Egyptian Journal of Chemistry
http://ejchem.journals.ekb.eg/

Green Thermoplastic Polymer Blends with Enhanced Antimicrobial Properties from Curcuma longa Nanoparticles

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Abstract

Ethylene vinyl acetate/ silicone rubber (EVA/ SR) polymer blends were prepared using the Brabender Plasticorder, then loaded with different ratios from curcumin nanoparticles and cured using dicumyl peroxide (DCP). The blends were investigated by Fourier Transform Infrared (FTIR) spectroscopy, Scanning Electron Microscope (SEM), and Differential Scanning Calorimetry (DSC). The mechanical properties are increased with increasing EVA content. 1-alkyl-3-methylimidazolium bromide [RMIM]Br Ionic Liquid (IL) and curcumin were selected as the hydrophilic and antibacterial for the green thermoplastic polymer blends. The combination of hydrophilic (IL) and antibacterial agent (curcumin) can kill the bacteria that found in contact with the surface of the polymers, and also avoid the adhesion of dead bacteria on the polymer surfaces. This study aims to produce, characterize, and assess the antimicrobial activity and cytotoxicity of polymer blends based on (EVA/ SR). The cytotoxicity of the prepared polymer blends towards the human tumor cell line BJ1 (normal skin fibroblast), indicated the safety of these products. On the other hand, the prepared polymer blends showed moderate antimicrobial potency towards the tested bacteria and fungi strains.

*Keywords:*Ethylene vinyl acetate, Silicone polymer, Curcumin, Antimicrobial agents, Cytotoxicity

1. Introduction

Physically combining two or more polymers results in polymer blends. The qualities of the blend are often better than those of the component homopolymers. Finding a method to make eco-friendly, natural antibacterial materials by combining active plant substances like curcumin has grown in popularity in the development of innovative medicines for both humans and animals.

The intrinsic ability of ethylene vinyl acetate (EVA) to be active against bacteria makes it a useful antibacterial agent. EVA is a widely used polymeric biomaterial with numerous uses, including mouthguards, catheters, prosthetic organs, and intravitreal devices. Excellent mechanical, weather and ozone resistance are displayed by ethylene vinyl acetate (EVA). Because of its simplicity in handling and processing, biocompatibility, and potential for drug delivery, EVA is also seen as a strong option for use in biomedical applications.

Due to its better functionality in the biological and industrial domains, silicone rubber (SR) has been the subject of substantial research. A polymeric substance with great weather and heat stability, strong biocompatibility, oxidation resistance, and dielectric properties is silicone rubber.

The use of silicone rubber in biomedical devices is common, and mixing two or more types of polymers is a useful and significant method for creating blends of polymers with properties that are superior to those of the individual constituents. This method is also useful for enhancing damping and physical properties.

Chemotherapy has been the most popular kind of

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EJCHEM use only: Received date 15 February 2023; revised date 04 April 2023; accepted date 10 April 2023
DOI: 10.21608/EJCHEM.2023.194168.7613

treatment for breast cancer and other cancers up to this point. However, this treatment method also kills some healthy cells. Some natural products have been utilized as alternative treatments for malignancies, including breast cancer, because of their broad spectrum of biological activities and low toxicity in animal models [1]. The main yellow pigment produced from turmeric (Curcuma longa) is called curcumin, and it is frequently used as a food flavor [2].

Due to its medical characteristics in Indian and Chinese medicine, curcumin has been extensively explored for its anti-inflammatory, anti-angiogenic, antioxidant, wound healing, and anti-cancer actions [3]. In addition, a lot of studies have demonstrated that curcumin has anti-proliferative and anticarcinogenic characteristics in a range of animal models and cell lines [4 -5].

Additionally, one of the most crucial fields of research to treat pathogens including Gram-positive and Gram-negative bacteria, fungus, algae, yeast, and some microorganisms that cause significant human diseases is the development of new, natural antibacterial drugs [6 -7].

Composites with antimicrobial activity are of great interest nowadays. Curcumin and quercetin nanoparticles exhibit anticancer influence against MCF-7 cells (breast cancer cell line) as well as antibacterial agents [8]. Their role in health status may be due to the presence of bioactive compounds as natural antioxidants. It is recommended to use them in dairy food as a preventative and to lower considerable side effects of cancer from chemical drugs.

It was used in cosmetics for its antiseptic, antimicrobial, anti-inflammatory, and antioxidant properties as well as in medical care for its health benefits.

Recent efforts by Ding et al [9] to create a medically useful hydrophilic and antibacterial silicone polymer through surface modification of silicone polymer produced good results in terms of biofilm resistance. It keep up strong antibacterial effectiveness even after a 48-hour cycle of E. coli bacterium suspension.

By using peroxide (Dicumyl peroxide, DCP) as a crosslinking agent, [10] create ethylene vinyl-acetate copolymer/silicone Blends with good two-way shape memory capabilities. Blends of silicone polymer and ethylene vinyl acetate were examined for their cure characteristics, morphology, mechanical properties, and aging characteristics [11].

By co-crosslinking, Ren et al., developed copolymer blends of methyl vinyl silicone polymer/olefin block copolymer blends that showed thermoresponsive shape memory characteristics [12].

The chemical and thermal resistance of silicone polymer/ethylene vinyl acetate blends was investigated [13]. Additionally, shape memory polymer composite coatings with improved mechanical and antibacterial properties were explored by Xin Wang et al. Qiang Wu examined the morphological evolution and rheological characteristics of PP/SR thermoplastic vulcanizate [14].

The aim of this study is to demonstrate and create an eco-friendly antibacterial blend using Ethylene vinyl acetate (EVA) and silicone Rubber (SR) and active plant extracts like curcumin. The cytotoxicity of the prepared polymer blends towards the human normal retina cell line (RPI-1) was investigated. Also, the prepared polymer blends used as an antimicrobial potency were tested towards bacteria and fungi strains to evaluate their antimicrobial activity.

EXPERIMENTAL

Materials:

Silicone rubber (SR) was supplied by GE Bayer Silicone (India) Pvt Ltd, Banglore, India, with 11 vol % of silica content and 0.45% of vinyl methyl silicone. EVA having 18% vinyl acetate content was supplied by Exxon Chemical Company (Houston, TX). The curing agent dicumyl peroxide (DCP) was supplied by Aldrich (Milwaukee, WI), pure grade, melting point (39-41oC) Mwt= 270.37g/mol, Aldrich product. Curcumin as Air-dried 500 gram Curcumin Turmeric (Curcuma longa) were purchased in the year (2022) from EL-Korma - The Egyptian Co. For Seeds, Oils & Chemicals, Cairo, Egypt. Curcumin nanoparticle with diameter ranges from 13-24 nm was prepared as in the previous work (15). Ionic liquid as 1-alkyl-3-methylimidazolium bromide [RMIM]Br ionic liquids that synthesis before [16, 17].

Preparation of samples:

The Brabender Plasticorder (C. W. Bra,

Instrument, Inc., Hackensack NJ; 230 V, 40 A) was used to prepare the blends with 1.50 phr of DCP for 5 minutes at 80oC and 70 rpm. The EVA and SR and their blends (EVA/SR (70/30), (50/50) and (30/70) were prepared. The blends (EVA/SR (70/30) and (30/70) with 5% ionic liquid with different concentrations of Curcumin (1, 5, 10%) were investigated. Every sample was prepared using a two-roll mixing mill (friction ratio, 1:1.4). The compounded samples were then compressed molded for 20 minutes at 160°C under 30 tones in an electrically heated hydraulic press (13).

Fourier transform infrared (FTIR):

The infrared spectrum was recorded by a JASCO FT/ IR 300 E, Fourier transform infrared (FTIR) Spectrometer (Tokyo, Japan), the IR analyses was done at the central laboratory of the National Research Centre of Egypt.

Mechanical properties:

According to ASTM D 412-98a, they were determined at room temperature using Zwick tensile testing machine (model-1425) and a crosshead speed of 500 mm/min. A cutter in the form of a dumbbell was used to cut blend films. Using a thickness gauge, the cross-sectional areas of the dumbbell specimens were precisely measured.

Differential scanning calorimetry (DSC):

Differential scanning calorimetry was performed using DSC131 evo (SETARAM Inc.,France). The operating temperature was from 25°C to 200°C with a heating rate 10°C/min.

Scanning electron microscope (SEM):

Using a scanning electron microscope, SEM/EDX, Philips XL30 (Japan), the homogeneity of the samples and the dispersion of the curcumin powder in the polymer matrix were examined.

Cytotoxic activity test:

The polymer blends loaded with curcumin as antimicrobial agents were tested towards the human tumor cell line: BJ1 (normal Skin fibroblast) to test their safety using MTT assay as follows:

Cytotoxicity Test:

The following steps were used to prepare the samples under examination:

a. Washing:

To successfully remove soil and leachable contaminants, strips from various polymer samples were washed in distilled water.

b. Drying and sterilization:

Prior to the experiment, washed strips were dried and sterilized by being suspended in 70% ethanol for 10 minutes.

c. Cell culture:

Human normal cell line BJ1 (normal Skin fibroblast) was cultivated and kept in RPMI1640 medium (Sigma-Aldrich, St. Louis, MO) supplemented with 10% fetal calf serum (FCS), 100 U mL penicillin, 100 IL mL streptomycin, and 2 mmol L-glutamine (Cambrex Bio Science, Verviers, Belgium) at 37 °C in a Cells from confluent cultures were used for the sub-culture after being treated with 0.2 g/ L of ethylene diamine tetraacetic acid (EDTA) in phosphate buffered saline (PBS).

d. Cytotoxicity of cells MTT assay:

By ISO standard 10993-12 [18], the polymer sample strips (10 mg polymer weight) were agitated for 48 and 72 hrs at 37°C while immersed in RPMI1640 media. The negative control samples, which only included the medium, were handled similarly. BJ1 (normal Skin Fibroblast) cells from a human cell line were diluted in fresh medium containing 2%, 5%, and 10% FCS before being seeded into 96-well plates (104 cells well 21). The media was removed from all wells after 24 hrs of incubation, replaced with polymer blends or control medium, and incubated for an additional 24 hrs before checking for cytotoxicity. The colorimetric MTT assay developed by Ghasemi et al. [19], and modified by Madar et al. [20] was used as a test for cell proliferation and survival assay. 20 mL MTT dye (5 mg/ mL 21 in PBS) was added to each well and incubated at 37°C, in air containing 5% CO_2 and at 95% relative humidity for 4 hrs in the dark. After incubation, the MTT was aspirated and the formazan product was dissolved in 100 mL of the acidified isopropanol (0.04 N HCI in isopropanol). The plates were shaken before the optical densities (OD) were measured at 570 nm wavelength. Each experiment included three tests for each polymer sample and control. To verify repeatability, each assay was performed at least twice more.

Antimicrobial activity:

Polymer blends were individually tested against a variety of certified reference strains of Gram positive, Gram negative bacterial pathogens. The current study includes three Gram-positive reference strains; B. cereus NCINB 50014, E. faecalis ATCC 19433, S. aureus NCINB 50080 and four Gram-negative reference strains; E. coli O157 ATCC 700728, S. Typhimurium ATCC 13311, and Shigella flexineri ATCC 12022 and V. parahaemolyticus ATCC 17802.

1.1 Agar Diffusion Test (ADT)

The antimicrobial activity was carried out using the qualitative method based on JIS L 1902:2008 [21] and Pinho et al. [22] with modification; using Muller Hinton agar (MHA) instead of Nutrient agar (NA).

The protocol of antimicrobial activity was performed after inoculation of tested reference strains onto nutrient broth incubated for 24 hrs/37°C. Then, 1ml from the inoculum (conc. 1×107 cfu/ml) was added to 15 ml sterile MHA warmed at 45–46°C. The inoculated solution was then poured into sterile petridish. The tested samples were previously prepared under aseptic conditions, each sample was cutted as square 1×1 cm using sterile scissor, then samples were placed over the surface of solidified MHA, then the plates were incubated for 24 hrs/37±°C. Evaluation of the antimicrobial activity was recorded based on the measurement of the zone of inhibition of bacterial growth. This is indicated by the halo zone formed around the edges of the samples and the bacteria growth under the samples.

Standard antibiotic Gentamycin (10µg/ml), was used as a control positive which show a halo zone of inhibition of microbial growth.

After incubation time, antimicrobial activity was estimated by estimating the zone of inhibition against the test microorganisms and was tabulated as (+), (++), (+++), and (++++). In case of negative inhibition, results are given as (-).

Antimicrobial activity of reference drug was expressed as inhibition diameter zones in millimeters (mm). The experiment was carried out in triplicate and the average zone of inhibition was calculated. The Zone of inhibition is measured using measuring caliber.

RESULTS AND DISCUSSION:

Fourier Transform Infrared (FTIR):

The recorded FTIR spectra in the 400–4000 cm^{-1} spectral region Figure (1). The stretching vibration absorption bands (v) for C=O groups at approximately 1737 cm^{-1} and the stretching vibration absorption of C-O at around 1159 cm^{-1} are visible. These bands are indicative of ester groups in EVA. The stretching vibration absorptions of Si-O are represented by the characteristic silicone rubber peaks at 1077 and 1013 cm^{-1}, whereas the peak at 795 cm^{-1} is the stretching vibration absorption of Si–CH_3, and the peak at 1259 cm^{-1} is specific to the stretching vibration absorption of methyl group (CH_3).

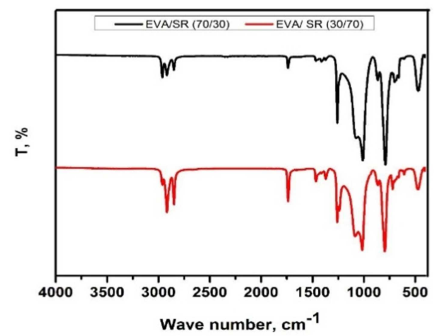


Figure 1: FTIR for EVA/SR with 5% ionic liquid and 10% Curcumin

The characteristic functional groups of EVA and SR blend after curing reaction included the additional characteristic absorption regions of C-H groups (2918 cm^{-1}) also these sharp peaks at 2920 and 2852.2 cm−1 attributed to CH Stretching, 1706 cm^{-1} for C=O, 1641 cm^{-1} for C=C stretching, 1464 for ν (OH), 1105 for C-O-C stretching modes, 723 for cis CH vibration of aromatic ring for the curcumin. Si-CH_3 symmetric deformation (1259 cm1) , Si-O-Si bonds (1067 cm^{-1} , 1009 cm^{-1}), Si-C (787 cm^{-1}) were observed for the EVA/SR (70/30) blend Particularly, as seen in Figure 1, there was a slight change in the Si-O-Si and Si-C stretching vibration in the magnified region, showing the silicone's efficient reaction with the EVA. This change was further apparent in the EVA/SR (30/70) as well. The C=N stretch is responsible for the noticeable peak at 1630 cm^{-1}, the C=C symmetric peak at 1571 cm^{-1}, and the C-N stretch for the ionic liquid is shown by the peak at 1165 cm^{-1} .

Scanning Electron Microscope (SEM):

The morphological properties of EVA/SR blends in Figure (2) shows the compatibility essentially increased without clear phase separation boundaries. This is due to the relative distribution of crystalline hard segments of (EVA) in the continuum of (SR) matrix. The EVA/ SR blends are heterogeneous in nature, while the addition of liquid-crystalline ionic liquid 5% [1-dodecyl-3-methylimidazolium Tetra fluoroborate [C12mim][BF4] increases the homogeneous distribution between the (EVA) and SR blend even when the IL content was 5%.

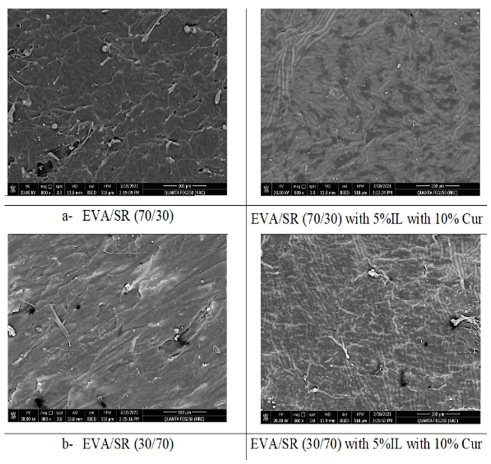


Figure 2: SEM for the EVA/SR blends of different composition without and with 5% IL and 10% Curcumin

This homogeneity reflects compatibility between (EVA) and (SR) matrices. Figure (a and b) appear that in EVA/SR (70/30), the minor SR component is good distributed within the major (EVA) component, and in EVA/SR (30/70), the (EVA) component is distributed as domains in the continuous (SR) matrix.

The Heat of mixing:

Schneier's equation [23, 24], which is stated as follows, has been used to calculate the heat of mixing to determine the compatibility of EVA/SR blends:

$$\Delta Hm = \{X1\ M1\rho1\ (\delta1-\delta2)2[X2 / (1- X2)\ M2\rho2 + (1-X1)\ M1\rho1]\ 2\}\ 1/2 \quad (1)$$

Where: X, ρ, and M are the weight fraction of polymer, density, and molecular weight of the monomer unit, respectively, δ is the solubility parameter of the polymer.

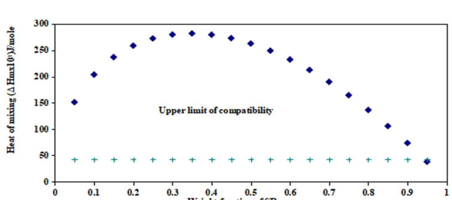


Fig 3: Heat of mixing as a function in a blend of silicon rubber and EVA

When the value of the heat of mixing of two polymers is under the value 41.85 x 10-3 J $mole^{-1}$, they are considered to be compatible, and incompatible if the value of heat of mixing is above this limit. Figure (3) shows the heat of mixing of silicon rubber and EVA blends as a function of composition. When the heat of mixing exceeds the upper limit of compatibility, polymer pairings are regarded as being incompatible. It is obvious that the SR/EVA blends are supposed to be incompatible blends, since the calculated values of the heat of mixing at all compositions were found to be in the range 151.44 x10-3 and 37.77 x10-3 J/mole. These values are greater than the compatibility's theoretical limit. This finding suggests that the mixture's macromolecules are in a disordered state, and as a result, these blends are considered to be thermodynamically incompatible.

Mechanical properties:

Mechanical characteristics like tensile strength and elongation at the break of EVA/SR blends samples cured with DCP are shown in Figures (4, 5 and 6).

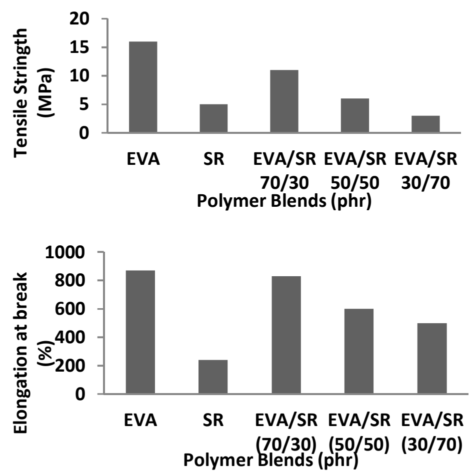


Figure 4: Mechanical properties of EVA/ SR blends (70/30) and (30/70)

It is clear that, the tensile strength measurements of the blend decreases with increasing the content of SR. On the other hand the same behavior is in the values of the elongation at break. Scanning electron micrographs, which confirm the morphology of the blends, have been used to explain the increase in mechanical capabilities of the blends with higher EVA content (10).

Figures (5, 6) show the effect of adding different concentrations of curcumin nanoparticles (1, 5, and 10 %) with 5% IL.

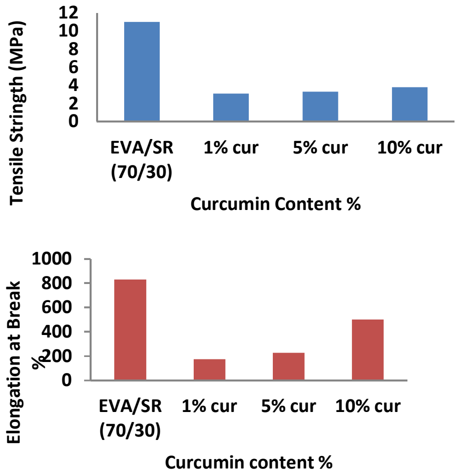


Figure 5: Mechanical properties of EVA/ SR blends (70/30) with Curcumin nanoparticles content

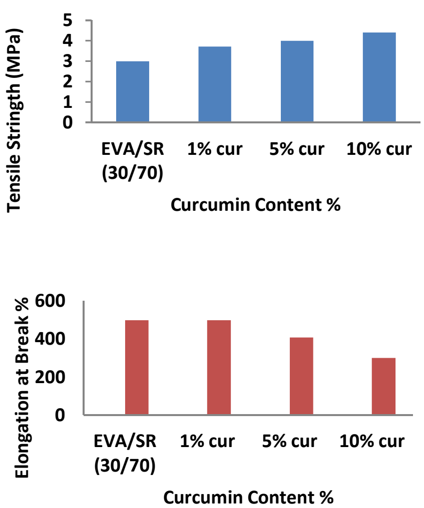


Figure 6: Mechanical properties of EVA/ SR blends (30/70) with Curcumin nanoparticles content

Tensile strength and elongation at break values for EVA/SR (70/30) were increased with increasing curcumin nanoparticles. In the case of EVA/SR (30/70), the tensile strength was increased but the elongation at break was decrease with increasing curcumin nanoparticles. This can be attributed to a good interface between EVA/SR and IL which helps in a good dispersion of the curcumin nanoparticles.

Differential scanning calorimetry (DSC) analysis:

Differential scanning calorimetry (DSC) was used to describe the thermal behavior of EVA/SR blends. The effect of degree of blending on the thermal characteristics of EVA/SR was examined using the DSC. It is interesting to show how the thermal transitions of EVA/SR blend to aspect the determination of whether the resulting blend samples miscible or not. The heating thermograms of EVA/SR are shown in figure (7).

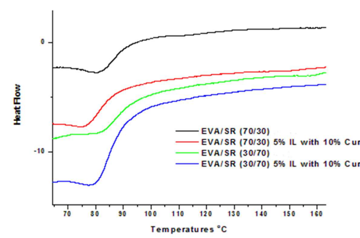


Figure 7: Differential scanning calorimetry (DSC) analysis

For EVA/SR 70/30%, the corresponding melting temperature values (Tm) 85.18 °C and for EVA/SR 30/70% 83.8 °C with increasing content of EVA the Tm decreases. This occurred because the disrupted the EVA chain structures' regularity and prohibited the movement of the polymer segments, making the regular arrangement of the molecular chains more difficult. Adding 5% ionic liquid and 10% curcumin as a natural product makes the Tm decreases for EVA/ SR 70/30% to 73 and 79 for the blends EVA/SR 30/70%.

Cytotoxic activity and antimicrobial testing of curcumin polymer blends:

1-Cytotoxic activity test

The effects of a cytotoxic on the prepared samples from the investigated polymer blends loaded with curcumin towards the human normal cell line: **BJ1** [normal Skin fibroblast] are illustrated in table (1).

The data in table 1 indicated that the investigated polymer blends loaded with curcumin gave negative results to the human normal Skin fibroblast [BJ1] cell line. It was found that these polymer blends are safe and nil in cytotoxic effect.

Table (1): Cytotoxic activity of different Polymer blends towards human normal Skin fibroblast [BJ1]

Sample Name	IC_{50} (µg/ml)
EVA/SR (70/30)	N.A.
EVA/SR (70/30) 5% IL with 10% Curcumin	N.A.
EVA/SR (30/70)	N.A.
EVA/SR (30/70) 5% IL with 10% Curcumin	N.A.
Negative control	N.A.

Where N.A.: No activity

2-Antimicrobial Activity

The results of tested Polymer blends loaded with curcumin towards the tested Gram positive & Gram negative bacteria are presented in table (2).

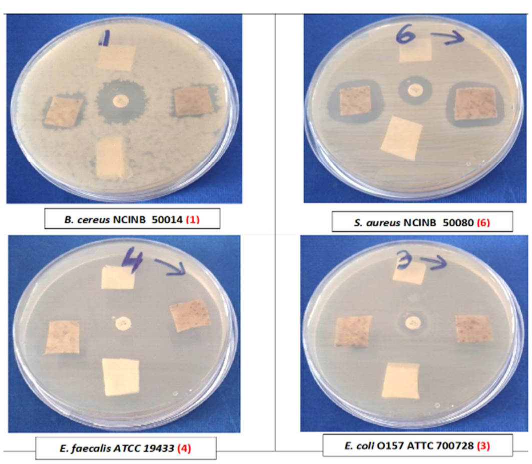
Table 2: Zone of inhibition of tested compounds using Agar Disc Diffusion Test (ADDT)

Material tested / *Tested Strains*	EVA/SR (70/30)	EVA/SR (70/30) 5%IL with 10% Curcumin	EVA/SR (30/70)	EVA/SR (30/70) 5%IL with 10% Curcumin	*Gentamycin (10 µg/ml)*
Gram positive bacteria					
B. cereus NCINB 50014 (1)	-	++	-	++	++++
S. aureus NCINB 50080 (6)	-	++++	-	++++	++++
E. faecalis ATCC 19433 (4)	-	+++	-	+++	++++
Gram negative bacteria					
E. coli O157 ATTC 700728 (3)	-	-	-	-	++++
S. Typhimurium ATCC 13311 (5)	-	-	-	-	++++
Shigella flexineri ATCC 120 22 (7)	-	+	-	++	++++
V. parahaemolyticus ATCC 17802 (11)	-	++++	-	+++++	++++

Key of inhibition scale: 0-5mm (-), 10-15mm (+), 15-20 mm (++), 20-25 mm (+++), 26-30 mm (++++), 31-35 mm (+++++)

The experiment was carried out in triplicate and the average zone of inhibition was calculated. The experiment was carried out in triplicate and the average zone of inhibition was calculated.

Results in table 2 and figure 8 showed that the investigated polymer blends loaded with curcumin gave promising results towards the tested G+ve and G-ve bacteria.



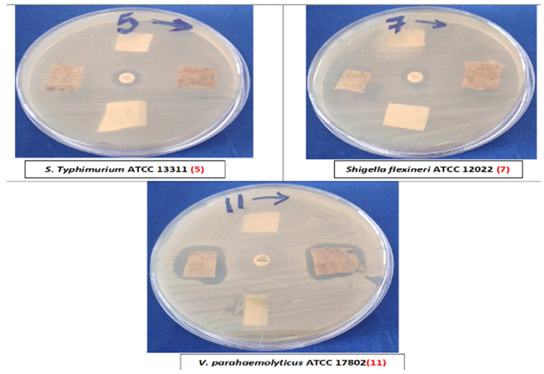


Figure 8: Zone of inhibition of the investigated polymer blends loaded with curcumin using Agar Disc Diffusion Test (ADDT)

Conclusion:

The green biopolymer composite was successfully prepared from EVA/SR blends without and with 5%IL and different contents from Curcumin 1, 5, and 10%. Due to their pleasant scent and potent antibacterial properties, these green blends may find usage in domestic products as well as in the fields of medicine, food packaging, coatings, and other industries. Studying of mechanical and thermal properties and the results show this may be believed to be due to a good interface between EVA/SR and IL which helps in a good dispersion of the curcumin nanoparticles.

Our present findings indicated that the cytotoxicity of the investigated polymer blends loaded with curcumin gave negative results to human normal Skin fibroblast [BJ1] cell line, demonstrating the polymer blends and its potential for use in biomedical applications such as wound healing, implants, and scaffolds. Moreover, Results indicated that the investigated polymer blends gave good promising results towards the tested G+ve and G-ve bacteria.

Funding information:

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of interest:

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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