Innovations

# Green Synthesis of Hafnium Oxide Nanoparticles using *Vaccinium Sect. Cyanococcus* and its Antimicrobial, Anti-Oxidant and Anti-Inflammatory Activity

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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 mediatedHafnium 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**

Nanotechnologies have paved the way to new approaches in various biomedical applications along with various other therapeutic applications.[1] Of late, scientists and researchers are fascinated with nanomaterials for their huge potential in the various fields of application such as biosensors, tissue engineering, deoxyribonucleic acid modification, cosmetics, drug delivery systems, and medical devices.[1,2]The electrical,catalytic, optical and other physical properties of nanoparticles (NPs) are influenced by their own parameters such as the size, morphology and surface characteristics.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 (Eg > 5 eV), a high dielectric constant (r = 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]

***Vaccinium*** is a common and widespread genus of shrubs or dwarf shrubs in the heathfamily (*Ericaceae*). The genus of *Vaccinium Sect.Cyanococcus* species was first described by Carl Linneaus.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.[5]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.[6]Anthocyanins are flavonoids which give a very good antioxidant property to the blueberries.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). [7]

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.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.[9]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.[10,11]

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.*Due to many of the benefits of the *vaccinium* plant extract, theaim was to broaden the various aspects and biomedical applications of these green synthesized hafnium oxide nanoparticles[12].

**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.


Figure:1.Preparation of nanoparticles using *Vaccinium sect. Cyanococcus* plant extract

(a) Powderwas weighed (b) Boiling of the Plant extract (c ) Hafnium oxide nanoparticles

**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.

**3.Evaluation of Anti-microbial 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. 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.

**4. Antioxidant Activity of green synthesized hafnium oxide nanoparticles.**

**(a) DPPH Assay :**

1 mL of DPPH (0.1 mM) methanol and 450 μg/ml of 50 mM Tris-HCl at 7.4 pH and it was incubated at room temperature in a dark room for 30 mins.To this various concentrations of Hafnium Oxide nanoparticles (10,20,30,40,5μg/ml) were added,the antioxidant activity of these Hafnium Oxide nanoparticles were at 517 nm.This measurement indicated the presence of antioxidant activity of these hafnium oxide nanoparticles.The percentage inhibition was calculated as = (Absorbance of control - Absorbance of sample/Absorbance of control) * 100.

**(b) Hydrogen Peroxide Assay :**

Various concentrations of the green synthesized hafnium oxide nanoparticles (10-50 μg/mL) were added to 1 mL of reaction mixture with 100 mL of 28 mM of 2-deoxy-2-ribose. To that 200 mL of ferric chloride, 200 μL of ethylenediaminetetraacetic acid, and 100 mL of ascorbic acid were mixed and was incubated for an hour at 37°C and the absorbance was measured at the wavelength of 532 nm against the blank solution.The formula used to calculate the hydroxyl radical scavenging activity (%) = ((A blank - A sample)/Ablank) × 100, where A blank is the absorbance of the control reaction (without sample), and A sample is the absorbance of the reaction with the sample.

**5.Anti-inflammatory Activity of green synthesized hafnium oxide nanoparticles :**

**(a) Egg Albumin Denaturation Assay :**

The anti-inflammatory activity of the green synthesized hafnium oxide nanoparticles was determined.To prepare this assay 0.2 mL of egg albumin (fresh),0.8 mL of phosphate buffered solution at pH 6.4 and 0.2% of DMSO were mixed. To this 0.5 mL of hafnium oxide nanoparticles were added at various concentrations ranging from 10-50μg/mL. The samples were incubated for 10 minutes at 37°C and then heated at 70°C in a water bath for an additional 20 minutes to induce denaturation of the egg albumin. After cooling the mixture, the absorbance was measured at 660 nm.Diclofenac sodium was used as a positive control for the study.

**(b) Bovine Serum Albumin Assay**

The anti-inflammatory activity of these green synthesized hafnium oxide nanoparticles was assessed using bovine serum albumin assay.1% of aqueous solution of bovine serum albumin was taken in five different test tubes.To these, various concentrations of these green synthesized hafnium oxide nanoparticles ranging from 10-50 μg/ml.The pH of the solution was stabilized using 1% of hydrochloric acid.The samples were then incubated at room temperature and were then subjected to heating at 55 degree celsius and were allowed to cool down.The absorbance was measured at 660 nm.Diclofenac sodium was used as control in this experiment.The following formula was used to determine the percentage inhibition.
% inhibition = Absorbance of control - Absorbance of sample / Absorbance of control) * 100.

**(c ) Membrane stabilization assay :**

The anti-inflammatory activity of these green synthesized hafnium oxide nanoparticles were determined using Membrane stabilization assay.First the RBCs were separated from the other blood components upon centrifugation at 3000 rpm at room temperature.The RBC suspension was prepared by slowly removing the supernatant and left behind RBCs were washed three times using PBS.In order to obtain 10% (v/v) RBCs suspension the RBCs were resuspended in Tri-HCl buffer solution.

Five different test tubes were taken and 1 mL of the RBC suspension were added to each test tube.The green synthesized hafnium oxide nanoparticles were added to each test tube and incubated at 30 minutes at room temperature.The tubes were then centrifuged at 2500 rpm at 5 minutes at 37 degree celsius to pellet the RBCs.

The percentage inhibition of hemolysis is calculated as follows = % inhibition = (OD control - OD sample/OD control) * 100.

**6.Characterization of green synthesized Hafnium oxide nanoparticles**

For studying the morphological characteristics of these green synthesized hafnium oxide, Scanning electron microscopy was employed using (SEM,JEOL,FE SEM IT-800,JEOL Ltd.Tokyo Japan).To study the optical properties of these hafnium oxide nanoparticles, the absorption characteristics of these hafnium oxide nanoparticles were ascertained via the utilization of double beam UV-visible spectrophotometer (ESICO-3375).

**Statistical analysis**

Data was tabulated and transferred to SPSS statistics for windows version for statistics analysis.A one-way ANOVA and Tukey was carried out between inter groups and intra groups at five different concentrations (10,20,30,40,50 μL).Any p value less than 0.05 was considered to be significant.

**Result**

**1.Visual observation**

The initial stage of Hafnium Oxide nanoparticles synthesis took place by showing the purple colour.As the reaction proceeded further,the color of the solution gradually changed to magenta.The completion of nanoparticle synthesis took place by the transformation of the color from purple to magenta.Using the Vaccinium sect cyanococcus as a reducing agent and eco friendly as well, the synthesis of hafnium oxide nanoparticles was confirmed.

**2.UV-visible spectrophotometry**

The optical properties of these green synthesized hafnium oxide nanoparticles were confirmed using Uv-visible spectroscopy.The Uv-visible spectroscopy revealed the prominent absorption peak at about 480 nm.At various time intervals of 1h,12h,24h,36h,48h,60h there were different absorption peaks having a band width of about 500 nm.There were stable and well defined production of hafnium oxide nanoparticles with *Vaccinium* as a promising source for fabrication of Hafnium oxide nanoparticles.There was synthesis of smaller sized nanoparticles and as the synthesis proceeded the larger sized nanoparticles tend to exhibit absorption peaks at longer wavelength.The location and intensity of absorption peak provides valuable insights into the size, morphology and composition of Hafnium oxide nanoparticles.

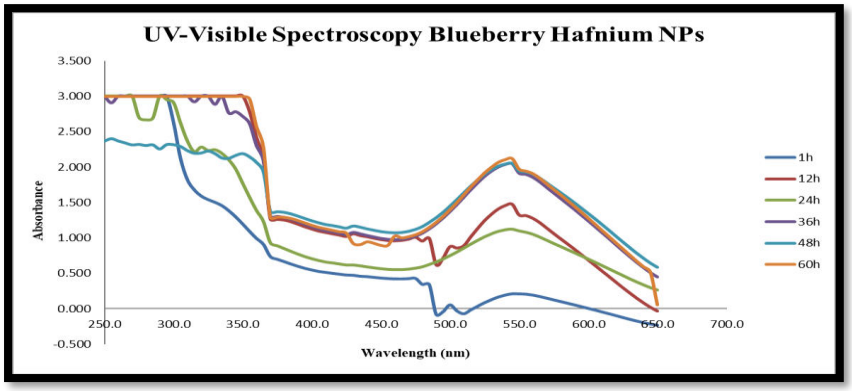



Figure:2.Uv-visible spectroscopy of *Vaccinium sect. Cyanococcus* synthesized Hafnium oxide Nanoparticles

**3.Scanning electron microscope (SEM)**

To study the morphological characteristics of these green synthesized hafnium oxide nanoparticles, these nanoparticles exhibited spherical shape and showed well aggregation of varying sizes.The approximately size of these hafnium oxide nanoparticles were determined to be 100 nm.Due to various factors like Van der waals forces, electrostatic forces of attraction and solvent evaporation effect, there was aggregation of these hafnium oxide nanoparticles which is a very common phenomenon during synthesis process.

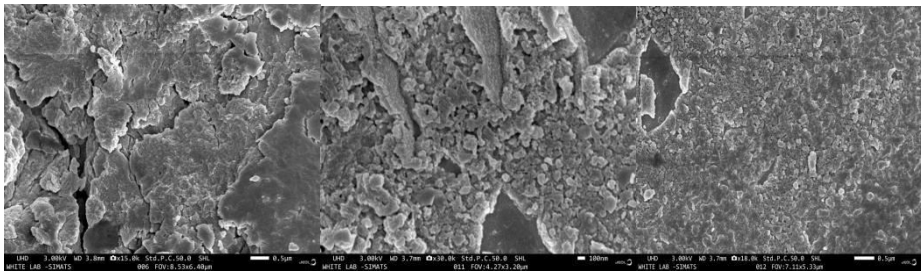
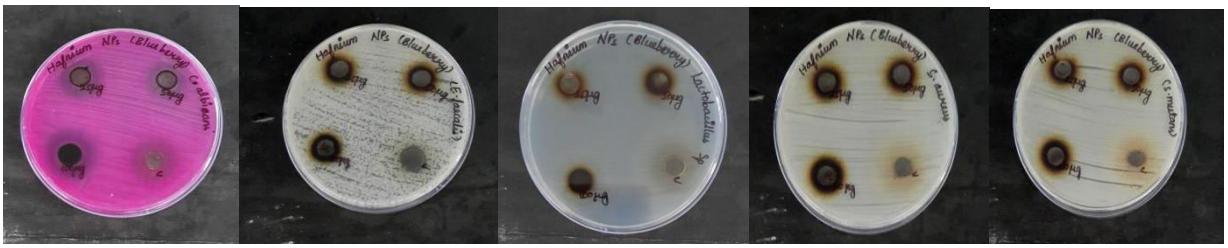


Figure:3.SEM images of *Vaccinium sect. Cyanococcus* synthesized Hafnium oxide Nanoparticles

**4.Estimation of Anti-microbial Activity**

Evaluation of antimicrobial activity using mueller hinton agar plates revealed the maximum zone of inhibition against *C.albicans* as 25 mm at 100μg/ml followed by 21mm at 50μg/ml, 20mm at 25μg/ml.The zone of inhibition against *S.aureus* was found to be 18 mm at 100μg/ml followed by 17 mm at 50μg/ml and 16 mm at 25μg/ml whereas the zone of inhibition against *S.mutans* was found to be 17 mm at 100μg/ml followed by 15mm at 50μg/ml and 14 mm at 25 μg/ml.Similarly,the zone of inhibition against *E.faecalis* was found to be 17mm at 100μg/ml, 16mm at 50μg/ml, 15mm at 25μg/ml whereas the zone of inhibition against *Lactobacillus sp.*was found to be least among all the micro-organisms having 14mm at 100μg/ml, 13mm at 50μg/ml, 12mm at 25μg/ml.



(a) (b) (c ) (d) (e)

Figure:4.Antimicrobial activity of *Vaccinium sect. Cyanococcus* synthesized Hafnium oxide Nanoparticles in oral pathogens namely (a) *C. albicans (b)E. faecalis (c ) Lactobacillus sp. (d) S. aureus (e) S.mutans*

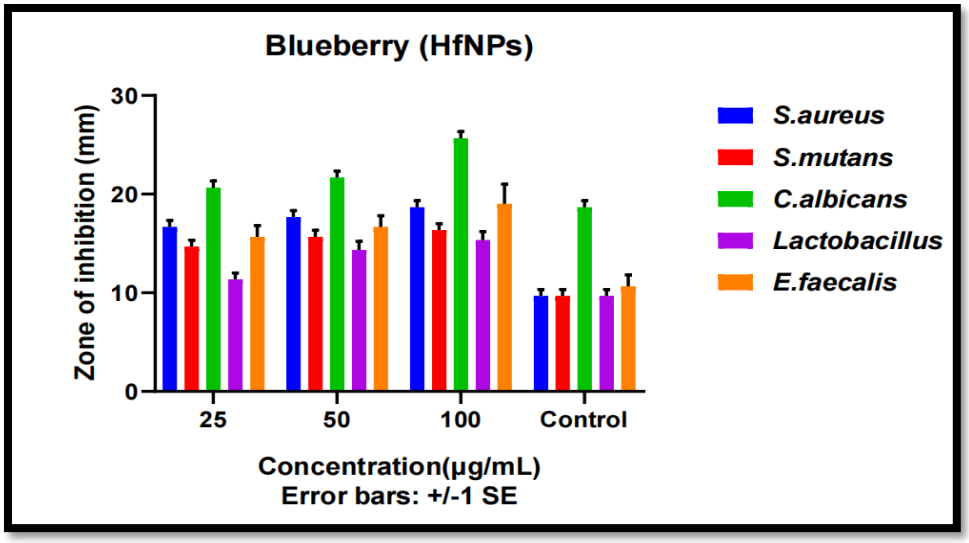
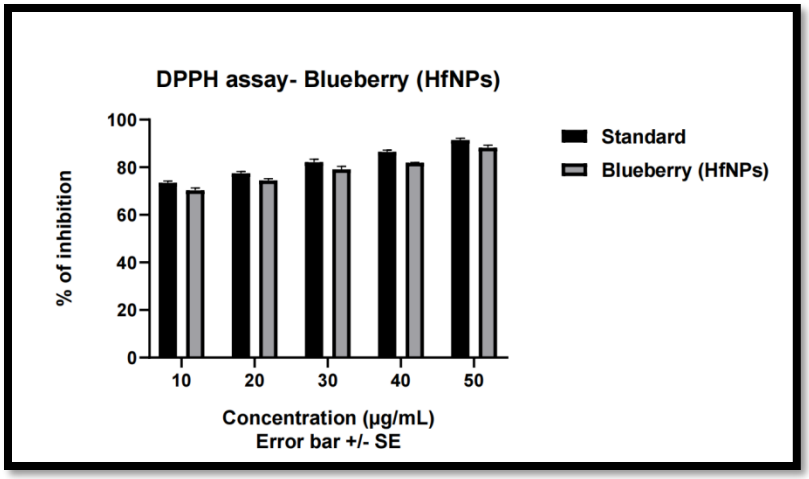


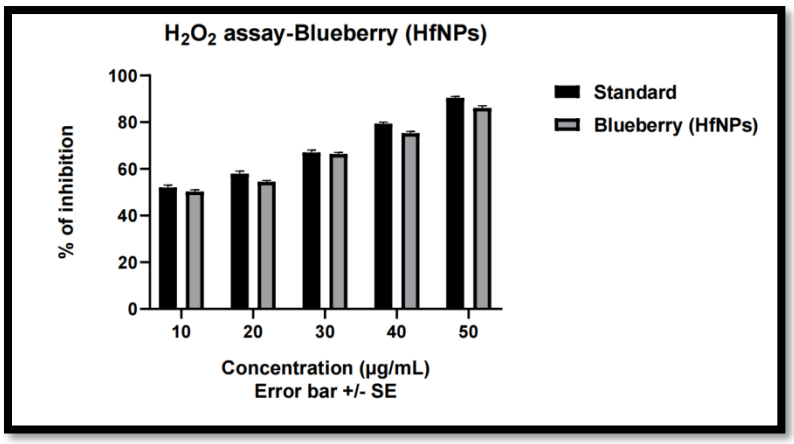

Figure:5.Estimation of Antimicrobial Activity of *Vaccinium sect. Cyanococcus* synthesized Hafnium oxide Nanoparticles

**5.Estimation of Antioxidant Activity**

In the different concentrations of these green synthesized hafnium oxide nanoparticles, 10µg/ml had a 72% inhibition, 20 µg/ml had a 76% inhibition in 30µg/ml had a 78% inhibition, 40 had a 80% of inhibition and 50 µg/ml had 90% of inhibition whereas the standard was taken as diclofenac sodium which showed the percentage inhibition of 78% in 10µg/ml, 80% in 20µg/ml, 80% in 30µg/ml, 82% in 40µg/ml and 85% in 50µg/ml.The antioxidant activity of these green synthesized hafnium oxide nanoparticles was also assessed using Hydrogen peroxide assay and it was found that there was percentage inhibition of 65% in 10µg/ml 70% in 20 µg/ml, 82% in 30µg/ml, 85% in 40µg/ml and highest percentage inhibition of 90% at a concentration of 50 mg/ml and all these values were found to be statistically significant (p value < 0.05).




(a)



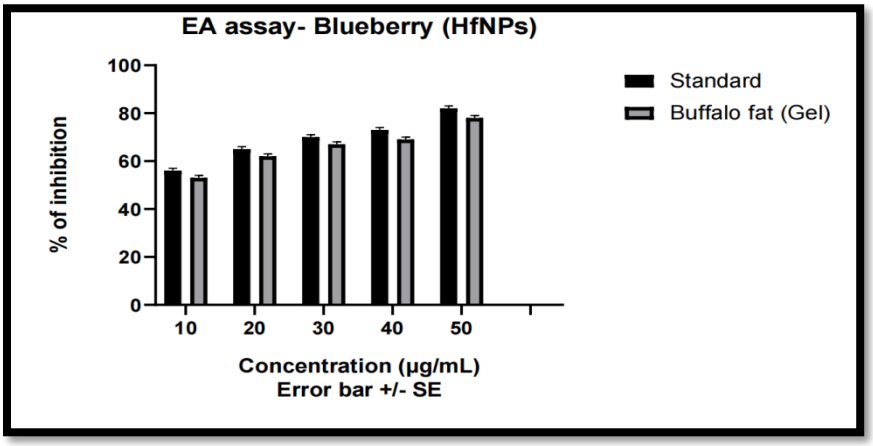

(b)

Figure:6.Estimation of Antioxidant activity of *Vaccinium sect. Cyanococcus* synthesized Hafnium oxide Nanoparticles using (a) DPPH assay , (b) Hydrogen peroxide assay

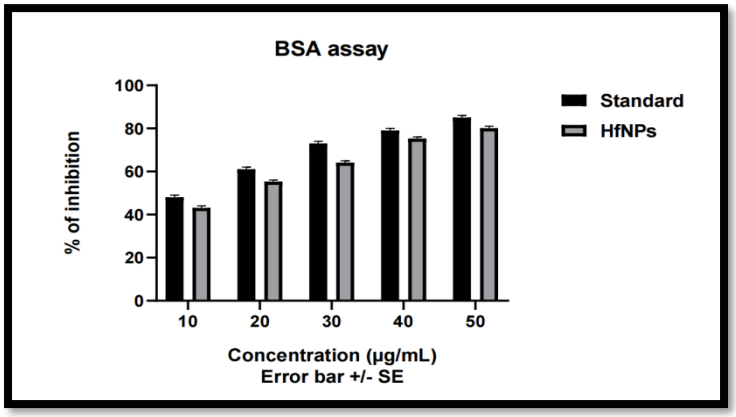
**6.Estimation of Anti-inflammatory Activity**

The results of egg albumin denaturation assay revealed a percentage of inhibition at a various concentration of 55% at 10 µg/mL,60 % at 20 µg/mL, 65% at 30 µg/mL, 70% at 40 µg/mL and 80% at 50 µg/mL whereas the results for the bovine serum albumin denaturation assay showed a percentage of inhibition at a various concentration of 10 µg/mL 42 % , 65 % at 20 µg/mL, 70 % at 30 µg/mL, 75 % at 40 µg/mL, and at 80 % in 50 µg/mL.These values indicated that these nanoparticles exhibited significant anti-inflammatory activity by inhibiting both egg albumin denaturation assay and bovine serum albumin denaturation.Moreover, the anti-inflammatory properties of these hafnium oxide nanoparticles were comparable to the standard diclofenac sodium at all tested concentrations.The results of Membrane

Stabilization Assay revealed a percentage inhibition of 55% at a concentration of 10 μg/mL, 60% at 20 μg/mL, 65% at 30 μg/mL, 70% at 40 μg/mL, and 75% at 50 μg/mL against 60%, 65%, 70%, 75%, and 80% for the standard, diclofenac sodium at same concentrations. These findings were found to be statistically significant and showed anti-inflammatory activity of these nanoparticles in the membrane stabilization assay.




(a)

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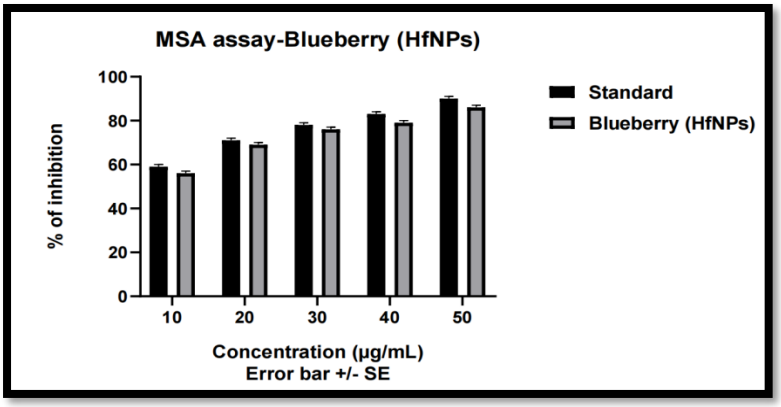


( c)

Figure:7.Estimation of Anti-inflammatory activity of *Vaccinium sect. Cyanococcus* synthesized Hafnium oxide Nanoparticles using (a) Egg albumin denaturation assay (b) Bovine serum albumin denaturation assay (c ) Membrane stabilization assay

**Discussion**

The present study had employed the green synthesis method of synthesizing hafnium oxide nanoparticles.The various characterisation techniques [13,14] and the visual observation of the successfully synthesized T.Chebula extract mediated copper oxide nanoparticles was evident in one of the study.[15] There was change in color from the initial golden brown to dark brown during the synthesis of CuONPs, similarly in the presentstudy 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.Also the Uvi-visible spectroscopy revealed the successful synthesis of Hafnium oxide nanoparticles in the present study.Many studies[16,17,18]have consistently demonstrated the multifaceted benefits of green tea extracts having shown its anti-inflammatory, antibacterial, antidiabetic, and notably, anticancer properties.

*Vaccinium*,which belongs to the *Ericaceae* family, have many health benefits and other pharmaceutical properties.They contain copper,folate,vitamin A and vitamin C as constituents which provide protection against heart disease and cancer.It helps to improve the mental health and helps in lowering the blood pressure.[18]To evaluate the antioxidant and anti-inflammatory activity of these green synthesized hafnium oxide nanoparticles, the present study provides insight into the therapeutic implications of these green synthesized Hafnium Oxide nanoparticles.

A prior study was conducted where SeNPs synthesized using *Theobroma cacao L.* bean shell extract demonstrated substantial antioxidant activity, as evidenced by their performance in both the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid).[19]The presence of bioactive compounds in *Theobroma cacao L.* bean shell extract likely contributed to the robust antioxidant properties observed in SeNPs[19].Similarly, in the present study, the *Vaccinium Sect.Cyanococcus* synthesized Hafnium oxide nanoparticles have shown remarkable antioxidant property.This can be explained due to the fact that they contain Phytonutrients namely vitamin A and vitamin C which render them this distinct antioxidant property.In the present study, the *Vaccinium Sect.Cyanococcus* synthesized HfONPs showed the consistent increase in their percentage inhibition with increase in their concentration and can be validated that these green synthesized HfONPs provide an anti-inflammatory effect, however previous studies[20,21,22] also depicted the similar results where the metal oxide nanoparticles successfully impart anti-inflammatory activity.

In the present study, the green synthesized HfONPs show the highest zone of inhibition in the *C.albicans* whereas *Lactobacillus* species were least inhibited by the nanoparticles.This could be explained by the fact that the blueberries contain gallic acid which is responsible for providing this characteristic feature of fungicidal action.

**Conclusion**

Within the limits of the present study, it can be concluded that the anti-inflammatory and antioxidant properties of *Vaccinium Sect.Cyanococcus* mediated hafnium oxide nanoparticles were found to be highest at 50µg/mL when compared to control groups.The presence of flavonoids such as anthocyanin,vitamin A and vitamin C in blueberries gives them a favorableantioxidant property.In the present study, the *Vaccinium Sect.Cyanococcus* mediated synthesis of hafnium oxide nanoparticles shows a promising antimicrobial activity against various oral pathogens predominantly in *C.albican* species.

These findings suggests that these green synthesized hafnium oxide nanoparticles have potential to act against precancerous lesions of the oral cavity such as candidal infections.Prosthetic rehabilitation of most of the acquired maxillary defects of head and neck requires careful supervision of the site.The green synthesized hafnium oxide nanoparticles in the present study with exceptional antimicrobial,antioxidant and anti-inflammatory activity can be used as an adjuvant therapy after the surgical resection at the defect site before the definitive treatment is advised at various follow up period throughout the treatment.

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