

THE ANTIMICROBIAL ACTIVITY AND CYTOTOXIC EFFECT OF MOMORDICA CHARANTIA MEDIATED HAFNIUM OXIDE NANOPARTICLES

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ABSTRACT:

Aim : The field of nanobiomedicine and nanotechnology are mostly inter-related in today's world. Functionalised Hafnium Oxide nanoparticles (HfONPs) being a single agent in HNSCC exhibit potent antimicrobial properties. The various health benefits of Momordica Charantia renders it a very good anti-oxidant and anti-cancer property. The aim of the study is to prepare a Momordica Charantia mediated hafnium oxide nanoparticles and to assess the antimicrobial activity and cytotoxic effect.

Materials and method: The Momordica Charantia extract was used in the synthesis of hafnium oxide nanoparticles. These green synthesised hafnium oxide nanoparticles using Momordica Charantia were tested for characterisation using X-ray diffraction and FTIR analysis and biomedical applications using antimicrobial activity in agar well diffusion method and cytotoxic effect in brine shrimp lethality assay.

Results: The Momordica charantia synthesised hafnium oxide nanoparticles showed maximum zone of inhibition of 18 mm in *C. albicans* at 100 µg/mL and the cytotoxic effect showed minimal toxicity at 80 µg/mL.

Conclusion: In the current study, Momordica Charantia synthesised hafnium oxide nanoparticles showed antimicrobial activity especially in *Candida albicans* and cytotoxic effect showed minimal toxicity.

Keywords: HafniumOxide, Momordica charantia, Green synthesis, Antifungal, Cytotoxic effect.

INTRODUCTION :

The field of Nanotechnology is emerging today having multiple applications as such in medicinal and chemical industry.¹ While managing other materials the field of nanotechnology may improve the prognosis of the diseases like cancer, heart disease, diabetes, and kidney diseases by their incorporation of nanoparticles to obtain medicinal benefits.² Metal Oxide nanoparticles have attracted new researchers because of their unique properties.³

Among various metal oxide nanoparticles used in biomedical research activity, Hafnium (Hf) oxide nanoparticle is the field of interest. Hafnium is known as the "little brother" of titanium and zirconium. It has a large band gap ($E_g > 5$ eV), a high dielectric constant ($\epsilon = 25$), a high material density (9.6 g/cm³), a high melting point (over 2700 °C) and chemical inertness, good dielectric properties and high chemical stability.³ It can exist in three polymorphic structures such as the monoclinic (P2₁/c) at low temperature, the tetragonal (P4₂/nmc) at around 2000 K and the orthorhombic Pnma at about 2870 K. The unique properties of hafnium is its excellent corrosion resistance in aggressive environments and a very large neutron absorption cross section. Other biomedical applications of Hafnium oxide nanoparticles include anti-inflammatory and antioxidant activities. By impeding DNA damage and attenuating various metabolic pathways, these nanoparticles reduce inflammation.⁴

The Hafnium oxide nanoparticles are frequently chosen because they are generally recognised as safe and less toxic compared to other metal oxide nanoparticle.^{4,5} Among the most commonly used methods for the synthesis of nanoparticles, the green synthesis method proves to be the safest and environmental-friendly and cost effective.⁶ Momordica Charantia belongs to the subtropical vine of family Cucurbitaceae, widely grown in Asia, Africa, and the Caribbean for its edible fruit. The other names include melon, goya, bitter apple, bitter gourd.⁷

Due to the presence of many bioactive compounds, some of which possess potent biological actions, this plant is used in folk medicine all over the world for the treatment of different pathologies, mainly diabetes, but also cancer, and other inflammation-associated diseases. Majority of cell lines and animal studies had been conducted using Momordica Charantia which had been used to show the various health benefits and medicinal aspects of this herb. Momordica Charantia also commonly known as Bitter Gourd contain many primary antioxidants and nutrients.¹³ They contain various phytonutrients namely lycopene, lignans, carotenoids, and reasonable amounts of vitamin A, zeaxanthin and lutein. All these phytonutrients help in fighting free radicals that are produced in our

body as a result of body metabolism. The present study was taken to carry out the antimicrobial activity of these Momordica Charantia synthesised Hafnium oxide nanoparticles. Also, to evaluate its toxicity effects, these green synthesised HfONPs were subjected to brine shrimp lethality test.

MATERIALS AND METHODS:

1. Synthesis of Plant extract

The Momordica Charantia powder was bought from the herbal shop in Chennai. Powdered Momordica Charantia was used in the present study. Accurately 1g of each powder was taken and then added to the 100 mL distilled water. It was then boiled at 50°C for around 20 minutes. The boiling helps in activating the phytochemicals present in the plant extract. The boiled extract was filtered using muslin cloth and filtrated extract was used to prepare the nanoparticles.



(a) Powder was weighed (b) Boiling of the Plant extract (c) Hafnium oxide nanoparticles
Figure:1. Preparation of nanoparticles using Momordica Charantia plant extract

2. Preparation of Hafnium Oxide Nanoparticles

0.016g of hafnium chloride was weighed and mixed with 90 ml of distilled water. Hafnium Chloride solution was mixed with 10 ml of Momordica Charantia extract and in between U-V reading was taken to confirm the synthesis of Hafnium Oxide nanoparticle. The synthesised hafnium nanoparticle was centrifuged using 8000 rpm for 10 minutes and pellet was stored and supernatant was discarded.

A. Evaluation of Antimicrobial Activity

Using the agar well diffusion method, the antimicrobial activity of the green synthesized Hafnium oxide nanoparticles was assessed. To prepare the Mueller hinton agar plates, the plates were first autoclaved at 121 degree celsius for 15-20 minutes. The plates were then sterilized and prepared. Different types of Oral pathogen namely (*S.aureus*, *S.mutans*, *Lactobacillus* sp., *E.faecalis*, *C.albicans*) were inoculated using sterile cotton swabs and 9mm well diameter using sterile polystyrene strip. The control group was taken as Momordica Charantia plant extract.

Then, these green synthesized hafnium oxide nanoparticles at varying quantities (25 µg/ml, 50 µg/ml, and 100 µg/ml) were added to the wells. The zone of inhibition (ZOI) was measured using the ruler surrounding the wells. The antimicrobial activity of these green synthesized hafnium oxide nanoparticles were assessed and recorded in mm.

A. Evaluation of Cytotoxic Effect Using Brine Shrimp Lethality Assay

The experiment is performed in a 6-well plate containing artificial sea water and adding 10 nauplii. By dissolving 35 g of sodium chloride in 1 L of distilled water using a bottle, artificial sea is created and the dried cysts are placed in them. At a temperature of 37 degrees celsius for 48h, an incubation is made under strong aeration and illuminations and the nauplii are hatched after the incubation period. The cytotoxic effect of HfONPs in brine shrimp is evaluated. The control group was taken as plant extract. The percentage of Lethality was calculated as follows :

Percentage of Lethality = $\frac{\text{Number of dead nauplii}}{\text{Number of dead nauplii} + \text{number of live nauplii}} \times 100$.

Different concentrations of Momordica Charantia mediated Hafnium nanoparticles ranging from 5 µg/mL, 10µg/mL, 20µg/mL, 40µg/mL and 80µg/mL, respectively were added in each well. The number of surviving shrimps are counted and taken into account after 24 h.

B. Characterisation of Momordica charantia synthesised Hafnium Oxide Nanoparticles

1.X-Ray Diffraction Analysis

To Evaluate the unit cell dimensions of the Momordica Charantia synthesised Hafnium Oxide Nanoparticles with its phase identification of a crystalline material, one of the most rapid analytical technique called as X-Ray diffraction analysis was done.

2.Fourier Transform Infrared Spectroscopy

The FTIR analysis was performed for synthesized Hafnium nanoparticles from Momordica Charantia extracts revealing diverse peaks at different wavenumbers in Hafnium nanoparticles.

D.Statistical Analysis

The Results were statistically analysed using Chisquare test using SPSS software version 22.P value less than 0.05 is taken significant.

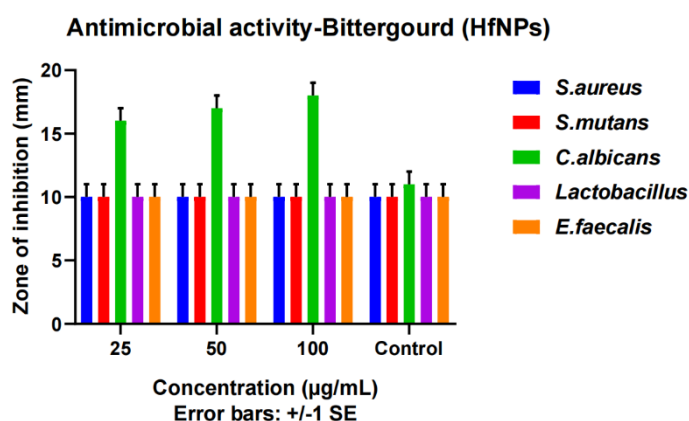
RESULT:

1.Estimation of Anti-microbial Activity

The green synthesised hafnium oxide nanoparticles exhibited a zone of inhibition of 9mm with a dilution of 100 µg/mL of nanoparticles against Streptococcus mutans, Streptococcus aureus, Lactobacillus sp. and E. faecalis while 9mm was the zone of inhibition for the standard for all of the above species. The zone of inhibition was found to be 17, 18, 19 mm for Candida Albicans at the concentration of 25, 50 and 100 µg/mL. The zone of inhibition was found to be 12 mm for the control group which was taken as plant extract.



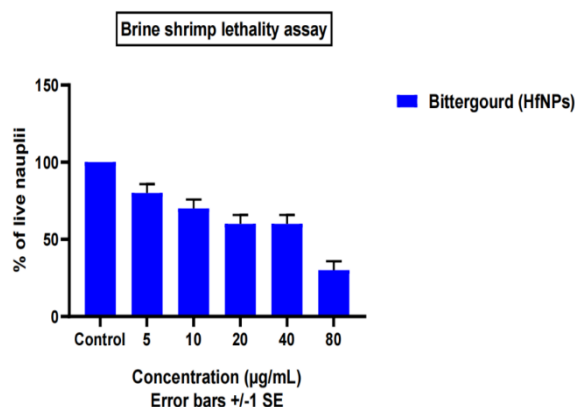
Fig.1: Anti-microbial Activity of Momordica synthesised HfNPs.



Graph.1: Zone of Inhibition of Momordica synthesised HfNPs

2.Estimation of Cytotoxic Effect

The results of the cytotoxic assessment indicated that different concentrations of the hafnium oxide nanoparticles exhibited varying effects on nauplii survival. At a concentration of 5 µg/mL, 10 µg/mL of Momordica mediated hafnium oxide, approximately 90% and 55% of the nauplii remained alive. Similarly, at concentrations of 20 µg/mL and 40 µg/mL, the HfNPs resulted in the preservation of approximately 60% of live nauplii. However, at a higher concentration of 80 µg/mL, only 40% of the nauplii survived. The control group was plant extract which showed 100% live nauplii.



Graph.2: Percentage of live nauplii in Momordica synthesised HfNPs

3.Characterisation of Momordica charantia mediated Hafnium Oxide Nanoparticles

1.X-Ray Diffraction analysis

The X-Ray diffraction peak showed the confirmation of hafnium oxide nanoparticles. Characteristic peaks were located at 49.33, 43.58, 42.38 degrees. The characteristics of these hafnium oxide nanoparticles presented with 14% crystalline phase and 85% amorphous phase. The XRD patterns of synthesized HfO nanoparticles with or without surfactant under the influence of microwave irradiation are shown in figure.2 and XRD peaks confirmed the formation of HfO in monoclinic phase.

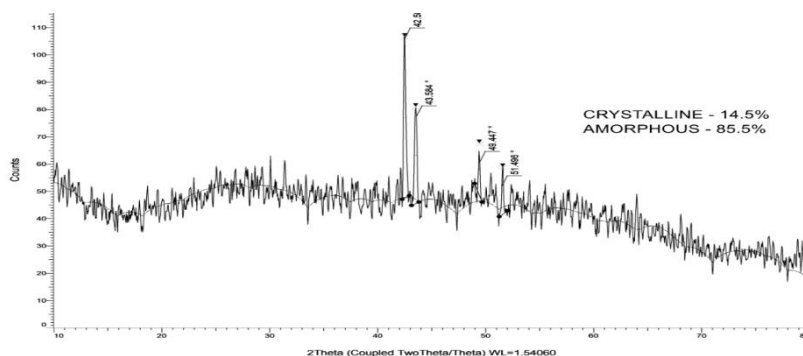


Fig.2:X-Ray Diffraction of Momordica synthesised HfNPs

2.Fourier-transform infrared spectroscopy (FTIR)

The notable peaks were observed at 3219.991 cm^{-1} , 1624.688 cm^{-1} , 1401.823 cm^{-1} , 1014.718 cm^{-1} . The broad peak observed at 3219.991 cm^{-1} was identified as a weak broad O-H stretching alcohol compound. The next peak was 1624.688 cm^{-1} which was recognised as strong C=C stretching α,β -unsaturated ketone. The other peaks were observed as 1401.823 cm^{-1} and 1014.718

cm^{-1} , which were identified as strong C-F stretching fluoro and strong C=C bending alkene compound respectively as functional groups present in the plant extract.

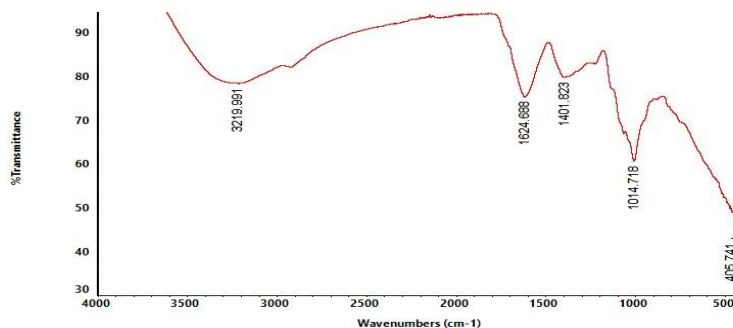


Fig.3:FTIR Analysis of Momordica synthesised HfNPs

DISCUSSION

In previous study, reported that silver nanoparticles with addition of curcumin assisted chitosan nanoparticle against *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, and *Candida Albicans* show zone of inhibition 20mm, 18mm and 16mm at concentration of 100 $\mu\text{g/mL}$ respectively. In previous research works^{10,11,12} highest zone of inhibition observed against *Staphylococcus aureus* (10mm), *Streptococcus* species (10 mm), and *Lactobacillus* species (10 mm) the inhibitory effect varied based on the concentration of the acid, with the highest zone of inhibition observed against *Staphylococcus aureus* (10mm), *Streptococcus* species (10 mm), and *Lactobacillus* species (10 mm) with lauric acid mention the sample name. On the other hand, the lowest inhibitory effect was found against *Escherichia coli* (4 mm) at the same concentration of dilution. Previous studies^{13,14,15} have also reported *Escherichia coli*, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, and *Serratia marcescens*, the antimicrobial effects of lauric acid, specifically against Gram positive streptococci but not as effective against Gram-negative bacilli such as the bactericidal activity of virgin coconut oil and lauric acid using an antibacterial disk diffusion test and reported that the bacteria-inhibiting zone was 0.17 μmol / 40 μL or 0.085 μmol / 40 μL of Lauric acid on plates inoculated with *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Streptococcus mutans*, and *Streptococcus sanguinis* and was greater than that of the paper disk containing 0.17 μmol 40 μL of virgin coconut oil.¹⁵ In the present study, the maximum zone of inhibition was shown by *C. Albicans* species of about 18mm. This can be explained due to the fact that the *Momordica Charantia* contains Recombinant alpha momorcharin which contain typical ribosomes inactivating proteins rendering this specific antifungal property. The antibacterial activity of coconut oil by agar well diffusion method using ciprofloxacin as a standard antibiotic^{16,17}.

There are two common techniques namely FTIR and UV-vis spectroscopy which offers detailed information about the local chemical environment. The XRD are sensitive to molecular vibrations and bonding characteristics thus complementing long range structural informations. XRD and spectroscopy enable a more holistic characterisation bridging the gap between atomic-scale and molecular scale interactions. In one of the study, the size of all three silver nanoparticles were smaller than their hydrodynamic diameters when viewed in X-ray diffraction technique. The presence of small discrepancy could be attributed to the fact that XRD analysis measures the crystallite size, which reflects the dimensions of the crystalline regions within the nanoparticles. The techniques like DLS method determines the hydrodynamic diameter of the nanoparticles, which accounts for the overall size of the particle, including both the nanoparticle core and its surrounding solvation shell or macromolecular components. Therefore, the hydrodynamic diameter typically appeared larger than the crystallite size observed in XRD analysis. In another study, by employing FTIR spectroscopy to analyze the silver nanoparticles, we confirmed the presence of specific functional groups and substantiated the plant extract's role as both a reducing and capping agent. There were variations in peak intensity or shifts in wavenumber suggested the involvement of these functional groups in the binding processes.

The *Momordica Charantia* mediated Hafnium Oxide nanoparticles showed significant antimicrobial activity in *C. Albicans* species when compared to other species. These green synthesised hafnium oxide nanoparticles showed moderate cytotoxic effect. These Green synthesised Hafnium Oxide nanoparticles were characterised using X-Ray Diffraction analysis which showed 14% crystalline phase and 85% amorphous phase and Fourier transform infrared spectroscopy analysis showing O-H stretching alcohol, α,β -unsaturated ketone, carbon, fluoro and alkene compound.

CONCLUSION

In conclusion, the *Momordica Charantia* synthesised hafnium oxide nanoparticles showed antimicrobial activity. These green synthesised Hafnium oxide nanoparticles were characterised using X-Ray diffraction analysis and FTIR analysis which confirmed the synthesis of hafnium oxide nanoparticles which were then tested for their cytotoxic effect. These green synthesised Hafnium oxide nanoparticles should be tested for various other biomedical tests like anti-inflammatory activity and anti-oxidant activity to render therapeutic actions.

CONFLICT OF INTEREST:

The authors have no conflicts of interest regarding this investigation.

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