



# Green Synthesis of TiO<sub>2</sub> Nanoparticles Using Root Extract of *Asparagus racemosus* (Shatavari) and Evaluation of its Antibacterial, Antioxidant and Antidiabetic activities

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## Abstract

Since ancient times, *Asparagus racemosus*, a traditional herb with various health advantages, has been used to cure a wide range of illnesses. Researchers sparked a lot of interest in the biological methods for producing nanoparticles (NPs). This study's objective was to synthesize titanium dioxide nanoparticles (TiO<sub>2</sub> NPs) utilizing the top-down nanofabrication method. *Asparagus racemosus* root extract was used to produce titanium nanoparticles utilizing green chemistry. The nanoparticles were characterized using UV-Visible spectroscopy, SEM (Scanning Electron Microscope), and EDX. According to the SEM images the particles are spherical in shape with an average size of 89.59 nm. The antibacterial activity was done with 3 bacterial strains i.e., *E. coli*, *Pseudomonas aeruginosa* and *Bacillus subtilis*. Antioxidant activity was evaluated by DPPH and phosphomolybdate assays, the calculated IC<sub>50</sub> was 85.18±0.55 µg/ml and 66.4±1.3 µg/ml respectively. The calculated IC<sub>50</sub> value for alpha amylase assay was 46.59±2.81 µg/ml. As per the outcomes it is concluded that the plant mediated titanium nanoparticle is very potent which can be used in pharmaceutical and biomedical areas.

**Keywords:** *Asparagus racemosus*; Nanoparticles; TiO<sub>2</sub>; SEM-EDX; Anti-oxidant and Anti-diabetic Activity

**Abbreviations:** SEM: Scanning Electron Microscope; Nps: Nanoparticles; TiO<sub>2</sub>: Titanium Dioxide Nanoparticles; DPPH: 2, 2-Diphenyl-1-Picrylhydrazyl; EDX: Energy Dispersive X-Ray; TCA: Trichloro Acetic Acid; HCL: Hydrochloric Acid; H<sub>2</sub>SO<sub>4</sub>: Sulphuric Acid; NaOH: Sodium Hydroxide; SPR: Surface Plasmon Resonance; ZOI: Zone of Inhibition.

## Introduction

*Asparagus racemosus* (Shatavari), also known as shatamuli, shatawari, satavar, shatavari, satmool, etc., is a

species of asparagus that is frequently found in various areas of India, the Himalayas, and the polar regions of Australia [1]. The demand for this plant has increased dramatically as a result of its extensive medical application. The plant's roots can either be fibrous or tuberous. Sharp pine-like needles make up the stem of this plant [2]. The flowers of this plant often have a white color and a spike-like stem. It produces berry-like fruit that comes in a variety of colors, including purple, black, and others. The picture of the plant has been shown in Figure 1.



**Figure 1:** Shows the leaves and roots of the plant *Asparagus racemosus*.

### Uses of *Asparagus racemosus*

One of the most significant herbal medicines in the world is this one. Using various components of this plant, numerous varieties of extract are produced [3]. This plant's root extract is used to treat a variety of female hormonal issues, including those related to reproduction. It has no negative side effects on nursing moms' infants or their own. Patients with colds and other illnesses are administered boiling water made from the plant's roots.

### Minimize Menopause

This herb has been used for centuries to cure a variety of conditions, including the female reproductive system. According to study conducted by modern experts, the *asparagus racemosus*, when taken with other medications, can lessen the symptoms of menopause in women. 117 women participated in a test that was done in 2018. Following a 12-week continuous course of this herb and a few others, those women reported reduced night sweats and hot flashes [4].

### Antianxiotic

This herb and several of its supplements have both historically been used to treat sadness and anxiety. Even though there is no proof that these effects have been observed in humans, studies have suggested that they may exist because they were tested on mice, suggesting that they may also apply to humans. According to certain studies, *Asparagus racemosus* reduces mice's anxieties through interacting with the serotonin hormone and certain acids, which together make up people's and mice's concerns. It has antidepressant properties [5].

### Antioxidants

Many herbs and medications have this quality. This property shields the body against several toxins and dangers

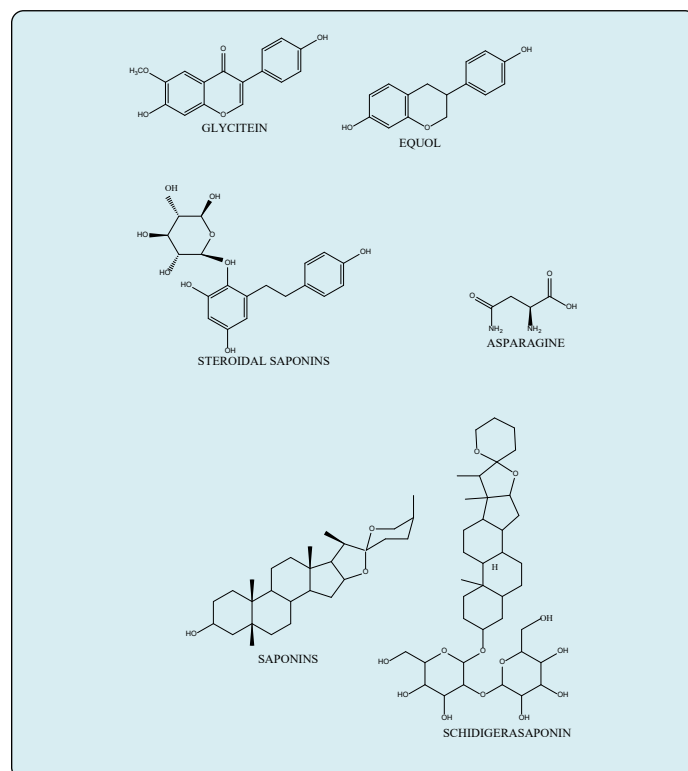
brought on by free radicals, which can seriously damage the body's cells and tissues and even result in the growth of cancerous cells. Thus, this feature also works well against oxo-stress, which can lead to a number of other disorders. We definitely need additional research in this area because numerous studies and evaluations have indicated that *Asparagus racemosus* contains certain antioxidants. Further research revealed that this plant had an antioxidant impact on rats. Asparagamine and racemsol, two different forms of antioxidants, are present in *Asparagus racemosus*. This plant has more saponins than average, which are also known for antioxidant property [6].

### Immunity

It is an ayurvedic herbal immune system booster. In 2004, a demonstration was carried out in which the animals were induced with the roots of *Asparagus racemosus* had acquired some antibodies to some strain of cough which is then distinguished with the un-induced animals. In contrast to the other animals, the ones who were stimulated recovered faster. The immunological response is thus demonstrated [7].

### Chemical Constituents of *Asparagus racemosus*

Its root was used to create polycyclic alkaloids, asparagmines, steroidal saponin, and schidigersponin. Shatavaroside A, B, and several isoflavone varieties have also been discovered there.



Nanometers (1–100 nm) are the units used to measure the diameter of nanoparticles, which are very small particles [8]. Both naturally occurring events and human activity can produce nanoparticles [9]. Manufactured nanoparticles could be helpful in a number of fields, including as engineering, catalysis, medicine, and environmental remediation [10]. These nanoparticles display a wide range of physical and chemical properties because of their nanoscale size and vast surface area [11]. This is as a result of their particular material properties and submicroscopic size. The substance known as titanium dioxide is created when one titanium atom and two oxygen atoms come together. As photocatalysts, titanium dioxide nanoparticles may harness the energy of light to speed up chemical reactions with other molecules at low temperatures [12]. Although there are other photocatalytic substances, researchers have found that titanium dioxide works best in sunlight [13].

The purpose of this study is to synthesize  $\text{TiO}_2$  nanoparticles using *Asparagus racemosus* root extract and to assess the antibacterial and phytochemical properties of these nanoparticles in vitro. According to the literature, it has been discovered that the Kumaon region of Uttarakhand has not yet conducted any research on antibacterial, in-vitro phytochemical screening, evaluation of antioxidant and antidiabetic activity.

## Materials and Methods

### Materials

**Plant Sample Collection:** The fresh roots of *Asparagus racemosus* (shatavari) were collected from Dineshpur U.S.N, Uttarakhand in the month of July. The plant often reaches a height of 1 to 2.1 meters, and its roots are the most important part and is edible and it may be fibrous or tuberous. The roots are spindle-shaped, fascicled, fleshy, light ash-colored on the outside and white on the inside [14]. It was firstly described in the year 1799.

**Chemicals:** Ethanol, distilled water, Sodium phosphate dibasic (heptahydrate), Trichloro acetic acid (TCA), Ferric chloride, Sodium phosphate monobasic (monohydrate), Lead tetra acetic acid, Copper sulphate, Mayer's reagent, glacial acetic acid, Potassium ferrocyanide, and Sodium bicarbonate was obtained from Hi Media Laboratories Pvt. Ltd. Sulphuric acid ( $\text{H}_2\text{SO}_4$ ), Hydrochloric acid (HCl), Ascorbic acid, and Sodium hydroxide (NaOH) were procured from Sigma Aldrich, India. Ammonium molybdate (tetrahydrate), and 2, 2-diphenyl-1-picrylhydrazyl (DPPH), were obtained from Central Drug House (P) Ltd. India. From Central Drug House (P) Ltd. in India, we obtained 2, 2-diphenyl-1-picrylhydrazyl (DPPH), ferric chloride, ammonium molybdate (Tetrahydrate), di-

nitro salicylic acid, alpha amylase, acarbose, starch, agar nutrient, and sodium phosphate buffer.

### Methods

**Preparation of root extract:** *Asparagus racemosus* (Shatavari) fresh roots were procured in Dineshpur (U.S.N), Uttarakhand, India. The roots were then completely rinsed with water and subjected to a 15-minute sonication, then washed once more. The roots were sliced into smaller pieces after being air dried at room temperature. After cutting, it was ground to a coarse powder and then dried for another 22 hours in a dark place. In order to prepare the soxhlet assembly, 120 ml of ethanol were used as the solvent, and 15 grammes of AR roots were measured and added to the soxhlet apparatus. Then held at 70 degrees for three hours as shown in Figure 2 [15]. Lastly, it is distilled using a distiller, and surplus ethanol is expelled, creating extract.

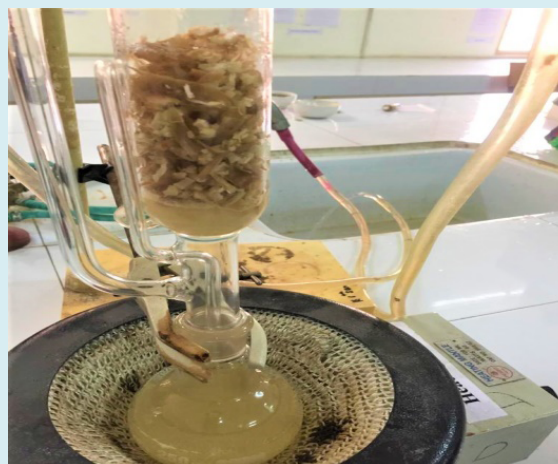
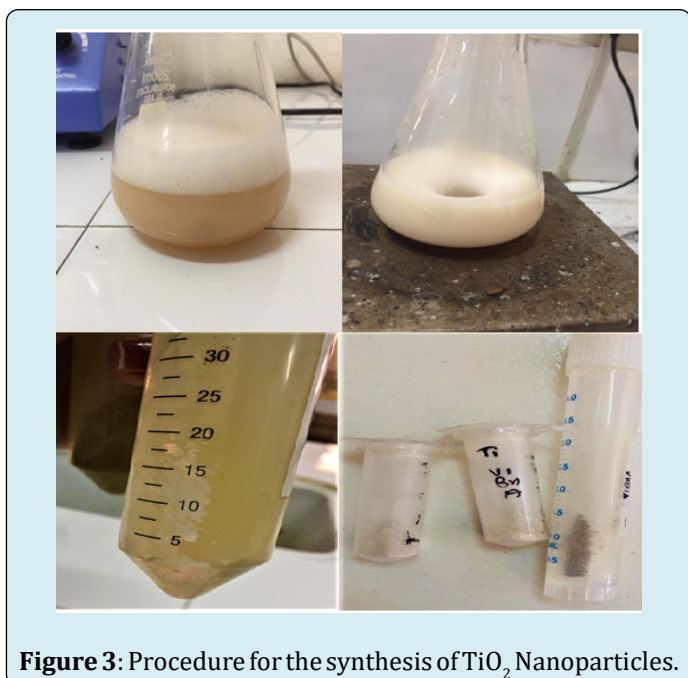


Figure 2: Extraction of plant material.

**Green synthesis of  $\text{TiO}_2$  nanoparticles by using root extract of *Asparagus racemosus*:** The newly obtained root extract was added to a 1.0M solution of titanium tetrachloride made in 80 ml of distilled water, which was then agitated for 3.5 hours at room temperature using a magnetic stirrer at 1700–1800 RPM. The hue had changed noticeably. It was pinkish white at first and then took on a curdy white color which is another sign of the reduction process. Now, the created nanoparticles were centrifuged at 8000 rpm for 30 minutes [16]. After being thoroughly rinsed three times with distilled water, the easily identifiable solid residue was finally dissolved in a small amount of ethanol and heated in an oven at 85° C before being collected in the vial as depicted in Figure 3.



**Figure 3:** Procedure for the synthesis of  $\text{TiO}_2$  Nanoparticles.

**Preliminary Phytochemical Screening Test:** According to the procedure opted by Bachhar, et al. [17] and Jayashree, et al. [18], the extract was carried out for phytochemical screening assays for the detection of plant secondary metabolites, tannins, saponins, flavonoids, terpenoids, alkaloids, cardiac glycosides, and carbohydrates.

- **Test for Saponins:** By adding a tiny amount of 2N HCl, shaking the extract with a small amount of water, and then adding a few drops of Mayer's reagent, the saponin content of root extract was ascertained. It is a sign that saponins are present if the foam formed [19,20].
- **Test for Alkaloids:** Heat 2 ml of root extracts with 10% NaOH solution to perform an alkaloid test. The presence of the white precipitate was considered evidence of alkaloids [21].
- **Test for Tannins:** Heat 2-3 ml of root extract with concentrated  $\text{HNO}_3$  in presence of ammonia. The development of white ppt. is a sign of tannins [22].
- **Test for Carbohydrate:** Fehling's A and B solution was heated and applied to the extract. The existence of carbohydrates is indicated by the orange-red color of the precipitate [23].
- **Test for Flavonoids:** The existence of flavonoids is shown by the appearance of green color after adding a few drops of a neutral ferric chloride solution to the alcoholic solution of the plant extract [24].
- **Test for Amino acid:** Add 3 drops of ninhydrin in 3 ml of plant extract and put it into a water bath for about 15 minutes, the formation of violet/purple color indicates the presence amino acids [25].

### Characterizations of Nanoparticles

- **UV Spectroscopy:** The characterization of *Asparagus racemosus* NPs was carried out by using UV spectroscopy (Shimadzu UV—1900 spectrophotometer). The UV-visible spectrometer, which has a wavelength range of 200-800 nm, was used to examine the first characterization of the nanoparticles. The synthesis of *Asparagus racemosus* NPs has been validated by the SPR (Surface Plasmon Resonance) band at 352 nm.
- **SEM-EDX:** The elemental composition of all the synthesized *Asparagus racemosus* NPs in the study was carried out by Energy Dispersive X - Ray Analysis (EDAX). The microstructural study was carried out by Scanning Electron Microscopy (SEM) using Scanning Electron Microscope EVO 18 (CARL ZEISS) for identifying structure for identifying the material property.

**Antibacterial Activity:** The antibacterial activity of *Asparagus racemosus*  $\text{TiO}_2$  nanoparticles was assessed using the disc diffusion method. Bacterial growth inhibition was left in place for the entire night. The bacteria used in this experiment were *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa*. These bacteria were daubed into the Nutrient Agar using Whatman filter sheets and tiny, circular discs of paper that were cut to a diameter of 6 mm and coated with a nanoparticle of  $\text{TiO}_2$ . The solutions were made by adding 5 ml of water to NP concentrations of 20%, 40%, and 60%, respectively. After the paper disc and plates had been autoclaved and sterilized, the bacteria culture was incubated in the incubator for roughly 24 hours at a temperature of 38°C.

### Antioxidant Activity

- **DPPH Assay:** According to the methodologies suggested by Santhoshkumar, et al. [26]. Sample stock solutions (1.0 mg/ml) were diluted to final concentrations of 20, 40, 60, 80, 100  $\mu\text{g}/\text{ml}$ , in ethanol and sonicated for 20 minutes. To analyze solutions of various concentrations, 1 ml of a 0.3 mM DPPH ethanol solution was added, and the mixture was left to react at room temperature. The absorbance readings were determined at 517 nm after 40 minutes. Using the following formula, the % radical scavenging activity was determined:

$$\text{RSA}(\%) = 100 \frac{(A_0 - A_1)}{A_0}$$

Where  $A_0$  was the absorbance of the control reaction (Without AR NPs) and,  $A_1$  was the absorbance of the sample. DPPH solution with ethanol was used as a negative control. The positive controls were those using the standard solutions. The  $\text{IC}_{50}$  values were determined by linear regression of plots, where the ordinate indicated the average percent of antioxidant activity from three different experiments and

the ordinate represented the concentration of the analyzed AR NPs.

- **Phosphomolybdate Assay:** *Asparagus racemosus* NPs was evaluated for antioxidant activity using phosphomolybdate assay according to the methodologies suggested by Sujatha, et al. [27]. Sample (20, 40, 60, 80, 100  $\mu$ l) and 1 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) were taken in the test tubes. The test tubes were capped tightly with aluminum foil and kept in a water bath for 1.5 hour at 95 °C. The samples were then cooled to room temperature and the absorbance was measured. The following formula was used to evaluate the % antioxidant activity.

$$\% \text{ Inhibition} = 100 \frac{(A_0 - A_1)}{A_0}$$

Where,  $A_0$  was the absorbance of the control reaction (Without *Asparagus racemosus* NPs) and,  $A_1$  was the absorbance of the sample.

**Antidiabetic activity:** The assay was carried out following the standard protocol with slight modifications. 600  $\mu$ l of *Asparagus racemosus* NPs with concentrations of 10, 20, 40, 60, 80, and 100 g/ml was mixed with 1.2 ml of starch in phosphate solution (pH 6.9) and 6.7 mM sodium chloride. 600  $\mu$ l amylase were added to start the reaction, which was then left at 37 °C. From the aforementioned mixture, 600  $\mu$ l were taken, 300  $\mu$ l of DNSA were added, and the mixture was then placed in a boiling water bath for 15 minutes (1 g of DNSA, 30 g of sodium potassium tartrate, and 20 mL of 2N sodium hydroxide were added). With 2.7 ml of water added to the reaction mixture, the absorbance was measured at 540 nm. In order to prepare blank, the enzyme solution was removed and replaced with 600  $\mu$ l of distilled water. Similar steps were taken to make the control, which represented 100% enzyme activity and contained no *Asparagus racemosus* NPs. Three times the tests were conducted using the same procedure [28]. The  $IC_{50}$  values were calculated from the graph by plotting the percentage of alpha-amylase suppression against the *Asparagus racemosus* NPs content. The  $\alpha$ -amylase inhibitory activity was calculated as follow:

$$\text{Inhibition (\%)} = (1 - A_s / A_c) \times 100$$

where  $A_s$  and  $A_c$  are the absorbance of the sample and the control respectively.

**Statistical Analysis:** MS-Excel and origin 9 pro were used for the calculations and graphs. Every experiment was done in triplicate and the results are presented in mean  $\pm$  standard deviation.

## Result and Discussion

### Phytochemical Analysis

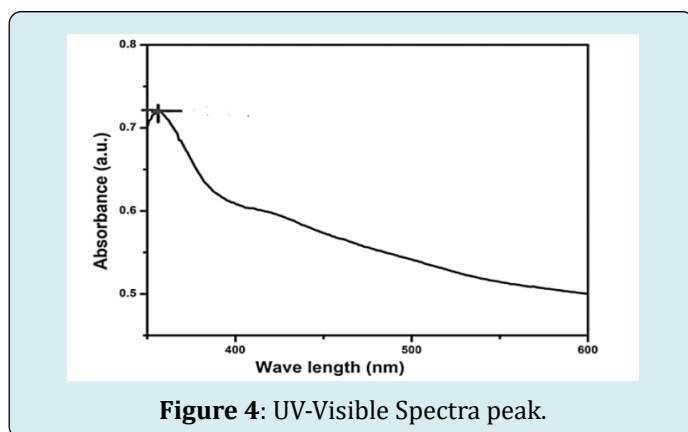
It has been noted that ethanolic root extract includes a variety of bioactive components, including primary and secondary metabolites. A preliminary phytochemical screening revealed the presence of alkaloids, saponins, steroids, and tannins. The use of root extract in the treatment of illnesses with probable bacterial aetiologies is therefore suggested and supported by our research. The results are represented in Table 1.

S. no	Phytochemical	Result
1	Saponin	+
2	Alkaloid	+
3	Tannins	+
4	Carbohydrates	+
5	Flavonoids	+
6	Amino acid	+
(+)= presence and (-)= absence		

**Table 1:** Results of Preliminary Phytochemical Analysis.

### UV-Visible Spectroscopy of $TiO_2$ NPs

Greenly produced  $TiO_2$  nanoparticles are analyzed using a UV-Visible spectrophotometer. The resulting graph shows the strong peak at 352 nm with an absorbance less than 1 very clearly. Significant peak at 352 nm in the wavelength range of 200 to 600 nm was discovered, providing evidence that  $TiO_2$  nanoparticle formation has occurred. The peaks in our results were comparatively extremely comparable to the peaks of conventional  $TiO_2$ , and they were also matched with the literature and numerous online sources [29,30]. The nanoparticle's UV peak is depicted in Figure 4.



**Figure 4:** UV-Visible Spectra peak.

### Scanning Electron Microscope (SEM) Analysis

It was utilized to determine the shape of a nanoparticle that was synthesized in a more environmentally friendly manner. The shape and size of the nanoparticles were confirmed by SEM pictures as shown in Figure 5; in accordance with the visual results, the particles were spherical, and the size was determined using a particle size analyzer [31]. The tiny circular particles were clustered together and tightly packed. The sample underwent EDX analysis as shown in

Figure 6, which confirms the presence of the component in the nanoparticles [32]. The most prevalent component in the sample was determined to be carbon, which was actually caused by the carbon tape used to hold the sample during examination. Titanium and oxygen were also identified in extremely substantial amounts, which suggests that  $\text{TiO}_2$  nanoparticles were present. The weight percentage of the constituents are given in Table 2.

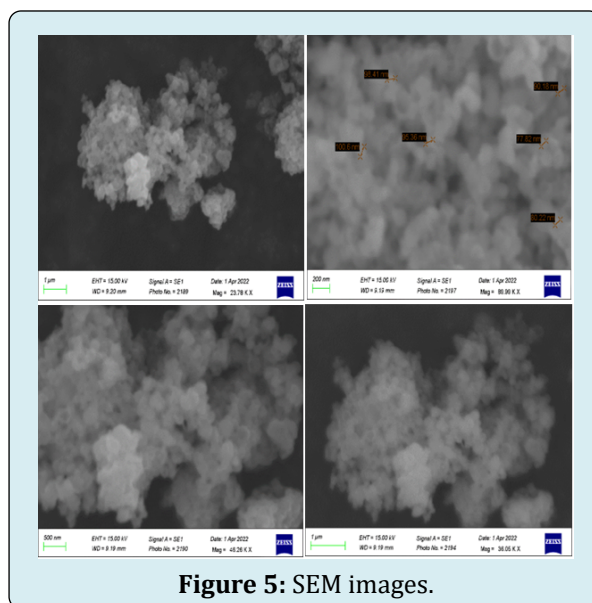


Figure 5: SEM images.

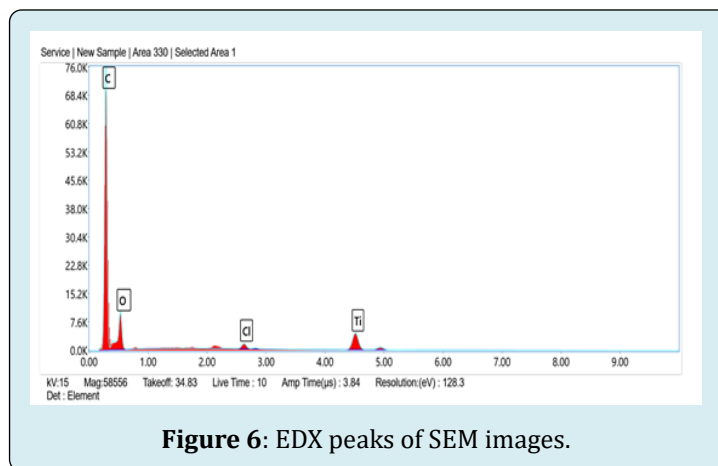


Figure 6: EDX peaks of SEM images.

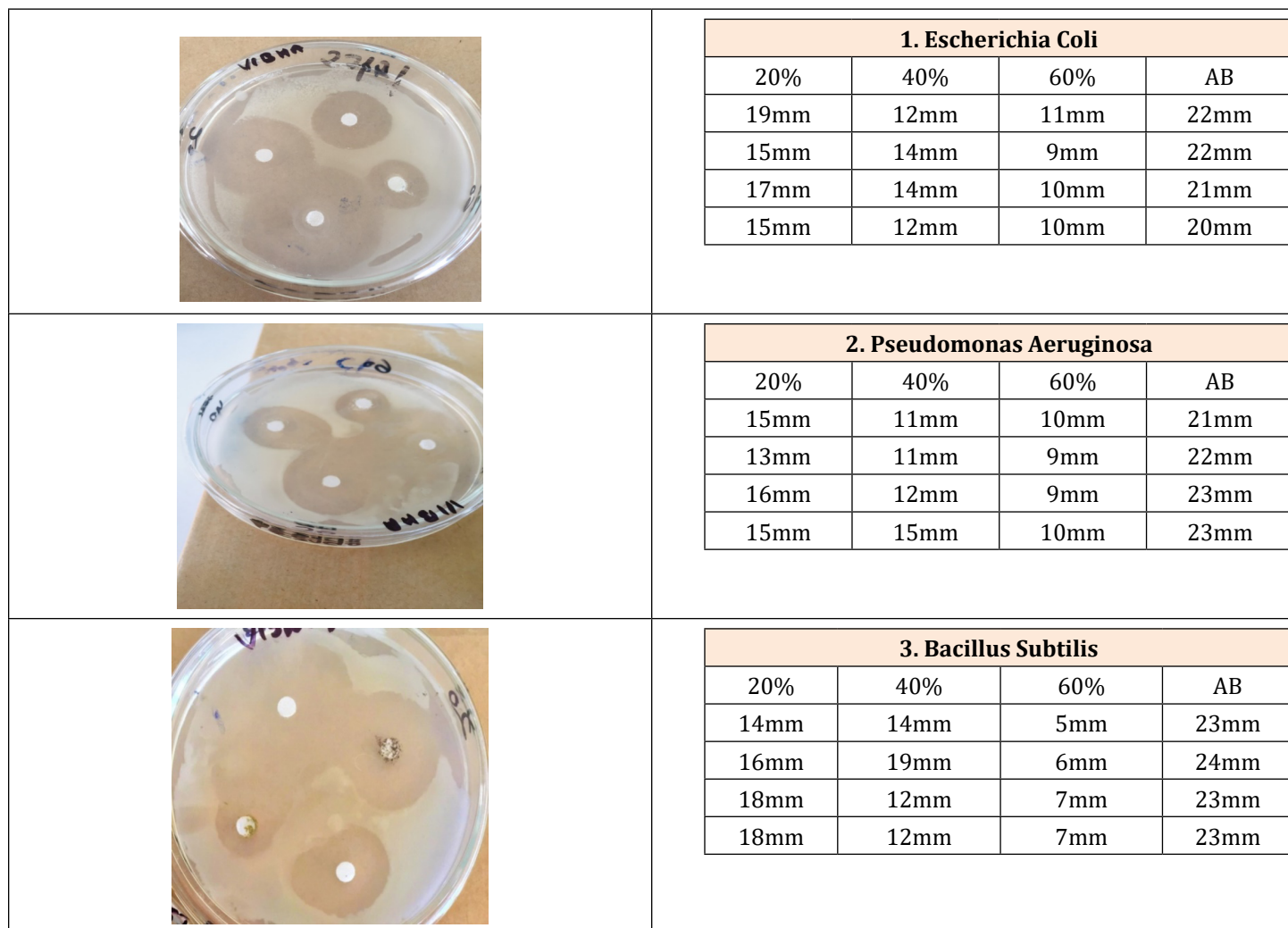
eZAF Result - Analysis Uncertainty: 99.00 %				
Element	Weight %	Atomic %	Error %	Net Int.
C	68.3	80.6	8.8	45265.6
O	16.6	14.7	11.6	5768.3
Cl	1.7	0.7	4.1	1420.5
Ti	13.5	4	2.7	5645.7

Table 2: Percentage of Nanoparticle constituents.

### Antibacterial Activity

The ethanolic root extract of *Asparagus racemosus* was tested for its antibacterial effects against a number of microorganisms, including Gram Positive *Bacillus subtilis*, Gram Negative *Pseudomonas aeruginosa*, and *Escherichia coli*. *Asparagus racemosus* root extract exhibits the highest level of effectiveness against all bacterial strains. By measuring the petri dish's zone of inhibition (ZOI) with a ruler, *Escherichia coli* (16.5 mm), *Pseudomonas aeruginosa*

(14.75 mm), and *Bacillus subtilis* (16.5 mm) in 20% concentration produced the greatest amount of inhibition. *Escherichia coli* (19mm) in a 20% concentration produces the greatest amount of inhibition. The overall results were highly encouraging, as seen in Figure 7, below, where the antibacterial activity was practically comparable to that of the usual antibacterial medication. This finally shows that the plant's root extract has good antibacterial properties.



**Figure 7:** Results of ZOI of the bacterias. 1. *Escherichia coli*, 2. *Pseudomonas aeruginosa*, and 3. *Bacillus subtilis*.

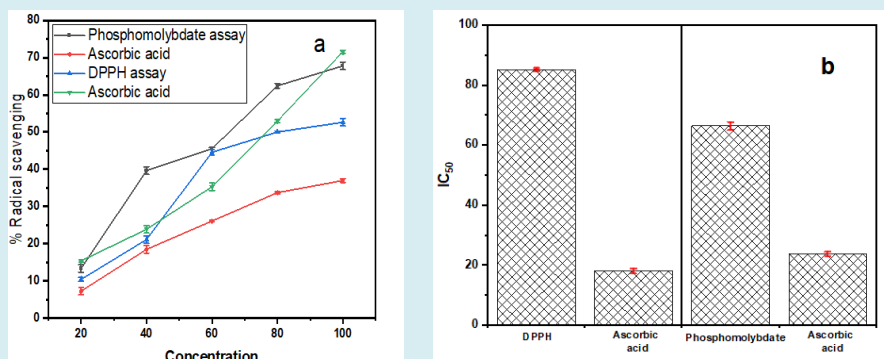
### Antioxidant Activity

The antioxidant potential of the nanoparticles was evaluated using two methods i.e., DPPH and Phosphomolybdate assays. In this study, antioxidant activity of the synthesized  $\text{TiO}_2$  nanoparticles from root extract of *Asparagus racemosus* has been evaluated and were found to be effective antioxidants. A visible color shift is seen in

the DPPH test as the concentration of the nanoparticle is increased from 20 to 100  $\mu\text{l}$ . As a result of the color shift, which suggests the nanoparticles' potential for reduction, the sample may be a powerful antioxidant. When the nanoparticle's  $\text{IC}_{50}$  value was compared to that for standard ascorbic acid, it was found that the nanoparticles had a lower value of  $\text{IC}_{50}$ , but the value was still very excellent, indicating that the nanoparticles have good antioxidant activity. The

calculated value of  $IC_{50}$  for nanoparticle is  $85.18 \pm 0.55 \mu\text{g/ml}$ . And for standard ascorbic acid is  $18.11 \pm 0.87 \mu\text{g/ml}$ . A lower value of absorbance indicates a good scavenging potential since the DPPH antioxidant assay is based on DPPH's ability to decolorize in the presence of antioxidants. In the phosphomolybdate assay, a dose dependent relationship has been seen as a function of increasing concentration. The calculated  $IC_{50}$  value is  $66.4 \pm 1.3 \mu\text{g/ml}$ . And for the standard ascorbic acid the  $IC_{50}$  value is  $23.8 \pm 0.68 \mu\text{g/ml}$  the

synthesized  $TiO_2$  nanoparticles shows a good antioxidant activity when compared to the standard ascorbic acid. Thus, these nanoparticles can be used for various applications in pharmacological and biomedical fields. Percentage radical scavenging activity as a function of concentration 20-100  $\mu\text{l}$  is presented in Figure 8a & b shows the difference between  $IC_{50}$  of two methods of antioxidant activity and their comparison with standard drug.

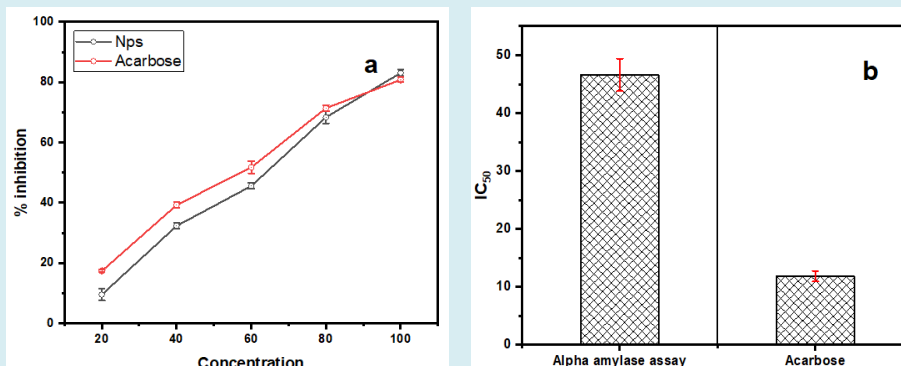


**Figure 8a:** Represents the dose dependent curve showing % of radical scavenging, **b:** Shows  $IC_{50}$  comparison of DPPH and Phosphomolybdate Assay with ascorbic acid as standard.

### Antidiabetic Activity

The antidiabetic activity was evaluated by using alpha amylase assay. The *Asparagus racemosus* NPs showed dose dependent  $\alpha$ -amylase inhibition with  $IC_{50}$  value  $46.59 \pm 2.81 \mu\text{g/ml}$ . The  $IC_{50}$  value of acarbose was found to be  $11.84 \pm 0.91 \mu\text{g/ml}$ . We have investigated that the *Asparagus racemosus* NPs can be used in the treatment of several diseases, and can also serve as anti-diabetic potential. Previously, this

beneficial and priceless plant NPs was not been investigated for its in vitro anti-diabetic activity in the northern region of India. It has clearly recognized the potential of anti-diabetic activity and anti-oxidant activity. A dose response relationship of the concentration of the sample vs. percentage inhibition of the alpha amylase is given in the Figure 9a & b shows the comparison of  $IC_{50}$  of standard drug and *Asparagus racemosus* NPs.



**Figure 9a:** Represents the dose dependent curve of the antidiabetic assay showing % of inhibition, **b:** Shows  $IC_{50}$  value of Alpha Amylase Assay with standard drug acarbose.

Concluding the outcomes of the study, it is noted that the synthesized *Asparagus racemosus* NPs are very potent as it

shows excellent biological activities. Various studies shows that the antioxidant activity of the extract of *Asparagus*



*racemosus* shows an  $IC_{50}$  value of  $\sim 78.15 \mu\text{g/ml}$  [33],  $4158.8 \mu\text{g/ml}$  [34],  $97.12 \mu\text{g/ml}$  [35], whereas the antidiabetic activity of the extract  $\sim 55.52 \mu\text{g/ml}$  [36], 0.9% inhibition [37], from these comparable outcomes of the various studies the synthesized nanoparticles in the present study is more potent as it possesses excellent outcomes than crude extract.

## Conclusion

The root extract of *Asparagus racemosus* was effectively used to synthesize titanium dioxide nanoparticles. The green synthesis approach was used in this process. Using SEM-EDX and UV-Visible spectroscopy, the synthesis of titanium dioxide nanoparticles was confirmed; the absorption peak in UV Spectroscopy denotes the presence of  $TiO_2$  nanoparticles. Finely powdered particles were formed as a result. Antibacterial activity was investigated using the disc diffusion method, *Asparagus racemosus* NPs showed a good antibacterial property in 3 bacterial strains and it also shows that a large number of bacteria were significantly suppressed by the sample. It shows a good antioxidant behavior as the  $IC_{50}$  value for DPPH and phosphomolybdate assay were  $85.18 \pm 0.55 \mu\text{g/ml}$  and  $66.4 \pm 1.3 \mu\text{g/ml}$  respectively. *Asparagus racemosus* NPs also has excellent antidiabetic potential, a good  $IC_{50}$  value of  $46.59 \pm 2.81 \mu\text{g/ml}$  have been shown in comparison with the standard drug acarbose (a widely used and marketed anti-diabetic drug). Concluding the outcomes of the study it may state that the nanoparticles are potent towards the activities performed and hence suitable for application in pharmacology and medicine.

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