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Abstract: Cellulose acetate (CA) is one of the most important esters of cellulose and it was analyzed by means of Fourier transform infrared spectroscopy (FT-IR), scanning electron microscopy (SEM) and EDX spectra. The FT-IR spectrum presented both the C=O and C-O stretching bands for acetyl groups. The EDX spectrum confirms the presence of C and O elements. The SEM image presented a spongy structure that is important for many applications. The antimicrobial properties of cellulose acetate solutions in acetic acid were determined in tests on two gram-negative species (Pseudomonas aeruginosa - ATCC 27853 and Escherichia coli - ATCC 25822), two gram-positive species (Staphylococus aureus - ATCC 29213 and Streptococcus pyogenes - ATCC 19615) and yeast species (Candida albicans - ATCC 10231). We have shown that samples of cellulose acetate solutions in acetic acid can be used to fight microbial and fungal infections. Of the gram-positive species tested, the strongest antimicrobial effect was observed against S. aureus. The diameter of inhibition zones of cellulose acetate solutions in acetic acid (P1 and P2) for S. aureus far exceeded inhibition zone both of reference substance (gentamicin) and of acetic acid solutions (M1 and M2), given values between 3.15 cm (P1) and 3.55 cm (P2). Also, the results suggested that the studied solutions (P1 and P2) had an antimicrobial effect pronounced for gram-negative species as P. aeruginosa, in which the P1 sample gave an inhibition zone of 2.95 cm, and the P2 sample achieved an inhibition of 3.15 cm. So, the antimicrobial activity of cellulose acetate solutions tested in vitro had a good antimicrobial effect, proportional to the concentration of the active substance.

Keywords: cellulose acetate, acetic acid, antimicrobial resistance, bacteria, antibiotic.

# **1. Introduction**

In recent years, antimicrobial resistance has become a serious public health problem [1, 2]. As a result, microbial contamination in hospitals and the development of drug resistance to various pathogenic bacteria (gram-negative and gram positive) have become a major cause of mortality. That is why, investigations into this problem and a search for solutions have been presented in many scientific articles [3-5]. It should be noted that, the researchers' attention in recent years [6-8] has been focused on finding chemically modified polymeric supports with biologically active groups which have antimicrobial properties. Polymeric biocides obtained by the chemical bonding of antimicrobial active groups on insoluble polymers have attracted a lot of interest due to the fact that they can be recovered and used for a longer period of time. In previous studies amino(hydroxy)phosphonic acid groups were functionalised onto styrene-divinylbenzene type polymeric materials and they had a decisive antimicrobial role [9, 10]. Antimicrobial polymers which are of the natural type are a significant alternative to man-made products with potential for application in many areas of human activity [11, 12]. Biopolymers are environmentally friendly bio-based materials made from renewable raw resources and which can be re-used [13]. A biopolymer is cellulose. It is one of the most widespread organic compounds in the world, produced mainly by plants. Semi-synthetic derivatives have been obtained from cellulose, which is widely used in the pharmaceutical and cosmetic industries.

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Cellulose derivatives are used in different types alone or in combination with other polymers, in different forms (e.g. films, membranes or fibers) [14], and different formulations prepared (e.g. buccal, ocular, vaginal, nasal and transdermal) in drug delivery systems [15]. A semi-synthetic derivative of cellulose would be cellulose acetate (CA), which is an ester of organic cellulose, which has uses in commercial products or in pharmaceutical investigations [16]. Thus, cellulose acetate (CA) is widely used for the preparation of semi-permeable, microporous and pH sensitive membranes [16].

The aim of this work was to integrate cellulose acetate and acetic acid into a simultaneous utilization to assess the antimicrobial efficacy and to show their effective use together. The study employed the cellulose acetate because it is a biodegradable and environmentally friendly material and with applications as a good drug delivery medium. The acetic acid is known to be used since old times against pathogen agents. The efficiency of the use of cellulose acetate solution in acid acetic was established for the antimicrobial evaluation against two gram-negative species (*Pseudomonas aeruginosa* - ATCC 27853 and *Escherichia coli* - ATCC 25822), two gram-positive species (*Staphylococcus aureus* - ATCC 29213 and *Streptococcus pyogenes* - ATCC 19615) and a species of yeast (*Candida albicans* -ATCC 10231). The study got a good incorporation of the cellulose acetate and acetic acid and brought synergistic effect in antimicrobial actions. Obtained results offer improving research and give a good method for antimicrobial assay with using cellulose acetate solution in acetic acid.

### 2. Experimental part

#### 2.1. Materials and apparatus

The following reagents were used for the preparation of cellulose acetate solutions (dissolved in glacial acetic acid and distilled water): cellulose acetate (cellulose acetate pure company ROTH, CAS number 9004-35-7, density 1.3 g/cm<sup>3</sup>; acute toxicity: oral LD50> 5050 mg/kg rat); 100% glacial acetic acid (Merck, 607-002-00-6, molar mass 60.05 g/mol). For antimicrobial testing, catalogue bacterial strains from two gram-negative bacterial species *Pseudomonas aeruginosa* - ATCC 27853 and *Escherichia coli* - ATCC 25822), two gram-positive species (*Staphylococus aureus* - ATCC 29213 and *Streptococcus pyogenes* - ATCC 19615) and a representative of the yeast group (a strain of *Candida albicans* - ATCC 10231) were used.

The FTIR spectrum (performed on KBr pills) of the cellulose acetate was recorded using the Jasco FTIR spectrophotometer (JASCO, Japan) in the range 4000-400 cm<sup>-1</sup>. The surface morphology of the cellulose acetate was studied by scanning electron microscopy (SEM) using a Quanta FEG 250 microscope equipped with an EDX ZAF detector (FEI Company, Netherlands).

The antimicrobial effect was determined by the agar diffusion disc method (according to the Standard Norms for Testing the Antimicrobial Sensitivity of Impregnated Discs) using impregnated discs [17].

#### **2.2. Preparation of the solutions of cellulose acetate**

**P1 sample**: 2% (w/v) cellulose acetate

In 40 mL solution (30 mL acetic acid and 10 mL distilled water, volume ratio 3:1), 0.8 g of cellulose acetate was dissolved. To obtain complete dissolution of the cellulose acetate the solution was heated slightly about 40  $^{\circ}$ C.

P2 sample: 2.13% (w/v) cellulose acetate

In 37.5 mL solution (22.5 mL acetic acid and 15 mL distilled water, volume ratio 1.5:1), 0.8 g cellulose acetate was dissolved. For complete dissolution of cellulose acetate, the solution was heated slightly about 40  $^{\circ}$ C.

#### 2.3. Preparation of the control solutions (M1 and M2) for tested solutions (P1 and P2)

M1 control for P1 sample: a solution in the volume ratio 3:1 (9 mL glacial acetic acid, 3 mL of distilled water) was prepared;

M2 control for P2 sample: a solution in the volume ratio 1.5:1 (9 mL glacial acetic acid, 6 mL of distilled water) was prepared.



#### 2.4. Experimental tests of the antimicrobial activity

The cellulose acetate solutions P1 and P2 were tested for antimicrobial activity along with the corresponding control solutions M1 and M2 (produced with the same dilutions as were used with the active substance (cellulose acetate) in P1 and P2 respectively).

The microbial strains chosen in this experiment, were two Gram negative bacterial species *Pseudomonas aeruginosa* - ATCC 27853 and *Escherichia coli* - ATCC 25822) and two Gram positive species (*Staphylococus aureus* - ATCC 29213 and *Streptococcus pyogenes* - ATCC 19615) and one yeast species, *Candida albicans* (ATCC 10231). Initially, 24 h cultures were prepared. Petri dishes with nutrient agar were inoculated with each of the microbial strains, using a sterile bacteriological loop to obtain isolated colonies on each dish. These colonies were incubated at 37°C for 24 h. From the resulting cultures, an isolated colony was sterile taken and was inoculated into 10 mL broth (liquid culture medium, consisting of: peptone; 10 g; NaCl 5.0 g; meat extract 5.0 g in 1000 mL of sterile distilled water; pH = 6.8). These tubes with the inoculated broth were also incubated for 24 h with the thermostat at 37°C. These microbial cultures were considered to be fresh and pure cultures and were used for tests. To obtain the pure culture of *Candida albicans* a similar procedure was performed, but the culture medium used was Sabouraud, and the incubation was carried out at 25°C for 48 h.

To perform the tests from each species cultures, broth was prepared with a cell density of  $1.5 \times 10^8$  CFU/mL, corresponding to an optical density of 0.5 on the McFarland scale. These bacterial and yeast dilutions were tested using the agar disk diffusion method. In order to actually test the antimicrobial activity, the culture media, nutritive agar and Sabouraud medium were poured into sterile Petri dishes, using one plate for each species. After the medium in each dish had solidified, the condensation which formed on plate cover was eliminated by holding the plates in the thermostat for 15 min (37 °C).



**Figure 1.** Petri dishes with microbial cultures and with the tested solutions (P1, P2, M1 and M2) by the disk diffusion method

Filter paper discs (about 6 mm in diameter) pre-impregnated with 10  $\mu$ L of each sample (P1 and P2) and of the control solutions (M1 and M2) were then placed on the surface of the nutritive culture medium. Also, on each plate with bacteria inoculums, a disc impregnated with the antibiotic, Gentamicin was placed, and for the yeast culture, nystatin was used. This was done to produce a comparative assessment of the antimicrobial effect (Figure 1).

The antimicrobial effect was assessed by measuring the diameter of the inhibition grow zone formed around the discs (including the paper disc - 0.6 cm). Determinations were performed three times, obtaining the average of the inhibition zones.



## **3. Results and discussions**

#### **3.1.** The characterization of cellulose acetate used for the antimicrobial study

The cellulose acetate has a structural uniformity by type  $\beta$ -1-4-linked polyglucan (Figure 2).



Figure 2. Structure of the cellulose acetate, where: Ac - acetyl group

In order to assess the use of cellulose acetate as an antimicrobial agent we have presented its analysis by EDX Quantification, SEM image and FTIR spectra.

The result of EDX analysis of the cellulose acetate (Table 1) indicated the presence of elements C and O in its structure.

C	of the cellulose acetate		
Sample	Cellulose acetate		_
/Elem.	Wt%	At %	_
С	49.66	56.78	_
0	50.34	43.22	_
	Sample	/Elem.     Wt%       C     49.66	Sample /Elem.Cellulose acetateVt%At %C49.6656.78

Table I. EDX Quantification (Standardless)
of the cellulose acetate

The cellulose acetate had a spongy structure (Figure 3), potentially important for its application in the fields of medical and food production.



Figure 3. SEM image of cellulose acetate

FTIR spectrum indicates some characteristic functional groups (Figure 4) that confirm the formation of cellulose acetate [18]. A band at 3440 cm<sup>-1</sup> is caused by O-H stretching. Both the absorption band at 1737 cm<sup>-1</sup> for C=O stretching and peak at 1240 cm<sup>-1</sup> for C–O stretching are consistent with acetyl groups. The absorption peak at 1320 cm<sup>-1</sup> was attributed to CH<sub>3</sub> vibration in the acetyl group.





Figure 4. FTIR spectra of cellulose acetate

#### **3.2.** Testing the antimicrobial activity

The antimicrobial activity of samples P1, P2 (cellulose acetate solutions), M1 and M2 (the control solutions) was tested by the agar disk diffusion method and the results are presented in Table 2 and Figure 5. Diameters of the inhibition zones (in cm) that indicated antimicrobial activity of the tested solutions and antibiotics, and their values are shown in Table 2. Comparisons in antimicrobial activity are shown as a percentage in Figure 5.

Microbial species tested	Diameter of the inhibition zone (cm)					
	Antibiotic / antifungal disc	Disc with P1	Disc with M1	Disc with P2	Disc with M2	
Pseudomonas aeruginosa	2.3	2.95	2.25	3.15	2.15	
Escherichia coli	2.1	2.15	1.85	2.45	1.95	
Staphylococcus aureus	2.4	3.15	2.40	3.55	2.65	
Streptococcus pyogenes	2.3	2.85	2.35	3.35	2.35	
Candida albicans	2.0	2.75	2.45	2.9	2.45	

It was found that all the tested solutions, both the samples containing cellulose acetate and the corresponding control samples, produced inhibition zones for bacterial and the yeast growth. In the case of samples containing cellulose acetate, these inhibition zones were even greater or approximately equal to those obtained with the antibiotic disc, or the antimycotic ones. The acetic acid solutions (M1 and M2), used as controls, also had an antimicrobial effect, but in all tested species this effect was less than that produced by the cellulose acetate samples. Of the gram-positive species tested, the strongest antimicrobial effect was observed against *Staphylococcus aureus*, with the sample P1 giving a 3.15 cm inhibition zone, and the sample P2 a zone of 3.55 cm.

Of the gram-negative species tested, the antimicrobial effect was more pronounced for *Pseudomonas* aeuginosa, in which the P1 sample gave an inhibition zone of 2.95 cm, and the P2 sample achieved an inhibition of 3.15 cm. Compared to the other two species, the P1 sample, with 2% cellulose acetate, formed a inhibition zone of 2.15 cm against Escherichia coli and 2.85 cm against Streptococcus pyogenes. The P2 sample with 2.13% cellulose acetate induced an inhibition zone of 2.45 cm for E. coli and 3.35 cm for S. pyogenes. For the yeast specie tested, Candida albicans, the diameter of the inhibition zones was relatively close in value for both cellulose acetate samples, namely 2.75 cm for the P1 sample, with 2% cellulose acetate and 2.9 cm for the P2 sample, with a cellulose acetate content of 2.13%.





**Figure 5.** Percent of microbial inhibition (%) of samples tested versus antimicrobial culture

A comparison between data for the antimicrobial action of the solutions tested (P1 and P2) and the antibiotic and the antimycotic used, is shown in Figure 5. The P2 cellulose acetate solution with a concentration of 2.13% compared to the antibiotic used (Gentamicin) had a 36.95%, higher antimicrobial effect against the *Pseudomonas aeruginosa* species and a 47.91% greater effect against *Staphylococus aureus*. Also, the antimicrobial effect of this P2 solution was greater than that of the antibiotic when used against the other bacterial species used for testing. Against the *Streptococcus pyogenes* species, the antibiacterial effect was 45.6% higher, but against *Escherichia coli*, the cellulose acetate solution P2 acted with an antibacterial effect of 16.6%. The antifungal effect against *Candida albicans* of the same solution (P2 - cellulose acetate 2.13%) was 45% higher compared to the antifungal drug used (nystatin).

The results of studies published so far show that cellulose acetate acquires antimicrobial activity dependent on the substance with which it is associated, and that this polymer is insoluble in water, but soluble in various solvents such as acetone, acetic acid, methanol, dimethyl acetamide, dimethyl-formamide, etc [19-21].

The novelty of this research consists in using cellulose acetate in combination with acetic acid in a antimicrobial study. Acetic acid was used, considering that it is a good solvent for cellulose acetate with the degree of substitution (DS) greater than 0.8 [14, 22, 23] and that its antimicrobial effect has long been known. So far, there are studies on the antimicrobial effects both for cellulose acetate and acetic acid, but they have been tested separately.

The antibacterial effect of acetic acid has often been investigated with the idea that it can be used as an antimicrobial agent in the poultry, beef or pork meat industry, in order to decontaminate and extend the shelf life of products and to control bacteria, such as *Salmonella* spp. or *Escherichia coli* [24].

The acetic acid solutions (M1 and M2 – control solutions) used in this study have presented an antimicrobial effect versus *E. coli.*; it was observed that the antimicrobial effect was higher in the case of the cellulose acetate solutions in acetic acid (P1 and P2 – tested solutions), exceeding even the effect of the antibiotic (Gentamicin) used as a reference substance.

In literature have been presented study on the antimicrobial activity of acetic acid on some antibiotic resistant bacteria strains such as *Streptococcus spp.*, *Staphylococcus aureus*, *E. coli*, *Pseudomonas aeruginosa* and *Proteus* spp. [25]. For this, the diffusion method was used and different concentrations of acetic acid (0.5%, 1%, 1.5%, 2% and 2.5%). At all concentrations tested, acetic acid was found to be



able to inhibit bacterial growth. Even at the low concentration of 0.5% acetic acid, a bacterial inhibition zone from 1.3 cm to 1.8 cm was obtained. At higher concentrations of 1% and 1.5%, the inhibition zone ranged from 2 cm to 2.2 cm respectively [25]. In our investigation were used same bacteria species that were described by Pundir and Pranay [25]. Also, it was observed the inhibition zone of acid acetic solutions (M1 and M2) which ranges between 1.85 cm (in *E. coli*) and 2.65 cm (in *S. aureus*), but the cellulose acetate solutions in acid acetic have more pronounced effect. The inhibition zones were as follows: for P1 between 2.15 cm and respectively 2.45 cm against *E. coli* and for the P2 was obtained 3.15 cm and respectively 3.55 cm against *S. aureus*.

Raftari et al. [26] reported that the action of acetic acid against the *S. aureus* is more pronounced than that against *E. coli*, at the tested concentrations of 1%, 1.5% and 2%. They also found that the inhibitory effect of bacterial growth was lower in *Clostridium spp*. The present study, the acetic acid solutions tested against *S. aureus* have shown a good antibacterial activity, similar or even higher than the antibiotic used as reference substance (gentamicin) and having the inhibition zone between 2.4 cm (M1) and 2.65 cm (M2). Instead, it has been remarked that the antibacterial activity of cellulose acetate solutions in acetic acid far exceeded the activity both of reference substance (gentamicin) and of acetic acid solutions (M1 and M2), given an inhibition zone between 3.15 cm (P1) and 3.55 cm (P2).

Thus, Liakos et al. [27, 28], using cellulose acetate fibers impregnated with essential oils from various aromatic herbs, tested the antimicrobial effect against *S. aureus, E. coli*, and *C. albicans*. Cellulose acetate fibers containing cinnamon, lemongrass and peppermint oils indicated high antimicrobial activity against *E. coli* and *C. albicans*. Also, Anitha et al. [29] have tested fibrous membrane of cellulose acetate impregnated with ZnO against methicillin-resistant *S. aureus* (MRSA), *E. coli and Citrobacter freundii*. Significant inhibition zones were observed for ZnO embedded CA. The diameters of these inhibition zones around the membranes were 27 mm, 22 mm, 14 mm for methicillin-resistant *S. aureus* (MRSA), *E. coli* and *Citrobacter freundii*, respectively. In this paper, the P1 and P2 solution showed similar and even better results compared to the ZnO-impregnated cellulose acetate fibrous membrane, which was studied by Anitha et al. [29]. For *S. aureus* the diameters of inhibition zone were 3.15 cm at P1 and 3.55 cm for P2, respectively. Also, for *E. coli* the diameters of the inhibition zone of P2 and P1 were 2.45 cm and 2.15 cm, respectively.

Cellulose acetate currently is the most commonly used as biopolymer and has multiple uses in the pharmaceutical industry and in medicine because it is stable, biocompatible and bioresorbable. Thus, studies on the antimicrobial effect of cellulose acetate solutions in acetic acid are of great relevance and importance [15, 16].

#### 4. Conclusions

The results of in vitro tests on the antimicrobial activity indicated that cellulose acetate solutions in acetic acid had a good antimicrobial effect, proportional to the concentration of the active substance. The results suggested that the antimicrobial effect of the solutions studied was superior to that given by the antibiotic and antifungal agents. The acetic acid used as a solvent for cellulose acetate had an antibacterial effect close to that of the antibiotic / antimycotic used, but much lower than that of the cellulose acetate samples (**P1**, **P2**). The results shown that the solutions studied had an increased antimicrobial effect against microorganisms compared to acetic acid. From the observed antimicrobial effect of samples **P1** and **P2**, it has been demonstrated that they can be used to counter microbial and fungal infections. Future research should be undertaken to establish the minimum inhibitory concentration of the two solutions and to highlight their potential for application in the medical field.

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