Environment and Ecology 43 (1) : 97—102, January—March 2025
Article DOI: https://doi.org/10.60151/envec/GXOX1694
ISSN 0970-0420

Anticancer Activity of ZnO Nanoparticles using *Averrhoa carambola* Leaves Extract

I. Srilega, M. Kavitha

Received 6 September 2024, Accepted 4 January 2025, Published on 27 January 2025

ABSTRACT

Averrhoa carambola, leaves are commonly used in ayurvedic and traditional Chinese medicine used for inflammatory skin disorders and fungal skin infection. The bioactive compounds are responsible for medicinal properties. *Averrhoa carambola* has proved to be effective in curing multiple diseases. *In vitro* cytotoxicity activity was done in Vero cell line. The synthesis of ZnO nanoparticles using aqueous leaves extract and aqueous ZnO extract changes was observed. The formation of ZnO nanoparticle was confirmed by UV-visible spectroscopy Fourier transform infrared spectroscopy (FT-IR). The bioactive compounds was analyzed in GC-MS. *In vitro* studies demonstrated that *Averrhoa carambola* inhibited the growth of different human cancer cells. Thus present study, no scientific report on anti-tumor activity of *Averrhoa carambola.* The project will be carried out on *Averrhoa carambola* as chemotherapeutic agent. Thus, it can be stated that this leaves is a suitable drug and can be further explored and exploited to meet the global demand for natural, cost- effective, and safer bioactive compounds.

Keywords ZnO nanoparticle, Cytotoxicity, Vero cell line, ZnO.

INTRODUCTION

The ZnONPs were synthesized and characterized using *Averrhoa carambola* leaves extract. Synthesized zinc oxide nanoparticles are primarily observed by the color change from white to pale yellow indicating the synthesis of ZnONPs. The zinc oxide nanoparticle showed peaks at 285 nm. Mohammed *et al.* (2022) reported the spectral absorbance peak at 335 nm of zinc oxide nanoparticles synthesized from *Clitorea ternatea* flower extract. Lopez and Herrera (1998) studied the maximum wavelength from 340 to 370 nm of ZnONPs synthesized from passion fruit peel extracts. The immediate appearance of white-yellowish color in peels extract indicates the formation of ZnONPs.

FT-IR measurements were carried out to identify the possible biomolecules responsible for the reduc-

I. Srilega[1]*, M. Kavitha[2]

[1]PhD Research Scholar, [2]Assistant Professor
[1,2] Department of Biochemistry, Annamalai University, Annamalai Nagar 608002, Tamil Nadu, India

Email: proffsrilekha@gmail.com
*Corresponding author

tion of the metal ions and capping of the reduced nanoparticles synthesized by *Averrhoa carambola* leaves extract. The present results showed a sharp absorption broadband peak of the leaves extract at 2173 cm^{-1} and ZnONPs at 1637 cm^{-1}. The nanoparticles formed were linked to metabolites, phenol, proteins and terpenoids having functional groups such as aliphatic amines, aromatics, alkenes and 1° amines. Phenols possess high binding capacity to metals which indicates that they may be the capping agent for the metal nanoparticles. Mujahid *et al.* (2021) reported that stinking passion fruit peels extract showed the peak at 3486, 3209, 2980 cm^{-1} associated with the intermolecular hydrogen-bonded –OH group phenol. C=O amide band stretching at 1650 cm^{-1}, which participated in stabilization by developing amide group protein encapsulation and protected to aggregation.

MATERIALS AND METHODS

Cold extraction

Ten gram of sample was weighed and soaked in 100 ml of Aqueous. The extract was allowed to stand overnight and filtered using sterile filter paper. The filtrate was collected and incubated at room temperature for evaporation. Then measure the weight and find the yield by calculating.

Yield = Initial weight - final weight

Synthesis of zinc oxide nanoparticles

Twenty ml aqueous extract of *Averrhoa carambola* leaves were added to 80 ml of 0.01M zinc acetate solution at room temperature under constant stirring, 2N NaOH was added drop wise until to set pH 12.0 to synthesis ZnO nanoparticles. The stirring was continued for 2 hrs to form yellow to pale white precipitate. The nanoparticles were obtained by centrifugation at 10000 rpm for 20 min. Pellet was washed with distilled water thrice and air dried. The obtained copper and zinc nanoparticles powder was stored in the refrigerator for further analysis.

UV–visible spectrophotometer

The spectral response of synthesized ZnNPs was monitored by absorbance measurements carried out on UV–visible spectrophotometer in the wavelength range of 200-800 nm (Thermo Scientific–Evolution 201). It is commonly used for measuring thin film thickness in semiconductor manufacturing, materials science research, measuring the energy content of coal and petroleum source rock, and in forensic laboratories for the analysis of microscopic amounts of trace evidence as well as questioned documents.

Fourier transform infrared spectroscopy (FTIR) analysis

FT-IR spectroscopy deals with the region of the electromagnetic spectrum that is in light with a longer wavelength and lower frequency than visible light. It covers a range of techniques mostly based on adsorption spectroscopy. The FT-IR spectrum showed bio molecules involved in the synthesis and stabilization of nanoparticles. The sample was ground with potassium bromide (KBr) salt finally. The powder mixture is then pressed in a mechanical press to form a translucent pellet through which the beam of the light can pass to record a neat spectrum. FT-IR results were obtained from a Jasco 6300 spectrometer (ATR mode) in the range of 400–4000 cm^{-1}.

In vitro cytotoxicity using Vero cell lines

Minimal essential media (MEM) preparation

Weighed 9.5 g of MEM dissolved in 950 ml of pre-sterilized double distilled water and mixed well. Sodium hydrogen carbonate 2.2 g was dissolved in 50 ml of pre-sterilized double distilled water mixed well. Both bottles were sterilized at 15 lbs, 121 ^{0}C, for 15 min and allowed to cool at room temperature. 0.3 g of L-glutamine was weighed and dissolved in 10 ml of pre-sterilized double distilled water and mixed with MEM. 1 mg of each antibiotic was weighed (Streptomycin, Penicillin G and Amphotericin-B) and dissolved separately in 1 ml of pre-sterilized double distilled water mixed with MEM, pH was checked and adjusted to 7.2 - 7.4 with diluted HCL. Then MEM was syringe filtered and stored at 4^{0}C.

MTT

MTT = 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphen-

yltetrazolium bromide = 5 mg/ml in 1XPBS

MTT assay

Cell lines were procured from National Center for Cell Science, Pune (NCCS), India. The cells were maintained in Minimal Essential Media supplemented with 10% FBS with antibiotics. The cytotoxicity activity of samples on normal Vero (African green monkey kidney cell lines) and anticancer activity of samples on MCF7 (Human breast cancer cell lines). MEM and TPVG were brought to the room temperature. The tissue culture flask was observed to check the cell proliferation, cell degeneration, pH and turbidity. Discarded the medium and washed the cells with MEM medium for twice. 4 ml of TVPG solution was added over the cell flask allowed for 1-2 minutes. Discarded the TPVG solution and waited for 1-2 min for detach the cells. Then added 5 ml of 10% serum with MEM to the flask for break off the cell clusters by gently pipetting back and forth with pipette (Passage the cells). Added 20 ml of serum with MEM to tissue culture flask and passage well and transferred the 200 µl of the cells into 96 well plates. The plates were incubated at 37^0C for 5% CO_2 incubator for 72 h. Then, the samples were diluted with 0.1% DMSO and various concentrations of the samples were loaded in each well. The plates were kept at 37^0C for 5% CO_2 incubator. After 24 h the cell lines change in morphology was visualized and photographed using Phase Contrast Inverted Microscope at 40× magnification (Labomed). The sample solution was removed from all the wells and 20 µl of MTT reagent was added to that wells. Incubated at 37^0C for 4-6 h in dark and 1ml of DMSO was added. The viability of the cells was evaluated at 540 nm. Assay was carried out using different concentrations of samples to found IC_{50} values for 50% of cell viability and calculated using the following formula:

$$\% \text{ cell viability} = \frac{\text{A540 of treated cells}}{\text{A540 of control cells}} \times 100\%$$

Fig. 1. Synthesized of ZnONPs powder (A) Before synthesis and (B) After synthesis.

Fig. 2. Synthesized of ZnONPs powder.

RESULT

Synthesis of zinc oxide nanoparticle from *Averrhoa carambola* leaves extract

The color formation of pale white precipitate indicates the synthesis of ZnONPs (Figs. 1-2).

UV analysis

A zinc oxide nanoparticle showed the peaks at 285 nm. The intensity of the UV absorption was increasing with time of incubation of metals with leaves extract (Fig. 3). The peak in the UV spectra is caused by incident electromagnetic energy coupling into a surface Plasmon at the particle's contact with the surrounding medium. UV absorption is high photo stability, biocompatibility and biodegradability. ZnONPs can also be obtained with a variety of particle structures, which determine its use in new materials and potential application in a wide range of fields of technology.

FT-IR analysis

The FT-IR analysis was carried out to determine the bioactive molecules of nanoparticles which are present in the reduction, capping and stabilization. The leaves of *Averrhoa carrambola* aqueous extract possess characteristic vibrational peaks at 2173, 2007, 2000, 1635, 1556, 1406, 1074, 457, 441, 418 and 406 cm^{-1}, which correspond to functional groups of C–N stretch (aliphatic amines), C–H bend (alkanes), C–C stretch (in–ring) aromatics, –C=C– stretch alkenes, –C=C–stretch (alkynes), respectively. Synthesized ZnONPs corresponds to 1637 and 999 cm^{-1}, which correspond to functional groups =C–H bend (alkenes) and N–H bend (1° amines) confirmed that the phyto-constituents present in the leaves extract were responsible for the reduction of ZnO nanoparticles (Fig. 4).

Anticancer activity by MTT assay

In vitro cytotoxicity activity for Vero cell line

The various concentrations of nanoparticles 200, 100, 50, 25, 12.5, 6.25 and 3.12 µg/ml, vehicle control (DMSO) and control (without samples) were checked for toxicity using Vero cell line. With the decrease in concentration there is a significant increase in the % of cell viability, thus exhibiting a dose-dependent effect. 50% of cell viability was calculated as 200 µg

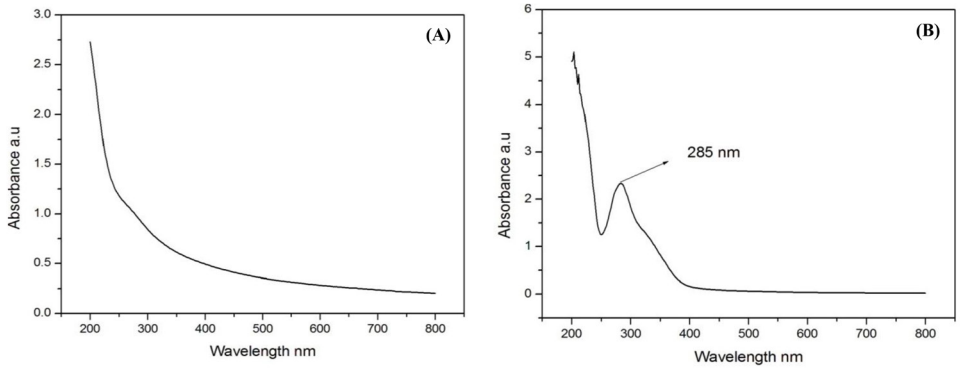

Fig. 3. UV visible absorption spectra of (A) *Averrhoa carambola* leaves extract and (B) ZnONPs.

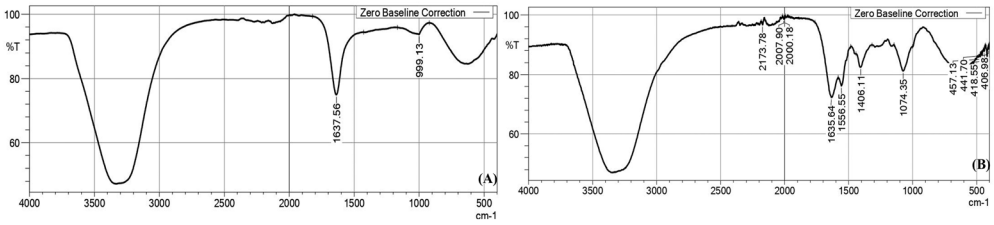

Fig. 4. FT-IR analysis of *Averrhoa carambola* (A) FT-IR analysis of *Averrhoa carambola* leaves extract and (B) FT-IR analysis of ZnONPs respectively.

for ZnONPs (Table 1). The result shows less toxicity property for ZnONPs.

In vitro anticancer activity for MCF7 cell line

The various concentrations of ZnNPs 200,100, 50, 25, 12.5, 6.25 and 3.12 µg/ml and vehicle control (DMSO) and control (untreated cells) were checked for anticancer activity in MCF7 cell line. For the cell lines, decrease in cell count was observed with increase in concentration of the samples. The IC_{50} for MCF7 cells treated with ZnONPs was found at 26.5 µg/ml. The result shows good anticancer property for ZnONPs (Tables 2-3).

Table 1. The cytotoxicity activity of ZnONPs against Vero cell line.

Concentration (µg/ml)	Absorbance (540 nm)	% Cell viability
1000	0.27	20.3
500	0.43	32.3
250	0.61	45.8
125	0.79	59.3
62.5	1.11	83.4
31.2	1.30	97.7
DMSO	1.33	100
Control cells	1.33	100

Table 2. The anticancer activity of ZnONPs against MCF7 cell line.

Concentration (µg/ml)	Absorbance (540 nm)	% Cell viability
1000	0.03	2.3
500	0.06	4.7
250	0.13	10.2
125	0.19	14.9
62.5	0.35	27.5
31.2	0.58	45.6
15.6	0.79	62.2
DMSO	1.25	98.4
Control cells	1.27	100

Table 3. IC_{50} values for Vero and MCF7 cell line.

IC_{50} values (µg) Content	Vero cell line	MCF7 cell line
Aqueous extract	300	37.4
ZnONPs	200	26.5

DISCUSSION

FT-IR analysis of aqueous *Olea europea* leaf extract indicated the presence of phytoconstituents such as amines, aldehydes, phenols and alcohols which were the surface active molecules stabilizing the zinc oxide nanosheets (Awwad *et al.* 2014). Paweena *et al.* (2022) studied the broad peak at 3400 cm^{-1} detected in the ZnONPs FT-IR spectrum corresponded to the stretching vibration of O–H in the water molecules adsorption onto the surface of zinc oxide nanoparticles.

Aljabali *et al.* (2022) studied that the MTT assay was performed to verify the anticancer effects of the ZnONPs synthesized from aqueous extracts of *Citrullus colocynthis* on MDA MB231/WT, MDA-MB-231/DR, MCF-7/WT and MCF-7/DR cell lines. The results were around two-folds higher than the MCF-7 cell line. A non-malignant Chang's liver cell line (MDA-MB-231) showed an IC_{50} values of 90 ± 3.46 µg in *Averrhoa bilimbi* fruit extract (Yan and Asmah 2017).

Our results exhibited more anticancer activity (IC_{50} value 26.5 µg) and less toxicity in Vero (IC_{50} value 200 µg) cell line when tested for ZnONPs. Similarly, Mohammed *et al.* (2022) revealed that IC_{50}

values of ZnONPs synthesized form *Clitorea ternatea* flower extract and doxorubicin (standard) were 8.2 ± 2.06 and 42.8 ± 1.15 µg respectively.

CONCLUSION

Present research showed the aqueous leaves extract of *Averrhoa carambola* was used to synthesized ZnONPs. The synthesized nanoparticle was characterized by UV and FT-IR. The nanoparticle was examined against microbial pathogens. Toxicity and anti cancerous activity of the sample was evaluated by Vero and MCF7 cell lines. Anticancer efficacy was found to be at low concentration compared to cytotoxicity. Therefore, nanoparticles were found as non toxic and it proves that it is safe for future studies. Hence the study has been proven that it could be recommended for pharmaceutical industry.

ACKNOWLEDGMENT

The author appreciates the Department of Biotechnology and Biochemistry. Plant authentication is done by the Department of Biotechnology Laboratory, Faculty of Biochemistry, Annamalai University, Chidambaram for synthesis, biological activities analysis in the Annamalai University, Department of Biotechnology and Biochemistry.

REFERENCES

Aljabali AAA, Obeid MA, Bakshi HA, Alshaer W, Ennab RM, Al-Trad B, Al Khateeb W, Al-Batayneh KM, Al-Kadash A, Alsotari S, Nsairat H, Tambuwala MM (2022) Synthesis, characterization, and assessment of anti-cancer potential of ZnO nanoparticles in an *in vitro* model of breast cancer. *Molecules* 27(6): 1827. https://doi:10.3390/molecules27061827

Awwad A, Albiss B, Ahmad AAL (2014) Green synthesis, characterization and optical properties of zinc oxide nanosheets using *Olea europea* leaf extract. *Advanced Materials Letters* 5(9): 520-524. https://doi:10.5185/amlett.2014.5575

Lopez RG, Herrera J (1998) Milk production from pastures and cassava (*Manihot esculenta*) or sweet potato (*Ipomoea batatas)* integral forage plant supplementation. *Cuban Journal Agricultural Science* 32(1): 29-32. https://www.feedipedia. org/node/551

Mohammed HEA, Afridi S, Khalil AT, Zia D, Shinwari ZK, Dhlamini MS, Maaza M (2022) Structural, morphological and biological features of ZnO nanoparticles using *Hyphaene thebaica* (L.) Mart. fruits. *Journal of Inorganic and Organometallic Polymers and Materials* 30: 3241-3254. https:// doi:10.1007/ s10904-020-01490-0

Mujahid K, Ware P, Shimpi N (2021) Synthesis of ZnO nanoparticles using peels of *Passiflora foetida* and study of its activity as an efficient catalyst for the degradation of hazardous organic dye. *SN Applied Science* 3: 528. https://doi:10.1007/s42452-021-04436-4

Paweena A, Netvichian R, Thiendedsakul P, Khaodhiar S, Tulayakul P (2022) Carcinogenic risk of Pb, Cd, Ni, and Cr and critical ecological risk of Cd and Cu in soil and groundwater around the municipal solid waste open dump in central Thailand. *Journal of Environmental and Public Health* 28: 3062215. https://doi: 10.1155/2022/3062215

Yan SW, Asmah R (2017) Anti-proliferation of MDA-MB-231 Cells by *Averrhoa bilimbi* extract is associated with G0/G1 perturbation and mitochondria-mediated apoptosis independent of p53. *International Food Research Journal* 24(3): 1331-1337. https://cabidigitallibrary.org by 2402:3a80:1956:cc7b:19f2:b492:3295:6c12