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Research Article

Characterisation of Vaccinium Sect.Cyanococcus Mediated Synthesis of Hafnium Oxide Snanoparticles and its Cytotoxic Effect against Fibroblast Cell Line

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ABSTRACT

Introduction

Green synthesized Hafnium oxide nanoparticles (HfONPs) can be prepared for various other applications to address oral health concerns like bacterial infections and inflammation. Hafnium oxide nanoparticles exert their antimicrobial, anti-inflammatory and antioxidant effects through mechanisms like producing reactive oxygen species and direct interaction with microbial cells.

Aim

The present research article describes the anti-microbial, anti-inflammatory and antioxidant activity of green synthesised of Hafnium oxide nanoparticles (HfONPs) using *Vaccinium sect.cyanococcus* extract.

Materials and Methods

The *Vaccinium sect. Cyanococcus* mediated Hafnium oxide nanoparticles were developed and tested for various applications.These Hafnium oxide nanoparticles were characterized using UV-visible spectroscopy and Scanning electron microscopy.The antimicrobial activity of HfONPs was assessed using Mueller hinton agar plates. The anti-oxidant was evaluated using 2,2-diphenyl-1-picryl hydrazyl (DPPH), hydrogen peroxide (H_2O_2) assay methods. The anti-inflammatory activity was determined through the egg albumin denaturation method, the bovine serum albumin denaturation method, and the membrane stabilization assay.

Results

The present study gave insight about the anti-inflammatory, antioxidant and antimicrobial activity. There was a positive outcome when testing for Antimicrobial activity showing maximum zone of inhibition in *C.albicans*.

Conclusion

The green synthesised hafnium oxide nanoparticles using *Vaccinium sect.cyanococcus* showed an excellent antimicrobial, anti-inflammatory and antioxidant activity suggesting its potential for oral application and other inflammatory conditions.

Keywords: Hafnium Oxide, Nanoparticles, Nanoscale, radiotherapy, Oral squamous cell carcinoma, neoplasm.

INTRODUCTION

Vaccinium is a common and widespread genus of shrubs or dwarf shrubs in the heath family (Ericaceae). The genus of Vaccinium Sect. Cyanococcus species was first described by Carl Linneaus.[1]The name Vaccinium was used in classical latin for a plant, possibly the bilberry or a hyacinth, and may be derived from the Latin bacca, berry, although its ultimate derivation is obscure. The taxonomy of the genus is complex, and still under investigation. Genetic analysis indicates that the genus Vaccinium is not Monophyletic. Blueberries as the common name of Vaccinium Sect. Cyanococcus contain anthocyanins, other polyphenols and various phytochemicals under preliminary research for their potential biological effects. Anthocyanins are flavonoids which give a very good antioxidant property to the blueberries.[2]Anthocyanins and anthocyanidins, as other polyphenols and flavonoids, possess the ability to act as free radical scavengers against harmful oxidants such as reactive oxygen and nitrogen species (ROS and RNS).With strong antimicrobial activity, silver nanoparticles (AgNPs) are widely

used as one of major ingredients in industrial, daily life, and healthcare related products.[3]Their antimicrobial ability is attributed to the strong oxidative activity of AgNP surfaces and the release of silver ions to biological environments. Both factors are thought to trigger a series of negative effects on the structures and functions of cells, which finally induce cytotoxicity, genotoxicity, immunological responses, and even cell death. Other health benefits of Vaccinium include presence of phytonutrients namely Vitamins A and Vitamin C which also render them an antioxidant property of protection of cells against disease free radicals.[4]This antioxidant property of the Vaccinium inhibit tumor growth, decrease inflammation and may help to slow down the other types of cancers such as esophageal, lung, mouth, pancreatic and colon cancers. The antimicrobial activity of vaccinium has been proven due to the flavonoid fraction especially presence of anthocyanins also the antiinflammatory activity of Vaccinium is having good response against lipopolysaccharide macrophages.[5]With the benefits of all these, Vaccinium sect. cyanococcus has been employed in the present study. Green synthesis has been proven to be the safer and more environment friendly when compared to other methods and has emerged as one of the best technique to produce nanoparticles, hence in the present study we had employed green synthesis method compared to various other methods.[6]

The main objective of the present study was to assess the antimicrobial, anti-inflammatory and antioxidant activity of these green synthesized hafnium oxide nanoparticles using Vaccinium sect. Cyanococcus.[7]Due to many of the benefits of the vaccinium plant extract, the aim was to broaden the various aspects and biomedical applications of these green synthesized hafnium oxide nanoparticles.

MATERIALS AND METHODS

1. Synthesis of Plant Extract

Vaccinium sect. Cyanococcus powder was brought in the Ayurvedic shop in Poonamallee. 1g of Vaccinium sect. Cyanococcus powder was weighed and mixed with 100 mL of distilled water. The mixed solution was placed in the heating mantle for 15 to 20 mins at 50 degree Celsius and the extract was filtered using the muslin cloth and was used to prepare the nanoparticles to check their applications.

2. Biosynthesis of Hafnium Oxide Nanoparticles

0.016 g of hafnium Chloride was mixed with 90 ml of distilled water and the 10 mL of plant extract was added and then alternately stirred and shaken at 900 rpm using a magnetic stirrer or an orbital shaker for room temperature. An observation was made to assess the color change of the solution (Figure:1).The progress of the reaction was routinely monitored by measuring the UV-visible spectra at specific time intervals up to the full 24h,48h,72h duration. The solution was then centrifuged at 8000 rpm for 10 minutes after centrifugation the solution was lyophilized to make it powdered form. The powdered nanoparticles were then stored in airtight container.These nanoparticles were characterized and tested for various biomedical application.

Figure: 1. Preparation of nanoparticles using *Vaccinium sect. Cyanococcus* plant extract Powder was weighed (b) Boiling of the Plant extract (c) Hafnium oxide nanoparticles

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2. Cytotoxic Effect

Brine Shrimp Lethality Assay

To prepare the solution, 2 grams of iodine-free salt was weighed and dissolved in 200 mL of distilled water.Six enzyme-linked immunosorbent Assay (ELISA) well plates were used for the experiment, and each wellwas filled with 10-12 mL of prepared saline water. Subsequently, 10 nauplii were added slowly to each well, with different concentrations of the CTLA nanogel (5 µg/mL, 10 µg/mL, 20 µg/mL, 40 µg/mL, and 80 µg/mL) added to the respective wells. The sixth well served as a control and did not receive the nanogel. The plateswere then incubated at room temperature for 24 hours, allowing the desired effects of the nanogel on thenauplii to take place. After 24 hours, the ELISA plates were carefully observed and counted for the number of live nauplii present and calculated by using the following formula: Number of dead nauplii/Number of dead nauplii + Number oflive nauplii × 100.

3. Characterisation of Vaccinium Sect. Cyanacoccus synthesised Hafnium Oxide Nanoparticles

The Vaccinium synthesised Hafnium Oxide nanoparticles are characterised using the following characterisation techniques:

1. X-Ray Diffraction Analysis

The X-Ray Diffraction Analysis is a rapid analytical technique used to determine the phase identification of a crystalline material and can provide information on unit cell dimensions of the Vaccinium Sect. Cyanococcus synthesised Hafnium oxide nanoparticles.

2. Fourier Transform Infrared Spectroscopy

An FTIR analysis was performed for synthesized Hafnium nanoparticles from *Vaccinium Sect.* Cyanococcus extracts. The FTIR spectra revealed diverse peaks at different wavenumbers in Hafnium nanoparticles.

RESULT

The cytotoxic effects of CTLA nanogel were evaluated using the brine shrimp lethality assay. This assay is commonly employed to assess the cytotoxicity of substances by measuring their impact on the survival of the brine shrimp nauplii. In the present study, a control group without any drug was maintained to establish a baseline for calculating the percentage of live nauplii. The results of the cytotoxicity assessment indicated that different concentrations of the nanogel exhibited varying effects on nauplii survival. At a concentration of 5 µg/mL of thiocolchicoside-lauric acid nanogel, approximately 90% of the nauplii remained alive. Similarly, at concentrations of 20 µg/mL and 40 µg/mL, the nanogel resulted in the preservation of approximately 70% of live nauplii. However, at a higher concentration of 80 µg/mL, only 60% of the nauplii survived.

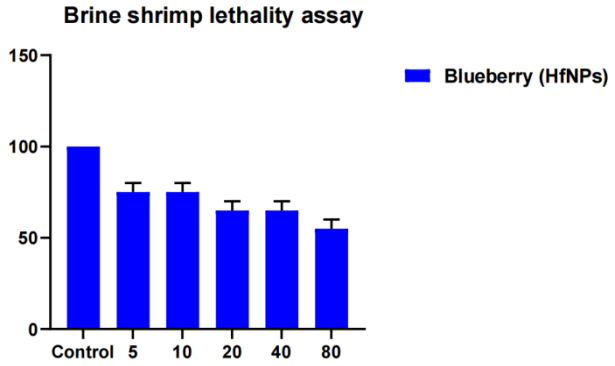

Figure: 2. Cytotoxic evaluation of *Vaccinium Sect. Cyanococcus* synthesised Hafnium oxide nanoparticles

X-Ray Diffraction analysis

It is a rapid analytical technique used to determine the phase identification of a crystalline material and can provide information on unit cell dimensions of the *Vaccinium Sect. Cyanococcus* synthesised hafnium oxide nanoparticles. The characteristics of these hafnium oxide nanoparticles presented with 14% crystalline phase and 85% amorphous phase. The X-Ray diffraction peak showed the confirmation of HfO nanoparticles. The XRD patterns of synthesized HfO nanoparticles with or without surfactant under the influence of microwave irradiation are shown in figure and XRD peaks confirmed the formation of HfO in monoclinic phase. Characteristic peaks were located at 49.33,43.58,42.38 degrees.

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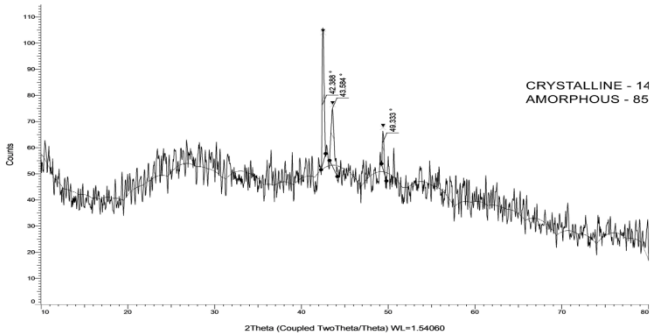

Figure: 3. X-Ray Diffraction Analysis of Vaccinium Sect. Cyanococcus synthesised Hafnium oxide nanoparticles

Fourier-Transform Infrared Spectroscopy (FTIR)

An FTIR analysis was performed for synthesized Hafnium nanoparticles from *Blueberry* extracts. The FTIR spectra revealed diverse peaks at different wavenumbers in Hafnium nanoparticles. The notable peaks were observed at 3204.712 cm−1, 1617.793 cm−1, 1358.189 cm−1, 1016.320 cm−1. The broad peak observed at 3204.712 cm−1 was identified as a weak broad O–H stretching alcohol compound. The next peak was 1617.793 cm−1 which was recognised as strong C=C stretching α,β-unsaturated ketone. The other peaks were observed as 1358.189 cm−1 and 1016.320 cm−1, which were identified as medium O=H bending phenol and strong C=F stretching fluoro compound respectively as functional groups present in the plant extract.

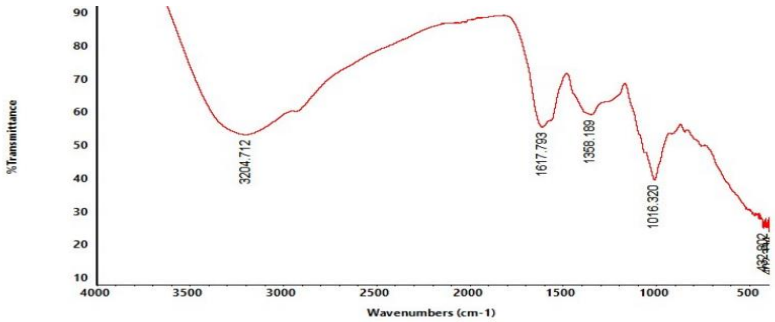

Figure: 4: FTIR analysis of Vaccinium Sect. Cyanococcus synthesised hafnium oxide nanoparticles

DISCUSSION

The present study had employed the green synthesis method of synthesizing hafnium oxide nanoparticles.[8]The various characterisation techniques and the visual observation of the successfully synthesized T. Chebula extract mediated copper oxide nanoparticles was evident in one of the study.[9]There was change in color from the initial golden brown to dark brown during the synthesis of CuONPs, similarly in the present study the presence of HfONPs was confirmed by the initial purple color and the transformation of purple to magenta color signified the completion of the green synthesis.[10]Also the Uvi-visible spectroscopy revealed the successful synthesis of Hafnium oxide nanoparticles in the present study. Many studies have consistently demonstrated the multifaceted benefits of green tea extracts having shown its anti-inflammatory, antibacterial, antidiabetic, and notably, anticancer properties.[11]

Overall, the cytotoxic activity results revealed a relatively low toxicity rate of the CTLA nanogel, which aligns with the findings of the current study. This indicates that the nanogel formulation exhibited minimal cytotoxic effects on brine shrimp nauplii, suggesting its potential safety for future applications. The cytotoxicity of AgNPs reinforced with O.tenuiflorum and S. rebaudiana. at 5 µl and 10 µl concentration, there was 0% of death of nauplii, 20 µl concentration 20% of death, at 40 µl concentration 20% of death of nauplii, and at 80 µl concentration 30% of death of nauplii.[12]By the above results, it is clearly understood that as the concentration increased the cytotoxicity of the nanoparticles increased, similarly the cytotoxicity of HfNPs mediated with *Vaccinium Sect. Cyanococcus* at various concentration of

5µg/mL,10µg/mL,20µg/mL,40µg/mL,80µg/mL was found to be increased with increase in concentration.[13]Current researches in analytical technology, particularly those associated with phytotherapy, have introduced a new era of anti-plaque therapies and natural products.[14]FTIR is a powerful analytical technique that allows for the identification of both organic and inorganic constituents. This provides valuable insights into the infrared spectra of solids, liquids, and gases. In order to help cap the AgNPs and identify the functional groups present in the biomolecules causing the bioreduction of Ag+, FTIR measurements were carried out. The presence of residual capping agent with the AgNPs was indicated by the FTIR spectra, which specifically showed main absorption bands at 3270.84 cm-1 and 3220.11 cm-1, 2915.48 cm-1, 2916.28 cm-1, 2839.85 cm-1, 2848.40 cm-1, 1703.60 cm-1, 1631.15 cm-1, 1450.63 cm-1 and 1453.09 cm-1, 1062.76 cm-1, 1040.90 cm-1, 1023.16 cm-1, and 1011.86 cm-1.[15]Similarly,in the present study, the FTIR analysis was done to specifically find the absorption bands at 3204.712 cm−1, 1617.793 cm−1, 1358.189 cm−1, 1016.320 cm−1.The XRD analysis of these *Vaccinium Sect. Cyanococcus* mediated Hafnium Oxide nanoparticles showed 14% crystalline phase and 85% amorphous phase.In one of the study[14,15], The NPs were indexed as crystalline silver having peaks at 2θ value of 38.12°, 43.83, 64.26° and 77.07° to the (111, 200, 220 and 311) planes of the face-centered cubic structure, respectively, are shown in XRD pattern.The findings of this study were correlated with the above study.

CONCLUSION

Hafnium Oxide Nanoparticles synthesized using extracts from *Vaccinium Sect. Cyanococcus* is one of the approach in developing antimicrobial agents and with nontoxic effect. It is one of the cost effective methods that may be very useful in future for nano-product development in biomedical applications.The Vaccinium Sect.Cyanococcus mediated Hafnium Oxide nanoparticles a versatile therapeutic agent show a multifaceted properties.

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