

## ***Prosopis Juliflora* Leaf Extract Mediated Synthesis Of Doped Tin Oxide Nanoparticles And Evaluation Of Antimicrobial Activity**

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### **ABSTRACT**

Nanomaterials are used in a wide range of industries, including storing energy, water treatment, nanomedicine, fuel cells, sensing, catalysis, optoelectronics, and tunable resonant devices. Varieties of metals used for the preparation of nanoparticles. SnO<sub>2</sub> is a prominent n-type wide-bandgap semiconductor, and due to the variety of controllable physicochemical features of SnO<sub>2</sub>-based nanostructures, they are emerging as one of the most significant classes. Therefore, the development of a more efficient nanomaterial with enhanced antimicrobial activity of great significance. The objective of this study was to synthesize 2% F doped Tin dioxide (SnO<sub>2</sub>) (FTO) nanoparticles using *Prosopis Juliflora* leaf extract and determine its antimicrobial activity against selected microbial strains. The characterization of synthesized nanoparticles were investigated by scanning electron microscopy, transmission electron microscopy, UV-Vis-IR spectroscopy. The grain sizes in the range of 16-26 nm were obtained. The antimicrobial activities of F- doped SnO<sub>2</sub> nanoparticles were studied against bacteria (*Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis*) and fungi (*Aspergillus flavus* and *Aspergillus niger*) using the standard disc diffusion method. The antimicrobial activity was increased directly proportional to dose-dependent manner. Experimental results demonstrated that 2% F doped Tin dioxide (SnO<sub>2</sub>) nanoparticles (F- doped SnO<sub>2</sub>) exhibited the good antimicrobial effects were observed.

**Keywords:** Nanoparticles, Characterization, *Prosopis Juliflora* leaf, F- doped SnO<sub>2</sub>, Antimicrobial activity.

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### **INTRODUCTION**

Nanotechnology is the study of nanoparticles, the size of its atomic and molecular aggregates is less than 100nm. Nanotechnology is one of the emerging fields because of its uses in various sectors of medicine, technology and in the field of research including electronics, optics, biomedical and material sciences. Nanoparticles are reduced to nano-scale size to influence their mechanical, chemical, structural, electrical, morphological, and optical properties (Rasmussen et al., 2010). Nanoparticles have larger structure with respect to their counterparts due to its small size. And this allows the possible applications of NPs in various biological fields such as biosensors, nanomedicine, and bio-nanotechnology (Ashe, 2011). Thus, nano-materials play an important role in the basic and practical sciences as well as in bio-nanotechnology (Zhang et al. 2012). SnO<sub>2</sub> is a prominent n-type wide-bandgap semiconductor, and due to the variety of controllable physicochemical features of SnO<sub>2</sub>-based nanostructures, they are emerging as one of the most significant classes. Many studies have recently reported the synthesis of SnO<sub>2</sub>-based nanocomposite materials, which can significantly improve performance (Gebreslassie and Gebretnsae, 2021). Elemental doping is one of the most

essential techniques for material modification. It is well known that fluorine is considered to be a highly efficient and inexpensive dopant in the field of materials. Fluorine is one of the most reactive elements with the highest electronegativity. F-doped ZnO has been synthesized by various techniques (Jiale Huo *et al.*, 2023).

Bacterial and fungal infections are a major cause of chronic infections and mortality. Antibiotics have been the preferred treatment method for bacterial infections because of their cost-effectiveness and powerful outcomes. Therefore, the development of more efficient material with enhanced antimicrobial activity is of great significance. Despite the great progress in antimicrobial development, many infectious diseases like intracellular infections are difficult to treat (Matthews *et al.*, 2010). Major reasons of difficulty are transportation through cell membranes, low activity in the cells, antimicrobial toxicity to healthy tissues and acquired resistance of infectious microbes (Deotale *et al.*, 2010). To address these issues, nanoscale materials have been emerged up as novel antimicrobial agents. Nanoparticles (NPs) are ideal forms of antimicrobial agents because these materials exhibit large surface to volume ratio and high reactivity in comparison to bulk form (Reddy *et al.*, 2007). Many NPs have antimicrobial properties and used to control drug-resistant microbial populations. Various inorganic metal oxide NPs viz., ZnO, MnO, TiO<sub>2</sub> and SiO<sub>2</sub> exhibit considerable antimicrobial activities and used in therapeutics, diagnostics and nanomedicine-based antimicrobial agents (Mukherjee *et al.*, 2011; Sobha *et al.*, 2010). synthesize 2% F doped Tin dioxide (SnO<sub>2</sub>) nanoparticles using *Prosopis Juliflora* leaf extract and determine its antimicrobial activity against selected microbial strains.

## MATERIALS AND METHOD

### Collection of plant materials

The leaves of *Prosopis Juliflora* were collected in January 2021 from Poondi, Thanjavur District, Tamil Nadu, India.

### Preparation of aqueous extract of *Prosopis Juliflora* leaf

The collected leaves were washed with double distilled water to remove dust particles and then dried in the shade for two days for remove wet condition. 20 g of leaf sample was mixed 100ml of deionized water and the mixture was boiled for 30 min. after cooling the leaf extract was filtered with whatman no.1 filter paper. The filtrate was stored at 4 degree C for further uses.

### Synthesis of F-doped SnO<sub>2</sub> nanoparticles using *Prosopis Juliflora* (PJ) leaf extract

For synthesis of F doped SnO<sub>2</sub> NPs, 0.1 M SnCl<sub>2</sub> • 2H<sub>2</sub>O solution and Ammonium fluoride were used as dopant precursors F (2%) was prepared with 80 mL water. Then 20 mL *Prosopis juliflora* leaf extract was added and 0.1 M NaOH solution added maintain pH level 6-7 to solution and kept under continuous stirring at 80°C for 2 h. The F-SnO<sub>2</sub> PJ NPs were then collected with annealing the sample in a furnace at 350 deg C for 3 h. F-SnO<sub>2</sub> nanoparticles collected and involved character study and application. Preparation F-SnO<sub>2</sub> nanoparticles by co-precipitate method.

## Characterization of nanoparticles

### UV and FTIR Spectroscopic analysis

The nanoparticles were examined under UV and visible spectrophotometer analysis. The nanoparticles were scanned within the wavelength starting from 200-1000 nm using Perkin Elmer photometer and also the characteristic peaks were identified. FTIR analysis was performed using Spectrophotometer system, which was used to detect the characteristic peaks in ranging from 400-4000 cm<sup>-1</sup> and their functional groups. The peak values of the UV and FTIR were recorded. Each and every analysis was repeated twice for the spectrum confirmation

### Scanning Electron microscopy (SEM)

The particle size and morphology of nanoparticles were analysed by ZEEISS-SEM machine. The dried form of silver nanoparticles were sonicated with distilled water, small droplet of silver nanoparticles were placed on glass slide and permitted to dry. The ZEEISS-SEM machine was worked at a vacuum of the order of 10<sup>-5</sup> torr. The accelerating voltage is 20 kV. The particle size of nanoparticles can be analyzed by using image magnification software compatible with SEM.

### Transmission Electron microscopy (TEM)

Transmission electron microscopy (TEM) technique was used to visualize the morphology of the nanoparticles. The make of the Transmission electron microscope (TEM; Philips model CM200) technique was to visualize the morphology of nanoparticles. The instrument was operated at an accelerating voltage of 200 kV with ultra-high-resolution of 0.2 nm and magnification of 2,000,000 X. TEM's grid size is 3 mm diameter which was prepared placing a 5 µl of the nanoparticle solutions on carbon-coated copper grids and drying under mercury lamp and then analyzed

### Determination of antimicrobial activity

The antimicrobial activity was performed by disc diffusion method followed by NCCLS (1993) and Awoyinka *et al.*, (2007). Antibigram was done by disc diffusion method using test sample. Petri plates were prepared by pouring 30 ml of NA medium for bacteria. The test organism was inoculated on solidified agar plate with the help of micropipette and spread and allowed to dry for 10 mins. The surfaces of media were inoculated with bacteria from a broth culture. A sterile cotton swab is dipped into a standardized bacterial test suspension and used to evenly inoculate the entire surface of the Nutrient agar plate while PDA used for fungal study. Briefly, inoculums containing of bacteria (*Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis*) and fungi (*Aspergillus flavus* and *Aspergillus niger*) were spread on agar plates. Using sterile forceps, the sterile filter papers (6 mm diameter) containing different concentration of sample (50, 100, 150 and 200 µl), 30 µl deionized water (control) and 30 µl Standard (Bacteria: Chloramphenicol and Fungi : Clotrimazole) solution were laid down on the surface of inoculated agar plate. The plates were incubated at 37 °C for 24 h for the bacteria.

### RESULTS AND DISCUSSION

UV-Vis spectroscopy is the most important technique and the simplest way to confirm the formation of nanoparticles. In the UV -Visible absorption spectrum of F-SnO<sub>2</sub> nanoparticle showed a clear absorption peak was observed at the wavelength 325 nm (Figure 1). Present study agreement with Lisnic *et al.* (2023) who reported that absorption peak was observed at the wavelength 350nm from Fluorine-Doped SnO<sub>2</sub> Thin films in solar cell applications.

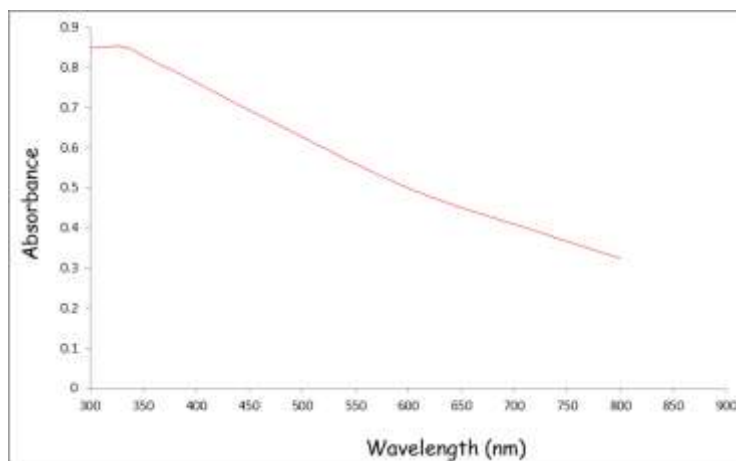
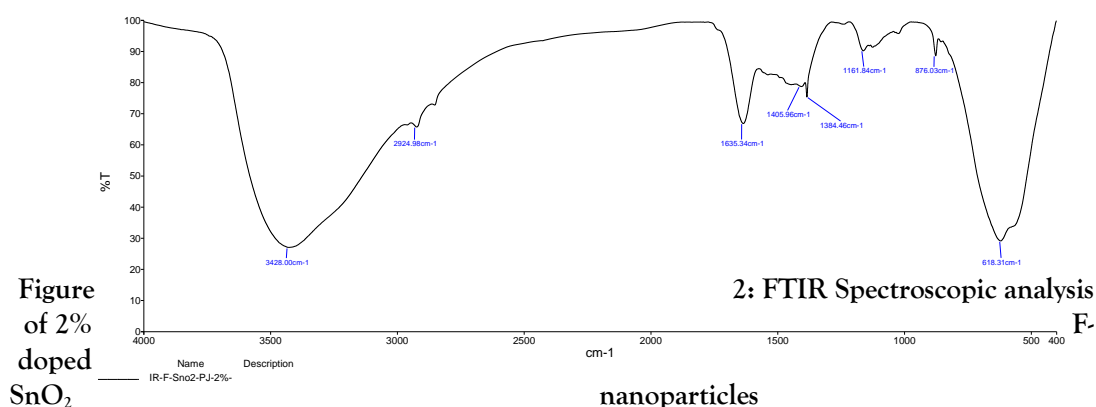


Figure 1: UV-Visible Spectroscopic analysis of 2% F-doped SnO<sub>2</sub> nanoparticles

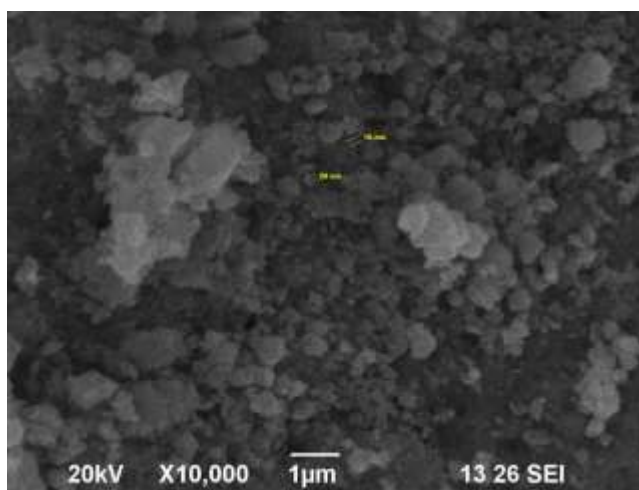
### FTIR Spectroscopic analysis

The FTIR spectra of F-doped SnO<sub>2</sub> films are illustrated in Figure 2. The spectrum is formed with several absorption peaks assigned to Vibrational modes of F-SnO<sub>2</sub> confirming its formation. The obtained spectra are comparable to the reported ones in the literature ((Kim and Riu, 2011; Kar and Kundoo, 2015). The spectrum shows several absorption peaks which confirm the formation of the material. Peaks (618 cm<sup>-1</sup>, 876 cm<sup>-1</sup>) between 500-1000 cm<sup>-1</sup> assigned to Sn-O and Sn-O-Sn vibration (Van Tran *et al.*, 2010). Small peaks (618.31 cm<sup>-1</sup>) between 600 - 1900 cm<sup>-1</sup> are due to Sn-OH vibration. An additional peak located at 876 cm<sup>-1</sup>, which becomes inconspicuous as fluorine concentration increases can be assigned to oxygen vacancies (V<sup>2+</sup>) presence in the O-Sn-O group (Zhang *et al.*, 2011). Broad peak (3428.00 cm<sup>-1</sup>) 3000- 3500 cm<sup>-1</sup> is due to O-H stretching vibration. Peak at 1161 cm<sup>-1</sup> occurs due to Si-O vibration due to substrate. FTIR spectra were obtained to identify the possible biomolecules in the extract responsible for predicting their role in nanoparticle synthesis and the reduction of ions as well as the capping agents for the stability of biogenic nanoparticle solution.



### Scanning Electron Microscopical (SEM)

SEM analysis was carried out to understand the topology and the size of the NPs, which showed the synthesis of higher density polydispersed spherical NPs of various sizes that crystalline nature of the nanoparticles (Figure 3). Most of the nanoparticles gathered and only a little of them were polydispersed and spherical in shape, when observed under SEM. To find out the particle size of the nanoparticles using histograms plotted on the obtained data to study the particle size distribution using ImageJ software and the size of the nanoparticles ranged from 16 to 26nm and the average particle size was found to be 23.39 ± 8.60 nm (Figure 4).



**Figure 3: Scanning Electron Microscopical (SEM) analysis of F-doped SnO<sub>2</sub> nanoparticles**

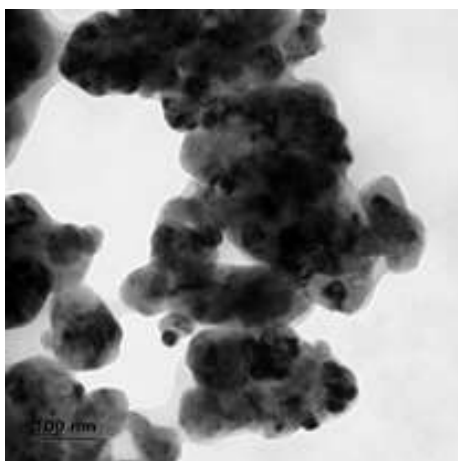


Figure 4: Histogram of showing particle size distribution of F-doped SnO<sub>2</sub> nanoparticles

#### TEM Analysis

TEM provides improved spatial resolution compared with SEM and enables a more in-depth analysis of nanoparticles. The TEM images of fluorine doped tin oxide nanoparticles are shown in Figure 5. It is observed that the morphology of nanoparticles is often polydispersed and spherical in shape. The close examination of the size of FTO particles reveals that it falls in nanoscale, and these particles are found to be agglomerated. In general, agglomeration occurs in the case of nanoparticles very easily because the surface forces such as Vander-Waals forces, capillary forces and electrostatic forces could overcome only against gravitational and inertial forces for particles in this size range (Pugh and Bergstrom, 1994). Similar reports observed in Senthilkumar et al. (2010).

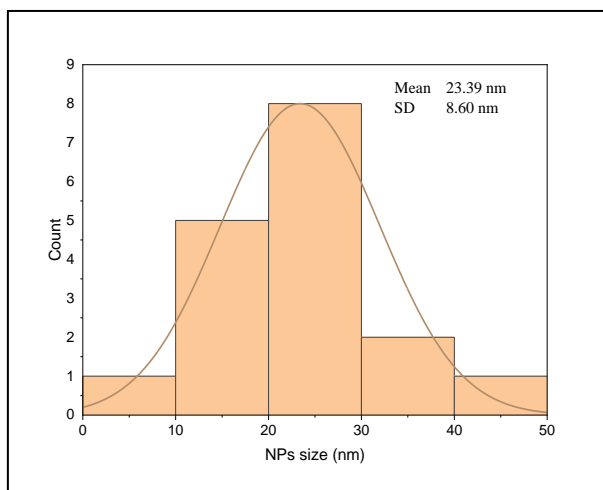


Figure 5: Transmission Electron Microscopical (TEM) analysis of F-doped SnO<sub>2</sub> nanoparticles

### Antimicrobial activity of F-doped SnO<sub>2</sub> nanoparticles

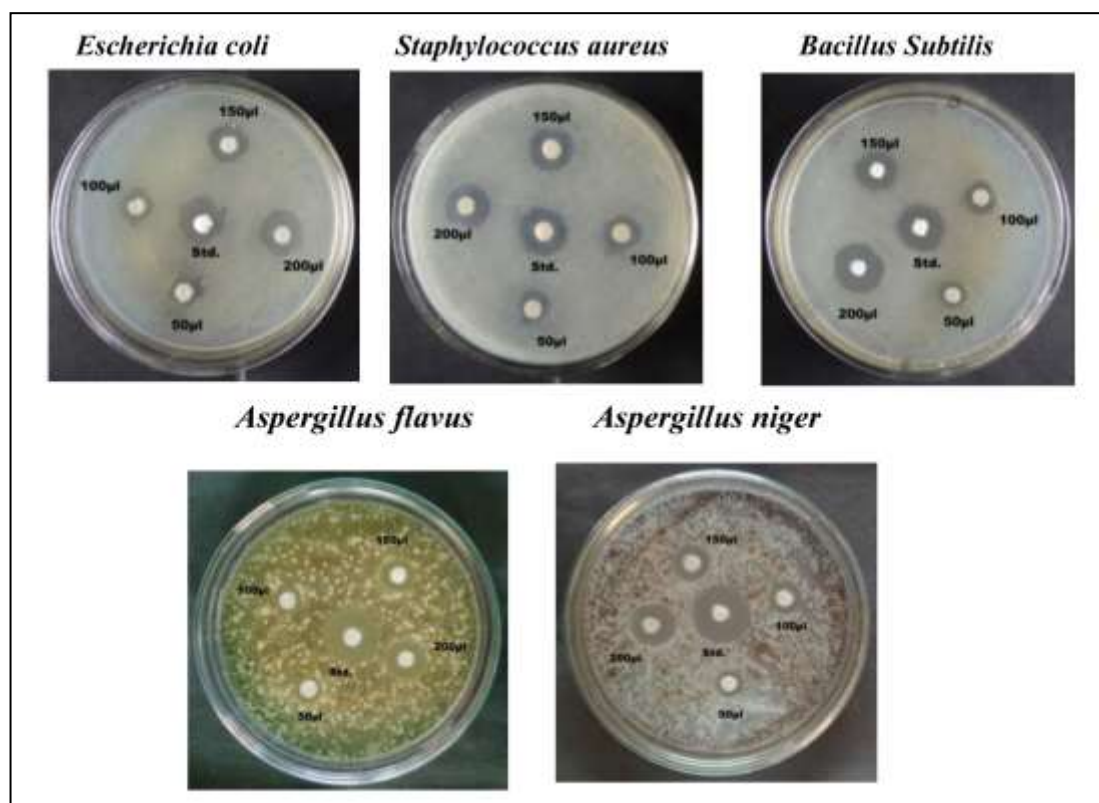
The 2% F-SnO<sub>2</sub> nanoparticles tested against bacteria (*Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis*) and fungi (*Aspergillus flavus* and *Aspergillus niger*) (Figure 6). The maximum zone of inhibition of 2 % F-SnO<sub>2</sub> nanoparticles against bacteria *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis* showed 10, 11 and 12 mm at the concentration of 200µl while the minimum was 4, 4 and 3 mm at the concentration of 50µl. The maximum zone of inhibition of 2 % F-SnO<sub>2</sub> nanoparticles against fungi *Aspergillus flavus* and *Aspergillus niger* showed 11 and 9 mm at the concentration of 200µl while the minimum was 3 and 3 mm at the concentration of 50µl. The zone of inhibition was increased with increase the concentrations were noticed. The antimicrobial activity of 2 % F-SnO<sub>2</sub> nanoparticles are nearest to the standard (Chloramphenicol and Clotrimazole) were observed (Table 1 and Plate 1). Among the various bacteria and fungal strains, the antimicrobial property of 2 % F-SnO<sub>2</sub> nanoparticle has potential against *Bacillus Subtilis* and *Aspergillus flavus*.

Table 1: Antimicrobial activity of F-SnO<sub>2</sub> nanoparticles against Bactria and fungal strains

S.No.	Microorganisms	Zone of Inhibition (mm in diameter)					
		Control	Standard*	50µl	100µl	150µl	200µl
	Bacteria						
1	Bacillus Subtilis	-	13	3	6	8	12
2	Staphylococcus aureus	-	12	4	7	9	11
3	Escherichia coli	-	11	4	6	7	10
	Fungi						
1	Aspergillus flavus	-	14	3	6	8	11
2	Aspergillus niger	-	16	3	5	7	9

\*Chloramphenicol (30 mcg); Clotrimazole (mcg/disc)

Figure 6: Antimicrobial activity of FTO against bacteria and fungal strains



Nanoparticles have attracted much interest because of their unique physical and chemical properties, which originate from the high area to volume ratio and elevated quantity of surface atoms. In fact, as the diameter decreases, the available surface area of the particle itself dramatically increases, and, consequently, there is an increase over the original properties of the corresponding bulk material. This feature makes nanoparticles superior and exceptional candidates for biomedical applications as a variety of biological processes occur at the nanometer level (Sharma et al. 2009; Prabhu and Poulouse 2012). Thus, nanoparticles hold incredible potential in various biomedical uses including antibacterial agent. Recently, many studies have demonstrated that different metal oxide nanoparticles exhibit biocidal action against bacteria and fungi. The antimicrobial activity of the nanoparticles is known to be a function of the surface area in contact with the microorganisms (Franci et al. 2015; Chiriac et al. 2016).

In the last years, bacterial resistance to bactericides and antibiotics has increased. Many organic antimicrobial agents are toxic to humans and other animals, furthermore, can be the cause of different allergic reactions (Rajawat and Qureshi 2012; Hossain et al. 2015). In order to solve this problem, inorganic antibacterial agents have attracted interest for bacteria control, due to their good safety, sustainability, heat resistance, and improved stability under harsh processing conditions. Currently, nanotechnology provides a sound platform for adjusting the physicochemical properties of numerous materials to generate effective antimicrobials (Beyth et al. 2015). Silver (Ag), gold (Au), titanium oxide ( $\text{TiO}_2$ ), copper oxide ( $\text{CuO}$ ), zinc oxide ( $\text{ZnO}$ ), and manganese oxide ( $\text{MnO}$ ) are the principal metal nanoparticles (NPs) used as antibacterial agents once their potent antibacterial effects are well known (Zhang 2015).

The antimicrobial activities of and F doped  $\text{SnO}_2$  nanoparticles ( $\text{F-SnO}_2$ ) were studied against bacteria and fungi using the standard disc diffusion method. In the present study 2% F doped  $\text{SnO}_2$  nanoparticles tested against bacteria (*Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis*). The antibacterial activity was increased dose-dependent manner. Experimental results demonstrated that 2% F-doped tin oxide nanoparticles ( $\text{F-doped SnO}_2$ ) exhibited the maximum antibacterial effect compared to standard. Similar reports agreed by Algethami et al. (2023) who examined the antibacterial activity against *Escherichia coli* and  $\text{CeO}_2\text{-SnO}_2$  composite nanofibers depicted excellent activity. Another study Assd et al., (2023) who showed that the most effective inhibitor of both Gram-negative and Gram-positive bacteria is  $\text{SnO}_2$  nanoparticles produced.

Metal oxide nanoparticles tend to have a negative zeta potential, which easily interacts with this surface potential according to the electrostatic force equilibrium. This significant bactericidal activity was due to the release of fluorine ions and Sn from F-doped  $\text{SnO}_2$  nanoparticles, and the permeation of the bacterial cell membrane by these ions. Ions can be released from the interaction between  $\text{SnO}_2$  NPs with the cell wall leading to the generation of reactive oxygen species (ROS) on the surface of the nanoparticles. The permeation of ions was possible due to the attraction of their positive charges to the negatively-charged cell wall. The binding of these ions destroyed the bacterial cell membrane. In addition, metal ions are involved in cross-linkage of nucleic acid strands by binding with DNA molecules of bacteria. This produces a disorder in the DNA structure, leading to protein denaturation and complete destruction of the bacterial cell (Reddy et al., 2007; Wang et al., 2015; Ahmad et al., 2017). The bactericidal effect of metal nanoparticles has been attributed to their small size and high surface to volume ratio, which allows them to interact closely with microbial membranes and is not merely due to the release of metal ions in solution (Ruparelia et al. 2008)

Fungi, which comprises diverse groups with different morphologies, such as unicellular yeasts and multicellular organisms. One promising solution as an antifungal agent is the use of nanoparticles (NPs). Many studies investigate this field of nanotechnology, but the

mechanisms of action involved in NPs use as an antifungal agent (Babele et al., 2018). In the present study 2% F doped SnO<sub>2</sub> nanoparticles tested against fungi (*Aspergillus flavus* and *Aspergillus niger*). Present study agreement with Jebril et al., (2020) who reported that metal nanoparticles synthesized with *M. charantia* and *P. guajava* extracts showed good antifungal capacity in concentrations of 20 ppm, inhibiting the growth of mycelium in fungi such as *A. niger*, *A. flavus*, and *F. oxysporum*.

Metal nanoparticle mediated fungal cell toxicity occurs through many mechanisms, including cell wall damage and change (such as ergosterol synthesis inhibition), gene regulation, interaction with reproductive structures and hyphae, and ROS production, causing cascades of damages such as lipid peroxidation and mitochondrial damage. This multi-faceted attack makes NPs an avenue worth investigating as potential future antifungal treatments (Slavin and Bach, 2022).

## CONCLUSION

The results of this study indicate that although the synthesized 2% F doped SnO<sub>2</sub> nanoparticles possess potent and desirable biological properties such as antimicrobial (Bacteria and fungi) activity, This is due to the large surface and smaller particle size of F-doped nanoparticles. Thus, the synthesized nanoparticles hold enormous potential for use in the cosmetic, nutraceutical and pharmaceutical industries.

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