ISSN 2664-4142 (Print) & ISSN 2664-6749 (Online)
South Asian Research Journal of Pharmaceutical Sciences
Abbreviated Key Title: South Asian Res J Pharm Sci

| Volume-5 | Issue-6 | Nov-Dec 2023 | **DOI: 10.36346/sarjps.2023.v05i06.002**

Original Research Article

Potent Effect of Plant Mediated Zinc Oxide Nanoparticles on Pathogenic Bacteria

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Article History
Received: 03.10.2023
Accepted: 07.11.2023
Published: 11.11.2023

Abstract: ZnO NPs are metal oxide nanoparticles that offer novel biomedical uses ranging from diagnosis to treatment. UV-VIS utilized to analyze nanoparticles derived from *Trifolium pratense* flower extracts to be able gain knowledge concerning the optical features of produced ZnO nanoparticles. Aggregation of smaller nanoparticles confirmed via SEM images. FT-IR test verified ZnO NPs-bacterial cell wall polypeptide and glycogen binding. Nanoparticles' shape was determined using plant extract and confirmed using (XRD) analysis. antibacterial efficacy of ZnO NPs was obvious on all species of bacteria that studied, the most effective of it was on gram ve $^{-}$ than gram ve^{+}.

Keywords: ZnO NPs, antibacterial activity, XRD, FTIR.

1. INTRODUCTION

Molecular engineering, with chemistry, biology, physics & other disciplines, nanoscience and nanotechnology are currently the most rapidly developing fields. Nanomaterials are goods created using nanotechnologies and comprise NPs having sizes ranging from 1 to 100 nm. Industries frequently utilize metal oxides & metal NPs as needs. Nickel, aluminum, copper, silver, iron, copper oxide, cerium dioxide, iron oxide, zinc oxide & titanium dioxide are a few examples of metal oxide and metal NPs that have become famous [1]. Physical, chemical, and biological processes can all be used to create NPs, but physical & chemical methods often need plenty of energy and is capable of producing poisonous or hazardous compounds, which can result in contagious threats [2]. Modern scientists have already created the biological or "green method" of synthesis, that utilizes (plant extract) with a low chemicals concentration to create a safe, economical, and less dangerous synthesis procedure. ZnO NPs have received more attention than other metal oxides as a result of their secure, low-cost preparation production method [3]. Among other classic techniques of producing nanoparticles, the green synthetic strategy for nanoparticles has been proposed. Also Among other conventional techniques of producing nanoparticles, the green synthetic approach was proposed. The biological ways of synthesizing nanoparticles, which use natural components (phytochemicals) found in plant extracts, have been recognised as an efficient and alternative method. Because of their having a variety of potential uses, ZnO NPs optimistic class of nanomaterials. According to reports, gram-negative, gram-positive and fungi are all sensetive to the antibacterial capabilities of nanoparticles of zinc oxide. Applications for ZnO NPs depending on the synthesis process used to make them, and these applications include antibacterial nanoagents for use in food, medicine, and textiles [4]. *Trifolium pratense* flower extract, which is used to synthesis ZnO-NPs, contains a variety of metabolites, including phenolic acids, anthocyanin and trace levels of carotene, tannins, vitamin C and essential oils. Efficient antibacterial action against strains of P. aeruginosa, S. aureus and E. coli is shown via ZnO NPs produced from T. Pratense [5]. The biosynthesized ZnO-NPs were found to cause tissue & cellular damage in different bacteria strains, making them be most efficient compared to traditional antibiotics against the bacterial strain [6]. The nanoparticles that are produced possess the ability for fighting certain bacterial isolates such as S. aureus and E. coli. anthocyanins, Phenolic acids, and trace levels of vitamins C and E, essential oils, carotene and tannins are found in *Trifolium pretense* L. (family: Leguminosae; section: Trifolium). Biochanin A, daidzein, formononetin and genistein are among estrogenic isoflavones found at high concentrations in *Trifolium pratense* L. [7]. These isoflavones provide benefits for the cutaneoprotective, osteoprotective effects and cardiovascularprotective. Many of these isoflavone effects have been attributed to their antioxidant or estrogenic properties [8]. Evaluation of ZnO NPs' antibacterial activity against harmful microorganisms is the study's main goal.

2. MATERIALS & METHODS

Plant Collection

Components of plants, like leaves, flowers, fruits, roots and stems, were extracted from healthy plants using sterilized scissors and soaked in 25% ethanol for 1-2 min to eliminate pathogenic spores and dust particles. Plant portions carefully cleaned by D.W before being thinly cut into fragments. For the preparation of broth solutions, five grams of finely chopped plant material were boiling (15) minutes of a 200 mL Erlenmeyer flask with 50 ml of double D.W extracts of plant boiling before filteration by filter paper of Whatman. Process of extraction done three times, the fresh extracts were used for further research.

Zinc Nitrate solution Preparation

In this investigation, (zinc nitrate hexahydrate) utilized as a source to produce nanomateriales. During experiments, a freshly produced aqueous solution of (Zinc Nitrate) (1 mM) utilized. Before usage, every glassware was carefully cleansed and washed in single-distilled water before being dried in a 110 °C oven.

Synthesis ZnO nanoparticles

By mixing the plant extracts with the zinc nitrate precursor solution, Zn^{2+} ions were reduced to ZnO. The mixture contains 1 mL from plant extract with 9 mL of 1 mM aqueous Zinc nitrate solution. Visual color change was used to evaluate the reduction of zinc nitrate.

Analysis of UV–Vis spectra

UV spectroscopy has been used to determine the sample's maximum absorption. Spectral analysis of UV & visible absorption at 280-800 nm was utilized to recognize optical characteristics of ZnO nanoparticles. It is utilized to measure a particle's maximal absorbance at a particular wavelength. In this type of spectroscopy, light is absorbed by the particles and indicates how the electrons are excited from their ground state to their excited state [9].

SEM

SEM utilized to analyse Zinc oxide nanoparticles morphology. It displays homogeneity, microstructure of the particle's coated surface & morphology as well as the distribution of the photocatalyst over the substrate surface. Once the impinging the electron beam makes interaction with the specimen., it determines the secondary electrons that are released [10].

FT-IR

This analysis utilized to evaluate binding features nanoparticles of Zinc oxide. Zinc oxide nanoparticle powdered was analyses using (FTIR) utilizing the {Perkin Elmer Spectrum 1000}spectra of the attenuated total mode with the spectral range of 4000-400 cm 1 at 4 cm.

XRD

It is an effective non-destructive method that provides details on the structure, size, and nature of crystals. XRD relies on monochromatic x-rays and constructive inference of crystalline samples [11].

Antibacterial efficiency

Well diffusion method

This method utilized to assess nanoparticles' antibacterial efficiency for gram negative & gram positive microorganisms. suspension of each bacteria sample diluted to {10^{-1}, 10^{-2}, and 10^{-3}} (1 ml of 108 cells/ml) before being spreading on a solid agar medium. A 6 mm sterile cork borer was used to prepare the wells. The amount of nanomaterial in each well varied, ranging from (ten to fifty microgram /milleter). Incubated of plates for (24 hours at 37°C). Zones of inhibition measured using meanSD values [12].

3. RESULTS AND DISCUSSION

Visible spectroscopy

ZnO solution sample utilized for record UV-Vis spectra was generated via ultrasonic dispersion it in pure ethanol. Peak absorption corresponds to a ZnO sample calcined at 500 °C, demonstrating high absorption at 280 nm. This is caused by electron transfers between the valence and conduction bands generate intrinsic band gap absorption in ZnO [13].

FT IR

The FTIR (fig 1 and fig 2) showed a few significant vibrational bands, like the OH, Zn-O, C-O, and so on, whic correlate to the ZnO NPs as well as bio-compounds exist on their surface. λmax of ZnO NPs in the UV-Vis spectrum determined at 283 nm utilizing double D.W as the blank. Also FT-IR revealed to changes & differences in peak locations, further supported the findings. In FT-IR spectroscopy, a sample's ability to absorb IR radiation is measured, utilizing a wavelength, the results of this measurement are displayed. IR spectra involves interpreting relationship between the chemical constituents in the sample and absorption bands. By utilizing this method, it is probable to recognize biomolecules present in extracts of plant that are essential for reduction & stabilization phases of environmentally friendly nanoparticles synthesis [14].

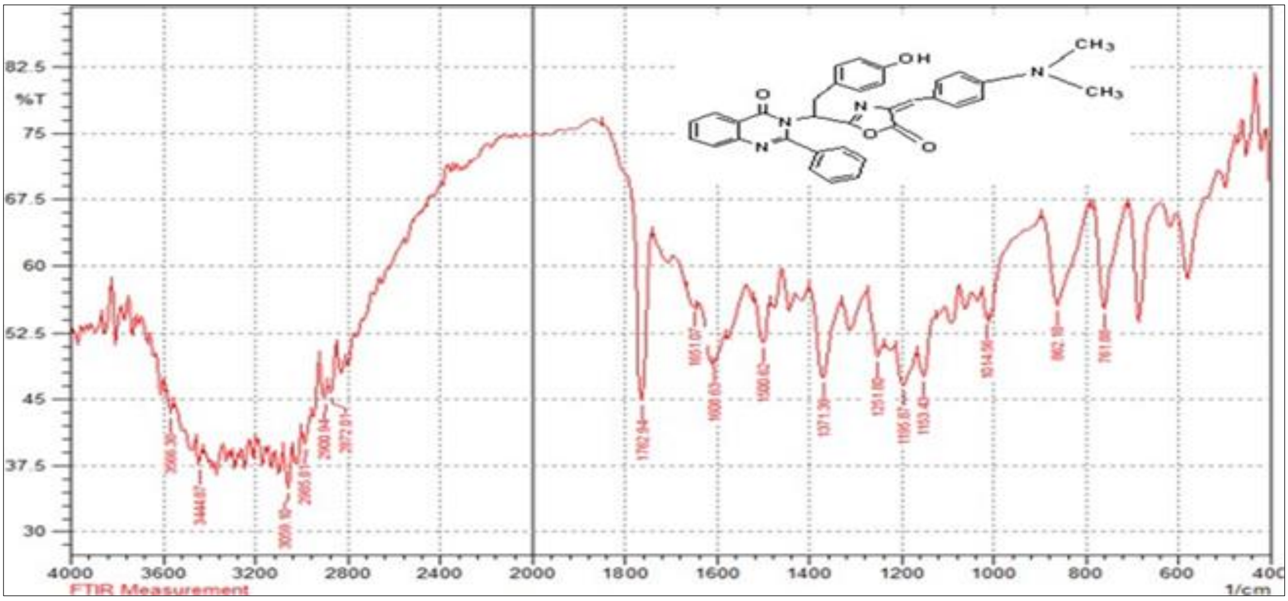

Fig. 1: FT-IR spectrum

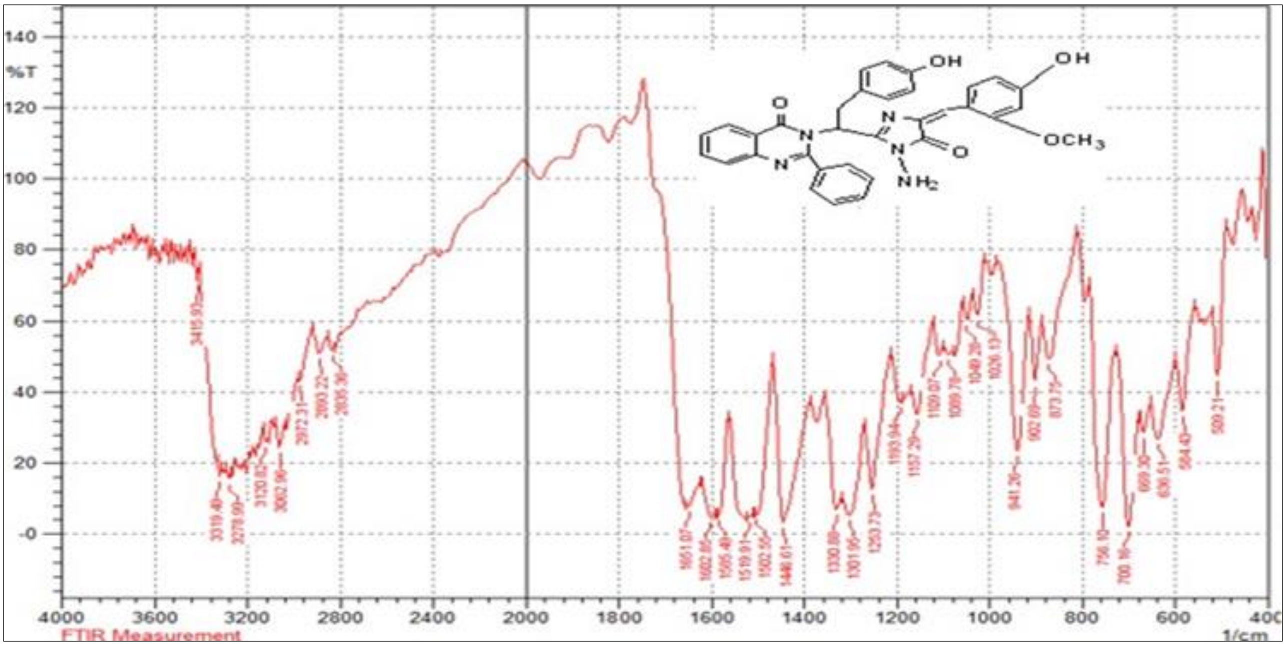

Fig. 2: FT-IR spectrum

The Fig (3) showed the color differential among all bacterial species when interact with zno nanoparticles as compared with control. The nanoparticles' smaller size makes it easy for them to pass through the membrane and destroy cells. The key characteristic of nanoparticles is their huge surface area, which allows them to firmly attach to bacterial cell surface and breakdown membrane, allowing intracellular components to flow out and killing the bacteria. Bacterial cell and nanoparticles' electrostatic interaction membranes may be the cause of nanotoxicity. Interaction between bacterial surfaces and ZnO nanoparticles. ZnO NPs having an important surface effect owing to their huge specific surface area. They interact with each other and make extensive contact with the bacterial cell membranes, damaging the membranes and releasing ion channels that cause internally ionic imbalances leading to death of the cells [15].

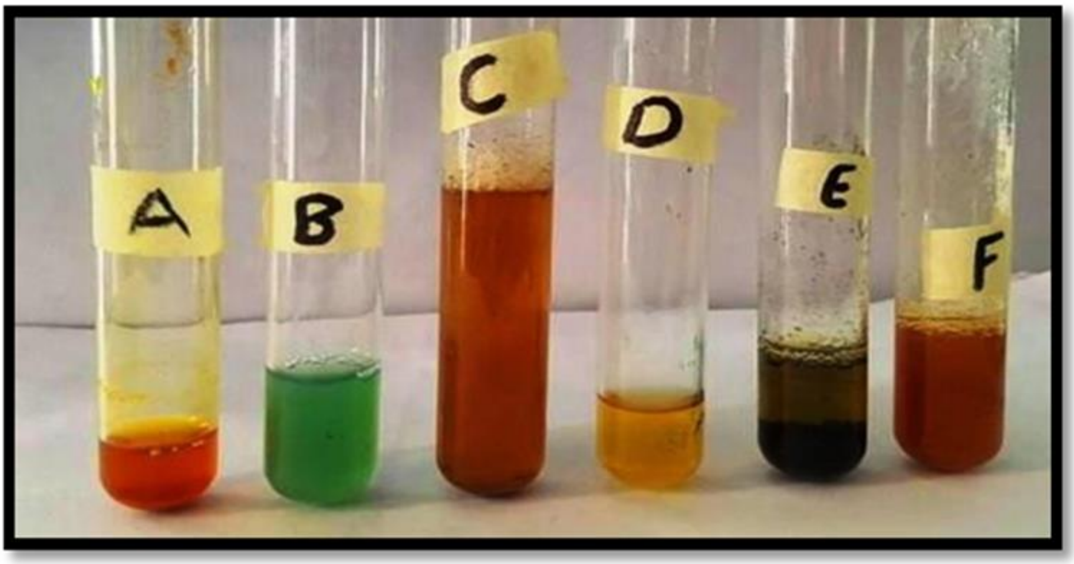

Fig. 3: Effects of nanoparticles on bacteria A-contol (+), B Strep. Pyogen, C S. aureus, D P. aerogenosa, E K. pneumonia F control (-)

XRD

The powder's crystallinity after being created using *Trifolium pratense* extract. Peaks of {(100), (002), (101), (102), (110), (103), (200), (112), (201), (004), and (202)}. Diffraction peaks show the produced (nanoparticles of ZnO) are substantially crystalline [16]. Green production of ZnO nanoparticles utilizing *Ocimum basilicum* [17] and *Agathosma betulina* [18] has produced the similar results. Utilizing the modified Scherrer equation, it was determined synthesized ZnO Nps having an average crystallite size about (24.53 nm).

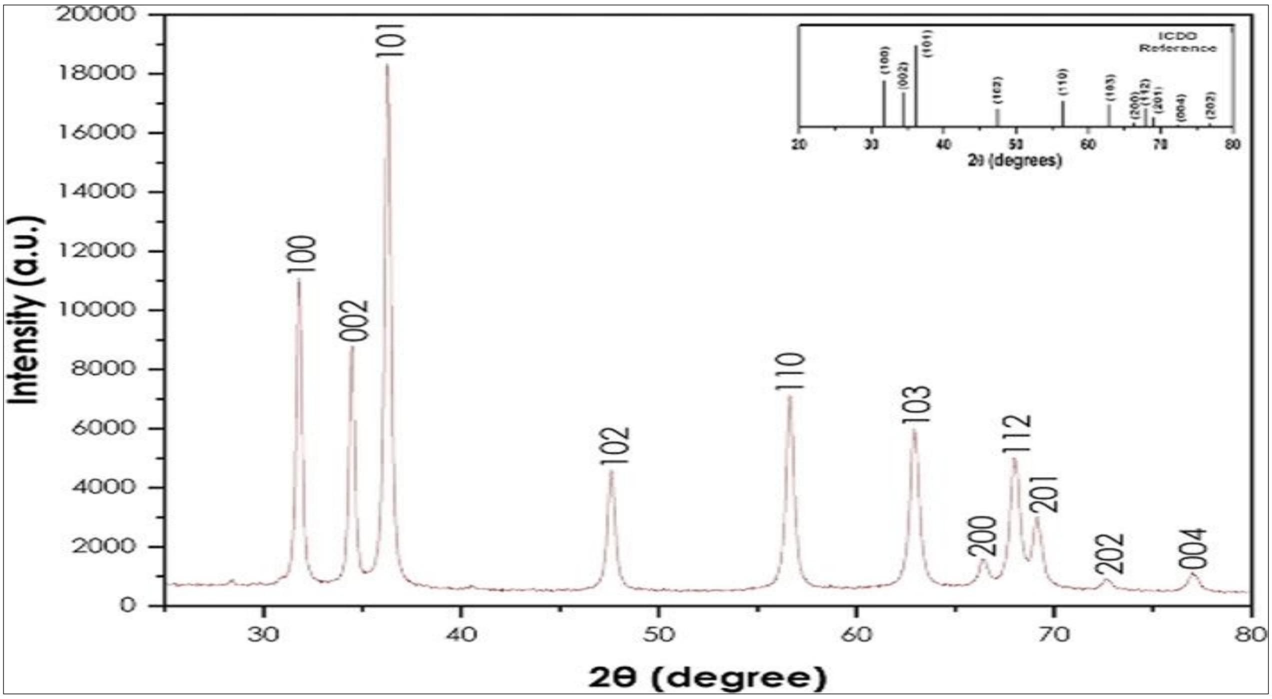

Fig. 4: ZnO nanoparticles X-ray diffraction pattern

Zno nanoparticles antibacterial activity

It is obvious from the Table 1 that all bacterial species were affected by different concentrations of zno nanoparticles, also it showed that P. aeroginosa and K. pneumonia bacteria were the most affected by the nanoparticles at different concentrations, as the concentration 50 µg /ml recorded high inhibition zones range than the other concentration, followed by 40 µg /ml,30, µg /ml20 µg / ml and finally 10 µg /ml. In comparison to gram ve+, gram ve- organisms have shown greater susceptibility to metal oxide nanoparticles [19]. Chemical & structural differences of cell membrane may be the cause of the difference in activity between gram positive & gram negative organism [20]. Due to this structural difference, peptidoglycan layers of gram positive bacteria are thicker than those of gram negative bacteria., making it difficult for nanoparticles to penetrate the membrane and cause reduced bacterial activity [21]. Not all microbial species and strains react to metal oxide nanoparticles in the same way [12]. The concentration of the nanoparticle is important in determining antimicrobial effeciency. Metal oxide nanoparticles' surface area that come into connection with bacterial cells is directly related to particle's. Zinc oxide nanoparticles antibacterial activity of ZnO NPs synthesized via green method might be described up as following: Mechanism for photocatalysis, When ZnO NPs get exposed to light electrons (e) in the valence band are stimulated when their energy exceeds the bandgap, allowing them for transfer to a conduction band and create a hole that is positively charged (H+).When H+ & e interact along with oxygen, OH & adsorbed water on the material's surface, they form OH, O2, and H2O2.H+ and OH have high oxidative characteristics, as they are capable of breaking Most of the chemical bonds of organic molecules, leading to breakdown of many microbe components and functioning as bactericidal agents. O2 has a large reduction capacity, which helps to explain its antibacterial effects [22]. Mechanism of ROS, Zinc Oxide NPs create ROS inside cells, which damages membranes of bacterial cells, promotes ZnO NP aggregation within bacteria, and ultimately results in bacterial death [23]. ZnO NPs have a significant surface effect due to their huge specific surface area. They interact with each other and make extensive contact with the bacterial cell membranes, damaging the membranes and releasing ion channels that cause internally ionic imbalance in the cells, followed by death [15]. Dobrucka and Dugaszewska reported that S. aureus and E. coli once cultivated in a doses of (125, 256, 516, and 1280 g/mL), inhibition zone diameters of ZnO NPs produced via *Trifolium pratense* flower extract (22, 26, 30, and 31 mm) respectively [24].

Table 1: ZnO nanoparticles antibacterial activity

Bacterial Isolates	**inhibition zone (mm)**				
	(10 µg /ml)	**(20 µg / ml)**	**(30 µg /ml)**	**(40 µg /ml)**	**(50 µg /ml)**
S. aureus	7	10	15	20	23
Strep.pyogen	6	9	10	12	19
P. aeroginosa	8	15	19	22	30
K. pneumonia	9	11	15	18	25

SEM analysis

The SEM image illustrates the ZnO NPs mainly aggregate into larger particles and spherical in shape. SEM pictures were captured at various high magnifications to check size & shape of the produced nanomateriales. Production of nanoparticles in their agglomerated form is confirmed by surface morphology. Several investigations have been carried out to study the relationship between ZnO surface shape and synergistic action. Majority of the particles discovered to be horizontal in form, and XRD confirmed this [25].

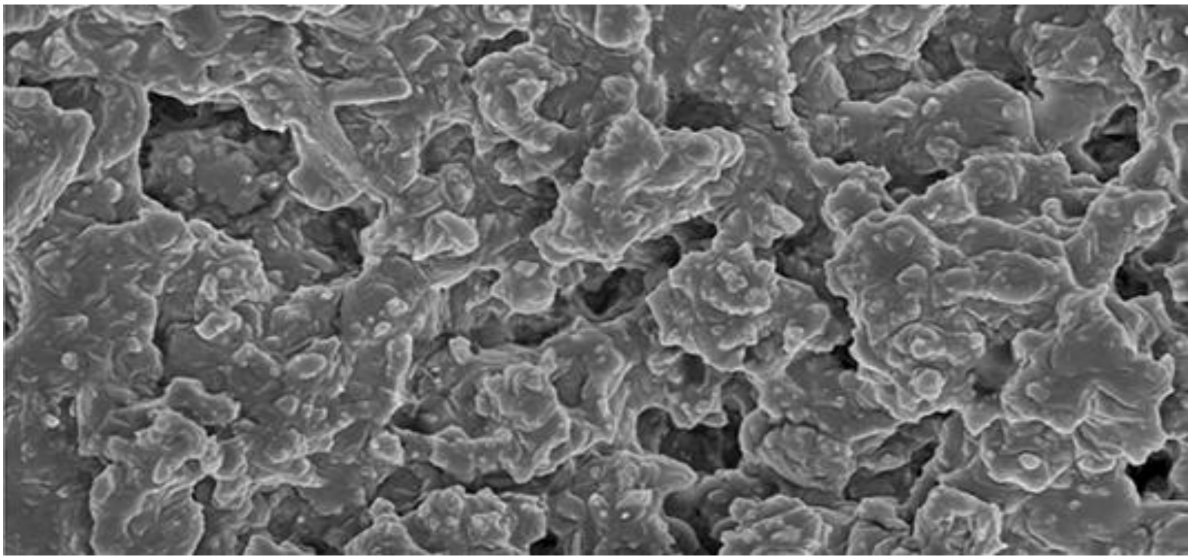

Fig. 5: SEM image of Zno nanoparticles

4. CONCLUSION

It is well recognized that ZnO nanoparticles via green method is substantially safer and more eco friendly than chemical manufacturing. The produced ZnO nanoparticles had significant antibacterial action against Strep. Pyogen, S. aureus, P. aerogenosa, and K. pneumonia. ZnO nanoparticles green synthesis utilizing *Trifolium pratense* flower water extract may also used as chemical alternative approaches.

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