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**CONTROLLING THE GROWTH OF GASTROINTESTINAL PATHOGENS USING
ROOT OF *Acorus calamus* MEDIATED ZINC OXIDE NANOPARTICLES**

**CHELLAKANNU M^{1*}, JAYASUDHA R¹, PANNEERSELVAM T¹ AND
RAJESHKUMAR S²**

1: PG and Research Department of Microbiology, Adhiparasakthi College of Arts and
Science, Kalavai – 632506, Vellore District, TN, India

2: Department of Pharmacology, Saveetha Dental College and Hospitals, SIMATS, Chennai-
600077, TN, India

***Corresponding Author: Chellakannu M: E Mail: chella1980@gmail.com;**

ssrajeshkumar@hotmail.com

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ABSTRACT

Zinc oxide nanoparticles play a major role in the field of nanotechnology for biomedical applications. In this present investigation, we have used *Acorus calamus* for the Green synthesis of Zinc oxide nanoparticles. The synthesized particles are characterized by its morphological analyses. The spherical, square and some agglomerated nanoparticles are found was analyzed and confirmed using Transmission Electron microscope, Scanning Electron microscope and Atomic Force microscope images. The nanoparticles are 10-90 nm in size in the all microscopic results the prepared nanoparticles are analyzed for its Antimicrobial activity against gastrointestinal pathogens such as *Pseudomonas spp*, *Escherichia coli*, *Salmonella spp* and *Vibrio spp*. Based on its zone of inhibition, was confirm the antibacterial potential of the Zinc oxide nanoparticles.

**Keywords: Zinc oxide nanoparticles; green synthesis; antibacterial activity;
gastrointestinal; *Acorus calamus***

1. INTRODUCTION

Zinc oxide is one of the best metal oxides used in nanoscale due to its exclusive with multitasking applications that can be electron mobility, optical electronic, semi

conductivity, transparency and biomedical properties. The synthesis and characteristic of zinc oxide nanoparticles are several biological methods using microorganism and plant extracts such as *Azadirachta indica* [1], *Agathosma betulina* [2], *Aloe Vera* [3] *Coptidis Rhizoma* [4], *Phyllanthus niruri* [5], *Pongamia pinnata* [6], *Trifolium pratense* [7], *Anisochilus carnosus* [8] and *Passiflora caerulea* [9], Bacteria such as *Rhodococcus pyridinivorans* [10], *Serratia ureilytica* [11], *Aeromonas hydrophila* [12],

algae *Chlamydomonas reinhardtii* [13], *Sargassum muticum* [14] and fungus *Aspergillus fumigatus* [15], *Candida albicans* [16]. *Acorus calamus* is one of the important medicinal plant coming under Acoraceae. The rhizome of the *Acorus calamus* most widely used as a traditional medicine in India. It has a lot of application in the field of pharmaceutical. The application of *Acorus calamus* shown in **Table 1**.

Table 1: Applications of *Acorus calamus*

S.No	Pharmacological Application	Reference
1	Neuropsychopharmacological effect	Dwivedi et al.,2012 [17]
2	Anti-diabetic Activity	Prisilla et al.,2012[18]
3	Anti-seizures Activity	Gopalakrishna et al.,2010 [19]
4	Antidepressant Activity	Tripathi et al.,1995 [20]
5	Neuromodulatory Effect	Vengadesh et al.,2009 [21]
6	Anticancer Activity	Gaidhani et al.,2009 [22]
7	Antioxidant Activity	Subathraa et al.2012 [23]
8	Antihypertensive Effect	Patel et al., 2012 [24]
9	Anti HIV Activity	Silprasit et al.,2011 [25]
10	Cytotoxic Effect	Rajkumar et al.,2009 [26]
11	Immunosuppressive Activity	Mehrotra et al.,2003 [27]
12	Radioprotection and DNA Repair Activity	Sandeep et al., 2010 [28]
13	Coronary Vasodilator Effect	Shah et al., 2012 [29]
14	Antispasmodic and Anti-diarrhoeal Effect	Gilani et al.,2006 [30]
15	Insulin Sensitizing Activity	Wu HS et al.,2009 [31]
16	Wound-healing Activity	Jain et al., 2010 [32]
17	Anti-inflammatory Activity	Kim et al., 2009 [33]
18	Synergistic Anthelmintic Activity	Merekar et al.,2011 [34]
19	Antihepatotoxic Activities	Palani et al.,2009 [35]
20	Anti-ischemic Heart Disease Activity	Mamgain et al.,1994 [36]
21	Antifungal Activity	Begum et al., 2007 [37]
22	Antibacterial Activity	Manikandan et al., 2010 [38]
23	Analgesic Effect	Hosen et al., 2011 [39]
24	Antipyretic Activity	Nethengwe et al., 2012 [40]
25	Bronchodilatory Activity	Shah et al., 2010 [41]

The objectives of the present study designed a green synthesis of Zinc oxide nanoparticles from *Acorus calamus* which is purely a green chemistry method and doesn't include any toxic chemicals and synthesized nanoparticle were characterized using UV-spectroscopy,

scanning electron microscope, atomic force microscope, transmission Electron microscope, electron dispersive analysis. We analyzed the zone of inhibition using bio synthesized nanoparticles and plant extract by antibacterial activity against gastrointestinal bacteria with was collected

from the clinical sample and the concentration of inhibition was analyzed by minimum inhibitory concentration. Finally, Antioxidant activities of ZnO nanoparticles are investigated using DPPH assay.

2. MATERIALS AND METHODS

2.1 Isolation and identification gastrointestinal pathogens

To test the antimicrobial activity of *Acorus calamus* the pathogenic organism was collected from the fecal samples of gastrointestinal disease affected the patient. The isolates were confirmed by morphologically and biochemical studies.

2.2 Collection of the plant extract

The plant powder (*Acorus calamus*) were collected from the Vellore market, on that 1 g of the powder and was dissolved in 100 ml of water then it was boiled for 10 minutes and it was filtered double time using Whatmann No. 1 filter paper. Finally collected extract was stored in the refrigerator for further use.

2.3 Phyto-synthesis and characterization of Zinc Oxide nanoparticles

Zinc nitrate was taken up to 1 M and it was dissolved in the 90 ml of the water then add 10 ml of the extract on that and it was kept in the shaker for 2 hours after 2 hours take reading on the UV spectroscopic 200nm-500nm then add 1 mM NaOH on that and again placed on the shaker for 3 hours after completion of 3 hours again take reading

on the UV spectroscopy and then centrifuge the solution, get the pellet and placed them in hot air oven for 100 °C for 1 hour .Finally collect the powder for characterization using SEM, EDX, TEM, and AFM.

2.4 Antibacterial activity of ZnO NPs

Antibacterial activities of the bio synthesized nanoparticles, plant extract and zinc nitrate were studied by agar well diffusion using Muller Hinton Agar (MHA) plates. MHA medium was prepared in Erlenmeyer flask and sterilized by autoclaving at 121°C for 15 minutes. After sterilization 20ml of the medium was added into each sterile Petri plate in an aseptic condition and allowed to solidify then cut the well to inoculate the biosynthetic solution. First, inoculate the 18 hours old culture broth on the medium by swabbing the culture on the medium using the sterile cotton swab. To that add 40µl of synthesized compound and plant extract by using micropipette. All the plates were incubated at 37°C for 24 hrs. After incubation, the plates were observed for the zone of inhibition.

2.5 Minimal inhibitory concentration

Minimum inhibitory concentration (Kirby Bauer method) used to determine the sensitivity of the microorganism to the specific antimicrobial agent. For this study the different concentration of the

nanoparticle loaded in the MHA medium containing plates into 20 μ l, 40 μ l, 60 μ l, 80 μ l, 100 μ l by using micropipette. All the plates were incubated at 37°C for 24 hrs. After incubation, the plates were observed for the zone of inhibition.

3. RESULTS AND DISCUSSION

3.1 Confirmation of selected pathogens

The pathogen includes *E.coli*, *Pseudomonas spp*, *Salmonella spp*, *Vibrio spp* was confirmed by Morphological, cultural, Biochemical studies shown in **Table 2-4**.

3.2 Green synthesis of zinc oxide nanoparticles

Green synthesis of zinc oxide nanoparticles using most widely used medicinal plant *A. calamus* shown in **Figure 1**. The changing colour from brown to whitish indicates the zinc oxide nanoparticles formation. The reaction between the phytochemicals present in plant extract may lead to colour changes [42].

3.3 Antibacterial activity of zinc oxide nanoparticles

In this present study, *Acorus calamus* (sweet flag) root was taken for the analysis of antibacterial activity against the gastrointestinal bacteria such as *E. coli*, *Pseudomonas sp*, *Vibrio sp*, *Salmonella sp*. Result obtained in the present study revealed that the tested medicinal plants

shown in **Figure 2 and 3**. Extract possess potential antibacterial against the selected pathogens. The antimicrobial activity of zinc oxide nanoparticles, *A. calamus* plant extract and zinc nitrate towards different bacterial pathogens were tested by the well and disc diffusion agar method. The presence of inhibition zone clearly indicates that the mechanism of the biocidal action of zinc oxide nanoparticles which involve disruption of the membrane with the high rate of generation of surface oxygen species and lead to the death of gastrointestinal pathogens. Interestingly, the size of the inhibition zone was different according to the type of pathogens, three different sources. When compared to zinc nitrate and plant extract the nanoparticles are showing the good zone of inhibition due to its highly efficient action against the pathogens by its reduced size [4, 43].

3.4 Characterization of green synthesized zinc oxide nanoparticles

The synthesized nanoparticles were characterized for its morphological investigation using various microscopic techniques.

3.5 Scanning Electron Microscope

The SEM image of the green synthesized zinc oxide nanoparticles is shown in **Figure 4**. This image indicated that Zinc oxide nanoparticles were predominantly spherical and a lot of agglomeration with other Zinc

oxide nanoparticles. The figure 5 EDX results revealed that the particles consisted of 87% of Zn and 9% oxygen and lower amount carbon Taken together, the SEM and EDX results confirm the presence of zinc oxide nanoparticles. The higher amount of phytochemicals of the *A. calamus* leads to the agglomeration and it confirmed in the EDX analysis [9, 43].

3.6 Transmission Electron Microscope

The TEM image confirmed the nanostructure and square, Rectangular, spherical and some undefined shape of zinc oxide with synthesized using *A. calamus* with the size being less than 30-70 shown in Figure 6. Aggregation was observed probably due to the high amount of phytochemical bind with the zinc oxide nanoparticles that usually occurs when synthesis carried out in plant extract [42].

3.7 Atomic Force Microscope

The size of the nanoparticles observed on the tip of the nanoparticle it was obtained as 10- 90 nm. The SEM and TEM images also showing more or less

same morphological identification. The clear aggregation of nanoparticles is identified in the all microscopic image analysis shown in Figure 7. The surface of the nanoparticles is showing some aggregation due to the plant extracts [9].

3.8 Minimal inhibitory concentration assay

Figure 8 shows the MIC of the zinc oxide nanoparticles against selected pathogens at different concentration. In that, Zinc oxide nanoparticles very vigorously reduce the growth of inhibition of *Vibrio* sp and *Pseudomonas* sp have the minimum zone of inhibition. The increased concentration of nanoparticles leads to the high zone of inhibition and lower concentration may lead to less activity in the microbial growth. But differences between the zone of inhibition in the 20, 40, 60, 80 and 100 µl concentration is not high. The size of the nanoparticles is the very major reason for the microbial growth control and the concentration is a minor reason was proved in this investigation [3, 4, 9].

Table 2: Confirmation of selected pathogens

Organisms	Gram staining	Motility	Capsular staining
<i>E. coli</i>	Gram (-ve),Rod	Motile	Non capsulated
<i>Pseudomonas sp</i>	Gram (-ve),Rod	Motile	Non capsulated
<i>Salmonella sp</i>	Gram (-ve),Rod	Motile	Non capsulated
<i>Vibrio sp</i>	Gram (-ve),Rod	Motile	Non capsulated

Table 3: Colony Morphology

Organism	Nutrient agar	Macconkey	Selective medium
<i>E. coli</i>	White, Pin headed colony	Lactose fermenting	EMB –green metallic sheen
<i>Pseudomonas sp</i>	Translucent pigment, bluish green colony	Non Lactose fermenting	Cetrimide green fluorescence colony
<i>Salmonella sp</i>	Cetrimide fluorescence green colony	Non Lactose fermenting	SS Agar Black colony
<i>Vibrio sp</i>	Small, circular, smooth colony	Non Lactose fermenting	TCBS Yellow colour colony

Table 4: Biochemical analysis

Test	<i>E.coli</i>	<i>Pseudomonas</i>	<i>Salmonella</i>	<i>Vibrio</i>
Indole	+	-	-	+
Methyl red	+	-	+	-
Voges-proskauere (VP)	-	-	-	+
Citrate utilization	-	-	+	+
Urease	-	-	-	-
Oxidase	+	+	-	+
Catalase	-	+	-	+



Figure 1: Green synthesis of ZnO nanoparticles using *A. calamus*

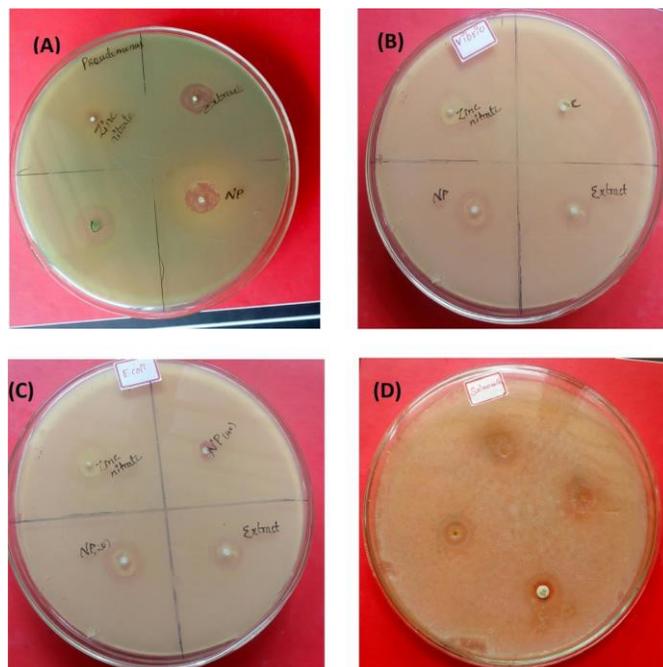


Figure 2: Antibacterial activity of ZnO NPs, Plant extract and ZnNo₃

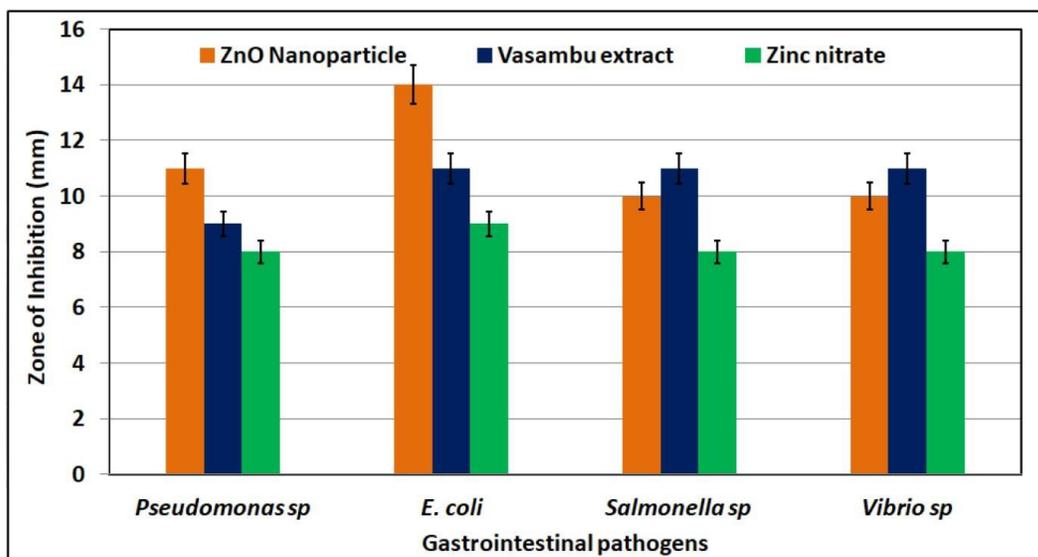


Figure 3: Graphical representation of antibacterial activity of ZnO NPs, Plant extract and ZnNo₃

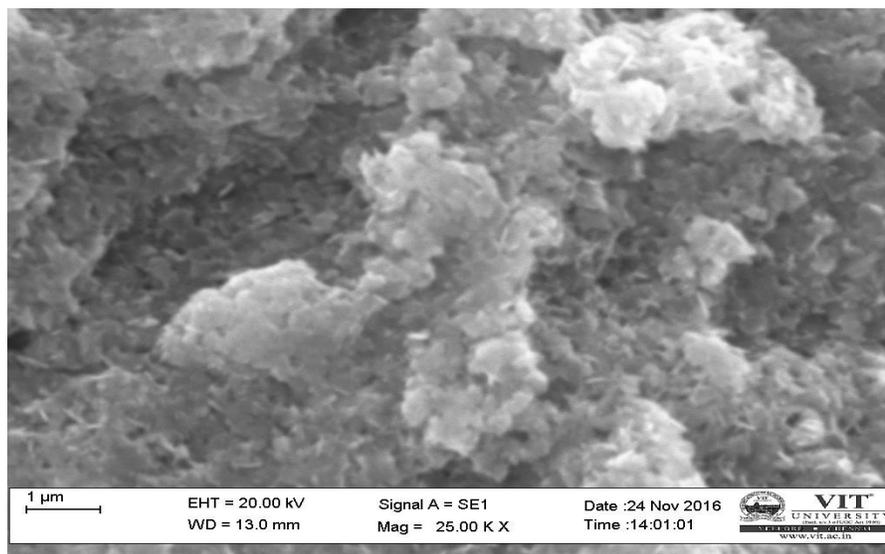


Figure 4: SEM image of ZnO nanoparticles

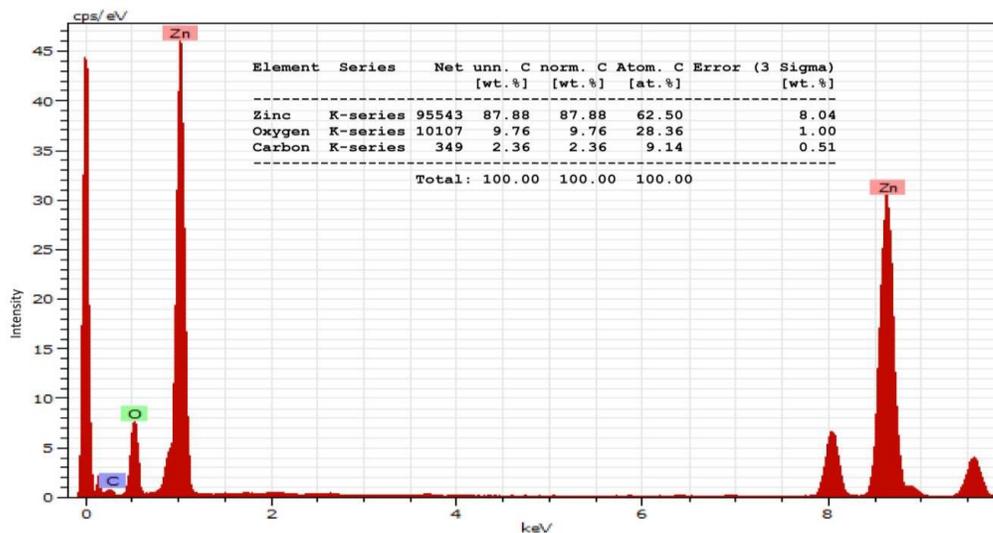


Figure 5: EDX spectrum of ZnO nanoparticles synthesized using *A. calamus*

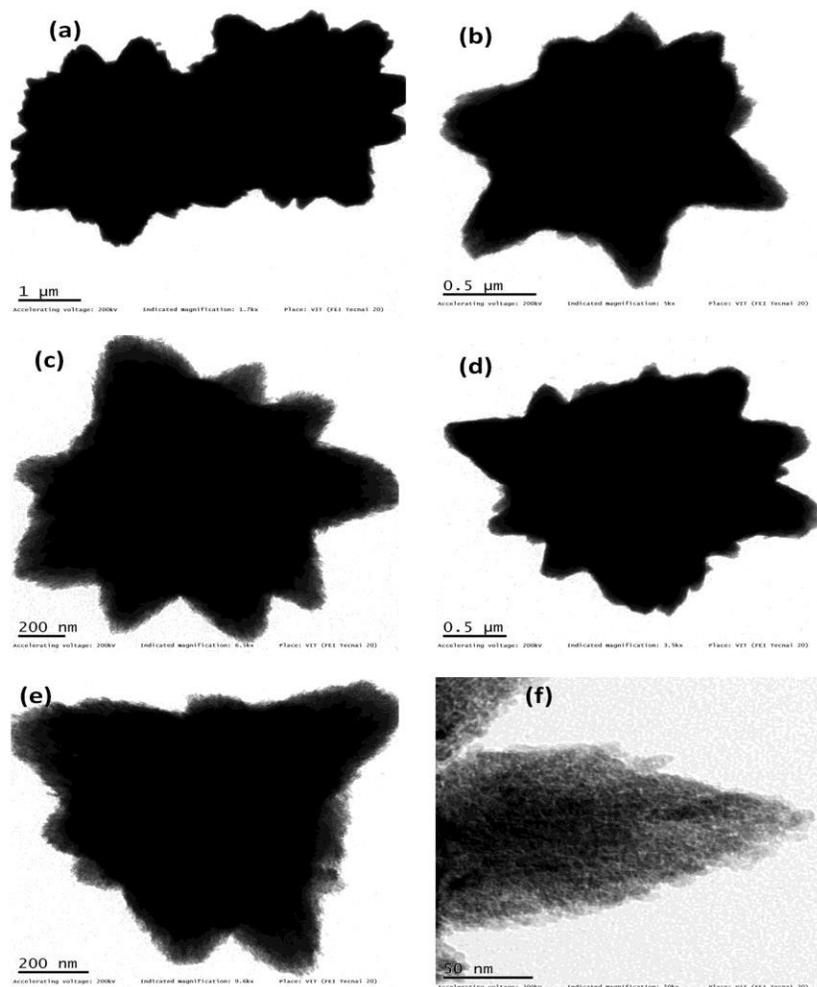


Figure 6: TEM images of ZnO nanoparticles

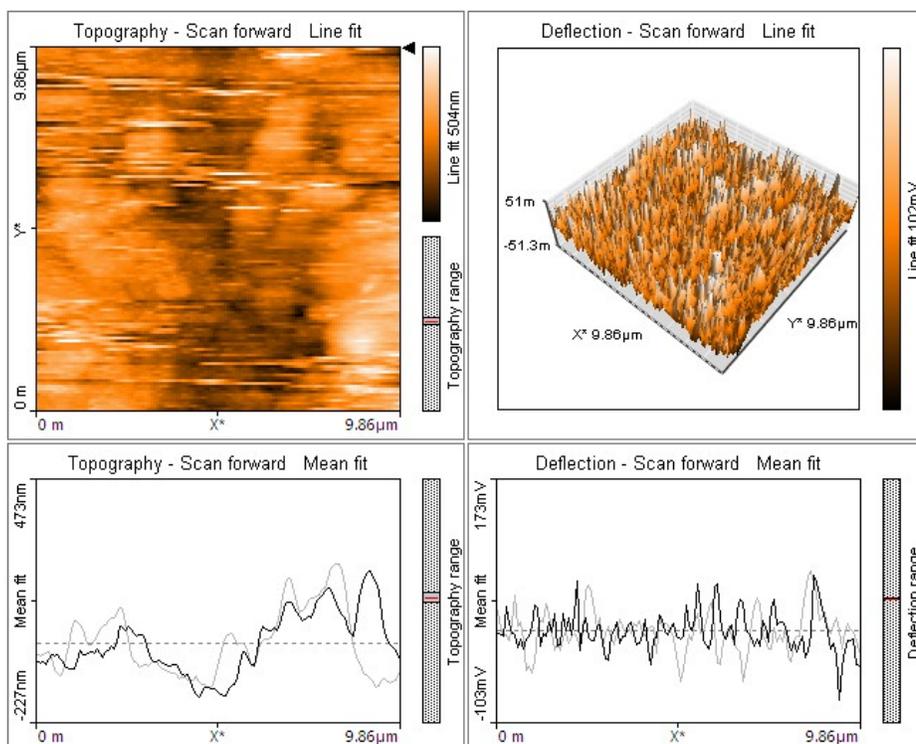


Figure 7: Atomic Force Microscope image of ZnO nanoparticles

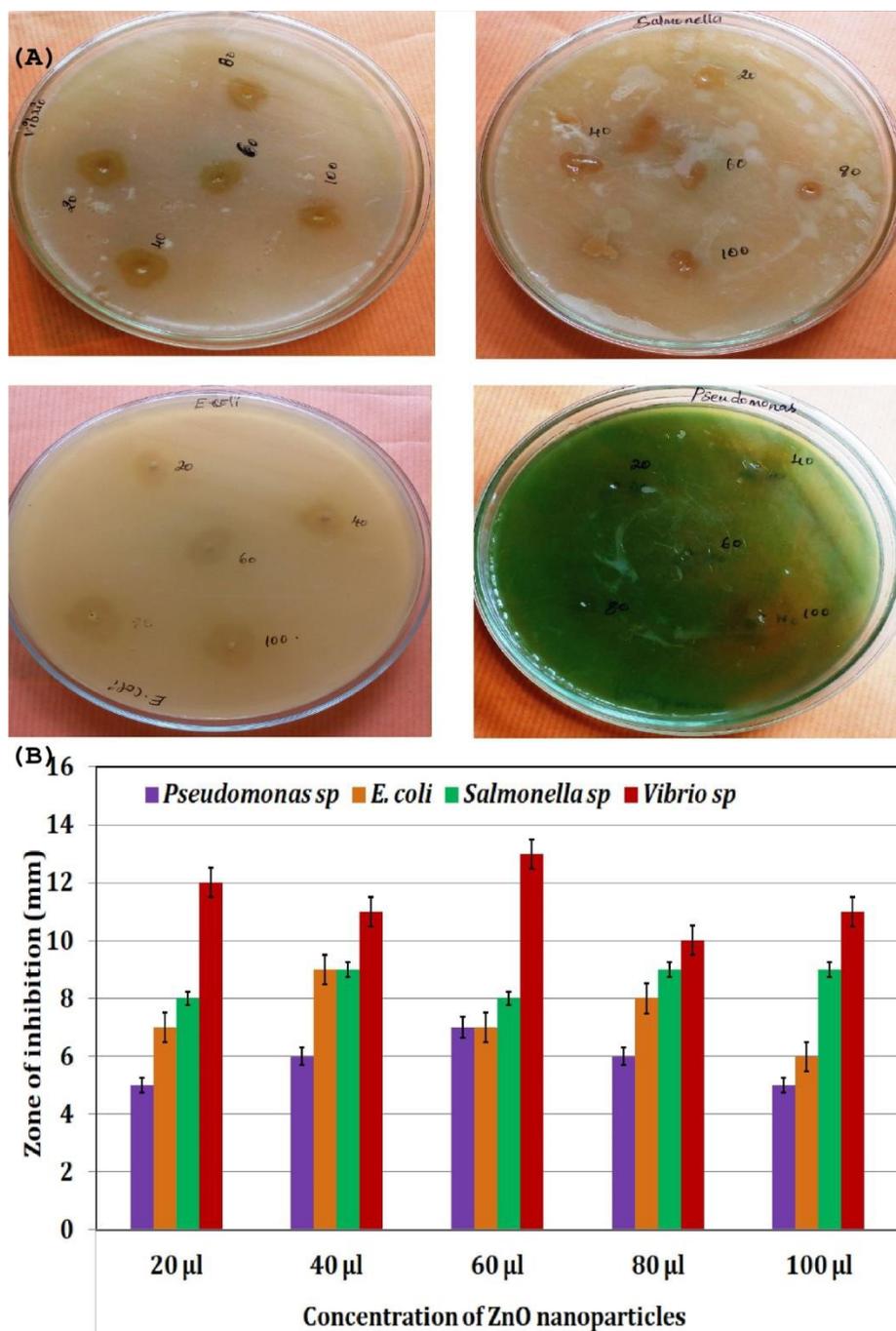


Figure 8: Growth of pathogenic bacteria in different concentration nanoparticles

CONCLUSION

A. calamus is well-known medicine for the stomach pain. In this study the herbal medicine *A. calamus* used for the zinc oxide nanoparticles synthesis. The results are confirming the zinc oxide nanoparticles characters. The prepare Zinc oxide nanoparticles are showing very good

bactericidal potential against the pathogens isolated from gastro intestinal such as *Pseudomonas sp*, *Escherichia coli*, *Salmonella sp* and *Vibrio sp* when compare to *A. calamus*. Our resultant nanoparticles are confirming the very good biomedical potential. In future herbal and nano-formulation is used as a medicine.

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