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Egypt. J. Chem. **Vol. 65,** No. SI:13B pp. 1135 - 1143 (2022)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Safety rubber vulcanizates containing natural product for toys industry

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Abstract

The present work aims to prepare nanocurcum and incorporate it into rubber formulations through the ordinary rubber mixer, and study the rheological characteristics of the prepared compounded rubber. Also, evaluate the physicomechanical properties of the vulcanizates as well as the cytotoxicity and the antimicrobial effects. The innovative product would open the door for advanced application in the field of antibacterial/antimicrobial children's toys and infant dummies. Natural and some synthetic rubber, including Ethylene propylene diene monomer (EPDM) and styrene butadiene rubber (SBR), were investigated for this study. Rubber and their ingredients as well as natural product applied antimicrobial agents such as nanocurcum were mixed in the ordinary rubber mixer. The rheological characteristics of compounded rubber were measured and the curing time was determined. The compounded rubber was vulcanized in a hydraulic press at a specified temperature and estimated cure time. The physicomechanical properties were evaluated. Additionally, the scanning electron micrographs (SEM) illustrated surface homogeneity of vulcanizate sample that demonstrate appropriate compatibility and dispersion of the material inside rubber chains. The rheological characteristics of natural rubber vulcanizates were superb and show no significant change in the presence of assessed natural product drug (nanocrucum). Moreover, the physicomechanical properties such as tensile strength and elongation were increased in the presence of nanocrucum depending on its nature and concentration. The same observation was obtained for styrene butadiene rubber and EPDM vulcanizates under investigation. On one hand, the cytotoxicity of the prepared vulcanizates towards the human normal retina cell line (RPI-1) indicated the safety properties of the prepared vulcanizates. On the other hand, the as-prepared nanocurcum rubber vulcanizates manifested moderate antagonistic activities against the examined bacteria and fungi strains and a significant inhibition in their growth was indicated.

Key words: Natural rubber, Ethylene Propylene Diene Monomer, Styrene-butadiene rubber, Nanocurcum, Cytotoxicity, Antimicrobial activity.

1. Introduction

Rubber is a polymeric material that can be employed in the preparation of network structures with outstanding elastic properties. Microbes, such as bacteria, fungi, and parasites are the critical sources of infections [1]. Consequently, infectious diseases result from pathogenic microbes capable to kill more people than any other single cause [2]. An antimicrobial is an agent used to kill microbes or inhibit their growth. Although numerous antimicrobial drugs have been developed to kill or inhibit microbes, many infectious diseases remain difficult to treat [3,4]. Antimicrobial polymers were discovered in 1965 [5] and have attracted considerable attention in both academic and industrial research. The medical, food and textile industries are three major areas of applied antimicrobials. Intriguingly, antimicrobial polymers demonstrate superior efficacy, reduced toxicity, minimized environmental problems, and greater resistance [6, 7]. Antimicrobial Rubber is a pro-active antimicrobial rubber that supplies residual protection against microbial contamination. Thereby, it eventfully reduces the necessity for frequent sanitization,

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Receive Date: 14 August 2022, Revise Date: 31 August 2022, Accept Date: 11 September 2022
DOI: 10.21608/EJCHEM.2022.156170.6761

disinfection and decontamination service. Additionally, the antimicrobial agent configures an integral part of the rubber compound. It is not simply superficial or liner. However, the original and antimicrobial synergistic properties always remain active. Therefore, any abrasion or wear to the surface of the rubber does not affect the efficacy of its antagonistic activity or bacteriostatic qualities. [8-10]. The rubber component of the innovative rubber formulation and cured articles is preferably selected from a rubber group consisting of natural and synthetic rubber, and a blend of them. It is important to consider the desired physical properties of the rubber article for the production of rubber goods. For instance, high molecular weight EPDM polymers tend to exhibit higher green strength, tensile strength, excellent heat resistance and lower compression set than lower molecular weight polymers' counterparts The research areas of antimicrobial polymers for toys and medical products are on the development and improvement of antimicrobial vulcanized rubber compounds [8]. Antimicrobial agents are the greatest contribution of the present century to create novel antibacterial rubber vulcanizates.

The organic antimicrobial agents from natural resources such as Curcuma, zingiber…etc, show antimicrobial potency with a wide range of activity against both Gram-positive and Gram-negative bacteria as well as fungi. [11]. Moreover, Curcuma is one of the most used traditional herbs to help treating bacterial infections in human medicine, veterinary practices, animal husbandry, agriculture, and aquaculture, among others [12 - 14].
The antimicrobial agents are incorporated in rubber formulation in an amount from ca. 0.001% - 20 % to total weight of the rubber formulation [8].

Children's toys, in particular, are known to harbor microbes where there grow different kind of moulds on the inside of a rubber duck or E.coli on dolls and infant dummies. Frequent contact with soiled hands and derelicts in cleaning contributes eventfully to product contamination. This can pose significant anxiety for parents, who have almost no control over the toys their children play especially with whilst at school and nursery. Antimicrobial rubber represent a very promising class of therapeutics with unique characteristics for fighting microbial infections.The present work aims to prepare nanocurcum and incorporate it into rubber formulations through the ordinary rubber mixer, and study the rheological characteristics of the prepared compounded rubber. Also, evaluate thephysicomechanical properties of the vulcanizates as well as the cytotoxicity and the antimicrobial effects. The innovative product would open the door for advanced application in the field of antibacterial/antimicrobial children's toys and infant dummies.

2. Materials and Methods

2.1. Materials

- Ethylene propylene diene monomer (EPDM), ethylene content 55%, the density is 0.86 g/cm^3 produces by Esso Chemi, Germany.
- Zinc oxide (ZnO) with specific gravity at 15^{o}C is 5.6.
- Stearic acid with specific gravity at 15^{o}C is 0.9-0.97.
- Elemental sulfur: with fine pale yellow powder and specific gravity is 2.04-2.06 at room temperature (25 ^{o}C± 1).
- N-cyclohexyl-2-benzothiazole sulphenamide (CBS) with specific gravity is 1.27- 1.31 at room temperature (25 ^{o}C± 1), melting point is 95-100 ^{o}C.
- Polymerized 2,2,4-trimethyl-1,2dihydroquinoline (TMQ) was used as antioxidant, purchased from Aldrich Co., Germany.
- Talc: Hydrous magnesium silicate, molecular weight: 379.27, purchased from Aldrich Co., Germany.
- Nanocurcum: Curcuma was purchased from local market and modified to nanocurcum according to the method identified in the experimental part.

2.2. Methods

2.2.1. Preparation of the investigated rubber compounds:

2.2.1.1. Synthesis of nanocurcum [15]:

Curcumin solution was prepared by dissolving curcuma powder in ethanol. Under ultrasonication conditions, this solution was added to boiling water in drop-wise mode. The solution was sonicated for about 30 min. Then the mixture was stirred at 800 rpm for about 20 min till the orange colored precipitate was obtained. Afterward, the supernatant was removed and the obtained pellet was used for the study.

2.2.1.2. Preparation of rubber vulcanizates:

Sample preparation

All rubber ingredients were mixed on a two roll mill (470 mm diameter and 300 mm working distance). The speed of the slow roll was 24 rpm with a gear ratio of 1:1.4.

2.2.1.2.1. Rheological characteristics [16]:

The rheological properties of compounded rubber were measured by Rheometer (MDR one [Moving Die Rheometer], USA). The curing time (tc90) was estimated.

2.2.1.2.2. Vulcanization process:

The compounded rubber mixes were vulcanized in a hydraulic press under a pressure of 4 MPa and temperature of specified for rubber type and the estimated curing time.

2.3. Characterization:

2.3.1. Mechanical properties [17]:

Tensile properties of the prepared rubber vulcanizates were tested with a Zwick tensile testing machine (1425) Germany according to ASTM D 412 at a crosshead speed of 500 mm/min. Dumbbell tensile specimens with 1-mm thickness were cut from the molded sheets with a Wallace die cutter, S6/1/6.A. Tensile strength, and elongation at break were measured at a crosshead speed of 500 mm/min, and the tests were performed at room temperature.

2.3.2. Swelling test:

The swelling percentage in toluene was determined in accordance with the ASTM D471-16a test method. The test pieces of thickness 1 mm were weighed and immersed in toluene at room temperature for 24 h and the weight swell percentage was determined until the equilibrium value reached. The specimens were removed after 24h, wiped with tissue paper to remove excess toluene, and weighed with an analytical balance. The weight swell percentage was then calculated as follows:

Swelling (%) = Swollen weight - Original weight/ Original weight ×100

2.3.3. Cytotoxic activity test

The rubber vulcanizates loaded with nanocurcum were tested towards the human tumor cell line: RPE1 [normal retina cell line] using MTT assay as follow:

2.3.3.1. Cytotoxicity Test:

The examined samples were prepared via the following steps:

a. ***Washing*:**

Strips from different rubber samples were washed with distilled water to remove soil effectively as well as leachable materials.

b. Drying and sterilization:

Washed strips were dried and sterilized by suspending them in 70% ethanol for 10 min before the experiment.

c. Cell culture:

Human normal cell line: RPE1[normal retina cell line] was cultivated and maintained 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 humidified atmosphere of 95% air and 5% CO_2. Sub-cultivation was performed with cells from confluent cultures treated with 0.2 g L ethylene diamine tetra acetic acid (EDTA) in phosphate buffered saline (PBS).

d. Cell cytotoxicity MTT assay:

The rubber samples strips (10 mg rubber weight) were immersed in RPMI1640 media and agitated for 48 and 72 h at 37°C according to ISO standard 10993-12 [18]. The control samples (negative control) which contained only medium, were similarly treated.

Human normal cell line: RPE1[normal retina]:cells were diluted in a fresh medium containing 2%,5%, and 10% of FCS and seeded into 96-well plates (104 cells well **2**1). After incubation for 24 h, the medium was aspirated from all wells and replaced by EPDM vulcanizates or control medium and incubated for another 24 h before checked 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 **2**1 in PBS) were added to each well and incubated at 37°C, in air containing 5% CO_2 and at 95% relative humidity for 4 h 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 measuring the optical densities (OD) at 570 nm wavelength. Three tests for each rubber sample and control were performed in each experiment. All assays were repeated at least twice to ensure reproducibility.

2.3.4. Antimicrobial activity

2.3.4.1. Toxicity test

Rubber vulcanizates loaded with nanocurcum was tested against a panel of Gram positive bacteria (*Staphylococcus aureus* ATCC 29213), Gram negative bacterial pathogens (*Escherichia coli* O157:H7), and *Candida albicans* NRRL Y-477 as the pathogenic fungi strain used for detection the toxicity of the investigated rubber vulcanizates. Antimicrobial tests were carried out by the agar well diffusion method [21 - 22] using 100 µL of suspension containing 1 x10^8 CFU/mL of pathological tested bacteria ,1 x10^6 CFU/ml of yeast spread on nutrient agar (NA) and Sabour and dextrose agar (SDA) respectively. After the media had cooled and solidified, wells (10 mm in diameter) were made in the solidified agar and each well was loaded with a piece of different rubber vulcanizates (5 mm^2) and placed on the solidified agar. The inculcated plates were then incubated for 1week at 37 °C. At the end of incubation time, the lethal effect of the tested rubber vulcanizates towards bacteria was evaluated by measuring the inhibition zone around the rubber vulcanizates pieces. Toxicity effect was expressed as inhibition diameter zones in millimeters (mm). The experiment was carried out in triplicate and the average inhibition diameter zone was calculated.

3. Results and Discussion

The objective of this work is to prepare antimicrobial safety rubber goods for toys, medical and industrial products using natural nanocurcum as antimicrobial agent.

3.1. Estimation of prepared nanocurcum using Transmission Electron Microscope (TEM):

The nanocurcum was prepared according to the method illustrated in the experimental technique part. The particle size was estimated using TEM.

Fig.1 shows the TEM image of nanocurcum particles. The micrograph shows spherical shape and their particles in the nano grade with diameter ranges from 13-24nm.

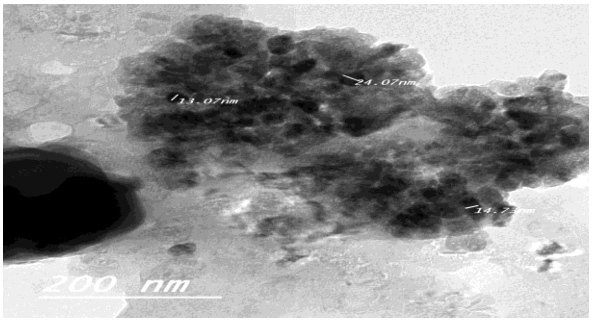


Fig. 1. TEM of the prepared nanocurcum

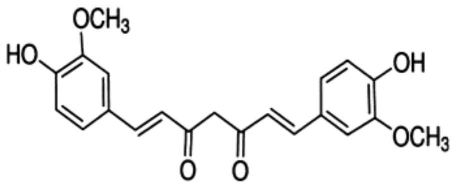


Fig. 2 .Structure of nanocurcumin

Nanocurcumin derived from Curcuma is an improved form of the compound with reduced particle size, improved delivery to the diseased tissue, better pharma-cokinetic properties, better internalization and reduced systemic elimination [23].

3.2. Rheological and physico-mechanical characteristics of the investigated natural rubber (NR):

The rubbers used are natural rubber and synthetic rubber as EPDM and SBR. The rubber and their additives as well as nanocurcum powder (as a natural resource antibacterial agent) were mixed together on the ordinary rubber mixer used in industry. The compounded rubber mixes were evaluated rheologically then vulcanized at their corresponding curing time. The physicomechanical and chemical properties were measured according to standard test methods. The formulations of natural rubber containing nanocurcum as well as the rheometric characteristics at 140ºC and the physico-mechanical properties of the rubber vulcanizates are illustrated in tables 1 - 3. It was found that, the cure rate index slightly decreased according to increasing nanocurcum concentrations that is confirmed by curing time or time at 90% cure, and the value of scorch time measurement. The nanocurcum as a natural product slightly affected the rheological properties of the compounded rubber, the maximum torque increased, while the minimum torque and time at 90% cure decreased (table 2) due to the nature and concentration of nanocurcum.

The physicomechanical properties of the investigated natural rubber vulcanizates are illustrated in Tables (2-3). It was observed that, tensile strength and elongation at break were increased in the presence of nanocurcum from concentration 10 – 30 phr and then decreased with increasing nanocurcum drug concentration to 40 phr. In addition, there is no great difference in change of modulus at different elongation (50 – 500%) from the formulation free of the nanocurcum drug (control) i.e. the mechanical properties are good.

3.3. Rheological and physicomechanical properties of the investigated synthetic rubber (EPDM &SBR):

The formulations of the investigated synthetic rubbers (EPDM and SBR) are presented in tables (4 and 7), respectively. The rheological properties of the compounded synthetic rubbers were determined at 152ºC using moving die Rheometer. The curing time was determined. The results are found in tables 5 and 8. It is clear that, the rheological properties of EPDM and SBR are good. So, there is no change in the minimum torque and thus maximum torque increased in the presence of nanocurcum for EPDM and SBR. The values of MH of EPDM are more than SBR depending on nature and type of rubber. The cure rate index changed and decrease depending on the type of rubber.

The compounded rubbers were vulcanized at their corresponding optimum cure time, and the vulcanizates examined and evaluated using mechanical testing machine at stretching rate 500 mm/min. The results were recorded in Tables 6 and 9. It is clear that, the elongation at break was increased for EPDM and SBR vulcanizates containing nanocurcum depending on their concentrations. The tensile strength was moderately increased. Therefore, the presence of natural nanocurcum gave and keeps good properties of rubber vulcanizates.

Table (1): Formulation of NR/ nanocurcum compounds

Ingredients (phr)	N0	NC1	NC2	NC3	NC4
NR	100	100	100	100	100
Stearic acid	2	2	2	2	2
ZnO	5	5	5	5	5
S	1.5	1.5	1.5	1.5	1.5
CBS	0.8	0.8	0.8	0.8	0.8
TMQ	1.5	1.5	1.5	1.5	1.5
Oil	2	2	2	2	2
Talc	30	30	30	30	30
Nanocurcum	0	5	10	20	30

Table (2): Rheometric characteristics NR/ Nanocurcum compounds

Sample	M_L	M_H	tc_{90}	ts_2	CRI
N0	0.09	4.54	14.47	10.58	25.71
NC1	0.05	5.58	13.35	7.52	17.15
NC2	0.05	6.33	12.39	6.83	17.99
NC3	0.05	6.52	11.01	4.18	14.64
NC4	0.04	7.33	9.86	3.09	14.47

Table (3): Mechanical properties of NR/ Nanocurcum vulcanizates

Sample	Tensile Strength (MPa)	Elongation at break %	Modulus 50% (MPa)	Modulus 100% (MPa)	Modulus 200% (MPa)	Modulus 300% (MPa)	Modulus 500% (MPa)
N0	4.38	1085	0.34	0.47	0.69	0.91	1.33
NC1	5.31	1500	0.42	0.55	0.74	0.95	1.56
NC2	6.45	1520	0.52	0.63	0.81	1.33	1.94
NC3	7.13	1540	0.64	0.69	0.89	1.58	2.03
NC4	7.38	1550	0.68	0.75	0.96	1.89	2.07

Table (4): Formulation of EPDM/Nanocurcum compounds

Ingredients (phr)	E0	EC1	EC2	EC3	EC4
EPDM	100	100	100	100	100
Stearic acid	2	2	2	2	2
ZnO	5	5	5	5	5
S	1.5	1.5	1.5	1.5	1.5
CBS	0.8	0.8	0.8	0.8	0.8
TMQ	1.5	1.5	1.5	1.5	1.5
Oil	2	2	2	2	2
Talc	30	30	30	30	30
Nanocurcum	0	5	10	20	30

Table (5): Rheometric characteristics EPDM/Nanocurcum compounds

Sample	ML	MH	Tc90	Ts2	CRI
E0	1.83	6.53	14.6	12.91	59.17
EC1	2.0	9.57	14.15	10.37	26.46
EC2	1.84	12.58	13.18	6.4	14.75
EC3	1.92	13.76	12.34	3.83	11.75
EC4	2.19	14.09	12.03	3.33	11.49

Table (6): Mechanical properties of EPDM/Nanocurcum vulcanizates

Sample	Tensile Strength (MPa)	Elongation at break %	Modulus 50% (MPa)	Modulus 100% (MPa)	Modulus 200% (MPa)	Modulus 300% (MPa)	Modulus 500% (MPa)
E0	2.14	375	0.85	1.13	1.46	1.81	1.89
EC1	2.63	570	1.57	1.80	1.96	2.47	2.51
EC2	2.81	620	1.66	1.85	2.58	2.66	2.70
EC3	2.41	530	1.65	1.82	2.35	2.25	2.27
EC4	2.14	530	1.6	1.80	2.30	2.04	2.09

Table (7): Formulation of SBR/Nanocurcum compounds

Ingredients (phr)	S0	SC1	SC2	SC3	SC4
SBR	100	100	100	100	100
Stearic acid	2	2	2	2	2
ZnO	5	5	5	5	5
S	1.5	1.5	1.5	1.5	1.5
CBS	0.8	0.8	0.8	0.8	0.8
TMQ	1.5	1.5	1.5	1.5	1.5
Oil	2	2	2	2	2
Talc	30	30	30	30	30
Nanocurcum	0	5	10	20	30

Table (8): Rheometric characteristics SBR/Nanocurcum compounds

Sample	ML	MH	Tc90	Ts2	CRI
S0	0.63	5.82	13.29	8.76	24.21
SC1	0.62	6.25	12.22	8.09	22.08
SC2	0.68	8.08	11.59	6.14	18.35
SC3	0.7	7.82	10.3	4.6	17.54
SC4	0.75	10.35	9.75	3.33	15.57

Table (9): Mechanical properties of SBR/Nanocurcum vulcanizates.

Sample	Tensile Strength (MPa)	Elongation at break %	Modulus 50% (MPa)	Modulus 100% (MPa)	Modulus 200% (MPa)	Modulus 300% (MPa)	Modulus 500% (MPa)
S0	3.32	520	0.81	1.10	1.64	2.18	2.55
SC1	3.89	650	1.05	1.22	1.78	2.66	3.14
SC2	3.88	630	1.03	1.20	1.74	2.64	3.12
SC3	3.55	630	1.00	1.89	1.69	2.61	3.02
SC4	3.51	600	0.95	1.77	1.51	2.41	2.98

3.4. Effect of nanocurcum on equilibrium swelling (Q) of the rubber vulcanizates:

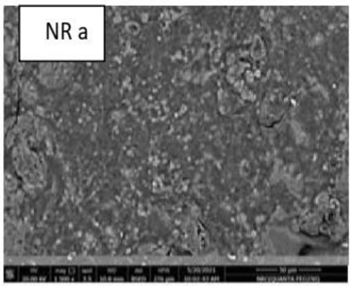
As shown in Table 10, there is no significant change in the swelling results, in absence and presence of nanocurcum, so the results are nearly to each other and the difference in results depends on the type of rubber (Table 10).

3.5. Morphological characteristic of the investigated rubber vulcanizates

SEM was used to study the essential morphological characteristic of the investigated rubber (NR, EPDM and SBR) vulcanizates in the presence of nanocurcum as a natural resource drug. Figures (2-4) illustrate the SEM micrographs of the tested samples. The micrographs depict the good dispersion and distribution of the nanocurcum drug inside rubber matrix.

Table (10): Equilibrium swelling of the different rubber vulcanizates in absence and presence of nanocurcum

Rubber type	Equilibrium swelling (Q), %	
	without nanocurcum	with nanocurcum
NR	250	247
EPDM	185	180
SBR	330	248



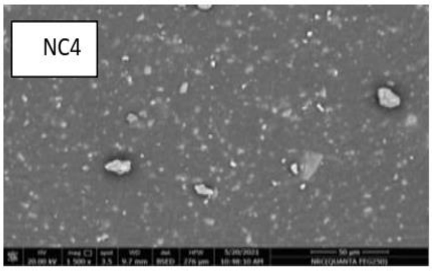


Fig. 2. SEM micrographs for NR in absence and presence of nanocurcum (N0, NC4).

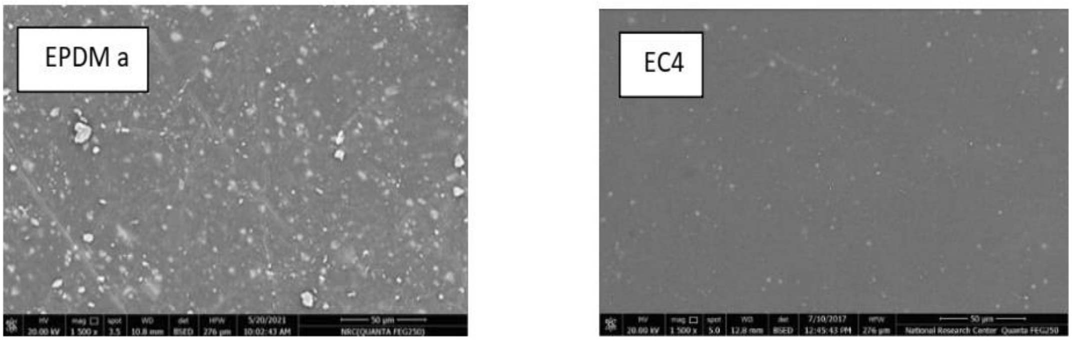


Fig. 3. SEM micrographs for EPDM in absence and presence of nanocurcum (E0, EC4).

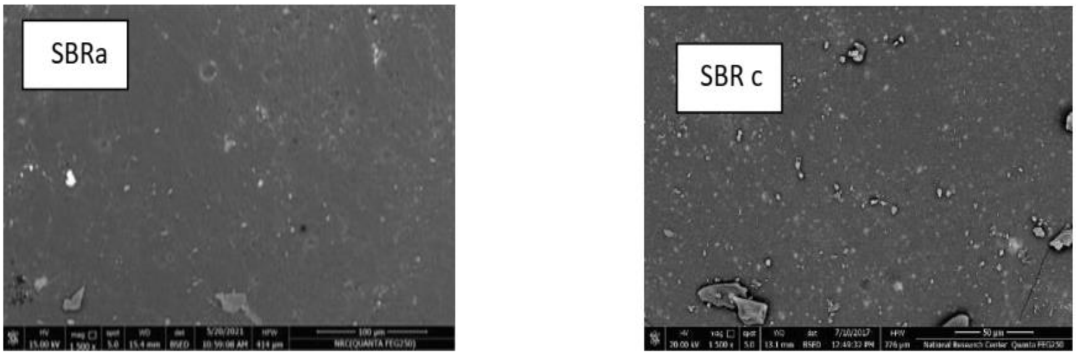


Fig. 4. SEM micrographs for SBR in absence and presence of nanocurcum (S0, SC4)

3.6. Cytotoxic activity and antimicrobial testing of nanocurcum rubber vulcanizates:

3.6.1. Cytotoxic activity test

The cytotoxic activity of the prepared rubber vulcanizates samples loaded with nanocurcum towards the human normal cell line: RPE1 [normal retina cell line] was studied and illustrated in table (11). It was found that they are safe and has no cytotoxic effect.

Table (11): Cytotoxic activity of different rubber vulcanizates towards human normal retina cell line [RPE1]

Sample Name	IC_{50} (µg/ml)
NR	N.A.
SBR	N.A.
EPDM	N.A.
NR/ C	N.A.
SBR/ C	N.A.
EPDM / C	N.A.
DMSO	N.A.
Negative control	N.A.

Where C: nanocurcum

N.A.: No activity

3.6.2. Antimicrobial activity results

The results of tested Rubber vulcanizates loaded with nanocurcum towards the tested Gram-positive & Gram-negative bacteria and fungi are presented in Table (15). The experiment was carried out in triplicate and the average zone of inhibition was calculated.

Results in Table (12) and figures 5,6 and 7 showed that the investigated rubber vulcanizates gave promising results towards Gram +ve and Gram -ve bacteria, while they showed no effect on tested fungi. Data in figure 5 represent the good effect of all tested rubbers (natural and synthetic) towards the Gram positive bacteria *Staphylococcus aureus.* Moreover, figure 6 indicated the moderate activity of all tested rubbers (natural and synthetic) towards the Gram negative bacteria *Escherichia coli.* On the other hand, figure 7 showed that all tested rubbers (natural and synthetic) had no activity towards *Candida albicans* fungi. These results are in accordance with other works [23- 24].

Table (12): Antimicrobial activity expressed as inhibition zone diameter in millimeters (mm) of different rubber vulcanizates towards the pathological strains based on diffusion assay.

Pathogen type			
Name	*Gram-positive bacteria*	*Gram-negative bacteria*	*Fungi*
Tested agent	*Staphylococcus Aureus* ATCC 29213	*Escherichia coli* O157:H7	*Candida albicans*

NR/C	12	14	N.A.
EPDM/C	16	14	N.A.
SBR/C	15	14	N.A.
Vancomycin	20	29	22
Cefodizime	18	26	25

Where C: nanocurcum
N.A.: No activity

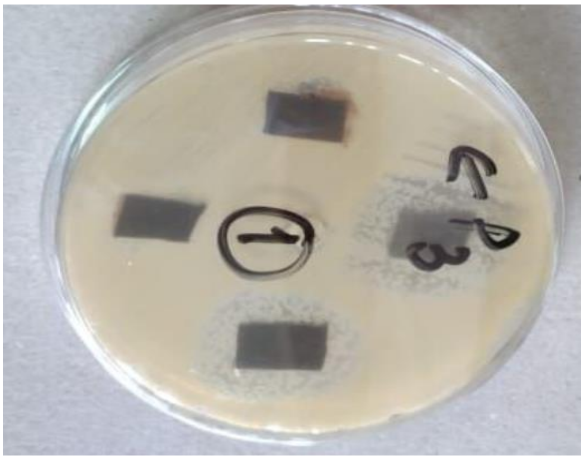


Fig. 5. Antimicrobial activity of nanocurcum/rubber vulcanizates towards *Staphylococcus aureus*

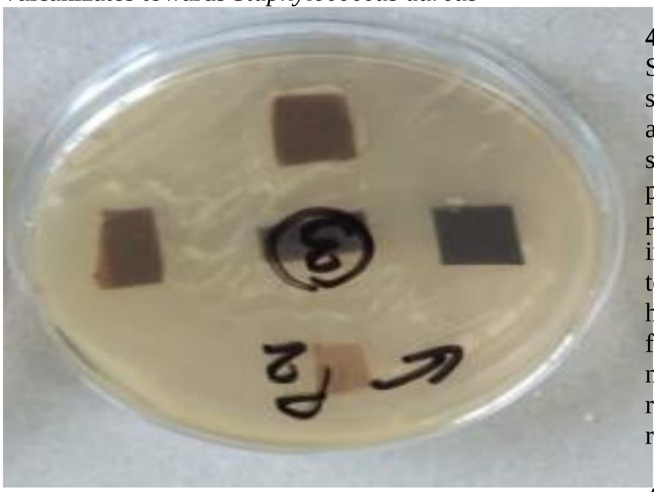


Fig. 6. Antimicrobial activity of nanocurcum/rubber vulcanizates towards *Escherichia coli*

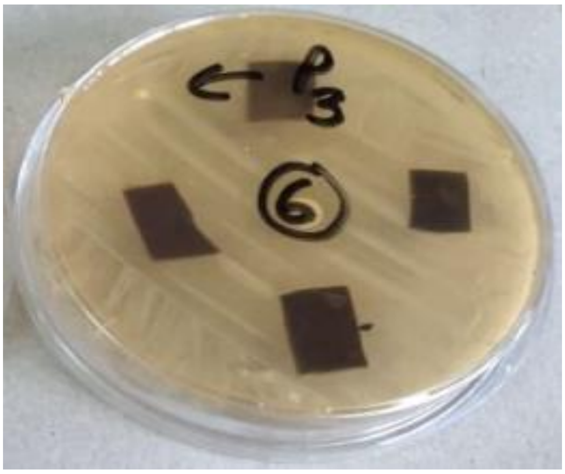


Fig. 7. Antimicrobial activity of nanocurcum/rubber vulcanizates towards *Candida albicans*

4. Conclusion

Safety rubber (natural or synthetic) vulcanizates were successfully prepared by incorporation of nanocurcum as a natural antimicrobial agent. Nanocurcum has no significant effect on rheological, mechanical properties and swelling properties (in toluene) of the prepared rubber vulcanizates. The cytotoxicity of the investigated rubber vulcanizates gave negative results to normal human retina cell line [RPE1]. On the other hand, promising antibacterial activity was remarked for the tested rubber vulcanizates containing nanocurcum. As a result, nanocurcum can b recommended as antibacterial agent for producing rubber products safe to human as rubber toys.

Acknowledgements
The present work is supported through the National Research Centre project No. 12010601.

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