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Green Antimicrobial Natural Rubber for Personal Protection Products

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Abstract

The objective of the present work is to prepare and evaluate green flexible antimicrobial natural rubber vulcanizates for application and manufacturing of personal flexible protection products as; swimming suits and diving allowance…etc. Natural rubber and their specific additives as well as some plant extracts Curcumin, Rosemary as natural plant of high bioactive phenolic compounds, and conventional pharmaceutical drug as Thiabendazole, were mixed through the ordinary rubber mixer. The rheological characteristics were estimated to determine the curing time. The compounded rubber was vulcanized at 142oC at curing time estimated from the rheological characteristics. The evaluation of the prepared vulcanizates was carried out, through identification with Fourier transform infrared spectroscopy (FTIR) . Specific surface area measurements and measuring the mechanical, swelling, cytotoxicity and antimicrobial properties. It was observed that the physico mechanical and chemical properties of all investigated vulcanizates are good before and after exposure to thermal oxidative aging at 90oC for 6 days. The cytotoxicity of the investigated rubber vulcanizates gave negative results towards normal human fibroblast cell line (BJ1) that confirms their safety. Moreover, antimicrobial activity results indicated that the release of different antimicrobial agents loaded on natural rubber vulcanizates inhibited the growth of different types of bacteria or fungi on the surface of rubber.

Key words: Natural Rubber, safety rubber products, Curcumin, Rosemary, Thiabendazole.

1. Introduction

Natural rubber (NR) is derived from the rubber tree Hevea braziliensis. It is made of isoprene units (C_5H_8) that are joined to form polymer chains, or cis-1,4-polyisoprene, together with traces of other organic impurities including biolipids and proteins. Gloves, rubber bands, threads, and other products employ NR extensively because of its extraordinarily high elasticity, strong strength, and remarkable resistance to many corrosive agents. The enormous number of double bonds in isoprene units. However, the abundance of double bonds in isoprene units makes NR highly susceptible to degradation by heat, oxygen, and ozone [1-3].

The rubber alone generally does not exhibit proper dimensional stability without additives. Some fillers and oils can be incorporated to rubber formulation in addition to the antimicrobial agents in order to provide flexural modulus for rubber vulcanizates [4-5].

The rheological, physico-chemical, mechanical, antimicrobial properties are very important purpose for evaluation rubber products [6].

Microbial growth in natural rubber is significant problems that reduces the material's quality, safety, and characteristics. Addition antimicrobial agent into natural rubber is an effective method to improve antimicrobial properties of rubber products. Consequently, natural rubber undergoes crosslinking and interaction with reactive antimicrobial agents to enhance its mechanical and antibacterial qualities. Natural rubber had antibacterial qualities can be used in medical applications and stop the growth of microorganisms during storage. Therefore, medical materials that have been combined with NR can be used in a variety of industrial items to eradicate bacteria and fungi [7-9].

Improving health care quality at individual and population levels requires identifying structures and processes that affect public outcomes, keeping the structures and processes that facilitate desired outcomes and altering or eradicating that inhibit desired outcomes [10-11].

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Essential oils are natural compounds extracted from plants, and have antimicrobial and antioxidant properties. Rosemary essential oil is one of materials that can mixed into an edible coating. Recently, Due to the presence of 1,8-cineole and camphor chemicals, which have antibacterial and antifungal qualities, in rosemary extracts and essential oils demonstrated that rosemary essential oil can provide a considerable growth inhibition of some bacterial strains such as *Lactobacillus acidophilus*, *Listeria monocytogenes*, *Escherichia coli*, *Pseudomonas fluorescens*, *Photobacterium phosphoreum* and *Shewanella putrefaciens*.

Curcumin is a phytochemical compound used as antioxidant, anti-inflammatory, and anticarcinogenic activities. Curcumin is obtained from the rhizome of the plant Curcuma longa; its color is golden yellow. It can be used for therapeutic applications according to their specific properties. Curcumin had also biological activity for various human diseases including cancer in the preclinical setting [12-14]. Thiabendazole (TBZ) is an interesting pesticide material and has fungicide action. So it can be used as conventional pharmaceutical drugs it [15].

The aim of the work is to prepare NR vulcanizates contained the investigated active agents like plant extracts as Curcumin , Rosemary and conventional pharmaceutical drugs as Thiabendazole, for application to manufacturing safety personal flexible protection products such as: swimming goggles frames, swimming and diving suits.

Also, evaluate their mechanical, chemical, cytotoxicity and antimicrobial properties.

2. Materials

- Natural rubber (NR), of the type SMR-20, density = 0.913, Mooney viscosity ML (1 + 4) at 100^{o}C = 60-90, and glass transition temperature Tg =-75oC, kindly obtained from Transport and Engineering, Company (TRENCO), Alexandria.
- N-cyclohexyl-2-benzothiazole sulphenamide (CBS), a light gray powder with a melting point of 95–100 ^{o}C and a specific gravity of 1.27–1.31 at room temperature (25 ^{o}C±1) provided from Sigma Aldrich.
- stearic acid and Zinc oxide as activators, with specific gravities of 5.55–5.61 and 0.90-0.97 at 15 °C, respectively provided from Sigma Aldrich.
- The elemental sulfur as vulcanizing agent that was applied as a fine, pale yellow powder with a specific gravity of 2.04–2.06 at ambient temperature purchased from Sigma Aldrich.
- Trimethyl-1,2dihydroquinoline (TMQ) polymerization that was employed as an antioxidant provided from Sigma Aldrich.
- Naphthenic oil, with specific gravity at 15^{O}C = 0 .94–0.96, viscosity at 100^{O}C= 80–90 poise and deep green viscous oil was supplied by Aldrich Company, Germany.
- Silica as reinforcing filler with specific gravity 1.95, pH 6.2 + 0.8, contained 82% precipitated silicon dioxide supplied from the Degussa, Germany.

2.1. Antimicrobial Agents

Pharmaceutical drugs or antibiotics as Thiabendazole is purchased from sigma Aldrich. Plant antimicrobial natural products Curcuma longa and Rosmarinus officinalis were purchased from local market and recognized by Prof. Dr. Sameh R. Hussein, the voucher specimens were placed at the National Research Center Cairo, Egypt's herbarium.

The Figure 1 indicates the chemical formula of Curcumin with $C_{21}H_{20}O_6$, Rosmarinic acid ,it is an ester of caffeic acid that occur in Rosmary as phenolic compounds. Its molecular formula is $C_{18}H_{16}O_8$ and Thiabendazole with molecular formula is $C_{10}H_7N_3S$.

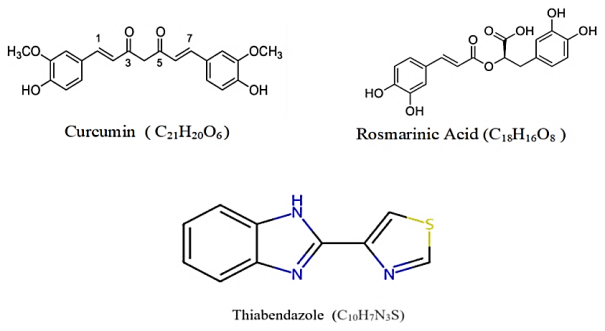


FIGURE 1 : Structural formulas of Curcumin, Rosmarinic acid and Thiabendazole

2.2. **Methods**

2.2.1. Preparation Of Plant Extracts

The plants under study Curcumin, Rosemary (250 g per plant) were collected from market at Mai 2024, The plants were defatted by using highly nonpolar solvents; *n*-hexane, ethyl acetate and finally with polar solvents ethanol (500 ml of each solvent)**.** The extraction process by using Soxhlet device, then each extract was separated by filtration using Whatman paper, and the solvents were evaporated using a rotary evaporator at 60 degrees Celsius. . The dried plant extracts (3.5, 9.64,13.5 gm) respectively, were later stored in a freezer at -2 to -8ºC

2.2.2. Quantitation of curcumin and rosemary from extract by using HPLC– DAD :

The plants were prepared with 100% methanol and quantitated with an HPLC system (Agilent 1100 series, Germany) equipped with a Zorbax Eclipse C_{18} column. The mobile phase consisted of water (A) with 1% formic acid and acetonitrile (B); the flow rete 1 ml/ min. The injection volume was 6 µl, detector used was UV at wave-length λ_{max} 424 nm.

2.2.3. Preparation of Rubber Samples:

Rubber, their additives and the investigated antimicrobial agents will be 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. The rubber was mixed with ingredients accordance with ASTM: D3182-07.

2.2.4. ulcanization Process

The compounded rubbers left overnight before vulcanization. All compounds will be compressed and molded using a hydraulic press (Mackey Bowley, C1136199) according to their respective cure time (e.g. Tc_{90}) that determined from rheological data obtained by Monsanto Rheometer (Tech-Pro, Cuyahoga Falls, OH, USA).

2.3. Characterization of Rubber Products:

2.3.1. Fourier transform infrared spectroscopy (FTIR) technique :

Infrared spectra of rubber vulcanizates with antimicrobial agen ts obtained with Jascow FTIR- 430 series infrared spectrophotometer equipped with KBr discs.

2.3.2. Mechanical properties

Tensile properties will be tested with a Zwick tensile testing machine (Z010) according to ASTM D 412. 500 mm/min is the crosshead speed of the machine. The Dumbbell shape specimens were cut from the molded sheets by Wallace die cutter with 1-mm thickness.

All the tests were performed at room temperature.

2.3.3. Swelling Test

The swelling test were carried out according to the standard test ASTM D471. The test samples of 1 mm thickness were weighed and submersed in toluene at room temperature. The samples were removed after 24h, wiped with tissue paper to remove excess toluene, and weighed with an analytical balance and the percentage weight change was determined until the equilibrium value reached.

2.3.4. Specific Surface Area Measurements (BET)

The most extensively used method for figuring out a solid or powder's surface area is BET-specific surface area. Natural rubber (NR) samples of dimension (1× 1× 1.5 mm) are first prepared by drying, with a flow of inert gas or vacuum atmosphere, to clear the surface from any contaminants. After that, the samples are heated to cryogenic temperatures to enable a probe gas to physically adhere to the sample's surface. The amount of gas needed to cover the sample's surface is calculated by measuring the volume of probe gas adsorbed. Next, the adsorption data are subjected to the Brunauer, Emmett, and Teller (BET) theory, which yields a specific surface area expressed in units of area per mass of sample (m2/g). Using a TriStar II 3020 Micrometrics analyzer, the BET analysis was performed on the prepared samples to evaluate their specific surface area, pore size distribution, and specific pore volume.

2.3.5. Rate of Release of Antimicrobial Substances Curcumin,Rosemary,Thiabendazole:

Cubic discs shaped or formulated from the prepare vulcanized rubber contained some prepared plant ex-tracts as antimicrobial agents such as Curcumin and Rosemary in addition to conventional pharmaceutical drugs as Thiabendazole.

The weight of the disc is about 0.5 g and its dimensions are 10 mm on the side and 1 mm on the thickness. The prepared discs were immersed in Petri dishes containing microbial culture media or normal human cells in incubators at 37°C.

2.4. Investigation of the Quality and Efficiency of the Prepared Antimicrobial Rubber Products:

2.4.1. Thermal oxidative aging

Accelerated aging was done in an electric oven at 90 ^{O}C for 6 days.

2.4.2. Bioassay Tests for the antimicrobial rubber products:

The activity of antimicrobial rubber formulations on normal human skin cell lines and different pathogenic microbes will be investigated to evaluate the safety and efficacy of these formulations for manufacturing of antimicrobial personal protection products [16].

2.4.2.1. Cytotoxicity activity assay

The newly prepared rubber vulcanizates containing antiviral and antimicrobial agents will be tested towards the normal human skin cell line: BJ1 Fibroblast using MTT assay [17].

2.4.2.2. Antimicrobial Activity

The Assessment of Antimicrobial Finished Products

The International Standard (ISO) 20743 method [18]: Determination of Antimicrobial Activity of Antimicrobial Finished Products", is designed to test the ability of final products that, over the course of 18 to 24 hours, have been treated with antimicrobial chemicals to stop the growth of germs and kill them. Three different quantitative test kinds are included in the official standard method: the "Absorption Method," "Printing Method," and "Transfer Method. According to the "Transfer Method," an agar plate's surface is inoculated, a test sample piece is weighed down on the inoculated agar for 60 seconds, and the sample is then incubated in humid circumstances for 18 to 24 hours to determine the final concentration of viable bacteria. Membrane-filtered bacteria are printed onto the sample using the "Printing Method" before it is incubated in humid conditions for one to four hours. This approach was adopted because there was a considerable demand for an ISO method to test the antibacterial activity of various final products, such as rubber and textiles. It is based on the Japanese Industrial Standard (JIS) method.

3. Results and Discussion

3.1. Characterization of plant extracts

From the HPLC analysis of ethanolic extract of Curcuma and Rosemary, the major compounds including curcumin (1.78%), were identified and quantified, along with two other curcumin derivatives. In Figure 2 HPLC chromatographic analysis of rosemary leaf ethanolic extract indicated that two main active ingredients are: carnosic and rosmarinic acids. In Figure 3

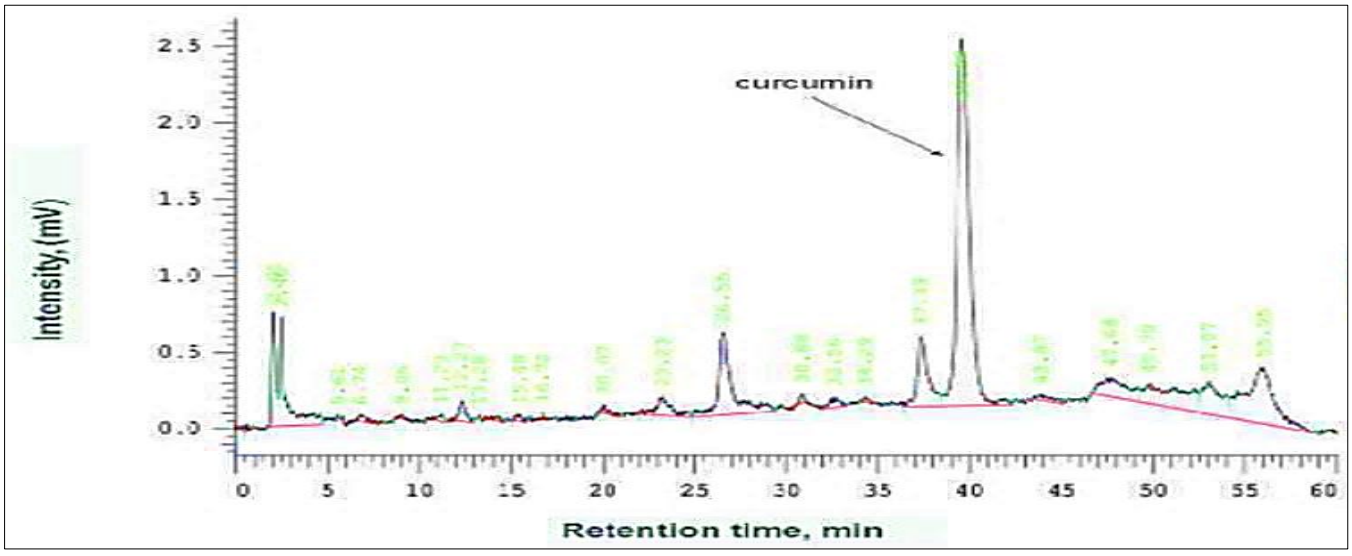


FIGURE 2: HPLC analysis of ethanolic extract of curcumin

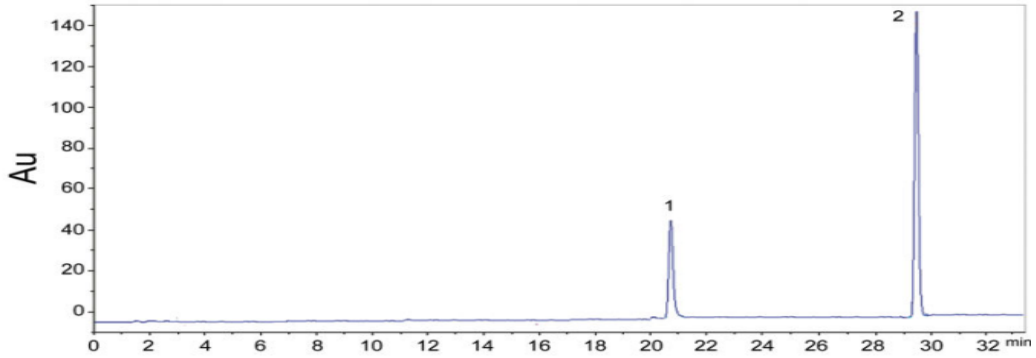


FIGURE 3: HPLC analysis of ethanolic extract of Rosemary

3.2. **Preparation of the investigated NR vulcanizates**

The formulations of NR vulcanizates under investigation are illustrated in Table 1

Table 1: The formulations of NR loaded with various concentrations of different antimicrobial agents

Sample keys / Ingredients, phr[a]	N0	Cur10	Cur20	Cur30	Ros10	Ros20	Ros30	TBZ	TBZ 7	TBZ 10
NR	100	100	100	100	100	100	100	100	100	100
Stearic acid	2	2	2	2	2	2	2	2	2	2
ZnO	5	5	5	٥	5	5	5	5	5	5
S	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
CBS[b]	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8
TMQ[c]	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Naphthenic oil	2	2	2	2	2	2	2	2	2	2
Silica	30	30	30	30	30	30	30	30	30	30
Cur	----	10	20	30	---	----	-----	----	----	-----
Ros	----	----	----	----	10	20	30	----	----	----
TBZ	----	----	----	----	----	----	----	5	7	10

Notes: a-Part per hundred parts of rubber, b-N-cyclohexyl-2-benzothiazole sulfonamide, c- Polymerized 2,2,4-trimethyl-1,2dihydroquinoline, Curcuma (Cur), rosemary (Ros) and Thiabendazole (TBZ).

3.3. Characterization of rubber products:

3.3.1. Fourier Transform Infrared Spectroscopy (FTIR) Technique:

The FTIR spectrum of rubber vulcanizates loaded with different antimicrobial agents. Figure 4 Basically, pure NR shows characteristic peak at 842 cm^{-1} represented =CH out of plane bending. Peak at 1375 cm^{-1} and 1432 cm^{-1} are characteristic of CH_2 deformation and at the region 2852- 2925 cm^{-1} represented the CH_2 symmetric stretching vibrations. There is no additional peak in the blends. The peaks at 2916 and 2845 cm^{-1} corresponding to C-H.

Curcumin's distinctive phenolic OH group is responsible for the stretching band that was visible in its FTIR spectra at 3392 cm^{-1}. Other stretching vibrations caused by C=O and C=C were also noticeable at 1712 cm^{-1} and 1510 cm^{-1}, respectively. The vibration bending of the O-H and C-H methylene groups was detected at 3492 cm^{-1} and 2944 cm^{-1}, respectively, in the FTIR spectrum of rosemary oil. The characteristic peak at 1720 cm^{-1} is attributed to the C=O carbonyl group.

The FTIR spectrum of Thiabendazole with NR was similar and there is no chemical bonds between NR and Thiabendazole . The contraction vibration absorption peak of C═C was at 1691 cm^{-1}. However, the characteristic absorption peak of Thiabendazole was not appeared because of Thiabendazole molecules holding and keeping between NR cavities formed due to cross linking through the rubber chains. So, FTIR analysis confirmed the investigated antimicrobial agents inside NR matrix. They are physically bonded with NR.

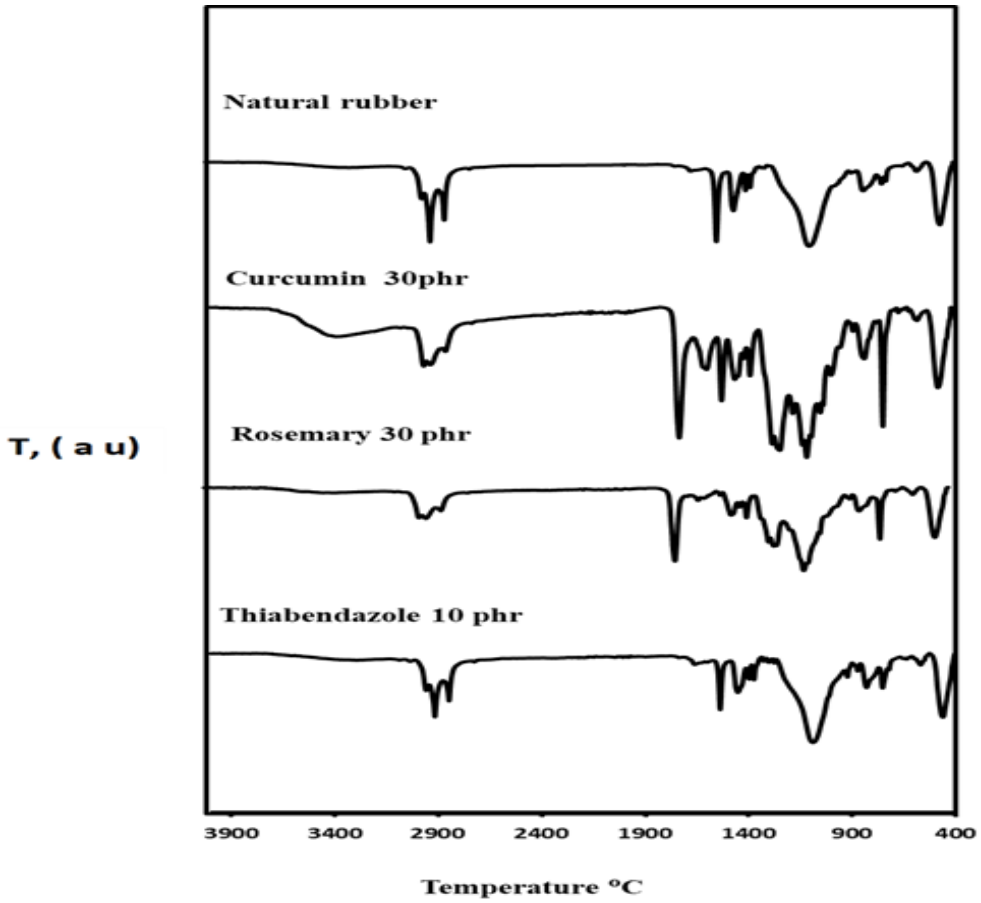


FIGURE 4: The FTIR spectrum of NR and NR loaded with 30 phr Curcumin, 30 phr Rosmary and10 phr Thiabendazole

3.3.2. ***Rheometric Characteristics***

The rheometric characteristics of the examined NR vulcanizates are illustrated in Table 2.

A moving die rheometer was used to measure the compounded NR rheological characteristics at 142°C.The findings are listed in Table 2. The rheological characteristics of NR were determined to be good. The rheological data that was gathered was used to determine the cure time. In the case of NR-contained plant extract (Cur & Ros), the minimum and maximum torque reduced according to their concentrations; nevertheless, the addition of TBZ, a standard pharmaceutical medicine, enhanced the minimum torque. NR, ML and MH values are higher than those of NR combined with Cur and Ros, suggesting a minor decline in the cure state; however, NR incorporated with TBZ has higher MH values [21]. The rate of cure for NR combined with TBZ, Cur, and Ros increases. Additionally, overall studies showed a reduction in optimal cure time (tC90) and Scorch time (tS2), resulting in good acceptable rheological properties [22]. It indicates improved curing efficacy and processability, which produced high-quality products [23].

The presence of fillers that aid in the curing reactions activation causes the Cure Rate Index (CRI) to rise [24]. The vulcanization of the rubber formulations occurred at the expected optimal cure time. Therefore, the antibacterial characteristics of the compounded natural rubber are improved by the use of plant extracts and traditional pharmaceutical medications, which have no adverse effects.

Table 2: Rheometric characteristics of NR loaded with various concentrations of ethanol extract of plants and conventional pharmaceutical drugs as antimicrobial agents

Sample keys / Rheological properties	N0	Cur 10	Cur 20	Cur 30	Ros 10	Ros 20	Ros 30	TBZ 5	TBZ 7	TBZ 10
Minimum torque (M_L), dN_m	0.28	0.23	0.14	0.14	0.22	0.04	0.06	0.32	0.37	0.37
Maximum torque (M_H), dNm	6.80	3.49	2.17	1.06	5.84	4.49	5.11	11.52	11.37	11.21
Scorch time (t_{S2}), min	28.81	24.19	23.59	12.39	20.13	15.37	13.81	7.06	6.95	6.13
Optimum Cure Time (t_{C90}), min	41.90	38.60	33.28	18.39	29.55	24.83	24.79	20.95	17.42	15.57
Cure rate index (CRI)	7.63	6.96	10.32	16.67	10.62	10.80	9.11	7.20	9.55	10.59

3.3.3. The physicomechanical properties

3.3.3.1. Mechanical properties

The examined tested of mechanical characteristics for the investigated vulcanizates are demonstrated in Table 3.

Table 3: Mechanical properties of NR vulcanizates loaded with various concentrations of different antimicrobial agents

Sample keys	Tensile Strength (MPa)	Elongation at break (%)	Modulus, 50% (MPa)	Modulus, 100% (MPa)	Modulus, 200% (MPa)	Modulus, 300% (MPa)	Modulus, 500% (MPa)
N0	6.42	1077	0.29	0.45	0.74	1.03	1.67
Cur10	5.76	1168	0.40	0.52	0.70	0.92	1.38
Cur 20	4.66	1110	0.29	0.40	0.59	0.86	1.20
Cur 30	3.19	990	0.27	0.40	0.48	0.78	1.14
Ros 10	5.59	1607	0.55	0.71	0.91	1.10	1.51
Ros 20	7.23	1684	0.66	0.79	1.02	1.24	1.71
Ros 30	8.63	1691	0.78	0.92	1.18	1.43	2.03
TBZ 5	11.88	1219	0.41	0.61	0.97	1.32	2.04
TBZ 7	13.94	1311	0.49	0.73	1.21	1.72	2.71
TBZ 10	14.66	1800	0.55	0.75	1.05	1.34	1.95

All the results show that, the tested NR vulcanizates had good mechanical properties. It was shown that, the mechanical properties were improved; tensile strength and elongation at break; were raised in presence of plant extracts and conventional pharmaceutical drugs; and there is no great difference in the change of modulus at different elongation (50 – 500%) compared the control one [25]. So, the investigated antimicrobial agents can help to improve the mechanical properties because they have good dispersion and consequently gave good homogeneity in the rubber vulcanizates [26]. There for there is no effect the strong colour and odours of Curcuminand Rosmary.

3.3.3.2. Chemical properties

Equilibrium swelling

The equilibrium swelling properties are demonstrated in Figure 5.

The equilibrium swelling of the investigated NR vulcanizates containing the antimicrobial drugs are illustrated in Figure 5. It was shown that, all NR vulcanizates had good equilibrium swelling. The swelling percentage decreased at low concentrations of Cur (10, 20 phr) , and (10, 20 and 30 phr) of Ros and also in existence of TBZ. The reduction in the swelling percentage was due to the presence of (Cur, Ros and TBZ) which prevented the penetration of toluene in the prepared samples. So, the swelling percentage reduced and the cross linking density increased and the properties of rubber products improved [27].

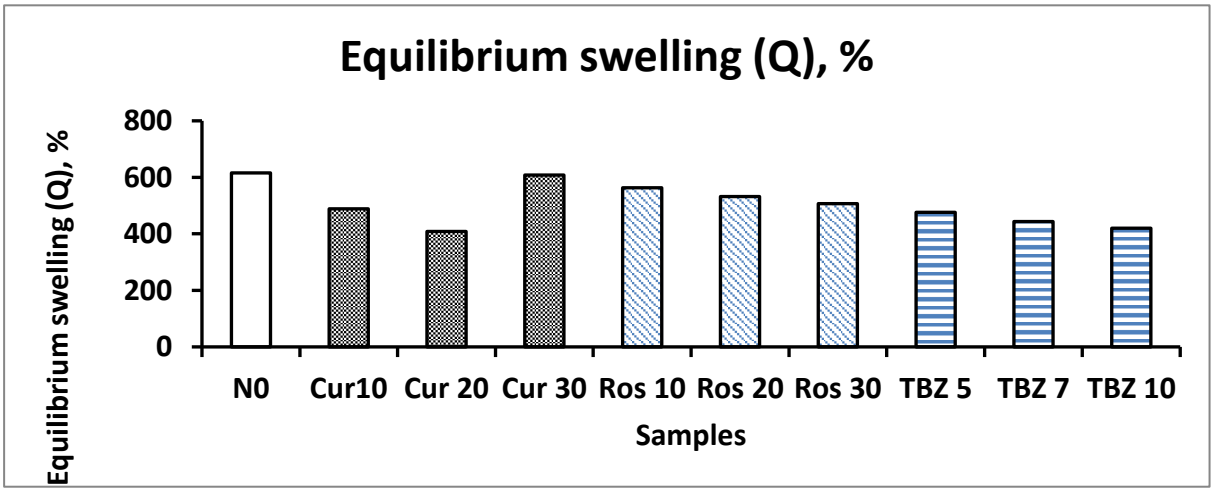
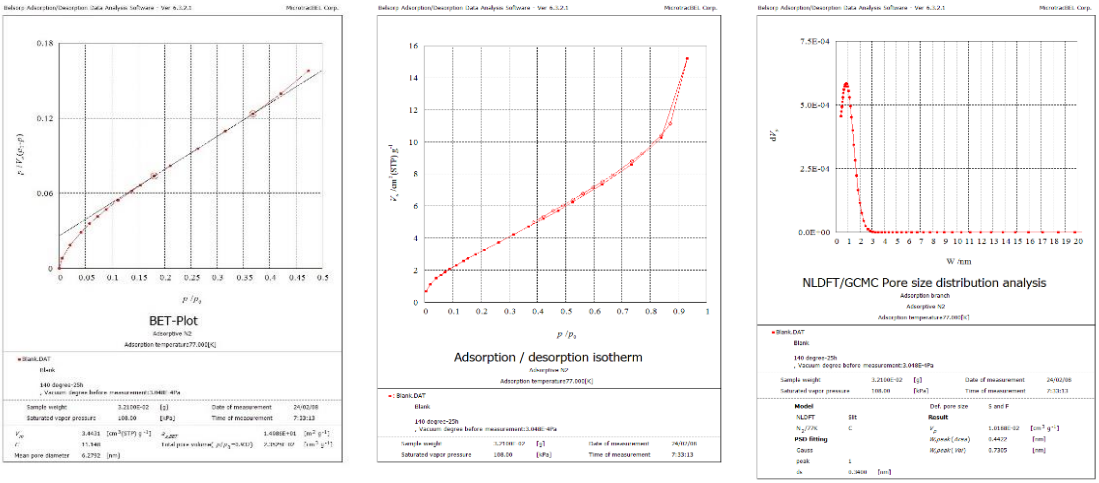


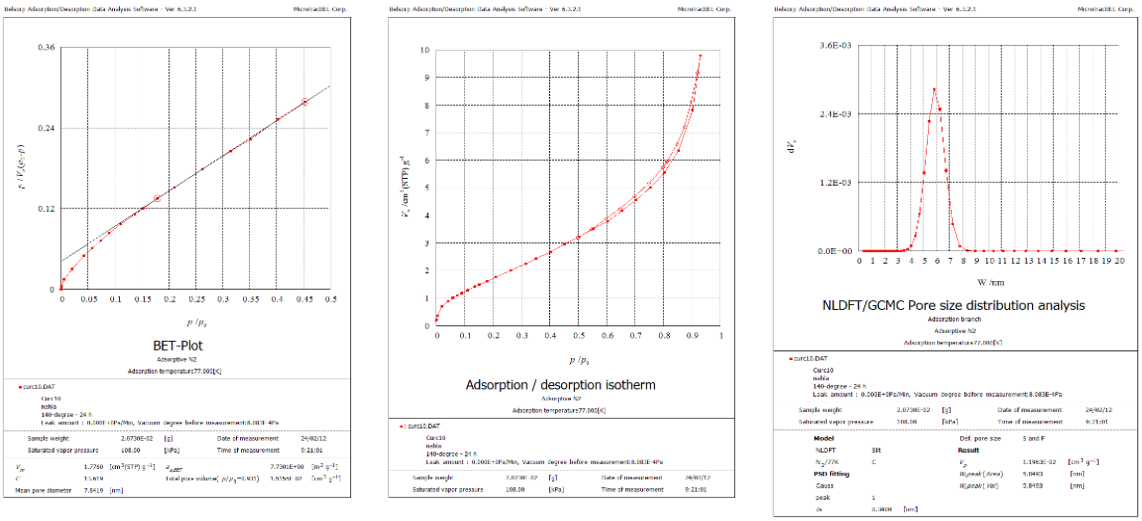
FIGURE 5: The swelling data results of NR vulcanizates loaded with various concentrations of different antimi-crobial agents

3.3.3.3. Surface area measurement (BET)

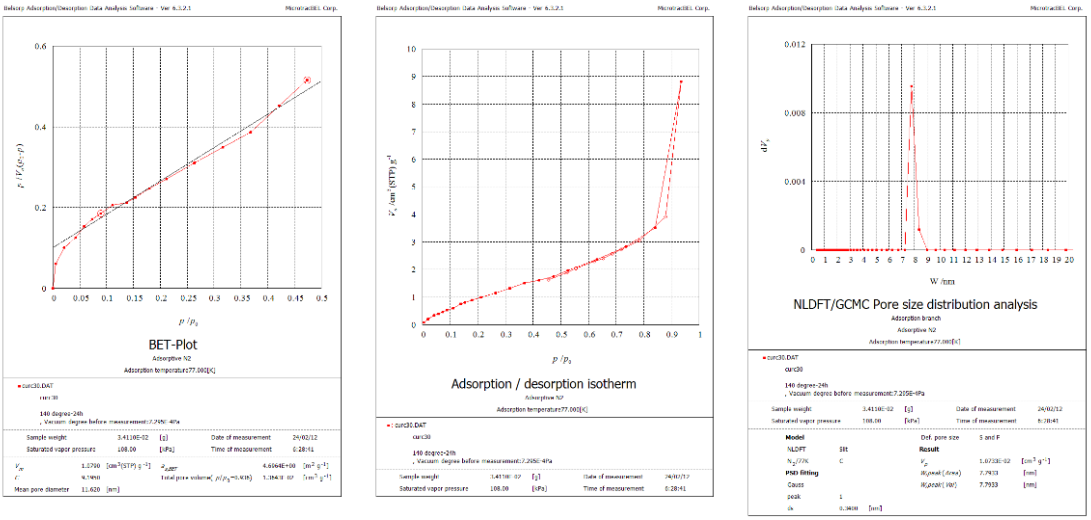
Results of surface area measurement (BET) are illustrated in Figures 6-8



NR blank sample

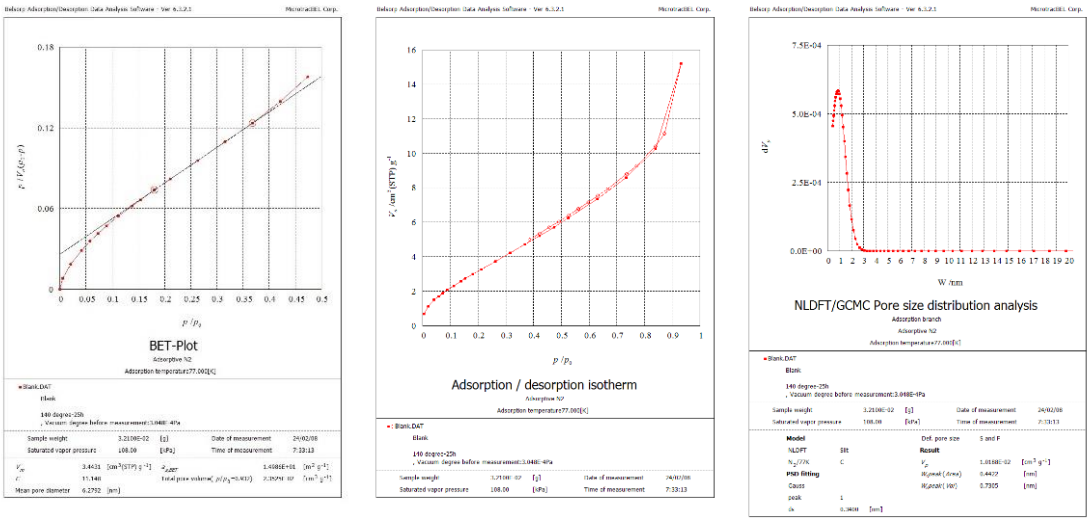


NR containing Curcumin 10 phr

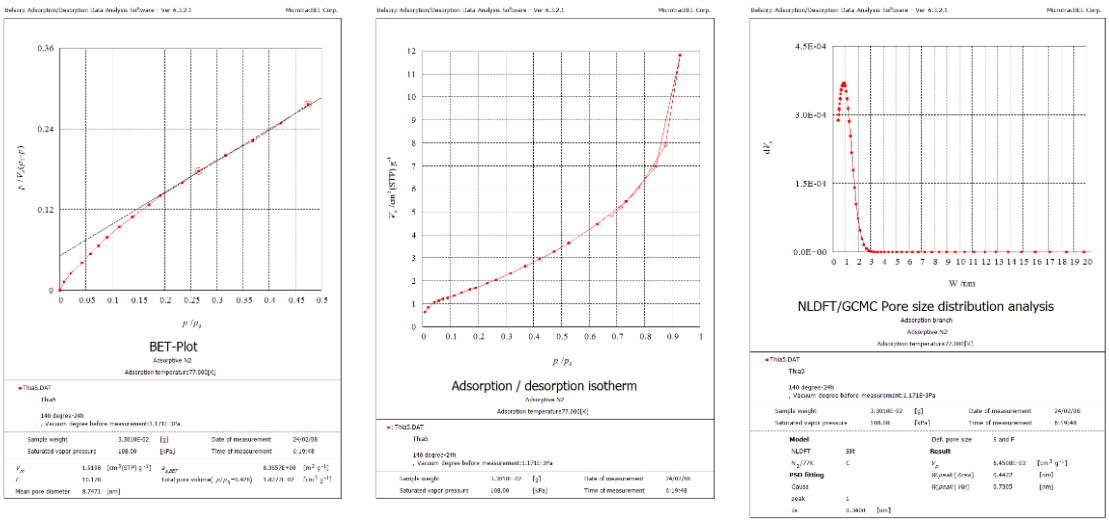


NR containing Curcumin 30 phr

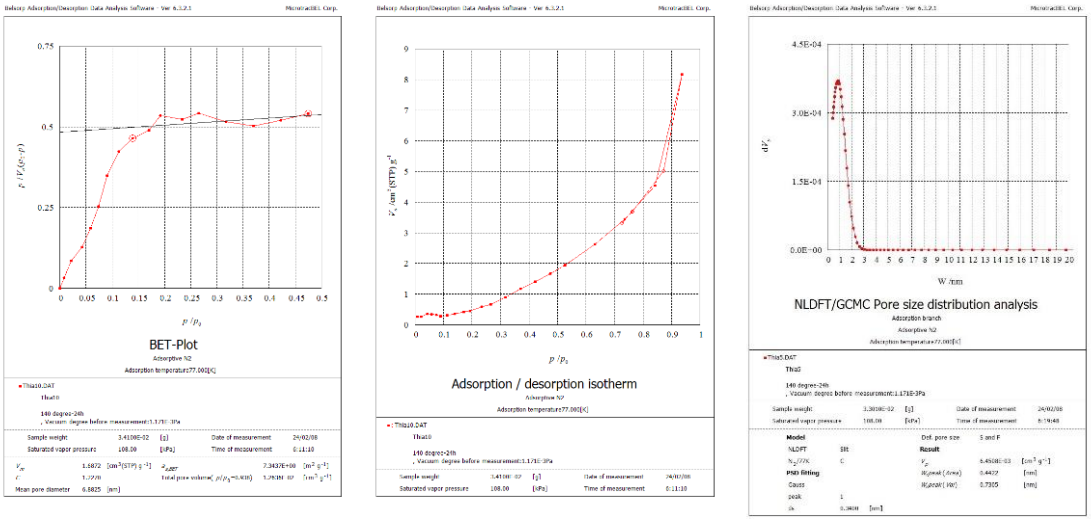
FIGURE 6: Analysis of specific surface area and pore structure of NR vulcanizate (blank sample) and NR vulcanizates loaded with Curcumin (BET-Plot, adsorption/ desorption isotherm , pore size distribution)



NR blank sample

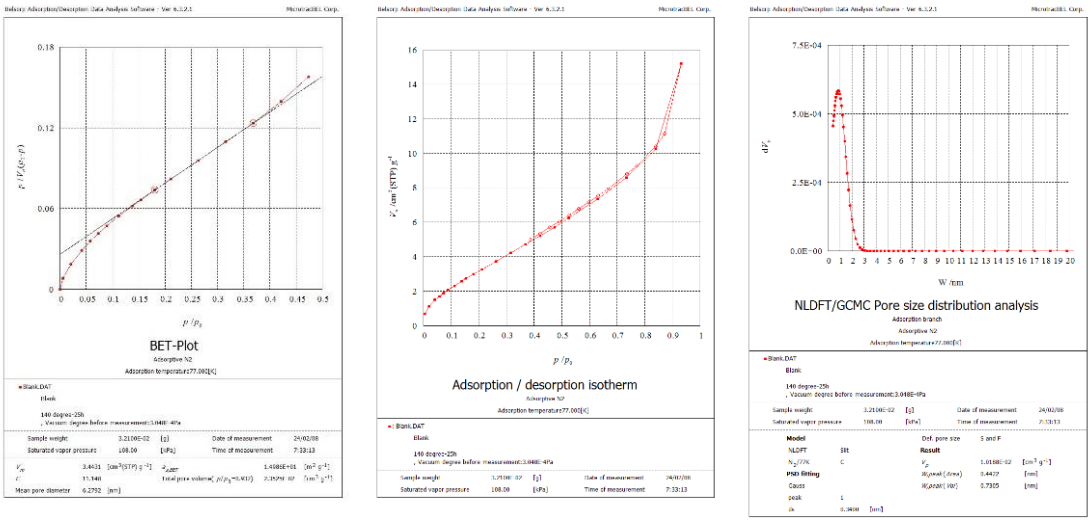


NR containing TBZa 5 phr

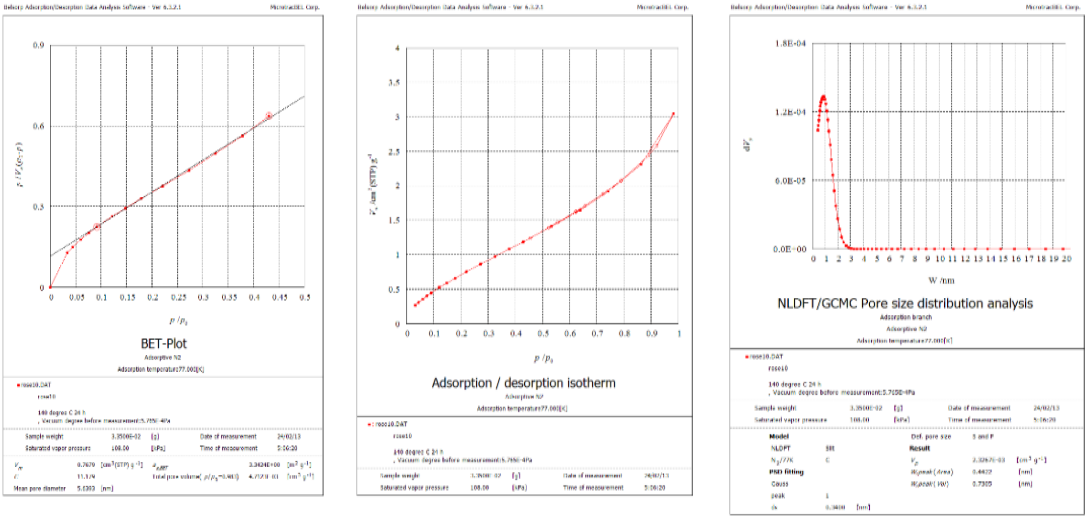


NR containing TBZa 10 phr

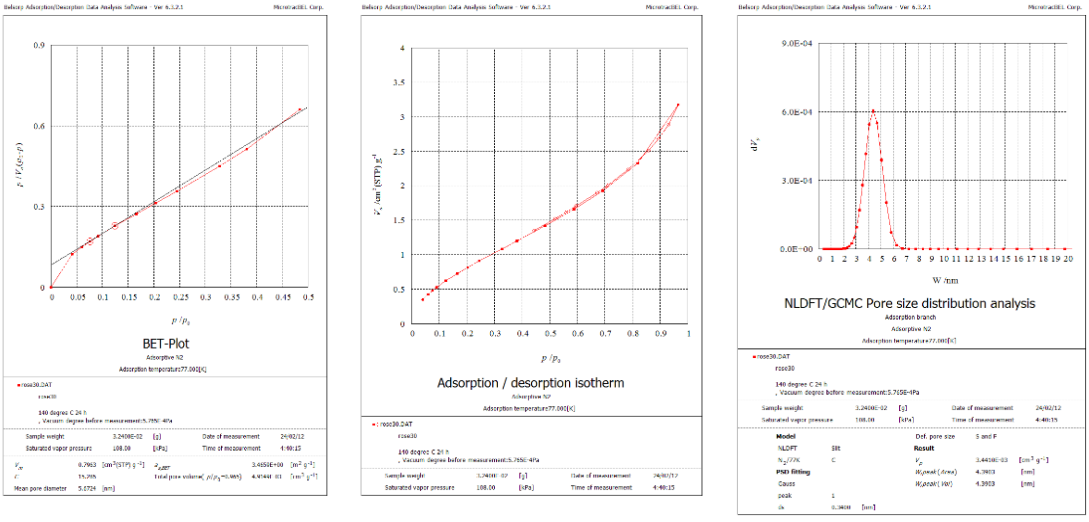
FIGURE 7: Analysis of specific surface area and pore structure of NR vulcanizate (blank sample) and NR vul-canizates loaded with TBZabendazole (BET-Plot, adsorption/ desorption isotherm , pore size distribution).



NR blank sample



NR containing Rosemary 10 phr



NR containing Rosemary 30 phr

FIGURE 8: Analysis of specific surface area and pore structure of NR vulcanizate (blank sample) and NR vulcanizates loaded with Rosemary (BET-Plot, adsorption/ desorption isotherm , pore size distribution)

The specific surface area and the total pore volumes of the investigated rubber vulcanizate are presented in Table 4. From Table 4, it is obvious that the specific surface area (a_s, $m^2.g^{-1}$) of each sample of the prepared NR vulcanizates decreases by integration and increasing the concentration of Curcumin, rosemary and TBZ into the NR rubber vulcanizates. These results suggest that all investigated antimicrobial agents have been successfully physically loaded to NR matrix. However, among all antimicrobial agents investigated, rosemary possesses the highest effect on increasing the pore volume between crosslinks in the NR vulcanizates (49.149×10^{-2}), and consequently decreasing the specific surface of the pores ($3.64\ m^2.g^{-1}$) in the NR vulcanizates [28].

Table 4 Specific surface area of the NR vulcanizates loaded with various concentrations of plant extracts as antimicrobial agents determined by BET

Sample designation	Total pore volume ($cm^3.g^{-1}$) × 10^{-2}	a_s, BET($m^2.g^{-1}$)
Blank	2.325	14.986
Cur10	1.5155	7.7301
Cur 30	1.3643	4.6964
Ros 10	47.1234	3.3424
Ros 30	49.149	3.4659
TBZ 5	1.8272	8.3557
TBZ 10	1.2636	7.3437

3.4. Evaluation of the quality and efficiency of the prepared antimicrobial rubber products:

3.4.1. Thermal oxidative aging

The resistance of the rubber vulcanizates to the thermal oxidative aging is a prerequisite for better product service performance. To evaluate the ability of adding plant extracts and conventional pharmaceutical drugs as antimicrobial agents on the NR vulcanizates to tolerate retard the aging process of NR composites, tensile properties of the prepared composites after exposure to aging were determined. It was observed from Figure 9 that, all investigated NR vulcanizates containing bioactive materials; (Cur, Ros and TBZ) exhibited a higher resistance to thermal oxidative aging i.e. they have a good properties before and after aging at 90 °C for 6 days. There is no great differencebetween the values of tensile strength and elongation at break comparing to the control one. These results might be due to the nature of the active materials that provided long term protection by inhibiting radicals and retarding the aging [29-30].

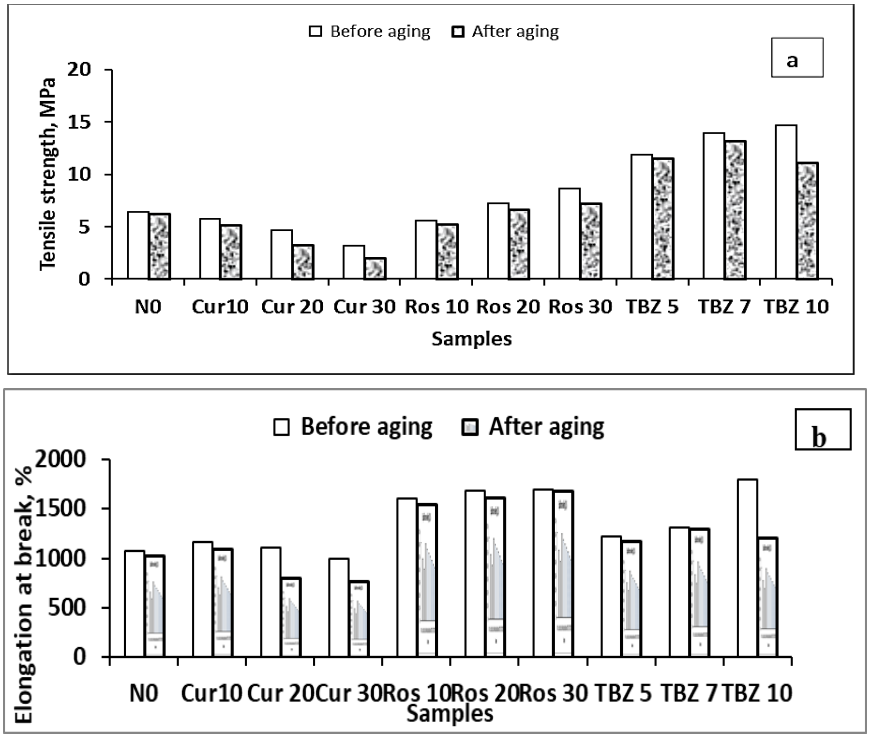


FIGURE 9: The Physico-mechanical properties of NR vulcanizates loaded with various concentrations of differ-ent antimicrobial agents before and after 6 days aging at 90 °C. a-Tensile strength, b- Elongation at break

3.4.2. Bioassay Tests for the antimicrobial rubber products

3.4.2.1. Cytotoxic effect

The cytotoxic effect of the investigated rubber vulcanizates loaded with different antimicrobial agents was tested towards the normal human skin cell line: **BJ1 Fibroblast** and illustrated in Table 5 and Figures 10 - 14.

Table 5: Cytotoxic activity of different Rubber vulcanizates towards human normal BJ1 Fibroblast cells

Sample Name	IC_{50} (µg/ml)
NR	**N.A.**
Cur	**N.A.**
Ros	**N.A.**
TBZ	**N.A.**
DMSO	**N.A.**
Negative control	**N.A.**

Curcumin (Cur), Rosemary (Ros) and TBZabendazole (TBZ). N.A.: No activity.

The data in Table 7 and Figures 10-14 indicated that investigated rubber vulcanizates with different anti-microbial agents are safe and have nil cytotoxicty. Moreover, there is no effect the strong colour and odours of Curcumin and Rosmary on the normal human fibroblast cell line (BJ1) under investigation.

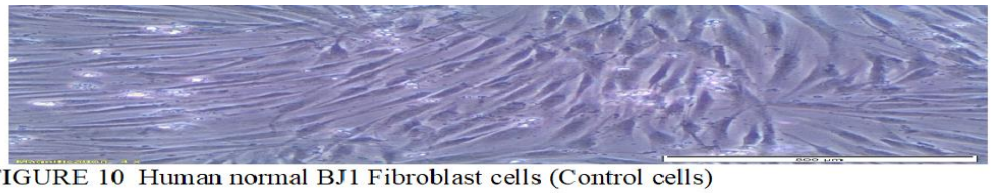


FIGURE 10 Human normal BJ1 Fibroblast cells (Control cells)

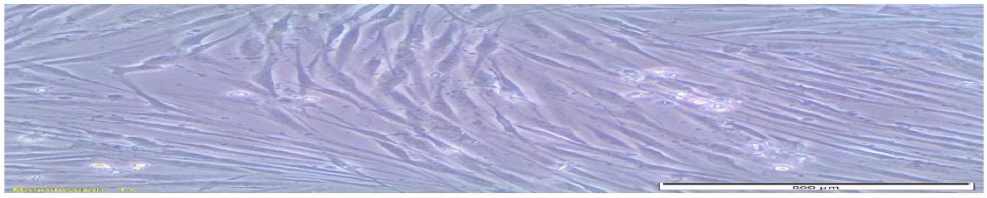


FIGURE 11 Cytotoxic activity of NR vulcanizates (Negative control)

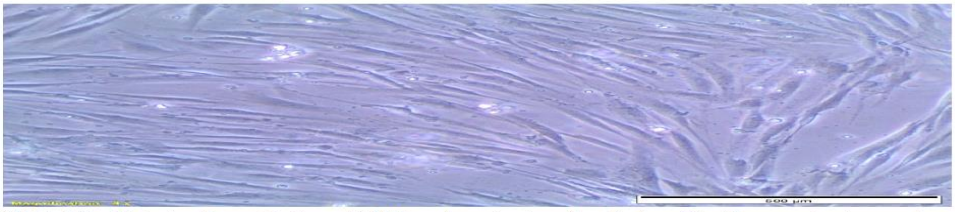


FIGURE 12 Cytotoxic activity of NR vulcanizates loaded with Curcumin towards BJ1 cells

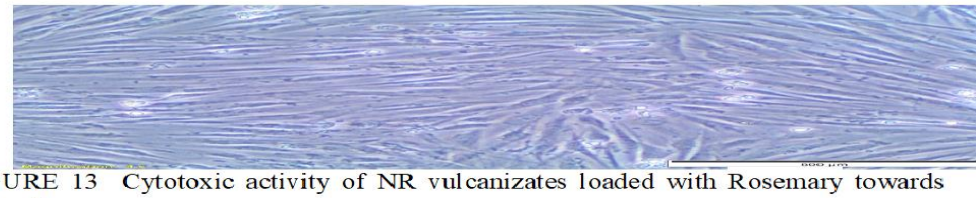


FIGURE 13 Cytotoxic activity of NR vulcanizates loaded with Rosemary towards BJ1 cells

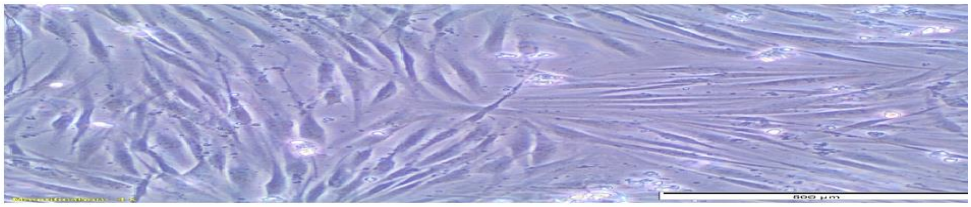


FIGURE 14 Cytotoxic activity of NR vulcanizates loaded with TBZ towards BJ1 cells

3.4.2.2. Antimicrobial activity

The results of tested Rubber vulcanizates loaded with different antimicrobial agents towards the tested Gram-positive & Gram-negative bacteria and fungi are presented in Figures 15-17.

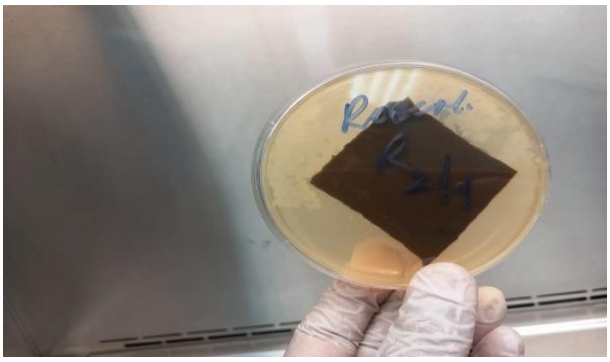


FIGURE **15:** Antimicrobial activity NR vulcanizates loaded with Rosemary

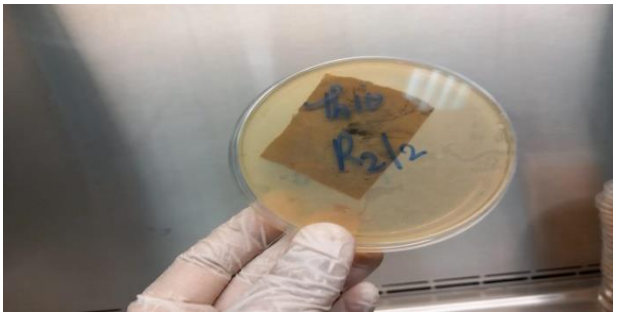


FIGURE **16:** Antimicrobial activity NR vulcanizates loaded with TBZabendazole

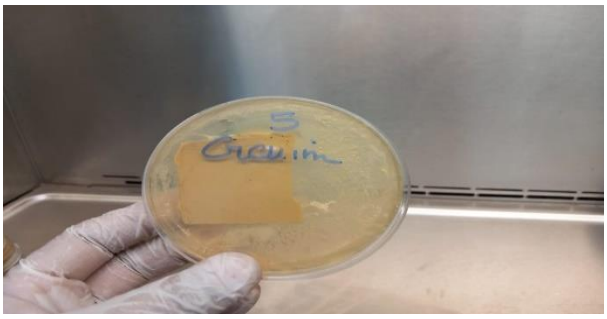


FIGURE **17:** Antimicrobial activity NR vulcanizates loaded with Curcurmin

Results in **Figures 15-17** indicated that the release of different antimicrobial agents loaded on natural rubber vulcanizates inhibited the growth of different types of bacteria or fungi on the surface of rubber. Also, the strong colour and odours of Curcumin and Rosmary indicated no effect on the tested bacteria and fungi

4. Conclusions

Natural Rubber (NR) vulcanizates containing plant extracts from Curcuma, Rosemary and Thiabendazole can be applied to produce safety green antimicrobial products. The mechanical properties (Tensile strength, elongation at break and modulus at different elasticity) are good before and after exposure to thermal oxidative aging at $90^{O}C$ for 6 days. The cytotoxicity of the investigated rubber vulcanizates gave negative results to normal human fibroblast cell line (BJ1). Promising results were obtained as antimicrobial rubber products, so there are significant inhibition in the growth of different types of bacteria and fungi on the surface of rubber.

So, it is recommended to fabricate and manufacturing swimming suits and diving allowances as a type of personal protection products as well as other safety rubber articles from natural rubber vulcanizates containing Curcuma, Rosemary and Thiabendazole.

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