EVALUATION OF ANTIMICROBIAL EFFICACY OF COUMARIN DERIVATIVE UMBELLIFERONE - ZINC OXIDE NANOPARTICLE FROM *Aegle marmelos* FRUIT PULP

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Abstract

In this study zinc oxide nanoparticle were synthesized using *Aegle marmelos* fruit pulp compound Umbelliferone. The phytochemical analysis of the *Aegle marmelos* fruit pulp extract confirms the presence of alkaloids, flavonoids, Phenols, Terpenoids and Tannins. The synthesized Umbelliferone - zinc oxide nanoparticle morphological and optical properties were characterized by using XRD, UV, FTIR, TEM, DLS and PL Spectra techniques. The crystal size of the nanoparticles was found at 30.85nm in XRD. The in vitro antibacterial activities of the synthesized UMB- ZnO NPs were tested against both Gram positive and Gram negative bacteria like *S. aureus, S. pneumoniae, B. subtilis, K. pneumoniae , E. Coli, S. Dysenteriae* was analyzed using the agar well diffusion method. The antibacterial activities of UMB-ZnO NPs showed good results with the antibiotic standards. From this the synthesized Umbelliferone- Zinc oxide nanoparticle using *Aegle marmelos* fruit pulp has promising approach for developing new drug products with efficient antibacterial properties.

Key Word: *Aegle marmelos, Zinc Oxide, Umbelliferone, Antibacterial, XRD, FTIR.*

INTRODUCTION

Aegle marmelos has been known to be one of the most important medicinal plants of India. It belongs to the Rutaceae family and it has also known as Bengal quince, Golden Apple, or Bael. In Ayurveda, the fruit is known to balance kapha and vata dosha [1]. The parts of the *Aegle marmelos* plant possess biological and pharmacological activity against various chronic diseases such as cancer and cardiovascular and gastrointestinal disorders. More than 100 phytochemical compounds have been isolated from various parts of the plant, namely phenols, flavonoids, alkaloids, cardiac glycosides, saponins, terpenoids, steroids, and tannins [2].

Bioactive compounds found in Aegle marmelos fruit (marmelosin, umbelliferone, luvangetin, aurapten, psoralen, marmelide, tannin, riboflavin, aegeline, β-carotene, lupeol, and eugenol) have a range of biological activities, such as antihelminthic, antibacterial, antiulcer, antispasmodic, artemicide, antidiarrhea, astringent, antiproliferative, anticancer, anti-inflammatory, and antioxidant [3].

Compounds produced from plants are currently employed extensively as anticancer agents [4]. Umbelliferone is a coumarin derivative that is found in *Aegle marmelos*. Umbelliferone is a phenolic compound, and it acts as a potential metal chelator, free radical scavenger, and powerful chain-breaking antioxidant [5]. Umbelliferone is a coumarin derivative found in numerous plant families, including the Rutaceae and Apiaceae. Umbelliferone (UMB) is also known as 7-hydroxycoumarin, hydrangine, and skimmetine. It is mostly utilized in sunscreens and optical brighteners in textiles [6].

The chemical formula for umbelliferone (UMB) is C9H6O3. It appears as needle-like, yellowish-white crystals that are highly soluble in dioxane and ethanol but very slightly soluble in hot water. 10 It has a melting point range of 230-233°C and a molecular weight of 162.144 g/mol. Ultraviolet radiation at various wavelengths is significantly absorbed by UMB. Its maximum absorbance occurs at 325 nm in acidic environments and shifts to 365 nm in alkaline solutions. With emission maxima at 460 nm, fluorescence excitation peaks at 330 nm in acidic solutions and 370 nm in alkaline ones [7].

According to Kaur and Kapoor [8], the fruit pulp of *Aegle marmelos* contains free radicals. Reactive oxygen species (ROS) are a harmful group of chemicals created during aerobic metabolic activities in human cells. These radicals are associated with various ailments. The potential therapeutic effects of UMB in diabetes, cardiovascular or neurodegenerative diseases, inflammatory disorders, various cancer types, and microbial infections have gained increasing interest in the development of its synthetic derivatives with beneficial pharmacological activities [9].

Nanotechnology emerging

Recent researchers are drawn to ZnO NPs due to their significant antibacterial, antioxidant, and anticancer properties [10]. It can be used to deliver an extensive range of medications into multiple organs, including the liver, brain, spleen, lungs, and lymphatic system [11]. Greenly synthesized nanoparticles are cost effective, sustainable ecofriendly. It Biofunctionalized ZnO NPs shows good biocompatablity and stability and also it can provide less toxic, avoids high energy and low cost. Recently many studies suggested that ZnO NPs had strong antimicrobial activity [12].

The U.S food and Drug Administration (FDA) identifies ZnO as a (GRAS – generally recognized and safe substance. It is also used in sunscreen due to this ultraviolet rays scattering properties, drug delivery and wound healing applications [13]. *Aegle marmelos* can enhance both antioxidant and photocatalytic properties of ZnO nanoparticles, reinforcing the rationale behind the nanoparticle synthesis method [14]. The present study was aimed to evaluating the phytochemical potential and antimicrobial activity of *Aegle marmelos* fruit pulp compound Umbelliferone encoded with Zinc oxide nanoparticle (UMB – ZnO NPs).

Materials and methods

Collection of the sample

Aegle marmelos (L.) Correa fruit pulp were collected from a temple in sampathnagar , Erode, Tamil Nadu, India and was identified and authenticated by Dr. S. S. Hameed, Scientist-E, Botanical survey of India, Southern regional Centre, Coimbatore, Tamil Nadu, India.

Chemicals and reagents

Umbelliferone (purity 98% w/w) purchased from SRL Chemicals. All chemicals are AR grade.

Resources and Preparation of Fruit Extract

The *Aegle marmelos* fruits were collected from the Kolli Hills, Namakkal district, Tamil Nadu, India, located at coordinates 11.2485° N latitude and 78.3387° E longitude, as shown in Figure 1. After harvesting, the fruits were carefully cleaned with distilled water. They were then finely sliced into small pieces and dried for 2weeks in a shaded area at 30°C. After drying, the fruits were crushed into a fine residue, sifted, and stored. For the extraction, 10 g of the fine residue underwent Soxhlet extraction using distilled water. The obtained

extract was centrifuged at 7000 rpm for 10min using a REMI centrifuge. The solution was then filtered using a filter cloth (Whatman No. 42) and kept at 4°C for further investigation.



Figure 1: Pictorial representation of *Aegle marmelos*fruit

Umbelliferone-ZnO NPs were synthesized using the chemical precipitation method

One gram of ZnO nanoparticles (ZnO NPs) was initially dispersed in 50 mL of aqueous medium prepared using double-distilled water (DD H_2O). The dispersion was subjected to continuous stirring to obtain a uniform suspension. Separately, Umbelliferone was dissolved in DD H_2O to achieve a final concentration of **500 µg/mL** and subsequently added dropwise to the ZnO NP suspension under constant stirring. The resulting Umbelliferone–ZnO NP mixture was sonicated for **2 h at 60°C** to facilitate efficient surface coating and enhance intermolecular interactions between Umbelliferone and ZnO NPs. Following sonication, the mixture was placed on a rotary shaker and incubated for **12 h** to ensure homogeneous coating and stabilization of Umbelliferone on the nanoparticle surface.

The obtained Umbelliferone–ZnO NPs were then collected and washed repeatedly with DD H_2O and ethanol to remove unbound Umbelliferone and residual impurities. The suspension was centrifuged at **4000 rpm for 15 min at 1°C**, and the resulting pellet was carefully separated. Finally, the purified Umbelliferone–ZnO NPs were dehydrated by drying at **200°C**

for 2 h, yielding a stable powdered nanocomposite suitable for further physicochemical and biological characterization.

Characterization analysis

The obtained Umbelliferone-ZnO NPs was characterized by using X-ray diffractometer (XRD). The diffraction patterns were recorded in the 2θ range of between 25o and 80o for Umbelliferone-ZnO NPs. Energy Dispersive X-ray Spectrometry (EDX) was employed to examine the Umbelliferone-ZnO NPs. The morphologies of Umbelliferone-ZnO NPs were analyzed using the TEM, which was used at an increasing voltage of 200 kV. The spectrum of the FTIR was noted in the wavenumber range of 400–4000 by using a spectrometer. The absorbance of Umbelliferone-ZnO NPs was studied between 200 and 1100 nm by Lambda 35 spectrometer. Photoluminescence (PL) spectra were taken using the spectrometer.

Antibacterial activity

The antibacterial activity of the prepared UMB - ZnO NPs were employed by using the agar well diffusion method and tested against gram positive (*S. aureus, S pneumonia, Bacillus subtilis)* and gram negative (*Klebsiella pneumonia, Shigella dysenteriae, E. coli*) bacterial strain. 100 mL of a fresh culture containing 1×10 8 CFU/mL of bacteria was spread onto the Mueller Hinton Agar (MHA) plates using the sterile swab. The petri-plate was tested at 10 µl, 20 µl and 30 µl concentration of the UMB-ZnO NPs nanomaterials dispersed in dimethyl sulphoxide (DMSO). Zone of inhibition levels (mm) was measured subsequently for 24 h at 37 °C. For positive control, standard antibiotic Streptomycin (10 µg disc) was used.

Antifungal activity

Antifungal activity was determined using potato dextrose agar by using a well diffusion method against the test fungi (*C. albicans*). First, the test strain was transferred into potato dextrose broth (PDB) and incubated at 30 °C. Subsequently, a well-loaded with 10 µl, 20 µl and 30 µl of test samples UMB-ZnO NPs nanomaterials was placed onto the inoculated plates using sterile forceps and incubated at 30 °C for 24 h under visible light. Zone of inhibition levels (mm) was measured subsequently for 24 h at 37 °C. For positive control, standard antibiotic Streptomycin (10 µg disc) was used.

Result and Discussion

Physiochemical properties of Ethanolic extract of *Aegle marmelos*

The extract exhibits a very high moisture content of 52.8% indicating the fresh nature of the pulp or a high water rentention capacity.Carbohydrates are the most abundant macronutrient, accounting for 40.78%. The total energy content is 168.81Kcal.The crude protein and crude fat contents are relatively low, at 0.86% and 0.25%, respectively. The crude fibre content is 2.88%. The total ash content, representing the mineral, is 2.21% with a very low acid-insoluble ash of 0.22%.

The table 1 shows physiochemical properties of Ethanolic extract *Aegle marmelos* fruit pulp.

S. NO	PARAMETERS	UNITS	RESULTS
1	Total Ash	%	2.21
2	Acid-insoluble ash	%	0.22
3	Moisture	%	52.8
4	Crude Protein	%	0.86

5	**Crude Fibre**	**%**	2.88
6	**Crude Fat**	**%**	0.25
7	**Carbohydrate**	**%**	40.78
8	**Energy**	**K Cal**	168.81

Toxicity Study

The toxicity study of the umbelliferone compound ADMET and CYP were tested by using SwissADME. It obeys the insilico toxicity assay ADMET.

CHARACTERIZATION RESULTS

XRD

XRD analysis carried out to analyze the crystalline structure of the Umbelliferone-ZnO NPs was shown in the figure .From the spectrum nine peak were observed at 2θ values15.87°, 18.41°, 27.84°, 29.74°, 32.77°, 34.21°, 36.03°, 47.32°, 56.36°, and 62.64° in the XRD pattern of synthesized NPs. These peaks were corresponding to the (100), (002), (101), (102), (110), (103), (200), (112) and (201).The crystal lattice planes of hexagonal wurtzite structure of ZnO NPs respectively. These values were matched with JCPDS card no 36-1451. The peaks between 2θ values between 10° to 25° confirms the presence of Umbelliferone. Furthermore, the average crystalline size of Umbelliferone –ZnO NPs was calculated by using the Debye –Scherrer equation which was approximately 30.85nm. The XRD results indicates previous studies and matched towards both high and small intensity spectra with an average crystalline size using Debye Scherrer's formula [15][16]. From the broadening of the XRD peaks the crystalline size (d) of the nanoparticles was calculated using Debye Scherrer's formula.

$d = k\lambda / \beta\cos\theta$

Where,

λ = is the wavelength of x-Rays (1.5406Å)

β = is the full width in radiations at half maximum (FWHM) of diffraction peaks

θ = is the diffracted angle of X-Rays pattern

The crystalline size of biologically synthesized UMB-ZnO NPs was 30.85nm calculated usingScherrer'sformula.

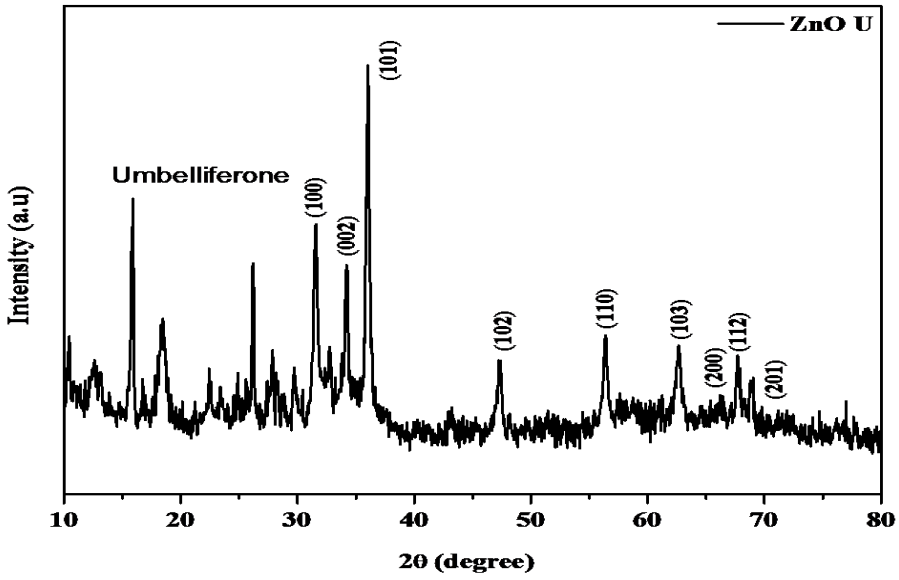


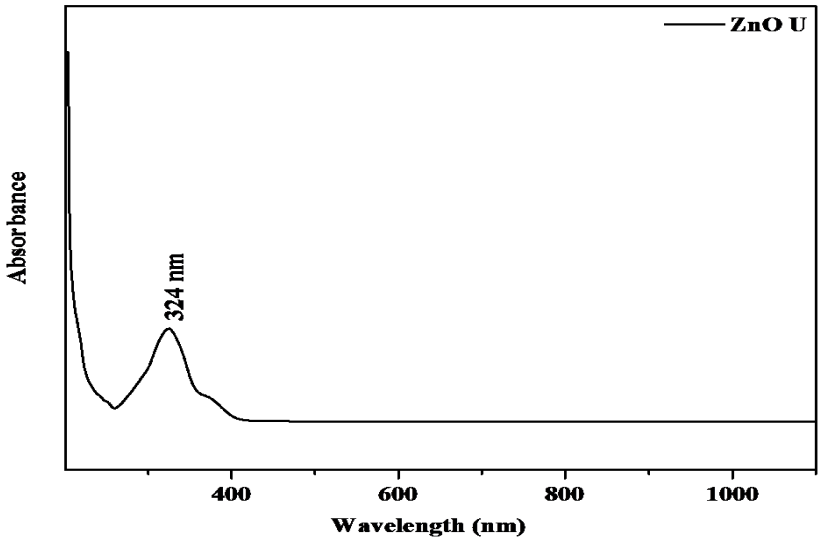
Figure 2 : XRD spectra of UMB – ZnO NPs showing the various diffraction peaks

UV visible spectroscopy

UV-Vis spectroscopy was used to observe the spectral characteristics of synthesized Umbelliferone – ZnO NPs .The peak in the spectrum indicates the wavelength at which the compound absorbs the most light. For zinc oxide nanoparticles (ZnO NPs) and related compounds the absoption peak is typically observed in the UV Region, often between 300

nm to 400 nm. The successful formation of Umbelliferone – ZnO NPs was confirmed through this technique, which presented a strong peak at 324nm, as shown in the figure 2 [18] Generally umbelliferone has strong ability to absorb UV light. From the strong absorption peak it was confirmed that the presence of umbelliferone in synthesized ZnO NPs.

Figure 3: UV-Visble spectroscopy of UMB- ZnO NPs



Fourier Transformed infrared spectroscopic (FTIR)

FTIR analysis is a valuable tool for identifying the functional groups present in biomolecules responsible for the stabilization and reduction of ZnO NPs. The broad peak centered 3395 and the peak at 3184 are characteristic of O-H stretching vibrations. This suggests the presence of hydroxyl groups, likely due to absorbed moisture or water molecules on the surface of the ZnO, or possibly O-H groups from organic residues. 1570 and 1512 these bands are often associated with asymmetric stretching of carbohydrates ions (COO^-), suggesting the presence of carboxylic acid residues bound to the ZnO surface.1461, 1381,

1323, 1235. These multiple peaks are typical of C-H bending vibrations and C-O stretching further confirming the presence of organic components. The strong absorption bands at 479 and 457 are characteristic of the stretching vibrations of the Zn-O bond in the zinc oxide crystal lattice [19].

Figure 4: FTIR spectra of Umbelliferone - ZnO nanoparticles

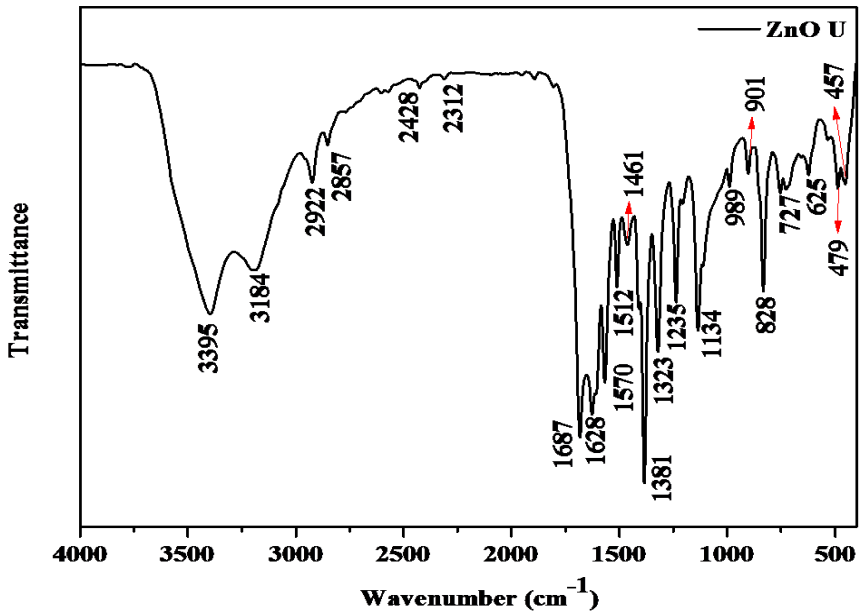


Table 2 depicts the FTIR functional group of UMB – ZnO NPs

S. No	UMB-ZnONPs absorption peak (cm^{-1})	Functional groups/ chemical bonding
1.	3395,2922	O-H stretching
3.	2857	C-H stretching
4.	1687,1682	C-H Bending
5.	1570 ,1512	N-O Stretching
6.	1381	C-H Bending
7.	1323,1325	O-H Bending
8.	1134	C-O Stretching
9.	989	C=C Bending
10.	828	C-H Bending
11.	727	C-CI Stretching

Energy dispersive X-ray

Energy dispersive X-Ray analysis employs to determine elemental composition of materials. Prominent peaks are observed for Zinc (Zn) and Oxygen (O), indicating the primary components of the material. The text in the image explicitly states that the present study confirms the Zinc Oxide nanoparticle. The elemental composition of the nanoparticles was analayzed using EDX as depicted in demonstrated that oxygen (32.21%) and Zinc (18.00%) were the dominant elements present [20].

A smaller peak for Carbon (C) is also visible. This is likely an extrinsic element, possibly originating from the sample preparation process, the adhesive used to mount the

sample, or residual organic material. The presence of strong Zn and O peaks confirms the successful synthesis or identification of Zinc Oxide (ZnO) as the main material. The relative intensity of the peaks provides qualitative information about the abundance of each element.

Figure 5: Graph Represents the EDX results of UMB –ZnO NPs

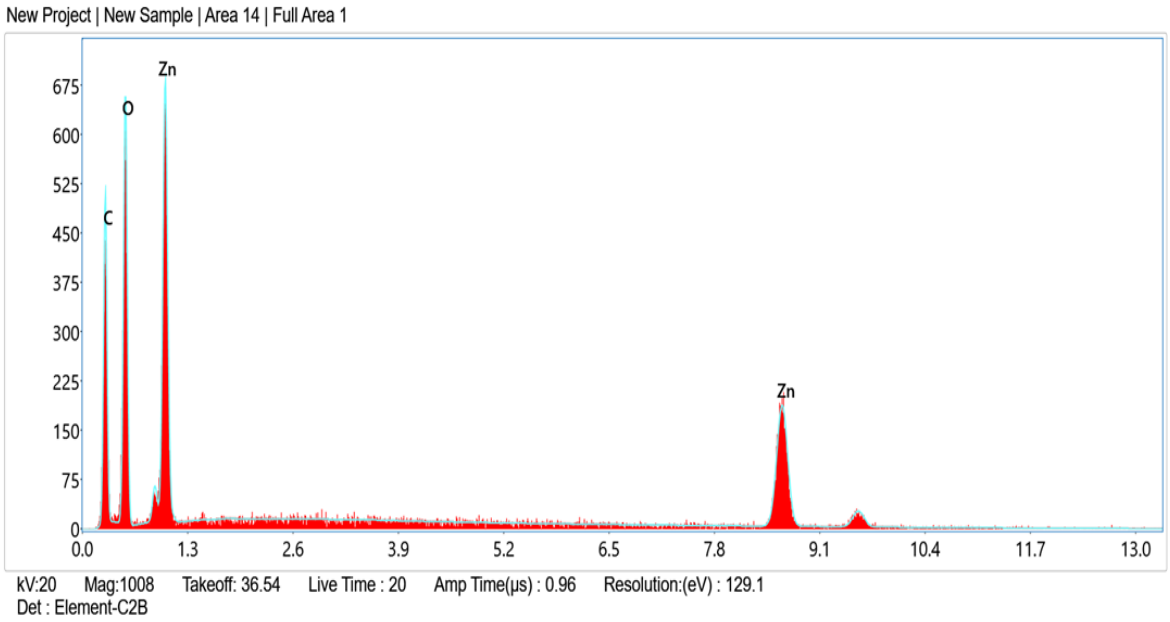


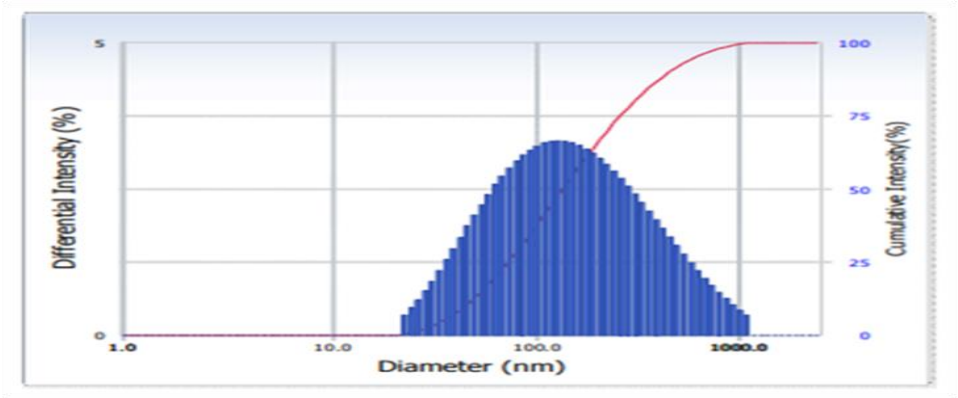
Table 3 shows the EDX results of UMB – ZnO NPs

Element	Weight %	Atomic %	Error %	Net Int.	R	A	F
C K	49.79	64.43	12.16	131.62	0.9078	0.0952	1.0000
O K	32.21	31.29	11.81	194.53	0.9182	0.0905	1.0000
Zn K	18.00	4.28	5.02	152.95	0.9787	0.9954	1.0792

DLS

The particle size of the synthesized nanoparticles was determined by dynamic light scattering (DLS) measurement technique which is used to determine the particle size by the random changes in the intensity of light scattered from a solution. This technique is also called as photon correlation spectroscopy (PCS) which is based on the laser diffraction method with multiple scattering technique employed to study the average particle size of zinc oxide nanoparticles. The DLS graph Umbelliferone-Zinc oxide nanoparticle revealed that the particle size of ZnONPs was in the range of 141.60nm as shown in Figure. [21]

Figure 6 shows DLS graph of UMB – ZnO NPs

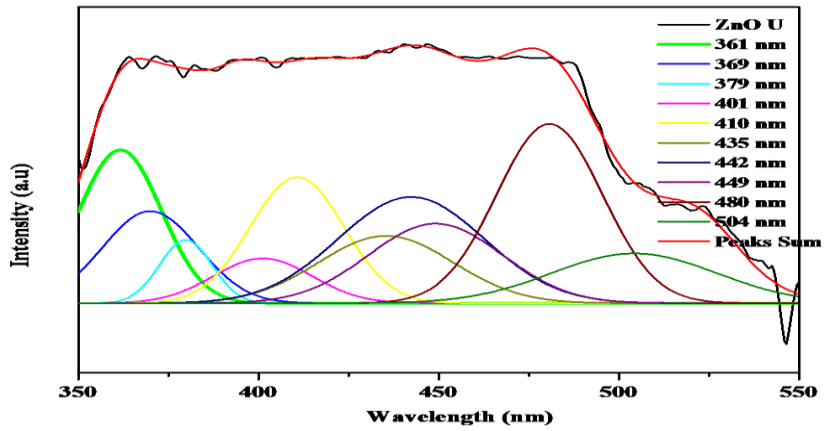


PL Spectra

Photoluminescence spectroscopy is an important tool to study the optical properties of semiconducting materials. Umbelliferone- Zinc oxide PL Spectrum exhibits key emission features in both the Ultraviolet and visible regions, as detailed by the spectral data and multi-peak fitting. The peaks shows a dominant, sharp emission band in the UV range and a broad, less intense emission spanning the visible range. The primary peak emission is observed,composed of multiple overlapping peaks centered around 361nm to 379nm.Such overlapping peaks at various wavelengths, including 361nm, 369nm, 379nm 401nm, 410nm, 435nm, 442nm, 449nm, 480nm and 504nm.The most intense visible peak is around 480 nm.

The peak spectrum has been deconvoluted into individualGaussian peaks to identify the specific contributing emission mechanisms. The presence of both strong near band edge emission and defect related visible emission is common in ZnO materials. The relative intensities of these bands can provide insights into the concentration and type of defects present in the sample [22]

Figure 7 shows the PL Spectra of UMB - ZnO NPs

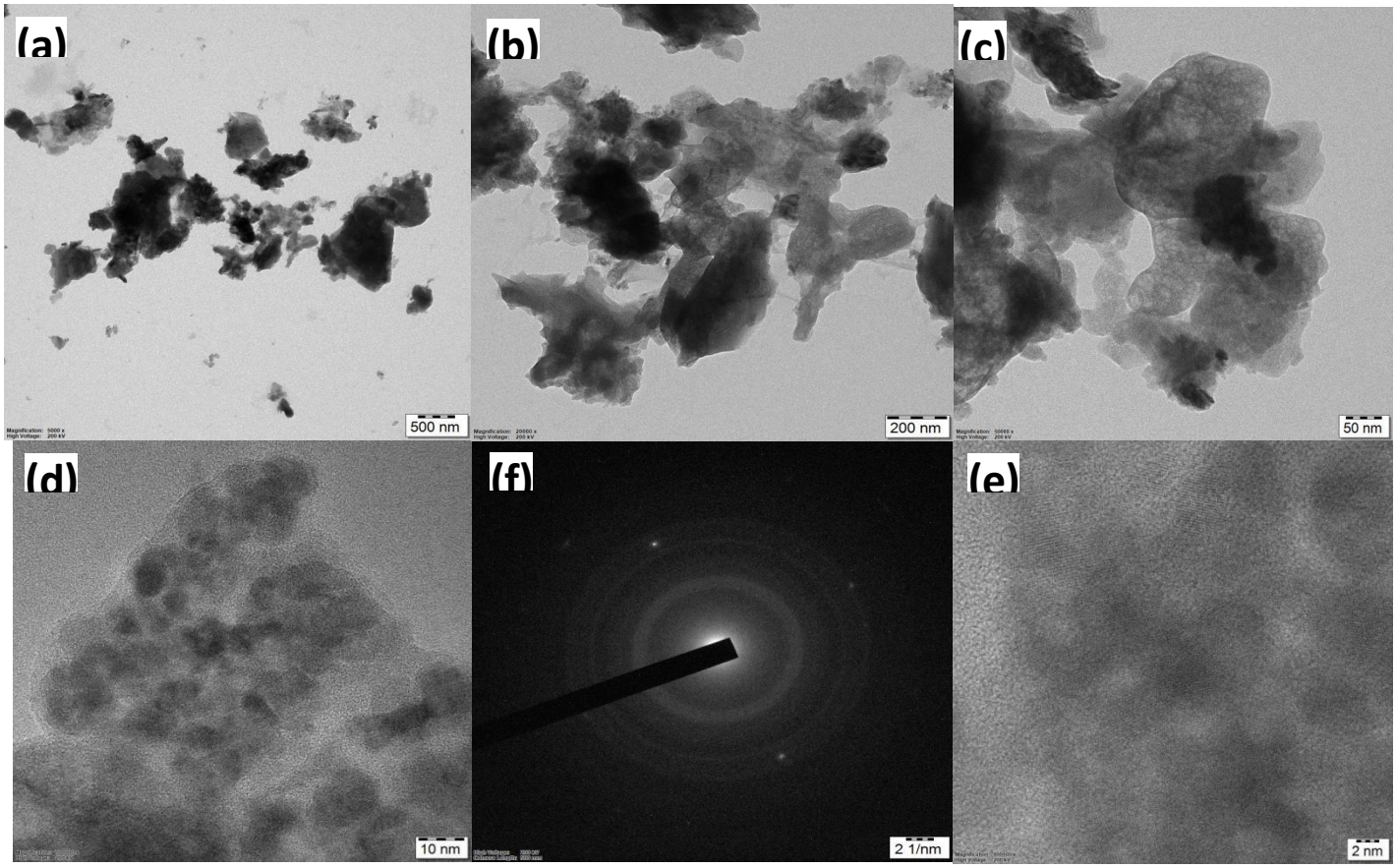


TEM

Transmission Electron Microscopy analysis was done using a Hitachi HF 3300 TEM machine. The size of the UMB-ZnO NPs was found. Images appear in the Transmission Electron Microscopy (TEM) which scale bars of 500nm, 200nm, 50nm, 10nm, 2nm respectively. The synthesized Umbelliferone zinc oxide nanoparticle appear to have a roughly spherical or irregular shape. The transmission electron microscopy images labelled display clusters of small particles. The individual particles do not exhibit a distinct, uniform geometric shape like rods, cubes, but rather then appears as agglomerated masses that are generally rounded or undefined in form [23].

Image shows a selected area electron diffraction (SAED) pattern with concentric rings rather than sharp, distinct spots. This pattern is characteristic of a polycrystalline or amorphous material, which is consistent with the visually observed irregular or non- uniform shapes sizes of the nanoparticles. Image f pattern indicating a crystalline structure.

Figure 8 shows TEM and SAED analysis of the UMB - ZnO NPs



3.3.1 Antimicrobial activity

The antimicrobial activity of the UMB-ZnONPs against various pathogens including *S. aureus, S. pneumoniae, B. Subtilis, K. pneumoniae, E. Coli, S. dysenteriae and fungi C.albicans* was analyzed using the well diffusion method.. The antimicrobial effectiveness of UMB–ZnO nanoparticles (UMB–ZnO NPs) is mainly due to a synergistic mechanism that includes the production of reactive oxygen species (ROS) by ZnO and the bioactive

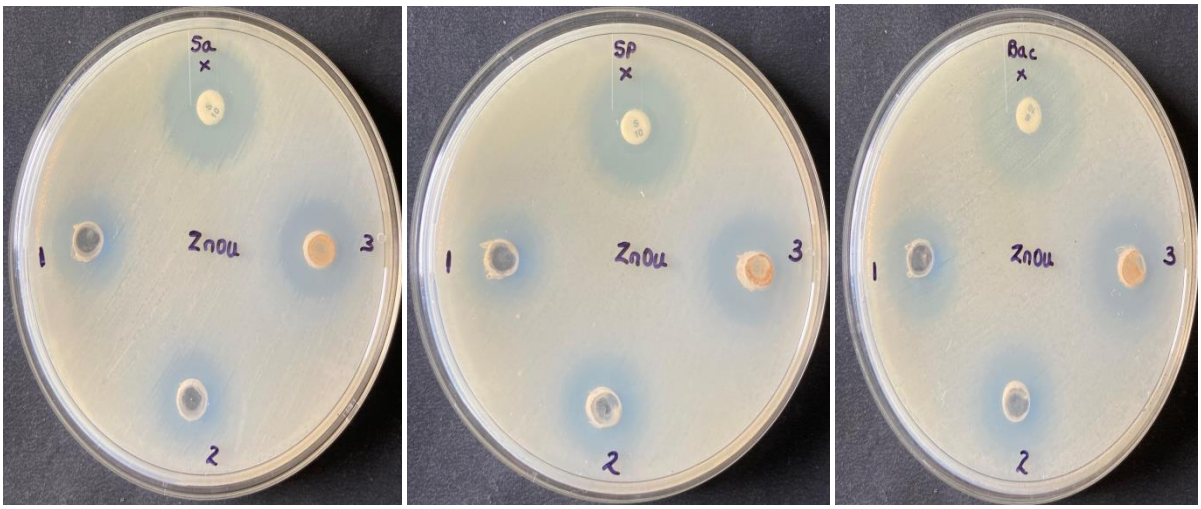
properties of Umbelliferone. When ZnO NPs come into contact with microbial cells, they produce reactive oxygen species (ROS), such as superoxide radicals, hydrogen peroxide, and hydroxyl radicals. These ROS cause oxidative stress. These ROS damage the membranes of bacterial and fungal cells by causing lipid peroxidation, which makes them more permeable and causes the contents of the cells to leak out. At the same time, the release of Zn^{2+} ions disrupt important metabolic pathways and enzymatic activities. Umbelliferone increases the effectiveness of antimicrobial drugs by getting through the cell wall of microbes and stopping the production of nucleic acids and proteins. The oxidative damage and metabolic disruption together cause DNA damage, enzyme inactivation, and eventually cell death. The treatment with the different concentrations of the synthesized NPs showed considerable antimicrobial activity against all the tested pathogens, which evidences the antimicrobial activity of the UMB-ZnO NPs.

The antimicrobial results of the UMB- ZnO NPs was confirmed by the results of standard antibiotic streptomycin. The maximum zone of inhibition was found on the S. *aureus, K. pneumonia, E. coli, and C. albicans* strains [24]. The antimicrobial study of Umbelliferon-ZnO NPs shows similarity [25] in zone of inhibition.

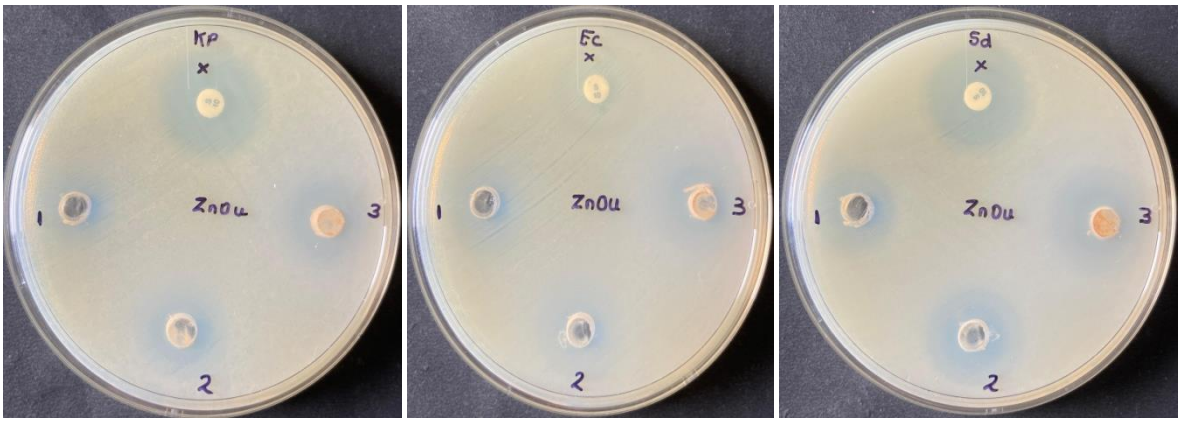
The present study shows zone of inhibition in various concentrations of UMB-ZnO NPs 1mg/ml, 2mg/ml, 3mg/ml *S. aureus* (17mm, 18mm, 22mm), *S. pneumoniae* (15mm, 18mm, 22mm), *B. Subtilis* (16.5mm, 19mm, 23mm), *K. Pneumoniae* (12mm, 17mm, 21mm), *E.coli* (13mm, 18mm, 22mm), *S. dysenteriae* (16mm, 19mm, 23mm), and *C.albicans* (16mm, 18mm, 22mm) [26].

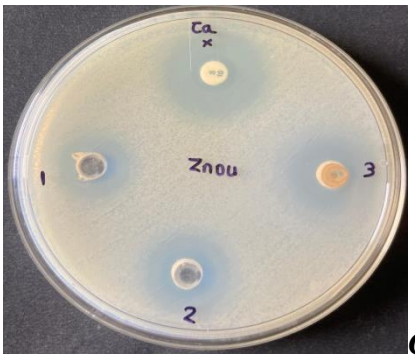
Figure 9 Depicts Agar Well Diffusion method of UMB-ZnO NPS Antimicrobial activity

Salmonella aureus ***Streptococcus pneumoniae*** ***Bacillus Subtilis***



Klebsiella pneumonia ***Shigella dysenteriae*** ***E.Coli***

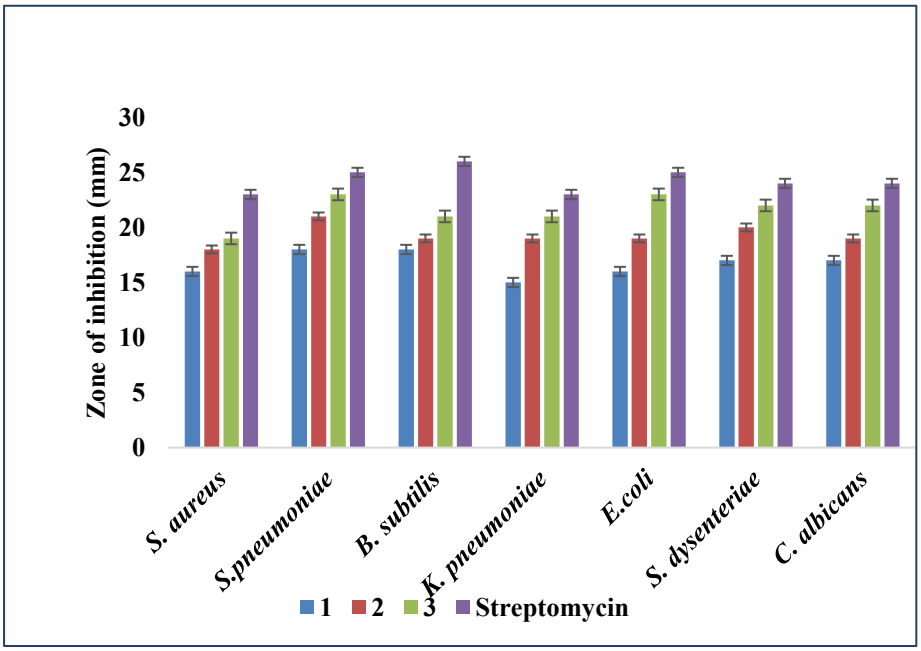




Candila albicans

Table 4 represents the Agar well diffusioin method result of UMB – ZnO NPs

S. No	Bacteria/Fungal names	Zone of Inhibition			
		UMB- ZnO NPs 1mg/ml	UMB-ZnO NPs 2mg/ml	UMB-ZnO NPs 3mg/ml	Standard Streptomycin
1.	*S. aureus*	17	18	22	24
2.	*S.pneumoniae*	15	18	22	25
3.	*B. subtilis*	16.5	19	23	29
4.	*K. pneumoniae*	12	17	21	25
5.	*E.coli*	13	18	22	24
6.	*S. dysenteriae*	16	19	23	26
7.	*C. albicans*	16	18	22	28



Graph represents the Antimicrobial activity of UMB – ZnO NPs

Conclusion

This paper has shown that a novel and sustainable method of green synthesis of Zno NPs with *Aegle marmelos* fruit pulp extracts can be achieved successfully. The findings of this study suggest that the umbelliferone dopped zinc oxide nanoparticle exhibited good antimicrobial activity against bacterial pathogens which indicates the immense potential of *A. marmelos* fruit pulp compound Umbelliferone as effective antimicrobial agent that can be used in various modern medicines. Additionally, the current work presents the cost effective and eco friendly procedure for the synthesis of zinc oxide nanoparticle by utilizing a renewable natural resource. Therefore, this study will be helpful in the development of value-added products from *A. marmelos* plants for biomedical, pharmceutical, biotechnological and nanotechnological-based industries and also could assist the discovery of new and effective herbal medicines to fight against drug-resistant bacterial infections using green routes. In future in vivo and in vitro studies are required for better understanding of toxicological aspects.

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