

RESEARCH PAPER

Antimicrobial of Binary Nano-Composite ZnO/V₂O₅: Green Synthesis

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ABSTRACT

Antimicrobial Binary Nano-Composite ZnO/V₂O₅ is synthesized through modified green sol-Gel method, that's depend on extract solution of Reishi mushroom. Calcination process have been done through different temperature degrees that's ranged from 300 into 700 °C. Characterization of Binary Nano-Composite ZnO/V₂O₅ is achieved through band gap measurements, X-ray diffraction, field emission scanning electron microscopy, and FT-IR spectroscopic techniques. Antimicrobial activity of Binary Nano-Composite ZnO/V₂O₅ is tested towards two different types of gram-negative (E. coli) and gram-positive (S. aureus) bacteria. The band gap value of binary nano-composite is equal to 3.424 eV. X-ray diffraction data show that binary nano-composite crystals is orthorhombic amorphous forms with average crystal sizes of 29.63nm. According to FE-SEM measurements that show the Crystal size of binary nano-composite is obeyed into nano scale, since the grain size ranges from 29.63 to 30.38nm. The synthesized binary nano composite have high antimicrobial activity toward the bacteria at concentration ranges from (0.025, 0.05 to 0.1 mg/ml).

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INTRODUCTION

The nanotechnology approach is a great significantly importance today for many application industries, that's including the pharmaceutical, cosmetics and medical industries and so on. The conditions necessary for processing nano materials, such as acidity, PH control, concentration of reactants, and mixing temperature, can be controlled [1]. Green synthesis has been on the rise recently because it is less expensive, safer, and environmentally friendly and non-polluting side residual material [2]. One of the most common methods in down-top approaches of nanoparticle synthesis is sol-

gel method due to their conditions is controlling in an open system vessel and can be modified under centered requirements of chemical reactions [3]. Reishi mushroom is one of natural products that's can be used in preparing the nanoparticles materials to reduced using of chemical pollutant agents in high concentrations such as Sodium hydroxide. Reishi mushroom contains 400 different biological compounds, including terpenoids and polysaccharides. Terpenoids are known for their antibacterial and anti-inflammatory effects [4].

In medicine and other industry, there many uses applications are found for nano particles of zinc oxide powder with their nano composites

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and it is considered one of an essential material due to safety, wide range applicable and low cost [5]. Zinc oxide nanoparticles have shown antibacterial activity and zinc oxide is currently being studied as an antibacterial agent [5]. One consideration for the use of zinc oxide nanoparticles as an antibacterial agent is that zinc oxide particles are not toxic to human cells. At the same time zinc oxide nanoparticles are toxic to T cells at concentrations higher than 1.2 mM [6]. Zinc oxide nanoparticles exhibit antimicrobial properties against *E. coli*, *Staphylococcus aureus*, bacteriophages, and various skin-specific pathogens, whether exposed to UV light or no [10, 7]. It was observed that by mixing zinc oxide with vanadium oxide, which has distinct photo catalytic and antibacterial properties, these two nano composites have recently been shown developed. On the other hand, the binary nano composite of ZnO/V₂O₅ shows the ability of a tremendous approach in photo catalytic processes as well as antibacterial processes that cause diseases [12,13]. The process of integrating nanoparticles (NPS) with plants, fungi or their metabolites in the green synthesis process. Other nanoparticle oxides are also considered essential materials in many applications. Vanadium oxide is one of the widely available compounds because it is non-toxic, economically beneficial and low-cost, so it can be exploited in the green synthesis process and effective activity against bacteria [8].

The recent work tend to synthesis a binary nanocomposite of ZnO/V₂O₅ at different ratio and at different temperature degree of calcination. The sol-gel method will modify through using a natural product as Reishi mushroom to minimized the pollutant residual and also finding suitable conditions for method of synthesis. Characterization will be done through some of microscopic and spectroscopic methods. At last, antimicrobial investigation will be test under different types of bacteria.

MATERIALS AND METHODS

Reishi mushroom extract

The dried mushrooms were taken well from locally markets of Iraq and ground into powder using a grinder. After that, the ground powder was collected and placed in a large 1000ml beaker. Hot distilled water was placed in the beaker with the addition of 50ml of ethanol to speed up the extraction process. The mixture was stirred and

left to cool. After that, the beaker was stored after covering it well in a dark place to avoid photo-oxidation and left for two weeks, stirring the mixture daily continuously. The mixture was filtered using a clean cloth and the filtrate was filtered well. The resulting extract was stored after filtering well in a dark glass container for later use [9-11].

Preparation of binary nano-composites

Different ratios have been used of zinc oxide with vanadium oxide were taken (1:1, 1:2, and 2:1). 0.1M of hydrated zinc nitrate (99.00_103.00% B/4-6, M.I.D.C., Dindori, Nashik, India) was mixed with 0.1M of vanadium oxide (pure 99.8 % BATCH No.170159) in 100ml distal water beaker, this ratio is 1:1. After that, 100ml of the previously prepared Reishi mushroom extract was added to the volumetric beaker with a magnetic stirrer and placed on a heater at 65°C. A basic solution of sodium hydroxide (Reagent World purity 97%) with a concentration of 2.5 molar was prepared. After that, the solution was placed in a volumetric burette in order to dropwise on the reaction mixture with a magnetic stirrer. The basic solution with the zinc oxide and vanadium oxide complex and after the titration process was completed, the solution was left to cool slightly and then filtered using a glass funnel and filter papers. The filtrate was washed with hot distilled water for several times to remove impurities and obtain a purer filtrate. The filtrate was taken and dried in a drying oven at a temperature of 100 for 3 hours. The filtrate was taken, ground, and converted into a powder for roasting at temperatures of 300-700°C and laboratory measurements were carried out. The same procedure is repeated for other composites ratio (1:2 and 2:1).

Spectral measurements

The band gap of nanocomposite is determined according to Tauc Plot method, which is based on measuring the material's optical absorbance and using the Tauc equation to calculate the band gap value. It is used to measure the band gap of a solid material (typically a semiconductor material) using a UV-VIS Spectrophotometer. The energy gap is calculated from the relationship as in Eq. 1 [12].

$$(\alpha h\nu)^n = A(h\nu - E_g) \quad (1)$$

Since that α : Absorption coefficient, $h\nu$:

Photon energy, E_g : Energy gap (band gap), A : Constant and n is a degree that's Depends on the type of electron transition where $1/2$ for the allowed direct transition and 2 for the indirect transition. the measuring the energy gap using the spectrophotometer Japanese Shimadzu UV-2600 Double Beam, Light Source D2 (UV) and W (VIS). The powder sample is place in a thin film on a quartz glass carrier [12]. The infrared measurements of prepared nanocomposite samples have been done using (Perkin Elmer Two, USA). The signal is being recorded from time to frequency (absorption spectrum) using a Fourier transform, Outcome: An FTIR spectrum that plots the transmittance (or absorbance) against wavenumber (cm^{-1}).

To examine the crystalline structure of Nano composite powder of ZnO/V₂O₅, the detection process is carried by X-ray diffraction technique (Diffracto meter for X-rays, Shimadzu (Japan) XRD-6000), Cu K α ($\lambda = 1.54 \text{ \AA}$). The instrument operates based on Bragg's Law: $n\lambda = 2d\sin\theta$ [9] since λ Wavelength of the X-ray (usually Cu K α = $1.5406 \text{ \AA}$), d : Spacing between atomic planes in the crystal, θ : Diffraction angle, n : Order of diffraction (usually $n = 1$). X-rays diffract at angles particular to the crystal structure when they strike a crystalline sample. The substance is identified by measuring these angles to comparable with standers values and crystal size determination. The crystal image is determined by Field Emission Scanning Electron Microscope (FE-SEM) Instead of using a conventional thermionic electron gun, the FE-SEM is a sophisticated kind of scanning electron microscope that uses a Field Emission Gun (FEG). This makes high-resolution imaging possible. Extremely high magnification, up to millions of times, also Surface analysis of nanostructures. The sample is coated be electrically conductive or sputter-coated with carbon or gold. Adhered with conductive adhesive to a metallic stub.

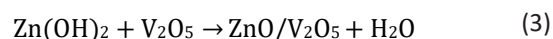
Antimicrobial activity

The Antimicrobial activity of binary nano composites particles have been tested against gram-negative (E.coli) and gram-positive(S.aures) bacteria. Sterile petri dishes were prepared. Then, 1 ml of gram-negative E. coli bacteria was cultured in a culture medium with Broth

solution for 24 hours. 100 microns were added to 5 ml of normal saline. The bacterial medium is groaned in the previously prepared dish. Holes were made in the dish according to the prepared ratios of the compound. These holes were filled with the Four different concentrations ratios of the nanocomposite that's calcinated at 400°C to examine the effect of these ratios on bacteria (1-4) (0.025-0.1gm/ml). the dish was placed in a sterile incubator for 24-48 hours. In the same way, positive bacteria were prepared. At lasted diameters of Bacterial zone is measured.

RESULTS AND DISCUSSION

The green synthesis is depended on the activity of extracted solution Reishi mushroom that's mixes with both hydrated zinc nitrate vanadium pentoxide in the presence of dropwise sodium hydroxide solution into the mixture. According to the Sol. Gel. method pathway, the reaction mixture is converted into white precipitate of zinc hydroxide in the presence of vanadium pentoxide as in Eq. 2.



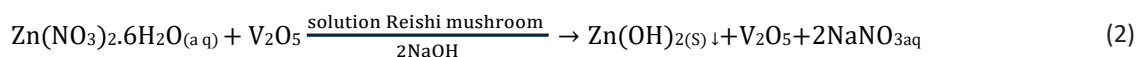
At last reaction progresses the zinc hydroxide will convert into zinc oxide in the presence vanadium oxide to yield the composite with yellow-orange precipitate as in Eq. 3.

The material's optical band gap energy is ascertained using the spectrum. Optical Band Edge The marked point indicates a wavelength of 362 nm, where strong absorption begins. The band gap energy is calculated using the Eq. 4 (Fig. 1).

$$E_g = \frac{1240}{\lambda} = 1240/362 = 3.424\text{eV} \quad (4)$$

The material is a broad band gap semiconductor, appropriate for applications in the following fields, as shown by the computed optical band gap of about 3.42 eV. The graph's band gap shift indicates a change in the band structure, most likely brought on by doping or the creation of heterojunctions [10-12].

FTIR spectra confirmed the presence of



Zn–O and V–O bonds as shown by the Fig. 2 (A1, A2, A3) Respectively (1:1, 1:2, 2:1) several characteristic peaks that indicate the presence of specific functional groups in the sample. The most prominent of these peaks is the distinctive peak 604 and 471.94 cm⁻¹. These are associated with metal–oxygen (M–O) bonds, such as Zn–O or V–O, which support the presence of metal oxides. The most prominent peaks are shown in Fig. 1, according to the different calcinated temperatures that's ranged from 300 °C into 700 °C. the Peak

frequency at ~1111.1 cm⁻¹ that's indicate V=O vibrations (a double bond between vanadium and oxygen). Two peak frequencies at ~870.80 cm⁻¹ and ~740.98 cm⁻¹ May result for a different vibration of V–O or Zn–O bonds respectively. Strong peak at ~547.47 cm⁻¹ This peak is typical of metal oxides, especially Zn–O, and indicates the presence of zinc oxide. this spectrum supports the presence of metal oxides of nano composite the optimum temperature was chosen at 400C° for all prepared proportions (1:1, 1:2, 2:1) Respectively [13-15].

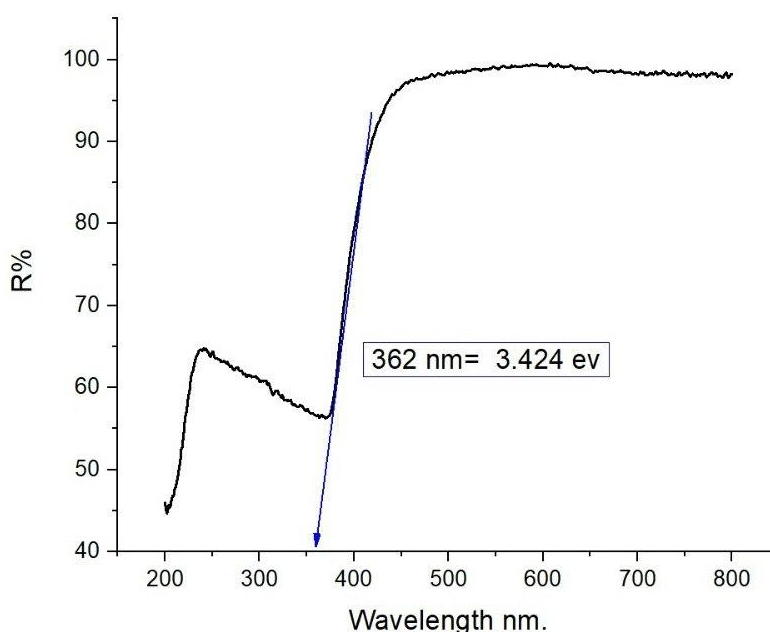


Fig. 1. band gap energy of binary nano composite ZnO/V₂O₅.

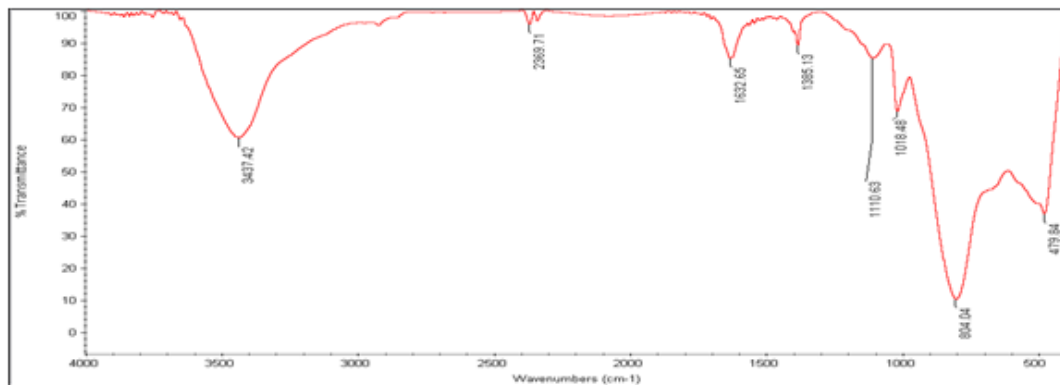
Table 1. Particle size determination by XRD of Binary nano composite ZnO/V₂O₅.

Calcination Temperature	Ratio 1:1 2 ^θ [Th]	Crystal size nm	Ratio 2:1 2 ^θ [Th]	Crystal size nm	Ratio 1:2 2 ^θ [Th]	Crystal size nm
300°C	10.0	8.1	21.0	8.1	10.0	8.1
	11.0	8.1	13.0	8.2	11.0	8.1
	7.50	8.0	15.0	8.4	13.0	8.2
	15.50	8.3	13.0	8.2	10.0	8.1
400°C	13.0	8.2	10.5	8.1	8.0	8.0
	10.5	8.1	7.5	7.9	7.5	8.0
	10.1	8.1	14.5	8.2	17.5	8.4
	13.0	8.2	13.0	8.1	15.5	8.3
500°C	15.5	8.3	11.0	8.0	14.5	8.2
	14.5	9.1	14.5	8.2	14.0	8.7
	13.0	8.2	13.0	8.2	13.0	8.2
	10.0	8.1	10.0	8.1	15.0	8.2
600°C	13.0	8.2	14.5	8.2	14.5	8.1
	14.0	8.7	13.5	8.2	13.0	8.2
	14.1	8.2	13.0	8.1	20.5	8.5

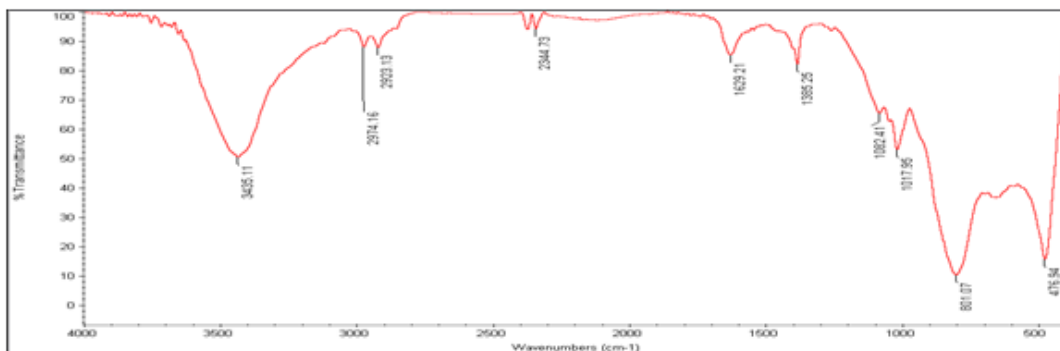
Particles size is determined through XRD patterns confirmed the presence of both ZnO and V₂O₅ phases, indicating successful composite formation as shown in Fig. 2(B1-B3)

Comparison with the JCPDS database, the main

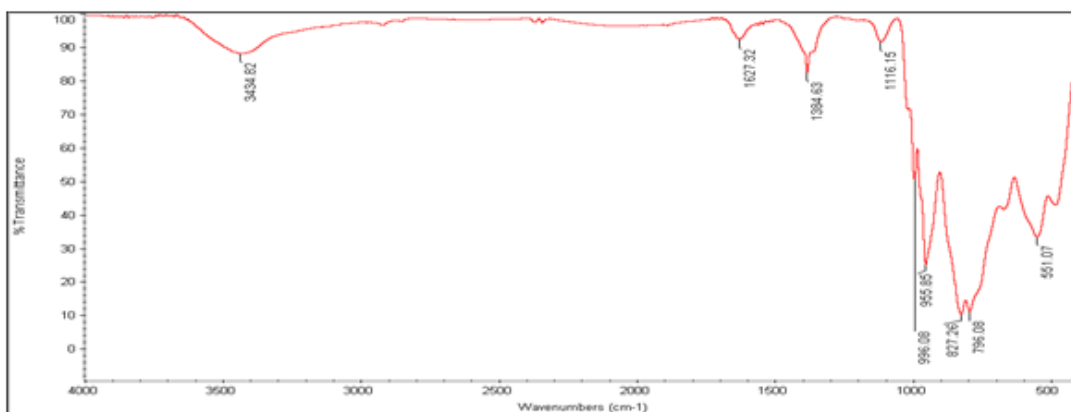
peaks present at 2 θ (31.7°, 34.4°, 26.0°) are those indicating the formation of highly crystalline nano zinc oxide and nano vanadium oxide are also shown through the peaks at 2 θ (15.3°, 20.3°) indicating the presence of V₂O₅. These peaks correspond to the



A1



A2



A3

Fig. 2. The optimum temperature was taken as 400°C for three prepared (1:1,1:2,2:1) proportions, respectively.

hexagonal wurtzite structure of ZnO. Verification of ZnO/V₂O₅ composition, no appreciable extra peaks emerge, suggesting the lack of important impurities or secondary phases; The presence of peaks at the stated angles is identical to ZnO in its nano scale state. Superior crystal structure: Well-ordered ZnO crystals are indicated by sharp, high-intensity peaks. The ability to determine crystal size, the size of nano crystals can be determined

using Scherrer's equation $D = k \lambda / \beta \cos \theta$ [16-19].

FSEM analysis shows the formation of nano composites and the size of the nano materials (C1, C11-C2, C22-C3, C33) prepared according Three ratios of this compound (1:1,1:2,2:1) at the optimum temperature degrees (400 °C) as respected at Fig. 4. Respectively.

The Table 2. shows irregular clusters consisting of nano plates, which are common in V₂O₅. In

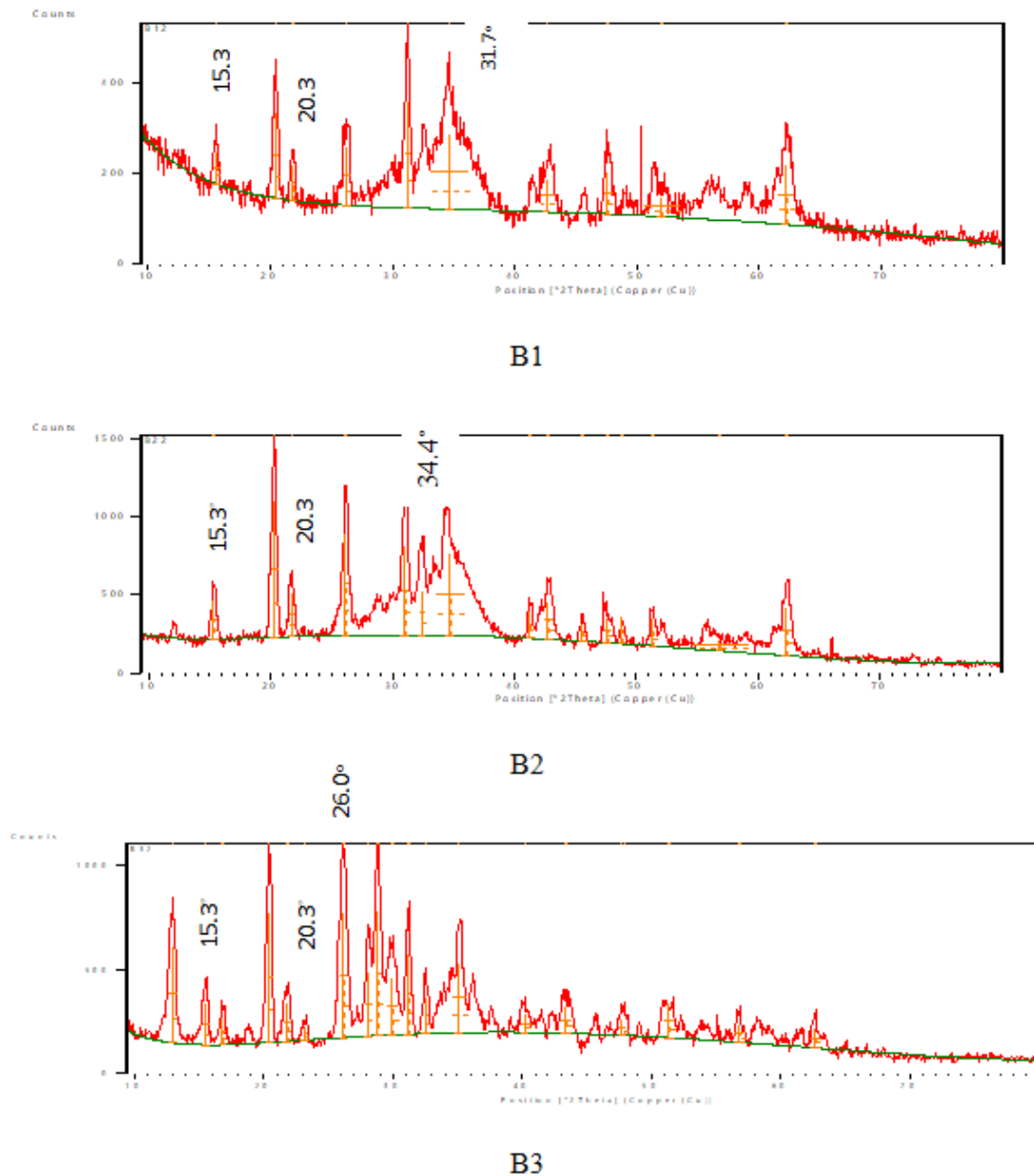


Fig. 3. X-ray diffraction pattern of binary nanocomposite at 400°C calcination temperature with different ratio. for three prepared (1:1,1:2,2:1) proportions, respectively.

addition, some small particles can be seen in the form of rods or spheres, which is a typical pattern for ZnO nano crystals with a needle-shaped shape. The grain size ranges from (29.63,36.89, to 30.38nm) [20,21].

Antimicrobial activity

The antimicrobial activity of binary nano composite ZnO/V₂O₅ crystal was selected from best nanosized crystal that's occurs at calcinated at 400 °C. Activity have been tasted against Gram-

negative bacteria E.coli and gram-positive S.aures bacteria, it showed effectiveness and inhibition of the growth of these species, according to pre-prepared concentrations and four concentrations. Four different concentrations were prepared (0.025 to 0.1 mg/ml) for each prepared ratio of this compound (1:1,1:2,2:1) as follows Fig. 5. shows image of the effectiveness against gram-positive and gram-negative bacteria, (D1, D2, D3) and (D11, D12, D13) respectively.

To estimation antimicrobial activity sensitivity,

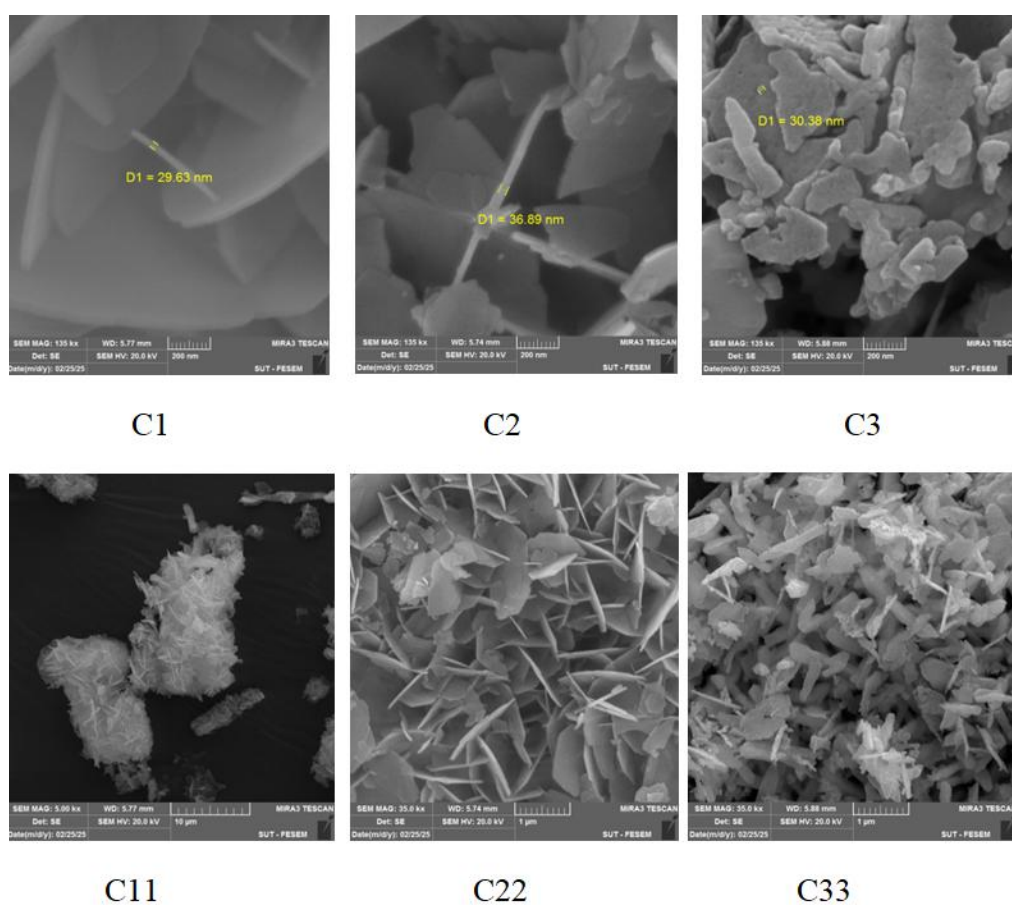


Fig. 4. Images of FE-SEM of binary nano composite ZnO/V₂O₅ at different ratio.

Table 2. Particle size determination by FE-SEM of Binary nanocomposite ZnO/V₂O₅.

Calcination Temperature	Ratio 1:1	Ratio2:1	Ratio1:2
300°C	25.47nm	32.43nm	24.04nm
400°C	29.63nm	36.89nm	30.38nm
500°C	49.81nm	35.55nm	36.48nm
600°C	139.02nm	157.93nm	33.25nm
700°C	28.44nm	35.94nm	59.79nm

the present study employed an agar gel diffusion method to evaluate the antibacterial activity of ZnO/V₂O₅ nanoparticles in vitro against *S. aureus* and *E. coli*. Thus, the antibacterial activity was assessed using the growth-free inhibitory zones encircling the wells. In overall, the ZnO/V₂O₅ combination shows encouraging antibacterial efficacy against the two microorganisms under investigation. As Fig. 5 shows, the zone of inhibition (mm) on ZnO/V₂O₅ for *E. coli* and *S. aureus* is 17 mm and 26 mm at 0.1 µg/mL, respectively, it can be observed from Table 3, that there is strong antibacterial activity in the ZnO/V₂O₅ combination. The most effective dose was also determined and found at concentration (0.1mg/ml at 2:1) In

the inhibition zone of about (17mm and 26mm) respectively gram-negative bacteria and gram-negative bacteria, Inhibition test was performed within 24 hours.

CONCLUSION

Binary nanocomposite of zinc oxide vanadium oxide is synthesized at different ratio with different calcinated temperature by using the environmentally friendly green method according to Reishi mushroom extract. The band gap value of binary nanocomposite equal to 3.424eV. the FT-IR spectra indicated to form metallic bond presence in the structure on nano composite. The crystalline size according into the x-ray FE-SEM technique



D1-D3 Gram-positive bacteria Staphylococcus aureus



D11-D33 -Gram-negative bacteria E. coli

Fig. 5. Antimicrobial activity estimation of binary nano composite ZnO/V₂O₅ crystal calcinated at 400 °C.

Table 3. Antimicrobial investigation of Binary nano composite ZnO/V₂O₅ cacinated at 400 °C against the bacterial of *E.Coli* and *S.aureas*.

Concentration (mg/ml)	Zone inhibition mm <i>E.Coli</i>	Zone inhibition mm <i>S.aureas</i>
0.025mg/ml	7mm	13mm
0.05 mg/ml	13mm	20mm
0.075 mg/ml	15mm	22mm
0.1 mg/ml	17mm	26mm

proving the prepared crystal size at nanosized is scaled from (29.63, 36.89, 30.38 nm) According to the proportions (1:1, 1:2, 2:1) respectively. The size and shape of the nanoparticles were also confirmed by FSEM The grain size ranges from (29.63, 36.89, to 30.38 nm). Nanocomposite gives high antimicrobial activity for both positive and negative bacteria through 24 hours, and it was found that the best inhibited dose concentration was 0.1 mg/ml for both types of bacteria.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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