An Elsevier Indexed Journal ISSN-2230-7346

*Journal of Global Trends in Pharmaceutical Sciences*

# CYTOTOXIC AND ANTIBACTERIAL ASSAY OF ZINC OXIDE NANOPARTICLES IN COMPARISON WITH *JUSTICIA ADHATODA* LEAF EXTRACT

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**ARTICLE INFO**

**Key Words**

Zinc Oxide nanoparticles, Ultraviolet-visible spectroscopy, X-ray diffraction, Scanning electron microscopy, Energy dispersive spectroscopy, Antibacterial activity, Cytotoxicity.

## ABSTRACT

The aim of the present study is to find out the comparative effect of ethanolic leaf extract of *Justicia adhatoda* and *J. adhatoda* mediated Zinc Oxide nanoparticles on cytotoxicity of A549 cell line and antibacterial activity on selected bacterial pathogens. Phytochemicals were analysed through standard qualitative tests. The green synthesized ZnO nanoparticles were characterised by ultraviolet- visible spectroscopy, Fourier transform infrared spectroscopy, X-ray diffraction, scanning electron microscopy and energy dispersive spectroscopy.The synthesised ZnO nanoparticles showed spherical morphology with an average size of 35.94nm. The antibacterial assay was carried out by well diffusion method on selected bacterial pathogens such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus pyogens*, *Escherichia coli* and *Klebsiella pneumonia* . During the antibacterial study the ZnO nanoparticles exhibited significant zone of inhibition when compared to the ethanolic leaf extract of *J.adhatoda*. Further the cytotoxicity assay were carried out on A549 lung cancer cell line respectively Both leaf extract and green synthesized Zinc Oxide nanoparticles exhibit a potential role in cytotoxicity effect against A549 cells. The cytotoxic effect was maximum at a concentration of 100µl where it showed 90% mortality. *Justicia adhatoda* leaf extract mediated ZnO nanoparticles can be considered as biosafe, eco-friendly and highly effective antibacterial and active cytotoxic agent for the management of bacterial infections and lung cancer.

## INTRODUCTION

Cancer and pathogenic diseases are the two major threats that affect the entire human population globally. The invention of synthetic drugs for their treatment has also become an important area of concern. The global cancer burden is estimated to have risen to 18.1 million new cases and 9.6 million deaths in 2018 [1]. Radiotherapy, chemotherapy and surgery are some of the cancer treatments which are used to improve patient's life but, these have significant side effects [2]. Infectious diseases continue to present a major public health challenge in developing countries. Conventional medical

drugs have specific targets, but typically also cause significant side effects [3].

Nanotechnology offers unique approaches to control a wide variety of biological and medicinal processes that occur at nanometre length and it is believed to have a successful impact on biology and medicine [4] [5]. Nanoparticles usually have a size in between 0.1 to 100 nm. It possesses a larger percentage of atoms at the exposed surface, and can readily penetrate in to cells [6]. The bio reduction of metal nanoparticles by combinations of biomolecules found in plant extracts such as vitamins, proteins, enzymes etc., are chemically complex, at the same time eco-friendly [7].

Zinc Oxide nanoparticles have drawn attention in the recent years because of its unique optical and chemical behaviours which can be easily altered by changing the morphology. Zinc Oxide nanoparticles has tremendous biological applications like biological sensing, biological labelling, gene delivery, drug delivery and Nano medicine along with antibacterial, cytotoxic, antifungal and anti-diabetic activities[8]. Medicinal plants constitute an important component of flora and are widely distributed all over the world. *Justicia adhatoda* otherwise called as *Adhatoda vasica* (Family: Acantheceae) is one of the widely distributed medicinal plants in Indian subcontinent and was used since ancient times in traditional medicine systems. The plant have significant antibacterial and anti-inflammatory properties and also exhibits expectorant, antiulcer and anticancer activities and blood purifying qualities. ZnO has recently achieved special attention regarding potential antimicrobial applications due to unique optical, electrical and chemical properties. It has a strong inhibitory and antibacterial effects as well as broad spectrum of antimicrobial activities. ZnO is considered as a viable treatment option for antimicrobial activities and studies have shown that the pathogenic strains are less resistant against ZnO nanoparticles [9].

Plants and plant derived products have proved effective and safe in the treatment and management of cancers. Many natural products and their analogues have been identified to have potential anticancer properties[10]. Green synthesized ZnO nanoparticles have high toxic effects towards cancer cells. The cytotoxicity, oxidative stress and mitochondrial dysfunction are due to solubility of ZnO nanoparticles and subsequent increase of intracellular ($Zn^{2+}$) level. Induction of oxidative stress is the major mechanism of ZnO nanoparticle cytotoxicity[11].

## MATERIALS AND METHODS

### MATERIALS

Pure and analytical grade chemicals were used in this study. Zinc acetate ($ZnC_4H_6O_4$) and Sodium hydroxide (NaOH) was purchased from Isochem Laboratories, Angamaly, Kochi were used as raw materials and are used without further purification.

### METHODS

**Plant Collection And Authentication:** Healthy, disease-free and matured leaves of *Justicia adhatoda* of Acanthaceae family were collected from local areas of Palakkad district of Kerala, India and the taxonomical identification of the plant was confirmed by the Botanical Survey of India (BSI) , TNAU Campus, Coimbatore.

**Preparation of Leaf Extract:** Authenticated plant material of *Justicia adhatoda* was washed thoroughly in tap water and further in distilled water to remove dirt from the surface. The washed leaves were subjected to shade drying for 7- 10 days. The shade dried leaves were made in to coarse powder in mixed grinder[12]. The leaf powder was mixed with 90% ethanol in a ratio of 1:5 in a beaker and was stirred using a magnetic stirrer for 5-10 min.. The mixture was kept for 24 h and then filtered twice using regular filter paper.

**Green Synthesis Of Zinc Oxide Nanoparticles:** For the synthesis of nanoparticle 43.8 g (1Mm) of zinc acetate was mixed in 100 ml of water in a conical flask to this 50 ml of *Justicia adhatoda* leaf extract was added drop by drop by continuous stirring in magnetic stirrer, thus the colour of the solution changes from colourless to green. Later freshly prepared NaOH 5 ml (8 g in 100ml) was added

drop wise in to the solution until the colour changes from green to milky white. The solution was then subjected to centrifugation at 1000 rpm for 10 mins. The precipitate was separated out from the supernatant and was spread in a petri plate and was kept for drying in room temperature. The dried particles were used for further analysis.

## CHARACTERIZATION

**Phytochemical screening of ethanolic leaf extract:** Phytochemical screening of freshly prepared ethanolic leaf extract of *Justicia adhatoda* was carried out by following the standard procedures for analysing various phytochemicals such as alkaloids, quinone, carbohydrates, cardiac glycosides, flavonoids, saponins, triterpenoids, sterols, phenols, fatty acids and tannins

**Visual inspection:** The reduction of metal ions was roughly monitored by visual inspection of the solution by colour change.

**UV-Vis Spectroscopy (UV):** The formation of Zinc Oxide nanoparticles by the reduction of $Zn^{2+}$ ions was observed by measuring the UV-Visible spectrum of the reaction solution.

**Fourier Transformed Infrared Spectroscopy (FTIR):** The FTIR of plant leaf extract and the plant mediated nanoparticles were achieved in a Shimadzu FTIR spectrophotometer by registering amplitude wave lengths ranging from 4000- 400 $cm^{-1}$ to determine the mode of interaction between the leaf extract and the nanoparticle surface.

**X-ray Diffraction (XRD)**

The crystallographic analysis of the samples was performed by powder XRD. The XRD patterns were recorded in a scanning mode on an X'pert PROPAN analytical instrument operated at 40 kV and a current of 30mA with Cu α radiation (λ = 1.54060 A°). The diffraction intensities were recorded from 35° to 79.93° in 2θ angles. The diffraction intensities were compared with the standard JCPDS files. The average size of the particle and the average size of the particles can be estimated using the Debye-Scherer equation.

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

Where, D is the thickness of the nanocrystal, 'k' constant, 'λ' wavelength of X-rays,'β' width of the half height of the reflection after correction for the instrumental broadening at Bragg's angle 2θ, 'θ' Bragg's angle.

**Scanning Electron Microscopy (SEM):** The Morphological characteristics of green synthesised Zinc Oxide nanoparticles were obtained through SEM operated at 4Kv, magnification 196 X.

**Energy Dispersive Spectroscopy (EDS):** Elemental analysis of nanoparticles was carried out using EDS instrument (PANDIAN) in a resolution of 60 A˚, operated at 10 Kv with a magnification of 5 K which determines the elemental composition and purity of the sample.

## ANTIBACTERIAL STUDIES OF ETHANOLIC LEAF EXTRACT AND GREEN SYNTHESIZED ZINC OXIDE NANOPARTICLES

The antibacterial activity of plant materials and copper nanoparticles was evaluated using agar well diffusion method [13]. Pure cultures of each bacterial strain were sub cultured in nutrient broth on a rotary shaker at 200 rpm for 24 hours at 37ºC. For preparing Mueller Hinton agar (MHA) plates, the MHA medium was boiled to dissolve completely and sterilised by autoclaving at 15 lbs pressure (120ºC) for 30 minutes. After sterilization, 20 ml of MHA media was poured into sterile petri dishes and kept at room temperature for solidification. Then, each strain was swabbed uniformly onto the individual Mueller Hinton agar plates using sterile cotton swabs. Well of 6 mm diameter were made on Muellller Hinton agar plates using sterile cork borer and 50 µl of plant extracts and 50 µl of Zinc Oxide nanoparticles were added to each well. The plates were incubated overnight at 37 ºC and the results were observed by the presence of bacterial growth inhibition zone around the sample loaded well and their diameters (mm) were measured using measuring scale. The assay was performed in triplicate.

## COMPARITIVE CYTOTOXICITY STUDY OF ETHANOLIC LEAF EXTRACT AND GREEN SYNTHESIZED ZINC OXIDE NANOPARTICLES

.Cytotoxicity test determine the cell death by qualitative and quantitative analysis. A549 Lung adenocarcinomic cell line was cultured using MEM medium supplemented with fetal bovine serum and incubated at 37°C. The cultured A549 cells were plated separately on 96 flat bottom well plates. Then, the cells were treated with different concentrations of 5, 25, 50, 75 and 100 µg/ ml in DMSO solution such that the final concentration of DMSO in media is not more than 0.5%, so that it didn't affect cell survival. The plates were then kept in $CO_2$ incubator at 37°C for 24 hrs. Blank contain only cell suspension and control well contain 0.5% DMSO plus cell suspension. After incubation period, MTT (5µg/ ml) was added to the incubated cells, then further it was incubated for another 4 hrs at 37°C in the presence of 5% $CO_2$ atmosphere. The plates were covered with aluminium foil to protect it from light. MTT was reduced in metabolically active cells to yield an insoluble purple formazan product. Cell viability was noticed by the conversion of tetrazolium salt MTT to a coloures formazan by mitochondrial dehydrogenases. Colour development was measured using micro plate (ELISA) reader at 570 nm (Biorad 680). Absorbance value of each test compound can be seen. The cell viability and cytotoxicity are calculated using the following formula.

Cytotoxicity = [(control – Treated)/ Control]* 100, Cell viability = (Treated / Control)* 100

## STATISTICAL ANALYSIS

Data for the antibacterial activity and cytotoxicity were analysed through analysis of variance (ANOVA) using SPSS 20. Version. Significant effects of treatments were determined by F values ($P < 0.05$).

## RESULTS

### PHYTOCHEMICAL ANALYSIS OF PLANT LEAF EXTRACT

The results of phytochemical analysis of the ethanolic leaf extract of *Justicia adhatoda* are given in Table 1.

### UV-Vis Spectroscopy

UV spectrum of zinc oxide nanoparticles shows strong peak at 342 nm. The zinc oxide nanoparticles exhibit an intense absorption peak due to the surface plasmon around 342 nm which really indicates the formation of zinc oxide nanoparticles (Fig.1).

### Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectra of *Justicia ahatoda* leaf extract (Fig. 2[a]) and *Justicia adhatoda* mediated Zinc Oxide nanoparticles (Fig. 2[b]) were recorded in order to identify the functional groups involved in the formation of nanoparticles. FTIR spectrum of leaf extract exhibited major absorbance bands at 3402.43 (O-H), 2858.51 (C-H), 1635.64 (C=O), 1419.61 (C-C), 1338.60 (N-O), 1242.16 (C-O), 759.95 (C-Cl) and 597.93 (C-Br) $cm^{-1}$ . The FTIR spectrum of Zinc Oxide nanoparticles gave major absorbance peaks at 3217.27 (O-H), 3194.12 (C-H), 1662.64 (C=O), 1570.06 (C-C), 1342.46 (N-O), 1026.13 (C-O), 829.39 (C-Cl) and 617.22 (C-Br) $cm^{-1}$.The ZnO peaks was noted at 474.49 $cm^{-1}$. The O-H stretching observed at 3402.43 in *Justicia adhatoda* was shifted to 3217.27 in *Justicia adhatoda* mediated Zinc Oxide nanoparticles. In addition to this, the C=O stretch appeared at 1635.64 was shifted to 1662.64 in nanoparticle. The shift in the peaks confirmed that plant extract reduced Zinc Oxide for the formation of nanoparticles.

### X-Ray Diffraction

Fig 3. Shows the X-ray diffraction of Zinc Oxide nanoparticles which represents that the particles are crystalline in nature. The intensive diffraction peak at 2θ value of 31.772° from the (100) lattice plane of the face centred cubic (fcc) unequivocally indicates that the particles are made of pure Zinc oxide. In the diffraction spectrum three more additional bands were observed at 34.420, 47.541 and 69.094 which corresponds to the (102), (110) and (201) lattice plane of Zinc Oxide nanoparticles. The size of the zinc oxide nanoparticles was 35.94 nm.

### Scanning Electron Microscopy

Scanning Electron Micrograph of Zinc Oxide nanoparticles revealed that the particles were more or less spherical in nature. The synthesized ZnO NPs showed individual particles as well as agglomerated particles. The

agglomeration is due to the intermolecular interactions (Fig. 4).

**Energy Dispersive Spectroscopy**

Energy Dispersive Spectrum of Zinc Oxide nanoparticles showed strong signal at 1 keV in the zinc region and this confirms the presence of Zinc Oxide nanoparticles (Fig. 5). The elemental analysis revealed that the zinc was present in major constituent percentage. No other peaks obtained in this graph which confirms that there were no impurities in the fabricated Zinc Oxide nanoparticles.

**CYTOTOXICITY STUDY OF *JUSTICIA ADHATODA* LEAF EXTRACT AND *J. ADHATODA* MEDIATED ZINC OXIDE NANOPARTICLES**

Cytotoxicity study of *Justicia adhatoda* leaf extract and *J. adhatoda* mediated Zinc Oxide nanoparticles were evaluated by MTT assay. The assay reveals the amount of MTT reduction by mitochondrial dehydrogenase and assumed that the cell viability was proportional to the production of purple formazan. The morphological changes of leaf extract and nanoparticle treated with cell line were observed under inverted phase contract microscope.

**CYTOTOXICITY EFFECT OF LEAF EXTRACT OF *JUSTICIA ADHATODA* ON A549 CELL LINE**

The cytotoxicity effect of both *Justicia adhatoda* leaf extract (Fig. 10) and *J. adhatoda* mediated Zinc Oxide nanoparticles (Fig. 6) were evaluated *in vitro* against A549 cell line at five different concentration (5. 25, 50, 75 and 100 µg/ ml) by MTT assay. The recorded results represented that percentage viability of cells decreased with increasing concentration of leaf extract and nanoparticle against A549 cells. The ethanolic leaf extract of *Justicia adhatoda* exhibited cytotoxicity percentages of 79.7, 87.9, 88.2, 89.5 and 90.5 at corresponding concentrations of 5, 25, 50, 75 and 100 µg. The *J. adhatoda* mediated Zinc Oxide nanoparticles exhibited cytotoxic percentages of 80.9, 82.8, 87.3, 87.6 and 88.9 at corresponding concentrations of 5, 25, 50, 75 and 100µ (Fig. 7). Hence, it was clearly demonstrated that both leaf extract and green synthesized Zinc Oxide nanoparticles exhibit a potential role in cytotoxicity effect against A549 cells.

**DISCUSSION**

Metal oxide nanoparticles represent a new class of important materials that are increasingly being developed for use in research and health-related applications. Highly ionic metal oxides have a wide variety of physical and chemical properties.An emerging integrative approach to treat infectious diseases is using nanoparticle. Advantages of nanomedicine delivery methods include better disease targeting, especially for intracellular pathogens, ability to cross membranes and enter cells, long duration drug action, reduced side effects and cost savings [3]. Recently, the green synthesis of biogenic nanoparticles from plants and microbial sources has become an emerging field due to their safer, eco-friendly, simple, fast, energy efficient, low cost and less toxic nature [2]. The green synthesis of ZnO NPs provides an environmental friendly, simple and efficient route for nanoparticle synthesis [14].

The phytoconstituents of ethanolic leaf extract of *Justicia adhatoda* also showed positive results for the presence of alkaloids, anthraquinones, cardiac glycosides, flavonoids, saponins, phytosterols, triterpenoids, polyphenols, carbohydrates, quinones, phenols, steroids and sterols, tannins, terpenoids, proteins and cardiac glycosides. The present result coincide with the earlier report [15].

The formation of nanoparticles was primarily confirmed by the colour change of the reaction mixture from light yellow to milky white, in the present experiment the colour change observed during the addition reducing agents such as *Justicia adhatoda* leaf extract and sodium hydroxide to the zinc acetate solution by continuous stirring. [16]Elizabeth Varghese and Mary George (2015) reported such colour change during their study. Hence the present result coincide with earlier findings.

The ZnO-NPs synthesised from *Cochlospermum religiosum* were characterised by UV-Vis absorption Spectrophotometer showed an absorption peak at 305 nm at room temperature [17]. In the present study a UV spectrum of Zinc Oxide nanoparticles shows strong peak at 342 nm due to surface plasmon.

The peak shift of ZnO NPs in the present study may be due to the difference in reducing agents used.

The FTIR results of present study revealed the shift in peaks which confirmed that the leaf extract acted as a reducing as well as stabilizing agent for the fabrication of Zinc Oxide nanoparticles. The results showed the existence of various functional groups in which the O-H stretching observed 3402.43 in *Justicia adhatoda* was shifted to 3217.27 in *Justicia adhatoda* mediated Zinc Oxide nanoparticles. In addition to this the C-H stretch (alkanes) at 2858.51 in leaf extract was shifted to 3194.12 in nanoparticles. The C=O stretch at 1635.64 in leaf extract was shifted to 1662.64 in nanoparticles. The N-O stretch appeared at 1338.60 was shifted to 1342.46 in nanoparticles. The alkyl halides C-Cl at 759.95 and C-Br at 597.93 in leaf extract were shifted to 829.39 and 617.22 in nanoparticles respectively . [18]Parthasarathi and Thilagavathi, 2011 studied the FTIR spectroscopy of ZnO NPs. The results gave a sharp peak of Zn-O bond at the range of 450-500 $cm^{-1}$. The above result was similar to the data obtained in the present study, in which the peak was obtained at 474.49 $cm^{-1}$.

Khursheed *et al*., 2016 [19] high purity and crystalline nature of *Aloe vera* extract mediated ZnO NPs by XRD analysis. The distinctive Bragg's reflections at 2θ values of 31.84˚, 34.45˚, 47.59˚ and 69.21˚ which corresponds to (100), (002), (102), (110) and (201) lattice plane of Zinc oxide nanoparticles were observed which were compared with standard JCPDS file. The above result coincides with the present study which determines that the ZnO NPs synthesized by *Justicia adhatoda* leaf extract was pure and crystalline in nature. The distinctive 2θ values where found at the angles 31.57˚, 34.53˚, 47.31˚, and 69.09˚ which corresponds to (100), (002), (110) and (201) lattice plane respectively.The size was focused to be 35.94 nm. In the present study the SEM analysis of the green synthesized ZnO NPs were more or less spherical in morphology. The morphology of bio-fabricated ZnO NPs was studied using SEM analysis by Mahendra *et al*., 2017 [17]. The resultant images of SEM showed aggregates of ZnO NPs which are hexagonal in shape. The above results concludes that the Zinc Oxide nanoparticles exist in varied morphological structures based on the difference in methods, reducing agents and stabilizing agents used in ZnO NPs synthesis. EDS pattern appear in the form of intense peak of Zn and O confirming the formation of ZnO NPs. In previous research the elemental analysis revealed the existence of Zn at 1keV [20]. Similar kind of elemental analysis was observed in the present study in which a sharp peak of Zn was observed at 1keV which confirms the presence of high purity of synthesized Zinc Oxide nanoparticles.

ZnO NPs have been widely used in therapeutics due to its high antimicrobial properties. It is considered as bio-safe, biocompatible with unique ability like structure dependent properties, electrical and thermal transport properties [21]. The ZnO NPs synthesized by *Justicia adhatoda* gave significant antibacterial activity among selected bacterial pathogens such as, the zone of inhibition in *Klebsiella pneumonia* was 26 mm, 33 mm in *Pseudomonas aeruginosa*, 25 mm in *E. coli*, 34 mm in *Staphylococcus aureus* and 32 mm in *Streptococcus pyogenes*.

Mariappan *et al*., 2011 [22]studied the cytotoxicity and antimicrobial efficiency of ZnO NPs prepared by wet chemical method and got significant results in human myeloblastic leukimia cells (HL60) and in selected Gram-positive bacteria (*Staphylococcus aureus*). The present experimental results revealed that the *Justicia adhatoda* mediated Zinc Oxide nanoparticles also have severe cytotoxic effect on A549 cell line as well as towards selective gram positive and gram negative bacterial pathogens. The presence of antimicrobial substances in higher plants is well established. Antimicrobial potential of leaf extract of *Justicia adhatoda* has significant contribution. The methanolic leaf extract of *Justicia adhatoda* gave maximum inhibition zone of 22 mm and 24 mm in *Staphylococcus aureus*, 16 mm and 18 mm in *Pseudomonas aeruginosa*. The leaf extract has the better antibacterial activity[23]. When compared to the above experiment the present study shows that the results were comparatively higher. The cytotoxic activity of ethanolic leaf extract of *Justicia adhatoda* and ZnO NPs were studied in different

concentrations and it gives severe effect on A549 cancer cell line. With increasing concentration the cytotoxicity also increases. Both leaf extract and nanoparticle have shown severe cytotoxic effects at 5µg, 25µg, 50µg, 75µg and 100µg concentrations. With increasing concentration there was a significant decrease in cell viability. *Justicia adhatoda* was found to be used by traditional healers for treatment of respiratory problems. The anti-inflammatory effects of this plant prove that it is beneficial in the treatment of inflammatory lung diseases [24].

Table 1: Qualitative phytochemical analysis of Ethanolic leaf extract of *J. adhatoda*

| **Phytochemical** | **Test Adopted** | **Ethanolic Extract** |
|---|---|---|
| Alkaloids | Mayer's Test | + |
| Steroids | Chloroform test | + |
| Sterols | Liermann-Buchard test | + |
| Flavonoids | Ferric chloride test | + |
| Terpenoids | Knollar's test | + |
| Saponins | Foam test | + |
| Phenol | Ferric chloride test | + |
| Triterpenoids | Salkowshi test | - |
| Volatile oils | Microsublimation vanillic test | - |
| Fatty acids | Filter paper test | - |
| Glycosides | Keller Killiani's test | + |
| Terpenoids | Knollar's test | + |
| Proteins | Xanthoprotein test | + |
| Tannins | Ferric chloride test | + |
| Cardiac glycosides | Keller Killiani's test | + |

+ : Present, -: Absent, *J. adhatoda*: *Justicia adhatoda*

Table 2: Zone of inhibition (mm) of ethanolic leaf extract of *Justicia adhatoda* and Zno nanoparticles against selected bacterial patogens

| **Sl. No** | **Name of Bacterial Pathogens** | **Zone of inhibition (mm) (Mean±SD)** | |
|---|---|---|---|
| | | **LE** | **ZnO NPs** |
| 1. | Klebsiella pneumonia | 11.3±1.25 | 12.5±0.57 |
| 2. | Pseudomonas aeruginosa | 05.6±0.57 | 11.66±0.57 |
| 3. | Escherichia coli | 08.4±0.58 | 11.0±1.56 |
| 4. | Streptococcus pyogenes | 07.8±1.15 | 13.26±0.57 |
| 5. | Staphylococcus aureus | 07.6±0.57 | 13.22±0.57 |

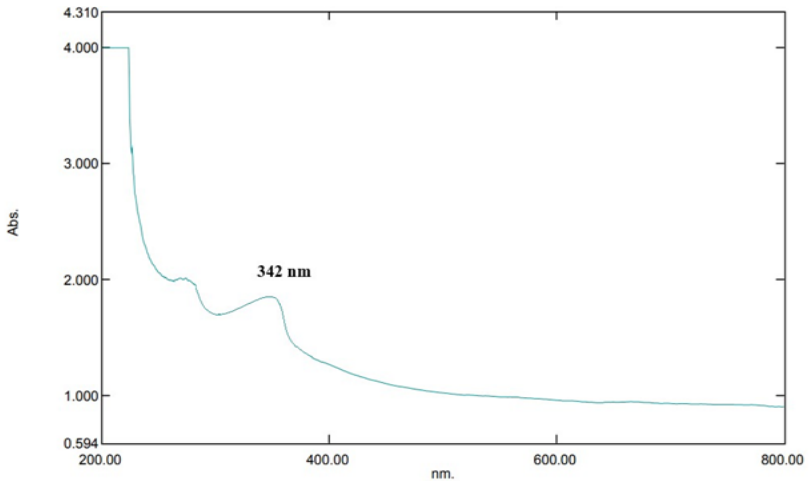
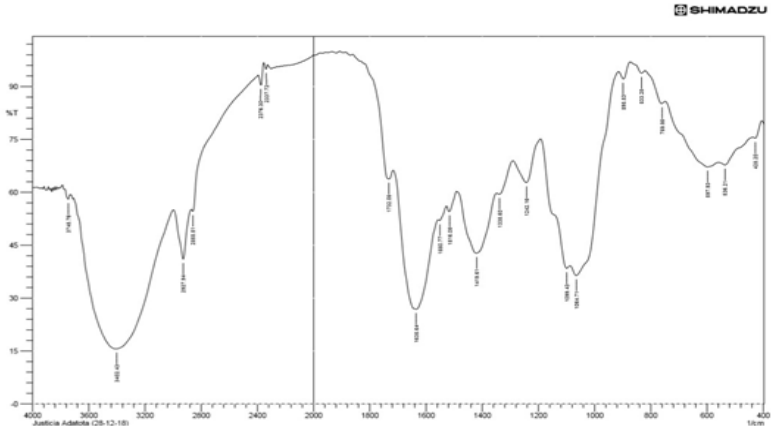
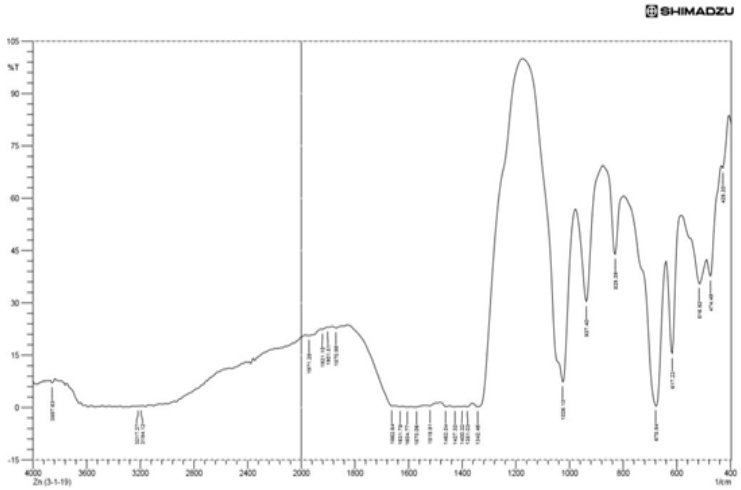



Fig 1: ultraviolet visible spectrum of *Justicia adhatoda* leaf extract




(a)




(b)

Fig 2: Fourier transform infrared spectra of (a) *Justicia adhatoda* leaf extract and (b) green synthesised Zinc Oxide nanoparticles

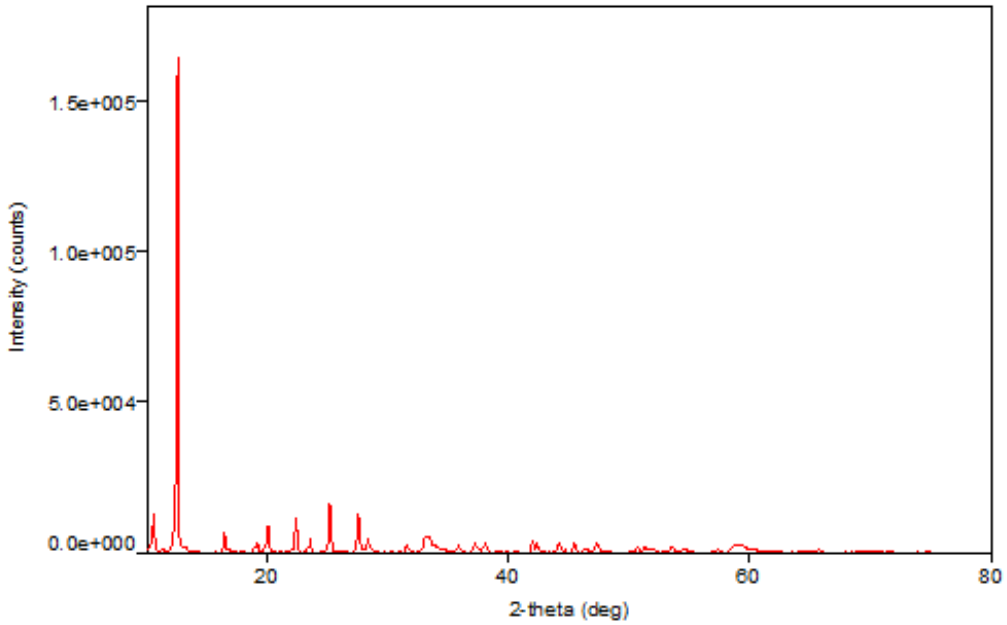



Fig 3: X-ray diffraction spectrum of *Justicia adhatoda* leaf extract mediated Zinc Oxide nanoparticles.

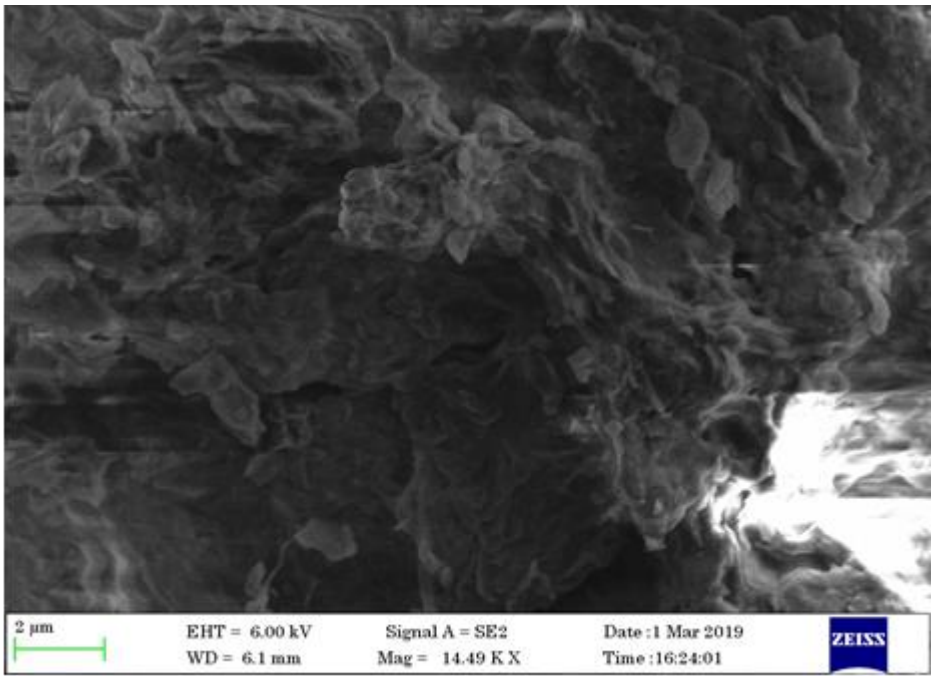



Fig 4: Scanning electron micrograph of *Justicia adhatoda* leaf extract mediated Zinc Oxide nanoparticles.

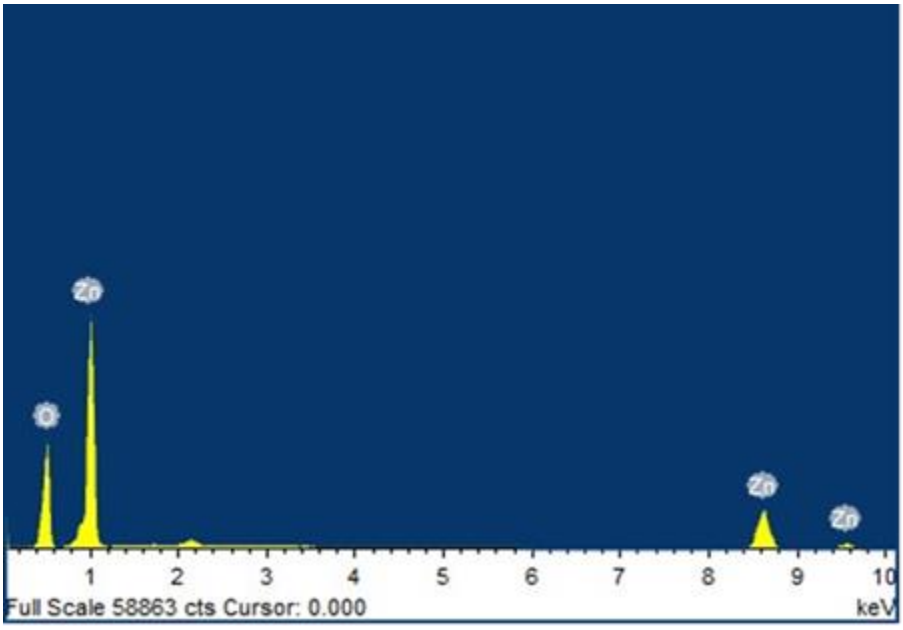



Fig 5: Energy dispersive spectroscopy of *Justicia adhatoda* leaf extract mediated Zinc Oxide nanoparticles

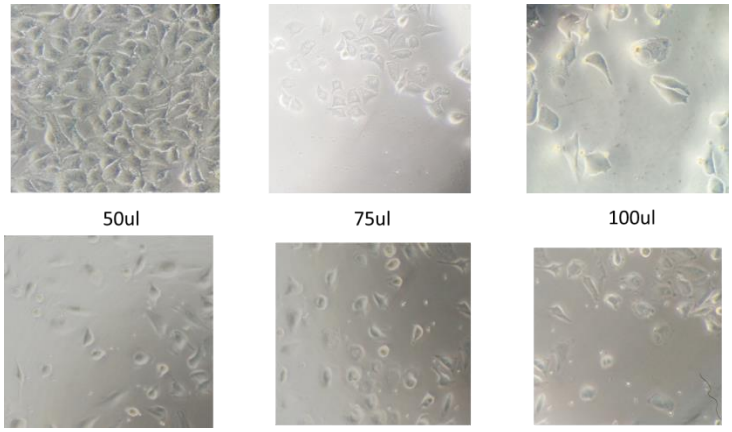



Fig 6: Cytotoxic study of ethanolic leaf extract of *Justicia adhatoda* at different concentrations on A549 cell line

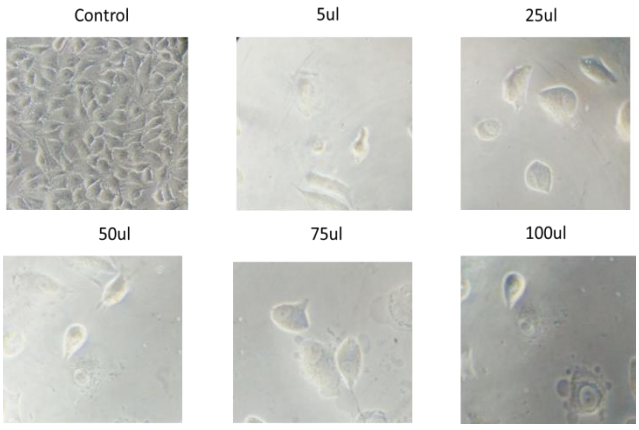



Fig 7: Cytotoxic study of *J.adhatoda* mediated zinc oxide nanoparticles at different concentrations on A549 cell line

## CONCLUSION

Tremendous improvement has been made in the development of cancer and bacterial disease treatment but still these remains the leading cause of death in the world. There are several medications available in the market to treat these deadly diseases but still no drug is found to be fully effective and safe. The major drawback is the toxicity of the established drug. Nanotechnology has now gained the attention of researchers as it is found to be a very effective alternative for commercial drugs. Utilising plant materials for the synthesis of nanoparticles have created greater applicability in therapeutics as it is considered as eco-friendly and cost effective method for the synthesis of nanoparticles. Zinc Oxide nanoparticles are considered as biosafe material which has potential antibacterial and cytotoxic properties.

The comparative study on *Justicia adhatoda* leaf extract and *Justicia adhatoda* mediated zinc oxide nanoparticles have shown potential antibacterial activity against selected pathogens and severe anticancer properties against A549 (Lung cancer) cell line. The present findings clearly indicates that the *Justicia adhatoda* leaf extract as well as *J. adhatoda* leaf mediated Zinc Oxide nanoparticles possess potential therapeutic activities against lung cancer and bacterial infections.

## ACKNOWLEDGMENT

The authors are grateful to the management of PSG College of Arts and Science, Coimbatore for encouraging and providing facilities for completing this work.

## REFERENCES

[1] World Health Organization. Latest global cancer data: Cancer burden rises to 18.1 million new cases and 9.6 million cancer deaths in 2018. IARC.

[2] Hamed Barabadi, Muhammad Ovais, Zabta Khan Shinwari and Muthupandian Saravanan, Anti-cancer green bionanomaterials: present status and future prospects, *Green.Chem.Lett.Rev.*10(2017)**:**285-314. https://doi.org/10.1080/17518253.2017.1385856.

[3] Iris Bell R., Gary Schwartz, E., Nancy Boyer, N., Mary Koithan and Audrey Brooks, J, Advances in integrative nanomedicine for improving infectious disease treatment in public health, *Eur. J. Integr. Med.* 5(2012)**:** 126-140.

[4] West J.L. and Halas N.J, Applications of nanotechnology to biotechnology, *Current Opinion in Biotechnology*. 11(2000)**:** 215-217.

[5] Zandonella C., Cell nanotechnology: The tiny toolkit, *Nature*. 423(2003): 10-12.

[6] Ran Mo, Tianyue Jiang and Zhen Gu, Recent progress in multidrug delivery to cancer cells by liposomes, *Nanomedicine*. 9(2014): 1117-1120.

[7] Siavash Iravani, Green synthesis of metal nanoparticles using plants, *The Royal Society of Chemistry*. 13(2011): 2638-2650.

[8] Niranjan Bala, Saha S., Chakraborty, M., Maiti, M., Das, S., Basu, R. and Nandy, P., Green synthesis of zinc oxide nanoparticles using *Hibiscus subdariffa* leaf extract: effect of temperature on synthesis, anti-bacterial activity and anti-diabetic activity, *The Royal Society of Chemistry*. 5(2015): 4993-5003.

[9] Jehad Yosef, M. and Enas Danial, N., In Vitro Antibacterial Activity and Minimum Inhibitary Concentration of Zinc Oxide and Nano-Particles Zinc Oxide against sPathogenic Strains, *J. Health. Sci.* 2(2012): 38- 42.

[10] Om Prakash, Amit Kumar, Pawan Kumar, Ajeet, Anticancer Potential of Plants and Natural Products: A Review, *Am. J. Pharm. Sci*. 1(2013): 104-115.

[11] Muthuraman Pandurangan and Doo Hwan Kim, In vitro toxicity of zinc oxide nanoparticles: a review, *J. Nanoparticle Res.* 17(2015): 158,1-8.

[12] Wangkhem Sandhya Chanu and Kananbala Sarangthem, Phytochemical constituents of *Justicia ahatoda* Linn. found in Manipur, *Indian. J. Plant. Sci.* 3(2014): 2319-3824.

[13] Melda A, Azade A, Fatih E, Corina M C and Ibrahim I., Green synthesis of copper oxide nanoparticles using *Ocimum basilicum* extract and their antibacterial activity, *Fresrn. Environ. Bull.,* 26(2017): 7832-7837.

[14] Vidya. C., Shilpa Hiremath, Lourdu Antonyraj, M.N., Indu Venu Gopal, Aayushi Jain and Kokil Bansal., Green synthesis of ZnO nanoparticles by Calotropis Gigantea, *Int. J. Curr. Eng. Technol.***(Special Issue-1)** 2013**:**118-120.

[15] Jayapriya and Gricil da Shoba, F, Phytochemical Analysis, Antimicrobial Efficacy Determination of Bioactive Components from Leaves of *Justicia Adhatoda* (Linn), *As. J. Plant Sci. Res*. 5(2015.): 43-51.

[16] Elizabeth Varghese and Mary George, Green synthesis of zinc oxide nanoparticles, *Int. J. Adv. Res. Sci. Eng.* 4(2015): 307-314.

[17] Mahendra, C., Murali, M., Manasa, G., Ponnamma Pooja, Abhilash, M.R., Lakshmeesha, T.R., A. Satish, Amruthesh, K.N. and Sudarshana, M.S., Antibacterial and antimitotic potential of bio-fabricated zinc oxide nanoparticles of *Cochlospermum religiosum*, *J. Microb. Pathog.*110(2017): 620-629.

[18] Parthasarathi, V. and Thilagavathi, G., Synthesis and characterization of Zinc oxide nanoparticle and its application on fabrics for microbe resistant defence clothing, *Int. J. Pharm. Pharm. Sci.* 3(2011): 392-398.

[19] Khursheed Ali, Sourabh Dwivedi, Ameer Azam, Quaiser Saquib, Mansour Al-Said, S. Abdulaziz A. Alkhedhairy and Javed Musarrat, *Aloe vera* extract functionalized zinc oxide nanoparticles as nanoantibiotics against multi-drug resistant clinical bacterial isolates, *J. Colloid Interface. Sci.* 472(2016)**:** 145-156.

[20] Niranjan Bala, Saha, S., Chakraborty, M., Maiti, M., Das, S., Basu, R. and Nandy, P., Green synthesis of zinc oxide nanoparticles using *Hibiscus subdariffa* leaf extract: effect of temperature on synthesis, anti-bacterial activity and anti-diabetic activity, *The Royal Society of Chemistry*. 5(2015): 4993-5003.

[21] Gunalan Sangeetha, Sivaraj Rajeshwari and Rajendran Venckatesh, Green synthesis of zinc oxide nanoparticles by *aloe barbadensis miller* leaf extract: Structure and optical properties. *J. Materresbull.* 46(2011): 2560-2566.

[22] Mariappan Premanathan, Krishnamoorthy Karthikeyan, Kadarkaraithangam Jeyasubramanian and Govindasamy Manivannan, Selective toxicity of ZnO nanoparticles towards Gram positive bacteria and cancer cells by apoptosis through lipid peroxidation, *J. Nano.* 7(2011): 184-192.

[23] Alka Sharma and Ashwini Kumar, Antimicrobial activity of *Justicia adhatoda*, *World. J. Pharm. Res.* 5(2016): 1332-1341.

[24] Mohamed M, Abdulaziz M, Aleisa Salim S, Abdulaziz A, Othman A and Mohamed M., Thymoquinone attenuates diethylnitrosamine induction of hepatic carcinogenesis through antioxidant signalling, *Oxi. Med. Cell. Longev.*, 3(2010): 254-261.