Evaluation of biologically synthesized and characterized zinc oxide nanoparticles on *Propionibacterium acnes*

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ABSTRACT

ZnO nanoparticles exhibit strong antibacterial activities on broad spectrum of bacteria. The present study focused on the green synthesis of ZnO nanoparticles using the extract of spinach, garlic and ginger with the use of zinc acetate as precursor.Characterization of the nanoparticles was done by UV-Visible Spectrophotometry and Fourier Transform infrared spectroscopy. The inhibitory effect of ZnO nanoparticles against *Propionibacterium acnes* that causes acne was tested by agar well diffusion method.Among the synthesised nanoparticles ZnO nanoparticles prepared from the garlic extract shown the enhanced antibacterial property against *propionibacterium acnes*, the zone of inhibition 1.9 cm.

KEYWORD*: Propionibacterium acnes,* ZnO nanoparticles, antimicrobial activity, agar well diffusion.

INTRODUCTION:

Nanotechnology is emerging as a rapidly growing field with its application in science and technology for the purpose of manufacturing new materials at the nanoscale level [1]. Nanoparticles can be synthesized using chemical, physical and biological methods[2].Green methods of synthesis uses plants, bacteria, fungi, yeast, enzymes and others[3].The use of green synthesis method by the researchers is rapidly increasing due to usage of less toxic chemicals, eco-friendly nature and one step synthesis of nanoparticles [4].In biosynthesis method, plant extract has been used as reducing, capping and stabilizing agents for the synthesis of ZnNPs due to their reducing properties. Zinc nanoparticles have proved to be most effective as it is exhibit strong antibacterial activities and observed that smaller zinc oxide particles were more toxic to the Microorganisms than the bigger particles[5]. Acne is one of the most common skin diseases, affecting many individuals.*Propionibacterium acnes* is involved in the pathogenesis of acne [6] .*P. acnes* is an anaerobic-aerotolerant diphtheroid-like Gram-positive bacillus that resides in pilosebaceous follicles of the skin and is also found in the conjunctiva , oral cavity, intestinal tract , and external ear canal [7]. Antibiotics (like tetracyclines, macrolides) and antimicrobial agents (such as Benzoyl PeroxideBPO) have been used epicutaneously to treat acne for several decades and are still widely prescribed for acne patients, suffering from mildto moderate acne. However,since the 1980s, numerous reports of increasing *Propionibacterium* species resistance worldwide have

been published [8].*Propionibacterium acnes* have shownresistance to tetracycline etc.Our aim of the study is to synthesis ZnO Nanoparticles from the Spinach, Garlic, and Ginger and to check their antibacterial activity against *Propionibacterium acnes*.

MATERIAL AND METHODOLOGY

2.1 Plant collectionand preparation of plant extract:

For synthesizing zinc oxide nanoparticles, leaves of spinach *(Spinacia oleracea*), garlic *(Allium sativum)*cloves, ginger (*Zingiber officinale)* were collected from local market. The leaves of spinach, garlic cloves and ginger were washed with water in order to get rid of the dust particles and then washed 2–3 times with de-ionized water. The garlic cloves and ginger were peeled off and again washed with water. The spinach leaves, garlic cloves, ginger were crushed with mortar pestle. 40 g of paste was collected in a beaker containing 200ml of distilled water. The solution was boiled at 70°C for 30 min. The leaf extract was allowed to cool at room temperature, was filtered through Whatman number-1 filter paper, and the filtrate was stored for further experimental use.

2.2 Chemicals:

Zinc acetate and sodium hydroxide chemicals were used during this experiment for green synthesis of zinc oxide nanoparticles.

2.3 Collection of microbes:

Propionibacterium acnes was collected from the Microbiology laboratory from Department of Biotechnology,Meerut Institute of Engineering and Technology, Meerut. Three antibiotics (Vanomycin, gentamicin,ciprofloxacin) antibiotic were used.

2.4Bio-synthesis of zinc oxide nanoparticles (ZnONPs) from spinach, garlic and ginger

Zinc acetate(0.2M)was dissolved in distilled water (200ml) using magnetic stirrer. While stirring, NaOH(0.2M) solution was slowly added into the Zinc acetate solution with 40 mL of plant extract . After completion, it was kept for incubation period of 2 hour on magnetic stirrer. After this, the solution was left for 16-18 hour.The precipitate was separated from the reaction solution by centrifugation at 10000 rpm at 4°C for 10 min and pellet was collected. Pellet was dried using a hot air oven at 80°C. In this process, the spinach *(Spinacia oleracea*), garlic *(Allium sativum)* cloves, ginger (*Zingiber officinale)* extract acts as the reducing and stabilizing agent. Zinc oxide nanoparticles were obtained gradually by the erosion and chemical degradation of plant extract.

2.5 Characterization of CH-ZnONPs

The ZnO nanoparticle were characterized using

JASCO-V-530 UV-VIS spectrophotometer. The scanning range for the samples is 350-700 nm. FTIR analysis was also done for the same using SHIMADZU. FTIR Analysis was evaluated to observe the chemical properties and amount of component present in materials.

2.6 Antibacterial activity

Antibacterial activities of ZnONPs were observed against acne bacteria by disc diffusion test along with its combination with antibiotics. Molten and cooled media was poured in sterilized petridishes. The plates containing the acne micro-organism and ZnONPs were incubated at 37 º C for 24 hr. The plates were examined for evidence of zones of inhibition, which appear as a clear area around the disc. The diameter of such zones of inhibition was measured using a meter ruler in centimeter.

RESULTS AND DISCUSSION

Comparative assessment of antibacterial activity of green synthesized zinc oxide nanoparticles with commercially available antibiotics such as gentamicin,ciprofloxacin,vanomycin was done along withsyngerstic effect of commercially available antibiotics with synthesized zinc oxide nanoparticles.

3.1. Green synthesis of ZnO nanoparticles

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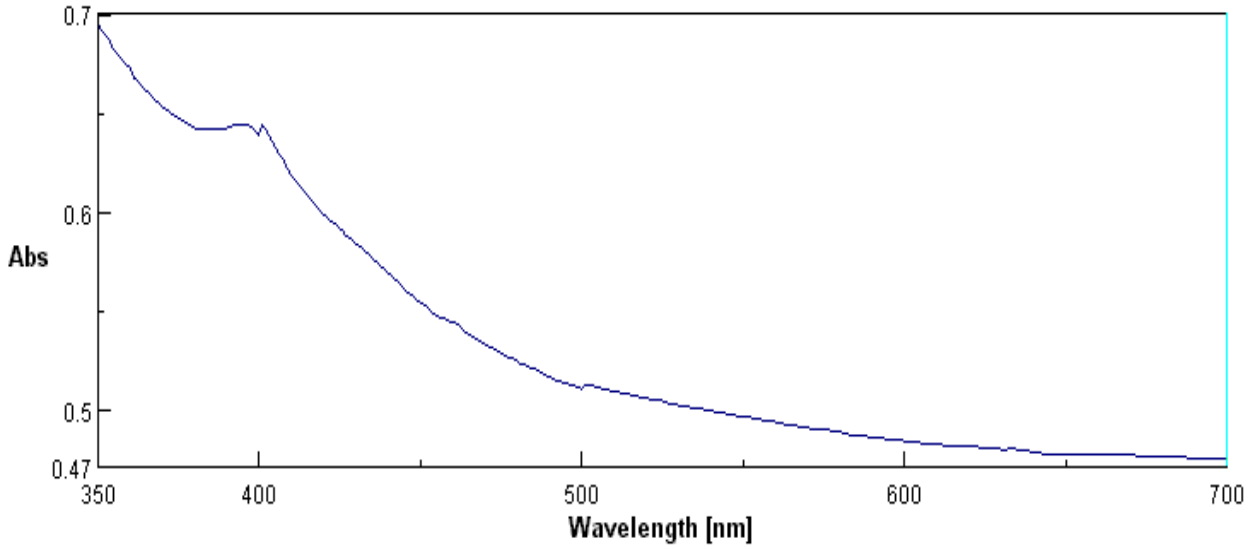
Figure 1:Extract of Ginger, Garlic and Spinach respectively

Figure 2: Green synthesis of zinc oxide nanoparticles

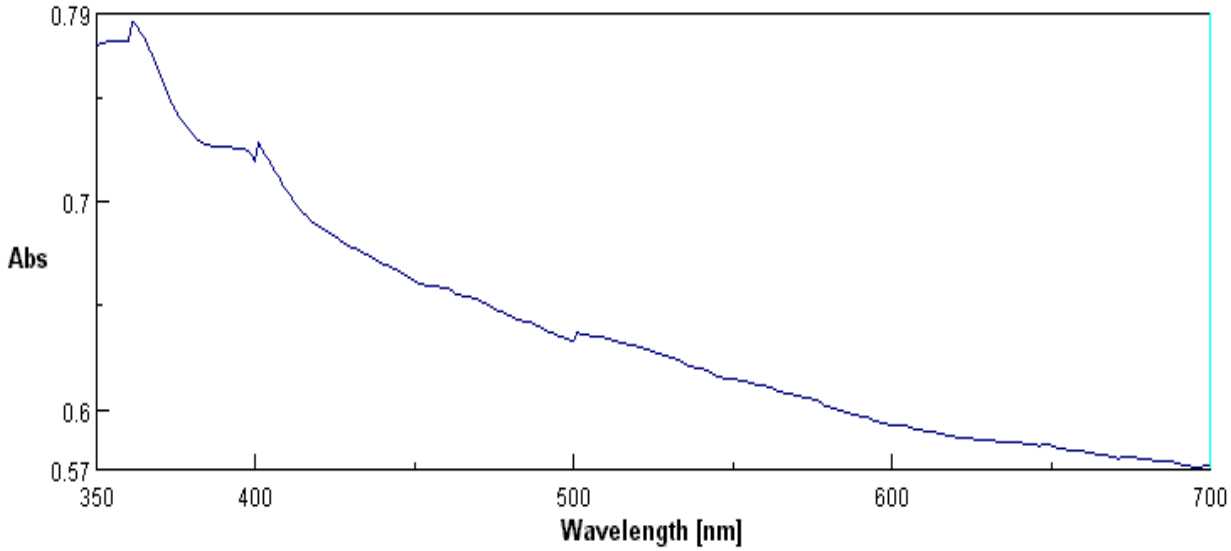
3.1 Characterization of biologically synthesized ZnONPs

UV-Vis spectra for biologically synthesized ZnO nanoparticles have spectrum range between 390- 420 nm. The data for UV-Vis graph and FTIR analysis confirmed the

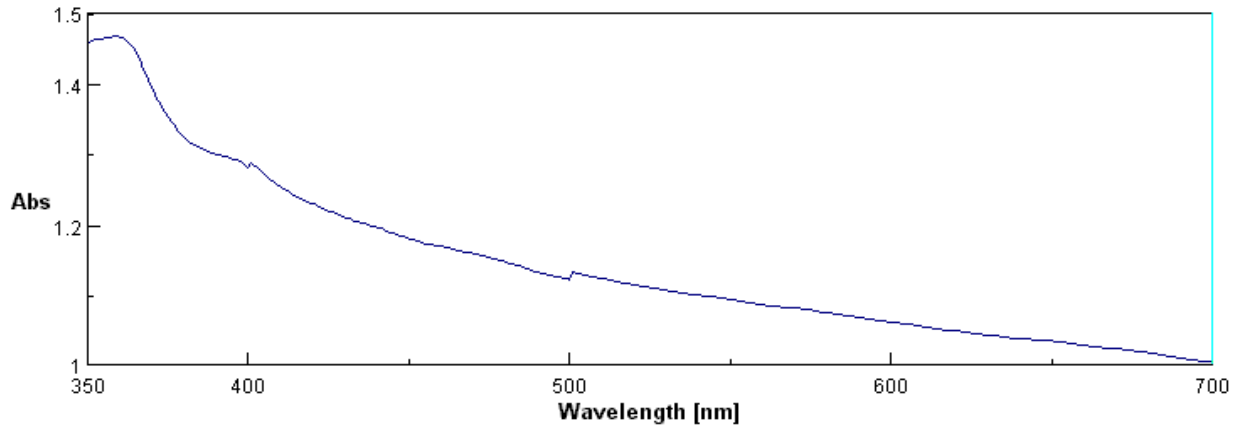
synthesis of zinc oxide nanoparticles (Graph-1, 2, 3, 4, 5, 6).The maximum peaks were observed at 415nm, 410nm and 412 nm for Bio –ZnO NPs synthesized from spinach, garlic and ginger.



Graph-1: UV-VIS Spectrophotometer of 0.2 M ZnO Nanoparticles prepared from Spinach



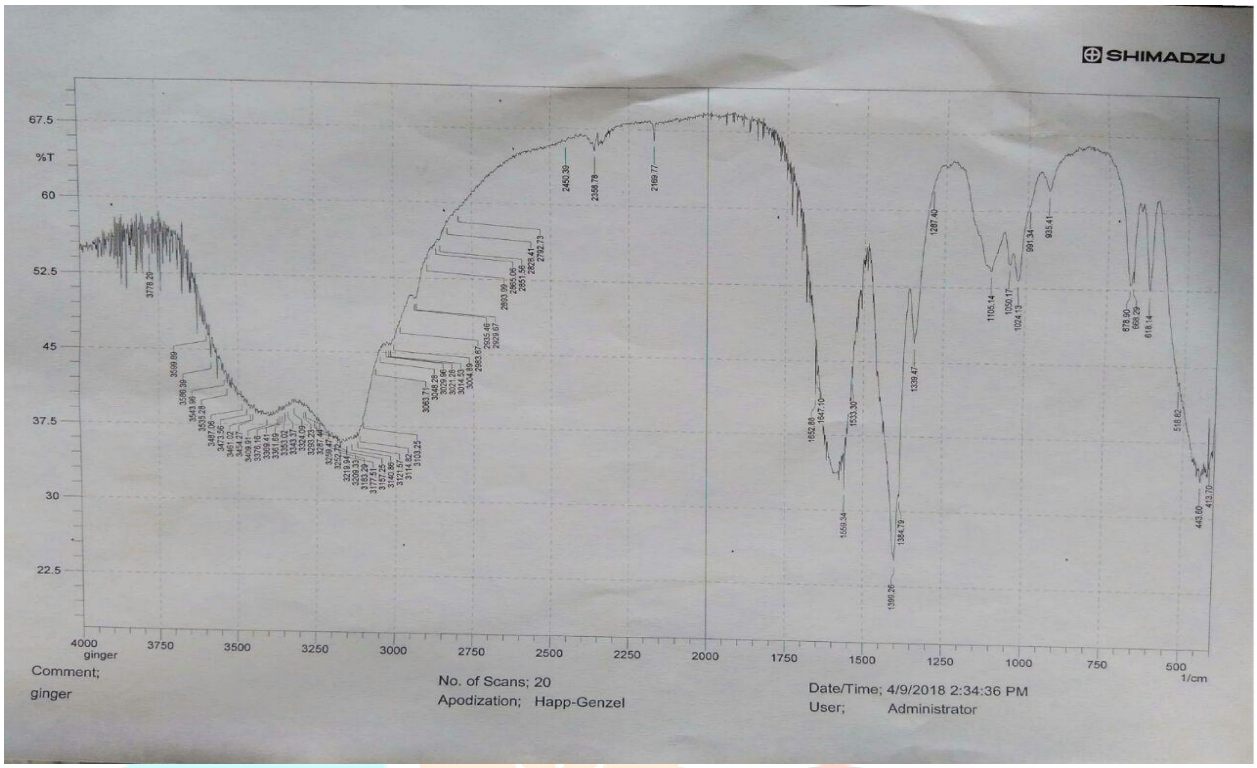
Graph-2: UV-VIS spectrophotometer of 0.2 M ZnO Nanoparticles prepared from Garlic



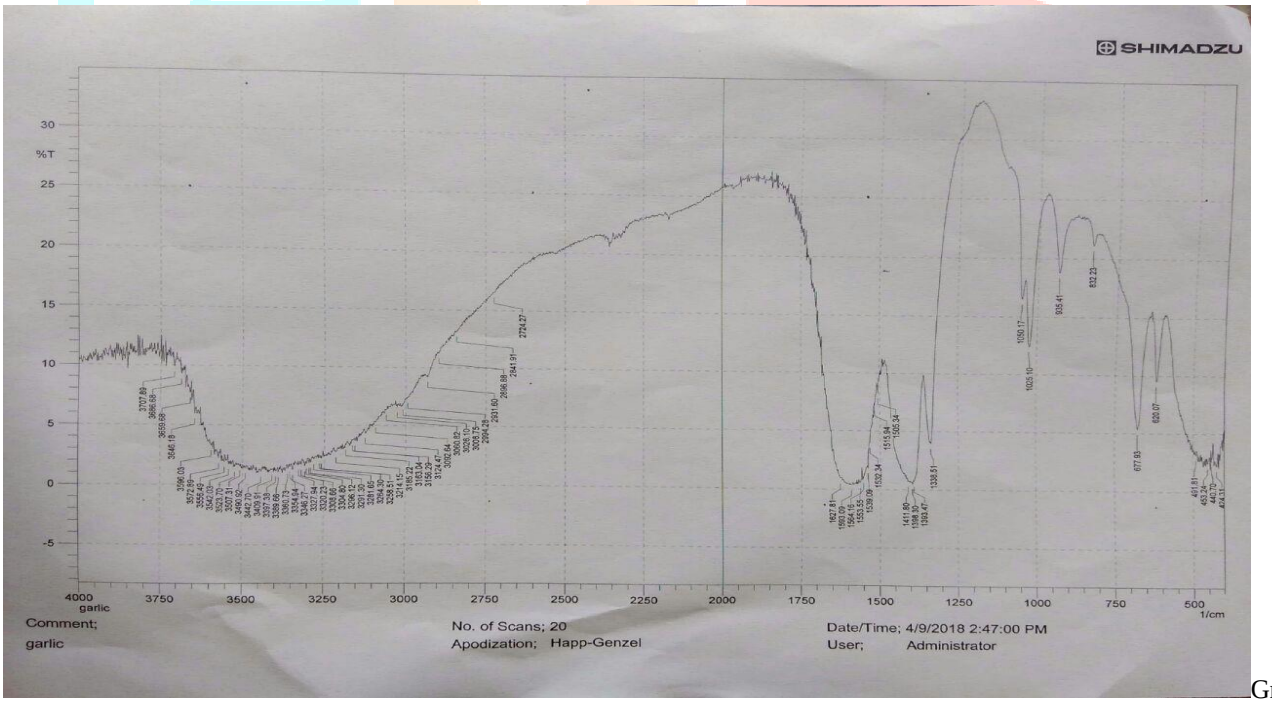
Graph-3: UV-VIS Spectrophotometer of 0.2 M ZnO Nanoparticles prepared from ginger

3.2 FTIR analysis of ZnO nanoparticles

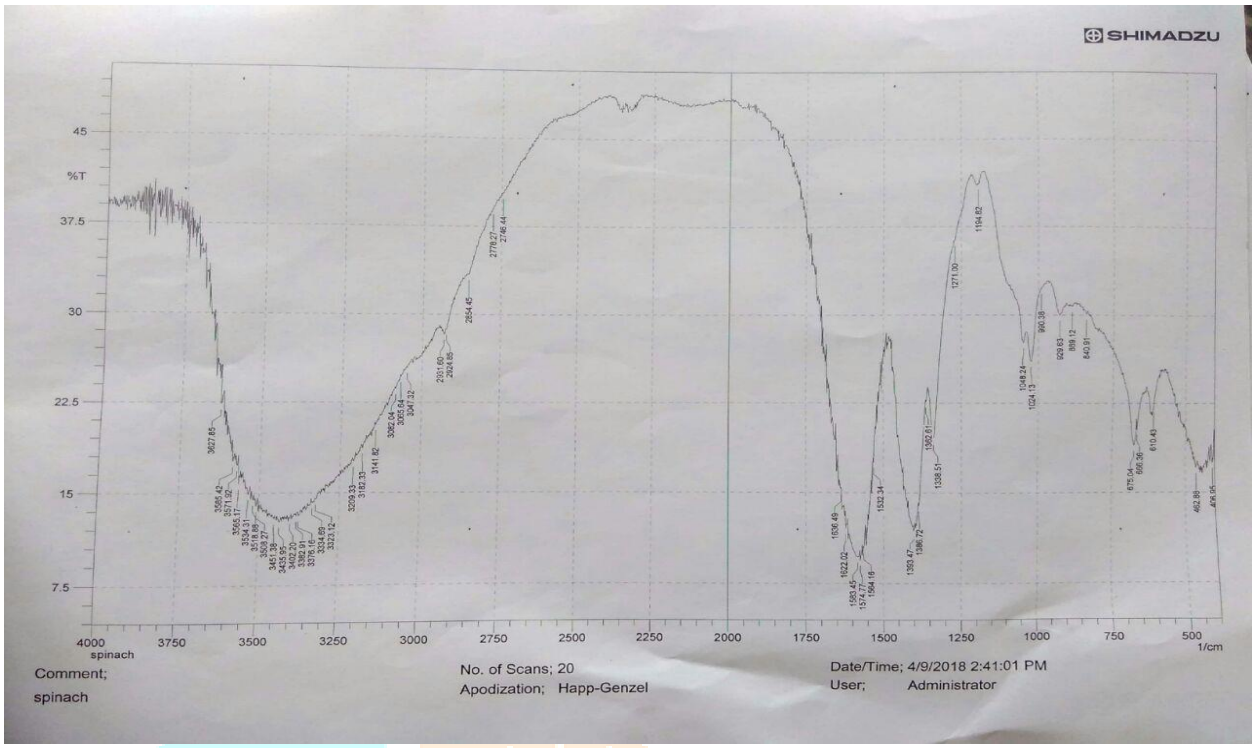
The spectrum of interference pattern by FTIR analysis in the wavelength of 500-4000 cm^{-1}. Graph- 4, 5, 6 clearly shows that absorption band of ZnO nanoparticles nearer to 1338cm^{-1}.



Graph-4: FTIR of 0.2M of ZnO nanoparticles prepared from ginger .The peak at 1384.26cm^{-1} correspond to Zn-O stretching.



Graph-5: FTIR of 0.2M ZnO nanoparticles prepared from garlic .The peak at 1398.30cm^{-1} correspond to Zn-O stretching



Graph-6: FTIR of 0.2 M ZnO nanoparticles prepared from spinach .The peak at 1386.72cm^{-1} correspond to Zn-O stretching

3.3 Antibacterial test for zinc oxide nanoparticles and antibiotics against bacteria

(a) (b) (c)

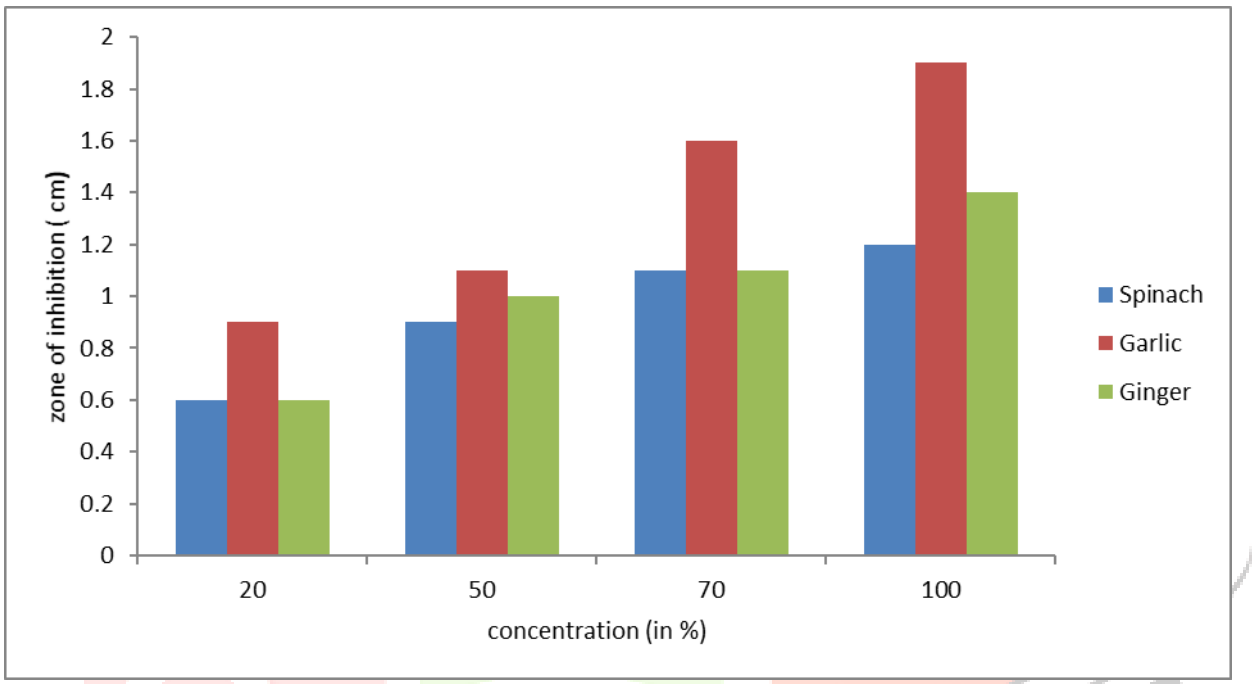
Figure -3: showing Antibacterial activity of ZnONPs prepared from garlic, spinach, ginger respectively as shown in (a),(b),(c).

Figure-4: Antibacterial activity of antibiotics against bacteria and Antibacterial activity of ZnONPs + antibiotic complex

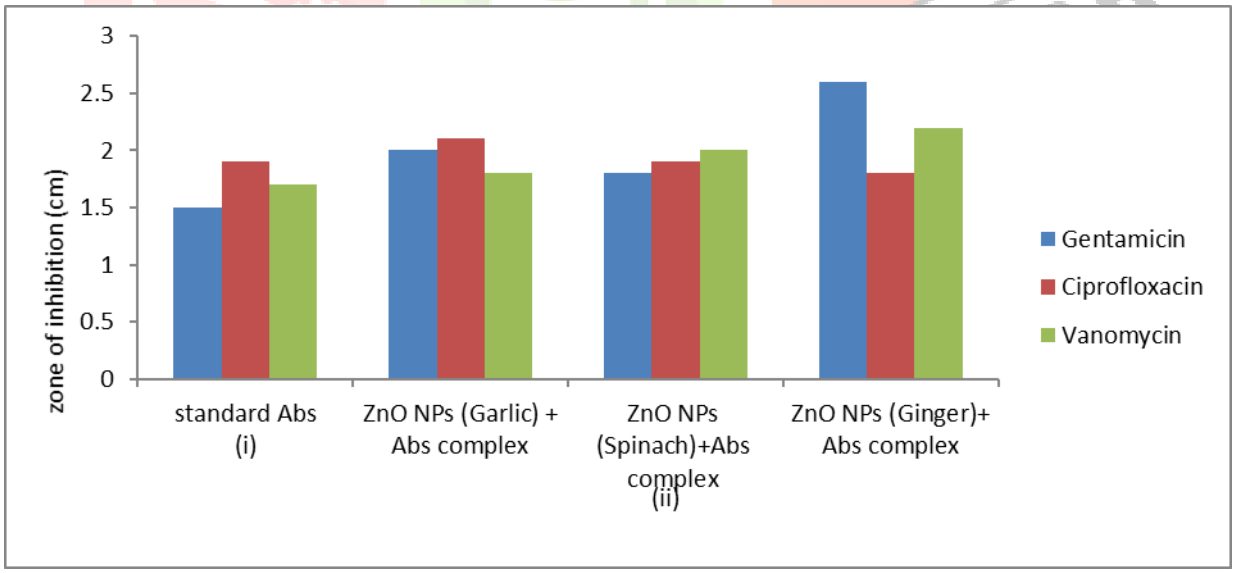
The highest antibacterial activity against *Propionibacterium acnes* is shown by the ZnO nanoparticles prepared from garlic extract as compared to other ones (Spinach, Ginger) as shown in figure 3.The decreasing order of antibacterial activity of biologically synthesized ZnO nanoparticlesis

Garlic > Ginger>spinach.

As observed from figure 4, the highest antibacterial activity is in case of ciprofloxacin followed by vanomycin and gentamicin. Moreover, the concentration (100%) of ZnO Nanoparticles +antibiotic complex are more efficient against Propionibacterium *acnes*.



Graph-7: Showing relationship for zone of inhibition (cm) of biologically synthesized different ZnONps



Graph-8: Showing relationship between:(i)standard antibiotics and zone of inhibition (cm) for *Propoinibacterium acne* and (ii) biologically synthesized ZnONPs + antibiotics complex and zone of inhibition (cm) for *Propoinibacterium acnes.*

CONCLUSION

In this work, Zinc Oxide nanoparticles were biologically synthesized using Garlic, Ginger and spinach extract and characterized by using UV-Vis spectroscopy and FTIR. We have also evaluated antibacterial property of biologically synthesized nanoparticles against *Propoinibacterium acnes.* The highest antibacterial activity against *Propionibacterium acnes* is shown by the ZnO nanoparticles prepared from garlic extract (zone of inhibition- 1.9 cm) as compared to other ones (Spinach, Ginger).Moreover, the concentration (100%) of ZnO Nanoparticles +antibiotic complex are more efficient against *Propionibacterium acnes.*The present study concludes that a novel way of treating acne would be the use of biologically synthesized zinc oxide nanoparticles as a *Propoinibacterium acnes* are becoming resistant towards antibiotics.

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