RESEARCH PAPER

Biodegradable PLA/ZrO_2/Curcumin Nanocomposite Films: Synthesis, Characterization, and Antimicrobial Activity

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ABSTRACT

The poly (lactic acid) (PLA) nanofilm reinforced with zirconia nanoparticles (synthesized in green) and curcumin was created by solvent casting to form a biodegradable composite nanofilm. The X-ray diffraction identified the amorphous structure of PLA and the determination of the monoclinic reflectance of zirconia at the 2θ = 28.18° and the crystalline peak of curcumin at the 2θ = 28.18° with crystalline size of nanometers. As can be seen with the scanning electron microscopy (FE-SEM), there was an even surface with no cracks and a well- dispersed zirconia and curcumin nanoparticles integrated into the PLA structure. Microscopic observations revealed that there were fine nanoscale structures and topography which were slightly rough, which was evidence that the nanofillers were in good compatibility with the polymer chains. Nanoscale dispersion was detected by EDX analysis and it predominantly had carbon and oxygen signals with low level of zirconium content (0.07%). Fourier transform infrared spectroscopy (FTIR) analysis revealed the presence of functional groups such as the PLA ester (C=O at 1719 cm^{-1}), the C=C aromatic curcumin (1510 cm^{1}) and the Zr -O (592 cm^{1}). The colony-forming unit (CFU) assay of antimicrobial activity showed a high level of biological activity, a 100 percent inhibition of Staphylococcus aureus and Candida albicans, and an 84 percent reduction of Escherichia coli colonies, and the synergistic effect of zirconia and curcumin in the PLA film.

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INTRODUCTION

Biodegradable polymers have recently become the focus of the vast amount of attention because of the necessity to diminish the climate pollution, as well as to minimize the microplastic accumulation as the sustainable alternative to the petroleum plastics in the recent years. Poly (lactic acid) (PLA) is one of these types of thermoplastic polymers which is biocompatible, biodegradable, and easily processed and therefore, can be used in biomedical and packaging products. [1,2]. Nonetheless, pure PLA has a range of inherent limitations that can be summarized as brittle nature, low thermal stability, and poorest functionality as an antioxidant or antimicrobial, limiting its high-tech use [3].

Nanoparticle reinforcement has come out as the way forward to counter these limitations. Zirconia nanoparticles (ZrO_2) are especially the desirable ones because they possess a strong level

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of chemical stability, mechanical strength, and biocompatibility [4].

Moreover, green methods of synthesizing nanoparticles like using aloe vera extract offer greener methods of synthesizing zirconia nanoparticles, thus avoiding the use of chemicals and improving biocompatibility [5]. Curcumin is a natural polyphenolic compound derived in rhizome of Curcuma domestica (Zingiberaceae) that has the strong antioxidant, anti-inflammatory, and antimicrobial properties, whereas its limitation is low solubility and instability in its direct use. Carcinogen is also more stable and releases in a controlled manner when used in the form of nanoparticles within polymeric matrices [6].

Thus, the association of green-synthesized zirconia with curcumin in a PLA film should lead to synergistic properties: zirconia should have a nucleating and enhancing effect, whereas curcumin should have bioactivity, at least in the form of antioxidant properties. Solvent-casting offered a simple path to the construction of such hybrid membranes with a fairly uniform thickness and a uniform distribution of nanoparticles [7,8].

Recent studies have highlighted the potential of PLA-based films. For example, PLA/ZnO composite films produced by electrospinning demonstrated significantly improved antibacterial properties, thermal stability, and mechanical durability, with potential applications in food preservation [9]. Solution-cast PLA films containing Curcuma longa extract (a natural source of curcumin) showed improved thermal behavior and antimicrobial function, indicating their suitability for active packaging such as cheese packaging [10]. More recently, the incorporation of green-synthesized oxides such as ZrO_2 into PLA matrices has shown improvements in thermal performance and surface reactivity, expanding the range of functional applications of bio-based nanofilms [7].

MATERIALS AND METHODS

Poly (lactic acid) (PLA, granular) and zirconium nitrate [$Zr(NO_3)_4$, white powder] were purchased from Sigma-Aldrich, UK. Tetrahydrofuran [THF, $(CH_2)_4O$, colorless liquid] was purchased from Merck, Germany. Commercially obtained curcumin nanoparticles were used as received. All chemicals were of analytical grade and used without further purification.

Plant Extract Preparation

Aloe vera leaves were washed, cut, dried, and ground into a powder. One gram of the powder was added to 50 ml of distilled deionized water in a 250 ml beaker. The mixture was stirred at 800 rpm at 50°C for one hour, and then filtered to obtain the extract.

Preparation of Zirconia Oxide Nanoparticles

Zirconium oxide nanoparticles were synthesized using a green method employing aloe vera extract. A 1 M zirconia nitrate solution in 50 ml of distilled deionized water was stirred at 800 rpm at 50°C for 30 minutes until clear. 50 ml of aloe vera extract was then added, and the mixture was stirred at 800 rpm at 50°C for 1 hour. The pH was adjusted to 9, which led to the colour change and the intensification of the viscosity. The solution was filtered, dried at 100 C ° over a period of 6 hours and then calcined at 650 C ° in 1 hour. The following product was ground to get nano zirconia oxide particles. Fig. 1 shows the process of synthesis.

PLA/ZrO_2/Curcumin Film Preparation

The film was prepared via solvent casting. 0.9 g poly(lactic acid) was dissolved in 20 ml THF using a magnetic stirrer. 0.1 g nano zirconia oxide was then

Fig.1. The synthesis process of nanozirconia.

added and homogenized. Subsequently, 0.05 g nano curcumin powder was added and completely dissolved. The resulting solution was poured into a dish and left to dry. The film preparation stages are illustrated in Fig. 2.

Characterization

The structural properties of the membrane composed of Poly(lactic acid), nano-zirconia oxide, and curcumin were studied using X-ray diffraction (PHILIPS, PW1730, The Netherland). Field Emission Scanning Electron Microscopy & Energy Dispersive X-ray Spectroscopy (FE-SEM/EDX) type (TESCAN, MIRA3, Czechia).

RESULTS AND DISCUSSION

X-ray diffraction

X-ray diffraction (XRD) was performed to characterize the structural properties and crystallinity of the PLA-based film. Analysis of the diffraction patterns allows the identification of the amorphous nature of PLA, as well as crystalline phases of ZrO_2 nanoparticles and curcumin. Interplanar distances (d values) were calculated using Bragg's equation [11,12].

$$2d_{hkl} \sin\theta = n\lambda \qquad (1)$$

While the average crystallite size of the inorganic nanoparticles was estimated using the Debye-Scherrer equation [13].

$$D = (K\lambda)/(\beta \cos \Theta) \qquad (2)$$

Table 1 lists the structural properties of the PLA/ZrO_2/curcumin sample, and Fig. 3 displays its XRD patterns.

The XRD pattern of the PLA/ZrO_2/curcumin nanocomposite film exhibits characteristic amorphous behavior, evidenced by broad diffraction bands at 2θ between 15–25°, consistent with the amorphous nature of PLA [14]. Sharp reflections, attributed to monoclinic ZrO_2 (ICDD card No. 96-900-7486), appear at 2θ = 28.18° ($\bar{1}11$), 31.74° (111), 34.47° (200), and 50.06° (202). The values of the d-spacing are in good agreement with the database, indicating that the monoclinic ZrO_2 crystal structure has been retained in the PLA matrix [15]. The Debye-Scherrer equation is used to determine the crystal size; the size of the crystals is nanocrystalline, which shows the nature of the zirconia material

The X-ray diffraction pattern shows reflections that are typical of crystalline curcumin besides the zirconia peaks, with sharp peaks at 28.18° (110), 37.21 ° (200), and 43.50 ° (210), which were in agreement with previous literature [16]. The crystalline size was determined to be in nanometers which means that it is nanocrystalline. These peaks implied that even though curcumin has been encrypted in the PLA/ZrO_2 matrix, its stability and controlled release have been associated with its partial crystallinity [17]. The polycrystalline nanocomposite structure formed is an amorphous PLA matrix, which is flexible, using fillers to reinforce it, nucleating agent, zirconium oxide, and a bioactive crystalline curcumin.

Field Emission Scanning Electron Microscopy

Fig. 4a presents an image of an FE-SEM of a polymer film made of poly(lactic acid), zirconia oxide, and curcumin nanoparticles at the scale of 200 nm. The surface has a brighter band of the fine nanoparticles which is because of the homogeneous dispersion of the zirconia oxide and curcumin nanoparticles within the polymer matrix. Fig. 4b shows a wider view at a scale of

Fig. 2. The synthesis process of film.

500 nm, revealing a uniform but somewhat rough texture, confirming that the addition of curcumin enhanced the nanotopography without causing visible cracks [18].

The combination of inorganic (zirconium oxide) and organic (curcumin) nanoparticles results in interfacial interactions with the lactic acid chains. This morphology is beneficial for improving antioxidant, antibacterial, and cellular interactions due to the synergistic effects of zirconium oxide and curcumin. The fine and rough nanostructure indicates a well-integrated amorphous compound, and the solvent casting process achieved efficient dispersion of both nanofillers.

Energy Dispersive X-ray Spectroscopy (EDX)

The elemental composition of the PLA/ZrO_2/curcumin film was determined through energy-dispersive X-ray spectroscopy (EDX) analysis and was presented in Fig. 5. The characteristic carbon (72.91 wt%) and oxygen (27.03 wt%) peaks are observed in the spectrum, which is a symptom of carbon-rich polyester chains and ester oxygen groups of poly(lactic acid) (PLA).[19].

This is due to the lack of a clear curcumin signal as it has a similar elemental structure (carbon, oxygen and hydrogen) to PLA. The low concentration of zirconium (0.07 wt) indicates the high dispersion on the nanoscale [20]. Elemental mapping reveals a uniform distribution of carbon and oxygen and scattered zirconium speckles which are characteristic of oxide nanoparticles embedded into organic substances.

There was also the observation of gold peaks,

Table 1. Structural properties of PLA/ZrO_2/curcumin film.

2θ (Deg.)	FWHM (Deg.)	d_{hkl} STd.(Å)	d_{hkl} Exp.(Å)	Crystallite size (nm)	(hkl)
28.18	0.75	3.153	3.16	10.99	($\bar{1}$11) ZrO_2
31.74	0.73	2.83	2.82	11.28	(111) ZrO_2
34.47	0.66	2.60	2.60	12.56	(200) ZrO_2
50.06	0.72	1.82	1.82	12.23	(202) ZrO_2
28.18	0.81	3.15	3.16	10.16	(110) Curcumin
37.21	0.47	2.40	2.41	17.99	(200) Curcumin
43.50	0.33	2.09	2.08	26.08	(210) Curcumin

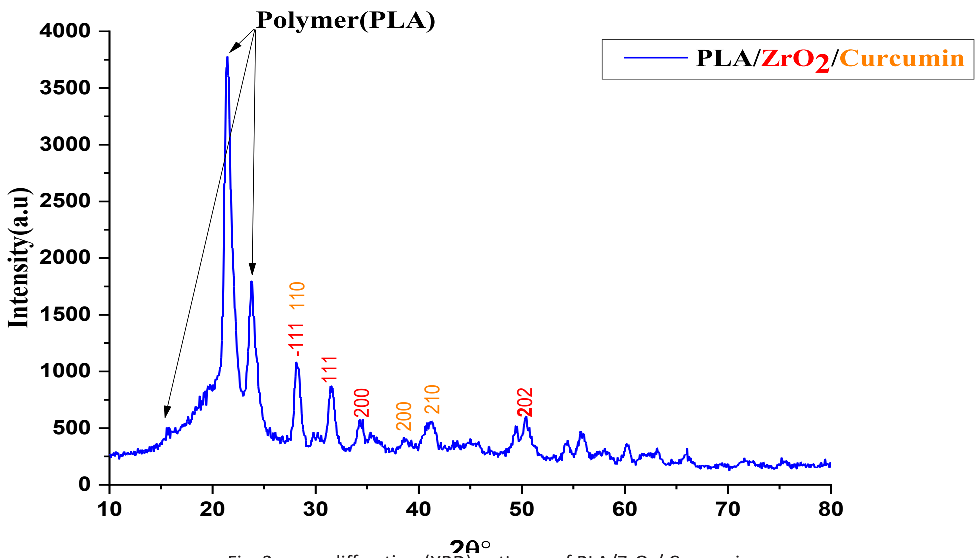

Fig. 3. x-ray diffraction (XRD) patterns of PLA/ZrO_2/ Curcumin.

which were a consequence of sputter coating done to enhance signal quality to this insulating sample [21]. These EDX findings are consistent with those of X-ray diffraction (XRD) on the PLA/ZrO_2/curcumin sample, which identified monoclinic zirconium oxide (ZrO_2) reflections and less intense, broader reflections of nanocurcum

Fourier transforms infrared (FTIR) spectroscopy

Fourier transform infrared (FTIR) spectroscopy (400-4000 cm^{-1}) identified functional groups and chemical bonds in the sample.

The PLA/ZrO_2/curcumin film's FTIR spectrum Fig. 6 revealed characteristic absorption bands. Peaks at 2938.7 and 2869.2 cm^{-1} indicated asymmetric and symmetric stretching of aliphatic C–H groups in PLA[22]. A weak band near 2095 cm^{-1} suggested conformational transitions of the ester carbonyl group [23]. The strong absorption at 1719.3 cm^{-1} was attributed to the ester carbonyl (C=O) stretching of PLA [22].

Curcumin was identified by aromatic C=C stretching at 1510.2 cm^{-1}, a well-known fingerprint of the molecule [6]. Methyl bending bands at 1463.9 and 1366.6 cm^{-1} confirmed PLA side groups [22]. Strong absorptions at 1292.4, 1232.5, 1158.3, and 1033.5 cm^{-1} corresponded to C–O and C–O–C stretching vibrations of PLA esters, potentially with contributions from enolic C–O in curcumin [6,22]. Finally, absorptions at 726.6 and 592.6 cm^{-1} are attributed to Zr–O–Zr stretching vibrations, a characteristic feature of zirconia nanoparticles [5].

Antimicrobial Activity

The antimicrobial activity test was conducted at the BPC center in Baghdad, Iraq, whereby the colony-forming unit (CFU) assay was done to measure the capability of the prepared film to kill or suppress the growth of bacteria and fungi. This

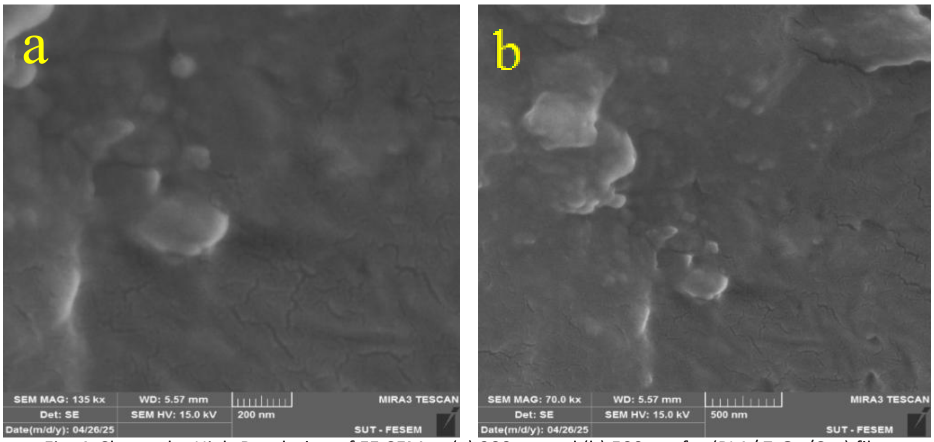

Fig. 4. Shows the High-Resolution of FE-SEM at (a) 200nm and (b) 500 nm for (PLA/ ZrO_2 /Cur) film.

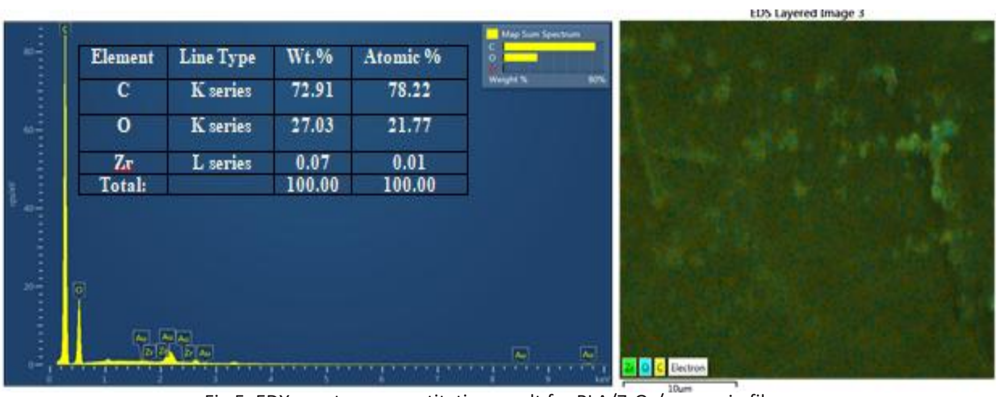

Fig.5. EDX spectrum-quantitative result for PLA/ZrO_2/curcumin film.

test involved the use of three microbial strains that included Staphylococcus aureus (Gram-positive), Escherichia coli (Gram-negative) and Candida albicans.

Fresh cultures of every microorganism were prepared and allowed to incubate in 37 °C at 24 hours in order to be viably optimized. The microbial suspension was then diluted to 1.5 x 10 ^{8} CFU/ml, based on the McFarland standard and then dilutions were further conducted to get pure and countable colonies. Microbial inoculator and the composite film were positioned (37°C, 30 minutes) in equal volumes (1:1) in sterile test tubes to approximate the contact time in the experiment necessary to estimate the killing efficiency. The results were incubated on sterile agar plates after 100 µLs of each mixture were deposited, and only the microbial suspension was used in the control plate without the added composite.

Each plate was incubated at 37 °C over a period of 24 hours after which visible colonies were obtained. Plates with 300 or more colony-forming units (CFU) were not analyzed since its limits were not the optimum range to accurately count the colonies on the plate. The colonies on the treated plates were compared to control plate and the removal efficiency (RE) was determined based on the following standard formula:

$$\text{Removal Efficiency (\%)} = \left(\frac{CFU_{control} - CFU_{Sample}}{CFU_{control}}\right) \times 100$$

Antimicrobial results

The polylactic acid (PLA)-based film, supplemented with green zirconia nanoparticles synthesized suing the aloe vera extract and curcumin nanoparticles, demonstrated an excellent antimicrobial activity in the colony forming unit (CFU) assay, as illustrated in Fig. 7, since it reduced Staphylococcus aureus and Candida albicans by 100 percent while showing an 84% reduction against Escherichia coli [24,25]. This high biological effect is explained by the interaction of the two active ingredients which becomes synergistic. The zirconia nanoparticles react directly with the surfaces as well as the membrane of microorganisms through their large surface area and surface charge resulting in membrane rupture, intracellular leakage and production of reactive oxygen species (ROS). These ROS degrade proteins, lipids and DNA eventually leading to irreversible damage of cells and cell death. Curcumin also helps in the inhibition of major cellular enzymes, and derails metabolic activity, and creates other oxidative stresses.

It is gram-negative and hence slightly less active

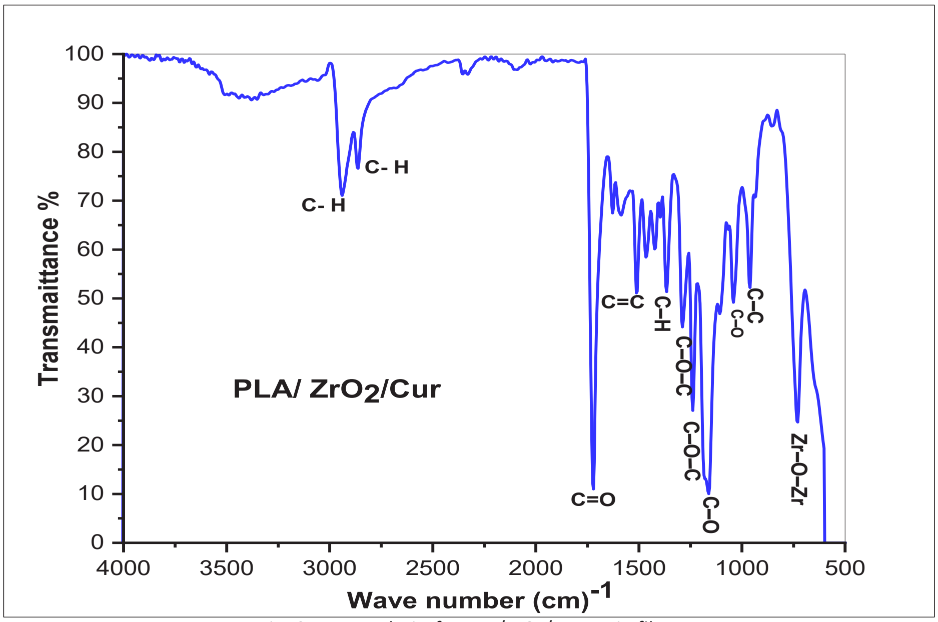

Fig .6. FTIR analysis, for PLA/ZrO_2/curcumin film.

against Escherichia coli. An outer-membrane which is rich in lipopolysaccharides, acts as an effective antimicrobial protection systemthat restricts penetration of organic compounds such as curcumin, which produces an inhibitory and not a bactericidal effect [26].

CONCLUSION

A biodegradable composite nanofilm based on PLA was prepared successfully in this study using solvent casting through green-processed zirconia nanoparticles with curcumin. The amorphous state of PLA and the maintenance of the monoclinic crystal form of zirconia as well as the typical crystalline reflectance of curcumin, which showed the development of a hybrid polycrystalline nanocomposite, were confirmed by X-ray diffraction (XRD) structural analysis. The surfaces were uniform, free of cracks, surface morphology could be examined through the scanning electron microscopy (FE-SEM), with evenly distributed zirconia and curcumin nanoparticles, where the EDX analysis showed a relatively good distribution of the filler with a low content of zirconia, which indicated excellent filler integration within the polymer skeleton. Additional support of the chemical interactions between PLA, zirconia, and curcumin was based on the Fourier transform infrared spectroscopy (FTIR) that revealed the presence of different functional groups. The antimicrobial analysis indicated that the PLA/ ZrO_2/curcumin film had a strong biological activity with a high (100) percentage of inhibition of Staphylococcus aureus and Candida albicans, and high (84) percentage of Escherichia coli growth decrease. This improved antimicrobial activity is explained by synergistic action of zirconia that has been reported to produce reactive oxygen species and damage microbial membranes and curcumin that adds more antimicrobial and metabolic-inhibiting roles.

Altogether, the findings enable concluding that the addition of green-processed zirconia and curcumin into PLA can enhance the structural, morphological, and antimicrobial properties of the latter to a considerable extent, which makes the resulting composite nanofilm a good candidate to be used in biomedical, packaging,

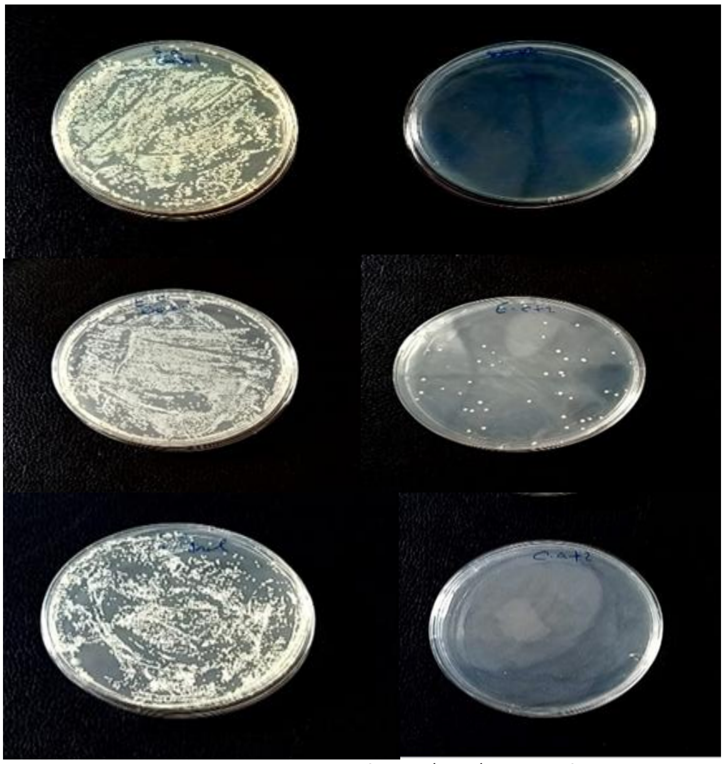

Fig .7. Antimicrobial activity, for PLA/ZrO_2/curcumin film.

and environmental-friendly applications.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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