Green Synthesis of Rod Shaped ZnO using Extract of Origanum majorana Leaf and Investigation for Antibacterial Applications

M Saini¹, A Mushtaq², S Yadav¹, S Rawat¹, Nutan Rani¹, K Gupta⁴, K Saini*¹

¹Department of Chemistry, Miranda House, University of Delhi, Patel Chest Marg, New Delhi-110007, India
²Department of Botany and Microbiology, H.N.B. Garhwal University, Srinagar-241674, Uttarakhand, India
³School of Life Sciences, Central University of Gujarat, Sector- 30, Gandhinagar-382030, Gujarat, India
⁴Department of Chemistry, Raj Rishi Government College, Alwar-301001, Rajasthan, India

* Corresponding author email address: kalawati.saini@mirandahouse.ac.in

Abstract. In this research article, we have fabricated the zinc oxide (ZnO) nanoparticles (NPs) with help of aqueous extract of leaf of Origanum majorana and studied their antimicrobial activity. Origanum majorana is one of medicinally important plant of Lamiaceae/Labiatae family. Green-approach of NPs has been favoured over traditional synthesis methods, as plant-based extracts have phytochemicals, which are non-toxic and biologically safe. The phytochemicals such as flavonoids, derivatives of phenol and polyphenolic biomolecules are found to be in aqueous leaf extract of Origanum majorana which used as capping and reducing agents. They include many functional groups such as -OH, -C=O and more which improve the physicochemical properties of NPs and consequently affect their targeting towards specific molecules. Plant mediated synthesis of ZnO NPs with fresh leaf extract of Origanum majorana is also simple, quick and provides a vast array of functionalised NPs of particular size and morphology. Herein, ZnO NPs have been prepared with 3 mL of 25% (w/v) of leaf extract of Origanum majorana (Maruva). These synthesized NPs have been characterized using PXRD (Powder X-ray diffraction), FT-IR (Fourier transform-infra-red spectroscopy), UV-Visible spectroscopy, SEM (Scanning electron microscopy) with EDS (Energy dispersive spectroscopy). UV-Visible spectrum shows maximum absorbance at 379.75 nm and energy band gap have been evaluated 2.84 eV using tauc plot. Obtained PXRD pattern shows hexagonal wurtzite crystalline structure which is similar as reported in the literature (JCPDS No-36-1451). The morphology of synthesized NPs has been obtained with SEM images. These NPs are rod shaped with width calculated approximately 90 nm-125 nm and length 0.5μm-1.2μm respectively. The EDS analysis shows the 51.41% of zinc (Zn) and 48.49% of oxygen (O)elemental composition of fabricated nanoparticles. Antimicrobial activity has been performed on gram negative and gram positive microbes with the help of broth dilution method. These synthesized NPs shows very great bactericidal activity against Staphylococcus
aureus, Pseudomonas aeruginosa, Escherichia coli and Streptococcus pneumoniae respectively. Minimum inhibitory concentrations (MIC) have been investigated 175 μg/mL for Pseudomonas aeruginosa, 125 μg/mL for Escherichia coli and 100 μg/mL for both the Staphylococcus aureus and Streptococcus pneumoniae respectively.

Keywords: Green approach; ZnO NPs; Extract of leaf; Origanum majorana; Antimicrobial activity; MIC

1. Introduction

ZnO NPs are very inexpensive, stable and biocompatible. These NPs have been investigated for various biomedical uses like as antibacterial, antifungal, anticancer, drug delivery bio-imaging and wound healing due to their physic-chemical properties [1-5]. ZnO NPs also have been used in many miscellaneous applications like as for supercapacitors, UV-shielding materials, nano-generators, functionalized air filters, gas sensing, optoelectronic as well as photocatalytic applications [6-14]. It has been investigated that the green approach of synthesis of NPs are more beneficial over the conventional chemical wet method, where the hazardous chemicals are used in their synthesis. These hazardous chemicals absorbed on the surface of the NPs that can cause toxic impact in biomedical applications. Recently, ZnO NPs have been prepared with help of extract of different parts of plants (leaf, seeds, bark, stem, fruits peels) and used in microbial activities [15-18]. Asif et al have also synthesized ZnO NPs using cyanobacteria [19]. Herein, a comparative study has been performed with commercial supplied ZnO NPs and green synthesized ZnO NPs regarding biocompatibility. Ann et al has done antibacterial study on Staphylococcus aureus, Psuedomonas aeruginosa and Streptococcus pyogenes using ZnO structures [20].

Antibacterial efficiency has been improved using coral-like ZnO/C-ZnFe2O4 hierarchical structure by Gu et al [21]. Herein, antibacterial study has been done on Escherichia coli and Staphylococcus aureus respectively. The optimized sterilization efficiencies have been investigated 98.0 % for Staphylococcus aureus and 99.4 % for Escherichia coli respectively. The outcomes of ZnO NPs on prohibition rate of Staphylococcus aureus and Escherichia coli have been studied by Pranjali et al [22]. Where, abovementioned ZnO NPs have been modified with help of polyethylene glycol (PEG). This study has been done by dispersing these modified ZnO NPs in peritoneal dialysis (PD) fluid at two concentrations such as 0.5mg/mL and 1 mg/mL respectively. Nano–bio interface interactions also have been studied using PD fluid by Guleria et al [23]. A Comparative study has been demonstrated on antibacterial and photocatalytic activities using ZnO NPs, also ZnO NPs based nanocomposite with therapeutic drug 9-aminoacridine hydrochloride hydrate (9AA-HCl) for anticancer activity [24]. Herein, Mitra et al has investigated that these ZnO nanoconjugates with this therapeutic drug shows synergistic catalytic effect than the bare ZnO. Gil et al has investigated the physical and chemical interactions between microorganism (gram-negative and gram-positive) and nanoporous ZnO spheres [25].

Ray et al has reported excellent bactericidal efficacy with good cytocompatibility using Ag-NPs decorated on ZnO Nanoflower impregnated eggshell membrane [26]. Shape-dependent antibacterial activity of ZnO nanostructure has been determined against several pathogens by Visinescu et al [27]. Herein, the surface reactivity of ZnO nanostructures and interactions with microbial and mammalian cells have been investigated. Synergistic effect of ZnO NPs synthesized with Cassia auriculata Leaf extract has been investigated on microbes and cancer cell lines [28]. Toxicity of the ZnO nanopowders
has been studied on gram negative bacteria such as *Escherichia coli* and *Pseudomonas aeruginosa* through disk diffusion procedure [29], which is similar to well diffusion method. Where, micro-broth dilution procedure has been employed for investigation of MIC (minimum inhibitory concentration) value. Antimicrobial activity of the ZnO NPs synthesized with *Trifolium pratense* flower extract have been demonstrated on *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* respectively [30]. Herein, 100 μL of the ZnO NPs have been used at different concentrations of 1028 μg/mL, 516 μg/mL and 256 μg/mL and 125 μg/mL. This study has been performed with reference to standard antibiotic Gentamicin. Divya et al has investigated antibacterial application of ZnO NPs synthesized with Hibiscus rosa-sinensis leaf extract [31]. Divyapiiya et al has done antimicrobial study on *Murraya Koenigii* [32]. Gunalan et al has studied ZnO NPs which was synthesized by green method for antibacterial and antifungal applications [33]. A novel microbial route has been investigated by Jayaseelan et al for the fabrication of ZnO NPs [34]. Herein, these synthesized NPs have been used to study for the antibacterial and antifungal applications. The maximum zone of inhibition has been reported for these synthesized ZnO NPs at concentration of 25 μg/mL against *Aspergillus flavus* (19±1.0 mm) and *Pseudomonas aeruginosa* (22±1.8 mm) respectively.

The antibiotic effects and cellular internalization of superfine zinc oxide nanoparticles have been demonstrated on *Escherichia coli* bacteria by Brayner et al [35]. Herein, ZnO nanoparticles have been fabricated by forced hydrolysis of ionic Zn$^{2+}$ salts in diethylene glycol (DEG) medium. Where, *Escherichia coli* cells have been exposed with DEG and ZnO NPs, which leads to damage as well as disorganization of cell membrane of these bacteria. Herein, the disorganization in cell membrane of tested bacteria leads the increment in permeability of membrane, which causes the accession of ZnO NPs. in the membrane of bacterial cells. Memarzadeh et al have investigated 100% ZnO NPs and its composite 25% nanohydroxyapatite (nHA) coated 75% ZnO NPs for dental and orthopedic implants [55]. Herein, ZnO NPs have been reported for inhibition of bacterial adhesion and help osteoblast growth. Herein, nano-coated material has been studied for antibacterial activity. *Staphylococcus aureus* stains are used to expose to nano-coated glass substrate in saline buffer or bovine serum and their antibacterial activity has been determined. Herein, in vitro study has also been done on MG-63 cells, UMR-106 cells and human mesenchymal cell (hMSC) respectively. Above literature survey reveals that the green synthesized ZnO NPs and chemically synthesized ZnO NPs are highly investigated for their various applications.

In this paper, our research goal is to synthesized ZnO NPs with leaf extract of *Origanum majorana* (Maruva) and their study for antibacterial applications. These green synthesized ZnO nanoparticles are characterized with the help of FT-IR, PXRD, UV-Visible absorption spectroscopy, SEM and EDS respectively. Herein, we have done the antimicrobial study with these synthesized NPs on *Escherichia coli*, *Staphylococcus Aureus* and *Streptococcus Pneumoniae* and *Pseudomonase Aeruginosa* using broth dilution method.

Though, few research work has been reported on antibacterial activities with *Origanum majorana* leaf extract and its essential oil. But, according to our best knowledge, this is first time, we have examine antibacterial activity of ZnO NPs fabricated with leaf extract of *Origanum majorana*. In future, we would like to use these ZnO NPs as antibacterial agent in place of some selective antibiotic which are resistant for some pathogens. Literature survey reveals that green approach of preparation of ZnO NPs is beneficial over the conventional chemical methods and these NPs are non-toxic and biocompatible. These green synthesized ZnO NPs may also be utilized in food packing industry to inhibit the pathogen growth in the packed food.

So, this study opens a new gate for the researchers about the synthesis of NPs with leaf extracts of *Origanum Majorana and to explore them for various biomedical applications*. Specifically, our reported results in this paper indicate that ZnO NPs synthesized with leaf extract can be an optimum
coating material in future for orthopedic and dental implants which is stable, biocompatible and antimicrobial. Our, obtained MIC values for bacteria like *Escherichia Coli, Pseudomonas aeruginosa, Streptococcus pneumonia* and *Staphylococcus aureus* respectively, demonstrate that these synthesized ZnO NPs can also be used selectively for gram positive bacterial infection in the human beings in the future.

2. Materials and Methods

ZnSO₄·7H₂O (Zinc sulphate heptahydrate) and NaOH (Sodium hydroxide) have been bought from Indian pharmaceutical company (Central Drug House (P) Ltd – CDH, India). All reagents and chemicals are utilized analytical reagent (A.R) grade.

2.1. *Origanum majorana* Leaf Extract Preparation

*Origanum majorana* leaves were taken from home garden, AG-Block-476, Shalimar Bagh, New Delhi-110088, India. These leaves were washed by using double deionized water and dried in the oven. These leaves were dried for two hours at temperature of sixty degree centigrade. In next step, these leaves were grounded. Then, the 25 % solution was prepared in deionized H₂O. This 25 % solution of grounded leaves was heated at forty degrees centigrade with continuous stirring. Then, the prepared aqueous leaves extract was filtered utilizing the Whatman filter paper no.1 after that this freshly prepared extract was utilized in fabrication of zinc oxide nanoparticles.

2.2. Fabrication of ZnO NPs.

ZnO NPs were fabricated by green approach. Herein, 50 mL (0.2 M) of zinc sulphate heptahydrate (ZnSO₄·7H₂O) aqueous solution was prepared in standard volumetric flask. In next step, 50 mL (1.0 M) of sodium hydroxide (NaOH) aqueous solution and abovementioned 3 mL leaves extract of *Origanum majorana* and 50 mL (0.2 M) zinc sulphate solution were transferred into the standard conical flask of 250 mL. After that reaction was run on hot plate with magnetic stirrer. The reaction solution was stirred at 400 rpm at temperature of forty degree centigrade (313 K) for two hours. Later on, the synthesized ZnO NPs were separated through centrifugation. These synthesized zinc oxide nanoparticles were washed with 90 % alcohol. In last step, these washed zinc oxide nanoparticles were dried for two hours at temperature of sixty degree centigrade (333 K). All solutions were prepared in double deionized water.

2.3. Advanced techniques used for characterization of zinc oxide nanoparticles.

The optical properties were obtained using advanced UV-Visible spectrophotometer. The spectrum was recorded using the model (Spectromax M2e). The presence of functional group in the synthesized ZnO NPs were identified through Fourier transform infrared (FT-IR). Herein, Potassium bromide was employed as the standard substance. The crystalline nature and phase purity of the synthesized ZnO NPs (dried powder) were investigated by model D8 Discover diffractometer (λ = 0.154056 nm). Herein, the θ ranges from 10° - 80° with a step per second (0.02°). The morphological determination of zinc oxide (ZnO) nanoparticles were investigated using scanning electron microscope (JEOL Japan Mode: JSM 6610LV) at 30 kV accelerating voltage and Magnification: X5 to X 3,00,000. Herein, the chemical composition was acquired using software of energy-dispersive X-ray spectroscopy (EDS) that is coupled with SEM.

2.4. Antibacterial activities

The antimicrobial study of fabricated zinc oxide nanoparticles against the pathogens viz., *Streptococcus pneumonia, Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli* was
evaluated through broth dilution process (Garcia, 2010) [36]. A stock solution of 2mg/mL of nanoparticles was prepared by dispersing them in pre-sterilized deionized water by ultra-sonication. The varying concentration ranging from 0.2 mg/mL to 200 mg/mL were prepared from stock solution and were added to the test tubes containing varying amount of sterile Luria Bertani broth (Muniyan et al., 2017) [37]. 0.5 mL of overnight culture (0.5 McFarland turbidity standards) of test pathogens was added to these tubes aseptically. Then, the tubes were incubated for 24 h at 37 °C. The bacterial cultures without any test solution and the tubes with only sterile media were kept as positive and negative controls respectively. The results were obtained by recording the optical density at 600 nm of the inoculated broth. MIC was recorded as the lowest concentration of the test sample prohibiting the growth of the inoculated test pathogen.

3. Results and Discussions

3.1. UV-Visible Spectroscopic and FT-IR Analysis

Figure 1(a) shows the UV-Visible absorption spectrum of synthesized ZnO NPs. The range of UV-Visible spectrum has been taken from 200 nm to 1000 nm. Herein, a sharp absorption band has been obtained at 379.75 nm and also a broad absorption band at near infra-red region 969.36 nm for synthesized ZnO NPs. This obtained value of λ maximum has been found in good agreements with the reported peak at 380 nm by Khara et al [38]. Alike outcomes have also been reported by Jayaseelan et al and Padalia et al respectively [34, 39]. Herein, they have prepared ZnO NPs by extract of Aeromonas hydrophila and Ziziphus nummularia respectively. The tauc’s plot was made with the help of abovementioned spectrum data of fabricated ZnO NPs with Orignum majorana leaves, which is given in Figure 1(b). The energy band gap is obtained using the equation investigated by Tauc. This is given as below.

\[(a\hbar\nu)^2 = A(\hbar\nu - E_g)^{1/2}\]  (1)

Herein, the constant A is not affected by magnitude of hv. But the absorption coefficient (a) depends on nature of the synthesized material. Herein, band gap (E_g) has been determined via drawing intercept in (a\hbar\nu)^2 vs hv curve as given in Figure 1(b). From this curve, the energy band gap (E_g) value have been obtained 2.84 eV for as-synthesized ZnO. Approximately similar, energy band gap value has been found by Rani et al., where, the ZnO NPs have been prepared by leaf extract of Tabernaemontana divericata [6]. In similar way the value of (E_g) has been evaluated by Arvanag et al [40].

The FTIR spectrum for synthesized ZnO NPs is given in Figure 1(c). Herein, the -Zn-O- bands have been obtained from 640.87 cm\(^{-1}\) to 835.56 cm\(^{-1}\) because of zinc oxide (-Zn-O-) stretching. There are other bands present in FT-IR spectrum like as 939.50 cm\(^{-1}\)-1044.27 cm\(^{-1}\), 1384.96-cm\(^{-1}\) because of presence of aromatic -C-H bond, 1498.80 cm\(^{-1}\) because of presence of -C-O bond and 3299.63 cm\(^{-1}\) a strong broad in H\(_2\)O or -O-H stretching. These bands show the functional groups of phytochemicals. These phytochemicals are derivative of phenol (Thymol), monoterpane, trans-hydrate Sabineno, polyphenolic acids and flavonoids. These functional compounds are found due to presence of these phytochemicals in extract of Origanum majorana leaves which are involved in surface coating of zinc nanoparticles. Such type of data are investigated by Ramesh et al, Soto-Robles et al and Guerra-Boone et al respectively [47-48, 54]. These phytochemicals also governs the size and shape of green synthesized ZnO NPs.
3.2. Powder X-Ray Diffraction (PXRD) Analysis

The PXRD pattern of fabricated ZnO NPs has been depicted in Figure 2. The PXRD pattern of these NPs has been obtained using X-ray diffraction. The PXRD pattern (A) shows for fresh prepared ZnO NPs using using 3mL of aqueous leaf extract of Origanum majorana where as PXRD pattern (B) for same ZnO NPs after six months. These is no decrease in peak intensity of synthesized ZnO NPs after six months and obtained peak positions are same, it means synthesized ZnO NPs are very stable. The peaks intensity have been obtained at $2\theta = 31.67^\circ$, $34.31^\circ$, $36.14^\circ$, $47.40^\circ$, $56.52^\circ$, $62.73^\circ$, $66.28^\circ$, $70.42^\circ$, $74.09^\circ$, and $79.60^\circ$. 
67.91°, 69.03° and 72.48° respectively. The corresponding (hkl) values have been assigned to (100), (002), (101), (102), (110), (103), (200), (112), (201) and (004) respectively. These values indicate the successful synthesis of hexagonal wurtzite crystalline structure of ZnO NPs. These 2θ values are well indexed with the JCPDS No. 36-1451 [41]. This crystalline structure also matches with the reported structure JCPDS No.5-0664 [42].

![Figure 2](image-url)  
**Figure 2.** Powder X-ray Diffraction Pattern for ZnO NPs prepared by green approach(A) For fresh prepared Sample, (B) After Six months

3.3. **SEM and EDS Analysis**

The scanning electron micrograph of green fabricated zinc oxide nanoparticles with help of leaf extract of *Origanum majorana* is given in Figure 3. It is clear from these Figures 3 (a, b, c) that all reported ZnO NPs are of rod shaped. The width of rod has been obtained approximately from 90 nm to 125 nm and from 0.5 µm to 1.2 µm in length. Our results are good agreement with reported dimension of ZnO NPs by Mahdizadeh et al [43]. Herein, ZnO NPs have been fabricated by green method using rough shell extract of *Cucumis melo* inodorus. These rod shaped NPs have been found agglomerated. The width and length of synthesized ZnO NPs have been found approximately 75-125 nm and 0.5 µm -1µm respectively. Basri et al has reported the ZnO NPs having diameters is in the scale of 73–123 nm with flower rod shapes [44]. Nutan et al and Zhong et al have obtained rod shaped morphology of synthesized ZnO NPs [6, 9]. The EDS spectrum of prepared ZnO NPs have been demonstrated in Figure 4. The EDS results (in set of Figure 4) reveal the elemental composition of this fabricated ZnO NPs and also reveal about the purity of fabricated ZnO NPs. The percentage atomic ratio has been obtained for Zinc (Zn) 51.41% and for Oxygen (O) 48.59 % respectively. Approximately similar percentage ratio of zinc (Zn) and oxygen (O) have been obtained by Rani et al [6].
Figure 3. SEM images of ZnO NPs prepared by green approach (a) at 10,000 Magnification, (b) at 15,000 Magnification (c) at 30,000 Magnification
Figure 4. EDS Spectra of green fabricated zinc oxide nanoparticles.

### Table 1

| Element | Weight % | Atomic % | Net Int. | Error % |
|---------|----------|----------|----------|---------|
| O K     | 20.57    | 51.41    | 62.75    | 9.85    |
| ZnK     | 79.43    | 48.59    | 598      | 1.45    |

3.4. Antibacterial activities

The antibacterial results have been given in Table 1. Herein, we have studied the antimicrobial activity of nanoparticles on *Staphylococcus aureus*, *Streptococcus pneumonia*, *Escherichia coli* and *Pseudomonas aeruginosa*. Herein, gram-negative microbes are *Escherichia coli* and *Pseudomonas aeruginosa* while gram-positive microbes are *Staphylococcus aureus* and *Streptococcus pneumoniae*. This study has been performed using the broth dilution method. The MIC has been found 150-175 µg/mL for *Pseudomonas aeruginosa*, 100-125 µg/mL for *Escherichia coli*, and 76-100 µg/mL for *Staphylococcus aureus* as well as for *Streptococcus pneumoniae*. More MIC value means that a higher concentration is needed to inhibit the growth of microbes. Table 1 shows that these synthesized ZnO NPs are more effective against all clinical isolates except *Pseudomonas aeruginosa*. It means *Pseudomonas aeruginosa* shows more resistance against the synthesized ZnO NPs. Our results are in good agreement with reported antimicrobial study by Sana et al [17]. Herein, Sana et al has studied the antibacterial study at the concentrations of 25 μg/mL, 50 μg/mL and 100 μg/mL respectively. Similar study has been reported with ZnO NPs using *Peltophorum pterocarpum* flower-mediated synthesis in the literature [38]. Herein, these NPs are tested against four types of gram-positive microbes and four...
types of gram-negative microbes. This study has been done at two concentrations of ZnO NPs 100 mg/mL and 150 mg/mL respectively. Hazra et al has also evaluated the antimicrobial activity of ZnO NPs synthesized using extract of Aloe vera and MIC values have been determined in the range of 3-5 mg/mL [45]. It means, our results are best than the previously reported results. Similar types of results have also been reported only with essential oil, whole plant extract and extract of leaves of *Origanum majorana* and *Origanum compactum Benth* respectively [46,54]. Herein, reported very high values of MIC with only extract indicates that the impact of only extract has been found very less on antimicrobial activity as compared with our synthesized ZnO NPs. Herein, an elaborative Table 2 has been given which indicates the type of system, synthesis methodology, application and advantage of the respective system as compared to the reported system [17, 29-30, 33, 35, 38, 45, 49-53]. Herein, Barani et al. have reported same value of MIC (125 μg/mL) for *Escherichia coli* but they have reported very high MIC value (250 μg/mL) for *Staphylococcus aureus*.

**Table 1.** Minimum Inhibitory Concentration (MIC in μg/mL) of ZnO nanoparticles for various bacterial pathogens.

| Isolates                        | MIC Range |
|---------------------------------|-----------|
| *Escherichia coli* (E. Coli)    | 100-125   |
| *Pseudomonase Aeruginosa*       | 150-175   |
| *Staphylococcus Aureus*         | 76-100    |
| *Streptococcus Pneumoniae*      | 76-100    |

**Table 2.** Comparison of earlier reported ZnO nanoparticles with synthesized ZnO nanoparticles for antibacterial activities.

| S.N. | Nanoparticles | Plant extract/Bacteria used for biosynthesis | Shape/Size          | Organism/MIC value                   | References |
|------|---------------|--------------------------------------------|---------------------|--------------------------------------|------------|
| 1    | ZnO           | *Origanum majorana*                        | Rod shape/90-125nm  | *Pseudomonas aeruginosa*/175μg/mL    | Present work |
|      |               |                                            |                     | *Escherichia coli*/*125μg/mL         |            |
|      |               |                                            |                     | *Staphylococcus aureus*/*100μg/mL    |            |
|      |               |                                            |                     | *Streptococcus pneumonia*/*100μg/mL  |            |
| 2    | ZnO           | *Punica granatum L and Tamarindus indica* | Spherical shape/10nm and 14nm | *Escherichia coli*/*512μg/mL | 29         |
| No. | ZnO Source                  | Trace Metal Source            | Particle Size Shape | MIC/MIC Range                  |
|-----|-----------------------------|-------------------------------|---------------------|--------------------------------|
| 3   | Aloe Leaf broth             | -/40nm and 25nm               | Staphylococcus aureus/0.40mM Serratia marcescens/1.40mM Proteus mirabilis/1.0mM Pseudomonas aeruginosa/2.9µg/mL Staphylococcus aureus/2.4µg/mL Bacillus subtilis/3.0µg/mL Escherichia coli/3.0µg/mL Klebsiella pneumonia/4.0µg/mL Pseudomonas aeruginosa/5.0µg/mL Staphylococcus aureus/4.0µg/mL | 33 |
| 4   | Aeromonashy drophila       | Spherical/57-72nm             |                    |                                | 35 |
| 5   | Aloe vera                   | Rod shape/less than 100nm     | Bacillus subtilis/3.0µg/mL Escherichia coli/3.0µg/mL Klebsiella pneumonia/4.0µg/mL Pseudomonas aeruginosa/5.0µg/mL Staphylococcus aureus/4.0µg/mL | 45 |
| 6   | Cissus quadrangularis      | Spherical shape/75-90nm       | Mycobacterium smegmatis/50µg/mL |                                | 49 |
| 7   | Marinobacter sp. And Vibrio sp. | Spherical shape/~10nm and ~20nm | Escherichia coli/125µg/mL Pseudomonas aeruginosa/62.5µg/mL Staphylococcus aureus/250µg/mL Bacillus subtilis/500µg/mL Listeria innocua/1000µg/mL | 50 |
| 8   | Livistona jekinsiana       | Not uniform spherical, oval and hexagonal/3.6nm | Staphylococcus aureus/15µg/mL Escherichia coli/25µg/mL Bacillus subtilis/10µg/mL Klebsiella pneumonia/25µg/mL Staphylococcus aureus/31.25µg/mL Escherichia coli/62.5µg/mL | 51 |
| 9   | Gum Arabic                 | ~/180nm                      |                      |                                | 52 |
| 10  | Brassica rapa              | Irregular shape/27.48nm       | Escherichia coli/25µg/mL Bacillus |                                | 53 |
4. Conclusions

Rod shaped ZnO NPs was prepared with the help of 3 mL leaves extract of *Origanum majorana*. These green synthesized ZnO NPs are very stable, energy saving, low cost, biocompatible and more environmental friendly. These ZnO NPs was also characterized with the help of different advanced techniques like as UV-Visible absorption spectroscopy, FT-IR, EDS, PXRD, SEM and EDS respectively. These rod shaped ZnO NPs have also been utilized for antibacterial activity. The four microbes namely (*Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus* and *Streptococcus pneumonia*) were tested for the antimicrobial study. The obtained value of MIC with broth dilution method indicates that our prepared ZnO NPs are more effective towards the gram-positive microbes as compare to gram-negative microbes. This, in vitro study reveals that these ZnO NPs synthesized with leaves extract of *Origanum majorana* can be developed as a new nano-medicine for bacterial infection in future.

Acknowledgment

Kalawati Saini wants to present a sincere thanks to Principal, Miranda House, University of Delhi for providing laboratory for research work. The authors are appreciating to USIC, University of Delhi, India for getting SEM, EDS and PXRD of green fabricated zinc oxide nanoparticles. Kalawati Saini and Mona Saini also very grateful to DBT, New Delhi, India for providing fund (Ref. No. BT/IN/Indo/Foldscope/39/2015 dated: 20.03.2018). One of the author, Sapna Yadav thanks to Council of Scientific & Industrial Research (CSIR), India for providing fellowship (File Reference No. 08/700/(0004)/2019-EMR-1). Kalawati Saini would like to thank Dr. Amrita Tripathi Sheikh, Associate Professor, MH, DU for her constant encouragement and support.

Disclosure

The authors claims no conflicts of interest in publishing of this research article.

References

[1] Taşdemir A, Aydin R, Akkaya A, Akman N, Altinay Y, Çetin H, Şahin B, Uzun A and Ayyıldız E 2021 *Ceram. Int.* 47(14) 19362-19373.

[2] Mishra P K, Mishra H, Ekielski A, Talegaonkar S and Vaidya B 2017 *Drug Discov. Today* 22 1825–1834.

[3] Martínez-Carmona M, Gun’ko Y and Vallet-Regí M 2018 *ZnO Nanomaterials* 8 268.

[4] Xiong H M 2013 *Adv. Mater* 25 5329–5335.
[5] Bayrami M, Bayrami A, Habibi-Yangjeh A, Shafeeyan M S, Feizpoor S, Arvanaghi F M, Nourani M R Taheri R A 2020 *IET Nanobiotecnol*. 14(7) 548-554

[6] Rani N, Saini M, Yadav S, Gupta K, Saini K and Khanuja M 2020 *AIP Conf. Proc.* 2276 020042

[7] Li R, Yabe S, Yamashita M, Momose S, Yoshida S, Yin S and Sato T 2002 *Solid State Ion.* 151 235-241.

[8] Lu M P, Song J, Lu M Y, Chen M T, Gao Y, Chen L J and Wang Z L 2009 *Nano Lett.* 9 1223-1227.

[9] Zhong Z, Xu Z, Sheng T, Yao J, Xing W and Wang Y 2015 ACS Appl. Mater. Interfaces 7(38) 21538-21544

[10] Yu A, Qian J, Pan H, Cui Y, Xu M, Tu L, Chai Q and Zhou X 2011 *Sen. Actuators B Chem.* 158 9-16.

[11] Elilarassi R and Chandrasekaran G 2010 *Optoelectron. Lett.* 6 6-10.

[12] Lv T, Pan L K, Liu X J and Sun Z 2012 *Catal. Sci. Technol.* 2 2297-2301.

[13] Gao P, Ng K and Sun D D 2013 *J. Hazard. Mater.* 262 826-835.

[14] Salehi R, Arami M, Mahmoodi N M, Bahrami H and Khorramfar S 2010 *Colloid Surface B* 80 86-93.

[15] Li J, Li Y, Wu H, Naraginti S and Wu Y 2021 *Environ. Res.* 200 111433.

[16] Soltanian S, Sheikhbahaei M, Mohamadi N, Pabarja A, Abadi M F S and Tahroudi M H M 2021 *BioNanoScience* 11(2) 245-255.

[17] Sana S S, Kumbhakar D V, Pasha A, Pawar S C, Grace A N, Singh R P, Nguyen V H and Le Q V 2020 *Molecules* 25(21) 4896.

[18] Arvanaghi F M, Bayrami A, Yangjeh A H and Pouran S R 2019 *Mater Sci Eng C.* 97 397-405.

[19] Asif N, Fatima S, Md Aziz M A, Shehzadi, Zaki A and Fatma T 2021 *Bioorg. Chem.* 113 104999.

[20] Ann L C, Mahmud S, Bakhori S K M, Sirelkhatim A, Mohamad D, Hasan H, Seeni A and Rahman R A 2014 *Ceram. Int.* 40 2993-3001.

[21] Gu Y, Teng G, Jin X, Wang L, Qiang Z, Ma W and Zhang C 2020 *Indust. Eng. Chem. Res.* 59(24) 11219-11231.

[22] Pranjali P, Meher M K, Raj R, Prasad N, Poluri K M, Kumar D and Guleria A 2019 *ACS Omega* 4(21) 19255-19264.
[23] Guleria A, Meher M K, Prasad N, Poluri K M and Kumar D 2017 J. Phys. Chem. C 121(34) 18598-18607.

[24] Mitra P, Dutta D, Das S, Basu T, Pramanik A and Patra A 2018 ACS Omega, 3(7) 7962-7970.

[25] Lucas-Gil E, Leret P, Monte-Serrano M, Reinoso J J, Enríquez E, Campo A D, Cañete M, Menéndez J, Fernández J F and Marcos F R 2018 ACS Appl. Nano Mater. 1(7) 3214-3225.

[26] Ray P G, Biswas S, Roy T, Ghosh S, Majumder D, Basak P, Roy S and Dhara S 2019 ACS Sustainable Chem. Eng. 7(16) 13717-13733.

[27] Visinescu D, Hussien M D, Moreno J C, Negrea R, Birjega R, Somacescu S, Ene C D, Chifiriuc M C, Popa M, Stan M S and Carp O 2018 Langmuir 34(45) 13638-13651.

[28] Padalia H, Moteriya P and Chanda S 2018 BioNanoSci. 8(1) 196-206.

[29] Prashanth G K, Prashanth P A, Utpal Bora U, Manoj Gadewar M, Nagabhushana B M, Ananda S, Krishniah G M and Sathyanaanda H M 2015 Karbala Int. J. Mod. Sci. 1 67-77.

[30] Dobrucka R and Dugaszewsk J 2016 Saudi J. Biol. Sci. 23 517–523.

[31] Divya M J, Sowmia C, Joona K and Dhanya K P 2013 Res. J. Pharm. Biol. Chem. 4(2) 1137–1142.

[32] Divyapriya S, Sowmia C and Sasikala S 2014 World J. Pharm. Pharm. Sci. 3(12) 1635–1645.

[33] Gunalan S, Sivaraj R and Rajendran V 2012 Prog. Nat. Sci. Mater. Int. 22(6) 693–700.

[34] Jayaseelan C, Rahuman A A, Kirthi A V, Marimuthu S, Kumar T S, Bagavanna A, Gaurav K, Karthik L and Bhaskara Rao K V 2012 Spectrochimica Acta Part A. 90 78– 84.

[35] Brayner R, Ferrari-Iliou R, Brivois N, Djediat S, Benedetti M F and Fiévet F 2006 Nano Lett. 6(4) 866–870.

[36] Garcia L 2010 Broth Microdilution MIC Test, In Clinical Microbiology Procedures Handbook, 3rd Edition. ASM Press, Washington, DC. 25-41.

[37] Muniyan A, Ravi K, Mohan U and Panchamoorthy R 2017 World J Microbiol Biotechnol. 33(7) 1-12.

[38] Khara G, Padalia H, Moteriya P and Chanda S 2018 Arab. J. Sci. Eng. 43 3393-3401.

[39] Padalia H and Chanda S 2017 Nanomed. Biotechnol. 45(8) 1751-1761. DOI: 10.1080/21691401.2017.1282868

[40] Arvanag F M, Bayrami A, Habibi-Yangjeeh A and Pouran S R 2019 Mater Sci Eng C 97 397-405.
[41] Seo H K and Shin H S 2015 Mater. Lett. 159 265-268.

[42] Akhtar M J, Ahamed M, Kumar S, Khan M M, Ahmad J and Alkakayan S A 2012 Int. J. Nanomed. 7 845-851.

[43] Mahdizadeh R, Homayouni-Tabrizi M, Neamati A, Seyedi S M R and Afshar H S T 2019 J. Cell. Biochem. 10 1-10.

[44] Basri H H, Talib R A, Sukor R, Othman S H and Ariffin H 2020 Nanomaterials 10(6) 1061.

[45] Lakshmi V J, Sharath R, Chandraprabha M N, Neelufar E, Hazra A and Patra M 2012 J. Biochem. Technol. 3 (5) S151–S154.

[46] Charai M, Mosaddak M and Faid M 1996 J. Essent. Oil Res. 8(6) 657-664. (Online published in 2011).

[47] Ramesh M, Anbuvannan M and Viruthagiri G 2015 Spectrochim. Acta A. Mol. Biomol. Spectrosc. 136 864-870.

[48] Soto-Robles C A, Luque P A, Gomez-Gutierrez C M, Nava O, Vilchis-Nestor A R, Lugo-Medina E, Ranjithkumar R and Castro-Beltran A 2019 Results Phys. 15 102807.

[49] Sathappan S, Kirubakaran N, Gunasekaran D, Gupta P K, Verma R S and Sundaram J 2021 Proc. Natl. Acad. Sci. India, Sect. B Biol. Sci. 91(2) 289–296.

[50] Barani M, Masoudi M, Mashreghi M, Makhdoumi A and Eshghi H 2021 Int. J. Pharm. 606 120878.

[51] Baruah R, Yadav A and Das A M 2021 Spectrochim. Acta A Mol. Biomol. Spectrosc. 251 119459.

[52] Pauzi N, Zain N M, Kutty R V and Ramli H 2021 Mater. Today: Proc. 41 1-8.

[53] Khan M I, Fatima N, Shakil M, Tahir M B, Riaz K N, Rafique M, Iqbal T and Mahmood K 2021 Physica B Condens. Matter 601 412563.

[54] Guerra-Boone L, Alvarez-Romá R, Salazar-Aranda R, Torres-Cirio A, Rivas-Galindo V M, Waksman de Torres N, González G and Pérez-López L A 2015 Pak. J. Pharm. Sci. 28(1) 363-369.

[55] Memarzadeh K, Sharili A S, Huang J, Rawlinson S C F and Allaker R P 2015 J. Biomed. Mater. Res. A 103(3) 981-989.