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Invitro Biomedical Applications of *Grewia Tilifolia* Mediated ZnONanoparticles

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Abstract: Nanomedicine discoveries have gone very impressive modifications and pushing the techniques to the new heights to get best outcome particularly in cancer treatment. Among these innovations, Nanoparticles made by green synthesis gaining more attention due to their superior properties over chemical and physical methods. This is because, green technique avoids the use of expensive chemicals, consume less energy and generate eco-friendly products. The aim of the present work is to synthesis green-based zinc oxide nanoparticles from *Grewiatilifolia* leaf extract (GT-ZnONPs) and to conduct invitro studies against MCF-7 cells. Characterization techniques like X-ray diffraction (XRD), transmission electron microscopy (TEM), and Fourier-transform infrared spectroscopy (FTIR), have been employed to elucidate the structural, morphological, and chemical properties of *Grewiatilifolia*-mediated ZnONPs. These analyses confirm the effective synthesis of crystalline ZnONPs with controlled size, distribution and surface functionalization. The prepared nanomaterials exhibited also significant antibacterial activity against various selected pathogens, and showed potential antioxidant activities. Moreover, GT-ZnONPs showed notable mortality on MCF-7 breast cancer cells thus revealed the potential of GT-ZnONPs in cancer therapy. This paves a way for the synthesis of GT-ZnONPs for different biological and nutraceutical applications like delay aging, prevent chronic diseases, treat cardiovascular diseases.

Keywords: *Grewiatilifolia*, biosynthesis, zinc oxide nanoparticles, characterization, antimicrobial activity, antioxidant, DPPH,invitro cytotoxicity.

Introduction

In the 21st century, Nanotechnology has major impact on global scale due its size ranging from 1-100nm [1,2]. Nanoscience and technology are an interdisciplinary field, which needs a scientific outlook from diverse areas of science and technology. The principles of chemistry, physics, biology, and engineering are integrated into the study of nanoscience [3]. nanoscience provides the foundation

for understanding and manipulating nanoparticles, while nanoparticles serve as building blocks for advancing nanoscience and its applications.

Compared to other nanoparticles inorganic nanoparticles have unique properties like optical, electronic, magnetic, versatility, tunability, stability and durability. Therefore, many researchers focus on synthesising non-toxicity, water-friendly, biologically acceptable inorganic nanoparticles, because of its non-toxicity, water-friendly, biologically acceptable and highly stable inorganic nanoparticles [5]. Inorganic nanoparticles include various types of materials. They consist of elemental metals, metal oxides, and metal salts. Each type has unique properties and applications. Metal oxide nanoparticles are used in agriculture, material science, clinics, environmental management, etc [7,9,10]. These nanoparticles have many unique features that have made them an ideal candidate for antimicrobial therapy in nanomedicine [6,8]. For example, they have been recognized and used as genotoxicity materials to study their genotoxicity potential and their possible health risks in man through human health [11,12]. Metal oxide nanoparticles are also critically important for various modern technologies. Hence, due to their applications in a wide range, these metal oxide nanoparticles have drawn some potency in all spheres of life starting from agriculture to healthcare [15]. The synthesis of nanomaterials has been done through many methods suchas the Chemical, Physical, and green methods [4]. Among the green synthesis of nanoparticles, a best approach for synthesis is plant extract mediated nanoparticles which is most biocompatible, effective, low-cost and safe [13,14]. Furthermore, in plant extract mediated nano synthesis, plant extract itself acts as stabilizing and reducing agents in nanomaterials synthesis [16].

Recently, studies have proven that green synthesized zinc oxide nanoparticles (ZnO NPs) using a number of plant extract have got a lot of success as they displayed significant biological activities [17,18]. Free radicals can also be scavenged by these nanoparticles and on the other hand they perform anticancer action by enhancing apoptosis in cancer cells. Furthermore, a possible antidiabetic activity of the ZnO NPs was evidenced, considering the enzyme inhibitory and cytotoxic effects on some cell lines, pointing them as promising agents for the treatment of diabetes and cancer [19]. These demonstrated multilayered biological functionalities of these ZnO NPs, which reinforces their potential applicability in biomedical and nutraceutical materials. Plant extracts-mediated zinc oxide nanomaterials have an effective remedy for cancer, hepatitis, malaria, and other acute diseases [20, 21].

Grewiatilifolia is a flowering plant. The common name of *Grewiatilifolia* is Dhaman belongs to the family *Malvaceae*. It is a traditional medicinal plant used for diarrhoea, wound healing, skin diseases etc, [22]. The Dhaman fruit is also used to treat blood disorders, heart diseases and respiratory issues. *Grewiatilifolia* holds many phytomolecules, which makes the plant to have medicinal values against pathogenic diseases. The main advantage of using plant extract is to reduce the toxicity of nanoparticles and unnecessary usage of toxic chemicals. Thus, the

present study aimed to biosynthesis and characterize the *Grewiatilifolia* leaf extract-mediated ZnONPs and their applications.

Materials and Methods

Collection of plant and extract preparation

Plant material *G.Tilifolia* were collected from yercaurd Hills, Tamil Nadu. The disease-free plant part (leaf) was spread out and dried in the laboratory at room temperature for 8-10 days until they were easily broken by hand and grounded to a fine powder using an electronic blender. About 20 g of dried and powdered plant material (leaves) were soaked separately with 250 mL of the solvent alcohol, in a soxhlet apparatus for 24 hrs until complete extraction of the materials. The filtrate was stored in the refrigerator for further qualitative screening and nanoparticle preparations.

Phytochemical screening of methanol extract of *G.Tilifolia*

Phytochemical analysis is very important process to identify the bioactive compounds present in plant material. The following standard qualitative screening tests were carried to detect the various bioactive compounds present in methanolic extract of *G.Tilifolia*[24].

Detection of alkaloids

Extract was dissolved in dilute hydrochloric acid and filtered. The filtrate was used to test the presence of alkaloids.

a) Mayer's test: Filtrate was treated with Mayer's reagent. Formation of a yellow cream precipitate indicates the presence of alkaloids.

b) Wagner's test: Filtrate was treated with Wagner's reagent. Formation of brown/reddish brown precipitate indicates the presence of alkaloids.

Detection of Flavonoids

a) Lead acetate test: Extract was treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavanoids.

b) H_2SO_4 test: Extract was treated with few drops of sulphuric acid. Formation of orange colour indicates the presence of flavanoids.

Detection of Steroids

Liebermann-Burchard test: 2 ml of acetic anhydride was added to 0.5 mL of the extract, each with 2 mL of Sulphuric acid. The colour changed from violet to blue or green indicate the presence of steroids.

Detection of Terpenoids

Salkowski's test: 0. 5 mL of the extract was mixed with 2 mL of Chloroform and 3 mL of Concentrated Sulphuric acid was carefully added to form a layer. A reddish-brown colouration of the inner face indicates the presence of terpenoids.

Detection of Anthraquinones

Borntrager's test: About 0.2 mL of the extract was boiled with 10% hydrochloric acid for few minutes in a water bath. It was filtered and allowed to cool. Equal volume of chloroform was added to the filtrate. Few drops of 10% ammonia were added to the mixture and heated. Formation of pink colour indicates the presence of anthraquinones.

Detection of Phenols

a) Ferric chloride test: Extract were treated with few drops of 5% ferric chloride solution. Formation of bluish black colour indicates the presence of phenol.

b) Lead acetate test: Extract was treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of phenol.

Detection of Saponins

Froth test: About 0.2 mL of the extract was shaken with 5 ml of distilled water. Formation of frothing (appearance of creamy stable persistent of small bubbles) shows the presence of saponins.

Detection of Tannins

Ferric chloride test: A small quantity of extract was mixed with water and heated on water bath. The mixture was filtered and 0.1% ferric chloride was added to the filtrate. A dark green colour formation indicates the presence of tannins.

Detection of Carbohydrates

Fehling's test: 0.2 mL filtrate is boiled on water bath with 0.2 ml each of Fehling solutions A and B. A red precipitate indicates the presence of sugar.

Detection of Oil and Resins

Spot test: Test solution was applied on filter paper. It develops a transparent appearance on the filter paper. It indicates the presence of oils and resins.

Biogenic synthesis of ZnO nanoparticles and its characterization

To the 25 mL of plant extract 25 mL of 1mM solution of zinc acetate dihydrate was added. The reaction mixture was kept on magnetic stirrer for 1 hr. After 1 hr, 20 mL of NaOH was added to the solution and it was placed in magnetic stirrer for 3 h for the formation of white precipitate and for the complete reduction of zinc [25,26]. The prepared ZnO nanoparticles were collected and washed thrice with double distilled water and oven dried 70°C to get powdered ZnO nanoparticles. The biosynthesised GT-ZnONPs sample were stored at room temperature in

airtight container for further characterization and application studies [27,28,29]. The bio-synthesized GT-ZnONPs were confirmed with the help of characterisation studies like UV-visible, FT-IR, SEM, TEM andXRD etc.

DPPH Assay

The antioxidant activity of biosynthesised GT-ZnONPs was done by diphenylpicrylhydrazyl (DPPH) assay. Different volumes of (50, 250, 500, 750 and 1000 µL) synthesised GT-ZnONps were mixed with 1mL of 0.1mM DPPH solution. The reaction mixtures were incubated in dark condition at room temperature for 30 minutes. After 30 min, the absorbance of the mixture was read at 517 nm [30,31]. The % radical scavenging activity was calculated using standard formula.

$$\% \text{ RSA} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Invitro Antimicrobial activity

Antimicrobial activity of GT-ZnONPs was investigated against some selected pathogens by the disc diffusion method [32,33]. All the bacterial cultures (*E.coli, S.aureus*) and fungi cultures (*A.niger, C.albicans*) were purchased from MTCC, institute of Microbial Technology, Chandigarh, India. Muller Hinton agar (MHA) was used for screening invitro antimicrobial activity. The MHA plates were prepared with molten media (15 mL). The prepared MHA plates were solidified for 5 min and inoculum suspension (0.1%) was uniformly applied over MHA medium and allowed to dry. Different concentration (30, 40, 50 and 60 µL) of GT-ZnONPs was filled on 6 mm sterile disc. Disc with embedded sample was kept on the top layer of MHA medium and the plates were kept for incubation (37 °C) for 1 day. After the incubation, inhibition zones were observed around the disc, which shows the inhibition effect of GT-ZnONPs on selected pathogens. Then the inhibition zones were measured in mm scale.

Invitro cytotoxicity study of synthesised GT-ZnONPs

Invitro cytotoxicity study of biosynthesised GT-ZnONPs was studied with MCF-7 (Michigan cancer foundation-7) [34,35]. A single cell suspension was prepared from tripsin-EDTA. The diluted cell solution (density of 1×10^5 cells/mL) was prepared with 5 % FBS (Fetal bovine serum). Then 100 µL/well solutions were planted onto 96-well plates at a cell density of 10,000 cells/well and incubated at 37 °C for one day. The medium containing grown cells were inoculated with different concentrations (50, 250, 500, 750, 1000 µg/mL) of the ZnONPs and kept inside the incubator (37 °C, 5 % CO_2, 95 % air and 100 % relative humidity) for 24-48 h. Then 15 µL of MTT (3-[4,5-dimethylthiazol-2-yl] 2,5-diphenyltetrazolium bromide) was added to 5 mg/mL phosphate-buffered saline (PBS) solutions in each plate [36]. The plates were kept inside the incubator for 4 h at 37 °C. The yellow solution of MTT was reduced to the purple formazan crystals. Then, the crystals were

dissolved with 100 µL of solubilization liquid dimethyl sulfoxide and the optical density was recorded at 570 nm using a microplate reader (EMR500-Labomed).

Results and discussion

Chemical screening

Chemical screening is also known as phytochemical screening. The secondary metabolites present in *G.Tilifolia* was studied by standard phytochemical screening test. Phytochemical screening tests reveal the occurrence of alkaloids, flavonoids, steroids, phenols, saponins, tannins and carbohydrates, among various other constituents. These metabolites serve as effective capping agent for the synthesis of ZnONPs.

Table 1: Phytochemical screening of *GrewiaTilifolia* leaf extract

Phytochemicals	**Test**	**Result**
Alkaloids	Mayer's test	++
	Wagner's test	
Flavonoids	Lead acetate test	++
	H_2SO_4 Test	
Steroids	Liebermann-Burchard test	+
Terpenoids	Salkowski test	-
Arthroquinone	Borntrager's test	-
Phenols	Ferric chloride test	++
	Lead acetate test	
Saponin	Froth test	+
Tannin	Ferric chloride test	+
Carbohydrates	Fehling's test	+
Oils and Resins	Spot test	-

Note : (-) Negative=Absent, (+) Positive= Present

UV-Vis Analysis

The UV-Visible spectrum of alcoholic extract of *G.Tilifolia* and biosynthesised GT-ZnONPs were taken at the wavelength between 300 to 800 nm. *G.Tilifolia*

spectrum (Figure 1a) shows highest peaks at 344 nm and 611 nm. The peak at 344 nm indicates the presence of conjugated double bonds or aromatic rings. The peak at 429 nm suggests the presence of carbonyl and azo compound. Absorption at 503 nm indicates certain organic dyes. Absorbance peak at 611 nm falls in the visible region. This indicates that the alcoholic extract contains starch, protein and antioxidant compounds. These compounds are responsible for the reduction of zinc (II) ions. In the spectrum of biosynthesised GT- ZnONPs (Figure 1b), the maximum absorption peak was observed at 344 nm. The sharp absorption peak confirms the formation of ZnOnanoparticles[37,38].

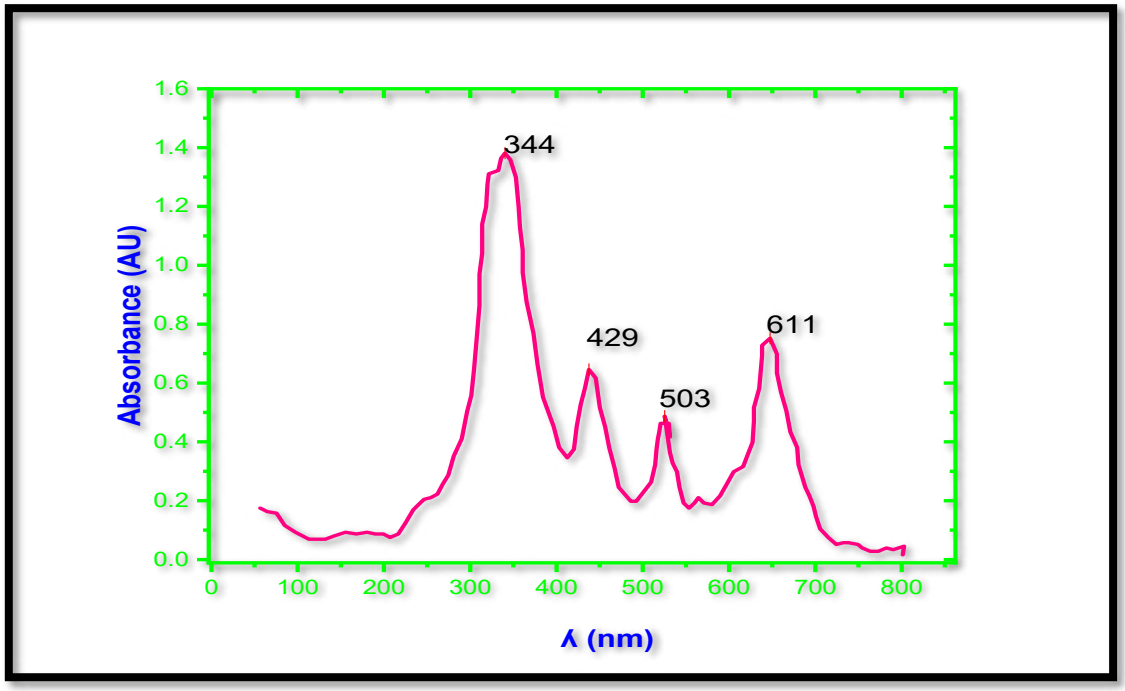

Figure 1a. UV Spectrum of *G.tilifolia*

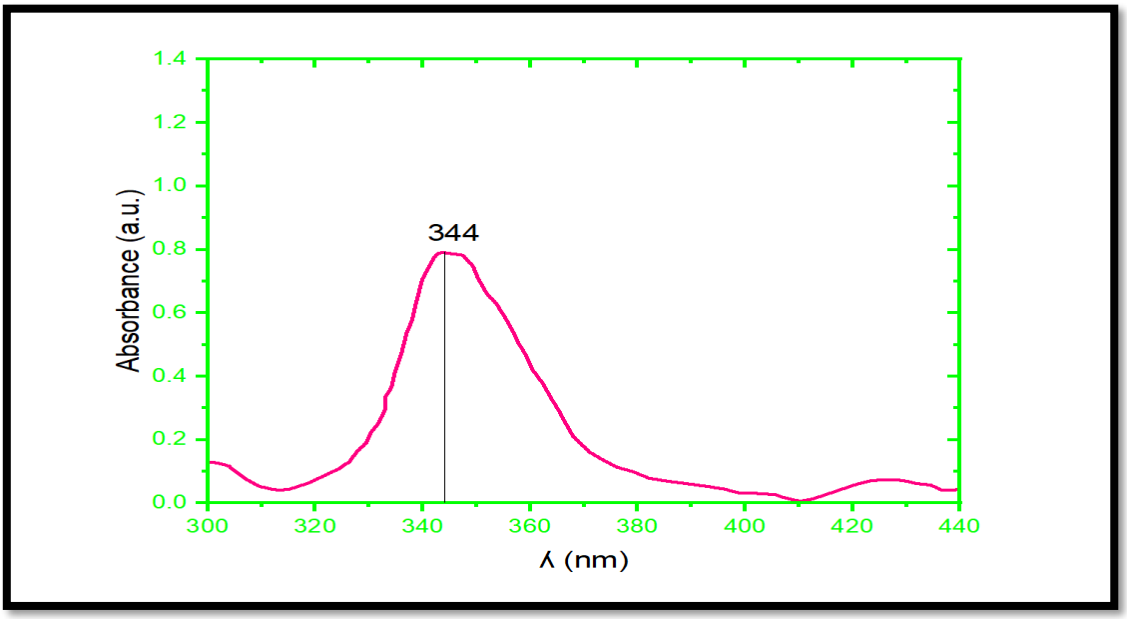

Figure1b. UV-visible spectrum of *G.tilifolia*

FT-IR analysis

The FT-IR spectrum of alcoholic extract of *G.Tilifolia* and biosynthesised GT-ZnONPs are shown in figures 2a and 2b. In figure 3a, Peaks are observed at 3340.71, 2978.09, 2893.22, 2831.50,1658.78,1327.03, 1273.02, 1087,1041, 879, 671, 470, and 424 cm^{-1}. The peak at 3340.71cm^{-1} is associated with OH stretching in alcohol. The peaks from 2978.09 cm^{-1} to 2831.50cm^{-1} is related to C-H stretching vibration in alkene group, the peak at 1658.78 cm^{-1} is attributed to N-H stretching in amines, the peak at 1327.03 is attributed to C-H stretching in alkane, the peaks at 1087$cm^{-1}$and 1041cm^{-1}indicates C-OH bonds in polysaccharides or in protein. The peaks at 470cm^{-1} and 424cm^{-1} denotes the C-H bending vibration in alkene. In FT-IR spectrum of GT-ZnONPs (figure 2b), Spectral peaks observed at 3132, 2841, 1990, 1677, 1381 and 841 cm^{-1}, confirms the aromatic C-H stretching, C-H stretching in alkanes, C=C stretching in alkene, substituted benzene ring system. The small peak at 649.84 cm^{-1} indicates the formation of hexagonal wurtziteZnO nanoparticles.

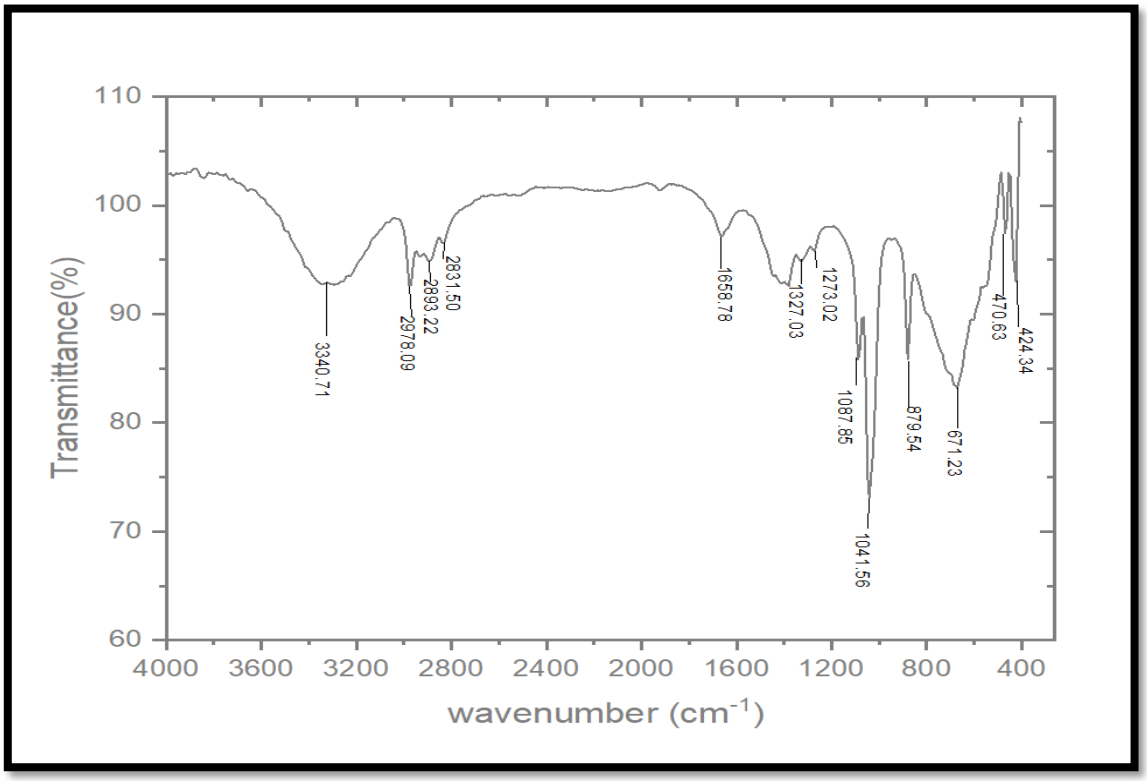

Figure2a. FT-IR *G.tilifolia*

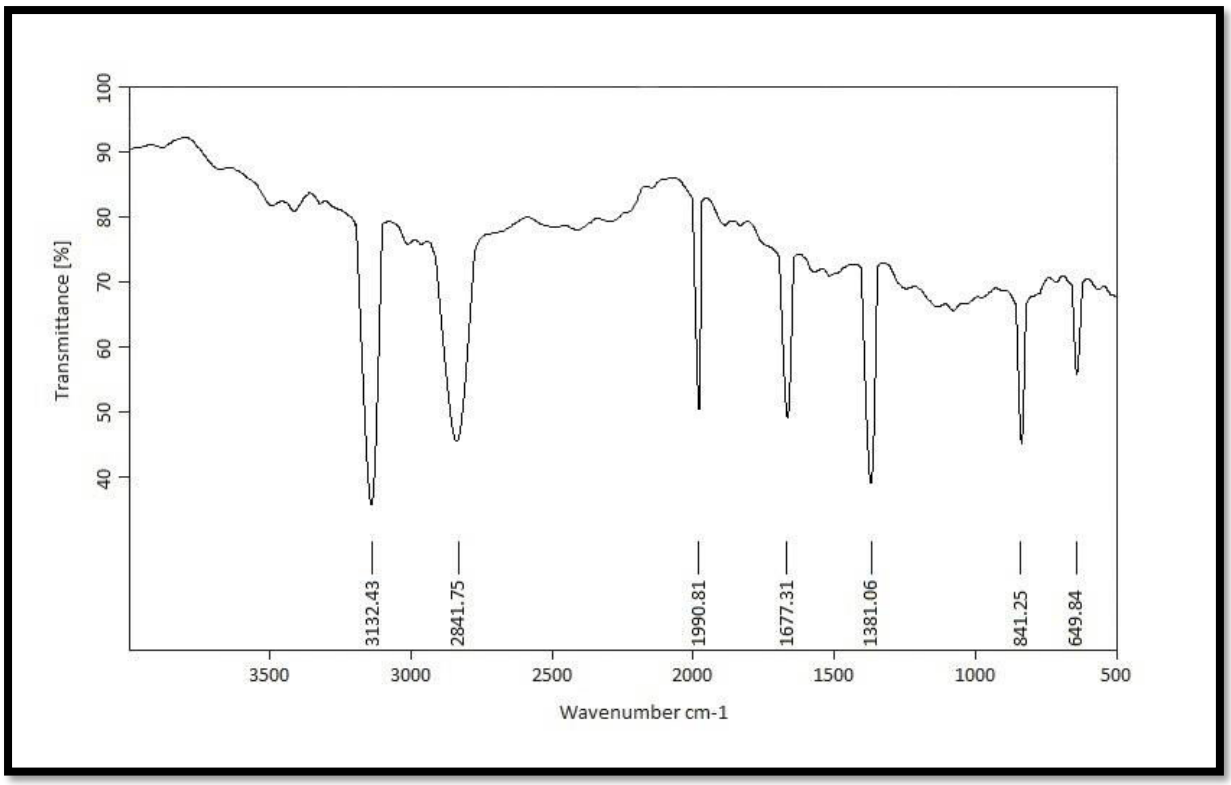

Figure2b. FT-IR GT-ZnO

SEM analysis

The scanning electronic microscope of biosynthesized GT-ZnONPs (figure 3a) shows that the particles are agglomerated and inclined together due to the presence of capping agent [39]. This capping agent stabilises the nanoparticles. Figure 3b is the EDAX analysis of GT-ZnONPs. The composition of zinc is 38.42% and oxygen is 18.15%.

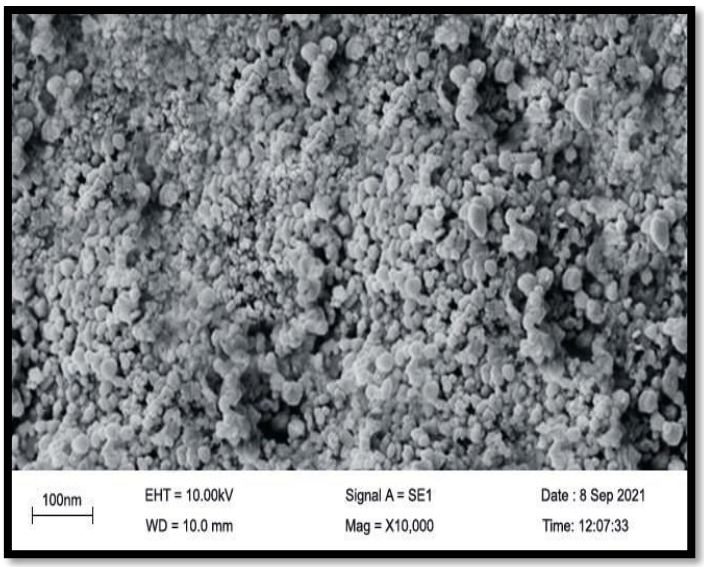

Figure3a. SEM image of GT-ZnO

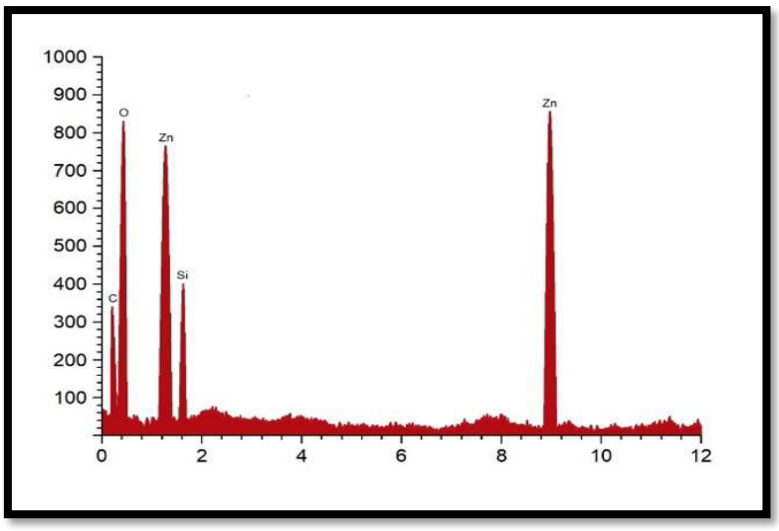

Figure3b. EDAX spectrum of GT-ZnO

XRD analysis

X-Ray diffraction study of biosynthesized GT-ZnONPswas shown in figure 4. *G.Tilifolia*mediated ZnONPsshowed diffracted peaks with different 2θ values. This result is well exactly matched with JCPDS-36-1451 data card. The values obtained from the diffraction peaks of GT-ZnONPs proves that the structure of synthesised ZnONPshas hexagonal wurtzite structure, and is closely matched with other articles [40,41]. Average crystalline size of GT-ZnONPs was found to be 23.45 nm using Debye Scherrer's equation and it is tabulated as follows (Table 2).

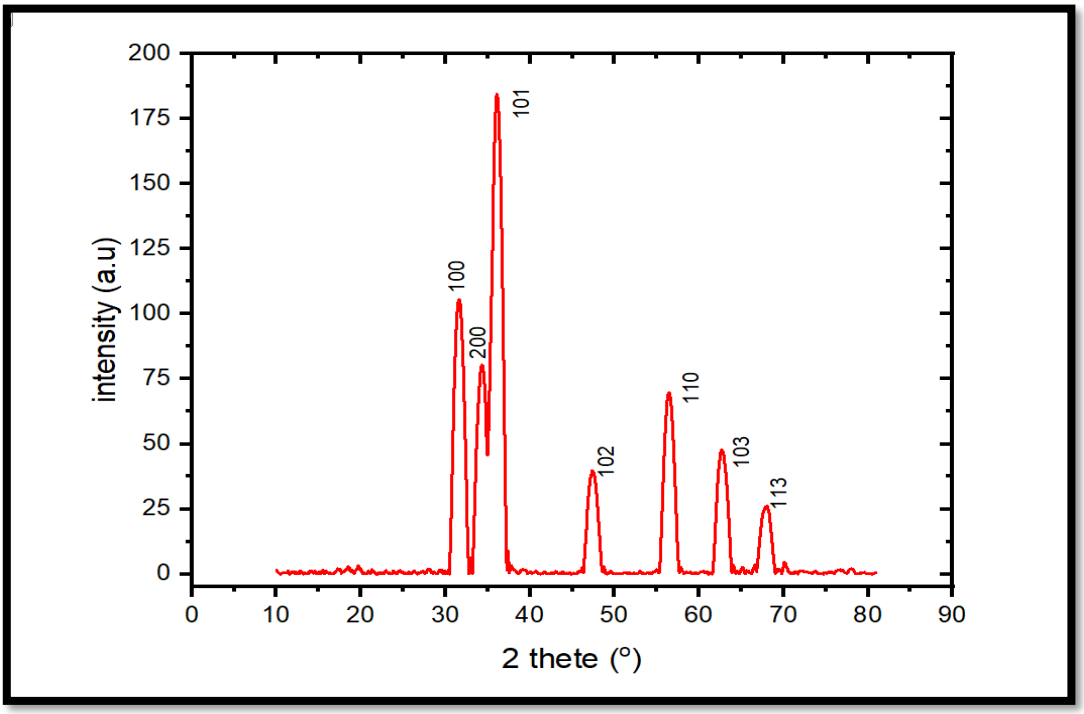

Figure4. XRD of GT-ZnO

Table 2. Average crystalline size of GT-ZnONPs

S.NO	2Θ	FWHM	D(Å)
1	31.81	0.4000	20.65
2	34.51	0.4000	20.79
3	36.31	0.4000	20.90
4	47.61	0.4000	21.70
5	56.66	0.6000	15.04
6	62.96	0.6000	15.52
7	66.48	0.3500	27.13
8	68.01	0.2000	47.91
9	69.11	0.4500	21.43

TEM analysis

The morphology and size of the biosynthesised GT-ZnONPs were confirmed with TEM analysis as shown in figure 5. This indicates that the synthesised nanoparticles are agglomerated, spherical, and hexagonal shaped particles. This is well supported with the XRD values.

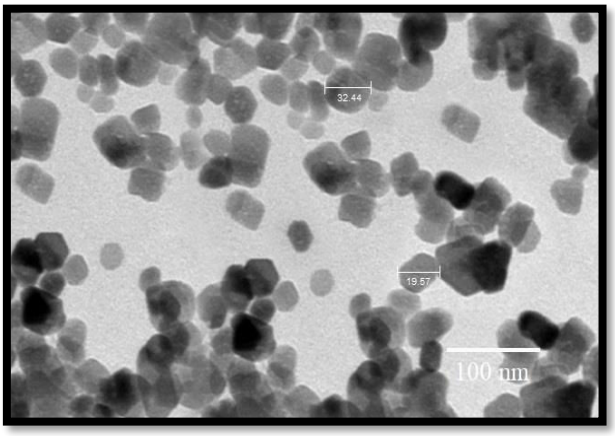

Figure5. TEM Analysis of GT-ZnO

Anti-oxidant activity- DPPH Assay

Anti-oxidants can be either man made or naturally obtained. It may prevent or delay the cell damage caused by some free radicals (unstable molecules). The antioxidant quality is tested in *G.tilifolia* mediated ZnONPs with DPPH assay. The deep purple colour of DPPH was changed to pale yellow by adding different concentration of test samples. Then, the absorbance of the samples was observed with UV-Vis Spectrometer at 517 nm and the data is noted in table 3. The scavenging effect of *G.Tilifolia* mediated ZnONPs are shown in figure 6. The results

confirm that on increasing the concentration of GT-ZnONPs shows good anti-oxidant activity.

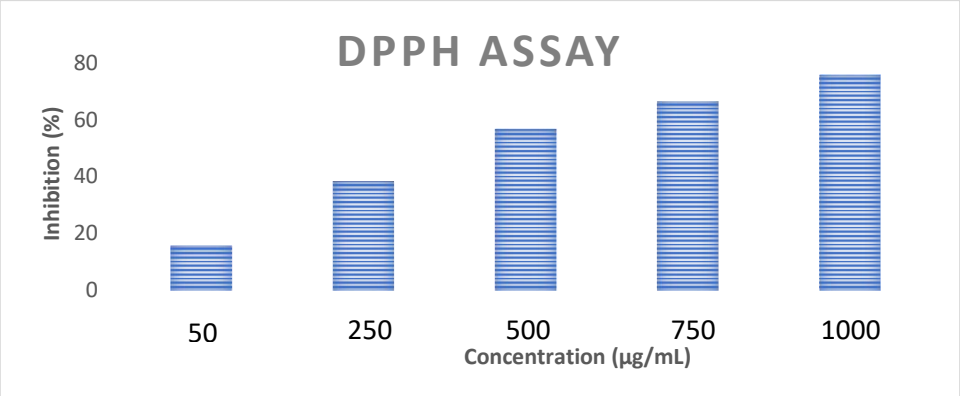

Figure 6. Anti-oxidant activity of GT-ZnO

Table 3. %RSA and IC50 of GT-ZnONPs

Concentration µg/ml	sample	% RSA	IC50
50	0.438	15.76	2.95
250	0.321	38.46	16.48
500	0.225	56.73	33.38
750	0.175	66.34	50.29
1000	0.126	75.76	67.19

Cytotoxicity Study of ZnO nanoparticles

The in vitro anticancer study of GT-ZnONPs against MCF-7 breast cancer cell line and the percentage of cell inhibition efficiency was done by MTT assay. Some structural variation like cell shrinkage has been observed, when different concentration (50, 250,500, 750, 1000 µg/mL) GT-ZnONPs were added to the MCF-7 cell line. The structural changes (intracellular suicide) as shown in figure 7 confirms that the MCF-7 breast cancer cell line growth was reduced with a higher concentration of GT-ZnONPs. Growth of cell line (apoptosis) were completely inhibited with 1000 µg/mL of GT-ZnO NPs. A measure of effectiveness of GT-ZnONPs on killing cancer was found to be 611.66 µg/mL. This cytotoxicity studies reveals that on increasing the dosage of ZnONPs reduces the cell viability. From the resultant data, biosynthesised GT-ZnONPs could become a drug to challenge the deadly disease cancer in the future.

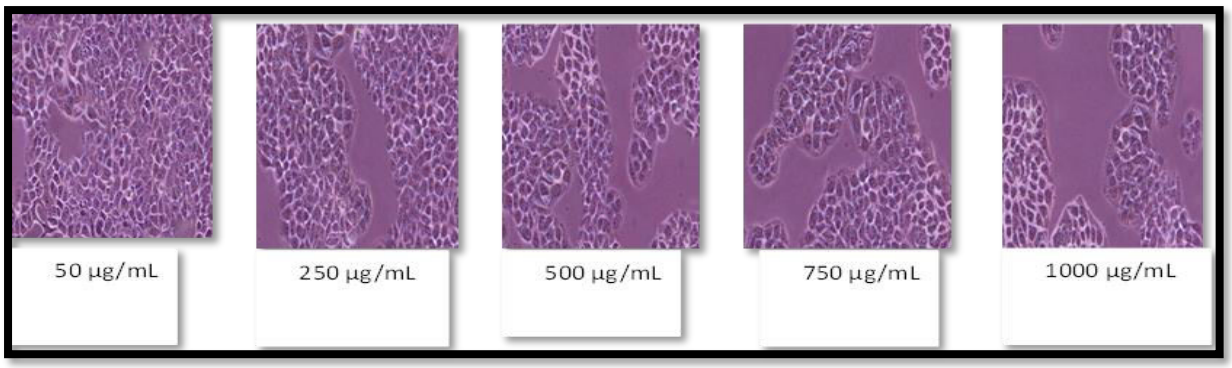

Figure7. Invitro cytotoxicity of GT-ZnO

Table4. Cell viability and CTC50 value of GT-ZnONPs

Concentration (µg/mL)	CTC_{50}	$\%CTC_{50}$	Cell Viability (%)
50	20.45	611.66	79.55
250	39.26		60.74
500	44.38		55.62
750	56.85		43.15
1000	66.26		33.74

Antimicrobial activity

Antimicrobial activity of *G.Tilifolia* mediated ZnONPs were studied against some pathogens like *E.coli, S. aureus, A. niger* and *C. albicans* using disc diffusion method [42,43]. Images 8a and 8b represents the antimicrobial activity and its graphical representation of GT-ZnO against bacteria and fungus. Antibacterial and antifungal activity of plant extract was considered to be good if its MIC was above 50 mg/ml, moderate if MIC was from 30-40 mg/mL and poor if it is 10 mg/mL.The results of antimicrobial activity of GT-ZnONPs were tabulated below (Table 5). The plant mediated ZnO nanoparticles was found to possess better inhibition value for *E. coli* as 20 mm for 60 µg/mL when compared with standard Chloramphenicol as 18 mm. The biosynthesised GT-ZnO was found to have better inhibition value for *A. niger* and *C.albicans* as 16 mm for 60 µg/mL when compared with standard Fluconazole as 19 mm. The biosynthesized ZnO nanoparticle from *G. tilifolia* shows very good susceptibility against E.*Coli, S.aureus,* and moderate inhibition efficiency against*A. niger, and C. albicans* at 60 µL. The inhibition zone increases, when the concentration of GT-ZnONPs increases.

E.coli *S. Aureus* *C.albicans* *A.niger*

Figure8a. Antimicrobial activity of GT-ZnO

Table 5. Antimicrobial activity of GT-ZnONPs

Organism	**Control**	**Concentration in µL**			
		30	**40**	**50**	**60**
Escherichia coli	18	10	12	13	20
Staphylococcusaureus	19	9	13	14	18
Aspergillusniger	20	11	12	13	16
Candida albicans	19	11	13	15	16

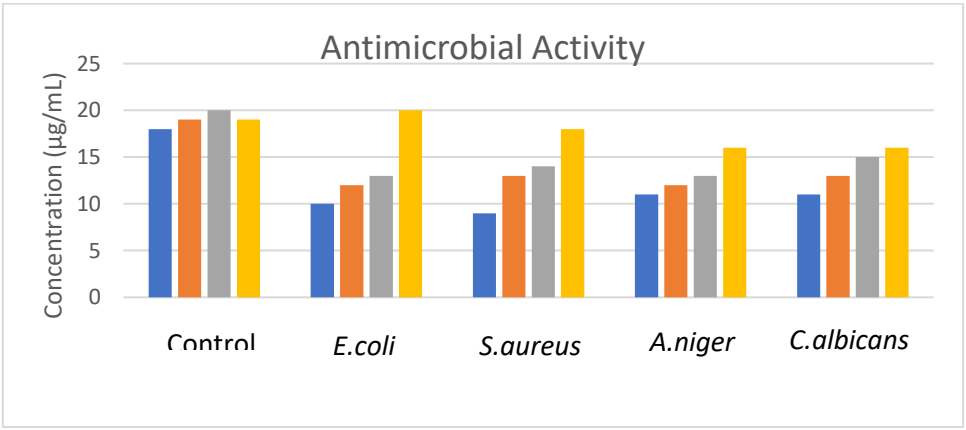

Figure8b. Graphical representation of antimicrobial activity of GT-ZnO

Conclusions

Nanoparticles has wide range of applications in many fields, particularly in medical field. But to synthesise the nanoparticles by chemical and physical methods need toxic chemicals and it will create many health hazards and also pollution in environment. To avoid such conditions, nowadays people are searching for lively products or plant-based products. This prompted me to do my research in natural way. Green synthesis of ZnONPs used in the research work was found to be eco-friendly, nontoxic, and less usage of chemicals than the physical and chemical methods. The phytochemicals present in the leaf extract itself help in the synthesis of nanoparticles. The functional groups that induce the nanoparticle synthesis were alcohols and phenols that are widely seen in secondary metabolite flavonoids The anti-oxidant and anti-cancer activities of *Grewiatilifolia*-mediated zinc oxide nanomaterials were excellently evidenced its pharmacological applications and also from the research the methanolic extract of *Grewiatilifolia* can act as a very good bio-reductant for the synthesis of zinc oxide nanoparticles. This suggests that GT-ZnONPs may have demand in biological and dietary supplements. Further studies needed to prove the potential effect of biosynthesised zinc oxide nanoparticles using *Grewiatilifolia* extract.

Limitation

The exact mechanisms by which the phytochemicals in *Grewiatilifolia* contribute to the reduction and stabilization of ZnONPs were not fully explained in this study. More detailed investigations into the specific roles of individual phytochemicals are needed. Future studies should include a broader range of biological evaluations, such as toxicity assessments in various cell lines and in vivo studies, to establish the safety and effectiveness of GT-ZnONPs.

Highlights

- Developed zinc oxide nanoparticles (GT-ZnONPs) using Grewiatilifolia leaf extract, promoting eco-friendly and cost-effective methods.
- Confirmed the effective synthesis of crystalline ZnONPs with controlled size and surface functionalization.
- Demonstrated significant antibacterial effects against various pathogens.
- Induced significant mortality in MCF-7 breast cancer cells, highlighting potential in cancer treatment.

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