Green Synthesis of zinc oxide nanoparticles using Quercus infectoria (galls) plant and study their biological activities against *Burkholderia cepacia*Isolated from diabetic foot ulcer and wounds

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ABSTRACT
Nanotechnology is one of the biggest research areas in modern science. Zinc oxide nanoparticles (ZnO NPs) are One of the most useful inorganic nanoparticles for treating topical infections, such as ulcerative skin and wounds. Quercus infectoria (galls) extract was used to create nanoparticles, which were then examined using a variety of techniques, including atomic force microscopy (AFM), scanning electron microscopy (SEM), energy dispersive analysis of x-rays (EDX), and X-ray diffractometer (XRD). The research thus offers a quick, easy, and effective way for making multifunctional ZnO NPS using Quercus infectoria. From diabetic foot ulcers and wounds, B. cepacia bacteria were isolated. A very good zone of inhibition was seen when the manufactured nanoparticles were tested against the pathogenic culture to this bacteria.

1. Introduction

Nanotechnology is defined as "science, engineering, and technological processes carried out at the nanoscale, which ranges from (1-100) nanometers."It is possible to apply nanoscience and nanotechnology to any other scientific subject, such as biology, chemistry, physics, materials science, and engineering (Darabdhara & Ahmaruzzaman, 2022). The usage of physical and chemical processes has significantly decreased because to the green manufacturing of nanoparticles.As a result of using less harmful chemicals, being environmentally benign, and producing nanoparticles in a single step, researchers are using green synthesis methods more and more frequently (M. Sundrarajan et al.,2015). Plants and their derivatives, as well as bacteria, fungus, algae, and yeast, are part of the biological system used to create nanoparticles in a sustainable manner.Infectoria Quercus belonging to the family Fagaceae, oak is known locally (Dar et al., 1976; Panahi et al.,2012). it is a tree that is semi-ever green and has ,grey bark. The scaly are alternating, elliptical to , rounded or wedge-shaped at the base, leathery, glabrescent, highly variable in size and colour, with typically wavy margins that have (4-8) crenate to saw-toothed lobes or whole leaf surfaces.Fruit is a glabrous, smooth nut known as an acorn that is largely encased in a scaly involucre called the cup or cupule, which has scales with a greyish pubescent appearance. (lim, 2012). The galls, also called as nut-galls, are almost spherical, greyish-brown, hard, corky, resinous excrescences that occur on immature branches. From the size of a huge pea to a small hickory nut, the excrescences range in size. According to (Soon et al., 2007).The extensively used Quercus infectoria galls have been utilized in Indian traditional medicine to treat gingivitis, toothaches, and as a dental powder.(Sharma et al.,2011) . Astringent, anti-diabetic, anti-tremorine, local anesthetic, antiviral, antibacterial, antifungal, larvicidal, and anti-inflammatory properties have also been pharmacologically demonstrated for the galls of Q. infectoria(Basri et al.,2005)B. cepacia can cause infections in humans and other animals, causing brain abscesses, "wetland feet", keratitis, Bcc is a major cause of morbidity and mortality in inpatients. It is most commonly reported in immunocompromised patients, especially those with cystic fibrosis (Ranjan, et al., 2017). B. cepacia is a rod-shaped, motile, catalase-positive, Gram-negative, non-spore-forming, and lactose-non-fermenting bacteria (Tavares, et al., 2020). They are regarded as a typical environmental species that have been isolated as free-living microorganisms, and they coexist closely with several animals, plants, ameobozoon hosts, and fungi (Stopnisek, et al., 2016; Xu, et al., 2016). Like many opportunistic pathogens, B. cepacia can establish an infection in any favorable environment

2. MATERIALS AND METHODS

2.1. Plant collection

The medicinal plant, Quercus infectoria, was procured from a nearby market. The galls were cleaned with deionized water, allowed to dry at room temperature, and then the samples were broken up and ground into powder using a grinder before being sieved to obtain only a fine powder. The powder was then stored in a dry container for use at a later time.

2.2. Preparation of the plant extract

Weighed 30 g of dry vegetable powder for each of the plant samples and mixed with 300 ml of 70% ethyl alcohol in a 500 ml glass beaker, closed with cotton and aluminum foil, placed in a shaking incubator, and left for 24 hours at room temperature, after which the mixture was filtered using several layers of liquid. Medical gauze to get rid of plankton after centrifugation at 3000 r/min for 10 minutes, then filter the extract using Whatman NO. 0.1 to obtain a clear solution for use andthe filtrate was stored for further experimental use

2.3. Synthesis of ZnO NPs

Zinc nanoparticles were synthesized from Quercus infectoria extract according to the method of (**P. Jamdagn et al.,2016**), mixing 10 mg of aqueous. Quercus infectoria extract with 100 mL of zinc oxide solution, concentration 1 mmol, and the mixture was poured onto heated on a hot plate. The magnetic shaker at a temperature of 45 C for 24 hours and accepting the color change of the mixture as preliminary evidence of zinc nanoparticle build-up, and 100 mL of the prepared 1 mmol zinc solution was kept for the purpose of accepting it as a control in some measurements

2.4. Characterization of biosynthesized ZnO NPs

Visible UV ZnO NPs were characterized using X-ray diffraction and spectroscopy. In the electron microscopy unit, SEM was utilized to characterize the morphology of nanoparticles. The microscope functioned with variable magnifications, low vacuum, a spot size of 4, and working distances of 5–10 mm at an accelerated voltage of 15 KV (Shafaghat, 2015). Utilizing Bruker EDS coupled with SEM, elemental analysis of a single particle was performed. EDS was utilized to do a point analysis with an accelerating voltage of 10 KV, a spot size of 5, and working distances of 10 mm (Shirley & Jarochowska 2022). The ZnO NPs were characterized using AFM. The FTIR spectrophotometer was used to measure the synthesized formulations transmittance.

2.5. Antibacterial activity of synthesized ZnO NPs

The antibacterial activity of synthesized ZnO NPs was performed by agar disc diffusion method against Burkholderia cepacia . Isolated from diabetic foot ulcer and wounds.Fresh overnight culture of each strain was swabbed uniformly onto the individual plates.The dipping swab was used to smear the entire surface of a Mueller Hinton agar dish. Pores (7 mm diameter) were then created using a sterile corkporer and filled with 100 µl of ZnO NPs at three concentrations (100, 200, 300) g/ml. The Petri dishes were then incubated at 37°C for 24 hours. The diameter of the growth inhibition zones in millimeters was measured to determine antimicrobial activity**(Rajeshkumar and Malarkodi.,2014).**

3. RESULTS AND DISCUSSION

3.1. UV-visible analysis

When ZnO NPs were analyzed using UV-vis spectrophotometry,an absorption peak at 360.5 nmwavelength was seen, showing that ZnO NPs werepresent in the reaction solution. figure (1).Visual inspection and UV-visspectroscopy measurementsof the surface plasmon resonance (SPR)band can both attest to the biogenesis of nanoparticles. Nanoparticles' single SPR banddemonstrates theirspherical form. (Abdulhassan, 2016).

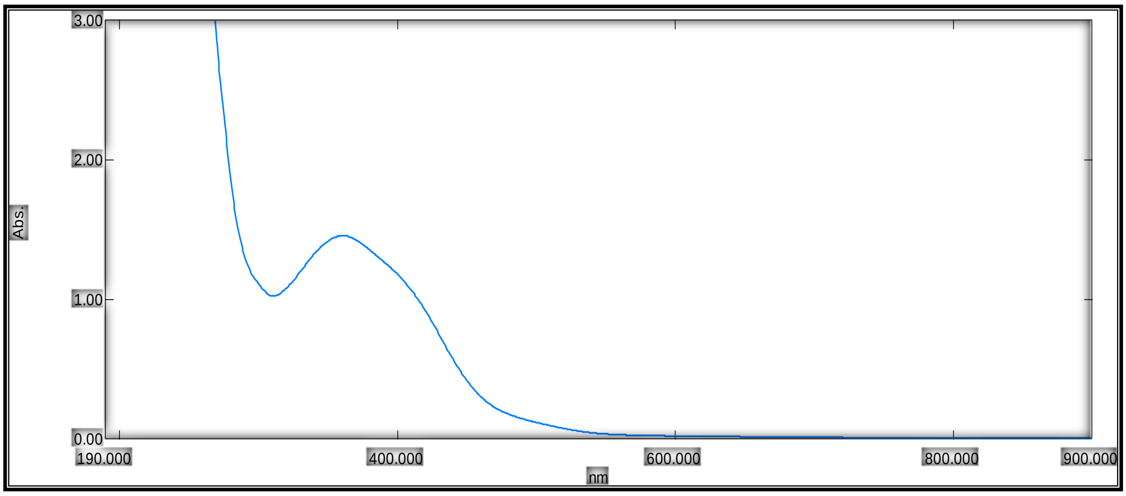


Figure (1):UV-visible spectroscopy analysis of ZINC NPs synthesis byQuercus infectoria

3.2.XRD analysis of nanoparticles

The XRD study confirms the structure of nanoparticles and the formation of narrow peak with the Bragg's angle suggests the crystalline nature of nanoparticle.Stabilization of the nanoparticles occurs by some capping agents which are confirmed by the sharp peaks (Shinwari, & Maaza, 2017). the average crystal size of NPs is calculated (Javed et al., 2016). In current study the results of average crystallite size of zinc NPs biosynthesized from Quercus infectoria (galls) plant were reached to(32.3) nm .Using the Debye–Scherrer equation

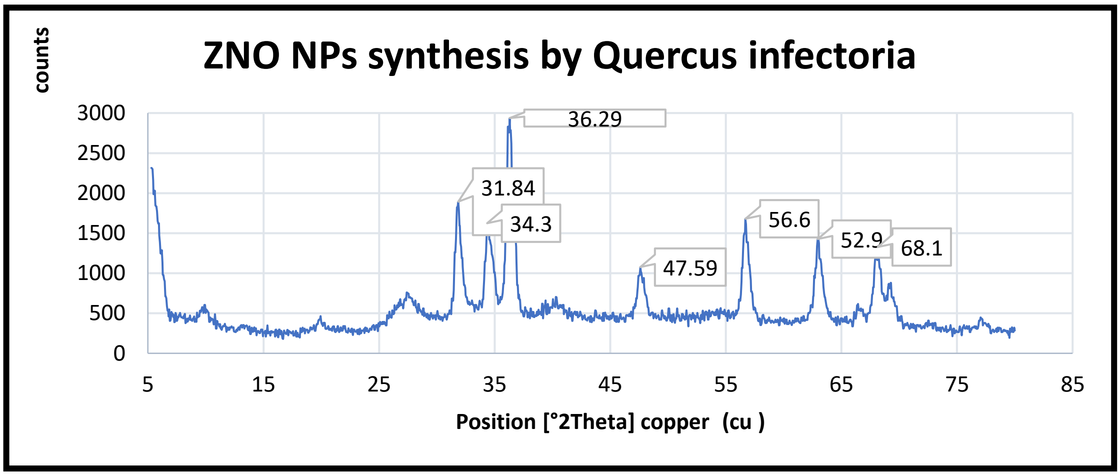


Figure (2) XRD analysis the size of Biosynthesized Zno nanoparticles Quercus infectoria

3.3. SEM and EDAX

SEM analysis is done to visualize shape and size of nanoparticle. Scanning electron microscope was used to determine the shape of Quercus infectoria capped ZnO NPs (Fig. 3). SEM images were seen in different magnification ranges like 500nm–200 nm which clearly demonstrated the presence of spherical shaped nanoparticle with mean average diameter of 70 nm (**S. Raut et al.,2013)**. (Fig. 4) shows the EDAX analysis, confirmed the presence of metallic zinc oxide in biosynthesized ZnO NPs. The composition obtained from EDAX analysis was Zinc (21.60%), Oxygen (36.77%), and Carbon (41.63%) (**N. Bala et al.,2015).**

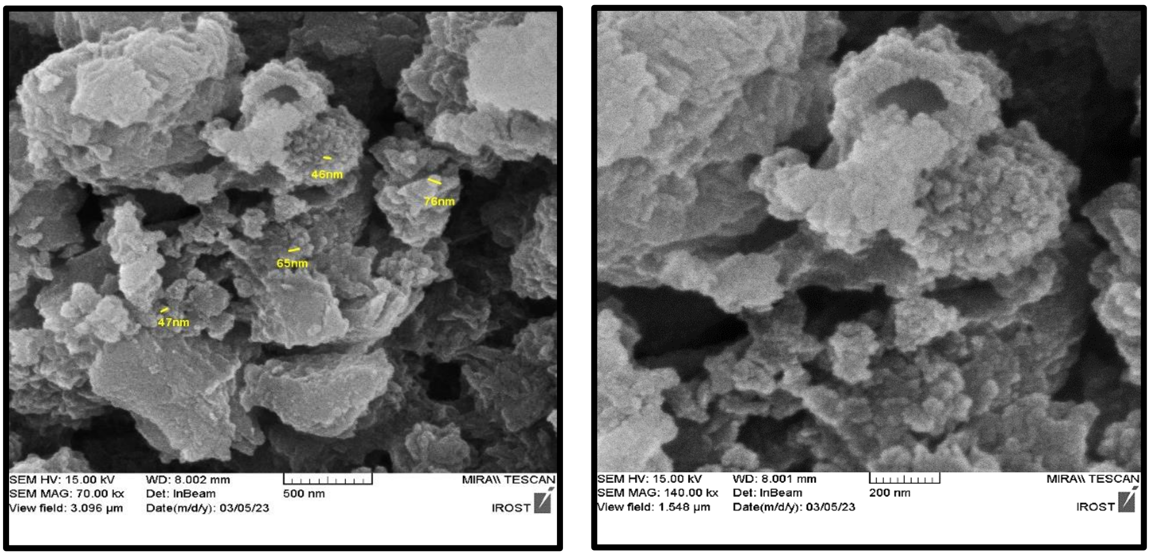
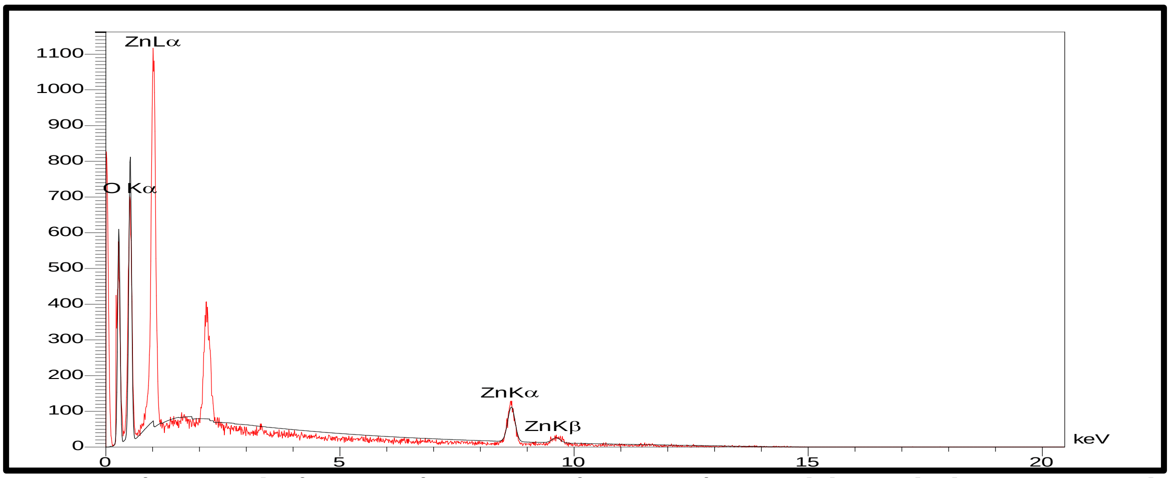


Figure (3): SEM Micrograph of biogenic Znic nanoparticle synthesized by Quercus infectoria

3.6. FT-IR analysis

Figure (4) EDS analysis of Zinc NPs synthesis by Quercus infectoria



The FTIR spectrum of aqueous leaf extract of (Quercus infectoria) (fig.5) and the resulted zinc nanoparticles figure (4-14).Predicts that a shift in the band from 3398.24 to 3315.74 cm-1 which attributed to the stretching vibration of O-H of alcohols and phenols,2343.55 to 2240.18 cm-1 band is attributed to the stretching vibration of C-H aliphatic, 1692.25 to 1610.61 cm-1 band is attributed to the stretchingvibration of carbonyl (C=O), 1554.52 to 1540.10 cm-1 band is attributed to the stretching vibration of C=C, 1420.09 to 1320.00 cm-1 band is attributed to the vibration of C-N, 1120.68 to 1118.75 cm-1 band isattributed to the vibration asymmetric of C-O, 1029.94 to 980.94cm-1band is attributed to the vibration symmetric of C-O, 879.19 to 830.89cm-1 band is attributed to the vibration of C-C skeletal (**S. Yedurkar et al.,2016**).

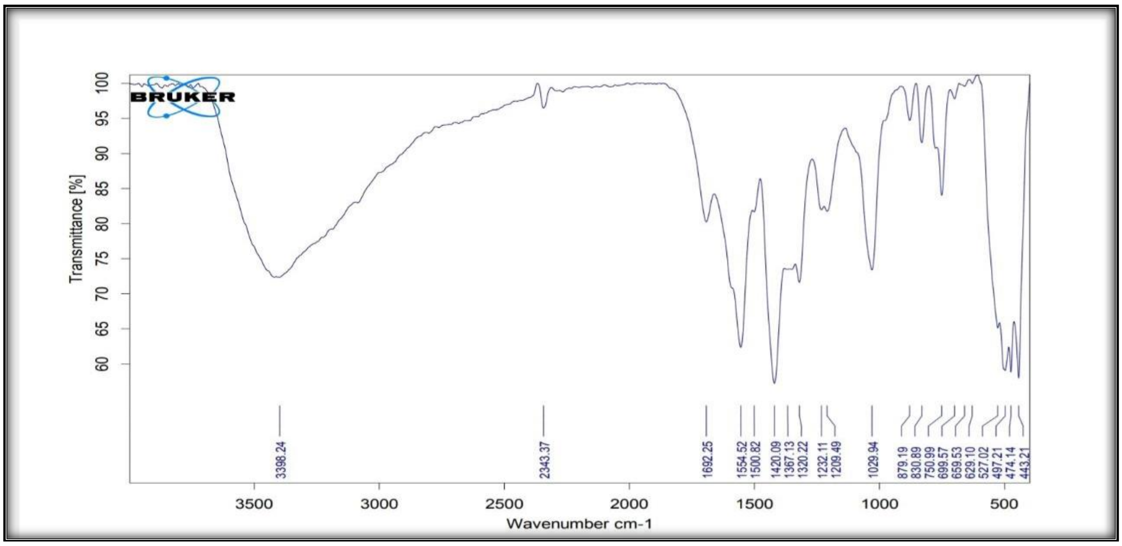


Figure (5): FTIR spectra of ZnO NPs synthesized by Quercus infectoria

3.7. AFM analysis

The AFM examination of the biosynthesized nanoparticles from Quercus infectoria revealed information about their morphology, average diameter, and roughness. As a result, it is possible to control the size and shape of the finished structure using the current density and etching time. The morphology of zinc NPs was documented, despite the fact that the lateral dimensions are affected by the form of the probe. Nanoparticle elevation may be determined with a high degree of accuracy and precision using height measurements **(V. Femi et al.,2011).**

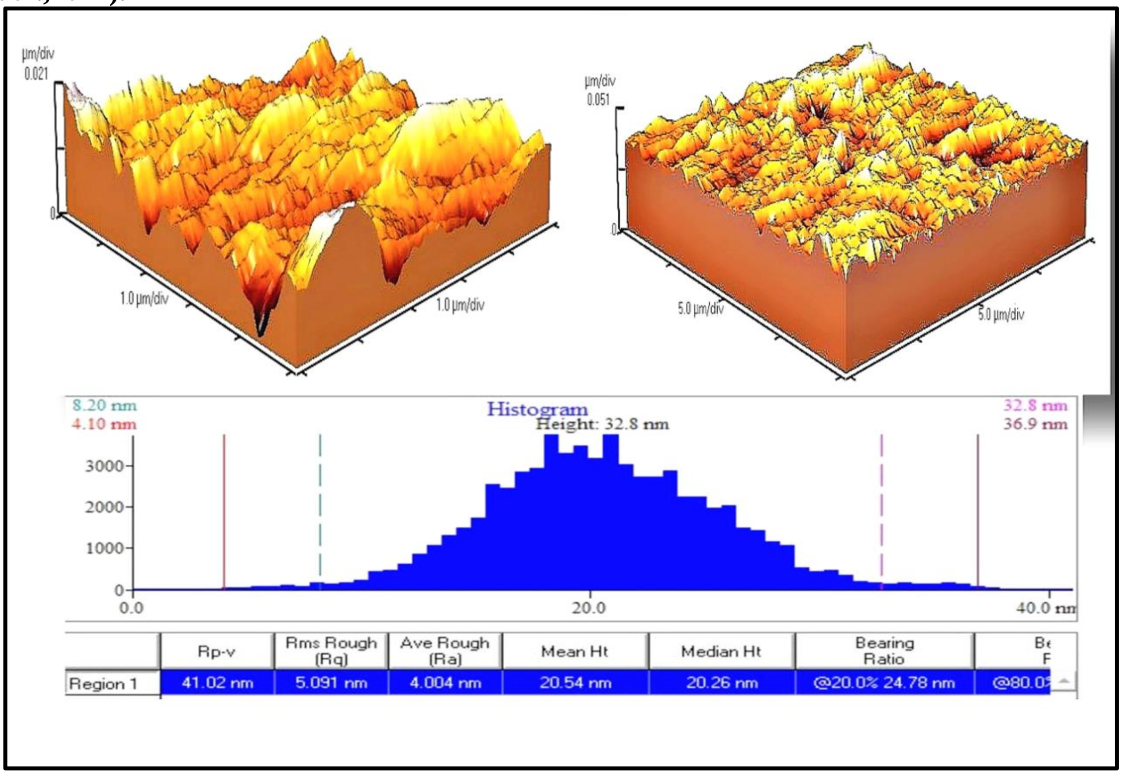


Figure (6) AFM analysis shows three-dimension image, and topography of biogenic ZnO NPs synthesis by Quercus infectoria

3.8. Antimicrobial activity

In this work, which comprised the biosynthesis of zinc oxide nanoparticles and evaluation of their efficiency against pathogenic bacteria isolated from diabetic foot ulcers,and wounds by three bis of all the isolation obtained The agar well diffusion method was used for detecting the antibacterial activity of biogenic NPs.ZnO NPs with different concentrations (300 ,200; 100)µg/ml .It was discovered that Quercus infectoria-produced ZNO NPs had the highest degree of inhibitory effect against B. cepacia (22) at a concentration of (300) g/ml in isolation No. 6 and the lowest inhibitory effect against B. cepacia (12). Different mechanism of action of nanoparti- cle against gram + and gram –bacteria has been already reported in previous literature because of difference in structural compo- sition. Zone of inhibition obtained using nanoparticle was much lower than the standard disc used which depicts the need of fur- ther engineering of nanoparticle to obtain desirable effects. The entire tests were done in triplicate. The zinc oxide nanoparticles are inhibit the microbial growth in in-vitro antimicrobial activities . (V. Mishra et al.,2015) and (Y. Xie et al.,2011) show table (1) and figure (7)

Table (1): Antibacterial activity of zinc nanoparticles synthesis by Quercus infectoria against Burkholderia cepacia

Concentration mg/ml	Inhibition zone rate (mm)									
	isolation number									
	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
100	9	10	12	13	12	15	14	14	11	12
200	10	13	15	14	15	17	17	16	13	14
300	12	15	17	18	18	22	21	20	15	20

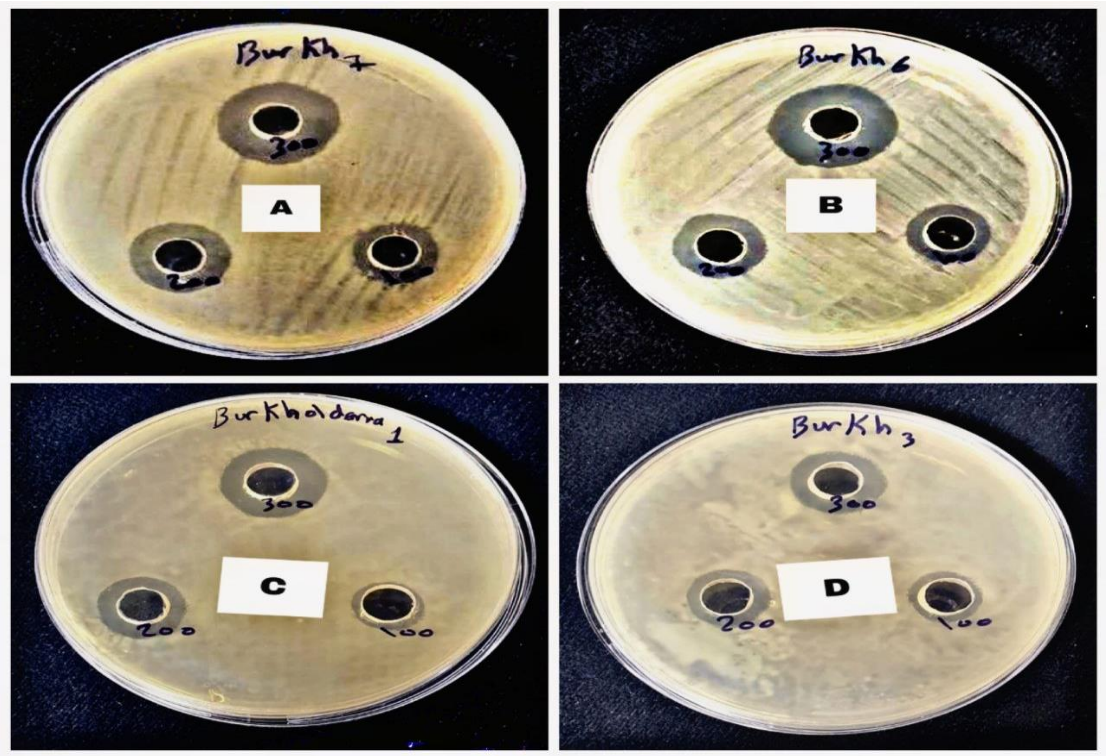


Figure (7): Antibacterial activity of zinc nanoparticles synthesis by Quercus infectoria against Burkholderia cepacia (A,B diabetic foot ulcer sample)(C,D wounds sample)

3.9.Antioxidant activity of biogenic ZnO NPs

The capacity of nanoparticles to scavenge DPPH free radicals was demonstrated in the study by examining the transformation of DPPH from its original (purple) hue to its current (yellow) color. With higher concentrations, ZnO NPs become more efficient at scavenging DPPH free radicals. The lowest inhibition was at a dosage of 0.12 mg/ml, whereas the maximum inhibition was at 1 mg/ml. According to the figure (8). The level of inhibition changes from one type of nanoparticle to another owing to an electron donation and DPPH acceptance (Kanipandian et al 2014;Bhakya et al 2015).

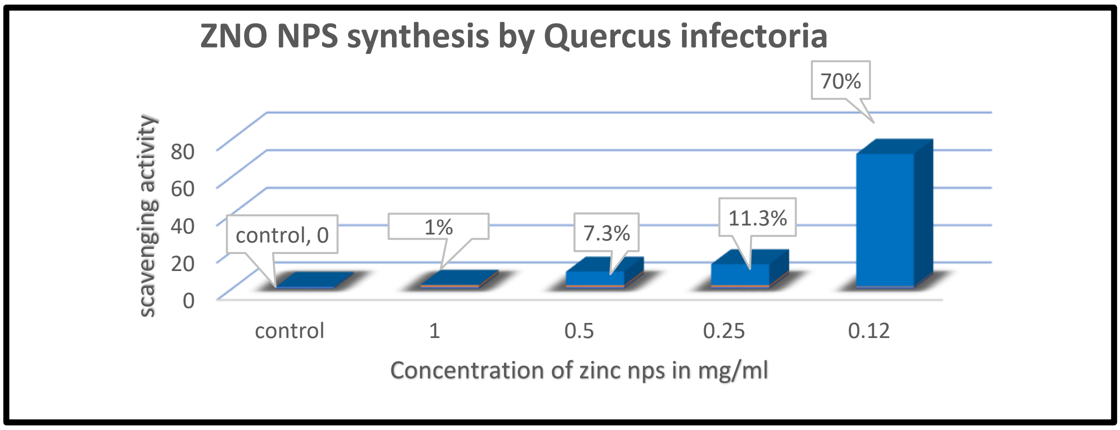


Figure (9): Antioxidant Activity of Biogenic ZNO NPsby DPPH assaysynthesis byQuercus infectoria

4- CONCLUSION

Green synthesis of nanoparticles used in this experiment is found to be eco-friendly, non-toxic and less usage of chemicals compared to physical and chemical method. The presence of phy- tochemicals in the leaf extract itself helps in the synthesis of metal oxide nanoparticle by inducing oxidation and reduction reaction.The functional groups of phytochemicals induce the nanoparticle synthesis were amines and alkanes that are widely seen in sec- ondary metabolites such as terpenoids, flavonoids, alkaloids, etc. As a preliminary confirmation, the rapid synthesis of ZnO NP was measured using the UV- Visible spectroscopy at a maximum ab- sorbance of 360 nm. Further the XRD analysis proved the crys- talline nature of the ZnO NP, while the EDX analysis confirmed the presence of zinc and oxide ions in the nanoparticles. The SEM anal- ysis of ZnO NP demonstrated the size approximately in the range of 30–70 nm. Also the anti-bacterial activity of ZnO NPs has proved that these can be used as potent anti-bacterial agent against diabetic foot ulcer and wounds infection.

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