Waste Onion Peel Extract Mediated Synthesis, Characterization, Antibacterial, Antidiabetic, Anticancer and Drug Loading Efficacy of ZnO Nanoparticles

Greeshma KP [1*], Thamizselvi R [2], Muthulingam [3]and Dhanya B Sen [4]

[1]*Department of Chemistry, Sri Ramakrishna College of Arts & Science (SRCAS), Coimbatore-641006, Tamilnadu, India;kpmgreeshma@gmail.com*

[2]*Department of Chemistry, Government Arts College, Coimbatore -641018, Tamilnadu, India;thamizslv@yahoo.co.in*

[3]*Department of Chemistry,1Sri Ramakrishna College of Arts & Science (SRCAS), Coimbatore-641006, Tamilnadu, India;muthulingam@srcas.ac.in*

[4]*Department of Pharmacy,Sumandeep Vidyapeeth Deemed to be University,Piparia, Vadodara. Gujarat. India;dhanyab1983@gmail.com*

Correspondance*kpmgreeshma@gmail.com

Abstract

Here in we proposed the structural and biological properties of ZnO nanoparticles from the extract of *Allium Cepa L* (Onion).This study aims to develop an anticancer drug doxorubicin-coated ZnO nanoparticles for the drug delivery system. The onion peel extract mediated synthesis of ZnO nanoparticles were initially characterized by UV-Visible spectroscopy, FT- IR, SEM-EDAX, and XRD studies.A characteristic peak at 384 nm revealed by UV-Visible spectroscopy and major functional groups were identified by FT-IR spectroscopy. The XRD studies and calculation using Debye Scherrer equation and identified the particle size of ZnO nanoparticles as 83.73 nm. It shows efficient antibacerial effect on *Escherichia coli and Staphylococcus aureus* by disc diffusion method and antidiabetic assay by Alpha –amylase inhibition studies. The same was investigated against anticancer studies on MCF-7 cell lines and its drug loading capacity using an anticancer drug doxorubicin.

Keywords: *Allium Cepa L*;ZnO nanoparticles; antibacterial and anticancer studies; doxorubicin.

Introduction

Recently, the need for materials with attractive electrical and biological applications has increased the scope of research and development in pharamceutical industry. Among such various micro and macro materials, nanosized particles with sizes 1-100nm undergo versatile applications in optical, electrical, and biomedical fields [1]. ZnO nanoparticle is a noble metal

nanoparticle with excellent surface morphological properties and it exhibits a vital role in nanomedicine, catalysis, imaging, sensor devices, and drug delivery system [2].The main advantage of nanostructured materials is their 2D and 3D confinement and their multiphase crystallinity. The type of size, shape, and other surface morphological properties of metal nanoparticles enhanced their potential activities and applications in various fields [3].

There are a number of different chemical approaches that can be used to synthesise nanoparticles, however the majority of these methods include the use of potentially toxic compounds, have prohibitively expensive production costs, or have quality control issues with the purifying processes [4].Nanoparticle production and biomedicine have benefited greatly from the emergence of cost-effective technologies (biological approach) that incorporate bacteria, fungi, plant extracts and waste materials [5]. In comparison to chemical approaches, the biological approach is both economical and energy-conserving because it uses various microorganisms to produce metallic NPs [6-8]. There is a wide range of potential uses for biologically produced nanoparticles in biomedicine and related domains [9-11]. Nanoparticles coated with biological molecules are more stable and compatible than those prepared using traditional chemical procedures [12-14]. As a less labor-intensive alternative to chemical synthetic techniques, the utilization of agricultural garbages or plants and their parts has also evolved [15]. ZnO nanoparticles have low toxicity and great efficiency, making them useful in the drug delivery system and showing powerful anticancer, antibacterial, antioxidant and antidiabetic activity [16]. As a member of the Amaryllidaceae family, medicinal properties of onion (Allium Cepa L.) have been recognized since antiquity [17]. It is not just used for flavor but also provides health advantages in humans and helps prevent cancer and other diseases by providing protective phytochemicals [18].

Onion has recently gained popularity because of the positive effects it has on human health. Therefore, there is a growing interest in using biological techniques to extract highly active components from these plants [19-20]. The majority of flavonoids in onions are located on their outer peel. Therefore, the synthesis of nanoparticles from extract of discarded onion peel has the potential to significantly alter biomedical research and is of great economic and environmental significance. Kumar Patra et al. [21] demonstrated biological activity of gold nanoparticles extracted from onion peel. Similarly, *Emblica officinalis* [Amla] fruit exctract exhibit extensively wide applications in green nanochemistry [22]. It acts as a good stabilizing agent in many nanomaterial syntheses. Malari *et al* in 2018 studied synthesis of Fe_2O_3 nanoparticles from *Phyllanthus Emblica* and its photo catalytic efficacy [23]. Ramesh *et a*l reported that Gooseberry plant extract is a widely accepted reducing and capping agent in

many metal oxide nanoparticle synthesis especially in silver nanoparticles [24]. From the reported study it was cleared that plant extract can replace organic solvents during the synthesis of metal oxide nano particles. The *Allium Cepa* extract has many bioactive components and these effective in anti-bacterial, anti-diabetic and anti-cancer applications [25].Synthesis of metal nanoparticles from the above mentioned extracts may enhance the properties and could be a promising application in nanomedicines [26]. In the pharmaceutical field, drug-coated nanoparticles have recently gained significant importance [27]. It can lessen the negative consequences of more common chemical processes. Numerous types of cancer are treated using the chemotherapeutic drug doxorubicin hydrochloride [28]. The development of cardiac myopathy is the principal drawback of this drug. Therefore, creating an inert carrier that is also environmentally beneficial may lessen eventual negative impacts [29]. In the the present work, biotemplate mediated preparation of ZnO nanoparticles using onion peel extract and its effect on anti cancer effecton MCF 7 cell lines and drug delivery effeciency using standard anticancer drug doxorubicin [30].

Materials and Methods

The chemicals used for the phytochemical analysis and synthesis of nanoparticles were procured from Sigma Aldrich. Waste Onion peel was collected from the nearby local market of Coimbatore, Tamil Nadu. Zinc acetate dihydrate, and Doxorubicin HCl were purchased from Sigma Aldrich, India. -Human Breast Adenocarcinoma (MCF7) used for this study was obtained from the NCCS (National Center of Cell Science), Pune, India. Dulbecco's modified Eagle medium (DMEM), Fetal Bovine Serum (FBS), 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) and Dimethyl sulfoxide (DMSO) were acquired from Hi-Media Pvt. Ltd. (Coimbatore, India)

Allium Cepa. L (Onion) peel extract preparation

The collected peels were cleaned, dried at 40°C, and then finely chopped. To obtain onion peel extract, weighed out 5g of shadow dried peels were finely powdered and boiled with distilled (100 ml) water about 15 minutes while stirring constantly. The onion peel extract obtained was filtered using Whatmann No.1 filter paper and cooled at room temperature. Thus, additional investigations were conducted with the obtained onion peel extract.

Qualitative Phytochemical Analysis

The extracts were treated with respective chemical reagents to identify and analyses bioactive components like alkaloids, amino acids, flavonoids, phenol, free radical scavengers and reducing sugars present in *Allium Cepa L* by standard methods [31-32].

Synthesis of Metal Nanoparticles

Onion peel extract (OPE) was taken in a burette and mixed to the 1mM aqueous solution of Zn $(CH_3CO_2)_2.2H_2O$(Zinc acetate dehydrate) in a conical flask with constant stirring at 330 rpm for around 24 h on a magnetic stirrer. The obtained solution was kept on sonicatior for about 30 minutes to obtain fine particles. The transformation of zinc acetate dihydrate into zinc oxide nanoparticles was denoted by a colour change from white to dark brown. The resulting OPE-ZnONPs solution was centrifuged at rpm for about 30 minutes. Supernatant liquid was drained off, and the remaining solid pellet was gathered and dried in a oven and muffle furnace to remove moisture content.

Characterization of synthesized nanoparticles

Different characterization methods such as Fourier-transform infrared spectroscopy (FT-IR Model-8400S Shimadzu) over the wavelength series of 400 $cm^{-1}$to 4000 cm^{-1} and UV-Visible Spectrophotometer (UV Model 1700 Shimadzu) readings from 300 to 800 nm were taken at regular intervals to track the reduction of Zinc and silver ions. X-ray powder diffraction analysis (XRD- BRUKER), Energy-dispersive X-ray (EDX) analysis were used to analyse the surface shape and percentage composition of the produced nanoparticle. Scanning electron microscopy (SEM- JEOL-6480) were used to study the morphology of the obtained particles.

Biological potential of synthesized nanoparticles

Antibacterial activity

The disc diffusion approach was used for the antibacterial effect on *Staphylococcus aureus* and *Escherichia coli*bacteria. About 70 µl of the test bacteria were brushed onto the Mueller Hinton Agar surface using a sterilised glass spreader. The sterile discs were loaded with 20 µl of the sample, Ceftazidime (5 g/disc), as a positive control, and DMSO, as a negative control. Once the plate had been incubated at 37°C for 24 hours, the zone of inhibition was measured and reported in millimetres [33].

Alpha –amylase inhibition assay

Alpha-amylase enzyme, which is made by mixing 27.5 g in 100 ml of distilled water, was added to 1 ml of the sample along with a 0.1% starch solution in a 16mM sodium acetate buffer. The colorimetric reagent was made by combining 96mMconcentrated 3,5-dinitro salicylic acid solution with sodium potassium tartrate solution. Following addition, the tubes were incubated for 3-5 minutes at 25^0C in an alkaline environment. The conversion of 3,5-dinitrosalicylic acid to 3-amino-5-nitrosalicylic acid served as a measure of the production of maltose. A UV-VIS spectrophotometer was used to estimate its wavelength at 540 nm

(Labtronics LT291). The formula below was used to analyse and calculate plant extract and nanoparticles made from it as an anti-diabetic test [34-35].

Calculation

$$\% \text{ of inhibition} \quad \frac{\text{Control} - \text{sample}}{\text{Control}} \text{ X } 100$$

Drug loading

In the first step, 10 mg of doxorubicin was solubilized in anhydrous DMF, and then drops of OPE-ZnONPs were added while continuously stirring using a magnetic stirrer for around 2 hours. After vigorous stirring, two days were spent dialyzing the resulting supernatant against deionized water to remove the DMF and obtain the OPE-ZnONPs coated with DOX.

Drug loading studies of DOX loaded OPE-ZnO NPs

This method was on the basis of an indirect method and involved measuring the drug concentration of the supernatant [42]. By measuring the UV absorbance at 254 nm with UV/visible spectroscopy, quantification of the drug's concentration was done in both the supernatant and the redisposed pellets. In order to investigate drug release in vitro, produced pellets were submerged in PBS buffer with a pH of 7.4 and 5. In an incubator shaker set to 37^0C and 120 rpm, the contents were shaken constantly. At regular intervals, 2 ml of the external medium was removed and discarded, and it was replaced with the same volume of freshly prepared PBS. After that, the amount of DOX that was released into the medium was measured at 254 nm, and the below given formula was used to identify drug release percentage [36].

$$\text{Drug release percentage} = \frac{\text{Amount of drug loaded}}{\text{Amount of drug released at time (t)}} \text{ X } 100$$

Cytotoxic studies

The anti-cancer properties of the nanoparticles loaded with DOX were evaluated using MCF-7 cell lines utilizing the 3-(4,5-dimethyl thiazole-2yl)-2,5-diphenyl tetrazoliumbromide(MTT) method. A cell culture medium termed DMEM-High Glucose, which contains foetal bovine serum and is utilised for cell culture, was utilized to grow and maintain the cells.The cells were grown on 96-well plates for around 24h, at the required 20,000 cells per well cell density. After adding the required amounts of the test agent, the plate

was allowed to incubate for 24 h at 37°C in a 5% carbon dioxide environment. The MTT reagent was added at a concentration of 0.5 mg/mL of the total volume after the initial incubation period, after which the used media was discarded. Afterwards,this mixture was incubated. When 100µl of solubilization solution (DMSO) had been added, the plates were shaken for a further 30 minutes and kept in the dark condition. The percentage cell viability can be calculated using the following equation.

$$\text{Percentage cell viability} = \frac{\text{Absorbance of treated cells}}{\text{Absorbance of untreated cells}} \times 100$$

Characterization of loaded nanoparticles

Following encapsulation, the drug sample was analysed by FT-IR (Perkin Elmer Spectrum RXI with KBr tablet) to recognize the functional groups present. On a Bruker system (XRD, D2 Phaser, and USA) with Cu K radiation at a mean wavelength of 1.54059 Å, we measured the average crystal size, phase constitution, and other structural information of the sample.

Results and Discussion

Phytochemical analysis and Antimicrobial Assay of synthesized OPE-ZnONPs

Onion peel extract has been shown to have flavonoids, phenolic substance, and free radical scavenging action in initial phytochemical tests. This indicates that it can be used as an eco-friendly method of converting Zinc acetate dihydrate into ZnONPs. After 24 hours of stirring, a dark brown colour indicates that the onion peel extract has been successfully used to reduce Zinc acetate dihydrate into OPE-ZnONPs. To completely convert Zinc acetate dihydrate into ZnONPs, incubation of reaction mixture for 24 hours was carried out. Table.1 shows a qualitative analysis of phytochemicals in *Allium Cepa L* extract.

Table 1. Results of qualitative analysis of phytochemicals in *Allium Cepa L*

Phytochemical	*Allium Cepa. L*	
	Aqueous	Ethanol
Alkaloids	-	-
Tannins	+	-
Flavonoids	+	-
Phenol	-	-

Carbohydrates	+	-
Sterols	-	-
Saponins	+	+

The literature values and the reported values agree with one another very well. Benitez et al. [43] used the outer layer of the onion to report the total phenol and flavonoid levels. Based on of the phytochemical studies, it is evident that the outer layer of the onion has the potential to act as a powerful oxidant and is able to get rid of free radicals in our body, thereby lowering the risk of cardiovascular disease and slowing the progression of tumours.

As shown in Table.2,3and Figure.1, prepared NPs were tested in an anti-bacterial assay against *E. coli and Staph. aureus* utilizing Ceftazidime (CAZ^{30}) as a reference antibiotic. OPE-ZnONPs are more sensitive to both microbes, as evidenced by their high antibacterial activity against the tested microbes.

Table.2. Antibacterial activity of OPE-Zn NPs with *Staphylococcus aureus*

Pathogen ***Staphylococcus aureus***	**Zone of Inhibition (ZOI in mm**			**Ceftazidime(CAZ^{30}) Disc**
	25µg	**50 µg**	**75 µg**	
OPE-ZnNPs	12.0 ± 0.73	16.0 ±0.32	15.0±0.709	8.0±0.0

Table.3. Antibacterial activity of OPE-Zn NPs with *E.coli*

Pathogen ***E Coli***	**Zone of Inhibition (ZOI in mm**			**Ceftazidime(CAZ^{30}) Disc**
	25µg	**50 µg**	**75 µg**	
OPE-ZnNPs	8.0 ± 0.73	6.0 ±0.32	13.0±0.709	8.0±0.0

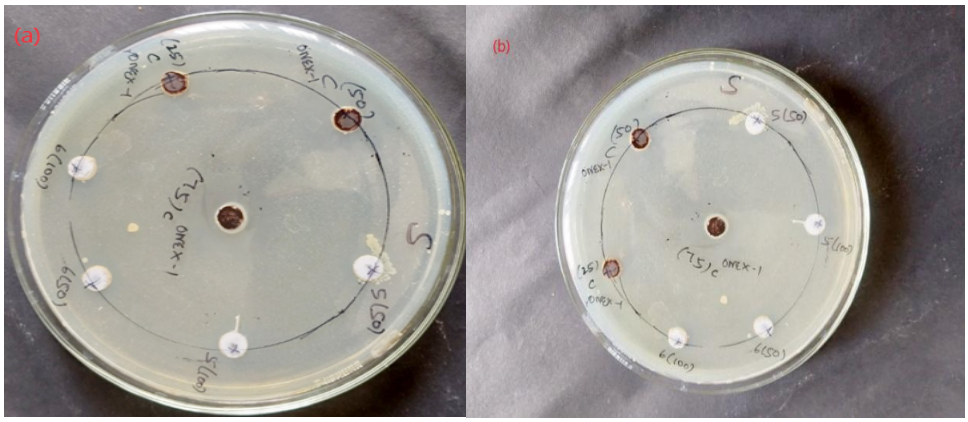

Figure.1. Anti-bacterial study of OPE-ZnO NPs (a) ZOI of *S. aureus (b)* ZOI of *E. coli*

Anti-diabetic Assay

Diabetes mellitus is mainly due to high glucose levels in the blood, this hyperglycaemia can be prevented by inhibiting carbohydrate digesting enzymes (alpha-glucosidase and alpha-amylase). Therefore the development of medicines with inhibitory activities may be helpful for preventing diabetics. The antidiabetic assay of OPE-Zn NPs depicted in Table 4: The results suggested that silver nanoparticles synthesized from *Emblica Officinalis* can reduce the activity level of the enzyme, which is significantly higher than zinc nanoparticles derived from *Allium Cepa.L.* Hence the plant-mediated nanoparticles prepared exhibit an enhanced antidiabetic potential against hyperglycemia. However Acarbose (an anti-diabetic drug) showed a better inhibitory activity indicating that the plant mediated nanoparticles developed poses such pharmacological properties that could prove an effective advancement in the diabetics mellitus research.

Table 4: Anti Diabetic Assay of plant extract and synthesized nanoparticles

Sample	***Emblica officinalis(EO-AgNPS)***	***Allium Cepa. L(OPE-ZnO)***
	∞-amylase inhibition (%)	**∞-amylase inhibition (%)**
Acarbose (Control)	68.78 ±0.03	68.78 ±0.03
Plant Extract	62.25 ± 0.03	45.70 ± 0.03
Nanoparticle	65.70 ± 0.03	54.79 ± 0.03

Structural analysis of synthesized Zinc Oxide nanoparticles

UV-Vis and FT-IR spectral analysis of synthesized nanoparticles

UV-Vis spectroscopy was utilized to verify the synthesis of ZnONPs; the solution of Zinc acetate dihydrate turned a dark brown upon addition of OPE, providing conclusive evidence for the transformation of Zinc acetate dihydrate into ZnONPs at room temperature. Fig 2 shows a sharp peak at 384 nm showing the presence of ZnO nanoparticles. The presence of secondary metabolites present in the Allium *Cepa.L*. functions as a reducing agent.

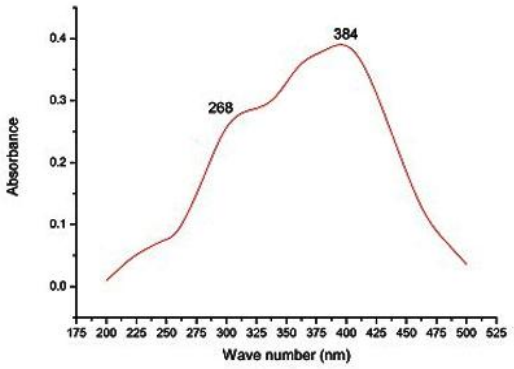

Fig: 2 UV-Vis spectral analysis of OPE-ZnO

FT-IR spectrum of OPE-ZnO indicated in Fig 3 confirms the existence of several functional groups in onion peel extract. The observed peaks at 1568, 1402 and 1113 cm^{-1} shows the existence of N-H, O-H and H bonds in amides. The band observed at 666 cm^{-1} clearly indicates the formation of metal oxygen bond ie; the formation of ZnO NPs

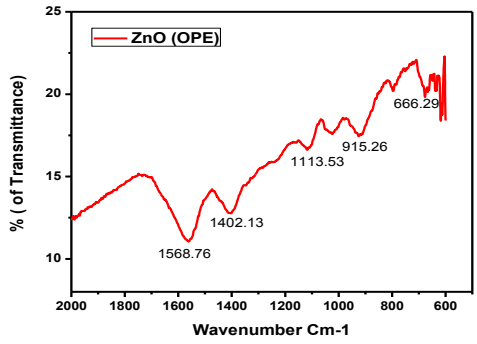

Fig:3 FT-IR spectra of OPE-ZnO

Elemental and surface morphological analysis of Zn and Ag NPs

SEM and EDAX analysis of OPE-ZnO NPs was depicted in Fig 4 and 5

respectively. The SEM images givesa crystal like morphology and percentage composition was identified by EDAX analysis. Electrostatic interactions between the biomolecules in the plant extract and the methods used to prepare the sample may contribute to the aggregate production of ZnO NPs. EDAX analysis was utilized to assess the elemental assessment of the ZnO NPs. Both analysis shows morphological and elemental confirmation of ZnO nanoparticles.

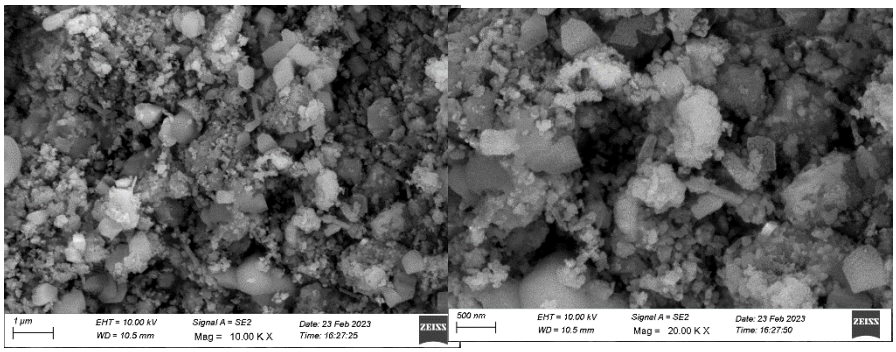

Figure 4: SEM morphological images of OPE–ZnO NPs

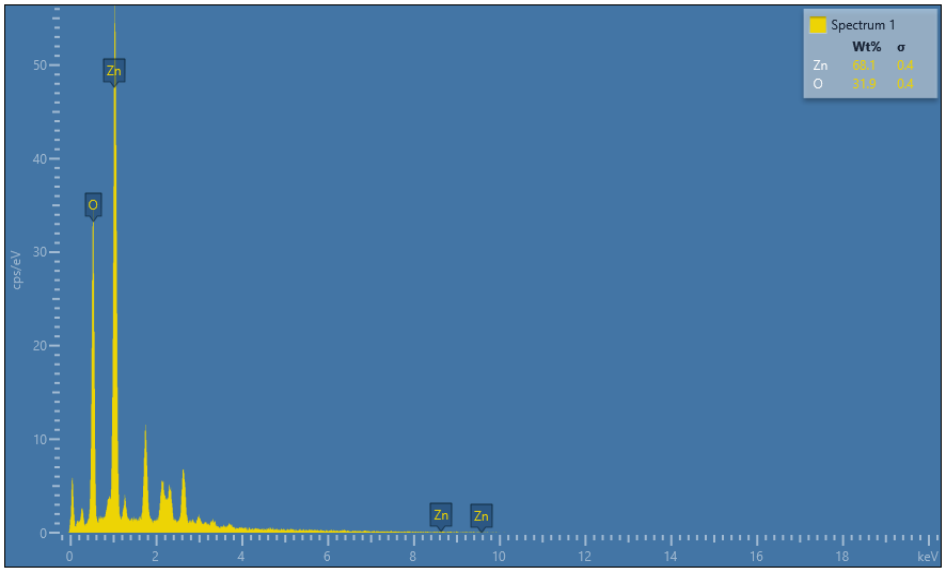

Figure 5: EDAX images of OPE–ZnO NPs

Fig 6 indicates the XRD arrangement of synthesized zinc and silver nanoparticles. In the XRD pattern of ZnO NPs revels six peaks indexed at (1 0 0), (0 0 2), (1 0 1), (1 0 2), (1 1 0), (1 0 3) (JCPDS card no.89-1397) and corresponds to known hexagonal wurtzite structure. The mean particle size was computed using Debye Scherrer equation and found to be 83.73 nm.

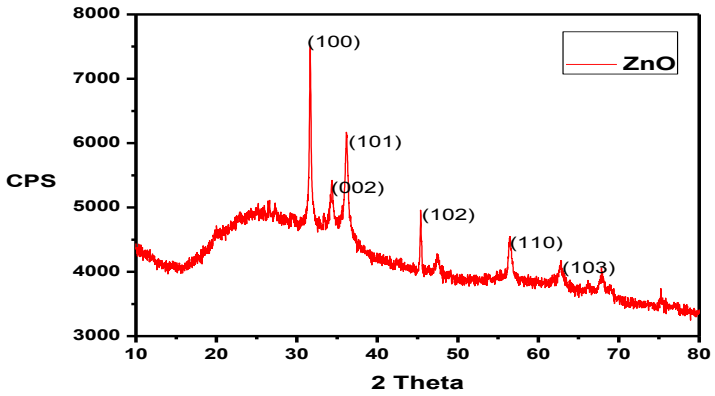

Figure 6: XRD pattern of OPE–Zn NPs

Characterization of doxorubicin loaded OPE-Zn NPs

The FT-IR spectrum of pure DOX and OPE-ZnNPs loaded with doxorubicin have been shown in Fig.7 and Fig.8 respectively. Doxorubicin loaded nanoparticles exhibited spectral peaks identical to those of free doxorubicin, including those at 2978cm-(C-H stretching vibrations), 887cm- (N-H wagging), 1265 (stretching of alcoholic O-H groups), 1388cm- (C-C stretching), and 671cm- (N-H wagging) [44]. The interaction of DOX with OPE-ZnNPs was demonstrated by a minor narrowing of the peaks matching to the stretching of the hydroxyl groups (3500-3200cm-), as compared with pure doxorubicin. Doxorubicin had a sharp peak at 1388cm-, demonstrating the presence of the drug in the OPE-ZnNPs. Encapsulation of doxorubicin on produced ZnNPs was further supported by the fact that the peak at 1010 in pure doxorubicin was significantly attenuated to 948 cm- in DOX loaded nanogel. The carbonyl peak at pure DOX at 1705cm^{-} also shifted to a lower position at 1651cm^{-} may be due to the formation of hydrogen bond between drug and nanoparticles. Yang *et.al* reported similar results in single walled carbon nanotube and ferrocene carboxylic acid [37].

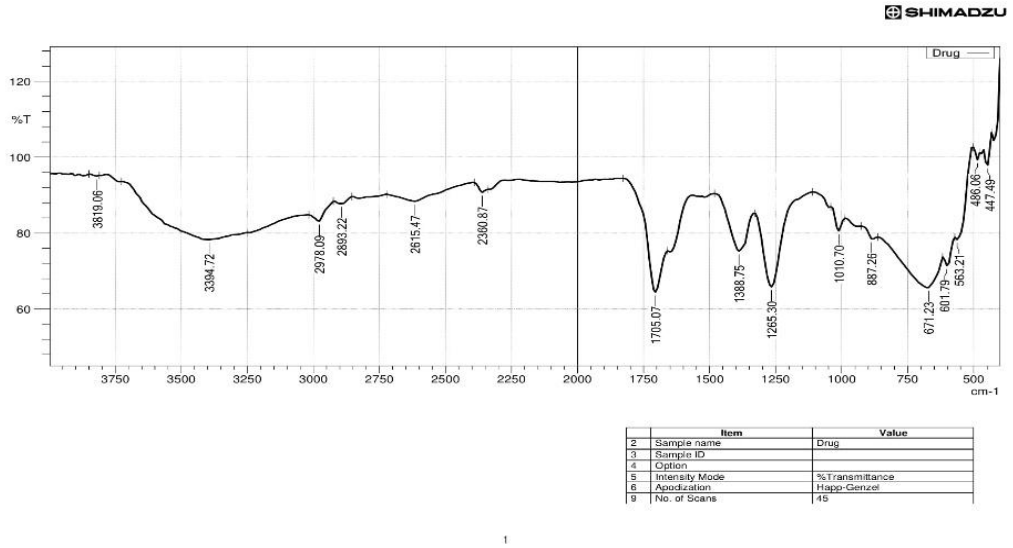

Figure 7: FTIR spectrum of drug alone

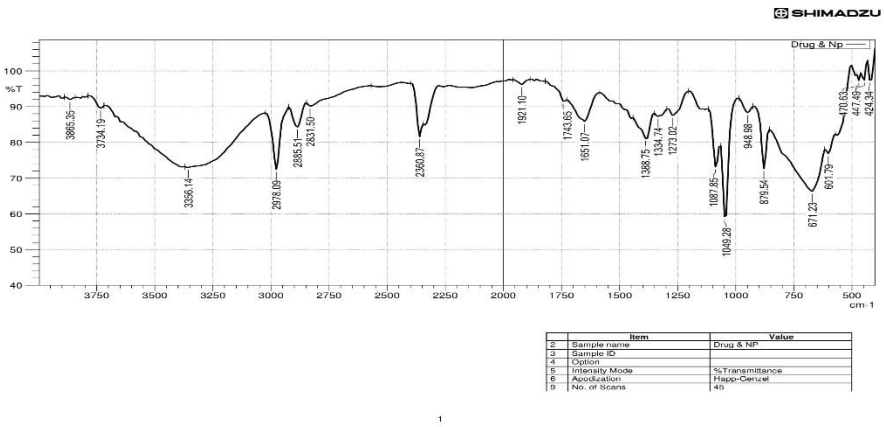

Figure 8. FTIR spectrum of Drug-Coated OPE- ZnONPs

Drug release study of Doxorubicin coated Metal nanoparticles

The entrapment efficiency of drug coated nanoparticle using 5ml of OPE-ZnO NPs and 0.01 ml of doxorubicin was found to be 32.6%.This results showed that maximum entrapment efficiency was observed in DOX coated nanoparticles. Invitro drug release was determined using dialysis bag method. After 24 hours of study, the total drug released in DOX-OPE ZnO NPs was about 41.2%.This indicates that anticancer drug doxorubicin coated ZnO nanoparticles can able to carry the drug and release on target position. This confirmed the application of green synthesized nanoparticles in drug delivery system.

In vitro cytotoxic studies against MCF-7 cell lines

Doxorubicin entrapped OPE-ZnNPs was evaluated for its *in vitro* cytotoxic studies against human breast cancer MCF-7 cell lines with diverse concentrations (12.5, 25, 50,100,200 µg/ml). Fig.9 depicts percentage cell viability of Doxorubicin coated OPE-EO AgNPs. In that case on comparing with standard drug it shows a high value for cell mortality and indicate its low possibility in drug delivery system. The cell viability percentage clearly confirmed its superior responses to cancer MCF-7 cancer cell line and its applications in drug formulation and drug delivery system. This appreciable result may be due to its nanoparticle size and which helps them for easy uptake and can block abnormal growth and activity of cancer cells.

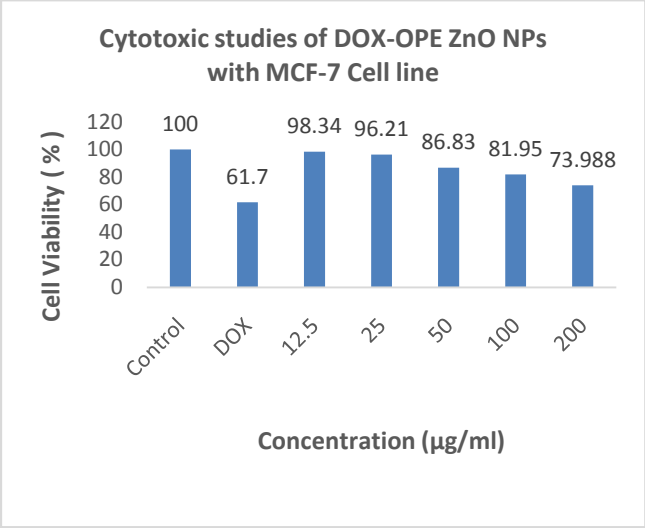

Figure 9: Cytotoxic studies of DOX-OPE ZnO NPs against MCF-7 Cell line

Conclusion

In this study, we described a green route mediated synthesis of zinc oxide nanoparticles from a variety of sources and proved the anti-microbial, anti-diabetic, and cytotoxic effects that these nanoparticles had on a selection of pathogens. Synthesized nanoparticles also encapsulated with chemotherapeutic drug doxorubicin and its loading efficacy was also evaluated. Biomediated synthesis of nanoparticles using a variety of environmentally benign materials is a well-established topic in the field of nanotechnology; yet, its application in drug loading is very uncommon and has not been thoroughly investigated. The use of doxorubicin-coated zinc oxide nanoparticles (NPs) in drug delivery systems is further confirmation of the pharmaceutical industry's widespread utilization of this technology. It is possible that the creation of these kinds of biodegradable nano carriers may lessen and minimize the undesirable interactions that

chemotherapeutic drugs have with normal active cells. In this investigation, we generated environmentally friendly nanoparticles by using waste onion peel extract. These nanoparticles can be stored up to 4 months without any coagulation or aggregation. The identified organic phytochemicals act as a strong reducing agent in both Zinc Oxide nanoparticle synthesis, this avoid the usage of chemicals. Anti-diabetic effects of the synthesized nanoparticle insight a promising research in the area of diabetics. Anti-bacterial activity that was efficient against both gram-positive and gram-negative bacteria was demonstrated by the nanoparticles that were synthesized. Additionally, we presented the results of an in vitro cytotoxic investigation conducted using MCF-7 cell lines and the percentage of cell viability. It was found that the produced nanoparticles might be employed for drug delivery after being applied to the encapsulation of doxorubicin under mild conditions. Therefore, the synthesized doxorubicin-loaded OPE-ZnO is a more suitable candidate for drug development. In further research, we will investigate the effect of these nanoparticles on breast cancer patients in vivo.

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Author Contributions: Conception and design of study, acquisition of data, analysis and drafting, KPG; Revising the manuscript critically for important intellectual content, KPG, R.T, S.M and D.B.S

Data availability: All data analysed and generated during this are included in this article.

Declarations

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Funding: Not applicable.

Consent to participate: Yes. All authors agreed to participate in this research.

Consent for publication: yes. All authors have approved the last version of the manuscript for its submission.

Competing interest: The authors declare no competing interest.

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