

**BIO-SENSING, ANTIMICROBIAL EFFICACY AND ANTIOXIDANT
ACTIVITY OF GREEN SYNTHESIZED SELENIUM NANOPARTICLES
FROM CREEPING TICK TREFOIL (*Desmodium triflorum*)**

PROJECT REPORT

Submitted in partial fulfilment of the requirements for the award of Bachelor of Science
degree in Biotechnology

By

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**SCHOOL OF BIO & CHEMICAL ENGINEERING
DEPARTMENT OF BIOTECHNOLOGY**

SATHYABAMA

**INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)**

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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of Sneha Shajan (41770032) who carried out the project entitled BIO-SENSING, ANTIMICROBIAL EFFICACY AND ANTIOXIDANT ACTIVITY OF GREEN SYNTHESIZED SELENIUM NANOPARTICLES FROM CREEPING TICK TREFOIL (*Desmodium triflorum*) under our supervision from January 2024 to April 2024.

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ABSTRACT

A solitary step ecological cordial methodology is utilized to synthesize selenium nanoparticles. The current review is to plan an eco-accommodating mode to quickly synthesize SeNPs through *Desmodium triflorum* leaf 's aqueous and ethanol extracts and affirming SeNPs synthesis by UV-Vis spectroscopy, FTIR and SEM. Our study centers around the unexplored combination of *Desmodium triflorum* and SeNPs and its different properties. The biomolecules found in plants induce the reduction of Se^{2+} particles from sodium selenite to SeNPs. UV-noticeable range of the aqueous medium containing selenium particles exhibited a peak at 400nm and the ethanol medium showed a top at 270nm. In addition, this study also aims to assess the anti- microbial efficacy and anti- oxidant activity of the SeNPs. The antimicrobial action was determined by taking Minimum inhibitory concentration of the green synthesized selenium nanoparticles against *Escherichia coli* and *Bacillus cereus*. The MIC value for *E.coli* is 0.5 $\mu\text{g}/\text{ml}$ and for *B. cereus* is 0.25 $\mu\text{g}/\text{ml}$. SeNPs are synthesized by natural and green methods utilizing plant extracts hence they are free from harmful chemicals and such synthesized nanoparticles play an important role in biomedical applications such as medications, drug delivery etc.

Keywords: *Desmodium triflorum*, SeNPs, Synthesis, Charecterization, MIC, Antioxidant activity, DPPH Assay, Glucose sensing activity.

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LIST OF ABBREVIATIONS

SeNPs	Selenium Nanoparticles
TEM	Transmission electron Microscopy
XRD	X- ray Diffraction
DLS	Dynamic light scattering
FTIR	Fourier Transmission Infrared Spectroscopy
SEM	Scanning Electron Microscopy
MIC	Minimum Inhibitory concentration
EDS	Energy-dispersive X-ray spectroscopy
UV	Ultra- violet
DW	Distilled water
DPPH	2,2-Diphenyl-1-picrylhydrazyl

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CHAPTER 1

INTRODUCTION

Past many years, metallic nanoparticles (Silver, Gold, Selenium, Zinc oxide, and Copper oxide) have been quickly combined through natural or green science techniques and these nanoparticles are delivered in unadulterated, non-poisonous and ecological agreeable mode by using high-energy sustainable materials to advances the exhibition and security in the nanoparticle creating processes. Creating plant-interceded nanoparticles is generally quicker as there is no requirement for keeping up with explicit circumstances in media and culture as it is expected for other natural organisms. The manufactured nanoparticles are as of now by and by in different fields like sun based energy transformation, catalysis, water treatment, medication and may support managing mechanical and ecological challenges. Nanotechnology is one of the most promising and innovative tools in the current scenario and has been acknowledged as “Key Enabling Technology” by the European Commission (Parisi *et al* ., 2015).

They also have higher solubility and surface reactivity than large size nanoparticles due to their large surface area. Selenium is a roundabout essential semiconductor and shows great photoelectrical properties, nonlinear optical properties, high photoconductivity and, surprisingly, synergist exercises towards natural hydration and oxidation responses. Selenium is one of the fundamental minor component in the body because of its anti-oxidative as well as pro-oxidative impact and has extraordinary significance in sustenance and medication. SeNPs certainly stand out because of their remarkable antimicrobial, anticancer, cell reinforcement and antibiofilm movement.

A few green techniques for basic SeNPs synthesis was accounted for to incorporate the union of SeNPs by microscopic organisms, parasites, and plants. Nowadays, SeNPs are acknowledged by numerous energetic specialists like researchers and suggested for use in different logical disciplines because of their less harmfulness and high steadiness. The nanoform of selenium is all-important for the research due to its high degree of absorption and low toxicity than its organic and inorganic forms (Bhattacharjee *et al.*, 2019). Additionally, it can very well be incorporated by various methodologies like physical, compound, and organic. As

per Wadhwani et al., articulation, organically intervened SeNPs are more secure, natural agreeable and financially practical when contrasted and different methodologies (synthetically and physical).

Simultaneously, reports are accessible on natural way to deal with produce SeNPs by means of plants part like dried leaf, seed, blossoms and bark. Leguminous plants have significant plant products and phytochemicals that are valuable as modern industrial, therapeutic, and agricultural unrefined components. Nevertheless, the majority of these legume species have not been completely taken advantage of for their chemical substance or naturally active compounds. One of the biggest tropical wild types of legume is *Desmodium*, with Southeast Asia as a significant focus of species diversification.

The *Desmodium triflorum* is a huge individual from the Papilionaceae (Fabaceae) family. The water decoction of *Desmodium* plants has been generally utilized in China to deal with different sicknesses like asthma, typhoid fever, irritations, intestinal sickness, juvenile ailing health, looseness of the bowels, pyrexia, and so on. The *Desmodium* plant extracts had many pharmacological properties, including working on cardiovascular and cerebrovascular capabilities and managing resistant framework as well as mitigating, antiparasitic, antidiabetic and antibacterial exercises.

The chemical literature uncovers that the species *Desmodium triflorum* are rich in polyphenols, flavonoids, sterols, triterpenes, and lessening sugars in all extracts. Studies have demonstrated that biomolecules like protein, phenols, and flavonoids not just assume a part in diminishing the particles to the nanosize, yet in addition assume a significant part in the covering of the nanoparticles. The cycle of SeNPs development within the presence of a covering specialist implies in the wake of adding reductant to response arrangement containing sodium selenite and covering specialist, Se particles decrease to neutralized Se (nucleation). Then Se cores structure groups and covering specialists coat Se bunches to deliver balanced out and uniform NPs.

We have taken a glance at the organization of different plants for conceivable presence of such particles and have distinguished *Desmodium* plants as a likely contender for synthesis of selenium nanoparticles. Extracts from this plant might act both as reducing and covering specialists or agents in SeNPs synthesis. Therefore, the central point of this current examination is to exhibit that the response of selenium particles with *Desmodium triflorum* extract brought about the extracellular arrangement of SeNPs at room temperature which was additionally collected by simple heat drying evaporation and to decide its biosensing, antimicrobial efficacy and anti-oxidant activity. The SeNPs are likewise answered to be nontoxic to human and best against microorganisms, infections, and other eukaryotic miniature life forms at extremely low fixation and with no aftereffects. We have likewise made an endeavor to figure out the utilization of these bioprospected plant-intervened incorporated SeNPs as possible antimicrobial agents.

The formation, size, shape, and function of the NPs are highly depends on physical, chemical and biological methods of their synthesis. Instrumentation techniques have developed considerably in the past decade to sufficiently characterize the physicochemical properties of nanoparticles including dynamic light scattering techniques for size determination, zeta potential measurements for relative particle charge, electron (scanning and transmission) microscopy to qualitatively analyze size and shape, nuclear magnetic resonance to accurately determine chemical structures, X-ray photoelectron spectroscopy to measure chemical composition, chemical and electronic states, mass spectrometry for mass, and elemental analysis and spectroscopy techniques that measure absorption, emission, or scattering of either wavelength or frequency.

An important note of caution while characterizing nanomaterials is to evaluate these properties under physiologically relevant conditions. (Oberdörster et al., 2005; Greish et al., 2012). For the detailed characterization of the nanoparticles, the 3 techniques of UV-visible spectroscopy, SEM, FTIR analysis had been used. Nanotechnology, the science of working with smallest possible particles, raises hopes for the future to overcome the difficulties.

CHAPTER 2

LITERATURE SURVEY

In a study by Shnoudeh *et al.*, 2019 the synthesis, characterization, and applications of metal nanoparticles was explained. Using their metallic characteristics scientists were able to synthesize metal NPs using top-down and bottom-up approaches including physical, chemical, and biological methods. Biological methods were found to be the most environmentally friendly and least toxic methods. Different analytical methods can be used to analyze and characterize the synthesized NPs, including, size, shape, the surface of the NPs, functional groups, size distribution, aggregation, dispersion, structure, binding hydrodynamics, and confirmation to have enough information to find the proper application for these NPs or proper functionalization. The main methods that can be used to determine the characteristics of NPs have been explained and summarized; they include optical, spectroscopic, and microscopic techniques such as UV spectroscopy, FTIR, SEM, XRD and DLS.

Similarly Ealias *et al.* 2017 did a review on the classification, characterisation, synthesis of nanoparticles and their application. Nanotechnology is improving our everyday lives by enhancing the performance and efficiency of everyday objects. It provides a clean environment by providing safer air and water, and clean renewable energy for a sustainable future. Nanotechnology has gained a wide attention where more investment is made for the research and development by top institutions, industries and organisations. Nanotechnology has established to be an advanced field of science where extensive research is carried out to implement the technology. It is being tested for various new applications to increase the efficiency and performance of the object or process and subsequently reduce the cost so that it is accessible for everyone.

And Santos Souza *et al.*, 2022 in another study determined the biosynthesis of SeNPs using combinations of plant extracts and their antibacterial activity. Antimicrobial resistance is a significant public health problem, and the use of nanotechnology to control bacterial growth has increased, including multidrug-resistant ones. In this context, nanoparticles of different elements, mainly metals, such as silver, gold, and iron, have been extensively researched due to their efficient antimicrobial activity. Another element that stands out is selenium, which

nanoparticles have high bioactivity and antibacterial action, although few studies report its use as a metallic nanoparticle. Therefore, the objective of this study was to perform the synthesis of SeNPs from extracts of three plants: *Allium cepa* (onion), *Malpighia emarginata* (acerola), and *Gymnanthemum amygdalinum* (boldo). The synthesized nanoparticles were characterized by Dynamic Light Scattering (DLS), scanning electron microscopy (SEM), Fourier-Transform Infrared Spectroscopy (FTIR). Antibacterial activity was evaluated by microdilution in broth, followed by the time-kill curve analysis.

In a study by Sentkowska *et al.*, 2023 showed the antioxidant properties of SeNPs synthesized using tea and herb water extracts. Green synthesis of selenium is also described in the literature. Generally, selenium precursor (most often Na_2SeO_3) is mixed with the plant extract solution at different ratios and stirred for different periods, sometimes with heating. During this process, the colour of the reaction media changes to red, indicating the formation of SeNPs. In the synthesis of SeNPs, the main goal is their formation with minimum particle size and maximum stability as a smaller size can guarantee more powerful antioxidant activities. In the present study, the potential of water extracts of popular black (Lipton Yellow label) and green tea (Lipton Green Tea Classic) as well as chamomile (*Matricaria recutita*) and mint (*Mentha*), commonly used to make herbal infusions, were compared in the biosynthesis of SeNPs. The dependence of the ratio of reagents on the properties of the obtained SeNPs has been investigated. The content of polyphenolic compounds, which can potentially act as selenium reducing reagents, was measured using liquid chromatography. The antioxidant activity of extracts themselves, as well as obtained nanoparticles, were determined using a hydroxyl radical scavenging assay. An attempt has been made to link the properties of the obtained SeNPs with the ratios of the reagents used for the synthesis.

As per the review of Bisht, *et al.*, 2022 on synthesis and biomedical applications of SeNPs states that Selenium is a trace and essential micronutrient for the health of humans, animals, and microorganisms. Recently, SeNPs attracted the interest of many researchers due to their biocompatibility, bioavailability, and low toxicity. Therefore, due to their higher bioactivity SeNPs are largely being used in various biomedical applications. Generally, SeNPs can be synthesized by physical, chemical, and biological methods. However, the biologically synthesized SeNPs

demonstrate greater compatibility with human organs and tissues. The effect of size, shape, and the method employed for their synthesis on their applications in biological systems has been explored by many researchers. This review discusses various synthesis methods employed for their preparation and highlights their applications in the biomedical field such as in the treatment of fungal, bacterial, and parasitic infections, cancer, and diabetes. They can also act as chemopreventive agents, anti-inflammatory agents, and antioxidants.

In a study of synthesis of SeNPs in the presence of polysaccharides, biopolymer from plants or animals, is the nurture, potential pharmaceutical and target for drug design. For example, Chitosan hydrogen selenites with low molecular weight has specific biological activities (anticancer activity, antibacterial activity and immunoenhancing effects). Chitosan hydrogen selenites have been prepared for antisarcoma tumor. Novel manufacturing or modifying techniques may improve the properties of polysaccharide and will get it to be more important for medical and food uses. In addition, water-soluble polysaccharides have widely been used as film formers, gelling agents, suspending agents, flocculants, binders, emulsifiers and stabilizer. The polysaccharides may modify the interface of nanoparticles, control the growth of nanoparticles and stabilize the nanoparticles solution. In our experiment, the SeNPs were prepared in the presence of water-soluble polysaccharides. The monodispersed spherical selenium colloids obtained were very stable in the solution and could keep unagglomeration at least for 6 months. In addition, SeNPs with various morphologies, such as nanorods and fractal-like shapes, were also obtained in certain experimental condition (Zhang *et al.*, 2004).

A recent study focuses on the biogenic current status and future prospects of SeNPs which are gaining importance in the field of medicine owing to their antibacterial and anticancer properties. SeNPs are biocompatible and non-toxic compared to the counterparts, selenite (SeO_3^{-2}) and selenate (SeO_4^{-2}). They can be synthesized by physical, chemical, and biological methods and have distinct bright orange-red color. Biogenic SeNPs are stable and do not aggregate owing to natural coating of the biomolecules. Various hypotheses have been proposed to describe the mechanism of microbial synthesis of SeNPs. It is primarily a two-step reduction process from SeO_4^{-2} to SeO_3^{-2} to insoluble elemental selenium (Se^0)

catalyzed by selenate and selenite reductases. Phenazine-1-carboxylic acid and glutathione are involved in selenite reduction. SefA and metalloid reductase Rar A present on the surface of SeNPs confer stability to the nanoparticles. SeNPs act as potent chemopreventive and chemotherapeutic agents. Conjugation with antibiotics enhances their anticancer efficacy. These also have applications in nanobiosensors and environmental remediation (Wadhvani SA, *et al.*, 2016).

In the present study, SeNPs were biologically synthesized by non-pathogenic, economic and easy to handle bacterium *Ralstonia eutropha*. The selenium oxo anion was reduced to SeNPs in the presence of the bacterium. The bacterium was grown aerobically in the reaction mixture. An extracellular, stable, uniform, spherical SeNPs was biosynthesized. The TEM analysis revealed that the biosynthesized SeNPs were spherical in shape with size range of 40–120 nm. XRD analysis showed that nanocrystalline selenium of pure hexagonal phase was synthesized. The formation of actinomorphic trigonal selenium nanorods was also observed. A mechanism of biosynthesis of SeNPs by *R. eutropha* was proposed. The biosynthesized SeNPs were investigated for their antimicrobial activity against potential pathogens. SeNPs showed excellent antimicrobial activity. (Srivastava N, *et al.*, 2015)

This study evaluated the antioxidant and antiproliferative activities of the crude extract and fractions of *Desmodium triflorum* (L.) DC. The total phenolic content, 1,1-diphenyl-2-picrylhydrazyl hydrate (DPPH) free radical scavenging activity, reducing power, total flavonoid content of *D. triflorum* were evaluated for the exploration of its antioxidant activities. It was compared with the antioxidant capacities of known antioxidants, including catechin, α -tocopherol, trolox and ascorbic acid. Among all fractions, ethyl acetate fraction was the most active in scavenging DPPH radicals, of which 0.4 mg was equivalent to $186.6 \pm 2.5 \mu\text{g}$ and $82.5 \pm 2.1 \mu\text{g}$ of α -tocopherol and trolox respectively. The total phenolic and flavonoid contents of the crude extract were equivalent to $36.60 \pm 0.1 \text{ mg}$ catechin and $45.6 \pm 0.6 \text{ mg}$ rutin per gram respectively. In the reducing power assay, 1.25 mg of crude extract was similar to $61.2 \pm 0.3 \mu\text{g}$ of ascorbic acid. For the assessment of the safety and toxicity of *D. triflorum*, LD₅₀ of the crude extract was greater than 10 g/kg when administered to mice through gastric intubation. The above

experimental data indicated that *D. triflorum* was a potent antioxidant medicinal plant, and such efficacy may be mainly attributed to its polyphenolic compounds (Lai, S.C., *et al.*, 2010).

A review on Shalparni (*Desmodium gangeticum* DC.) and *Desmodium* species (*Desmodium triflorum* DC. & *Desmodium laxiflorum* DC.)—Ethnomedicinal perspectives. Shalparni is one of the drug of Laghupanchamoola so of Dashamoola. Shalparni is mainly found to be used as one of the ingredient of Parnidwaya, Chatuparni or as Laghupanchmoola/Dashamoola. Shalparni is found widely used in Samhitas, its various single uses were reported in various chikitsagranthas. It possess Tikta-kashaya rasa, Ushna veerya, Madhura vipaka and Guru guna. It is found indicated in various conditions like, Jwara, Atisara, Shwasa, Chardi, Shwasa etc. Accepted botanical source of Shalparni is *Desmodium gangeticum* which belongs to family Fabaceae, is reported as endangered species (Singh S, *et al.*, 2015).

In a study by Gavalapu VR, *et al.*, 2013 the preliminary phytochemical screening and anthelmintic activity of *Desmodium triflorum* was explained. *Desmodium Triflorum* (Fabaceae/Leguminosae) is a medicinal plant is a very small terrestrial, annual, prostrate herb, up to 50cm long, slender branches rooting at nodes. Its leaves are small, alternate, stipulate and trifoliate Flowers are irregular, bisexual, very small and bright purplish blue in colour. This plant is found on a wide range of soils and most commonly in dry, distributed in lawns, waste places and along road sides in tropical countries including India, Srilanka, Philippines and Taiwan. The plant is easily available throughout the states of India.

The present investigation was aimed to study the efficacy of aqueous and methanolic extracts of plant *Desmodium triflorum* for potential antibacterial activity. To evaluate the efficacy of aqueous and methanolic extracts of plant *Desmodium triflorum* for potential antibacterial activity. Materials and Methods: Phytochemical analysis of *Desmodium triflorum* belonging to family Fabeacea was examined using aqueous and methanolic extracts. Methanolic and aqueous extracts of whole plant were investigated individually for antibacterial activity by agar disc diffusion method as well as by agar well diffusion method. Both the extracts were tested against selected species of *Staphylococcus aureus* (G+ve), *Micrococcus luteus* (G+ve),

Bacillus pumilus (G+ve), *Pseudomonas aeruginosa* (G-ve), *Pseudomonas fluorescens* (G-ve), *Escheria coli* (G-ve) to find the inhibitory activities of microbes at the dose level of 50 and 100 µg/ml. Result: The phytochemical analysis of aqueous and methanolic extracts confirmed the presence of alkaloids, steroids, tannins, saponins and flavonoids. Methanolic extract of *Desmodium triflorum* showed considerably high antibacterial activities against (G+ve) bacteria than aqueous extract. Conclusion: Although the active components were not isolated but antibacterial active plant principles such as flavonoids, alkaloids and tannins were observed in the extract. Methanolic extract of *Desmodium triflorum* possess effective antibacterial properties for (G+ve) bacteria (Sharma R, *et al.*, 2013)

In this study, we evaluated the In-vitro cytotoxic and anti-Vibrio cholerae activities of alcoholic extracts of *Desmodium triflorum* (L.) whole plant and *Terminalia citrina* (Roxb.) fruits. Cholera is recognized as an ancient diarrheal disease that is a continuous burden to public health for years in the developing and under-developed countries of Asia and Africa, where there are a population influx and poor water supply and basic hygiene. It is a water-borne bacterial disease caused by a facultative rod-shaped gram-negative anaerobic bacterium, *V. cholerae*. This bacterium is one of the most infamous enteric pathogens that belongs to the family Vibrionaceae. The primary route of transmission of the bacterium to the human body is via the consumption of contaminated water and food. The bacterium attaches itself to the human small intestine. Eventually, it releases cholera toxins or cholera toxin, produced by *tcp* gene of *V. cholerae*, which results in the production of the enzymatically active subunit that elevates intracellular cyclic adenosine monophosphate, leading to excessive loss of water and electrolytes from the body via vomiting and severe form of acute secretory diarrhoea. However, not all *V. cholerae* secrete cholera toxins strains. There are only two causative strains of *V. cholerae* for the disease that belongs to the serogroups O1 and O139 and have been responsible for massive cholera pandemics for years (Karim R, *et al.*, 2021)

The aim of the present study is based on the literature mining from the *Desmodium* species is to acknowledge the importance of flavonoids in human health as dietary food supplements and therapeutic uses. Secondary metabolites are an important part to play a major role in society and it was isolated from plant

flavonoids and useful in the treatment of various kinds of diseases in the human race. They are widely used as food and nutrition supplements as well as antioxidants. Traditionally, the *Desmodium* species are an important tool for the secondary metabolites to treat various diseases. *Desmodium triquetrum* (Fabaceae) one of the Indian medicinal plants is widely used in the treatment of asthma and inflammation. Three flavonoids isolated from *Desmodium triquetrum* Linn namely Baicalein, Naringin and Neohesperidin are useful as antioxidants, food and nutrition supplements, that help the body to function efficiently while protecting it against toxins as well stressors. The role of flavonoids may be due to the presence of the phenolic compound. Similarly, the flavonoids such as gangetin, gangetinin, desmocarpin and desmodin isolated from the species *Desmodium gangeticum* are responsible for antileishmanial, antioxidant, anti-arthritic, and immunomodulatory activities. Additionally, isolated flavanoids from the species *Desmodium triflorum* show antibacterial, antiepileptic, antifungal, and radioprotective activities (Singh V, *et al.*, 2024)

CHAPTER 3

AIM AND OBJECTIVES

3.1. AIM

- ❖ To synthesise and characterize SeNPs from the extract of *Desmodium triflorum* and to evaluate its biosensing property and antimicrobial efficacy and antioxidant activity.

3.2. OBJECTIVES

- ❖ To collect and prepare extracts of creeping tick trefoil plant *Desmodium triflorum*.
- ❖ To synthesize SeNPs from the plant extract.
- ❖ To characterize SeNPs using UV-Vis spectrophotometer, SEM, and FTIR.
- ❖ To evaluate Minimum Inhibitory concentration of SeNPs from *Desmodium triflorum* by Microplate assay method.
- ❖ To evaluate Anti-oxidant activity of SeNPs.
- ❖ To evaluate biosensing application of SeNPs especially by biological analyte glucose.

CHAPTER 4

MATERIALS AND METHODS

4.1 MATERIALS

Plant samples, glasswares, Sodium Selenite Anhydrous, 96 well titre plate, Resazurin dye, bacterial samples, pipettes, filter paper, rotary evaporator, hot air oven, incubator, UV vis spectrophotometer, SEM, FTIR equipments, distilled water, DPPH, Dextrose, etc.

4.2 METHODOLOGY

4.2.1. Preparation of plant extract:

Selection and collection of Creeping tick trefoil. The leaves (as shown in Fig 4.1) were washed, shade dried and ground into small pieces.



Fig 4.1: *Desmodium triflorum* (leaves)

Two Plant extracts were prepared: Aqueous extract and solvent(ethanol) extract. Aqueous extract was prepared by adding 250 ml of distilled water, 50g of plant sample in a 500ml conical flask and was boiled in heating mantle for half an hour. Solvent extract was prepared by adding 250ml of ethanol, 50g of plant sample in a 500 ml conical flask and was subjected for magnetic stirring for 24 hours. Then both the aqueous and solvent extracts were filtered by Whattman filter paper(as shown in Fig 4.2) .



Fig 4.2: Filtering the extract

4.2.2 Synthesis of nanoparticles

In 2 conical flask 250 ml of distilled water and 1M of Sodium Selenite Anhydrous (Na_2SeO_3) was added and was subjected for magnetic stirring for 10 minutes. To this 500 μl of plant extract was added respectively once in 2 hours for 2 times and was again kept for magnetic stirring for 24 hours (as shown in Fig 4.3 and 4.4). The colour change of the reaction mixture from uncoloured to yellowish confirms the synthesis of SeNPs.



Fig 4.3: SeNPs in plant aqueous extract.

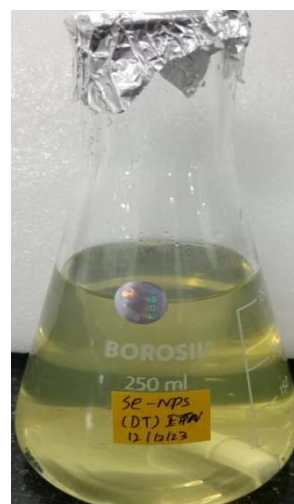


Fig 4.4: SeNPs in plant solvent extract.

The aqueous and solvent extract of 250 ml were then subjected for centrifugation. Selenium deposits as pellets, remove the supernatant in a separate

vial, the pellet obtained from this was kept in hot air oven and the supernatant was kept in rotary evaporator to reduce to paste like consistency. The obtained paste was kept in a petri plate and kept in hot air oven for 2 days and was scraped out as depicted in Fig 4.7.



Fig 4.5: Subjecting the extracts in rotary evaporator.



Fig 4.6: Both solvent and aqueous extracts reduced to paste.



Fig 4.7: The paste was dried in hot air oven and scraped out to get powder.

4.2.3 Characterization of SeNPs

4.2.3.1 UV- Visible Spectrometry

This method consists of a deuterium or tungsten lamp for the ultraviolet and visible region wavelengths, respectively, sample and reference beams, a detector, and a monochromator. Exposing the sample to UV light will give the UV spectrum. Cuvettes are used for sample holding and are kept inside the instrument for introducing samples to the light path. Glass, plastic, silica, or quartz cells can be

used as cuvettes. Plastic and glass cuvettes absorb wavelengths below 310nm as shown in Fig 5.1, so they cannot be used for absorbance studies below that wavelength. Therefore, quartz cuvettes are used for absorption measurements in the ultraviolet range as they are transparent to the wavelengths above 180 nm.

4.2.3.2 SEM

In scanning electron microscopy (SEM), an electron beam is directed toward the specimen instead of a light beam, as in the case of an optical microscope. A highly concentrated electron beam is shot from an electron gun located at the device's top. The two main electron gun types are field emission guns, which generate a strong electric field that rips electrons from the atom, and thermionic guns, in which the filament is heated until the electrons stream away. The SEM scans the surface of the sample with high- energy electron beams. Thus, SEM differs from conventional light microscopes as they uses light waves to create a magnified image. In SEM, when the electron beam strikes the specimen surface, it interacts with the surface (Titus *et al.* 2018).

4.2.4 MINIMUM INHIBITORY CONCENTRATION (MIC)

This assay consists in the determination of chemical agent spectrum of action, according to resistance of studied microorganisms. It was developed the determination of minimum inhibitory concentration (MIC) for every chemical agent, through the classic method of successive dilution. The minimal inhibitory concentration (MIC), which is a key indicator of an antimicrobial agent's potency, is defined as the concentration at which visible growth of bacteria is prevented under defined growth conditions. Resazurin dye is used as a redox indicator. Determination of MIC of SeNPs was done against *E.coli* and *Bacillus cereus*. For test take 50ul distilled water and 30ul MH broth and 10ul dye and 10ul of bacterial solution. NPs were dissolved in Muller Hinton broth (MHB) at twice the concentration of the final test. 100 µl of the NPs/MHB broth was dispensed in each well of Column 1, while Columns 2-10 contained 50 µl of MHB broth only. Column 11 contained 100 µl of diluted standardised inoculum(positive control), and Column 12 contained 100 µl of the medium broth (as a negative control to monitor sterility) A multichannel pipette was then used to transfer and mix NPs from column 1–10, resulting in 50 µl

NPs per well. The standardised microorganism suspension was then diluted by 1:100 in MHB broth. 50 µl of the bacterial suspension was then added to all wells containing nps and to the control wells. Then the dye resazurin (0.015 %) was added to all wells (30 µl per well), and incubated for 2–4 h for the observation of colour change. On completion of the incubation, columns with no colour change (blue resazurin colour remained unchanged) were scored as above the MIC value.

4.2.5 ANTI-OXIDANT ACTIVITY BY DPPH ASSAY

Antioxidants are molecules that fights free radicals in our body. Free radicals are compounds that can cause harm if their levels become too high in the body. They're linked to multiply illnesses, including diabetes, heart disease, and cancer. Our body has its own antioxidants defense to keep free radicals in check. antioxidants are also found in food, especially in fruit, vegetable, and other plant-based, whole foods. antioxidant preservatives also play a crucial role by increasing shelf life.

4.2.5.1.Determination of Antioxidant Activity Using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Method

In vitro antioxidant activities of the extracts were determined using the DPPH free radical scavenging assay This is a quick and easy method to analyse the scavenging potential of antioxidants. DPPH in oxidized form gives a deep violet colour in methanol. An antioxidant compound donates the electron to DPPH, thus causing its reduction and in reduced form its colour changes from deep violet to yellow. DPPH solutions show a strong absorbance at 408 nm appearing as deep violet colour. Scavenging of DPPH free radical determines the free radical scavenging capacity or antioxidants potential of the test samples, which shows its effectiveness, prevention, interception, and repair mechanism against injury in a biological system.

DPPH solution (0.1 M) was prepared by dissolving 0.39 mg of DPPH in a volumetric flask, dissolved in methanol, and the final volume was made 100 mL. Thus, prepared purple-coloured DPPH free radical solution was stored at –20°C for further use.

The antioxidant activity of the plant extracts against DPPH was determined using this method. A methanolic dilution of DPPH 1×10^{-4} M was prepared. Aliquots of 1 mL of each sample in the methanolic extract were collected and had 2 mL of

methanolic dilution of DPPH added. The mix was kept in the dark at room temperature for 16 min, and absorbance was measured at 408 nm in a UV-30 spectrophotometer. The blank was prepared with the methanolic dilution of DPPH samples were dissolved in methanol and the results were given as IC₅₀, which is the amount of extract required to scavenge the initial DPPH radical by 50%.

4.2.6 GLUCOSE- SENSING ACTIVITY

For the concentration of glucose assay the dextrose were taken for the test, because the dextrose is the identical form of glucose (Bienkowski *et al.*, 1987). The higher concentration to lower concentration of dextrose with SeNPs were added into separate tubes with distilled water (DW) by equivalent ratio like 1ml dextrose+ 1ml (DW) + 1 ml NPs were taken with different ratio for maximum glucose sensing technique. For the first uv take dextrose peak and NPs peak for reference. Then in second take 2ml of dextrose solution and 2ml of NPs solution in a cuvette and subject for UV. Thirdly, take 2ml of dextrose solution and 700ul of water and 1300ul of NPs(2ml) and subject for UV. Then for the fourth take 2ml dextrose and 800ul water and 1200ul NPs and subject for NP. Lastly for the fifth one take 2ml dextrose and 900ul water and 1100ul NPs and subject for UV. Observe and compare the results.

CHAPTER 5

RESULTS AND DISCUSSION

5.1 RESULTS

5.1.1 CHARACTERIZATION

5.1.1.1. UV spectrophotometer

Se (aqueous) 1stPeak is obtained at 400nm for SeNPs as shown in Fig 5.1.

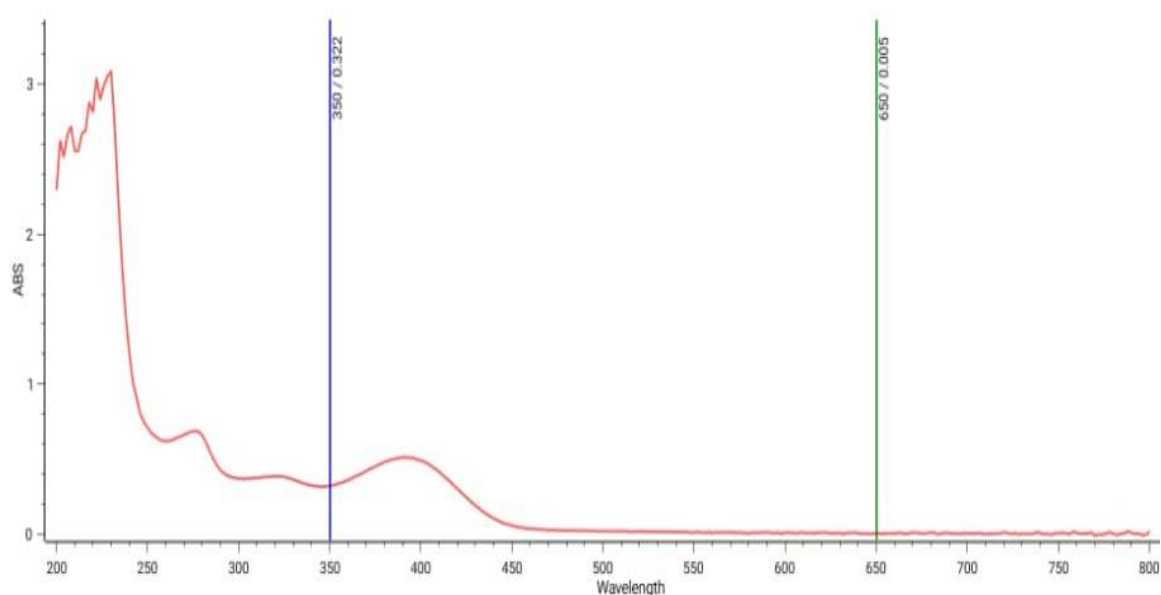


Fig 5.1: UV- Vis of SeNPs in aqueous extract

Se(ethanol)has highest peak at 4:1 dilution ratio(4NPs,1 plant) with peak at 270nm.

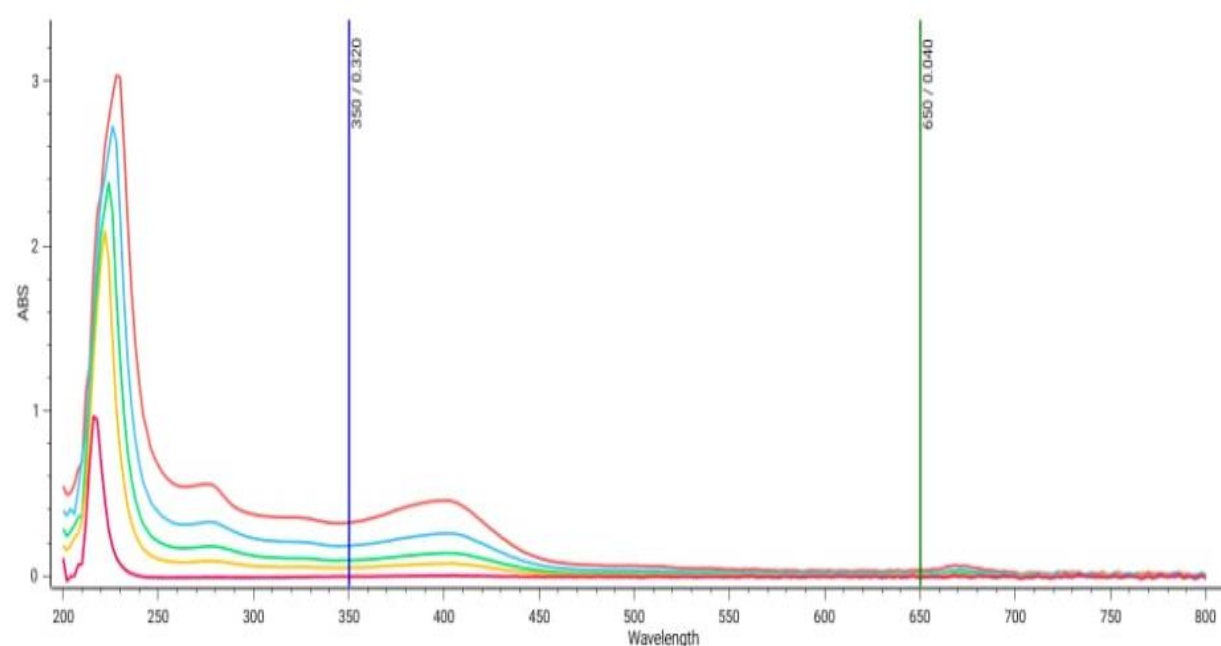


Fig 5.2: UV- Vis of SeNPs in ethanol extract

5.1.1.2. FTIR Analysis

The results of FTIR analysis were shown in the Fig 5.3. The FTIR spectrum shows the presence of -NH group, CO group, -OH group in the SeNPs represents the bioactive molecules might be responsible for the formation of SeNPs.

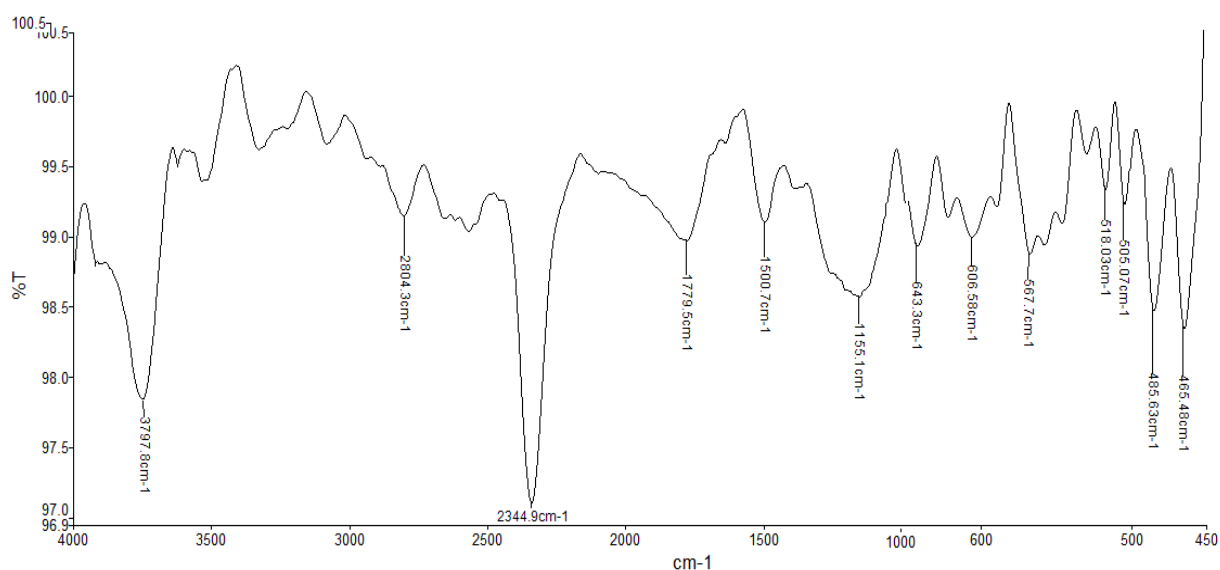


Fig 5.3: FTIR spectrum of SeNPs

5.1.1.3. SEM Analysis

The results of Scanning electron microscope analysis were shown in the Fig 5.4. The Image shows the SeNPs of spherical to elliptical shape formed with size measuring about 120-130nm.

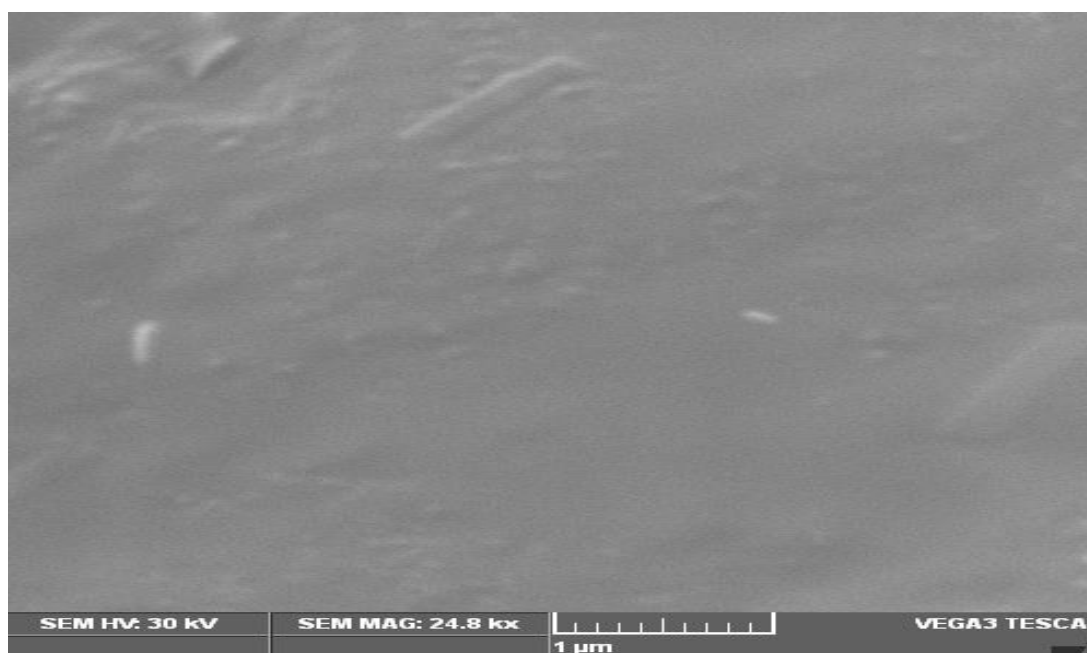


Fig 5.4: SEM image of SeNPs

5.1.2 MINIMUM INHIBITORY CONCENTRATION (MIC)



Fig 5.5: MIC of SeNPs before incubation



Fig 5.6: MIC of SeNPs after incubation

Active living bacterial cells causes reduction of resazurin (purple-blue) to resorufin (pink-colorless). Column 12 (Fig 5.6) confirms no contamination occurred while preparing the plate. The MIC values for *Ecoli* is 0.5ug/ml and for *Bacillus* is 0.25ug/ml.

5.1.3 ANTI-OXIDANT ACTIVITY BY DPPH ASSAY

Table 5.1. Antioxidant activity of SeNPs prepared from *Desmodium triflorum*

Concentration ($\mu\text{g/ml}$)	Absorbance at 517nm
Control	0.576
5	0.532
10	0.395
15	0.284
20	0.158
25	0.125

Half-maximal inhibitory concentration IC₅₀: 15.36 $\mu\text{g/ml}$

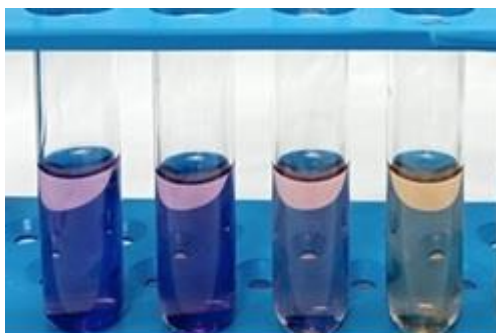


Fig 5.7: Antioxidant activity of SeNPs

5.1.4 GLUCOSE- SENSING ACTIVITY

Peak is obtained at 280 nm at 2:2 dilution ratio, 2ml dextrose + (700ul water + 1300ul NPs) as shown in Fig 5.8.

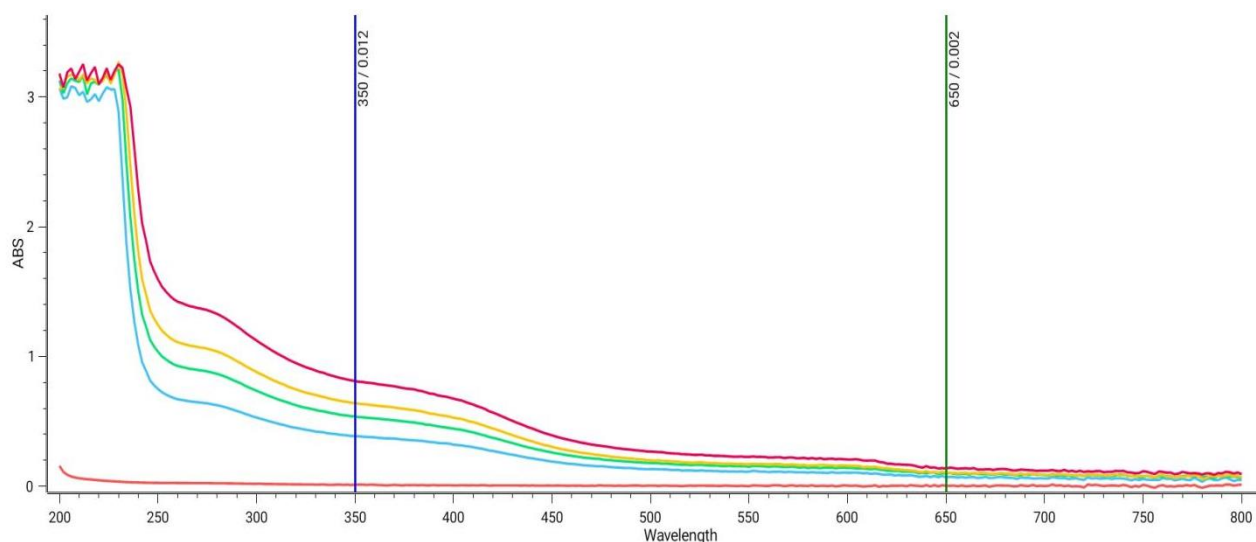


Fig 5.8: Glucose- sensing activity of SeNPs

5.2 DISCUSSION

In the study by (Nishant *et al.*,2015) SeNPs were biologically synthesized by non-pathogenic, economic and easy to handle bacterium *Ralstonia eutropha*.The reaction mixture with activated *R. eutropha* and sodium selenite was displayedUV–visible spectra showed the broad peak at 270 nm which confirms the formation of SeNPs.In the study by (Ramamurthy *et al.*,2013) the green synthesis of SeNPs was achieved by a simple biological procedure using the reducing power of fenugreek seed extract.The SEM images of SeNPs synthesized by different combinations were

oval in shape with smooth surface. The particle size was found to be around 50–150 nm and (Wenjing *et al.*,2017) in another study determined that the 1% green tea *Lycium barbarum* polysaccharides green tea-SeNPs showed high antioxidant activity with low EC50 value which is 22 μ M whereas here the mixture with SeNPs synthesized by *Desmodium triflorum* with both aqueous and ethanol extract had a peak of 400nm and 270 nm respectively(as shown in fig 5.1 and 5.2) and also the particle size of SeNPs synthesized by *Desmodium triflorum* is about 120 - 130 nm (as shown in fig 5.4.). So the SeNPs synthesized by *Desmodium triflorum* showed antioxidant activity through DPPH assay with IC50 value as 15.36 μ g/ml.

CHAPTER 6

SUMMARY AND CONCLUSION

SeNPs were synthesized by natural or green compound techniques and was characterized by UV Vis spectroscopy, FTIR and SEM. The MIC value obtained for *E.coli* is 0.5µg/ml and for *Bacillus* is 0.25µg/ml. The techniques for SeNPs synthesis utilizing plant extracts don't need the utilization of poisonous synthetic compounds, the precursors are effectively available, cheap and does not bother with any exceptional circumstances. That method of synthesis additionally empowers command over the size, shape and stability of nanoparticles. Such synthesized SeNPs display specific potential in biomedical applications, for example, disease treatment, designated chemotherapy, molecular diagnosis and drug delivery system. They could likewise be tracked down in reasonable applications as efficient antioxidants and antibacterial agents in the food and drug industry. A ton of work has been done emphasizing various utilizations of SeNPs in the innovation and horticulture sectors. The future possibilities of selenium nanoparticles incorporate the improvement of new quick and climate cordial technique for their combination to get nanomaterial with the comparing size, shape, and properties for the ideal application. It will require a superior comprehension of physical and chemical properties connected with the particular utilization of SeNPs. Because of their less harmfulness to typical cells, it is normal that the medications in view of selenium nanoparticles might be financially accessible.

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