Green Synthesis of Zinc Oxide Nanoparticles (ZnO-NPs) by Pterolobium hexapetalum (Roth) Santapau & Wagh Aqueous Leaf Extract and Its Assessment of Biocompatible, Antibacterial, DPPH Radical Scavenging, Anticancer and Larvicidal Activities: An Effective Eco-Friendly Approach

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Abstract

Pterolobium hexapetalum aqueous leaf extract (*P. hexapetalum*-ALE) was utilized as a reducing agent to synthesize zinc oxide nanoparticles (ZnO-NPs) and it's forms of white precipitate. The synthesized *P. hexapetalum* - ZnO-NPs (*Ph*-ZnO-NPs) were characterized by UV-Vis, XRD, IR, EDAX, and HR-TEM with SAED pattern. UV-visible spectra results showed an absorbance peak at 280 and 370nm for *Ph*-ALE and synthesized *Ph*-ZnO-NPs. The HR-TEM result shows a spherical in shape with a size of 10-93 nm by *Ph*-ZnO-NPs. X-ray powder diffraction (P-XRD) spectra confirmed the crystalline nature of the synthesized ZnO-NPs. Hemolytic activity of the synthesized ZnO-NPs was carried out against Human RBCs and it's found to be 0.6% at 100 µg/mL. The synthesized *Ph*-ZnO-NPs appeared excellent antibacterial and antioxidant activity against *P. aeruginosa* (17.8mm) and DPPH radical scavenging activity (IC_{50}:118.14µg/mL). *Ph*-ZnO-NPs exposed superior anticancer activity against breast cancer cell line (MCF-7) with an IC_{50} value of 22.91 µg/mL. Additionally, the synthesized *Ph*-ZnO-NPs exhibited an excellent larvicidal activity with LC_{50} & LC_{90} values of 4.178 and 16.157 mg/mL on *Cx. quinquefasciatus* larvae at 24h inspection. Thus, plant-based synthesized *Ph*-ZnO-NPs exhibit significant biological activities, and hence it can be considered a potential source for the development of innovative drugs.

Introduction

The success of nanotechnology has altered the scope and output of research owing to its assorted various applications [1]. Nanoparticles have been synthesized by various techniques, like as, physical, chemical and biological. Biological synthesis (microbes, algae, fungi and plants) gains more importance because of their it's less toxic, low cost, safe and simple when compared to other synthesis methods [2]. Metal oxide nanoparticles (MONPs) have been widely utilized for therapeutic purposes in the past many years. A few of the MONPs such as Fe_3O_4, TiO_2, CuO and ZnO have been explored by different biological activities. Amid these NPs especially, ZnO-NPs also have been potential applications in the field of medicine like drug delivery and also antibacterial, antioxidant, anticancer and larvicidal applications. In recent times, several studies have documented the biosynthesis of ZnO-NPs such *Knoxia sumatrensis* [3], *Garcinia cambogia* [4], *Labeo rohita* [5], *Spondias pinnata* [6].

In everyday life, adherence and multiplication activity of bacterial strains cause extremely irresistible sicknesses among humans as well as animals. The important complication is the improvement of microbial resistance against commercial antibiotics in the medicinal zone [7]. Subsequently, to overcome the problem, there is a need for the development of a new drug. Medicinal plants with nanotechnology are fundamental sources to annihilate the microorganism. In earlier document, the synthesized ZnO-NPs using *Conyza canadensis*; Bioflavonoid rutin compound displayed significant antibacterial activity against human bacterial pathogens [8, 9].

Oxidative pressure was generated when there is an unreasonable free radical formation or low antioxidant resistance, which prompts a change in biomolecules causing morphology and functional changes [10]. Antioxidants are very important in the performance of entire bio-systems. Numerous

medicinal plants with nanotechnology have been explored to find the inventive application as antioxidants drugs [11].

Information on mosquito involvement in environment and control is of opportune significance to battle the spread of numerous mosquito-borne diseases [12]. Since mosquito-borne sicknesses now don't have effective vaccines or medicinal drugs. The utilizing of synthetic insect sprays such as, diflubenzuron, organophosphates, organochlorine and carbamates has been considered a valuable technique to manage insects. Utilization of these insecticides develops resistance in mosquitoes, and they are unsafe and destructive [13]. Resistance to mosquitoes, the insecticides used against mosquitoes are risky to other's lives on the earth, hence there is a necessity to find the eco-friendly, drugs. In the current study, we explored the synthesis of ZnO-NPs using *Pterolobium hexapetalum* aqueous leaf extract and characterize the NPs using various techniques. Furthermore, we examined the, in vitro studies such as hemolytic, antibacterial, antioxidant, anticancer and larvicidal activities of *Ph*-ZnO-NPs.

Materials And Methods

Collection and Extraction

Pterolobium hexapetalum leaves were collected in September-2021 from Paali hills, Thoppur, (latitude-11.941996 and longitude-78.054291), Dharmapuri district, Tamil Nadu, India. The *Ph* leaves were cleaned, washed with distilled water (D. H_2O) and then dried for fourteen days in room temperature. The dried leaves were powdered in a sterilized electrical blender. Plant powder (10g) was weighed and added in 100mL D. H_2O and then it was heated for 30mins at 70 ºC. The Whatman No.1 filter paper was used for extract filtration and stored at 4 ºC by further assessment.

Green Synthesis of ZnO-NPs

The method followed by Vijayakumar et al. [14] with little modification was used in this study. 80 mL of 2M zinc acetate dehydrate solution was added into 20mL of *Ph*-ALE and then stirred for 3 h at 80 ºC. After 1 hour the NaOH solution (2 M) was added drop by drop to adjust the pH level at 12. The change in color and formation of white precipitates, indicate the formation by ZnO-NPs. The white precipitate was obtained and dried. After, the dried materials were calcinated for muffle furnace (350 °C at 3 h).

Characterization Study

The formation of *Ph*-ZnO-NPs was confirmed by Ultraviolet visible spectroscopy (LI-UV-7000 wavelength at 200-800nm). Crystalline nature was analyzed by X-Ray Diffraction (XRD-Rigaku, Japan) and 2 theta ranges in 20º-80º. Functional groups present in the *Ph*-ZnO-NPs were identified using Fourier transform infrared spectroscopy (FT-IR-Perkin Elmer-L1600300, Liantrisant, USA). Further the NPs size and morphology was analyzed through high-resolution-transmission electron microscopy (HR-TEM-FEI Tecnai, F30).

Hemolytic Assay

Methodology of Rajapriya et al. [15] with slight modification has been used for this study. Blood sample (1st author) was obtained and citrated to avoid coagulation. 3mL of blood was centrifuged at 1600 rotate per minutes (RPM) for seven minutes. The plasma was discarded and the pellets containing Red Blood Cells (RBCs) were saved. Further, the RBCs were cleaned with sterile phosphate-buffered saline for 5 times (PBS-6mL, pH-7.4). The RBCs were diluted with PBS solution. The different dosages of *Ph*-ZnO-NPs (50 and 100µg/mL) were mixed with RBCs by vortex. All the test samples were kept in an incubator at 25 °C for 1h. Triton X 100 (2%) was used as a positive and negative control for PBS. The test samples were then centrifuged at 3000 rpm for 5mins and 0.1 µL of supernatant was obtained. The absorbance was recorded using a UV-Vis Spectrophotometer at 545nm. The hemolysis percentage was calculated as shown below.

$$\text{Hemolysis (\%)} = \frac{\text{Sample OD - Negative control}}{\text{Positive control - Negative control}} \text{ X } 100$$

Antibacterial Study

The synthesized *Ph*-ZnO-NPs were demonstrated of *Bacillus cereus, Enterococcus faecalis, Pseudomonas aeruginosa* and *Escherichia coli* using disk diffusion method of Loo et al. [16]. The bacterial cultures were swabbed on Petri-plates and then dried for 10 min. The synthesized *Ph*-ZnO-NPs dosages of 25 and 50µg were used in this study along with Chloramphenicol used as a positive control (30µg/disc). The tested plates were incubated for 24h at 37 ºC and the bacterial growth was measured in millimeters (mm).

Antioxidant Study

DPPH assay of the synthesized *Ph*-ZnO-NPs were determined as described by Shimada et al. [17] In brief, five hundred (500 µL) of DPPH solution (0.1M) dissolved in methanol was added to different concentrations of synthesized *Ph*-ZnO-NPs (20-100 µg/mL) and transferred to an incubator (30mins). Ascorbic acid was used as a positive control. After 30 min the mixed solution absorbance was measured at 517nm. The inhibition percentage was calculated as below,

$$\text{DPPH \% of Inhibition} = A_0\text{-}A_1/A_0 \text{X } 100$$

A_0-Control (ascorbic acid), A_1-ZnO-NPs.

Anticancer Activity

Cell culture & Viability

Breast cancer cell-line (MCF-7) was collected from NCCS-Pune, India. The MCF-7 cell line was grown in Dulbecco with DMEM medium (Sigma Aldrich, USA). Cell viability assay was reported by Mosmann, [18]. Ninety-six well plates (3×10^3cells/wells) were used for the seeding of MCF-7 cells and it is incubated at 37 °C, for 24h with different concentrations of ZnO-NPs (6.5 to 100 µg/mL) to treat the cancer cells during the incubation period. Cell viability was calculated (MTT 10 µL for 4 h at 37° C) after 24h exposure. Dimethyl Sulfoxide (DMSO) was used for dissolving the treated cells. The ELISA instrument (2.0-Epoch-USA) was used to measure (OD-540nm-reference: 630nm) crystal formation. The treated cell morphology images were captured and its calculation is given below.

$$\text{Cell viability \%} = \text{OD of ZnO-NPs/ OD of Control (Untreated) X 100}$$

Larvicidal Activity

Ae. aegypti, *An. stephensi* and *Cx. quinquefasciatus* 4^{th} instar larvae were collected from the Centre for Medical Entomology (CRME), Madurai, Tamil Nadu and then preserve it for study by providing feed. The assay was trailed by World Health Organization WHO, [19] a standard protocol with few changes according to the technique for Loganathan et al. [3]. Larvae (4^{th} instar) of twenty numbers were added from all paper cups (200mL). The concentration of *Ph*-ALE and synthesized *Ph*-ZnO-NPs (5-25 mg/L) was included. The die larva had been counted after 24h post treatment then mortality % was obtained from average of n=3. Abbott, [20] was used in correction of larval mortality.

Statistical Analysis

The data was analyzed by mean ± SD. Larval mortality was calculated probit analysis for finding out LC_{50}, LC_{90} and chi-square values using SPSS software.

Results

Visual Observation and UV-Vis spectroscopy analysis

Ph-ALE in synthesized ZnO-NPs was affirmed by the color changes (yellow to white precipitate). Fig. 1a. showed that the UV-Vis absorbance peak at 280 by *Ph*-ALE. The synthesized *Ph*-ZnO-NPs illustrated a UV-Vis absorption peak at 370nm due to Surface Plasmon Resonance (SPR), which confirms the synthesis of ZnO-NPs (Fig. 1).

FT-IR

FT-IR spectra of *Ph*-ALE and synthesized *Ph*-ZnO-NPs appeared the presence of seven (07) functional groups (Fig. 2 a&b). The characteristic peak at 3447.02 cm^{-1} is related to O-H group (Alcohols, phenols)

followed by 1429.47 cm^{-1} can be attributed to the OH (Carboxylic acids), 865.14 cm^{-1} represents CH (1,2,4-trisubst benzenes), 557.73 cm^{-1} can be assigned to the C-C=O (Aldehydes), 498.26 cm^{-1} linked to the NO_2 group (Nitro compounds), 457.99cm^{-1} for C-O-Cgroup (Ethers) and the strong characteristic peak at 434 cm^{-1} (Table. 1) indicate a formation ZnO-NPs.

XRD study

XRD analysis (Fig. 3). displayed that the 11 diffraction values were presented at 2 theta ranges with indexed of 31.83º(100), 34.50º(002), 36.32º(101), 47.53º(102), 56.72º(110), 62.94º(103), 66.57º(200), 68.06º(112), 69.19º(201), 72.84º(004), and 77.05º(202) by the synthesized *Ph*-ZnO-NPs. These results noticed the configuration of ZnO-NPs and its hexagonal phase (JCPDS-NO:89-0511) and also the synthesized NP's average crystalline size of 60nm (Scherrer's formula).

HR-TEM with SAED Pattern and EDAX

The synthesized *Ph*-ZnO-NPs shape and size were checked by HR-TEM and it disclosed the spherical and few particles were a cluster in shape with an average size of 10-93 nm Fig. 4a. The EDAX spectra of *Ph*-ZnO-NPs proved the strong Zn signal value as well as another element of Oxygen was present as shown in Fig. 4b.

Hemolytic activity

Biocompatible study of the synthesized *Ph*-ZnO-NPs was done to determine the hemolytic activity against human RBCs. In this study, *Ph*-ZnO-NPs appeared less than 5% of hemolytic activity. Fig.5, exhibited the treated images of the synthesized ZnO-NPs against Human RBCs. This study, indicates that the concentration of the synthesized ZnO-NPs (50 and 100µg/mL) was increased while gradually increasing the lysis percentage. The highest lysis percentage was observed from 0.6% at a higher concentration of 100µg/mL followed by 0.4% at 50 µg/mL. From these outcomes, we propose that the synthesized *Ph*-ZnO-NPs can be utilized in the domain of biomedical applications.

Antibacterial activity

The synthesized *Ph*-ZnO-NPs proved the strong antibacterial activity using human pathogens. A total of two dosages were used in this study, such as 25 and 50 ug/mL (Fig. 6). The highest zone of inhibition was seen in *P. aeruginosa* (17.8mm), followed by *E. coli* (15.0mm) and *E. faecalis* (14.5mm) at a higher concentration of 50 µg/mL (Table.2). A certain level zone of inhibition was noted by plant extract to chosen pathogens.

Antioxidant potential

Ph-ZnO-NPs were tested against the DPPH assay and it expressed superior antioxidant activity. Ascorbic acid (standard) and synthesized ZnO-NPs showed the maximum inhibition percentages of 57.10%;

53.45% was noticed from 125µg/mL dosages as shown in Fig.7 with IC_{50} values of 102.98 µg/mL; 118.14 µg/mL respectively.

Anticancer activity

For IC_{50} value was obtained by the synthesized *Ph*-ZnO-NPs and it was found to be 22.91µg/mL against the MCF-7 cell line. This study confirmed excellent anticancer activity against MCF-7 cell line. The cell viability of MCF-7 cells was decreased with the increasing concentration of *Ph*-ZnO-NP & the treated images (Fig. 8a,b).

Larvicidal activity

Larval bioassay was performed for *Ph*-ALE and *Ph*-ZnO-NPs against *Ae. ageypti*, *An. stephensi* and *Cx .quinquefasciauts* (4^{th} instar) larvaes at 24h surveillance. In this study, the synthesized ZnO-NPs proved tremendous larvicidal activity. The maximum mortality percentage (100%) was observed in *Cx. quinquefasciatus* treated with synthesized *Ph*-ZnO-NPs at a higher dosage (25 mg/mL). Though, the various concentrations of *Ph*-ALE revealed a low mortality percentage obtained by *Cx. quinquefasciatus* at a higher concentration of 25mg/mL *Ph*-ALE. Results noticed that the mortality percentage was higher when increasing the synthesized ZnO-NPs dosages. For the LC_{50} and LC_{90} values were recorded in *Cx. quinquefasciatus* (7.640 and 53.165 mg/mL) by *Ph*-ALE at 24h exposure. Similarly, synthesized *Ph*-ZnO-NPs have shown LC_{50} and LC_{90} values of 4.178 and 16.157 against *Cx. quinquefasciauts* (Table.3).

Discussion

Ph-ALE of the synthesized ZnO-NPs was affirmed by the color changes (yellow to white precipitate). In concurrence with our results, *Azadirachta indica* and *Costus igneus* [21, 22].The green synthesis of the Ph-ZnO-NPs was confirmed by UV-Vis spectroscopy & its absorption peak at 370nm due to Surface Plasmon Resonance (SPR). Similarly, the synthesized ZnO-NPs using aqueous leaf extract by *Cynara scolymus* [15]. The functional groups like as, alcohols, phenols, carboxylic acids, 1,2,4-trisubst benzenes, aldehydes, nitro compounds, ethers and the strong characteristic peak at 434 cm^{-1} indicate a formation ZnO-NPs [23]. Similarly, the ZnO-NPs synthesized by *Momordica charantia* & *Solanum nigrum* leaf extract displayed the presence of six & five functional groups [24, 25]. XRD analysis demonstrate that the presence of 11 diffraction value at 2 theta ranges with crystalline size of 60nm (Scherrer's formula), which was comparable earlier documented on ZnO-NPs by *Cassia fistula* and *Spondias pinnata* [26, 6] The result from HR-TEM analysis of the synthesized *Ph*-ZnO-NPs displayed same results from synthesized ZnO-NPs by *D. tortuosa* leaf extract [27]. The EDAX elemental analysis of the synthesized *Ph*-ZnO-NPs was revealed that the strong Zn signal value were present which is very similar to Abdelmigid et al. [28].

A biocompatible study of the synthesized *Ph*-ZnO-NPs displayed less than 5% of hemolytic activity was recorded. According to 5% of hemolysis is recommended by biomaterials [15]. The related work was observed from ZnO-NPs by *Costus igneus* [22].

The common mechanisms of action for antibacterial activity proposed to date are direct damage of ZnO-NPs to the cell membranes finally cells died [29]. An earlier report, registered varying degrees of antibacterial activity according to the type of pathogens, synthesis method, and dose depending on ZnO-NPs [28]. In the present study, *Ph*-ZnO-NPs authonticated strong antibacterial activity and also the best zone of inhibition was perceived by *P. aeruginosa* (17.8mm). Similarly, the synthesized ZnO-NPs of *Beta vulgaris* [30]. In another report, *Cassia alata* leaf extract in synthesized ZnO-NPs indicated dose-dependent activity [31]. Moreover, the synthesized ZnO-NPs of bioflavonoid rutin displayed moderate antibacterial activity [9]. The present data acted as potential activity against the experimental bacterial pathogens and can be utilized as another source of drugs.

Antioxidant is a substance. It can be protect the oxidation of additional compounds or neutralize free radicals and also it based medicine formulations are utilized by the prevention as well as treatment of a lot of complex diseases [32]. The present study the antioxidant assay displayed various levels of antioxidant activity of *Ph*-ZnO-NPs. Similarly, the synthesized ZnO-NPs using *Knoxia sumatrensis*; *Carica papaya* leaf extract appeared excellent DPPH activity with IC_{50} values of 95.80µg/mL & 104.9 µg/mL [3, 33]. The high antioxidant performance of *Ph*-ZnO-NPs makes them very useful in therapeutic application.

Breast cancer cell line (MCF-7) was test at different concentrations (6.5 to 100µg/mL) of *Ph*-ZnO-NPs utilized MTT assay at 24h observation. This result depicts the dose-dependent anticancer activity. Similarly, the synthesized ZnO-NPs by *Pongamia pinnata* leaf extract [34]. In Fact, the action mode of ZnO-NPs against cancer cell lines may induce ROS species and then finally cells are damaged. In previously, more exploration revealed that the cytotoxicity impact of ZnO-NPs utilizing the biosynthesis approach against the MCF-7 cell line has significant anticancer activity [4, 35]. This study indicates that the synthesized *Ph*-ZnO-NPs were performed effectively, and they could be used as an anticancer drug in near future.

Nano-pesticide is an agrochemical amalgamation used to overcome the human health problem caused by regular pesticides [36]. Presently, we have investigated the larvicidal effect of *Ph*-ALE and *Ph*-ZnO-NPs against *Ae. ageypti*, *An. stephensi* and *Cx .quinquefasciauts* (4th instar) larvaes at 24h examination. Commonly, the fourth instar larvae are recommended to be containing more immune competency than the younger larvae [37] Several types of materials such as, surfactants, organic polymers and minerals which fall in the nanometer size have been utilized for nano-pesticides formulation [38]. In this study, the synthesized *Ph*-ZnO-NPs proved significant larvicidal activity. Results noticed that the larvae toxicity was proposed a concentration-dependent manner for tested species. Similar concentration-dependent larvicidal efficacy was noticed from synthesized ZnO-NPs using *Cuscuta reflexa* [39]. In recent times, the larvicidal activity of synthesized ZnO-NPs using *Elettaria cardamomum* and *Pleurotus djamor* indicated superior larvicidal activity against *Cx. quinquefasciauts* larvae [13, 40]. In this investigation, the

synthesized ZnO-NPs show potential larvicidal action and the results can be useful to synthesize nano-based mosquito drugs, due to being less hazardous to the environment.

Conclusion

In this study, ZnO-NPs were effectively synthesized using *P. hexapetalum* aqueous leaf extract as a biological material. The characteristic for UV-Vis absorption peak at 370nm proved the configuration of ZnO-NPs. XRD pattern indicates the presence of 11 diffraction values. The HR-TEM result shows spherical nanoparticles with few particle cluster shapes. FT-IR study revealed the functional groups presence in the synthesized ZnO-NPs due to enormous secondary metabolites related to NPs synthesis. Further, antibacterial & antioxidant activity of *Ph*-ZnO-NPs displayed predominant activity against *P. aeruginosa* as well as DPPH assay. In addition, the anticancer activity of *Ph*-ZnO-NPs expressed significant activity against MCF-7 cell-line. Besides, the synthesized *Ph*-ZnO-NPs revealed strong larvicidal activity against *Cx. quinquefasciatus* larvae. Thus, this plant medicated synthesis of ZnO-NPs can fill in as a compelling pharmacological tool in biomedical area for different applications like beauty care products, drug transporters, and coatings in clinical gadgets.

Declarations

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Availability of Data and Materials All the data presented in the manuscript are the original work of the authors.

Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Author Contributions SL and DGP conceived and designed the research. SL Lab related work & KMM hemolytic work. SL and DGP data analysis. The manuscript corrected for SL, DGP, KM & RS. all authors read and approved the final manuscript.

Compliance with Ethical Standards

Conflict of interests: The authors have no conflict of interests to declare.

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References

1. Şuhani MF, Băciuţ G, Băciuţ M, Şuhani R, Bran S (2018) Current perspectives regarding the application and incorporation of silver nanoparticles into dental biomaterials. Clujul Medi 91: 274. https://doi.org/10.15386/cjmed-935
2. Singh J, Dutta T, Kim KH, Rawat M, Samddar P, Kumar P, (2018) Green synthesis of metals and their oxide nanoparticles: applications for environmental remediation. J Nanobiotech 16: 1–24. https://doi.org/10.1186/s12951-018-0408-4
3. Loganathan S, Shivakumar MS, Karthi S, Nathan SS, Selvam K (2021) Metal oxide nanoparticle synthesis (ZnO-NPs) of *Knoxia sumatrensis* (Retz.) DC. Aqueous leaf extract and It's evaluation of their antioxidant, anti-proliferative and larvicidal activities. Toxicol Rep 8: 64–72. https://doi.org/10.1016/j.toxrep.2020.12.018
4. Sivakamavalli J, Pandiselvi K, Park K, Kwak IS, (2021) *Garcinia cambogia* assisted synthesis of zno nanoparticles coupled with chitosan for antibacterial, antibiofilm, cytotoxic, anticancer and ecotoxicity assessment. J Clust Sci 1–16. https://doi.org/10.1007/s10876-021-02032-5
5. Thangapandiyan S, Monika S, (2020) Green synthesized zinc oxide nanoparticles as feed additives to improve growth, biochemical, and hematological parameters in freshwater fish *Labeo rohita*. Biol Trace Elem Res 195: 636–647. https://doi.org/10.1007/s12011-019-01873-6
6. Ahlam AA, Shaniba VS, Jayasree PR, Manish Kumar PR (2021) *Spondias pinnata* (lf) Kurz leaf extract derived zinc oxide nanoparticles induce dual modes of apoptotic-necrotic death in HCT 116 and K562 cells. Biol Trace Elem Res 199:1778–1801. https://doi.org/10.1007/s12011-020-02303-8
7. Ventola CL (2015) The antibiotic resistance crisis: part 1: causes and threats. Pharmacol Ther 40: 277.
8. Ali J, Irshad R, Li B, Tahir K, Ahmad A, Shakeel M Khan ZUH (2018) Synthesis and characterization of phytochemical fabricated zinc oxide nanoparticles with enhanced antibacterial and catalytic applications. J. Photochem. Photobiol. B, Biol. 183: 349–356. https://doi.org/10.1016/j.jphotobiol.2018.05.006.
9. Bharathi D, Bhuvaneshwari V (2019) Synthesis of zinc oxide nanoparticles (ZnO-NPs) using pure bioflavonoid rutin and their biomedical applications: antibacterial, antioxidant and cytotoxic activities. Res. Chem. Intermed 45: 2065–2078. https://doi.org/10.1007/s11164-018-03717-9
10. Lourenço SC, Moldão-Martins M, Alves VD (2019) Antioxidants of natural plant origins: from sources to food industry applications. Molecules 24: 4132. https://doi.org/ 10.3390/molecules24224132
11. Xu DP, Li Y, Meng X, Zhou T, Zhou Y, Zheng J, Li HB (2017) Natural antioxidants in foods and medicinal plants: Extraction, assessment and resources. Int J Mol Sci 18: 96. https://doi.org/10.3390/ijms18010096
12. Benelli G, Beier JC (2017) Current vector control challenges in the fight against malaria. Acta Tropica 174: 91–96. https://doi.org/10.1590/0037-8682-0542-2018
13. Vinotha V, Yazhiniprabha M, Raj DS, Mahboob S, Al-Ghanim KA, Al-Misned F, Vaseeharan B (2020) Biogenic synthesis of aromatic cardamom-wrapped zinc oxide nanoparticles and their potential

antibacterial and mosquito larvicidal activity: An effective eco-friendly approach. J Environ Chem Eng 8: 104466. https://doi.org/10.1016/j.jece.2020.104466

14. Vijayakumar S, Vaseeharan B, Sudhakaran R, Jeyakandan J, Ramasamy P, Sonawane A, Faggio C (2019) Bioinspired zinc oxide nanoparticles using *Lycopersicon esculentum* for antimicrobial and anticancer applications. J Clust Sci 30: 1465–1479. https://doi.org/10.1007/s10876-019-01590-z
15. Rajapriya M, Sharmili SA, Baskar R, Balaji R, Alharbi NS, Kadaikunnan S, Vaseeharan B (2020) Synthesis and characterization of zinc oxide nanoparticles using *Cynara scolymus* leaves: Enhanced hemolytic, antimicrobial, antiproliferative, and photocatalytic activity. J Clust Sci 31: 791–801. https://doi.org/10.1007/s10876-019-01686-6
16. Loo YY, Rukayadi Y, Nor-Khaizura MAR, Kuan CH, Chieng BW, Nishibuchi M, Radu S (2018) In vitro antimicrobial activity of green synthesized silver nanoparticles against selected gram-negative foodborne pathogens. Front Microbiol 9: 1555. https://doi.org/10.3389/fmicb.2018.01555
17. Shimada K, Fujikawa K, Yahara K, Nakamura T (1992) Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. J Agric Food Chem 40: 945–948. https://doi.org/10.1021/jf00018a005
18. Mosmann T (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J immunol Method 65: 55–63. https://doi.org/10.1016/0022-1759(83)90303-4.
19. World Health Organization (WHO) (2005). Gneva. .
20. Abbott WS (1925) A method of computing the effectiveness of an insecticide. J Econ Entomol 18: 265–267. https://doi.org/10.1093/jee/18.2.265a
21. Vijayakumar S, Divya M, Vaseeharan B, Ranjan S, Kalaiselvi V, Dasgupta N, Durán-Lara EF (2021) Biogenic preparation and characterization of ZnO nanoparticles from natural Polysaccharide *Azadirachta indica*. L. (neem gum) and its clinical implications. J Clust Sci 32: 983–993. https://doi.org/10.1007/s10876-020-01863-y
22. Vinotha V, Iswarya A, Thaya R, Govindarajan M, Alharbi NS, Kadaikunnan S, Vaseeharan B (2019) Synthesis of ZnO nanoparticles using insulin-rich leaf extract: Anti-diabetic, antibiofilm and anti-oxidant properties. J Photochem Photobiol B Biol 197: 111541. https://doi.org/10.1016/j.jphotobiol.2019.111541
23. Hussain A, Oves M, Alajmi MF, Hussain I, Amir S, Ahmed J, Ali I (2019) Biogenesis of ZnO nanoparticles using *Pandanus odorifer* leaf extract: anticancer and antimicrobial activities. RSC advances 9: 15357–15369. https://doi.org/10.1039/C9RA01659G
24. Gandhi PR, Jayaseelan C, Mary RR, Mathivanan D, Suseem SR (2017) Acaricidal, pediculicidal and larvicidal activity of synthesized ZnO nanoparticles using *Momordica charantia* leaf extract against blood feeding parasites. Exp Parasitol 181: 47–56. https://doi.org/10.1016/j.exppara.2017.07.007
25. Thomas S, Gunasangkaran G, Arumugam VA, Muthukrishnan S (2022) Synthesis and characterization of zinc oxide nanoparticles of *Solanum nigrum* and its anticancer activity via the

induction of apoptosis in cervical cancer. Biol Trace Elem Res 200: 2684–2697. DOI: 10.1007/s12011-021-02898-6

26. Pai S, Sridevi H, Varadavenkatesan T, Vinayagam R, Selvaraj R (2019) Photocatalytic zinc oxide nanoparticles synthesis using *Peltophorum pterocarpum* leaf extract and their characterization. Optik 185: 248–255. https://doi.org/10.1016/j.ijleo.2019.03.101
27. Selim YA, Azb MA, Ragab IHM, Abd El-Azim M (2020) Green synthesis of zinc oxide nanoparticles using aqueous extract of *Deverra tortuosa* and their cytotoxic activities. Sci Repor 10: 1–9. https://doi.org/10.1038/s41598-020-60541-1
28. Abdelmigid HM, Hussien NA, Alyamani AA, Morsi MM, AlSufyani NM, Kadi HA (2022) Green synthesis of zinc oxide nanoparticles using pomegranate fruit peel and solid coffee grounds vs. chemical method of synthesis, with their biocompatibility and antibacterial properties investigation. Molecules 27: 1236. https://doi.org/10.3390/molecules27041236
29. Ray PG, Biswas S, Roy T, Ghosh S, Majumder D, Basak P, Dhara S (2019) Sonication assisted hierarchical decoration of Ag-NP on zinc oxide nanoflower impregnated eggshell membrane: evaluation of antibacterial activity and in vitro cyto compatibility. ACS Sustain Chem Eng 7: 13717–13733. https://doi.org/10.1021/acssuschemeng.9b01185
30. Pillai AM, Sivasankarapillai VS, Rahdar A, Joseph J, Sadeghfar F, Rajesh K, Kyzas GZ (2020) Green synthesis and characterization of zinc oxide nanoparticles with antibacterial and antifungal activity. J Mol Struct 1211: 128107. https://doi.org/10.1016/j.molstruc.2020.128107
31. Happy A, Soumya M, Kumar SV, Rajeshkumar S, Sheba RD, Lakshmi T, Nallaswamy, VD (2019) Phyto-assisted synthesis of zinc oxide nanoparticles using *Cassia alata* and its antibacterial activity against *Escherichia coli*. Biochem Biophys Rep 17: 208–211. https://doi.org/10.1016/j.bbrep.2019.01.002
32. Chaves N, Santiago A, Alías JC (2020) Quantification of the antioxidant activity of plant extracts: Analysis of sensitivity and hierarchization based on the method used. Antioxidants 9: 76. https://doi.org/10.3390/antiox9010076
33. Dulta K, Koşarsoy Ağçeli G, Chauhan P, Jasrotia R, Chauhan PK (2022) Ecofriendly synthesis of zinc oxide nanoparticles by *Carica papaya* leaf extract and their applications. J Clust Sci, 33: 603–617. https://doi.org/10.1007/s10876-020-01962-w
34. Malaikozhundan B, Vaseeharan B, Vijayakumar S, Pandiselvi K, Kalanjiam MAR, Murugan K, Benelli G (2017) Biological therapeutics of *Pongamia pinnata* coated zinc oxide nanoparticles against clinically important pathogenic bacteria, fungi and MCF-7 breast cancer cells. Microb Pathog 104: 268–27. https://doi.org/10.1016/j.micpath.2017.01.029
35. Boroumand Moghaddam A, Moniri M, Azizi S, Abdul Rahim R, Bin Ariff A, Navaderi M, Mohamad R (2017) Eco-friendly formulated zinc oxide nanoparticles: induction of cell cycle arrest and apoptosis in the MCF-7 cancer cell line. Genes 8: 281. https://doi.org/10.3390/genes8100281
36. Jasrotia P, Nagpal M, Mishra CN, Sharma AK, Kumar S, Kamble U, Singh GP (2022) Nanomaterials for postharvest management of insect pests: current state and future perspectives. Front Nanotech

3: 811056. https://doi.org/10.3389/fnano.2021.811056

37. Deepak P, Sowmiya R, Ramkumar R, Balasubramani G, Aiswarya D, Perumal P (2017) Structural characterization and evaluation of mosquito-larvicidal property of silver nanoparticles synthesized from the seaweed, *Turbinaria ornata* (Turner) J. Agardh 1848. Artif Cells Nanomed Biotechnol 45: 990–998. https://doi.org/10.1080/21691401.2016.1198365
38. Tripathi M, Kumar S, Kumar A, Tripathi P, Kumar S (2018) Agro-nanotechnology: a future technology for sustainable agriculture. Int J Curr Microbial Appl Sci 7: 196–200.
39. Soni N, Dhiman RC (2020) Larvicidal activity of Zinc oxide and titanium dioxide nanoparticles synthesis using *Cuscuta reflexa* extract against malaria vector (*Anopheles stephensi*). Egypt J Basic Appl Sci 7: 342–352. https://doi.org/10.1080/2314808X.2020.1830236
40. Manimaran K, Balasubramani G, Ragavendran C, Natarajan D, Murugesan S (2021) Biological applications of synthesized ZnO nanoparticles using *Pleurotus djamor* against mosquito larvicidal, histopathology, antibacterial, antioxidant and anticancer effect. J Clust Sci 32: 1635–1647. https://doi.org/10.1007/s10876-020-01927-z

Tables

Table 1 FT-IR analysis of the synthesized *Ph*-ZnO-NPs.

Wave number (CM^{-1})/Vibrations	**Intensity**	**Group Compound**	**Functional Group**
3447.02/ Stretch	Medium	O-H	Alcohols, Phenols
1429.47/bending	Medium	OH	Carboxylic acids
865.14/ Stretch	Very Strong	CH	1,2,4-trisubst benzenes
557.73/ Stretch	Strong	C-C=O	Aldehydes
498.26/ Stretch	Medium/ Strong	NO_2	Nitro compounds
457.99/ bend	Medium/ Strong	C-O-C	Ethers
434.02/stretch	Strong	Cl-C=O	Acid chlorides

Table 2 Antibacterial activity of the synthesized *Ph*-ZnO-NPs.

Microorganisms	Zone of inhibition (mm)				
	Positive Control (Chloramphenicol)	Plant Extract (25 (µg/mL)	Zinc nitrate	ZnO-NPs	
				25 (µg/mL)	50 (µg/mL)
B. cereus	19.4 ± 0.8	7.0 ± 0.5	7.5 ± 0.5	10.8 ± 0.5	11.0 ± 0.9
E. faecalis	15.3 ± 0.8	6.0 ± 0.1	00 ± 00	12.3 ± 0.3	14.5 ± 0.7
P. aeruginosa	16.8 ± 0.6	8.0 ± 0.3	00 ± 00	13.9 ± 1.6	17.8 ± 0.6
E. coli	19.8 ± 1.1	7.5 ± 0.57	00 ± 00	12.0 ± 0.5	15.0 ± 0.4

Value are given as Data (Mean ± SD) (n=3) represent the zone of bacterial growth inhibition (mm).

Table 3 Larvicidal activity of the *Ph*-ALE and the synthesized *Ph*-ZnO-NPs against 4th in-star larvaes of *Aedes aegypti, Anopheles stephensi* and *Culex quinquefasciatus* at 24h exposure.

Types of Larvae	Samples	LC_{50} (mg/mL) (LCL-UCL)	LC_{90} (mg/mL) (LCL-UCL)	χ^2	*Df*
Aedes Aegypti	Plant extract	14.274 (11.157-18.847)	113.814 (58.256-570.691)	1.59	13
	ZnO-NPs	8.982 (6.840-10.868)	43.563 (30.782-83.337)	1.939	13
Anopheles stephensi	Plant extract	13.688 (10.433-18.299)	125.801(60.651-815.782)	2.523	13
	ZnO-NPs	6.247 (3.919-8.084)	36.338(25.622-73.086)	4.234	13
Culex quinquefasciaus	Plant extract	7.640 (4.978-9.762)	53.165 (33.752-143.884)	3.289	13
	ZnO-NPs	4.178 (2.511-5.542)	16.157 (13.160-22.128)	6.499	13

LC_{50}: Lethal concentration kills 50% of the exposed larvae, LC_{90}: Lethal concentration kills 90% of the exposed larvae, LCL: Lower confidence limit, UCL: Upper confidence limit, χ^2 Chi-square value, *df*, degrees of freedom.

Figures

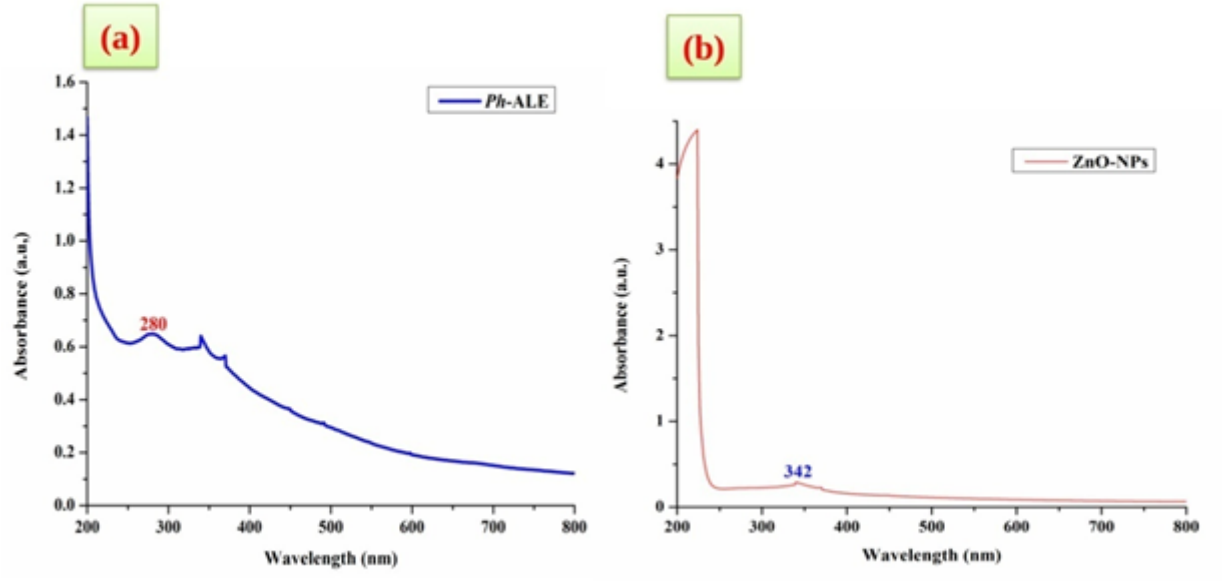

Figure 1

UV-visible absorbance spectra of **a** *P. hexapetalum*-aqueous leaf extract (*Ph*-ALE), and **b** Synthesized *P. hexapetalum*-ZnO-NPs (*Ph*-ZnO-NPs).

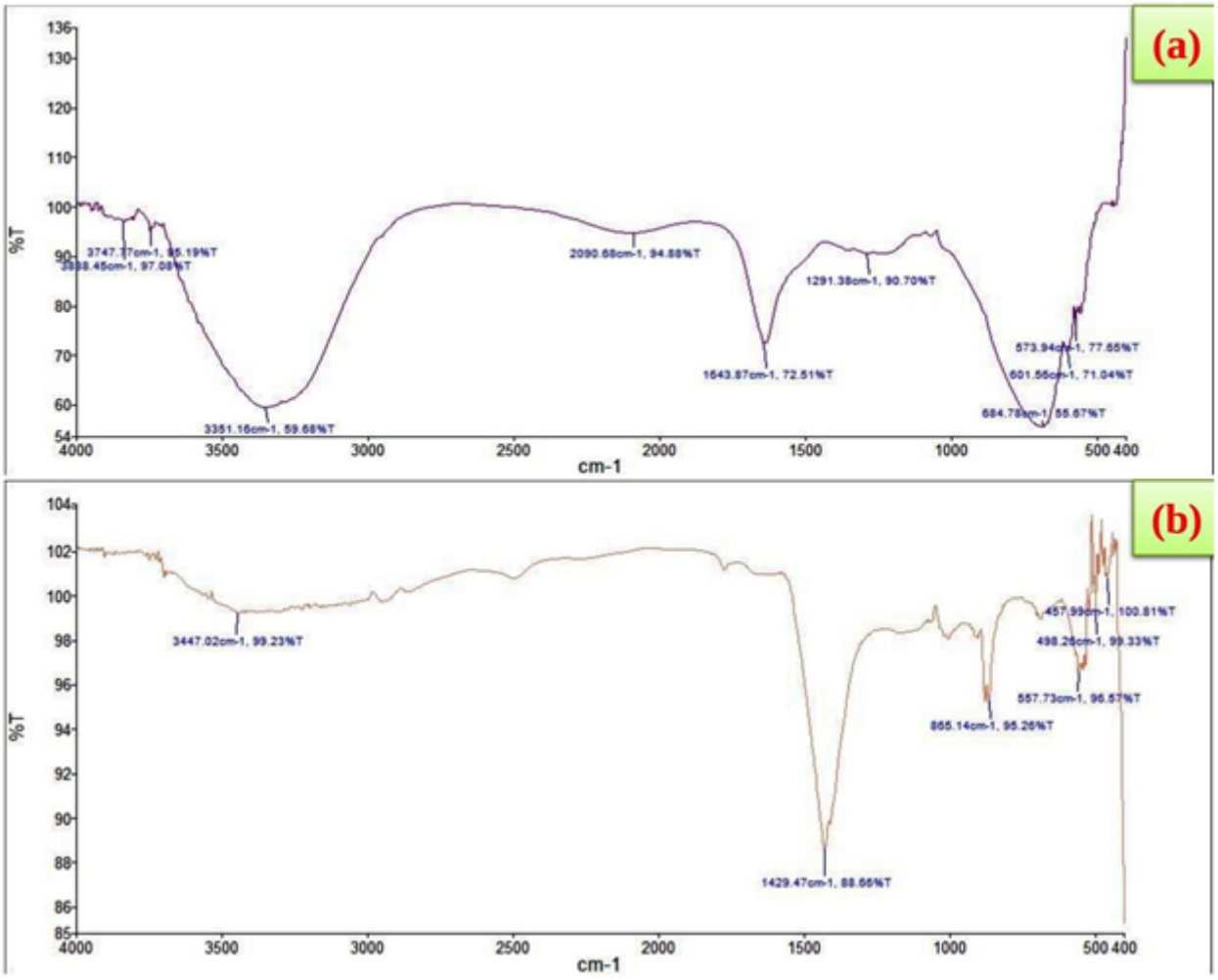

Figure 2

a FT-IR analysis of *Ph*-ALE **b** Synthesized *Ph*-ZnO-NPs.

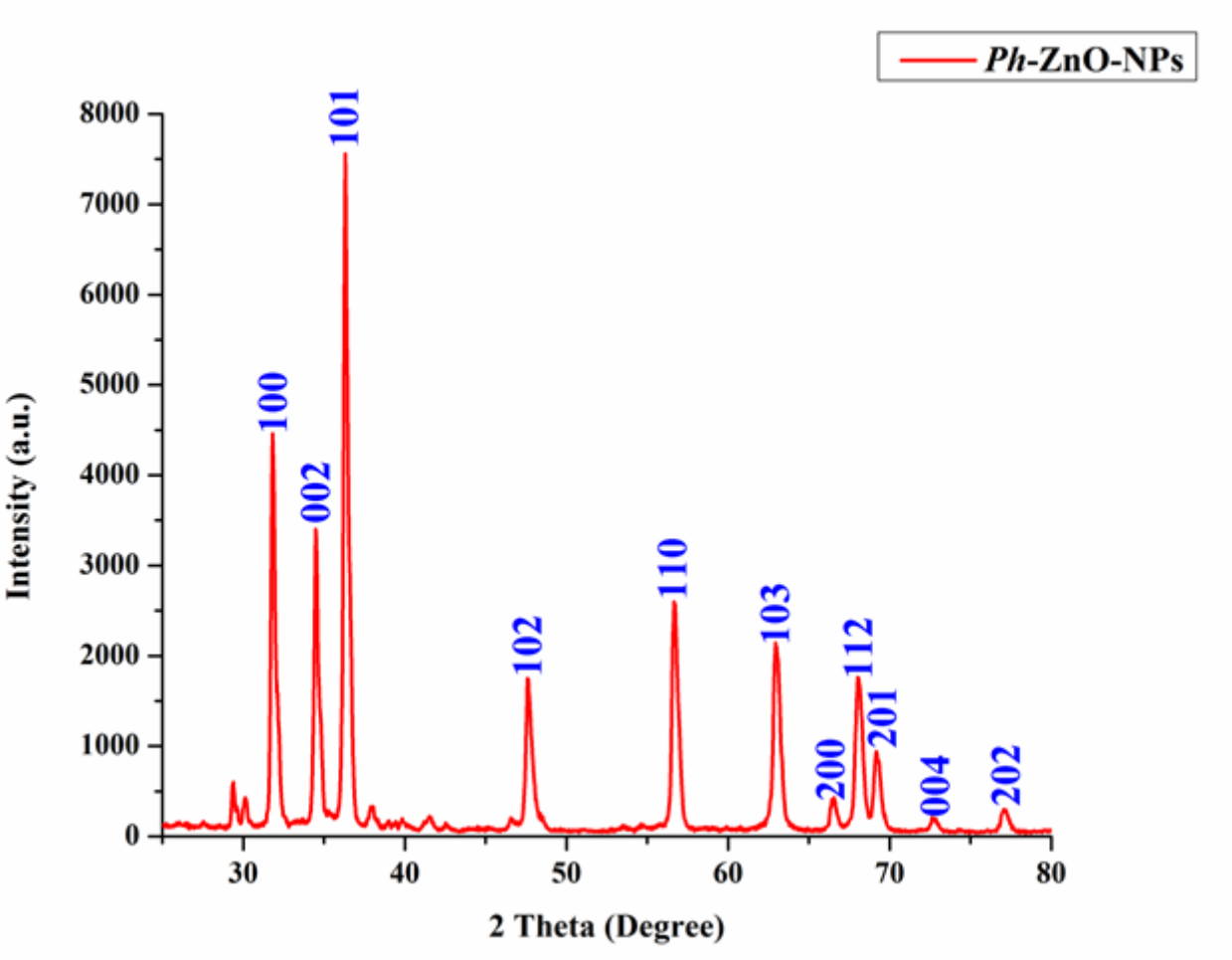

Figure 3

XRD patterns of the synthesized *Ph*-ZnO-NPs.

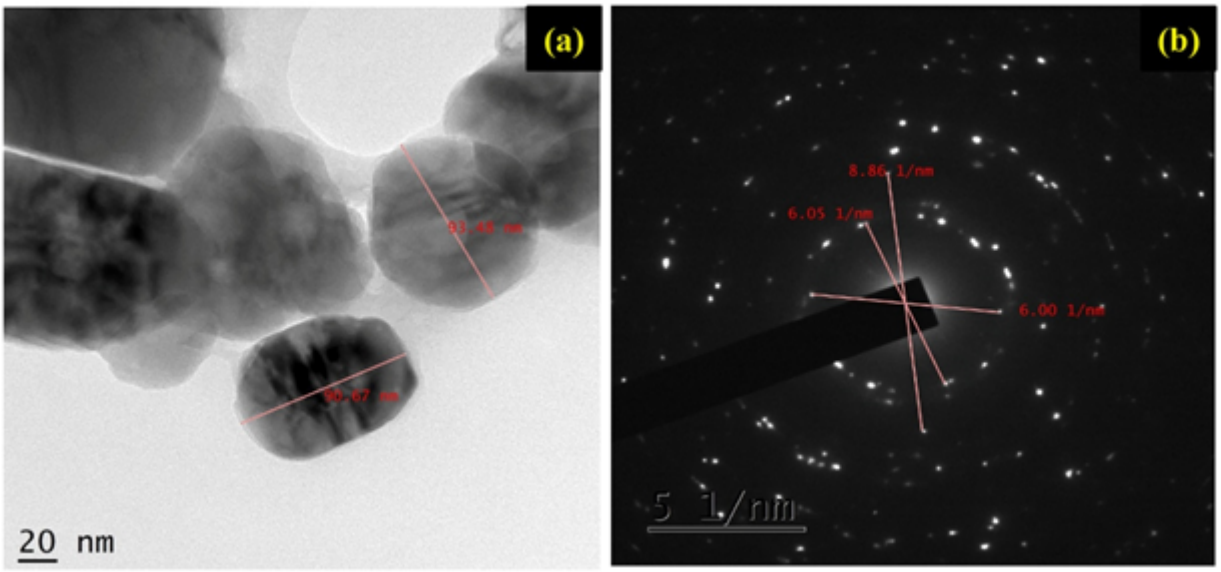

Figure 4

a HR-TEM morphological analysis of the synthesized *Ph*-ZnO-NPs. **b** SAED pattern by the synthesized *Ph*-ZnO-NPs.

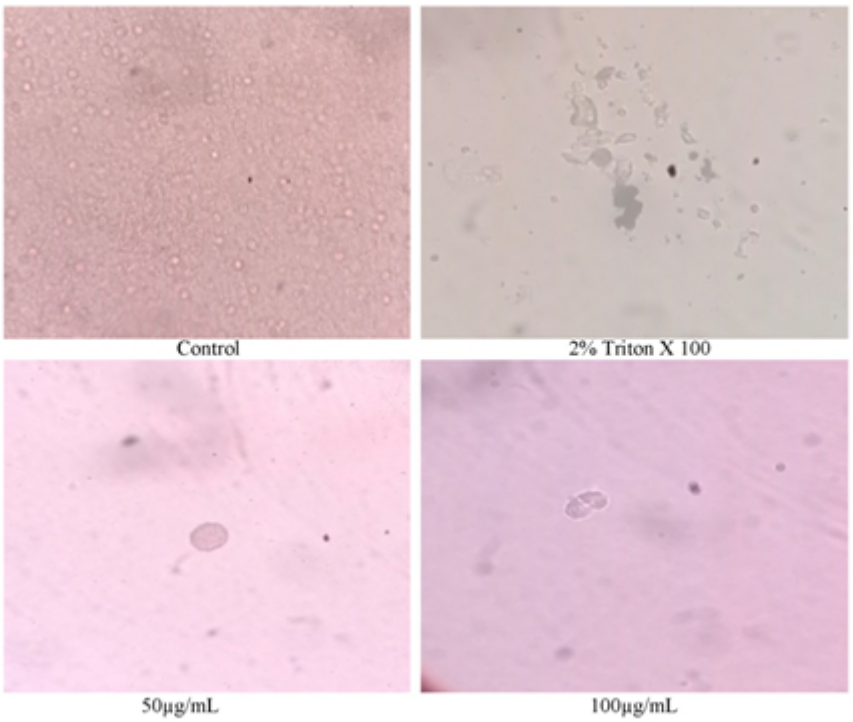

Figure 5

Hemolytic activity of the synthesized *Ph*-ZnO-NPs. Control and various concentrations (50 and 100µg/mL) of the synthesized *Ph*-ZnO-NPs treated on Human RBCs. The positive control was used for Triton X 100 (2%). The RBCs morphology was captured by light microscope (magnification 40x).

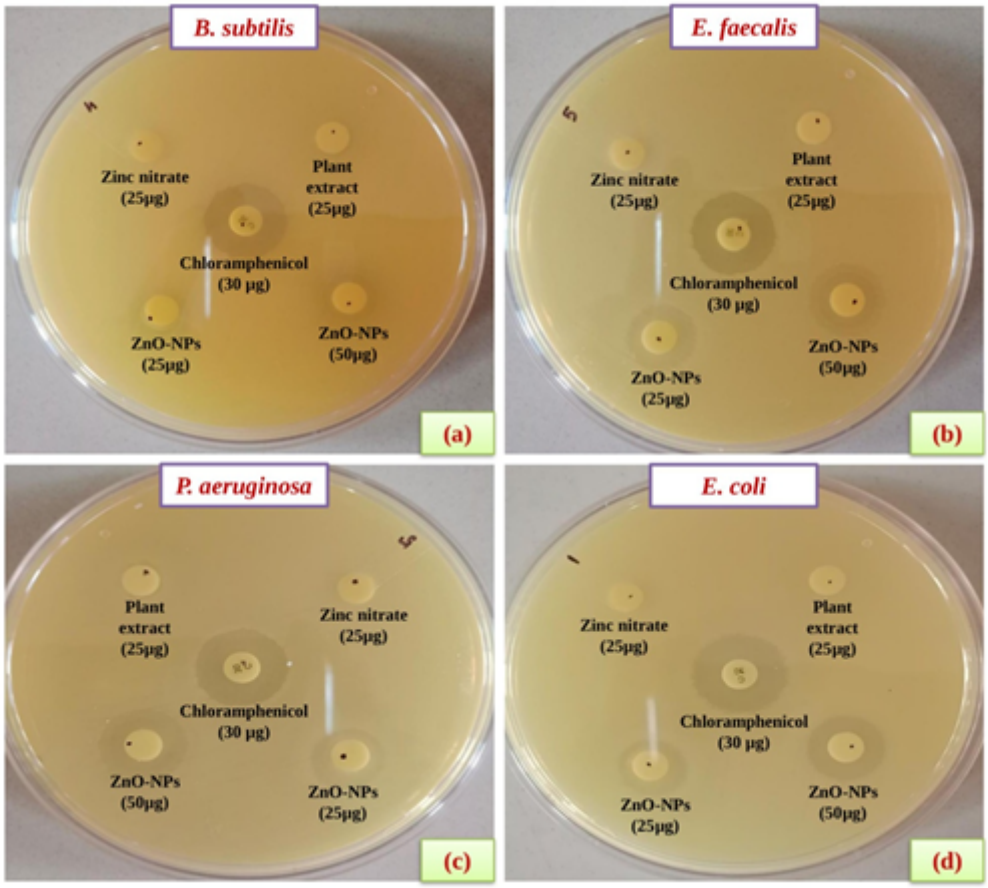

Figure 6

Antibacterial activity of the synthesized *Ph*-ZnO-NPs **a** *B. subtilis* **b** *E. faecalis* **c** *P. aeruginosa,* and **d** *E. coli.*

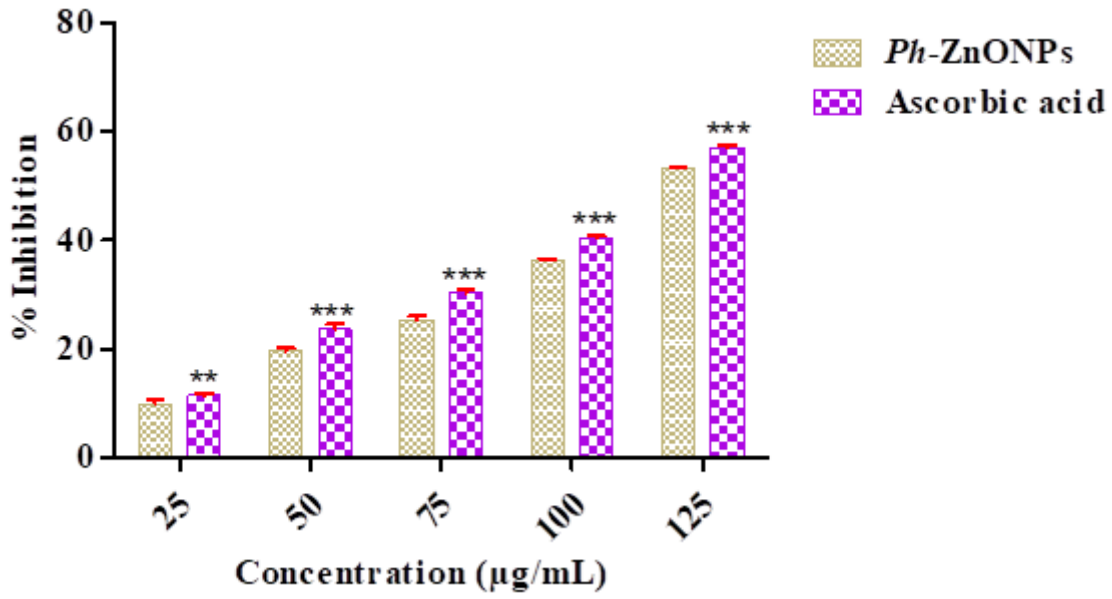

Figure 7

Antioxidant activity of the synthesized *Ph*-ZnO-NPs. (a) DPPH radical scavenging activity. The values are expressed as mean ± SD values and analyzed by Two-Way analysis of variance (ANOVA). Asterisk (**, ***) indicates significant different among treatments with respect to control ($P < 0.01$ and $P < 0.001$).

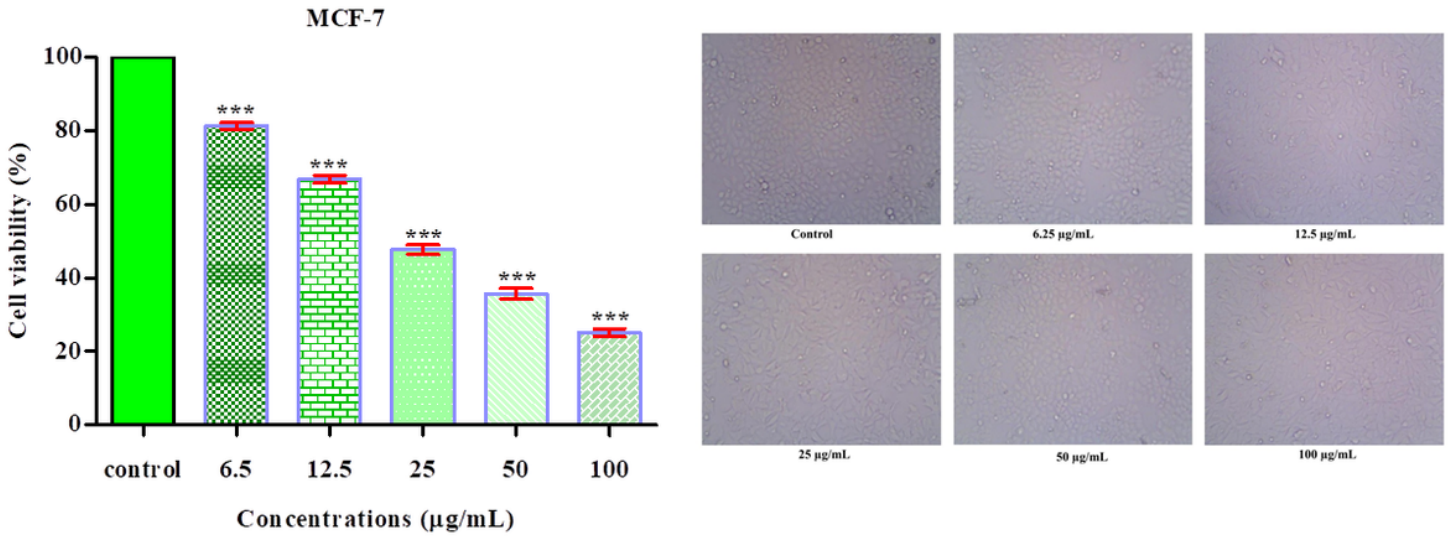

Figure 8

a MTT assay confirming the anticancer effects of the synthesized *Ph*-ZnO-NPs against breast cancer cell line (MCF-7). The values are expressed as mean ± SD values and analyzed by One - way analysis of variance (ANOVA). Asterisk (***) indicates significant different among treatments with respect to control ($P < 0.001$).

b The anticancer activity (MCF 7 cell-line) of the synthesized *Ph*-ZnO-NPs. Control and various concentrations by the synthesized *Ph*-ZnO-NPs (6.25-100µg/mL). The MCF-7 cell morphology was captured from a confocal microscope (340 pixels).