

Comparative Study of Antibacterial and Antifungal Activities of Vanadium Oxide Nanoparticles and Punica Granatum Extract

Rubina Ansar¹, Naeemullah², Muneeba Zubair², Muhammad Ahsan^{2*}, Inamuddin³, Sayyad Fayaz Ahmad Shah^{2*}, Sania Javed⁴, Tariq Jamal⁵

¹Department of Community Medicine, Gajju khan Medical college swabi, Pakistan

²Department of chemistry, Abdul Wali Khan University, Mardan 23200, KPK, Pakistan

³Institute of chemical sciences, University of swat, swat, Pakistan

⁴Horticulture department, Agriculture University, Peshawar, Pakistan

⁵Department of Biochemistry, University of Malakand, Dir Lower, Pakistan

*Corresponding Author

Muhammad Ahsan &
Sayyad Fayaz Ahmad Shah

Department of chemistry, Abdul
Wali Khan University, Mardan
23200, KPK, Pakistan.

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Abstract: Researchers are becoming more interested in the eco-friendly bio-synthesis of vanadium oxide nanoparticles because they could be used in many areas of science and technology. The Punica granatum peel extract was used to prepare vanadium oxide nanoparticles (V_2O_5 -NPs) by treating $VOSO_4 \cdot H_2O$ with it. The synthesized particles was characterized using SEM, FTIR, and UV visible spectroscopy. The synthesized particles and extract were then tested for their ability to kill bacteria and fungi by testing them against species like Escherichia coli, Pseudomonas fluorescens, Bacillus subtilis, Staphylococcus aureus, Pseduomonousaliginoza, Candida albicans, Rhizopus oryzae, Aspergillus Niger, Aspergillus Parasiticus, and Aspergillus flavus. The comparison of antibacterial and antifungal activity against microbes was carried out and it was found from the growth curves of bacteria and fungi that the vanadium oxide nanoparticles showed more zone of inhibition than the Punica granatum peel extract.

Keywords: Vanadium Oxide, Antibacterial, Antifungal

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1. Introduction

It is now widely accepted that the effectiveness of antibiotics is limited by differences in the bacterial structure of antibiotic-associated bacteria. This situation will occur as a result of the misuse and overuse of antibiotics in humans and animals, which will result in the emergence and spread of antibiotic-resistant bacteria worldwide and will cause a slightly greater problem for the public. Therefore, alternative treatments must be found to solve the problem of anti-inflammatory drugs. A promising new area is the use of nanotherapeutics to combat the immune system in malignant diseases. It is even claimed that brain cells can be specifically targeted using nano drug delivery platforms and side effects can be minimized. These products can bypass the immune system and provide treatments such as antibiotics to prevent the infection from developing resistance [1]. Nanomaterials have unique properties that distinguish them from many common ones [2]. The unique properties of nanoparticles, especially silver nanoparticles (AgNPs) and gold nanoparticles (AuNPs), include their unique surface area, tunable size and shape, and the ability to cross the barrier of objects, making them attractive for many applications [3]. From a biomedical perspective, AuNPs have unique properties such as their small size, non-toxicity, and lack of resistance to antibiotics, making these materials useful for drug

delivery [4]. In addition, AgNPs exhibit good therapeutic effects, including antibacterial, antifungal, anti-inflammatory, anti-inflammatory, and antioxidant potential, thus positioning them as drugs has many delivery possibilities [5]. However, noble metal nanoparticles have some important problems such as high cost, advanced storage methods and cytotoxicity; Vanadium (V) is an element that attracts a lot of attention [6]. Vanadium pentoxide (V_2O_5) is a vanadium oxide with outstanding properties such as structural stability, strong electrical conductivity and the ability to be used as other materials in biological media. V_2O_5 nanoparticles (VNP) have antioxidant, cytotoxic, anticancer and antidiabetic activities [7]. In addition, VNPs can improve the bioactivity of plant extracts, including antibacterial, photocatalytic and antioxidant capacity, thus enhancing their efficacy. Vanadium nanoparticles (VNPs) are chemically synthesized by the sol-gel method using sodium metavanadate as a precursor and cetyltrimethylammonium bromide (CTAB) as a surfactant [8]. Modern technology has not been proven to meet these standards, but the demand for synthetic methods continues to increase. Recently, filamentous fungi have been used as reducing agents and stabilizers to biosynthesize VNPs [9]. The use of plant extracts for nanoparticle synthesis has many advantages, such as rapid production, high yield, and easy scaling up for commercial applications, making it a promising method for nanomaterial

synthesis. Furthermore, VNPs can undergo structural changes due to changes in temperature during synthesis. Previous studies have shown that changes in temperature can cause changes in the VNPs crystal plane, leading to different properties. [10]

In contrast, green synthesis uses biological materials, including plant extracts, bacteria or fungi, to produce nanomaterials. The use of plant extracts in nanoparticle synthesis is preferred over bacteria or viruses due to the variety of plant materials, cost-effectiveness, environmental safety, non-toxicity and ease of preparation. The ability to reduce the ion capacity and stabilize nanoparticles depends on the amount of polyphenols and other metabolites entering the plant extract [11]. Plant extracts promote the biosynthesis of many metal nanoparticles that are more resistant to the environment and allow the production of nanoparticles of different sizes and shapes to be controlled. The name "power fruit" is associated with pomegranate (*Punica granatum*), a fruit known for its unique healing properties and health benefits for consumers. The peel of the pomegranate (*P. granatum*), which constitutes about one-third of the fruit weight, is removed after harvest. Agricultural wastes have recently attracted great attention due to their high availability and low cost. Pomegranate peel contains high levels of phenolic compounds and is considered a source of antioxidants. In addition to punicalagin, gallic acid, and ellagic acid found in pomegranate peels, there are also chlorogenic acid, caffeic acid, punicalagin, and other phenolic components such as apigenin, quercetin, pelargonidin, and nanoparticles of pomegranate A. and B. [12]. More importantly, there are very few studies on the synthesis of VNPs alone and the study of their biological effects; when available, VNPs are often used as additives together with other metals or oxides. This limits the use of VNPs that could be effective biocides and the understanding of their effectiveness. Therefore, the research model for the preparation and evaluation of VNP bioactivity including the work of Suma et al. (2020) [13], Shabani et al. (2021) [14], Hardy et al. (2023) [15]. However, except for Shabani's research group using *F. oxysporum* cell-free filter for VNPs biosynthesis, other VNPs synthesis methods are chemical or physical, which may have adverse effects in terms of morphological and biological properties of the material. In this sense, botanical extracts act as green herbs and stabilizers due to their rich content of plant compounds. In addition, their anti-inflammatory, antiviral and DNA-splitting abilities have been studied in clinical trials. Significant evidence can be provided that its target works like other products used in biomedicine and pharmaceuticals [16].

In the current study antimicrobial activities were assessed for biosynthesize vanadium oxide nanoparticles from pomegranate peel extract.

2. Materials and Methods

2.1 Chemicals

Punica granatum was purchased from local market of swat. The Hydrated Vanadium sulphate ($\text{VOSO}_4 \cdot \text{H}_2\text{O}$), was purchased from sigma Aldrich.

2.2 Collection of *Punica granatum*

The fruit of the pomegranate (*P. granatum*) was procured at a local market in swat Pakistan, thoroughly rinsed with potable tap water to eliminate contaminants, and the peels and seeds were hand

removed. The fresh *P. granatum* (100 g) was diced into small fragments and blended in 500 mL of purified water utilizing a high-speed blender (800ES blender, 230 V, 50 Hz, 330 W, model BB90E, USA) for 10 minutes at ambient temperature. The dispersed *P. granatum* mixture was subjected to boiling for 45 minutes under stirred conditions. The mixture was centrifuged at 15,000 rpm for 15 minutes at 4 °C, after which the supernatant of the *P. granatum* extracts was filtered, collected, and kept at 4 °C for subsequent use [17].

2.3 Synthesis of vanadium oxide nanoparticles

The biosynthetic method was employed to produce vanadium oxide (V_2O_5) nanoparticles. Consequently, 100 mL of pomegranate peel extract was incrementally added (one drop per second) to 0.012 moles of 50 mL $\text{VOSO}_4 \cdot \text{H}_2\text{O}$ and stirred for 30 minutes. Subsequently, 40 mL of 1 N NaOH was incorporated into the solution. The pH value increased to 12. A dark green precipitate was acquired; it was subsequently cleaned with deionized water, used a centrifuge followed by decantation for all processes. Subsequently, it was dried for four hours at 110 °C in the oven and then calcined for four hours at 300 °C. A yellowish powder of vanadium oxide nanoparticles was obtained [18].

2.4 Characterization

A Shimadzu Corporation made FTIR spectrophotometer was used to identify the functional groups in polymeric nanocomposite. SEM analysis were done to know about the structures of polymeric nanocomposite and to know about the elemental analysis of the synthesized polymeric nanocomposite an Oxford EDX (Inca-200) was used.

2.5 Evaluation of antimicrobial activities

The agar well diffusion technique was employed to evaluate the antibacterial efficacy of the extract and Vanadium oxide (Vanadium oxide). It required 100 ppm of the culture from each strain to inoculate a Petri dish containing Muller Hinton agar for bacteria and potato dextrose agar for fungi. A pristine cork drill was employed to create four apertures in each plate. The initial well contains DMSO as a negative control. The second well contains sulfamethoxazole for bacterial inhibition and clotrimazole at 100 ppm for fungal inhibition as a positive control. The third well contains an extract at 100 ppm, whereas the fourth well contains Vanadium oxide at 100 ppm. Petri plates were incubated at 37 °C for 24 hours to assess antibiotic activity. For antifungal action, they were maintained in an incubator at 28 °C. We determined the minimum inhibitory concentration (MIC) of the nanoparticles against several illnesses and obtained significant results [19].

3. Results and Discussion

3.1 UV-spectrum of Vanadium oxide nanoparticles

The UV-Visible spectrum was employed to examine the optical properties of the synthesised V_2O_5 nanoparticles. Figure 1 illustrates the UV-Vis absorption spectrum of the biosynthesized V_2O_5 nanoparticles dispersed in deionized water. The transition holes process between V and O resulted in the emergence of an absorption peak in this spectrum at 379 nm, along with the quantum confinement feature. The energy gap was determined using the equation $E_g = 1239.83 / \lambda$, yielding a value of 3.2713 eV.

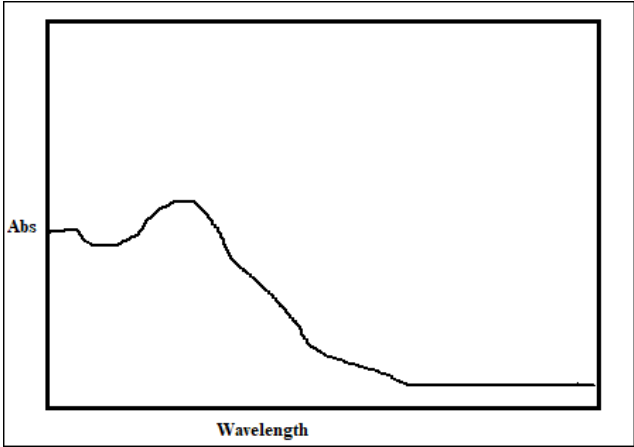


Figure 1. UV-spectrum of Vanadium oxide nanoparticles

3.2 FT-IR of Vanadium oxide nanoparticles

FT-IR spectrum analysis was conducted on the vanadium oxide, as depicted in Figure 2. The distinctive V_2O_5 peaks were identified at 1056.13 and 827 cm^{-1} , respectively. The numbers pertain to the stretching vibration of terminal oxygen bonds, $V=O$ (827 cm^{-1}), and the vibration of doubly coordinated oxygen (bridge oxygen) bonds, $V-O-V$ (1056.13 cm^{-1}). The bands at 597.94 and 420.48 cm^{-1} were attributed to the $V-O$ stretching mode.

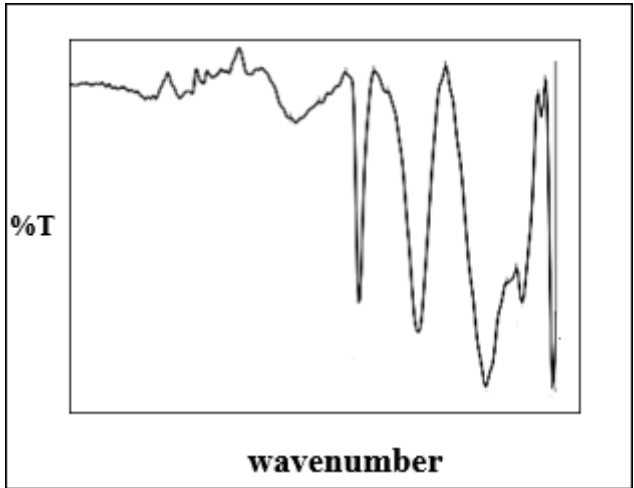


Figure 2. FT-IR of Vanadium oxide nanoparticles

3.3 SEM of Vanadium oxide nanoparticles

Figure 3 displays scanning electron microscopy images of the V_2O_5 . The V_2O_5 consists of schistose grains with irregular sizes.

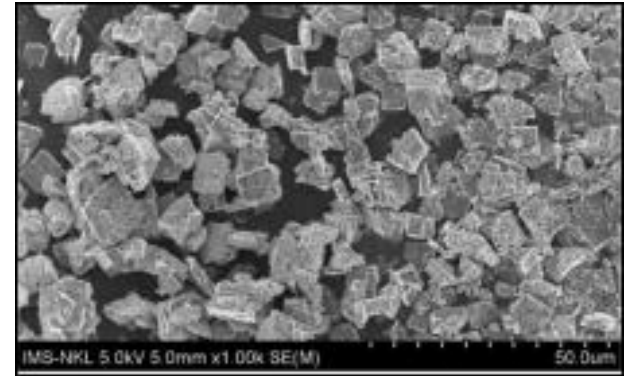


Figure 3. SEM of Vanadium oxide nanoparticles

3.5 Antibacterial activity

The Agar well diffusion method was employed to assess the antibacterial efficacy of the extracts and Vanadium oxide against pathogenic microorganisms. At a concentration of 50 mg/mL of extract, the zone of inhibition (ZOI) recorded in Table 1 was 6.2 mm for *E. coli*, 8 mm for *P. fluorescens*, 12 mm for *S. enterica*, 7 mm for *B. subtilis*, and 8 mm for *S. aureus*. Vanadium oxide can effectively eliminate pathogens due to their stability and efficacy against both gram-positive and gram-negative bacterial cells. The application of Vanadium oxide resulted in a zone of inhibition of 13 mm for *E. coli*, 14 mm for *P. fluorescens*, 15 mm for *S. enterica*, 9 mm for *B. subtilis*, and 10 mm for *S. aureus*. The antimicrobial outcomes are illustrated graphically in Figure 4. The bacterium examined exhibited the greatest zone of inhibition against *S. enterica* for both the extract and the Vanadium oxide. Smaller Vanadium oxide can eliminate more germs than larger ones due to their ability to engage with a greater number of surfaces and their enhanced absorption capacity. This study indicates that Vanadium oxide are effective against *Salmonella enterica*. The MIC of the extract and Vanadium oxide was also reported. The nanoparticles exhibited significantly lower minimum inhibitory concentrations than the extracts. The results unequivocally demonstrated that the synthesised nanoparticles possess potent antibacterial activities. It can serve as an effective source for developing novel pharmaceuticals that combat resistant pathogens. Table 2 presents the MIC values identified for several strains.

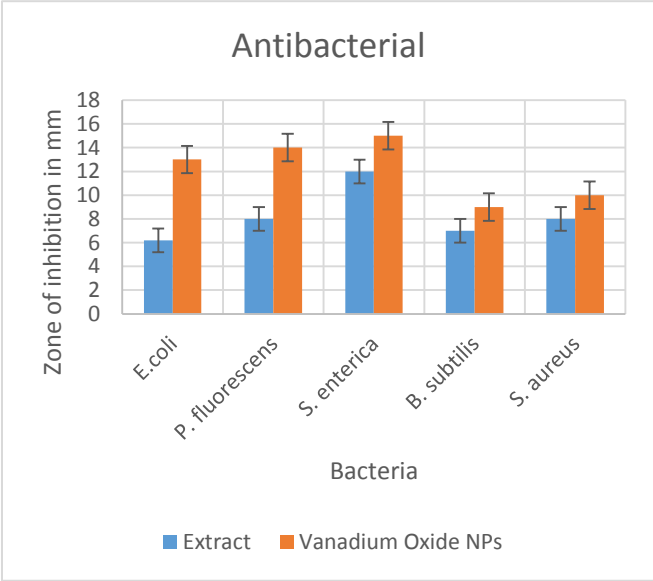


Figure 4. Error bar graph of antibacterial activity of extract and vanadium oxide nanoparticles

Table 1: Zone of inhibition (mm)		
Strain	Extract	Vanadium Oxide NPs
E.coli	6.2	13
P. fluorescens	8	14
S. enterica	12	15
B. subtilis	7	9
S. aureus	8	10

Table 2: MIC of extract and V₂O₅ NPs

Strain	Extract(mg/mL)	V ₂ O ₅ (mg/mL)
E.coli	12	5
P. fluorescens	13	3.6
S. enterica	4	4
B. subtilis	12	4
S. aureus	4	2.6

3.6 Antifungal activity

The Agar well diffusion method was employed to evaluate the efficacy of the extracts and Vanadium oxide against pathogenic microorganisms. Fifty mg/mL of extract was required to eradicate *C. albicans* (6.3 mm), *A. niger* (9.0 mm), *R. oryzae* (9.2 mm), *A. paharisticus* (7.1 mm), and *A. flavus* (9.2 mm) following incubation. Vanadium oxide (Vanadium oxide) are employed to exterminate live organisms due to their stability and efficacy against a broad spectrum of fungal cells. The inhibition zones for Vanadium oxide (50 mg/mL) measured 7.6 mm for *C. albicans*, 12 mm for *A. niger*, 15 mm for *R. oryzae*, 12 mm for *A. paharisticus*, and 13.5 mm for *A. flavus* (Table 3). The antifungal results are illustrated graphically in Figure 5. The extract exhibited the maximum zone of inhibition (ZOI) against *R. oryzae* and *A. niger*, while the Vanadium oxide had the highest ZOI alone against *R. oryzae*. Due to their increased surface area for interaction and enhanced absorption, smaller Vanadium oxide can be more efficacious in eradicating fungus compared to larger counterparts. The MIC of the extract and nanoparticles was also reported. The nanoparticles exhibited much reduced MICs compared to the extracts. The results unequivocally demonstrated that the synthesised nanoparticles possess potent antifungal activities. It can serve as an effective source for developing novel pharmaceuticals that combat resistant pathogens. Table 4 presents the MIC values identified for several strains.

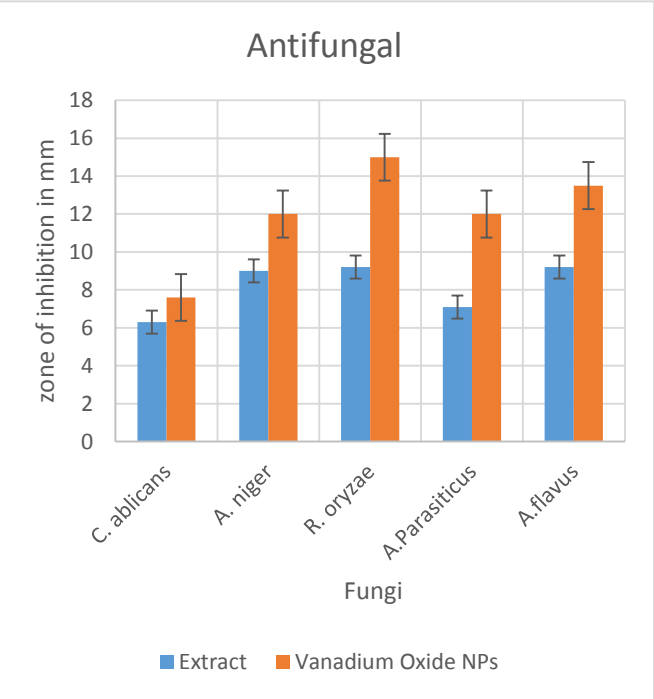


Figure 5. Error bar graph of antifungal activity of extract and vanadium oxide nanoparticles

Table 3: Zone of inhibition (mm)

Strain	Extract	Vanadium Oxide NPs
C. albicans	6.3	7.6
A. niger	9	12
R. oryzae	9.2	15
A.Parasiticus	7.1	12
A.flavus	9.2	13.5

Table 4: MIC of extract and V₂O₅ NPs

Strain	Extract (mg/mL)	V ₂ O ₅ NPs (mg/mL)
C. albicans	15	2.7
A. niger	16	4.0
R. oryzae	12	3.8
A.Parasiticus	13	3.6
A.flavus	165	4.3

Conclusion

The results indicated that significant quantities of Vanadium oxide (Vanadium oxide) were generated, as confirmed by UV-visible spectroscopy. We observed the emergence of a Surface Plasmon Resonance (SPR) peak at 379 nm, corresponding to the Vanadium oxide. In conclusion, our data indicate that plant peel extracts yield viable Vanadium oxide. The Vanadium oxide were characterized using UV, FTIR, and SEM techniques. The synthesised particles and extract were subsequently evaluated for their efficacy in eradicating bacteria and fungi by conducting tests on species such as *Escherichia coli*, *Pseudomonas fluorescens*, *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas alginosa*, *Candida albicans*, *Rhizopus oryzae*, *Aspergillus niger*, *Aspergillus parasiticus*, and *Aspergillus flavus*. The growth curves of bacteria and fungi indicated that Vanadium oxide exhibited greater antibacterial activity than the extract.

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