed with the gram-negative bacilli *Klebsiella pneumoniae* and the least zone of inhibition (10.5±0.5) was at 20 µg/ml concentration with *Enterococcus faecalis* as indicated in Table3 and Figure 7. Among the bacterial strains, both gram-negative bacteria *Escherichia coli* and *Klebsiella pneumoniae* showed significant susceptibility toward the silica nanoparticle. The antifungal activity was also observed as shown in table 3 and was concluded to be good in comparison as the activity increased with increasing concentration comparable to the commercially available antifungal agent.

Table 3: Antimicrobial assessment of Silica Nanoparticle

S. No	**Pathogens**	**Zone of Inhibition (mm) Concentration (µg/ml)**					**Positive Control (Antibiotic)**	**Negative Control**
		20	40	60	80	100		
1.	*E.coli*	30.83±0.28	31.16±1.25	32.16±1.89	32.83±0.28	43.08±0.28	40.08±0.14	0
2.	*K. pneumoniae*	15.25±0.25	15.33±0.25	20.5±0.5	70.5±0.25	70.5±0.5	40.08±0.14	0
3.	*E. faecalis*	10.5±0.5	12.25±0.25	14.5±0.5	23.25±0.25	35.5±0.5	40.08±0.14	0
4.	*S. saprophyticus*	18.25±0.25	20.5±0.5	22.5±0.25	23.5±0.5	30.5±0.76	43.16±0.28	0
5.	*C. albicans*	16.25±0.25	16.25±0.25	20.5±0.5	24.25±0.25	28.25±0.25	40.08±0.14	0
6.	*A. flavus*	16.5±0.5	16.5±05	20.5±0.5	24.25±0.25	28.5±0.5	40.08±0.14	0

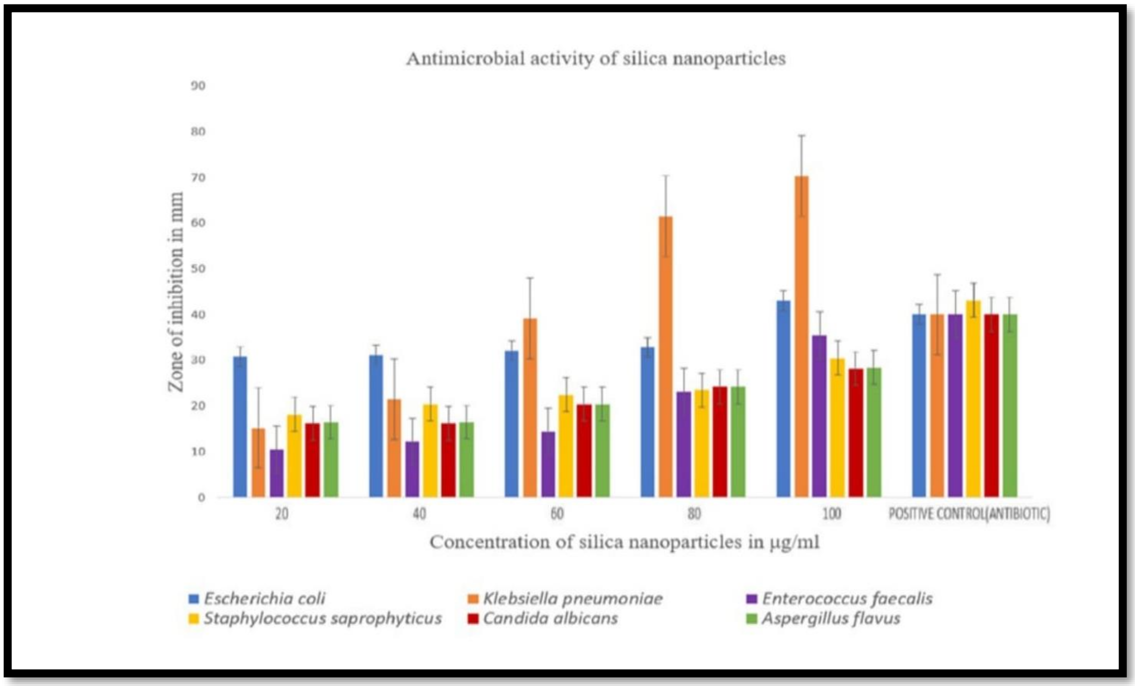

Figure 7: Antimicrobial activity of the silica nanoparticles

Discussion

Silica nanoparticles have great potential in the medical field owing to their tunable physicochemical properties like size, shape, surface functionalization, unique properties, and surface patterns since these nanoparticles have a wide range of applications and are in demand in the food industry, biomedical applications, and other economic sectors. Conventional techniques for producing silica nanoparticles involve the use of chemicals and physical techniques. Stobers technique, which was discovered in 1962 involves the use of tetraethyl orthosilicate as the surfactant, as well as a mixture of ethanol and water via hydrolysis and polycondensation [1]. Another approach of synthesizing silica nanoparticles is through the Sol-gel method that offers silica nanospheres of controlled particle size synthesized via hydrolysis of silica precursor leading to a silicic acid formation followed by condensation and the final product is Si-O-Si. The external parameters that manipulate the morphology of silica nano powders are the aging time and calcination temperature. [2, 3]. As a result, under high fabrication conditions, there are fewer, lower-quality silica nanoparticles with a regulated shape, as well as hazardous byproducts.

As an alternative to traditional methods, biological systems are currently used as nano factories for fabricating nanoparticles, a process that is not only environmentally benign but also has unlocked their potential in the realm of biomedical applications. Green synthesis using microbes, and extracts from plant and animal sources mostly contains proteins and polyphenols which replaces the chemical agents lowering the toxicity. The current study employed the bioleaching approach to create environmentally beneficial silica nanoparticles by combining fly ash and the fungus *Fusarium oxysporum*. An excellent and easily accessible byproduct of coal combustion, fly ash is employed as a precursor for the synthesis of SiNPs. The most logical approach is to use fungal-assisted bioleaching, which not only separates these metals selectively but also transforms them into their nano form, which is naturally sealed by the protein that the microorganisms synthesize during the process. Similar research has been conducted employing fungi as bio templates in the creation of nanoparticles (10). Fungal nano factories belonging to genera *Fusarium, Aspergillus, Penicillium* and *Verticillium a*re mostly employed in synthesis of silica nanoparticles. The generation of nano silica through myco-nanotechnology is one of the newest and most promising methods due to the ease of obtaining biomass, the large number of enzymes involved in the bioleaching scale-up of the nanoparticles, the reduction of toxic metal ions to moderately toxic forms, and the shorter duration of bioleaching between the biomass and substrate. The synthesized bioleached SiNp was studied using Transmission Electron Microscopy JEOL-JEM 3010 at 200kV. The microscopic imaging confirmed the morphology of the silica nanoparticles. The sizes of the silica nanoparticles vary from 10- 20 nm, which have spherical shaped, aggregated which have a tendency to form a cluster as shown in the **Figure 2** respectively. The agglomeration of the SiNp was observed during the analysis (14). For further confirmation UV-Vis spectroscopic characterization was performed for the bioleached silica nanoparticles the absorbance at 216nm indicating the presence of aromatic amino acids such as Phenylalanine, Tyrosine and Tryptophan and these amino acids were the major component of enzymes secreted by the *Fusarium oxysporum* during the process of bioleaching with fly ash (15).

The bioleached silica nanoparticles were characterized by X-ray powder diffraction (XRD) to analyze the structure of silica nanoparticles. The X-ray diffraction analysis of synthesized bioleached silica nanoparticles showed different peaks corresponding to different components of fly-ash (such as Al, Si, Fe, Mg and Ca). This confirmed the presence of silica nanoparticles. The characterization by Fourier transform infrared (FTIR) spectroscopy for the identification of organic, inorganic, and polymeric materials utilizing infrared light for scanning the nanoparticle sample were performed the peak at 3450 cm-1 represents the Si-O-Si asymmetric stretching vibrations, 2080cm-1 indicates the and H-O-H bending, 1650 cm-1 represents the C=C stretching vibrations, 1420 cm-1 denotes the =CH bending vibrations, 1130 cm-1 represents the -C-O-C- vibrations, and 710cm-1 represents the stretching vibrations of -C-H bonds which confirms synthesized silica nanoparticles.

Testing for *in vitro* toxicity is essential for assessing nanoparticles and providing insights into their potentially harmful effects. Before these biomedical nanoparticles are employed in medicine, toxicity and adverse effects need to be excluded because they can be powerful therapeutics and are likely to interact with cells [16]. The present investigation focuses on the synthesized silica nanoparticles, which were tested against human peripheral blood employing a hemolytic assay and a chromosomal aberration assay. The defined concentrations of silica nanoparticle (20, 40, 60, 80, 100 µg /ml) was subjected for evaluation. The percentage of hemolysis for the defined concentration of the silica nanoparticles sample was considered to be non-hemolytic. [17]. The chromosomal aberration assay was performed to analyze the chromosomal breaks, increases or decreases in chromosomal number, deletion, and growth ability in the culture against the bioleached silica nanoparticles with defined concentrations (20, 40, 60, 80, and 100µg/ml) exposed to the human peripheral blood. The chromosomes were isolated, casted, and stained, and 100 metaphases were scored and analyzed under each concentration. Microscopic examinations indicated no structural or numerical abnormalities. 80 µg/ml and 100 µg/ml, showed no structural aberration but numerical deviation in 10^{th} and 24^{th} spreads were observed. All four concentrations were determined to be non-genotoxic, demonstrating that the synthesized silica nanoparticle was not toxic.

Antimicrobial susceptibility testing was done to evaluate the efficacy of the green synthesized silica nanoparticle by agar well diffusion method was performed against gram positive bacteria, gram negative bacterial and fungal strains as shown **Figure (5,6,7)**. The zone of clearance was observed in all the bacterial and fungal strains at varying concentrations as shown in the **(Figure 9).** Among the bacterial strains, both gram negative bacteria *Escherichia coli* and *Klebsiella* *pneumoniae* showed significant susceptibility towards the silica nanoparticle. The antifungal activity was also observed and was concluded to be good in comparison with the commercially available antifungal agent [18, 19]. The outcome of the study indicated that, when compared to commercially available antibiotics and antifungal agents, the microbially synthesized silica nanoparticles had significant activity against both bacteria and fungi.

Conclusion

The present research demonstrates the extracellular production of silica nanoparticles through microbial synthesis using fly ash and mesophilic fungus *Fusarium oxysporum* bioleached in ambient conditions. The synthesis of silica nanoparticles was confirmed by the sample material's size, shape, phase, and chemical composition. This information corresponded to the specific role that the secondary metabolites of the mesophilic fungi played in the bioleaching and synthesis processes. The evaluation of the silica nanoparticles' *in vitro* toxicity assessment and antimicrobial properties revealed that the green synthesized Si Np was not toxic, and the current study found that it exhibited strong activity against Gram- negative bacteria when compared to commercially available antibiotics, suggesting that it is an effective antimicrobial agent. The molecular mechanism governing silica nanoparticles' antibacterial effect has to be investigated more thoroughly as part of the research's future goals.

Acknowledgement

We take this opportunity to express my deep sense of gratitude to all my professors and the staff of the Department of Biomedical Sciences and Sri Ramachandra Institute of Higher Education and Research for providing us the facilities to conduct our research.

REFERECNCES:

1. McNamara K, Tofail SA (2017). Nanoparticles in biomedical applications. *Advances in Physics*. 2(1):54-88.
2. Yang Y, Zhang M, Song H, Yu C (2020). Silica-based nanoparticles for biomedical applications: from nanocarriers to biomodulators. *Accounts of Chemical Research*. 53(8):1545-56.

3. Khandel P, Shahi SK (2018). Mycogenic nanoparticles and their bio-prospective applications: current status and future challenges. *Journal of Nanostructure in Chemistry*. 8(4):369-91.
4. Bitar A, Ahmad NM, Fessi H, and Elaissari A (2012). Silica-based nanoparticles for biomedical applications. *Drug discovery today*. 17(19-20):1147-54.
5. Karande SD, Jadhav SA, Garud HB, Kalantre VA, Burungale SH, and Patil PS (2021). Green and sustainable synthesis of silica nanoparticles. *Nanotechnology for Environmental Engineering*.6(2):1-4.
6. Kuzmin PG, Shafeev GA, Bukin VV, Garnov SV, Farcau C, Carles R, Warot-Fontrose B, Guieu V, and Viau G (2010). Silicon nanoparticles produced by femtosecond laser ablation in ethanol: size control, structural characterization, and optical properties. *The Journal of Physical Chemistry*. 114(36):15266-73.
7. Halbus AF, Horozov TS, and Paunov VN (2019). Strongly enhanced antibacterial action of copper oxide nanoparticles with boronic acid surface functionality. *ACS applied materials & interfaces*. 11(13):12232-43.
8. Michailidis M, Sorzabal-Bellido I, Adamidou EA, Diaz-Fernandez YA, Aveyard J, Wengier R, Grigoriev D, Raval R, Benayahu Y, D’Sa RA, and Shchukin D (2019). Modified mesoporous silica nanoparticles with a dual synergetic antibacterial effect. *ACS applied materials & interfaces.* 9(44):38364-72.
9. Greish, K., Thiagarajan, G., Herd, H., Price, R., Bauer,H., Hubbard, D., Burckle, A., Sadekar, S., Yu, T.,Anwar, A., Ray, A., and Ghandehari, H. (2012). Size and surface charge significantly influence the toxicity of silica and dendritic nanoparticles. *Nanotoxicology*. 6(7):713–723. doi:10.3109/17435390.2011.604442.
10. Shadab Ali Khan, Imran Uddin, Sana Moeez, and Absa Ahmad (2014). Fungus-Mediated Preferential Bioleaching of Waste Material Suchas Fly-Ashasa Means of producing Extracellular, Protein Capped, Fluorescent and Water Soluble SilicaNanoparticles. *Plos One*. 9(9);1-7.
11. Jong hoon C, Vytas R, Victoria MH, Peter LG, and Richard A. Malinauskas (2011). Physicochemical characterization and in vitro hemolysis evaluation of silver nanoparticles. *Toxicol Sci*.123(1):133-43

12. Ana PAG, Adriana CG, Diego AA, and Luiz RC (2014). Genotoxicity and DNA repair: A Practical approach. *Humana Presss*. 115-39. https://doi.org/10.1007/978-1-4939-1068-7_7
13. Reinheimer JA, Demkow MR and Candioti MC (1990). Inhibition of coliform bacteria by lactic cultures. *Australian Journal of Dairy Technol*ogy. **45**: 5 – 9.
14. I.A. Rahmana, P. Vejayakumaran, C.S. Sipaut, J. Ismail, and C.K. Chee (2009). Size-dependent physicochemical and optical properties of silica nanoparticles. *Chem. Phys*. 114 328–332.
15. Wen J, Yang K, Liu F, Li H, Xu Y, and Sun S (2017). Diverse gatekeepers for mesoporous silica nanoparticle-based drug delivery systems. *Chemical Society Reviews*. 46(19):6024
16. Sumitha, R., Banu, N., Vijayalakshmi, S., and Krithika, V. (2021). *In vitro* Toxicity Evaluation of Sterol Isolate from Echinoderm *Stellaster equestris* against Human Peripheral Blood. *International Journal of Pharmaceutical Investigation*. 11(2), 184–188. https://doi.org/10.5530/ijpi.2021.2.33.
17. Khan, S. A., Uddin, I., Moeez, S., & Ahmad, A. (2014). Fungus-Mediated Preferential Bioleaching of Waste Material Such as Fly - Ash as a Means of Producing Extracellular, Protein Capped, Fluorescent, and Water-Soluble Silica Nanoparticles. *PLoS ONE*. 9(9), e107597. https://doi.org/10.1371/journal.pone.0107597
18. Salehi S, Shandiz SA, Ghanbar F, Darvish MR, Ardestani MS, Mirzaie A, and Jafari M (2016). Phytosynthesis of silver nanoparticles using Artemisia marschalliana Sprengel aerial part extract and assessment of their antioxidant, anticancer, and antibacterial properties. *International journal of nanomedicine*. 11:1835.