



Anticancer and Antimicrobial Activity of Biosynthesized *Pergularia daemia* Leaves Mediated Zinc Oxide Nanoparticles

A. AGILA^{1*}, R. DAKSHAYANI², J. ROSALINE VIMALA¹, M. STELLA BHARATHY¹,
S. MARGRAT SHEELA¹, S. VIMALA¹ and M. NIVETHA¹

¹Department of Chemistry, Holy Cross College (Autonomous), Affiliated to Bharathidasan University, Tiruchirappali-620002, Tamil Nadu, India.

²Properitor, VRD consultancy, Thanjavur-613007, Tamil Nadu, India.

*Corresponding author E-mail: agila@hcctrichy.ac.in

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ABSTRACT

This study explores the medicinal potential of *Pergularia daemia*, a perennial vine abundant along Indian roadsides, focusing on its leaves have diverse therapeutic properties in traditional medicine. This research investigates its unexplored potential in application of drug delivery and the synthesis of zinc oxide nano-particles from its leaves. PD mediated Zinc Oxide nanoparticles were synthesized by precipitate method. The qualitative phyto chemical analysis of PD leaves extract was carried out and the extract showed the presence of carbohydrates, phenols, flavanoids, alkaloids and terpenoids. The synthesized zinc oxide nanoparticles were characterized by UV–Visible spectroscopy, Fourier transform Infrared spectroscopy, X-ray diffraction Analysis and scanning electron microscope. The results from characterization suggest that the synthesized compound was ZnO in nano scale which was in oval shape and with high purity. The synthesized zinc oxide nano particle could be a huge application in medical field, pharmaceutical and biotechnology in near future.

Keywords: Pergularia daemia, Zinc oxide, Nanoparticles, Characterization and phytochemicals.

INTRODUCTION

Pergularia daemia, a member of asclepidaceae family, is a perennial vine commonly found along Indian roadsides. Its aerial parts possess diverse medical properties, serving as anti-helminthic, antiseptic and anti-venom agents, along with treating gastric ulcer and menstrual issues. Furthermore, its leaves are utilized in addressing ailments such as arthritis and respiratory conditions (Ananth *et al.*, 2021). Though the medicinal effect

of PD leaves were known for ages in medicinal and home cooking, its ability in drug delivery and synthesis of ZnO nano-particle from its leaves were still exploratory and this study is an attempt to explore the underexploited area.

Nanotechnology, encompassing nanoscience, involves the synthesis and utilization of nano-sized particles, typically ranging from 1 to 100nm, finding extensive applications across energy, chemicals, healthcare and cosmetic sector. Notably



among metal oxide nanoparticles like CuO, ZnO and Co₃O₄, titanium dioxide (TiO₂) nanoparticles stand out for their remarkable stability, insolubility in water and distinctive magnetic, thermal, optical and electrical characteristics (Stella Bharathy *et al.*, 2021) & (Agila, Aranganathan, Vimala, Joseph Devaraj Rosaline, Bharathy, Moses Stella, Dayana jeyaleela, Gnanasekar, sheela, 2022). Zinc oxide nano-particles (ZnONPs) exhibit remarkable therapeutic efficacy, particularly in combating and treating infectious diseases. Moreover, zinc oxide (ZnO) particles demonstrate wound-healing attributes, antioxidant capabilities and even display anticancer properties (Agila, Aranganathan, Dayana Jeyaleela, Gnanasekar, vimala Joseph devaraj Rosaline, Bharathy, Moses Stella, Sheela, 2022). Owing to its numerous health potential of ZnO, the production of ZnO nanoparticle becomes the major part of study from potential source.

MATERIALS AND METHODS

All the chemicals require for performing the synthesis of zinc oxide nano particle and analyzing its properties were done using chemicals procured from Sigma-Aldrich. The reagents used for analysis were of pure analytical grade and the water used were double distilled throughout the experiment.

Extract production using *Pergularia daemia* leaves

Pergularia daemia leaves (raw material) for synthesis of zinc oxide nanoparticle were procured from Sekkakudi, Sivagangai district, Tamil Nadu. 100 g of crushed dried leaves of PD was mixed with 70 mL methanol i.e. 70% methanolic solvent. The above solid-liquid ratio was mixed thoroughly using glass rod, covered by aluminium foil and allowed to stand for 4 days. Then the extract was filtered via whatmann No. 1 filter paper which was further used for phyto-chemical screening and synthesis of *Pergularia daemia* mediated zinc oxide nano particle.

Synthesis of Zinc oxide nano-particles from *Pergularia daemia* (precipitation method)

ZnO nanoparticles were prepared by mixing 95 mL of a 0.01M zinc acetate dihydrate solution with 5 mL of PD leaf extract in a sterile 250 ml flask. The contents were vigorously stirred (at 150 rpm) for an hour at 70°C. The white precipitate formed is known as green mediated PD zinc oxide nano particles. The above effluent was discarded, and the formed

white precipitate was transferred to 1.5 mL microfuge tubes and centrifugated at 3000 rpm for 30 min with distilled water. This washing process was carried out three times to achieve maximal impurity removal.

Phyto-chemical screening of extract

Various test on phytochemical screening was performed are for alkaloids using Mayer's test, Wagner's test, presence of carbohydrate by Molisch test, Fehlings test, Barfoed's test, Benedict reagent test, glycosidic reduction test, saponins, protein identification using Millon's test, Biuret test, amino acids by ninhydrin test, oils by saponification test followed by phenols, anthocyanin, terpenoids and flavonoids identification tests. All the analysis for phytochemical screening were performed based on method adopted from (Nithyatharani, 2018).

Characterization

UV-Visible

The UV-Visible absorption spectrum of synthesized nanoparticles reveals a recognized trend in absorption spectroscopy: a rise in band gap accompanies a reduction in particle size. The UV-Visible was performed at 400-700nm wavelength.

FTIR

FTIR analysis serves as a tool for discerning molecular compounds, operating through the measurement of infrared radiation absorbance by a sample. FTIR was performed in wavelength of 4000 to 500 cm⁻¹ with resolution of 4 cm⁻¹ and 32 scans.

XRD

This method was employed to assess both the structure and crystalline dimensions of the synthesized nano-particles recorded by XPERT-3. The data was collected in the 2θ range of 5-80°. The scan axis was goniometer, type continuous with goniometer radius 240mm.

SEM

The scanning electron microscope (SEM) stands as a prevalent method for analyzing nonmaterial's and nanostructures. SEM analysis was determined using VEGA 3 TESCAN microscope.

Anti-cancerous activity

The MBA-MB-231 breast cancer cell lines were sourced from the NCCS cell

repository in Pune, India. They were cultured in DMEM supplemented with 10% FBS, streptomycin (100 µg mL⁻¹ and penicillin (100 µ mL⁻¹) to prevent bacterial contamination, under humidified conditions with 5% carbon-di-oxide at 37°C.

MTT Test

MDA-MB-231 cell cytotoxicity was assessed using the (Ferrari *et al.*, 1990). MDA-MB-231 viable cells were harvested and counted using hemocytometer. They were then diluted in DMEM medium to a density of 1x10⁴ cells mL⁻¹ and seeded (96-well plates), allowing attachment over a 24-h incubation period. Subsequently, MDA-MB-231 cells were treated with varying concentrations ranging from 2.5 to 15 µg mL in each well. After wards, the drug-containing cells were washed with culture medium and MTT dye (5mg mL⁻¹ in PBS) and included to each well. The cells were then rested in incubator for an additional 4 h at 37°C to allow for the formation of purple precipitated formazan. The formed formazan crystals were dissolved in 100 µL of concentrated DMSO and the cell viability was assessed by measuring at 540nm using a multi-well plate reader. The results were presented as the percentage of viable cells compared to the control. The half maximal inhibitory concentration (IC₅₀) values were calculated and the optimal doses were analyzed at different time periods to determine their efficacy.

Antioxidant assay (DPPH)

To assess the antioxidant activity of the sample, stable DPPH free radical activity was utilized. Initially, a 500 µL ethanolic solution of DPPH (0.05 mM) was combined with 1000 µL of PD mediated zinc oxide nano particles. The freshly prepared DPPH solution was stored (4°C) in dark. The resulting mixture was vigorously shaken, followed by the addition of 96% ethanol (2.7 mL). Subsequently, the mixture was allowed to rest (5 min) and absorbance was examined spectrophotometrically (540nm). To establish a baseline, the absorbance was at zero using ethanol. Additionally, a blank was prepared with the same quantity of ethanol and DPPH. Each experiment was performed in triplicate to ensure accuracy. The radical activity of the tested analytes was calculated and expressed as a percentage of inhibition relative to the control.

DPPH activity inhibition (%) = [(A-B)/A] x 100

RESULTS AND DISCUSSION

The methanolic extract of leaves of PD was screened for their active phytochemical constituents. It is evident that location of sample procurement has difference in composition and our study is the first to report the qualitative phytochemical investigation of the leaves of PD from Sekkakudi, Sivagangai district, Tamil Nadu. The results were given in table 1 for the qualitative phytochemical analysis from leaves of PD for alkaloids, carbohydrates, glucose, saponins, protein, amino acids, fixed oils & fats, phenolic compounds, flavanoids, terpenoids, lignins and anthocyanin. All the analysis were done using methanol leaf extract of PD owing to the results obtained by (Nithyatharani, 2018). The findings of (Nithyatharani, 2018) revealed that the methanolic extract in comparison with other chemical extraction such as ethanol, petroleum ether, chloroform and aqueous had better result in qualitative analysis of PD leaf extract. The results of flavanoid & amino acids of our study found different from (Nithyatharani, 2018), these variations may be owing to the change in geographical location of species taken for analysis. The presence of saponins, terpenoids, flavanoids and alkaloids indicates that the leaf extract contains hypoglycemic activity reported similarly in (Nithyatharani, 2018). The presence of flavanoids and phenols indirectly represents the presence of high antioxidant capacity as well. From this we could able to say that the extract taken for ZnO synthesis has good antioxidant properties and could be effectively used in various pharmacological, cosmetic and drug delivery applications.

Table 1: Qualitative analysis of phytochemicals from Pergularia daemia

S.No	Phytochemicals	Results
1	Alkaloids	+
2	Carbohydrates	+
3	Glucose	-
4	Saponins	+
5	Protein	+
6	Amino acids	-
7	Fixed oils & fat	+
8	Phenolic compounds	+
9	Flavonoids	+
10	Terpenoids	+
11	Lignins	-
12	Anthocyanin	-

Characterization of PD mediated ZnO nanoparticles

The chemical method of ZnO nanoparticle

synthesis was done and its important to characterize the nanoparticles to verify that the obtained samples were ZnO without any impurities. The synthesized PDmediated ZnO nanoparticle were characterized by UV-Visible, FTIR, XRD, SEM and EDAX.

UV-Visible Spectral analysis

The Fig. 1 provided represents the UV-Visible spectra of ZnO from PD. The color change was observed in spectra from brownish green to white that indicates that the component extracted was ZnO nano particles. The absorbance band anywhere between 300 to 550nm represents the presence of zinc oxide nano particle reported by (Ghorbani *et al.*, 2015). Similarly our results were found to be in the range of 250nm to 550nm indicates the presence of ZnO nano particles. The strong absorption band was found at 401.30 which has much difference from the result obtained by (Talam *et al.*, 2012). Though this variation doesn't affect the conclusion, since they were within the range of ZnO synthesized and the variation could be due to different extraction techniques followed in both studies. This also suggests that from the UV-Visible spectra the synthesized nanoparticle possess monodispersed nature.

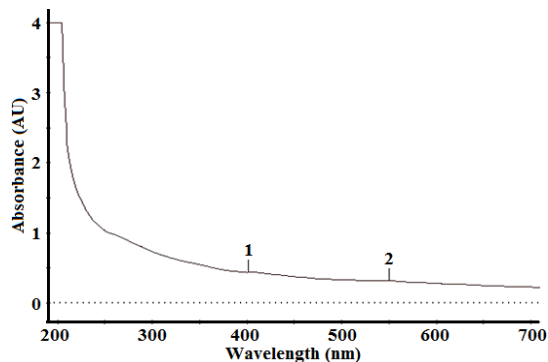


Fig. 1. UV-Visible spectra of ZnO extracted from *Pergularia daemia*

FT-IR Spectroscopy

FTIR study of PD leaves based synthesized nano-particle to identify the purity and chemical makeup of the zinc oxide nano-particle. The peak found at 1631 cm^{-1} corresponds to the zinc oxide Zn-O stretching and deformation vibration respectively. Similar results were found by (Fakhari *et al.*, 2019) at 1634 Zn-O stretching from *L.nobilis* extract synthesized nano particle. The peak at 1401 and 1051 cm^{-1} were also found to have O-C-O stretching vibration do adsorbed carbonate anion peak indirectly represents Zinc oxide based material

similar to results obtained by (Shamhari *et al.*, 2018). It also represents that the other components obtained were also related to ZnO nanoparticle and there were no impurities present in the extract.

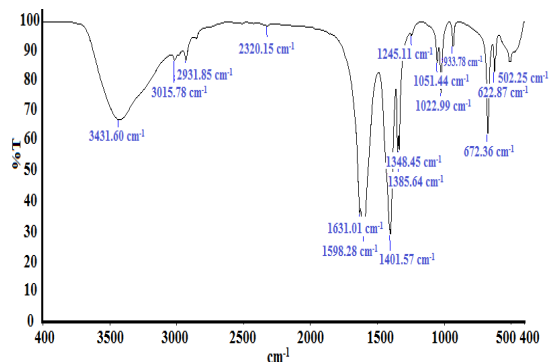


Fig. 2. FTIR Fingerprint of ZnO nanoparticle from *Pergularia daemia*

XRD

XRD represents the crystalline nature of synthesized nano particle as well as the purity of the nanoparticle. The Fig. 3 represents the XRD pattern of synthesized crystalline nanoparticle. And the presence of sharp peak at 30.78, 31.68 represent the spherical to the hexagonal phase of ZnO with high crystalline nature. Similar peak was observed by (Saraswathi *et al.*, 2020) for synthesizing nano-particle of ZnO from *Parthenium hysterophorus*. The other peaks obtained from XRD also indicate the presence of ZnO and showed that the compound extracted were with high purity and there are no impurities from the synthesized nanoparticle. The average particle size was reported to be in range of 200-300nm.

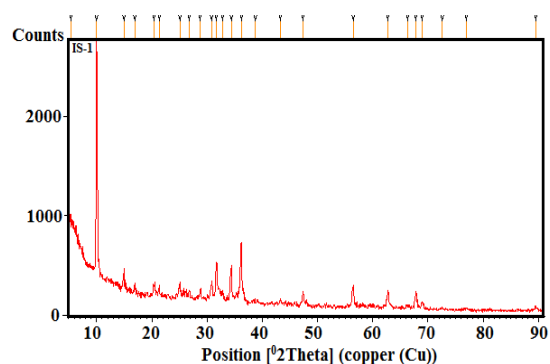


Fig. 3. XRD image of ZnO extracted from *Pergularia daemia*

SEM analysis

Figure 4 SEM image of synthesized Zinc oxide nano-particle under magnification of 40.0kX at 200nm represents the shape & size of nanoparticle. The particle synthesized were agglomerated, oval shaped and are not completely separated similarly

found by (Mohan & Renjanadevi, 2016), may be owing to the weak physical force. The particles formed were in the range of nano size (100-250nm) confirms the synthesized zinc oxide were in nano scale. This also shows that the synthesis of nanoparticle from precipitate method is suitable though the achieving of uniform particle size was difficult. The size was found to be oval may be because of the seed material used that is zinc acetate. The other synthesis method has also to be compared to this study to achieve better quality end product.

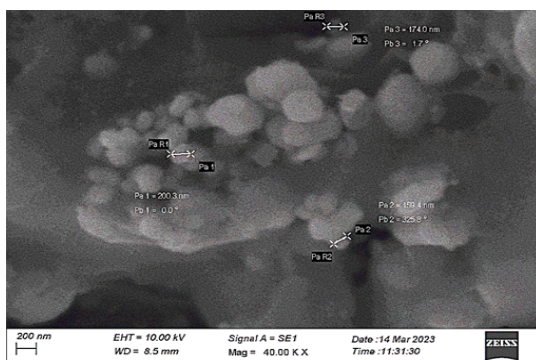


Fig. 4. SEM image of ZnO nanoparticle synthesized from *Pergularia daemia*

Table 2: Cell viability of extract using medicinal plants on MDA-MB-231 cell line by MTT assay

S. No	Tested sample concentration (µg/mL)	Cell viability (%) R1	Sample Cell viability (%) R2	Cellviability (%) R3	Average	SD
1	Control	100	100	100	100	0
2	50 µg/mL	70.19	73.39	75.96	73.18	2.89
3	100 µg/mL	65.75	67.45	70.93	68.04	2.64
4	200 µg/mL	60.65	65.27	64.78	63.57	2.54
5	300 µg/mL	50.67	45.90	55.85	50.81	4.97
6	400 µg/mL	45.90	40.26	43.63	43.26	2.84
7	500 µg/mL	32.83	35.16	29.71	32.57	2.73

Table 3: Antioxidant potential of ZnO synthesized from *Pergularia daemia*

S. No	Concentration(µg/mL)	Antioxidant activity DPPH%	
		Sample	Standard (Ascorbic acid)
1	20 µg/mL	40.36	59.63
2	40 µg/mL	51.37	63.30
3	60 µg/mL	57.79	66.05
4	80 µg/mL	63.30	68.80
5	100 µg/mL	66.05	73.39
	IC ₅₀	41.83	20.17

CONCLUSION

Thus the synthesis of ZnO nanoparticle through the precipitate method was successful, as evidenced by qualitative analysis and characterization. The results indicate the presence

Anti-cancerous assay

The synthesized ZnO nanoparticle from PD was evaluated for *in vitro* cyto toxicity using MTT assay for MDA-MB-231 breast cancer cell line. The dose-related reduction in viable cells was given in Table 2 for about 24 h of treatment of MB-232 cells with varied concentration of PD zinc oxide nano particle. The IC₅₀ value was estimated based on these results and found that around as the concentration of test samples increases there was subsequent reduction in cell viability of MB-231 and proves synthesized nano-particle was effective in reduction of MDA-MB-231 cells that has greatest anti proliferative activity besides helps in boosting health of breast cancer patients.

Antioxidant activity (DPPH)

From the Table 3, the antioxidant potential of ZnO synthesized were deliberates that an increase in concentration of ZnO (synthesized compared with standard) in the DPPH solution gives better result for synthesized sample than for the control. It declares that the antioxidant potential of synthesized nanoparticle found effective and could be good immune fighter.

of promising phytochemicals in *Pergularia daemia* and the produced high-purity ZnO nanoparticles in a oval shape. Overall, this research highlights the potential of *Pergularia daemia* as a valuable resource for pharmaceutical and nontechnological interventions, encouraging continued investigation into its medical properties and extraction methods.

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Conflict of interest

The author declare that we have no conflict of interest.

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