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Characterization and Evaluation of the Antibacterial Profile of ZnO Nps Synthesized by Liv-Pro-08 Ayurvedic Herbal Formulation (AHF)

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Abstract

This study was performed to determine the Zinc Oxide nitrate reducing potential of aqueous extract of Liv-Pro-08 polyherbal formulation and characterize the synthesized ZnO NPs and assess their antibacterial activity against pathogenic bacteria such as *Staphylococcus aureus Bacillus cereus, Escherichia coli, Klebsiella pneumonia,* and *Pseudomonas aeruginosa.* The results showed that the yellow-colored crystalline ZnO NPs were synthesized, and it was preliminarily confirmed by UV analysis by attained a predominant peak at 375 nm. Through Powder X-ray diffraction (PXRD) and Fourier-transform infrared spectroscopy (FT-IR) analyses, the crystalline structure and functional groups exist in the surface of ZnO NPs, and its size was measured as 2 to 5 µm through Scanning Electron Microscope (SEM) analysis, and morphology was found as spherical with the smooth-edged surface. The antibacterial potential of characterized ZnO NPs was assessed, and found significant antibacterial activity against pathogenic bacteria such as *S. aureus B. cereus, E. coli, K. pneumonia,* and *P. aeruginosa* with 100 µg concentration of various volumes (25, 50, and 75 µl) of nanoparticles. These results conclude that the aqueous extract of Liv-Pro-08 polyherbal formulation synthesized ZnO NPs might be used as an antibacterial agent against some common pathogenic bacteria.

Keywords: Liv-Pro-08; ZnO NPs; PXRD; FT-IR; antibacterial

Introduction

The global population's continuous rising generates stress over the research community since several unidentified clinical cases are finding every day (Heyland et al., 2013). Hence, the scientific community and researchers face more challenges to find and produce significant drug

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from various biological sources, including microbes and plants (Ahmed, et al., 2017). Significant percentage of medicines circulating in the globe are derived from plant sources. The traditional way of drug molecules synthesis, formulation, and medication could cause some adverse effects on human kind (Wangkheirakpam, 2018). Thus, the researchers are concerned about applying nanoparticles for the welfare of humans and the ecosystem (Roco, 2008). Since several literatures are stated that the nanoparticles have multidimensional usage, researchers recently received more attention by researchers from various fields such as medical, environmental, automobiles, etc. (Chen, 2008). Since they large surface area and fits into the various application based performance. There are several types of nanoparticles that have been reported by number of literatures, however, among them the metal-based nanoparticles receiving more attention due to its multidimensional application (Baeza, et al., 2017). They possess unique and significant traits such as chemical, physical, and biological natures (Mitragotri and Lahann, 2009). The metal nanoparticles synthesized through chemical based technologies are not receive much attention especially with the motive of biological application, since the chemical agents used to reduce the metal oxides into nanoparticle could converted to become a capping molecule and capped over the surface of the synthesized nanoparticle (Duan, et al., 2015). Hence that could act as a toxic agent to the organisms and cause some adverse side effects (Sak, 2012). Thus the researchers are paying their attention to use the plant as reducing agent to reduce the metal elements into metal nanoparticles. The plant extracts contain the number of most significant phytochemical ingredients, which could be served as a reducing agent to reduce the complex metal elements into less or nontoxic nanoparticles by capping (phytochemicals) over the large surface of the synthesized nanoparticle (Shanker, et al., 2016). The green synthesized nanoparticles are ecofriendly and renewable in the synthesis process and could produce more nanoparticle yield (Virkutyte and Varma, 2011). The phytochemicals such as alkaloids, flavonoids, terpenoids, tannins, terpenes, quinones, etc. could significantly reduce the metal elements nanoparticles and also could act as a copping component over the surface of the synthesized nanoparticle (Kisała, et al., 2017). Among various metal elements, the Zinc oxide (ZnO) based nanoparticle synthesis attain more attention since the significant quantity of Zn could be considered as essential elements for regular metabolic activity of body cells (Manna, 2012). Thus the nanoparticle synthesized from Zinc oxide possesses more application potentiality, especially in the medical field as drug carrier, antimicrobial properties. Surprisingly, various solvent extracts of individual plant species such as *Passiflora caerulea*, *Vitex trifolia*, *Azadirachta indica*, *Moringa oleifera*, *Lycopersicon esculentum*, *Artocarpus gomezianus*, *Aloe vera*, *Cinnamomum verum*, *Trifolium pretense*, *Bauhinia tomentosa*, *Moringa oleifera*, *Olea europaea*, *Matricaria chamomilla*, etc. have been reported by various authors as these plant species could produce zinc nanoparticle from Zinc Oxide nitrate ($Zn(NO_3)_2$) (Demissie, et al., 2020). This Zn nanoparticles have been used in various industrial activities such as nano-optical, electrical devices, food packaging, etc. The nanoparticle synthesized from various metal sources might possess significant antimicrobial

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properties against various pathogenic microbes such as *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumonia*, *Vibrio cholera*, *species of Penicillium*, *Aspergillus*, *Trichoderma*, etc . (Rani, et al., 2019). This initial report develops Liv-Pro-08 herbal (poly) formulation using fruits and seeds of fennel flower, African dream herb seeds, and cluster fig to synthesize the ZnO nanoparticle (Panda and Hazra, 2020). These ingredients were previously used individually to synthesize various nanoparticles, and these ingredients possess medicinal value (Manna, 2012). Hence this study was designed to synthesize the ZnO nanoparticle from $Zn(NO_3)_2$ using Liv-Pro-08 polyherbal formulation and characterize the nanoparticles by typical FTIR analysis. Furthermore, assess their antibacterial potential against some common pathogenic bacteria.

Materials and methods

Collection and processing of plant samples

The plant samples such as fruits and seeds of fennel flower, African dream herb seeds, and cluster fig were collected from Kolli hills and their surrounding Namakkal district of Tamil Nadu, India. The collected samples were used to develop the Liv-Pro-08 polyherbal formulation by following the typical protocol (Panda and Hazra, 2020). Briefly, the collected plant samples were immediately shifted to the laboratory and shadow dried at room temperature, then the pulverized using an electric mixer and filtered through the regular mesh to attain a refined form of powder. About equal concentrations of this mixture were mixed and stored in an air-tight container for further analyses.

Aqueous extract preparation

About 10 g of Liv-Pro-08 polyherbal formulation powder was dissolved in 100 mL of distilled water and incubated at 40 °C for 48 h in a shaker incubator with 120 rpm. After 48 h of incubation, the mixture was filtered using Whatman No. 1 filter paper, and filtrate was concentrated by kept at hot air oven (at 30 °C) to evaporate the moisture (Demissie, et al., 2020). Then the concentrated dark yellowish color extract was refrigerated at 4°C for further study.

Synthesis of ZnO nanoparticles

About 5 g of Zinc Oxide nitrate $Zn(NO_3)_2$ was used as the substrate to synthesize the ZnO nanoparticles using 50 mL of concentrated aqueous extract of Liv-Pro-08 AHF in a 100 mL conical flask and boiled at 60-80 °C. The $Zn(NO_3)_2$ was added when the temperature of the Liv-Pro-08 AHF extract reaches 60°C during boiling (Zak, et al., 2011). The boiling is continued until the solution came deep yellow-colored paste like substance. This paste was shifted to the ceramic crucible and heated by furnace approximately 400 °C for 2 h and finally, yellow colored powder was attained and subjected to characterization and application study.

Characterization of ZnO nanoparticles

UV–Vis spectrophotometer analysis

The synthesized ZnO nanoparticles were subjected to characterization study. Initially, UV spectral analysis was performed with the range of 200-800 nm at room temperature in 1 cm

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path quartz cell (Zak, et al., 2011). The attained absorbance values were documented and compared with published literature.

FTIR analysis

FTIR analysis possible presence of functional groups on ZnO NPs was characterized by following typical operating procedures (Zak, et al., 2011). Concisely, about 20 mg of synthesized nanoparticles were placed over the sampling point, and spectra were gathered in the range of 400–4000 cm^{-1} at a resolution of 4 cm^{-1}. The spectra were collected in transmittance mode in triplicate.

XRD and SEM analysis

The X-ray diffractometer was used to measure the X-ray diffraction pattern of ZnO NPs synthesized by aqueous extract of Liv-Pro-08 polyherbal formulation. The measurement was performed using Cu Kα radiation of wavelength λ = 0.1541 nm in the scan range 2θ = 20–90◦. The morphological appearance of synthesized ZnO NPs was studied by Scanning Electron Microscope analysis using JSM-IT500HR InTouchScope™ Scanning Electron Microscope, JEOL, Japan.

Antibacterial potential of ZnO NPs

The antibacterial efficiency of aqueous extract of Liv-Pro-08 polyherbal formulation synthesized ZnO NPs were tested with bacterial pathogens such as *Staphylococcus aureus Bacillus cereus Escherichia coli, Klebsiella pneumonia,* and *Pseudomonas aeruginosa* by following disc diffusion method on Mueller-Hinton agar plate (Fahimmunisha, et al., 2020). Briefly, about 0.1 mL of (1.5×10^8 CFU/mL) each test bacterial pathogens were inoculated on sterilized Mueller-Hinton agar Petri plates (in triplicates) following the spread plate method. Tetracycline (30µg) antibiotic disc and dimethyl sulfoxide (DMSO) wetted discs were used positive and negative control correspondingly. Meanwhile, ZnO nanoparticle discs were prepared by dissolving about 100 µg of ZnO NPs in 200 µL of DMSO. Various volumes (25, 50, and 75 µL) were added in sterile discs (6 mm diameter disc) and dried from this stock solution. The prepared discs were placed over the bacterial culture inoculated petri plates and incubated at 37°C for 24 h. After incubation, the zone of inhibition (in diameter mm) around each concentration's discs and each bacterial culture were measured and compared with positive control.

Results and discussion

The aqueous extract of Liv-Pro-08 polyherbal formulation can reduce the $Zn(NO_3)_2$ into ZnO NPs by boiling at 80°C and confirmed by the development of yellow-colored past and attained yellow color powder by furnace drying the paste at 400 °C for 2 h. The results achieved from the UV-Vis analysis confirmed that the reduction of $Zn(NO_3)_2$ into ZnO NPs. It was confirmed by individual predominant peak attained at 375 nm it corresponded to the ZnO NPs since it was characteristic to the essential band gap of Zn-O molecules. Moreover, a similar peak (at 378 nm) was reported as hexagonal structure (wurtzite) of ZnO NPs (Shamhari, et al., 2018).

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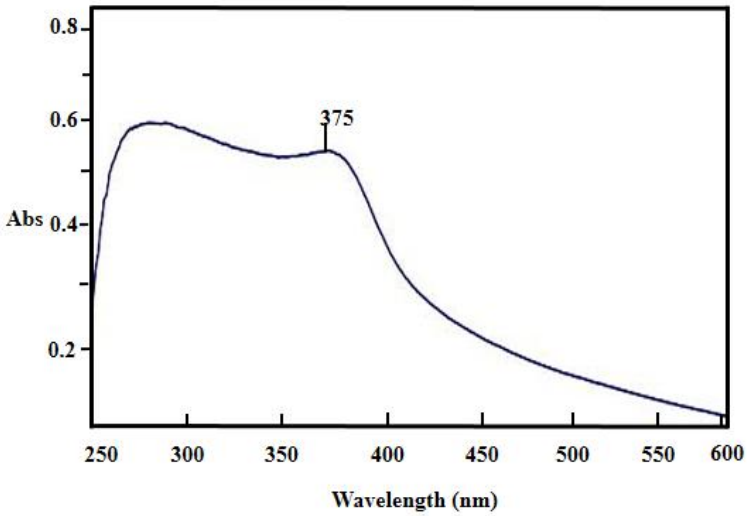
Sun, et al., (2011) found the ZnO NPs synthesized from biological sources hold a peak at 365 nm in UV visible spectrophotometer analysis. The ZnO NPs synthesized from solvent extracts of Euphorbia *Jatropa* latex showed absorption peaks at 360 and 365 nm in spectrophotometer analysis. These results indicate that the absorption peak for ZnO NPs synthesized from various biological sources could exist in the range of 355 to 380 nm (Akhil and Khan 2017).

Figure 1. UV-Visible spectrophotometer analysis of ZnO NPs



FT-IR analysis

Figure 2 denoted that functional group existence in the aqueous Liv-Pro-08 polyherbal formulation synthesized ZnO NPs has significant functional groups. These were confirmed by the peaks attained in the range of 573.33 to 3448.73 cm^{-1} attribute to the various stretching vibrations corresponding to ZnO NPs. In this study, the synthesized ZnO NPs possess an essential band at 573.33 cm^{-1} related to the Zn-O stretching vibration. Another band found at 1330 cm^{-1} that attributes the stretching vibration indicates Zn(CH3COO)2·2H2O combined with CH3 group. The band observed at 1637.56 and 2075.07 cm^{-1} were symmetric and asymmetric stretching (O-C-O) vibration corresponds to carbonate anion. The most influential band attained at 3448.73 cm^{-1} attributes to stretching vibration and that corresponding to hydroxyl group. Similarly, the hydroxyl group attributed band was recorded at 3417 cm^{-1} of ZnO NPs synthesized by absolute ethanol (Shamhari, et al., 2018). Obtained band patterns were confirmed that the synthesized ZnO NPs were partially pure form coated with reducing agents. Similar kind of result pattern was reported by Geetha, et al., (2016) and Jayaseelan, et al., (2012) on ZnO NPs synthesized from the plant source. This study band pattern confirmed that the existence of a surface functional group over the nanoparticle surface.

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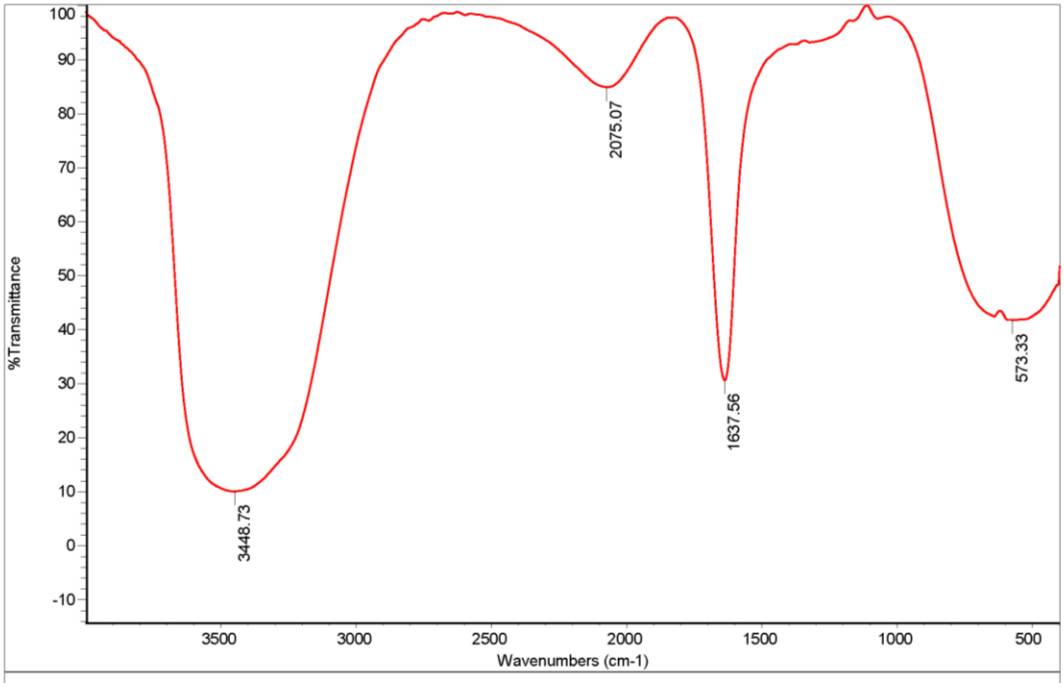
Figure 2. FT-IR analysis of functional groups present in the ZnO NPs synthesized by Liv-Pro-08 polyherbal formulation



XRD and SEM analyses of ZnO NPs

Figure 3 showed the powdered XRD with different diffraction peaks denotes the crystalline structure and size of the ZnO NPs synthesized by aqueous extract of Liv-Pro-08 polyherbal formulation. This analysis was performed at room temperature with the 2θ range of 0–80° and recorded many resolved diffraction peaks and prominent peaks found at (100), (002), (101), (012), (110), (103), (112), (201), (004), (202) and (113) crystallographic planes correspond respectively to the Bragg reflections at 2θ values of 31.8°, 34.5°, 36.3°, 47.6°, 56.7°, 62.9°, 66.0°, 68.0° 69.2°, 72.7° and 77.0°, which states that the synthesized ZnO NPs existed in pure form. The existence of passionate peaks at (100), (002), and (101) planes corresponds to crystalline and pure forms of ZnO NPs. A similar PXRD pattern was reported on ZnO NPs synthesized from the solvent extract of *Camellia sinensis* (green tea) (Senthilkumar and Sivakumar, 2014).

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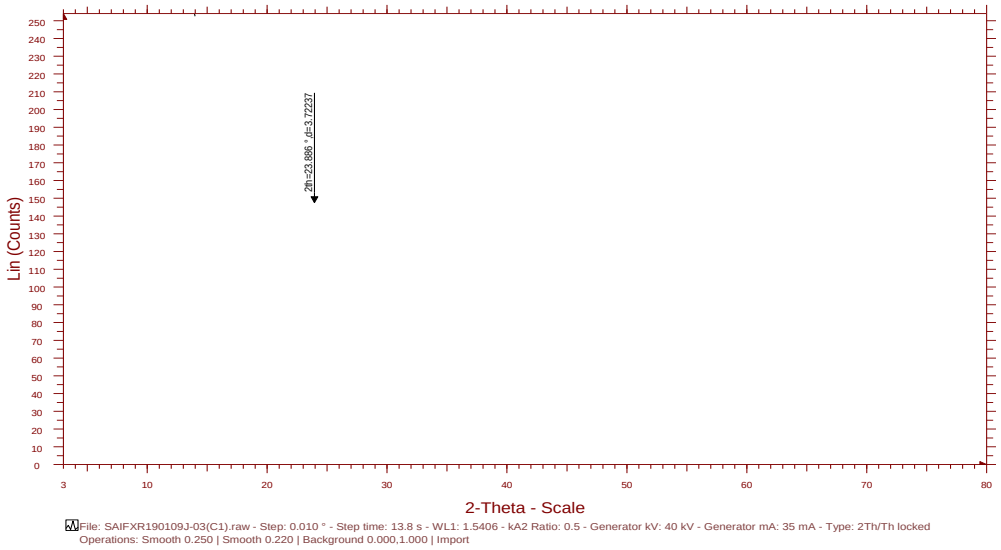
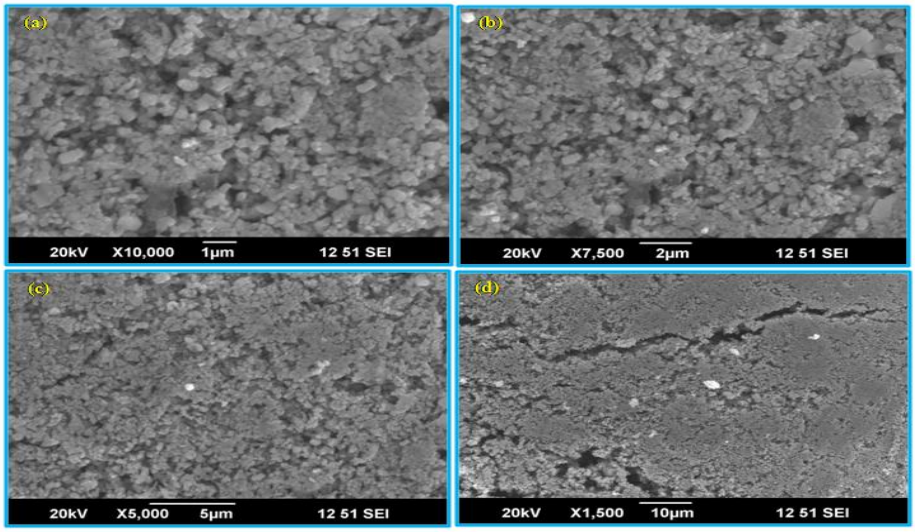
Figure 3. Powdered XRD analysis of ZnO NPs synthesized by aqueous extract of Liv-Pro-08 polyherbal formulation



The morphologies of ZnO NPs were observed by SEM analysis by various scaling such as 1, 2, 5, and 10 m (Fig. 4). The observed image represented that ZnO components' morphology was reduced and changed with calcination temperature at 700°C (calcined). The high resolution with 1, 2, and 5 µm scaling showed (Fig. 4) that the presence of ZnO NPs with 2 to 5 µm size. The morphology was appeared as spherical with a smooth external surface. Similarly, Pillai, et al., (2020) found spherical shaped less than 10 5 µm sized ZnO NPs synthesized by plant extracts.

Figure 4. SEM analysis of ZnO NPs with various scaling



Legend: SEM analysis of ZnO NPs **(a):** 1 µm **(b):** 2 µm **(c):** 5 µm **(d):** 10 µm

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Antibacterial potential of ZnO NPs

The antibacterial profile of aqueous extract of Liv-Pro-08 polyherbal formulation synthesized ZnO NPs was studied with some common pathogenic bacteria species by disc diffusion method with various volumes (25, 50, and 75 µl) with the concentration of 100 µg. The attained results showed that these volumes of ZnO NPs possess significant antibacterial activity against test bacterial pathogens such as *S. aureus B. cereus, E. coli, K. pneumonia,* and *P. aeruginosa* (Table 1). This result declared that this ZnO NPs could act against both gram-positive and gram-negative bacterial species. It could be directly related to the size of the particle and materials which capped over the surface of the nanoparticles (Bhuyan, et al., 2015). Moreover, the most volume of ZnO NPs possesses significant antibacterial activity against these test bacteria. At the volume of 25, 50, and 75 µl of 100 µg of ZnO NPs showed zone of inhibition as 2.5 ± 0.6 & 3.0 ±0.5; 5.0 ± 0.4 & 5.0 ± 0.5, and 6.5 ± 0.3 & 4.0 ± 0.3 mm in *S. aureus* and *B. cereus* respectively. Similarly the zone of inhibition as 3.5 ± 0.3 & 3.0 ± 0.5, 5.0 ± 0.5 & 4.5 ± 0.9, and 3.0 ± 0.5 & 6.5 ± 0.7 mm were measured against *E. coli* and *K. pneumonia* correspondingly. About 3.0 ± 0.5, 4.0 ± 0.7, and 6.0 ± 0.5 mm of a zone of inhibition was observed against *P. aeruginosa* at the volume of 25, 50, and 75 µl of 100 µg concentration of ZnO NPs and triplicates were performed. The obtained zone of inhibition results of most volume ZnO NPs was significantly compared with the standard. The possible way of bactericidal activity of ZnO NPs could be related to their size, since it can penetrate the membrane with ease, collapse the internal metabolic activity and another essential process such as active transport, enzymatic activity, etc. in bacteria (Kumar, et al., 2020; Soenen, et al., 2015).

Furthermore, the particles (aqueous extract of Liv-Pro-08 polyherbal formulation) capped over the surface of ZnO NPs might possess antibacterial activity. As these capped over the ZnO NPs it could reach inside the bacteria along with nanoparticle and cause death to pathogenic bacteria (Khezerlou, et al., 2018). This ZnO NPs could stimulate reactive oxygen species formation leads to generate oxidative stress and cause cell death (Guo, et Al., 2013). Obviously the nanoparticles possess excellent surface reactivity, hence it could bind over the cell wall of bacteria through electrostatic forces and cause damage on cell membrane and leads to release of intracellular contents and death (Ramalingam, et al., 2016).

Table 1. Antibacterial profile of ZnO NPs by aqueous extract of Liv-Pro-08 polyherbal formulation

S.No	Name of the bacterial pathogens	Zone of inhibition (mm) of positive control (tetracycline 30 cg)	Zone of inhibition (mm) various volume of synthesized ZnO NPs (100 µg concentration)		
		50 µl	25 µl	50 µl	75 µl
1	***Staphylococcus aureus***	8.5 ± 0.5	2.5 ± 0.6	5.0 ± 0.4	6.5 ± 0.3
2	***Bacillus cereus***	8.5 ± 0.2	3.0 ±0.5	5.0 ± 0.5	4.0 ± 0.3
3	***Escherichia coli***	10.5±0.4	3.5 ± 0.3	5.0 ± 0.5	3.0 ± 0.5

4	***Klebsiella pneumonia***	9.5 ± 0.7	3.0 ± 0.5	4.5 ± 0.9	6.5 ± 0.7
5	***Pseudomonas Aeruginosa***	11.0 ± 0.5	3.0 ± 0.5	4.0 ± 0.7	6.0 ± 0.5

The mentioned values are mean and standard error (±SE) of triplicates

Conclusion

The aqueous extract of Liv-Pro-08 polyherbal formulation has an excellent ZnO reducing potential and synthesize ZnO NPs, and it was confirmed by UV-Vis spectrophotometer analysis, FT-IR, PXRD, and SEM analysis. Initially, yellow-colored crystalline powder was derived from the synthesis process. Furthermore, a single predominant peak was recorded at 375 nm, which confirmed the reduction of Zinc Oxide nitrate into ZnO NPs. The PXRD and FT-IR analyses confirmed that the synthesized nanoparticles possess significant active functional groups over their surface with crystalline structure with less than 5 µm sized particles. Furthermore, this was confirmed by SEM analysis results, and it showed that the synthesized ZnO NPs were in the range of 2 to 5 µm size with a spherical shape with smooth edges. The antibacterial activity of this ZnO NPs was studied against some common pathogenic bacteria such as *S. aureus B. cereus, E. coli, K. pneumonia,* and *P. aeruginosa* with various volumes (25, 50, and 75 µl) of 100 µg concentration of nanoparticles. Surprisingly, the results showed that most of these ZnO NPs showed significant antioxidant activity against all these pathogenic bacteria species. These results concluded that the ZnO NPs synthesized by aqueous extract of Liv-Pro-08 polyherbal formulation might be considered for drug development against some common pathogenic bacteria.

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