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Biosynthesis, Characterization and Antibacterial Efficaciousness of Zirconium Oxide Nanoparticles Using Azadirachta Indica Extract

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Abstract:- Zirconium oxide (ZrO_2) nanoparticles were synthesized by biosynthesis technique using Azadirachta indica leaf extract. The synthesized ZrO_2 nanoparticles were subjected to X-ray diffractometer, Ultraviolet-visible spectroscopy, FTIR spectrophotometer,Field emission scanning electron microscopy and energy dispersive X-Ray spectrometer.The particle size, micro strain, dislocation density and the orthorhombic phase of prepared ZrO_2 nanoparticles were determined via XRD. Bandgap value of the ZrO_2 nanoparticles was found to be around 5.4 eV, using UV-visible spectroscopy. From FTIR, the functionalgroups were identified. FESEM results show a uniform distribution of the ZrO_2 nanoparticles. Zr and O elements in the synthesized samples are conformed by EDX. The synthesized ZrO_2 nanoparticles antibacterial efficacy to inhibit Escherichia coli, Pseudomonas aeruginosa, Enterococcus faecalis and Streptococcus mutans was investigated.

Keywords: Biosynthesis, Azadirachta indica, Orthorhombic, Bandgap, Antibacterial efficacy.

1. Introduction

Today, nanomaterials are extensively studied for their activities against various bacteria. Metal oxide nanoparticles have gained more attention in this field and also in photocatalysis, electrocatalysis, bio medicine, artificial implants and as adsorbants [1]. Zirconia (ZrO_2) is a crystalline dioxide of a multifunctional element, zirconium. It provides promising output against infectious diseases and exhibits antibacterial activity due to its low surface free energy. ZrO_2 is an important inorganic p - type metal oxide. ZrO_2 nanoparticles are wide band gap dielectrics with significant interest as they have many practical applications owing to their physicochemical property such as good corrosion resistance, microbial resistance, thermal stability, photo-catalytic activity, reusability and their excellent optical, mechanical and electrical properties [2, 3]. The physicochemical properties of zirconia depend on its crystalline phases. Pure ZrO_2 exhibits three phases namely, monoclinic, cubic and tetragonal. These crystal phases depend on synthesis temperature, precursor concentration, pH level of reaction and the reagents used [4]. Numerous techniques have been developed for the synthesis of ZrO_2 nanoparticles. Some of them are ball milling [5], hydrothermal [6-8], combustion method [9], microwave irradiation [10], co-precipitation [11, 12], solvothermal [13], sol-gel [14] and biological method [12, 15-18]. Among these, biological synthesis is preferable as it is an environmentally friendly method of producing nanoparticles. It uses natural materials such as plants extracts, microorganisms as reducing agents. Redox reactions occur during biosynthesis, and create metallic oxide nanoparticles when leaf extract transfers electrons to metal ions. This method produces high yield and high-quality products at low cost [19]. Recently,several reports on the use of plant extract to synthesis ZrO_2 nanoparticles. V. Sai Saraswathi et al. (2017) prepared zirconium oxide nanoparticles using Lagerstroemia extract. They illustrated the cytotoxicity activity against MCF-7 cancer cells. The results showed that the prepared ZrO_2 nanoparticles performs only at higher concentration [20]. Shinde et al. (2018) prepared monoclinic, tetragonal structured ZrO_2

nanoparticles by using the extract of Ficus benghalensis. The sample was cubic phased with crystallite size of 17nm. The nanoparticles had spherical morphology with particle size ranging upto 100 nm and they exhibited good photocatalytic activity [21]. N. Kavitha et al. (2020) mentioned the bio synthesis of ZrO_2 using by Fusarium solani extract towards dental coating application. The average crystalline size of ZrO_2 about 23 nm. The ZrO_2 nanoparticles showed 40-50 nm sized spherical shaped particles [22]. Subramanian et al. (2021) used biogenesis method to prepare ZrO_2 nanoparticles using Momordiga charantia leaf extrcat. They stated that ZrO_2 nanoparticles have strong antibacterial efficacy against Escherichia coli and Staphylococcus aureus [23]. Pragya Goyal et al. (2021) produced ZrO_2 nanoparticles use the extract of Helianthus annuus seed and evaluate its antimicrobial activity for biomedical applications. The results demonstrated that prepared nanoparticles had more active in Pseudomonas aeruginosa with inhibition zone 13.5 mm and poor activity in Staphylococcus aureus with inhibition zone 12 mm [16]. In the present work, we have used Azadirachta indica (Neem) leaves extract to prepare ZrO_2 nanoparticles. Azadirachta indica is a powerful reducing agent and stabilizer for making orthorhombic ZrO_2 nanoparticles. Azadirachta Indica leaves have vitamin C, E & K and is used in many biological and pharmacological applications. The leaves are known for its enhanced antioxidant activity, inhibition of bacterial growth [24].

2. Experimental Procedure

2.1. Materials

Azadirachta indica (neem) leaves are collected in nearby areas of Koodankulam, Tamil Nadu (India). Chemicals such as Zirconyl Nitrate Hydrate ($Zr(NO_3)_2$. H_2O) and acetone were analytical grade and directly used without any purification. Zirconyl Nitrate Hydrate was used as a precursor.

2.2. Preparation of Azadirachta indica leaf extract

Azadirachta indica (neem) leaves were washed with deionized water to get rid any dust and debris. After washing, the leaves were dried for 15 days at room temperature and ground into powder. Then 20g of ground powder and 200 ml of deionized water were boiled at 80 ºC for 30 minutes. Yellowish- brown color leaf extract was obtained. It was cooled, filtered and used for further work.

2.3. Biosynthesis of ZrO_2 nanoparticles

For the usual synthesis procedure, 50 ml of 0.2 M $Zr(NO_3)_2.H_2O$ is dissolved in deionized water. The solution was agitated for 30 minutes at room temperature in magnetic stirrer. Then added 10 ml of Azadirachta indica extract to the mixture. The mixture was heated at 70 ºC under constant stirring. The foamy gel was formed without the addition of any chemical reducing agent. Then, foamy gel was filtered using Whatman filter paper. The filtered samples were washed five times using deionized water and acetone to get rid of impurities and unreacted precursor in the sample. Using muffle furnace, the collected samples were calcinated for 3 hours at 700 and 900 ºC. The samples calcinated at 700 and 900 ºC are referred to as G1 and G2 respectively in this article. In figure 1 depicts the synthesis process schematic.

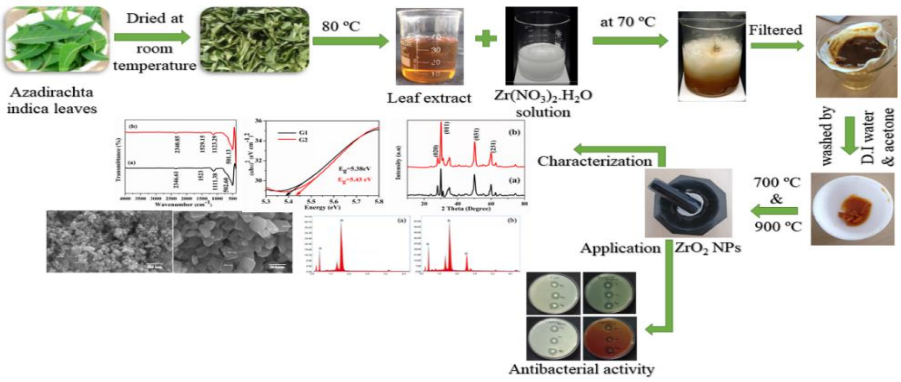

Figure 1. Biosynthesis and characterization of ZrO_2 nanoparticles

2.4. Characterization of Zirconium oxide nanoparticles

The calcined ZrO_2 nano particles were subjected to X-ray diffraction using Bruker Eco D8 Advance diffractometer with angle scan range of 15° to 80°. The instrument used Cu-Kα x-rays and operated at voltage and current 40kV and 25mA respectively. UV-visible absorption spectrum of the prepared samples was recorded by JASCO V-750 spectrophotometer with a wavelength range of 190nm-600nm. The prepared ZrO_2 nanoparticles were analyzed for the presence of functional group using FT/IR-46000 type A (ATR PRO ONE) spectrometer. The morphology and composition of the synthesized nanoparticles analyzed using Field emission scanning electron microscope. The SEM images and EDX data were recorded by SEM ZEISS microscopy operated at accelerating voltage of 20 kV. The antibacterial activity of bio-synthesized ZrO_2 nanoparticles was examined by four bacterial strains using Kirby-Bauer method. The synthesized ZrO_2 nanoparticles were investigated against Escherichia coli, Pseudomonas aeruginosa, Enterococcus faecalis and Streptococcus mutans.

3. RESULT AND DISCUSSION

3.1. Structural analysis

The XRD pattern of biosynthesized ZrO2 nanoparticles (calcined at 700 °C and 900 °C) shown in figure 2. It shows four sharp and strong peaks at 2θ= 27.96°, 29.98°, 50.03°, 59.94° (Figure 2(a)), 28.08°, 30.088°,50.19° and 60.02° (Figure 2(b)) which related to the formation of orthorhombic phase with planes of (020), (011), (031)and (231), respectively (JCPCDS: 87-2105). The crystalline size of the samples was found using Debye-Scherer formula,

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

where D- the particle diameter (nm), k- shape factor (0.9), λ- wavelength of X-ray (0.15406 nm), β - peak width at half maximum, θ-diffraction angle(degree) [25]. It found that when the calcination temperature raised 700 °C to 900 °C, the crystallite size of the particle was decreased from 16 nm to 14 nm. For gas sensing application, the small particles size of ZrO_2 was suitable [26].

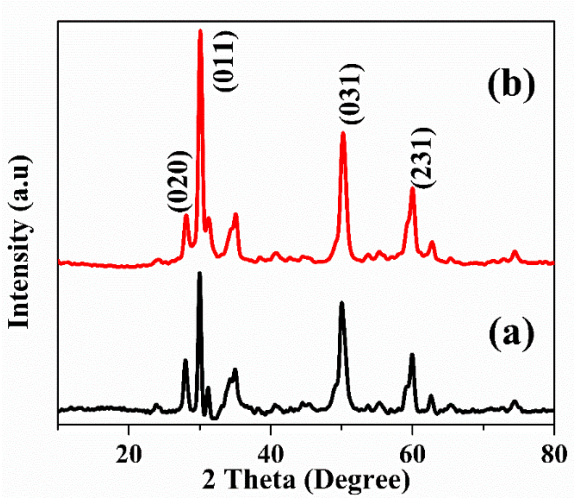

Figure 2. XRD patterns of ZrO_2 nanoparticles calcination at (a) 700 ºC and (b) 900 ºC

The dislocation density, micro strain of the prepared samples showed in Table 1.

$$\delta = \frac{1}{D^2}$$

$$\varepsilon = \frac{\beta}{4\tan\theta}$$

Where, δ - dislocation density (nm^{-2}), ε - micro strain.

The high intensity peak (011) has been indexed as orthorhombic structure phase of ZrO_2 (O- ZrO_2) with

lattice constant a= 5.587nm, b= 6.484nm and c= 3.329nm (JCPCDS: 87-2105).

Table 1. Crystallite size, phase micro strain and dislocation density of prepared ZrO_2 nanoparticles at calcined temperature 700 °C & 900 °C

Sample	D (nm)	Phase	ε x 10^{-3}	δ x 10^{-4}(nm^{-2})
ZrO_2 (calcined at 700 °C)	16.14	Orthorhombic	6.67	49.18
ZrO_2 (calcined at 900 °C)	14.42		7.35	58.49

3.2. UV-Visible analysis

For the analytical study of the prepared ZrO2 nanoparticles, the strong and prominent absorption peak at 212 nm and 214 nm was observed by UV spectroscopy (Figure 3 (a) & (b)).

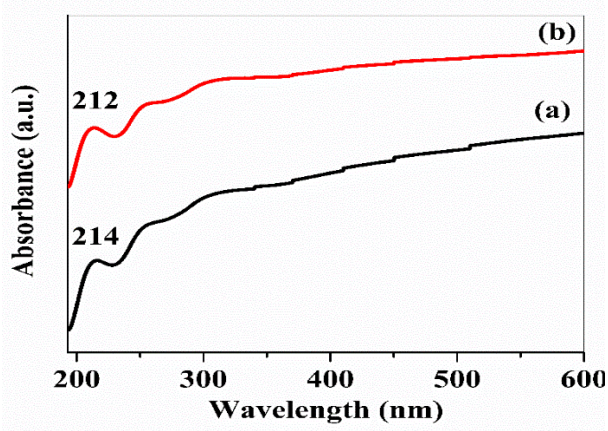

Figure 3. Optical absorption spectrum of ZrO2 nanoparticles calcined at 700 °C (a) and 900 °C (b)

The absorption is employed to found the band gap of the material. From the relationship,

$(\alpha h\upsilon)^2 = A\,(h\upsilon - Eg)^n$

the direct band gap energy can be determined.

Where, α - absorption coefficient, A - depicting constant factor, h - plank's constant, υ -frequency of the light, exponent n based on the nature of the transitions, which is used to differentiate direct and indirect transition. If a direct transition has n=1/2, indirect transition has n=2. The straight line of zero coefficient was used to determine the energy bandgap. The tangent intercept to the plot gives an approximation of the material's direct bandgap energy [25].

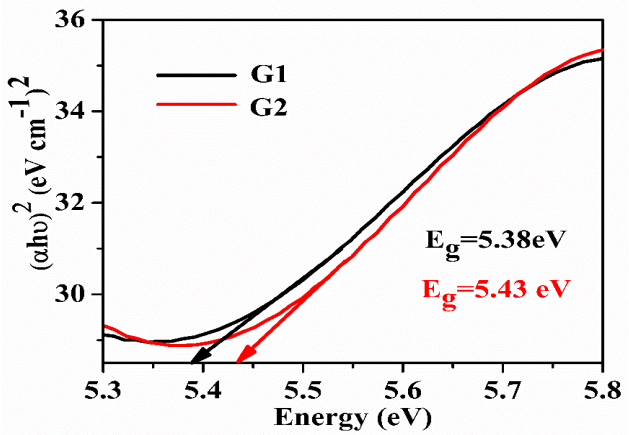

Figure 4. Tauc-plot of direct transition of ZrO_2 nanoparticles

The tauc's plot for biosynthesized ZrO_2 nanoparticles shown in figure 4. The calculated band gap value of ZrO_2 nanoparticles prepared at 700 ºC and 900 ºC were 5.38 eV and 5.43 eV respectively. Compared with the referred work the bandgap value is decreased [15]. So, it may use in power switching applications. The obtained energy gap value also confirms the ZrO_2 nanoparticles. The correlation between decreasing particle size and increasing bandgap energy are well provided for quantum confinement effect [27].

3.4. FT-IR analysis

Figure 5 (a) & 5 (b) shows FTIR spectra of ZrO_2 nanoparticles synthesized by biological method. Metal oxides give absorption bands in fingerprint region. Less than five absorption bands indicate the prepared ZrO_2 nanoparticles have small molecular weight and a simple organic compound.

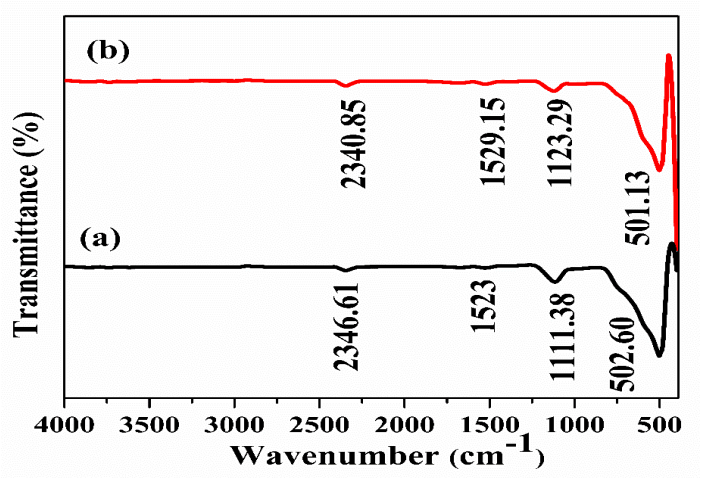

Figure 5. FT-IR spectra of ZrO2 nanoparticles calcination at (a) 700 ºC and (b) 900 ºC

Absence of broad band absorption informs there is no hydrogen bond in the sample. The peak at 502.60 cm^{-1} & 501.13 cm^{-1} corresponds Zr- O stretching vibrations, these confirms ZrO_2 formation [28]. The peak at 1523 cm^{-1} & 1529.15 cm^{-1} absorbed moisture and attributed to bending OH vibrations of water molecules[29]. The peak at 2346.61 cm^{-1} and 2340.85 cm^{-1} absorbed atmospheric CO_2 by metallic cations [30]. Zr-OH symmetric frequencies and C-H bonds bending vibrations in the species connecting are indicating the peaks at 1111.38 cm^{-1} and 1123.29 cm^{-1} [31].

3.5. SEM and Elemental analysis

The morphological detail of biosynthesized ZrO_2 nanoparticles were using FESEM.

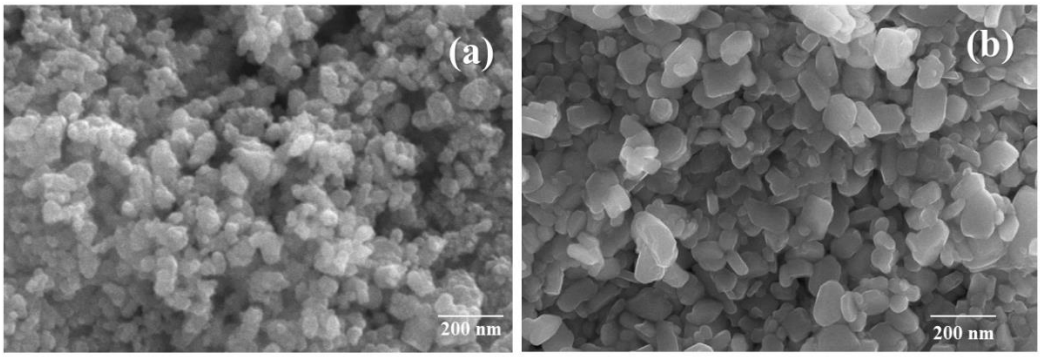

Figure 6. FESEM images of ZrO2 nanoparticles calcination at (a) 700 ºC and (b) 900 ºC

The surface morphology of biosynthesized ZrO2 nanoparticles at 700 ºC and 900 ºC are shown in figure 6 (a) & 6(b). SEM pictures reveals that the prepared samples consist of regular shape and smooth surfaces. Figure 6(b)

looks like a rectangular nano bar shape. The samples were contained flat and flaky shape with uniformly distributed.

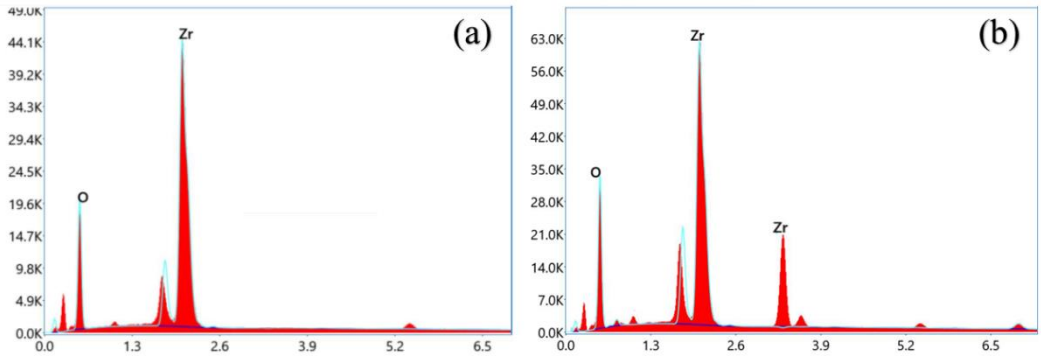

Figure 7. EDX Spectra of biosynthesized ZrO_2 nanoparticles calcination at (a) 700 ºC and (b) 900 ºC

EDX characterization used to found the elemental composition of prepared ZrO_2 nanoparticles. The EDX spectrum of ZrO2 nanoparticles shown in figure 7. It clearly shows the strong peaks reveals the presence of Zirconium (52.44 %) and oxygen (47.56 %) for synthesized samples calcined at 700 ºC (figure 7 (a)). For synthesized samples calcined at 900 ºC (figure 7 (b)) Zirconium (72.37 %) and oxygen (27.63 %). The result shows the change in weight percentage of Zr and O with increasing the calcined temperature. The formation of oxygen vacancies causes weight percentage of O decreases. The strongest peak at 2 keV confirmed the formation of nanoparticles [16].

3.6. Antibacterial efficaciousness of bio synthesized ZrO_2 nanoparticles

Microorganisms are the causative agent of different diseases. Escherichia coli and Pseudomonas aeruginosaare gram-negative bacteria, Enterococcus faecalis and Streptococcus mutans are gram-positive bacteria. These microorganisms colonize and become pathogens and progressively discovered in many diseases. This study to examine the efficacy of prepared nanoparticles against bacteria. The Kirby Bauer method was used to analyze the antibacterial efficaciousness of ZrO2 nanoparticles. Figure 8 illustrates the antibacterial efficaciousness of ZrO2 nanoparticles (at 700 ºC and 900 ºC) against various bacteria. The zone of inhibition is represented by the clear zone. The zones diameters are measured using vernier caliper in millimeter.

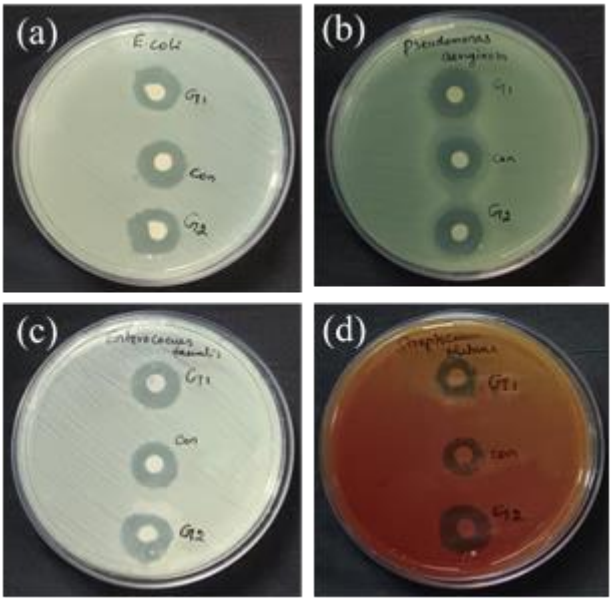

Figure 8. Zone of inhibition of ZrO2 calcined at 700 ºC and 900 ºC nanoparticles against various bacteria

The antibiotic exhibits the inhibition zone range from 10 mm to 20 mm for every examined bacterium. The bacterial dismissal of biosynthesized ZrO2 nanoparticles calcined at 700 ºC exhibits better outcomes against Pseudomonas Aeruginosa (18.3 mm) followed by Escherichia coli (17mm), Enterococcus faecalis (17mm) and Streptococcus mutans (14.5 mm). At the same time, ZrO2 nanoparticles calcined at 900 ºC shows higher antibacterial effect towards Escherichia coli (18.3 mm) followed by Enterococcus faecalis (18.2 mm), Streptococcus mutans (17.2 mm) and Pseudomonas Aeruginosa (17 mm).

Table 2. Antibacterial efficaciousness of ZrO_2 with different extracts

Extract	Bacterial Strains/ fungi	Antibacterial efficaciousness (Zone of Inhibition)	Ref.
Momordica charantia	Escherichia coli	11 mm	[23]
	Staphylococcus aureus	10 mm	
Helianthus annus	Escherichia coli	13 mm	[16]
	Pseudomonas aeruginosa	13.5 mm	
	Klebsiella pneumonie	12.5 mm	
	Staphylococcus aureus	12 mm	
Laurus nobilis	Aspergillus niger	14 mm using 50 µg/ml	[32]
	Klebsiella pneumoniae	13 mm using 50 µg/ml	
	Escherichia coli	12 mm using 50 µg/mL	
	Bacillus substilis	13 mm using 50 µg/ml	
	Staphylococcus aureus	12 mm using 50 µg/ml	
Melia dubica	Pseudomonas aeruginosa	17.15 mm by 50 µg/mL	[33]
	Streptococcus mutans	8.2 mm by 50 µg/ml	
Zingiber officinale	Streptococcus mutans	28 mm	[34]
	Staphylococus aureus	26 mm	
	Escherichia coli	27 mm	
Syzygium aromaticum	Streptococcus mutans	32 mm	
	Staphylococus aureus	28mm	
	Escherichia coli	31 mm	
Wrightia tinctoria	Bacillus subtilis	7 mm	[35]
	Pseudomonas aeruginosa	9 mm	
	Escherichia coli	12 mm	
	Staphylococcus aureus	10 mm	
Guettarda speciosa (For 0.02 M Solution)	Escherichia coli	10 mm	[36]
	Bacillus substilis	10 mm	

	Salmonella typhi	15 mm	
	Proteus vulgaris	5 mm	
	Pseudomonas aeruginosa	8 mm	
Tinospora cordifolia	Bacillus subtilis	36 mm	[37]
	Pseudomonas aeruginosa	32mm	
	Streptococcus mutans	28 mm	
	Escherichia coli	34 mm	
	Aspergillus fumigatus	34 mm	
	Aspergillus niger	32 mm	
Punica granatum	Staphylococcus aureus	15 mm using 75mg/L	[38]
	Escherichia coli	13 mm using 75mg/L	
	Candida albicans	31 mm using 75mg/L	
Azhadirachta indica	Escherichia coli	17 mm (at 700 ºC) 18.3 mm (at 900 ºC)	[present work]
	Pseudomonas aeruginosa	18.3 mm (at700 ºC) 17 mm (at 900 ºC)	
	Enterococcus faecalis	17 mm (at 700 ºC) 18.2 mm (at 900 ºC)	
	Streptococcus mutans	14.5mm (at 700 ºC) 17.2 mm (at 900 ºC)	

Both synthesized ZrO_2 nanoparticles are performs against gram-positive and negative bacteria and give better results especially against gram-positive bacteria. These findings show better agreement compared with the previous reports [16, 23, 34]. The mechanism of antibacterial efficaciousness of ZrO_2 nanoparticles is it penetrate the bacteria cell wall disrupting their proteins, metabolism and triggering molecular changes that inhibit cell division, resulting in cell death. Smaller size of the nanoparticles exhibiting enhanced penetration capabilities by a direct relationship with their diffusion over the bacterial membrane. Table 2 provides an investigation of antibacterial efficaciousness (zone of inhibition) that ZrO_2 nanoparticles prepared by different types of extracts. Azadirachta indica's parts exhibit antibacterial/antimicrobial by potentiality break the cell walls. The crude leaves extract of Azadirachta indica has steroids, alkaloid, saponins, phenolics, flavonoids, etc., [39]. These are widely used in biomedical and dental fields.

4. Conclusion

Azadirachta indica leaf extract used to synthesis of orthorhombic structured ZrO_2 nanoparticles. The characteristics of biosynthesized ZrO_2 nanoparticles were analyzed using by XRD, UV, FT-IR, SEM and EDX. The XRD peaks show the formation of orthorhombic structure. The average crystalline size of ZrO_2 nanoparticles decreased when increasing the temperature. UV-visible used to examine the optical properties. The FT-IR spectral analysis reveals the characteristics peaks for Zr-O stretching. The SEM analysis revealed the presence of nano

bars with uniform distribution. From antibacterial analysis the bio synthesized ZrO2 nanoparticles defeat both gram-negative and positive bacteria. Thus, this work creates a new approach of the nanoparticles in biomedical applications such as dental implants, bio-film formation.

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