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Preparation of Antimicrobial Efficient Rubber Vulcanizate Compounded with Jojoba Seeds Meal Powder

Abdelmohsen Soliman [1], Doaa Essamey El-Nashar [2], Aman I. Khalaf [2], Heba S. Kandil [2], Dalia E. El-Nashar [3], Azza Ward [4]

[1] Therapeutic Chemistry dept. National Research Centre, Dokki, Giza, Egypt
[2] Polymers and Pigment Department, Chemical Industries Research Division, National Research Centre, Cairo, Egypt
[3] National Center for Radiation Research and Technology (NCRRT), Atomic Energy Authority, Cairo, Egypt
[4] Microwave Physics and Dielectrics Department, National Research Centre (NRC), Giza, Egypt

In Loving Memory of Late Professor Doctor ""Mohamed Refaat Hussein Mahran""

Abstract
The goal of this work is to create antimicrobial rubber for industrial rubber products that are safe. Acrylonitrile butadiene rubber (NBR) was investigated for this reason. In a rubber mixer, rubber, ingredients, and the antimicrobial agent jojoba seed meal powder (JSMP) were mixed. The curing time, rheological characteristics, mechanical properties, dielectric properties, and antimicrobial potency of compounded rubber were examined. Scanning electron micrographs (SEM) revealed that the components were well-dispersed throughout the examined vulcanizates. According to rheology studies, there was no discernible change in the flow behaviours of the vulcanizates. It is important to keep in mind that, depending on their nature and concentration, jojoba seed meal powder (JSMP) can improve the desired physical characteristics of rubber products, including their chemical and mechanical characteristics (elongation at break, tensile strength, hardness, and equilibrium swelling). The dielectric results revealed that the presence of JSMPP caused an increase in the permittivity, dielectric loss, and conductivity of the resulting composites. It can be concluded that the presence of JSP is influential for good electrical performance for NBR/JSP compounds. Additionally, the test fungus and bacteria strains demonstrated good antimicrobial efficacy of the developed rubber vulcanizates.

Keywords: Acrylonitrile butadiene rubber (NBR), Mechanical properties, Jojoba , antimicrobial agents.

1. Introduction

Thousands of different species of medicinal plants are used throughout the world to treat various infections [1]. For instance, prior research demonstrated the antibacterial properties of extracts from *Euphorbia tirucalli* L., *Euphorbia hirta* L., and *Casuarina equisetifolia* Forest. Large amounts of research have been done to determine the scientific basis of these and other plants' use as antimicrobial agents [2]. From ancient times until the present, locals have used other plants, such as *Zingiber officinale*, as herbal remedies to treat inflammation [3]. The compounds in the plants have the potential to either produce a specific physiological effect on the human body or even function as antibiotics by attacking the cells of bacteria, which is why the plants are used medicinally [4]. The plant known as jojoba is *Simmondsia chinesisis*, a member of the Simmondsiaceae family. It is indigenous to southern Arizona in the United States. Native Americans found that the Jojoba plant's seeds yield over 45% weight percent of colourless, odourless, and oily material, and they recognised the plant's significant medicinal benefits [5]. Jojoba is being grown all over the world, including in Saudi Arabia and the deserts of Egypt, because of its high economic value [6]. A lot of research has gone into figuring out what jojoba's antibacterial qualities are [7]. Jojoba oil is made up of various tocopherols and oil sterols, giving it a special chemical structure [8]. Jojoba seeds also contain a sizable amount of tannin [9]. Along with alcohol monoesters, several triglycerides, stanols, and straight chains of C-20 and C-22 acids are present. There for JSMP had several aliphatic and aromatic compounds in its composition especially protein and phenolic compounds [10].

It is believed that flavonoids are responsible for the antibacterial qualities of jojoba oil [11]. Because crude J.O. may also act as a carrier for oxidation-sensitive substances like vitamin A, it was used as an ingredient in cosmetics and skin care products [12]. The purpose of the current study is to look into the anti-microbial activity of J.O. against different microorganisms at different concentrations.

*Corresponding author e-mail: amsolimannrc@gmail.com; (Abdelmohsen Soliman).
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Extensive biological and pharmacological investigations, based on the uses of jojoba oil in folk medicine, revealed that jojoba oil and its derivatives exhibit vast biological activities in different pharmaceutical forms, whether used topically or internally. These activities can be attributed to the unique chemical composition of the wax esters [13]. Most of the relevant activities were grouped in Figure 1.

Figure 1. Summary of the main biological activities of jojoba oil.

Rubber is a polymeric material that has exceptional elastic properties and can be used to prepare network structures. The primary causes of infections are microbes, including bacteria, fungi, and parasites .

Since they were discovered in 1965 [14], antimicrobial polymers have received a lot of attention from researchers in academia and industry. Three key industries that use applied antimicrobials are the food, textile, and medical sectors. Antimicrobial polymers are intriguing because they show increased resistance, decreased toxicity, less environmental issues, and better efficacy [15,16]. Antimicrobial Rubber offers enduring defence against microbial contamination and is a proactive type of antimicrobial rubber. The need for regular sanitization, disinfection, and decontamination services is thus ultimately decreased. When producing rubber goods, the intended physical characteristics of the rubber article must be taken into account. For example, compared to their counterparts with lower molecular weight polymers, high molecular weight NBR polymers typically show better green strength, tensile strength, exceptional heat resistance, and lower compression set. The development of antimicrobial vulcanized rubber compounds and their improvement are the focus of research on antimicrobial polymers for toys and medical products [17].

The aim of the present investigation is to prepare NBR vulcanizate containing jojoba seed meal powder (JSMP) and evaluate its effect on the mechanical, dielectric properties also antimicrobial activity of the investigated vulcanizates.

2. Experimental

2.1. Materials

Bayer AG, Germany supplied acrylonitrile butadiene rubber (NBR) with a 32% acrylonitrile content and a specific gravity of 1.17 ± 0.005 at 25°C. We purchased silica (Hi-Sil 233D) from PPG Industries Inc. in the Netherlands, Europe, with a particle size of 15 nm. The remaining ingredients in rubber, which were of industrial grade and commercially available, were sulphur (S), stearic acid (St. Ac), zinc oxide (ZnO), N-cyclohexyl-2-benzolthyazolsulfenamide (CBS), and trimethylquinoline (TMQ). The source of jojoba seed meal powder (JSM) was the Al Kanz oil extraction company located in Zagazig, Egypt. JSMP was ground in a laboratory grinder and then sieved through a 240 μm screen to create the JSMP powder (JSMP), which is a fine powder that is easily combined with rubber ingredients; Figure 2. [18].

FIGURE 1 Jojoba seed meal powder (JSMP) before (a) and after grinding and sieving for obtaining jojoba seed meal powder (JSMP) (b)

Table 1. Formulation of NBR loaded with different concentrations of JSMP

Ingredients (phr)[a]	**S**	**S1**	**S2**	**S3**	**S4**	**S5**
NBR	100	100	100	100	100	100
ZnO	5	5	5	5	5	5
Stearic acid	2	2	2	2	2	2
Jojoba seeds powder (JSMP)	---	10	20	30	40	50
CBS [b]	6	6	6	6	6	6
DOP [c]	3	3	3	3	3	3
S	2	2	2	2	2	2
TMQ[d]	1	1	1	1	1	1

a Part per hundred parts of rubber.
b N-cyclohexyl-2-benzothiazole sulfonamide.
c Dioctyl phthalate
d Tetramethylthiuram disulfide

2.2. Preparation NBR compounds

The effect of Jojoba seed meal powder (JSMP) was studied in NBR. The formulations used for preparation of NBR composites filled with different concentrations of Jojoba seed meal powder (JSMP) are shown in Table 1 where a laboratory roll mill of diameter 470 mm and width 300 mm was used to mix the NBR with other ingredients according to ASTM D3182. After mixing, the obtained composites were left overnight before curing process. At the end of the mixing cycle, the materials were collected and conditioned at a temperature of 25±1°C for 24 h before cure assessment.

2.3. Characterization

2.3.1. Techniques

2.3.1.1. Scanning electron microscope (SEM)

The surface morphology of JSMPP and prepared NBR composites was determined by scanning electron microscope, Quanta FEG-250.

2.3.1.2. Curing characteristics

The curing properties of the prepared NBR composites were determined in compliance with ASTM D 2084 using a single Moving Die Rheometer (TA Instruments, USA) at 152 ± ^{O} C for 30 minutes. The rheometric graph displayed the scorch time (ts2), optimum curing time (tc90), minimum torque (ML), and maximum torque (MH). The cure rate index (CRI) was calculated using the following formula using the tc90 and ts2 values, (CRI) were calculated as follows:

CRI = 100/ tc90-ts2 (1)

2.3.1.3. Mechanical properties

Using an electronic tensile testing machine (Zwick model Z010, Germany), the mechanical properties of the prepared NBR vulcanizate composites were measured in accordance with ASTM D412-06a in terms of tensile strength (MPa), elongation at break (%), and modulus at 100%. Using a Bareiss Shore A durometer (Germany), the shore hardness of the prepared composites was determined in accordance with ASTM D 2240 standard. Hardness was measured using the Shore A, durometer according to ASTM D2240.

2.3.1.4. Swelling test:

Equilibrium swelling of test was carried out in the solvent toluene. About 0.1– 0.2 g of each specimen was weighed in a weighing bottle, which was covered with toluene for 24 h according to the method described in standard test ASTM D3616. The swollen samples were weighed and then dried in an oven to a constant weight. The last weight was taken as the correct weight of the sample free from dissolved matter. The swelling percentage (Q %) of the samples was calculated as follows:

$$Q\% = (W\text{-}W0\ )/W0 \times 100 \qquad (2)$$

Where w and w0 represent the weights of the samples after swelling and free from dissolved matter, respectively. All these tests were performed at room temperature (25°C), and the reported results were averaged from a minimum of five specimens.

2.3.1.5. Dielectric measurements

The permittivity (dielectric constant) ε', loss factor tan δ and ac-resistance R_{ac} were measured at room temperature ~ 30 °C in a broad frequency range (0.1 Hz - 1 MHz). The permittivity ε' is calculated in accordance to; $\varepsilon' = cd/\varepsilon_o A$ where C is capacitance of the dielectric, d thickness of the sample A is area of electrode, and ε_o is permittivity of vacuum (8.85 x10^{-12} F/m). The loss factor "tanδ" is calculated based on $\tan\delta = 1/\ 2\pi\ f\ R_p C_p$ where δ is the loss angle, f is the frequency , R_p is the equivalent parallel resistance and C_p is the equivalent parallel capacitance respectively. Additionally the dielectric loss is calculated in accordance to; $\varepsilon'' = \varepsilon' * \tan\delta$. The measurement was computerized by interfacing the impedance analyzer with a personal PC through a GPIB cable IEE488. A commercial interfacing and automation software program "Lab VIEW" was used for data acquisition. The error in ε' and tan δ amounts to ±1 % and ±3 %, respectively. The temperature of the samples was controlled by a temperature regulator with (Pt 100) sensor. The error in temperature measurements amounts ± 0.5 °C. The samples were saved in desiccators to avoid moisture effects. However, ε′ and tan δ were re-measured once again after performing the experiment to test the measurements' reproducibility.

2.3.1.6. Antimicrobial activities of rubber vulcanizates

Rubber vulcanizates compounded with Jojoba seeds mills were individually tested against variety of certified reference strains of Gram positive, Gram negative bacterial pathogens. The current study includes two Gram-positive reference strains; ; *B. cereus* NCINB 50014, *E. faecalis ATCC 19433, S. aureus* NCINB 50080 and &. Three Gram-negative reference strains; *E. coli* O157 ATTC 700728, *S. Typhimurium* ATCC 13311 and *Shigella flexineri* ATCC 12022 and *V. parahaemolyticus* ATCC 17802.

Agar Well Diffusion Test (AWDT)

Antimicrobial tests were carried out using the agar well diffusion method [19-21]. Sterile plates of Muller Hinton agar (Oxoid) agar were prepared under aseptic conditions. After the media had cooled and solidified, wells of diameter 0.6 mm were aseptically formed in the agar, 6 wells per plate. Bacterial freshly prepared suspension was prepared using reference strains. Then using 100 µL of suspension containing 1×10^8 CFU/mL of pathological tested bacteria spread onto the surface of the Muller Hinton Agar plates. Then the material was cut under sterile conditions into squares of 1cm dimensions and after spreading of the reference strain the material is place onto the surface and pressed well to allow spread of active ingredient under study. The inoculated plates were then incubated for 24hrs at 37˚C for bacterial growth. Negative controls were prepared using DMSO and Ciprofloxacin (5µg/ml) was used as standard antimicrobials for testing antibacterial activity. 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 activities were expressed as inhibition diameter zones in millimeters (mm). The experiment was carried out in triplicate and the average zone of inhibition was calculated. Zone of inhibition is measured using measuring caliber.

3. Results and Discussion

The objective of this work is to prepare antibacterial safety rubber goods for toys, medical and industrial products using natural material as *Jojoba seed meal powder* powder (JSMPP) antibacterial agent.

3.1. Rheological and physico-mechanical characteristics of the investigated acrylonitrile butadiene rubber (NBR):

Acrylonitrile butadiene rubber (NBR) is the type of rubber used. On a typical rubber mixer used in industry, the rubber, additives, and meal powder (JSMPP) (a natural resource antibacterial agent) were combined. The rubber mixes that were compounded were assessed rheologically and vulcanized at the appropriate curing time. Using standard test procedures, the physicomechanical properties were measured. Tables 2 present the rheometric characteristics at 152ºC and the physico-mechanical properties of the NBR vulcanizates containing jojoba seed meal powder (JSMP). Due to the nature and concentration of (JSMP), the compounded rubber's rheological properties were slightly impacted as a natural product; torque values increased for both the minimum and maximum torque, and cure times at 90% decreased (table 2). The value of the scorch time measurement and the curing time or time at 90% cure both supported the finding that the cure rate index increased with increasing (JSMP) concentrations [22].

The physico mechanical measurements of NBR rubber compounds holding jojoba seed meal powder (JSMP) are illustrated in Table 3. From this Table, it was found that there is no great difference between the obtained data of the rheological and physico mechanical measurements of NBR compounds had (JSMP) and that without (JSMP). This indicates that the conventional (JSMP) used had no effect on their properties of the compounded NBR. In addition, there is no great difference in change of modulus at different elongation (50 – 500%) from the formulation free of the jojoba seed meal powder (JSMP) i.e. the mechanical properties are good [23].

Table 2. Curing characteristics of NBR loaded with different concentrations of JSMP

Sample code	S	S1	S2	S3	S4	S5
waste Jojoba seeds powder (JSP)	--	10	20	30	40	50
MH maximum torque, (dN.m)	5.7	6.06	6.18	7.35	7.56	7.84
ML minimum torque, (dN.m)	1.2	1.75	2.02	2.37	2.52	2.54
Ts2 scorch time, (min)	5.02	5.25	5.44	5.34	5.11	5.1
Tc90 optimum cure time, (min)	15.8	14.77	14.43	14.11	14	14
CRI cure rate index, (min^{-1})	9.27	9.5	10.01	10.04	10.11	10.10

Table (3): Mechanical properties of NBR loaded with different concentrations of JSMP

Sample code	Tensile Strength (MPa)	Elongation at break (%)	Modulus 50% (MPa)	Modulus 100% (MPa)	Modulus 200% (MPa)	Modulus 300% (MPa)	Modulus 500% (MPa)
S	2.2	500	0.73	1.07	1.49	1.78	2.4
S1	2.5	520	0.76	1.09	1.52	1.8	2.4
S2	2.8	550	0.9	1.3	1.6	1.9	2.5
S3	3	580	1.05	1.4	1.7	1.99	2.7
S4	3.5	600	1.1	1.6	1.9	2.2	3
S5	3.5	600	1.3	1.65	1.9	2.3	3

The mean results of each property was estimated as 5 replicates

3.2. Hardness of the investigated acrylonitrile butadiene rubber (NBR):

The results of hardness of the NBR vulcanizates are shown in Figure 3. It was noticeable from Figure 3 that the hardness of NBR vulcanizates containing jojoba seed meal powder (JSMP) were higher than the sample without jojoba seed meal powder (JSMP) The increase in the hardness of the samples containing JSMP was attributed to decreasing the elasticity of rubber chains when more JSMP particles got into the NBR matrix. As a result, the stiffness of these composites increased [18].

3.3. Equilibrium swelling (Q) of the investigated acrylonitrile butadiene rubber (NBR):

To further investigate the effect of adding JSMP on the NBR vulcanizates, the equilibrium swelling was determined. Figure 4 shows the result of adding JSMPP at different loadings onto the equilibrium swelling of the NBR vulcanizates. From this figure, it was observed that the samples containing JSMPP showed a decrease in the swelling percentage . This reduction in the swelling percentage was attributed to the presence of JSMP which prevented the penetration of toluene within the prepared samples, thus the swelling percentage reduced and rubber improved [18].

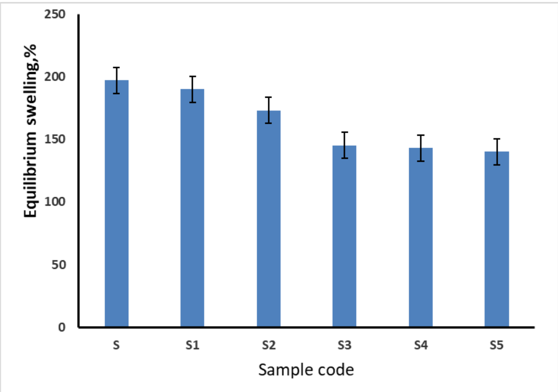


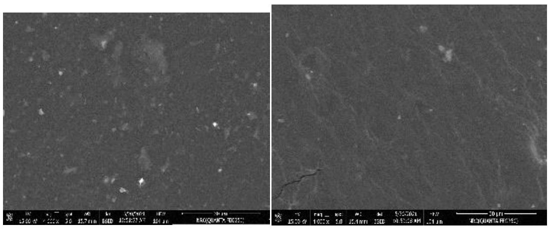
Fig. (4)Effect of different loadings of JSM on the equilibrium swellingof the NBR Vulcanizates

3.4. Morphological characteristic of the investigated rubber vulcanizates

Scanning electron microscope was used to examine and study the surface morphology of the prepared cured rubber samples NBR in the presence of jojoba seed meal powder (JSMP). Figures 5 (a&b) illustrate the SEM micrographs of the tested samples. The micrographs depict the good dispersion and distribution of the jojoba seed meal powder (JSMP) inside rubber matrix [18].

3.5. Permittivity (ε′) of JSP filled NBR compounds

The permittivity (ε′) at 100 Hz for JSP filled NBR compounds is depicted in Figure 6. Obviously, the values of ε′ get higher with the increase JSP loading (samples (S-S5). The values of ε′ increased from 14.554 corresponding to blank sample (S) to 33.5 for the sample containing 50phr JSP (S5) respectively. This result confirmed that increasing the loading of JSP has a positive impact on the permittivity. This might be attributed to the polarity of JSP [24].



(a) **(b)**

Fig.5. SEM micrographs of the NBR without JSMP (a), NBR loaded with 50phr JSMP (b)

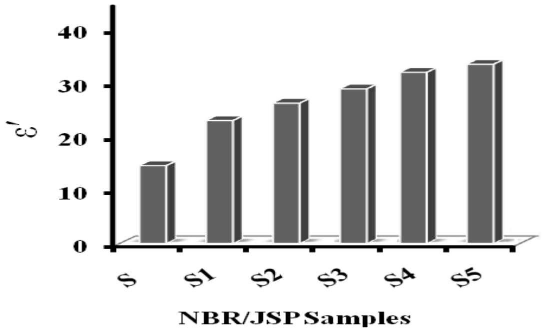


Figure 6: The permittivity (ε′) of NBR vulcanizates loaded with different JSP loadings (0, 10, 20, 30, & 50phr) is represented by samples (S-S5) at 30ºC, respectively.

Dielectric loss (ε") of JSP filled NBR compounds

Figure **7** represents the dielectric loss (ε") of NBR vulcanizates loaded with different JSP loadings. It was observed that the values of ε" increased by increasing JSP loading. This increase can be attributed to increased polarity of NBR/JSP compounds due to the presence of polar JSP [25].

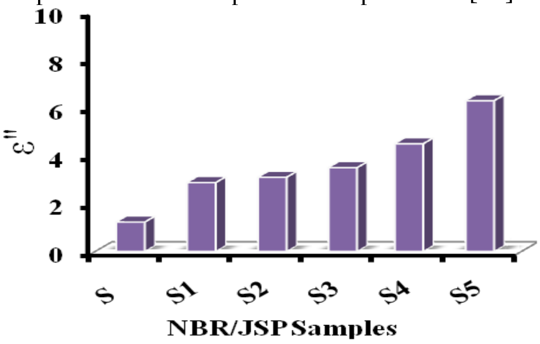


Figure 7: The dielectric loss (ε") of NBR vulcanizates loaded with different JSP loadings (0, 10, 20, 30, & 50phr) is represented by samples (S-S5) at 30ºC, respectively.

3.6. Conductivity (σ)of JSP filled NBR compounds

The conductivity (σ) values of NBR vulcanizates loaded with different JSP loadings are depicted in Figure **8**. It was noticed that the conductivity of NBR/JSP compounds increased with increasing JSP loading. This result might be due to increasing polarity of the system (NBR vulcanizates) as a result of the presence of polar JSP [26].

From these results it seems likely that, increasing JSP loading improves somewhat the polarity of the system and conductivity as well. It can be concluded that, the presence of JSP is influential for good electrical performance for NBR/JSP compounds.

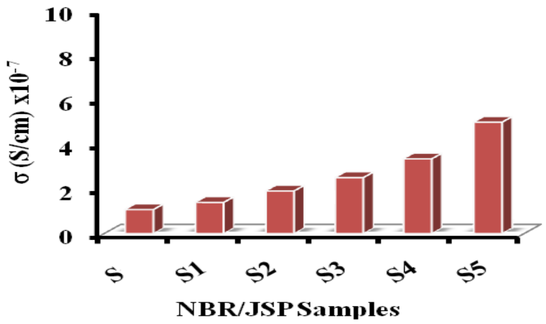


Figure 8: The conductivity (σ) of NBR vulcanizates loaded with different JSP loadings (0, 10, 20, 30, & 50phr) is represented by samples S-S5) at 30ºC, respectively.

3.7. Antimicrobial activities of rubber vulcanizates

Rubber vulcanizates compounded with Jojoba seeds mills were individually tested against variety of certified reference strains of Gram positive, Gram negative bacterial pathogens. Table 4 shows the outcomes of tests conducted on rubber vulcanizates containing Jojoba seeds mills against tested Gram positive & Gram negative bacteria and fungus. The average zone of inhibition was computed after the experiment was completed in triplicate. Findings in Table 4 and Figure 9 showed that rubber vulcanizates containing Jojoba seeds mills gave promising results as an antimicrobial agent.

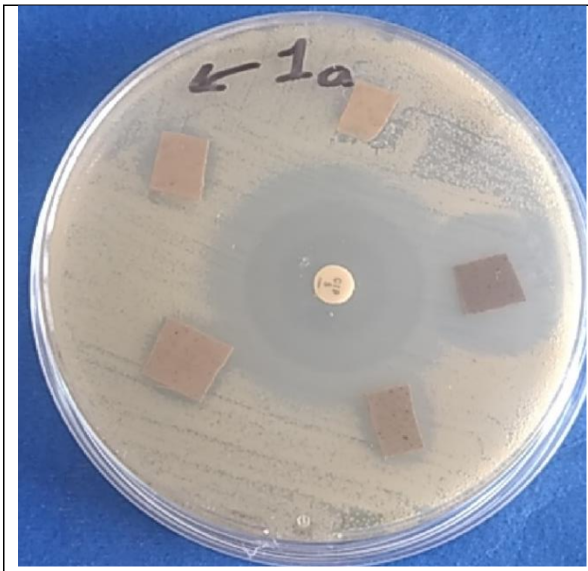
Conflicts of Interest

Authors declare that there are no conflicts of Interest.

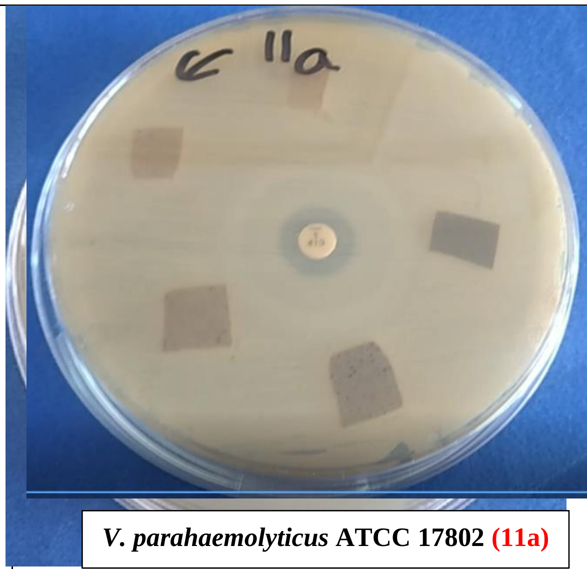
Table 4: Zone of inhibition of tested compounds using Agar Disc Diffusion Test (ADDT).

Material tested / ***Tested Strains***	***S***	***S_1***	***S_2***	***S_3***	***S_{55}***	***Ciprofloxacin (5 µg/ml)***
Gram positive bacteria						
B. cereus **NCINB 50014 (1a)**	-	+	++	++	+++	++++
S. aureus **NCINB 50080 (6a)**	-	-	-	-	-	++
E. faecalis ***ATCC 19433*** **(4a)**	-	-	-	-	-	+++
Gram negative bacteria						
E. coli **O157 ATTC 700728 (3a)**	-	-	-	-	-	+++
S. Typhimurium **ATCC 13311 (5a)**	-	-	-	-	-	++
Shigella flexineri **ATCC 12022 (7a)**	-	-	-	-	-	+++++
V. parahaemolyticus **ATCC 17802 (11a)**	-	-	-	-	-	+

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



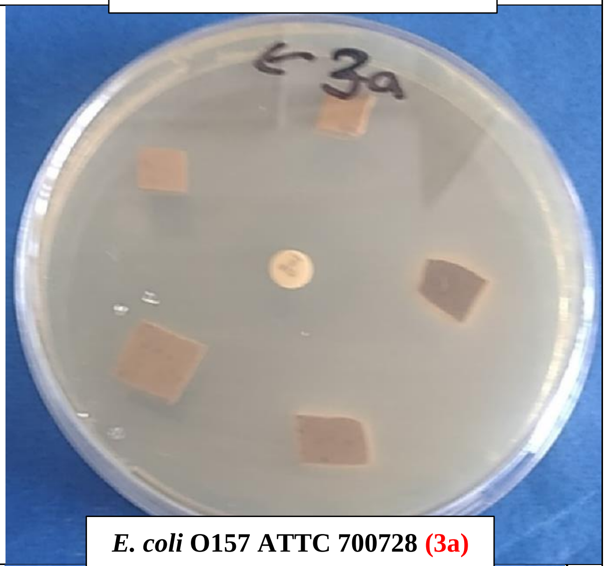
B. cereus **NCINB 50014 (1a)**



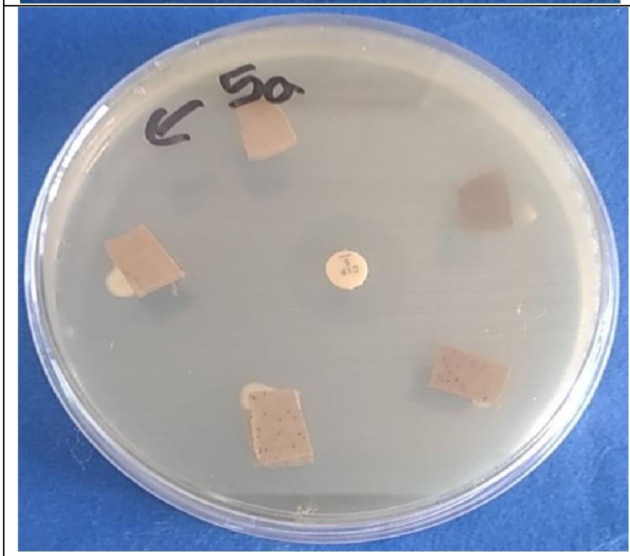
V. parahaemolyticus **ATCC 17802 (11a)**

S. aureus **NCINB 50080 (6a)**

E. faecalis ATCC 19433 **(4a)**



E. coli **O157 ATTC 700728 (3a)**



S. Typhimurium **ATCC 13311 (5a)**

low-cost natural products as effective antimicrobial agents in rubber goods.

Shigella flexineri **ATCC 12022 (7a)**

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