

GREEN SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF VANADIUM OXIDE NANOPARTICLES USING NYCTANTHES ARBORTRISTIS AQUEOUS LEAF EXTRACT

¹Madhuri Patil, ²Dr. Neetu Shrogar, ³Dr. Satish Ingale

Department of Chemistry

Pacific University, Pacific Hills, Airport Road, Pratap Nagar Extension, Debari, Udaipur, Rajasthan, - 313024 INDIA.

Corresponding Author: Ms. Madhuri Patil, Research Scholar, Pacific University, Udaipur.

Email: 19madhuriyp@gmail.com

The development of green nanotechnology has revolutionized the synthesis of nanoparticles by providing safer, more eco-friendly, and sustainable alternatives to conventional physical and chemical methods. Among the various metal oxide nanoparticles, vanadium oxide nanoparticles (V_2O_3 NPs) have attracted significant attention due to their diverse applications in biomedical, catalytic, and electronic fields. The present study focuses on the green synthesis of vanadium oxide nanoparticles using *Nyctanthes arbor-tristis*, a medicinal plant known for its bioactive compounds. This biogenic approach not only reduces the use of hazardous chemicals but also provides a cost-effective and scalable method for nanoparticle production. In this work, fresh leaves of *Nyctanthes arbor-tristis* were used to prepare an aqueous extract, which served as a reducing and stabilizing agent in the synthesis process. The reaction was initiated by mixing the plant extract with 100 mL of 5% NaOH solution and vanadium oxide precursor. A rapid colour change in the solution marked the formation of vanadium oxide nanoparticles, indicating a successful reduction of vanadium ions by the phytochemicals present in the leaf extract. This colour change served as a visual indicator of nanoparticle synthesis and was further confirmed by ultraviolet-visible (UV-Vis) spectroscopy. The biosynthetic route employed in this study proved to be rapid, simple, and environmentally friendly. UV-Vis analysis showed a characteristic absorption peak indicating the formation of V_2O_3 nanoparticles, confirming the optical properties and successful synthesis. The presence of plant-based biomolecules in the extract plays a dual role by not only reducing vanadium ions to nanoparticles but also stabilizing them and preventing aggregation. To further explore the structural and morphological characteristics of the synthesized nanoparticles, a series of analytical techniques were employed. Fourier Transform Infrared Spectroscopy (FTIR) was used to identify the functional groups involved in nanoparticle stabilization. The FTIR spectra revealed the presence of various biomolecules such as alcohols, phenols, carboxylic acids, and amines, which contributed to capping and stabilizing the nanoparticles. X-ray Diffraction (XRD) analysis confirmed the crystalline nature of the synthesized vanadium oxide nanoparticles. The XRD pattern exhibited prominent Bragg reflections corresponding to planes (012), (104), (110), (113), (202), (024), (116), (122), (214), (300), and (1010), which matched the standard data for face-centered cubic (fcc) V_2O_3 . These results verified the crystalline structure of the particles and their phase purity. Morphological studies were conducted using Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM). SEM images provided insight into the surface structure and general shape of the particles, while TEM offered higher-resolution images that revealed uniform dispersion of nanoparticles. TEM analysis showed that most particles were spherical to slightly irregular in shape and ranged in size from approximately 1 to 500 nm. Although a generally homogeneous distribution was observed, some degree of agglomeration occurred, which is common in plant-mediated syntheses. Energy Dispersive X-ray Spectroscopy (EDX) was performed to confirm the elemental composition of the nanoparticles. The EDX spectra confirmed the presence of vanadium, along with oxygen and trace elements derived from the plant extract. These results validated the successful synthesis of vanadium oxide nanoparticles with minimal impurities. The synthesized nanoparticles were also evaluated for their antimicrobial properties. The antibacterial activity of V_2O_3 nanoparticles was tested against several clinical bacterial isolates. Results indicated that the nanoparticles exhibited stronger antibacterial effects than the crude plant extract, demonstrating broad-spectrum efficacy. This enhanced activity is likely due to the small size and increased surface area of the nanoparticles, allowing better interaction with microbial membranes.

KEYWORDS: Green synthesis, Vanadium oxide nanoparticles, UV, FTIR, XRD, SEM, TEM, EDX and Antimicrobial Activities.

INTRODUCTION

Nanotechnology has become a rapidly growing field of research, with widespread applications in various industries¹ influence extends across sectors such as daily life, defence, aerospace, cosmetics, and medicine^{2,3,4}. This emerging discipline focuses on the synthesis and application of nanomaterials to improve human life. Due to their extensive use in physicochemical and biological processes, nanomaterials have attracted significant interest. Several metallic elements, including silver⁵, gold⁶, palladium⁷ and copper⁸, have been utilized in nanoparticle fabrication. Among the various synthesis methods, green-plant extraction has gained attention for being an eco-friendly and non-toxic alternative^{9,10,11}. In this article, we present a green synthesis technique for vanadium oxide nanoparticles (V_2O_3 NPs) using the leaf extract of *Nyctanthes arbor-tristis*, a plant from the Oleaceae family. Commonly known as Parijat, coral jasmine, harsingar, and queen of the night, this plant offers a sustainable and eco-friendly approach to nanoparticle fabrication¹². *Nyctanthes arbor-tristis* Linn. is native to Southern Asia, extending to Peykan in Northern Pakistan and Nepal. It is also found in Northern India and the southern regions of Thailand. This plant thrives in rocky terrains on dry hillsides and as an undergrowth in dry deciduous forests. In India, it grows in the outer Himalayas and is distributed across Jammu and Kashmir, Nepal, Eastern Assam, Bengal, and Tripura, extending to Central India up to the Godavari River in the south.^{13,14} Due to its diverse bioactive properties, including anti-inflammatory, antifungal, antidiabetic, antioxidant, antimicrobial, antileishmanial, antipyretic, and antinociceptive effects, *Nyctanthes arbor-tristis* has been selected for this study.^{15,16,17,18} However, no prior research has explored its potential role in the biosynthetic pathway for synthesizing vanadium oxide nanoparticles. Vanadium metal is known for its diverse therapeutic applications, including bone augmentation, tumor suppression, cardioprotective effects, and antimicrobial properties. Additionally, it plays a crucial role in diabetes management by mimicking insulin activity.¹⁹ In this study, we report an eco-friendly method for synthesizing vanadium nanoparticles using an aqueous extract of *Nyctanthes arbor-tristis*, which acts as a potential source of natural reducing and stabilizing agents. To thoroughly analyse the biosynthesized vanadium oxide nanoparticles, we employed UV-visible spectroscopy, X-ray diffraction (XRD), and transmission electron microscopy (TEM). Zeta potential analysis demonstrated that the nanoparticles-maintained stability for up to three months. Additionally, we utilized size-optimized vanadium oxide nanoparticles for further applications. Optimization of various parameters like time period of reaction, solvent and quantity of plant extract were done to achieve a good yield of nanoparticles. Various UV-VIS, FTIR, XRD, EDX and SEM etc. were used to characterize the synthesized vanadium oxide nanoparticles through physicochemical methods. Surveyed the influence of surfactant and the influence of calcination temperature on the shape, size and morphology using these techniques.



Fig.1. Photograph of *Nyctanthes arbor-tristis* leaves

MATERIALS AND METHODS:

Preparation of plant extract

Leaves of *Nyctanthes arbortristis* L. collected randomly from Thane (Dombivli) district area of Maharashtra State, India. These plants offer a wide range of medicinal properties and are readily available in our locality. The plants were identified and studied according to their families. Leaves were washed with tap water to get rid of all dust and unwanted particles, the process were repeated for 2–3 three times. Shaded dried at room temperature for 2–3 days and used for the preparation of powder with the help of a mechanical grinder. The powder was sieved using a 20-mesh sieve to obtain uniform size powder and stored in airtight glass bottles. 5 grams of air-dried powder was taken in 100 ml distilled water in a conical flask, and then kept on a magnetic stirrer at 500-600 rpm for 30 min. Later the mixtures were cooled to room temperature. The obtained leaf extracts filtered using Whitman number 41 filter paper. Leaf extract was obtained by filtering the mixture and either directly used in the synthesis of Vanadium nanoparticles

Preparation of vanadium nanoparticles

To prepare the reaction, begin by measuring 100 ml of the extract and pour it into an appropriate reaction vessel or container. Next, measure 100 ml of a 5% sodium hydroxide (NaOH) solution and add it to the same vessel. After that, carefully weigh out 18 grams of vanadium oxide (V_2O_5) and introduce it into the mixture. Once all components are added, begin stirring the mixture while gently heating it to a warm temperature. Continue stirring under these conditions for approximately 30 minutes, ensuring thorough mixing and interaction of the components. After this initial warming period, remove the heat and allow the mixture to continue stirring at room temperature for an extended duration of four hours. This ensures that the reaction progresses fully and evenly. Keep in the refrigerator for four days. The resulting dark blue precipitate was washed three times with distilled water and subsequently centrifuged at 12,000 rpm for 15 minutes. The solid residue was then separated using Whitman filter paper, dried thoroughly, and stored in a clean vial for further chemical characterization and assessment of its biological activity.

CHARACTERIZATION OF THE SYNTHESIZED SILVER NANOPARTICLES

UV-Vis Spectroscopy

The synthesis of vanadium oxide nanoparticles was monitored by measuring the optical density of the reaction mixture using a double-beam UV-Vis spectrophotometer (Model 2203). This spectroscopic technique is effective for detecting the formation of metallic nanoparticles. Absorbance spectra were recorded across the wavelength range of 200–800 nm, allowing observation of characteristic peaks associated with nanoparticles formation and confirming the progression of the synthesis process.²⁰

Fourier-Transform Infrared Spectroscopy (FTIR)

FT-IR Analysis of Fourier-Transform Infrared Spectroscopy (FT-IR) To characterize the surface structure of the vanadium oxide nanoparticles, Fourier-Transform Infrared (FT-IR) spectroscopy is used to identify the phytochemical compounds responsible for the reduction and stabilization during the biosynthesis of silver sulfide nanoparticles. Fourier transform infrared (FTIR) spectra of the dried extract and nanoparticles were recorded using an FTIR spectrophotometer (Bruker, Germany) in the wavelength range of 4000 to 650 cm^{-1} ^{21,22}

X-Ray Diffraction (XRD) Analysis

X-ray diffraction (XRD) was employed to identify the crystalline phases and determine the structural properties of the synthesized nanoparticles. This technique also offers valuable insights into the oxidation states and the crystalline nature of the particles over time. X-rays are generated when high-energy electrons strike a heavy metal target, such as copper. A diffractometer equipped with a $\text{Cu K}\alpha$ radiation source ($\lambda = 1.54056 \text{ \AA}$) was used. As these electrons interact with atomic nuclei, they are deflected by electrostatic forces, resulting in an energy loss that is emitted as X-rays. The resulting diffraction patterns were analyzed using Bragg's Law to elucidate the crystal structure of the nanoparticles.^{23,24} The resolution of the measurements was 0.0001° .

Diffractograms were recorded over a 2θ range of 20° to 90° .

Energy Dispersive X-ray (EDX) Analysis

Energy Dispersive X-ray (EDX) spectroscopy was employed to analyse the elemental composition of the sample. This method was particularly used to verify the presence of vanadium within the particles and to identify additional elements. Beyond simply detecting which elements are present, EDX also allows for an estimation of their relative concentrations. For sample preparation, the particle solution was diluted 1:100 with water, and 10 μL of the diluted solution was deposited onto a carbon stub and left to air dry²⁵. Spectral data were acquired at an accelerating voltage of 20 kV over a 19-second collection period. Elemental mapping was performed using pseudo-colour imaging to visualize the spatial distribution of the detected elements. The analysis was conducted using a JEOL JSM 6360 scanning electron microscope fitted with an energy dispersive X-ray analyzer.

Transmission Electron Microscopy (TEM) Analysis

X-ray diffraction (XRD) analysis was conducted using a Rigaku Miniflex II diffractometer equipped with a Cu K α radiation source ($\lambda = 1.54056 \text{ \AA}$). The scanning was carried out in the 2θ range of 20° to 90° , with a step size of 0.05° and a scan time of 2 seconds per step. The obtained XRD pattern confirmed the crystalline nature of the synthesized vanadium oxide nanoparticles. Further analysis of particle size and morphology was performed under different magnifications, providing additional insight into the structural features of the nanoparticles.²⁶

Antimicrobial activity

Procurement of Agar Cup (Well Diffusion) Method

Pure cultures of *Escherichia coli* and *Staphylococcus aureus* were maintained on sterile Nutrient Agar (NA) Petri plates and selected to evaluate the antimicrobial activity of *Nyctanthes arbor-tristis* leaf extract. For the experiment, sterile Petri plates were used along with a 6 mm sterile cork borer to prepare wells in the agar medium. The bacterial inoculum was prepared in sterile broth and evenly spread across the agar surface using sterile cotton swabs to ensure uniform distribution. Micropipettes with sterile tips were used to dispense the extract into the wells. All plates were incubated at 37°C for 24 hours to allow bacterial growth and interaction with the plant extract. Zones of inhibition were then measured to determine the antibacterial effectiveness of the extract against both bacterial strains. This method allowed for a controlled, sterile environment to accurately assess the antimicrobial potential of the biosynthesized material.^{27,28}

Testing of antibacterial activity

Sterile, molten Nutrient Agar ($\sim 20 \text{ mL}$) was poured into sterile Petri plates and allowed to solidify at room temperature. *Escherichia coli* and *Staphylococcus aureus* cultures were grown to the logarithmic phase (4–6 hours) and evenly spread over the agar surface using sterile cotton swabs to form a uniform bacterial lawn. Wells (6 mm diameter) were punched into the agar using a sterile cork borer, and the agar plugs were removed with sterile forceps. Each well was labelled according to sample concentration and controls. Serial dilutions of the test samples were prepared, and 50–100 μL of each was introduced into the respective wells. Positive (ciprofloxacin) and negative (solvent) controls were also added. Plates were left at room temperature for 30 minutes to allow diffusion, then incubated inverted at 37°C for 18–24 hours before measuring zones of inhibition.

RESULTS AND DISCUSSION

V_2O_3 nanoparticles were synthesized from plant extract of *Nyctanthes arbor-tristis* using a green synthesis approach. Various physicochemical methods such as UV-VIS, FTIR, XRD, SEM, TEM, EDX and Antimicrobial were used for characterizing synthesized catalysts. Using these techniques, the effect of surfactant and the effect of calcination temperature on the dimension, nature and morphology were Explored.

UV-visible spectroscopic analysis

UV-visible spectroscopy is a very useful and reliable technique for the primary characterization of synthesised vanadium oxide nanoparticles which is also used to monitor the biosynthesis and stability of vanadium oxide nanoparticles in aqueous solution. Vanadium oxide nanoparticles are known to exhibit a UV-visible absorption maxima in the range of 200-800 nm. The result obtained from UV-visible spectra showed the absorption peak approximately at 457 nm for freshly prepared leaf extract, due to the

surface plasmon resonance absorption band along with free electronic vibrations of V_2O_3 Nps in resonance with a light wave.

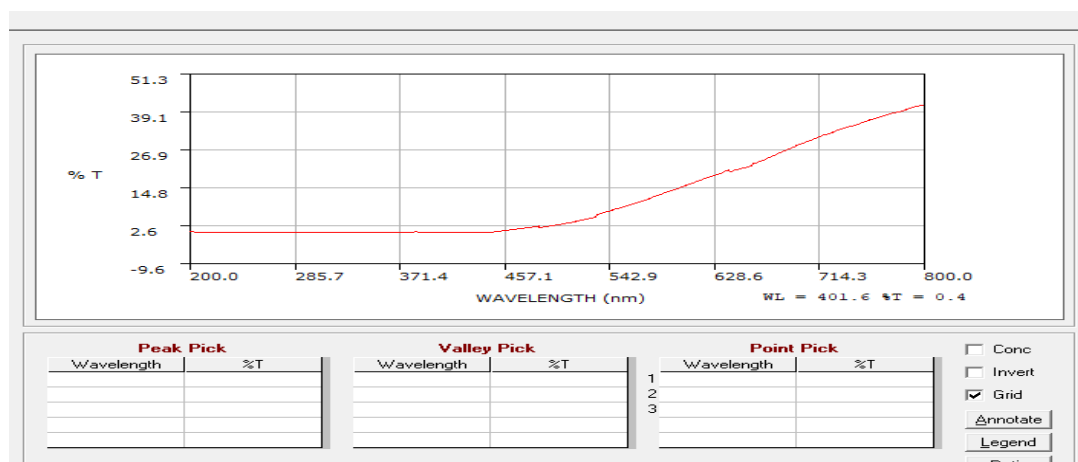


Fig 2: UV-Visible spectra of biosynthesized vanadium oxide nano particles

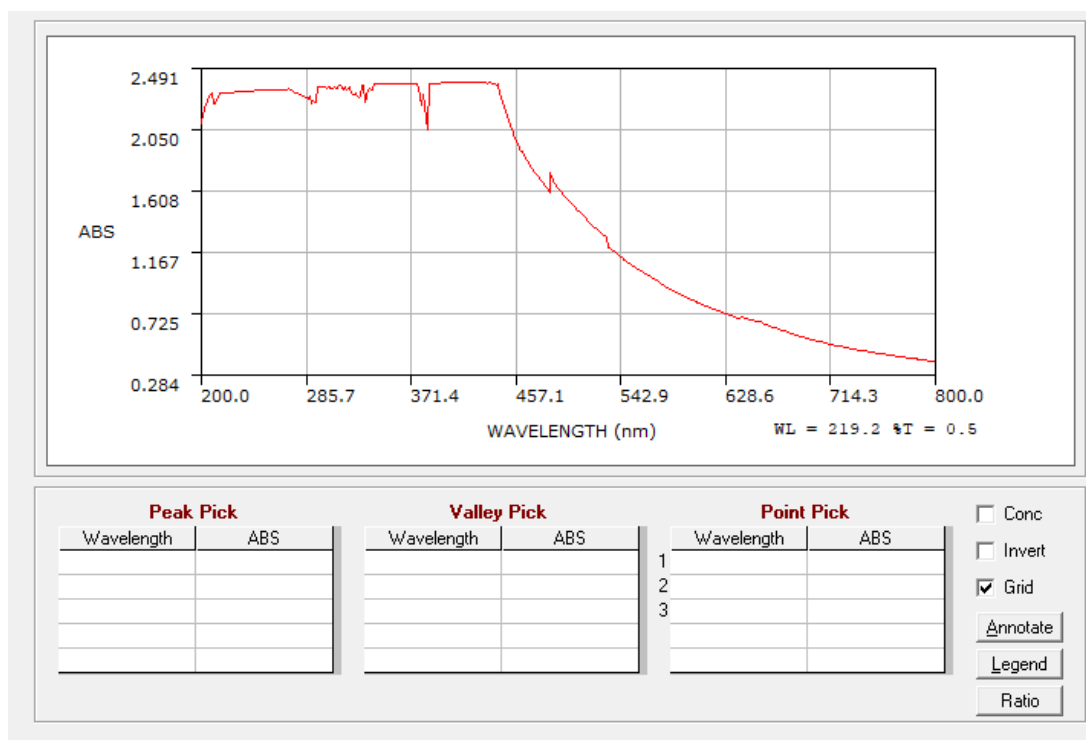


Fig 3: UV-Visible spectra of biosynthesised vanadium oxide nano particles

Fourier transform (FT-IR) spectroscopic analysis

FTIR studies Fig. 4 depicts surface contents of vanadium oxide nanoparticles (prepared using *Nyctanthes arborescens* Leaves extract) identified using FTIR spectroscopy. This unique technique is used to discover functional groups surrounding or capping on the surface of vanadium oxide nanoparticles.²⁹ The figure also confirms that the synthesized vanadium oxide nanoparticles are reduced with *Nyctanthes arborescens* Leaves extracts. The observed peaks are more prominent when *Nyctanthes arborescens* Leaves extracts are used to reduce vanadium oxide nanoparticles. The bands at 1000 cm^{-1} is observed from the present $V=O$ bond.

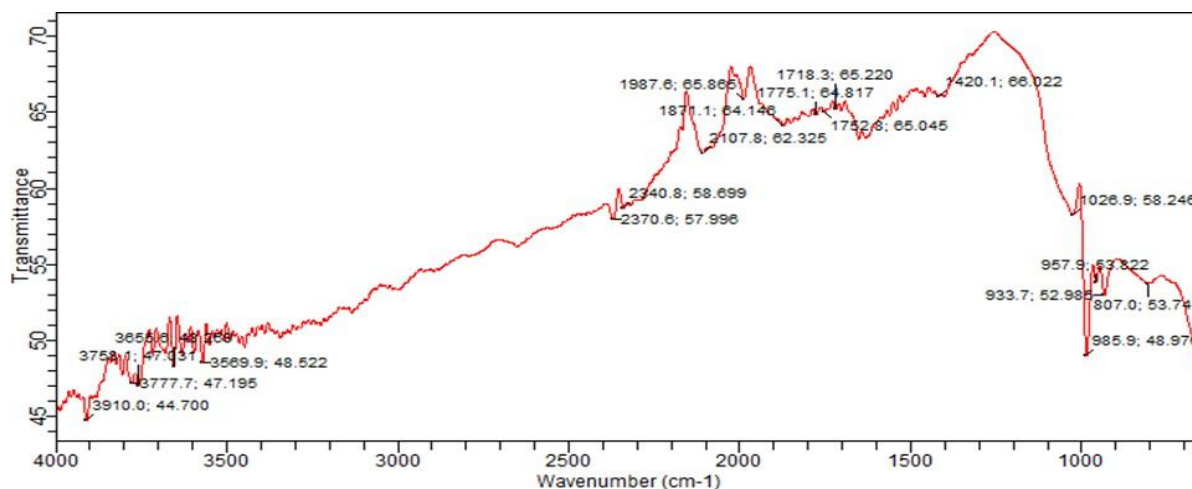


Fig 3: FT-IR spectra of biosynthesised vanadium oxide nano particles

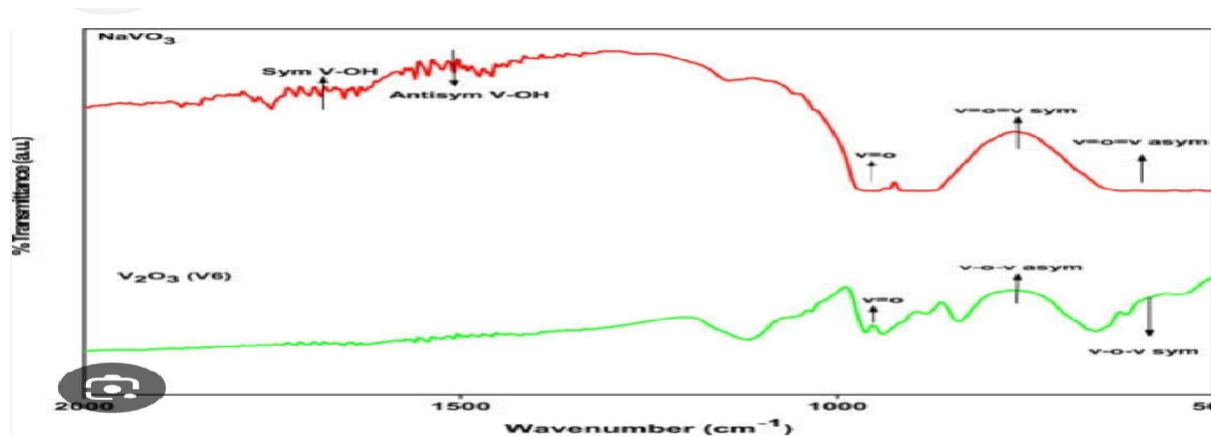


Fig 4: FT-IR spectra of biosynthesized vanadium oxide nano particles

Scanning Electron Microscopy (SEM) Analysis

Scanning electron microscopy is used to study the morphology of synthesized nanoparticles. The SEM of the image shows agglomerates of vanadium oxide nanoparticles. The size of observed nanoparticles is different between 1 to 500 nm. The SEM images are given below in figure 5.

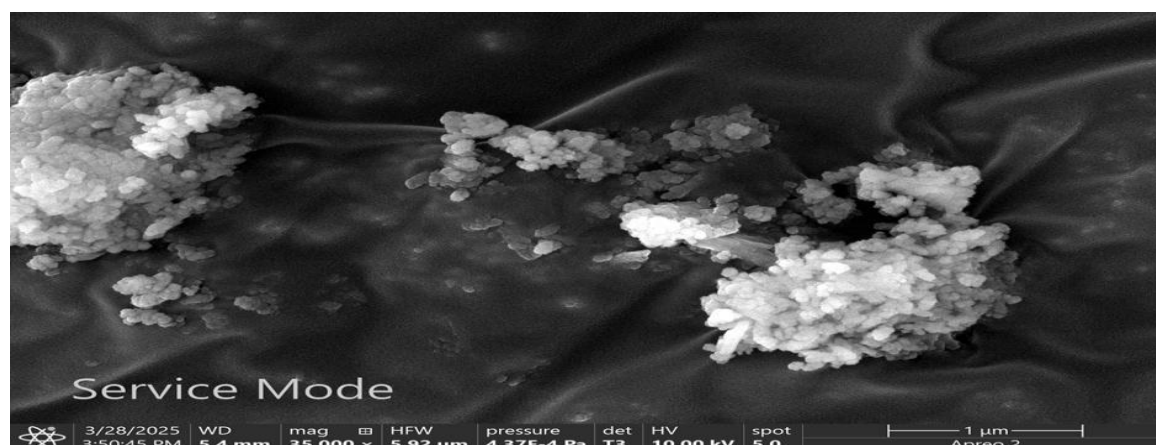


Fig:5 SEM Image of biosynthesised vanadium oxide nano particles

X-Ray Diffraction analysis (XRD) Analysis

The crystallographic structure as well as lattice properties of biosynthesized silver nanoparticles was evaluated by X-ray diffraction measurement. Fig.6 illustrates the XRD patterns of the biosynthesized vanadium oxide nanoparticles obtained from *Nyctanthes arbortristis* L. Figure 7 shows the XRD pattern for V_2O_3 nanoparticles synthesized from *Nyctanthes arbortristis* L plant extract. The 2θ values of the standard vanadium oxide sample are 24, 34, 36, 41, 45, 50, 55, 59, 63, 65, 71 corresponding to the Bragg reflections (012), (104), (110), (113), (202), (024), (116), (122), (214), (300) and (1010) respectively and confirmed the crystalline nature of the synthesized V_2O_3 NPs.

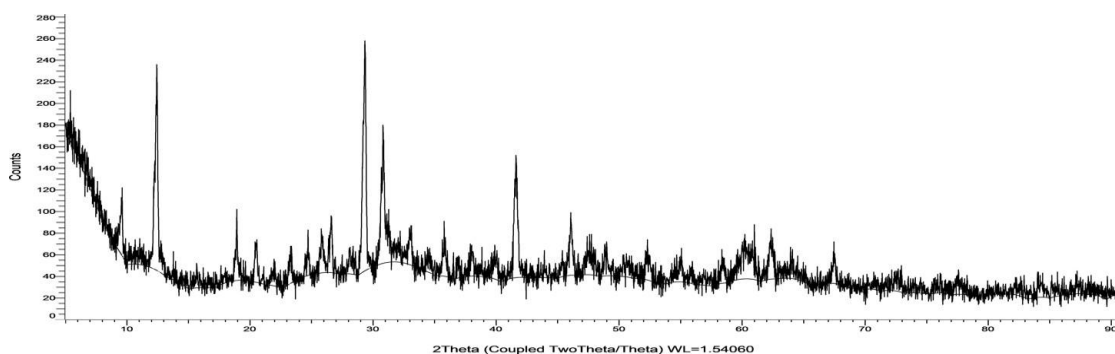


Fig 6: X-Ray diffraction pattern of vanadium oxide nano particles

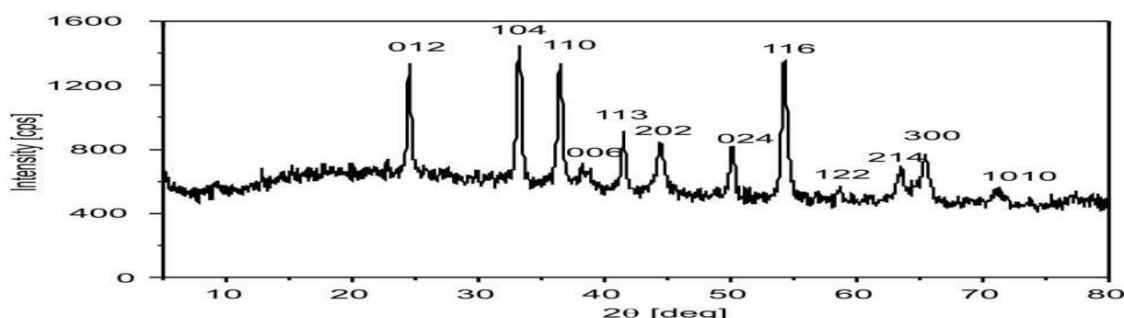


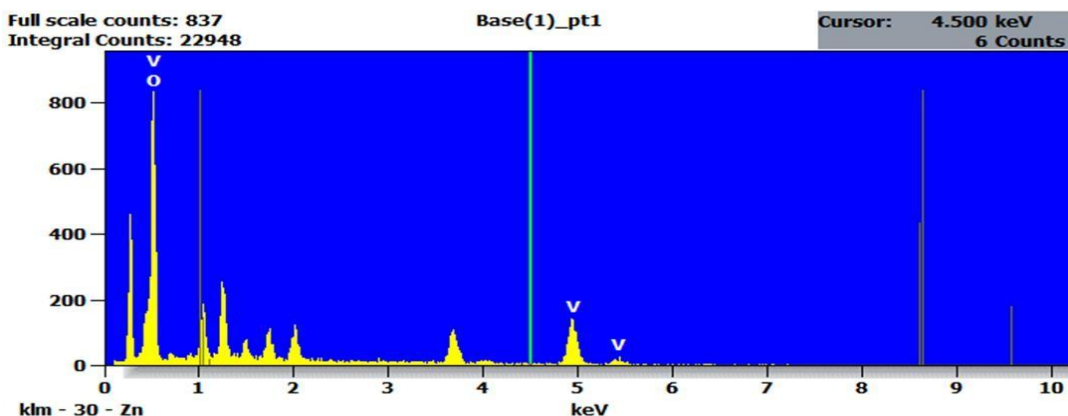
Fig. 2. X-ray diffraction pattern of the obtained V_2O_3

Fig 7: X-Ray diffraction pattern of vanadium oxide nano particles

Energy Dispersive X-ray spectroscopy (EDX) Analysis

This spectroscopy confirmed the presence of the signal characteristic of elemental silver. Figure 8 shows the Energy dispersive absorption photographs of derived v_2o_5 nps. All the peaks of vanadium are observed and are assigned. Vanadium nano crystallites display an optical absorption band peak at approximately 4.5keV. Which is typical of the absorption of metallic vanadium nano crystallites due to the surface. And O elements are also seen.

Fig.8: EDX spectra of vanadium oxide nano particles which shows the presence of V, O



ELEMENTS

1.1 Transmission electron microscope (TEM) Analysis

TEM micrographs of V_2O_3 nanoparticles Figure show rod-shaped particles with sizes ranging from 50 to 500 nm, along with their corresponding size distribution histogram."

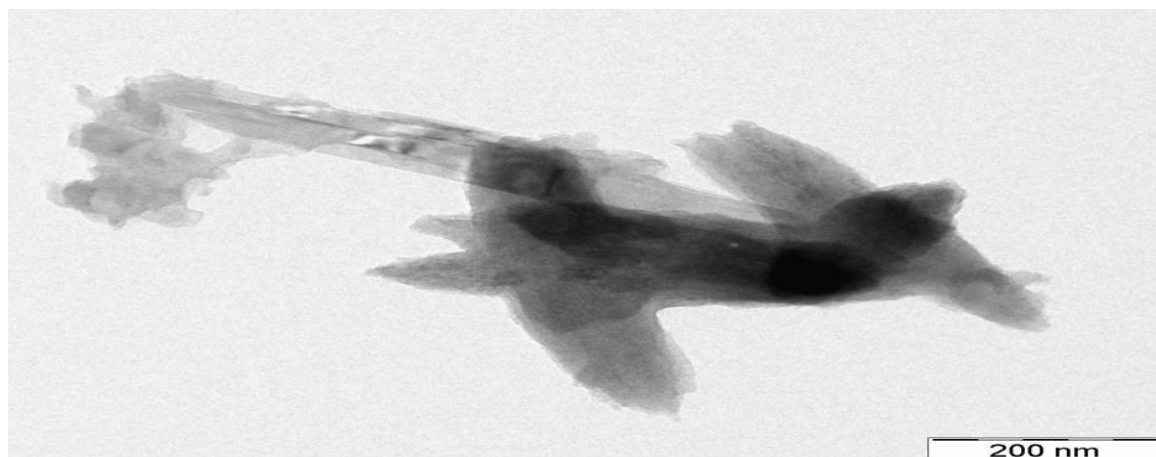


Fig 9: TEM Images of biosynthesized vanadium oxide nano particles

Antibacterial Activity

The antibacterial activity of vanadium oxide nanoparticles of samples is tested against both gram negative and gram-positive bacteria such that the inhibition zone increases (shown in figure). This was performed in presence of ciprofloxacin which is an effective antibiotic for various diseases. The result shown were remarkable it shows that the effect of ciprofloxacin increased 38mm in inhibition of *E. coli* bacteria and 43mm in *Staphylococcus aureus*.

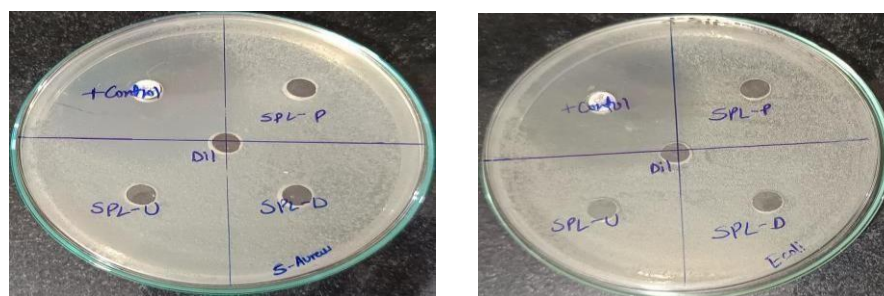


Fig 10: Antibacterial Activity Images of biosynthesised vanadium oxide nanoparticles after 24 hours incubation

The agar cup diffusion method was employed to evaluate the antibacterial activity of the three samples on Nutrient agar. After incubation at the appropriate temperature, the plates were thoroughly examined for the zone of inhibition. The results demonstrate that samples exhibit no antibacterial activity against *E. coli* and *S. aureus*.

CONCLUSIONS

A rapid and efficient method for synthesizing stable vanadium oxide nanoparticles using *Nyctanthes* leaf extract has been successfully demonstrated. The structural and morphological characteristics of the synthesized nanoparticles were analyzed through various techniques, including XRD, FTIR, SEM, TEM, EDX, and UV spectroscopy. The antimicrobial activity of the nanoparticles was evaluated against *Escherichia coli* and *Staphylococcus aureus*, yielding positive results that support their potential use in biomedical applications. The synthesis process is highly cost-effective, requiring minimal expenses and producing no harmful chemical emissions into the atmosphere. Additionally, the method offers a high yield of nanoparticles. This green synthesis approach, utilizing *Nyctanthes* leaf extract, presents a novel

and practical technique for preparing vanadium oxide nanoparticles using inexpensive and readily available materials. The process is not only simple and time-efficient but also environmentally sustainable, aligning with the principles of green chemistry. Employing plant-based systems such as *Nyctanthes* leaves as biological nano factories has garnered increasing attention due to their economic feasibility and potential for sustainable nanoparticles production.

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