UNIVERSIDAD DE INVESTIGACIÓN DE TECNOLOGÍA EXPERIMENTAL YACHAY

Escuela de Ciencias Biológicas e Ingeniería

Antimicrobial Properties of Plant Fibers for Biomedical Applications

Trabajo de integración curricular presentado como requisito para la obtención del título de Ingeniera Biomédica

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Dedication

This work is dedicated to my beloved mother and sister Andrea for supporting me in this crazy idea of studying science.

Karla Miño Toro

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Karla Miño Toro

Resumen

Actualmente, en el mundo de los materiales existe una creciente demanda por biopolímeros que reemplacen a sus pares sintéticos. Una alternativa podría ser fibras plantas, las cuales son mayormente usadas por sus características de disponibilidad, biocompatibilidad y degradabilidad.

Sin embargo, propiedades antimicrobiales de las fibras de plantas no han sido reportadas, generalmente este biopolímero es modificado con un agente antibacterial, metales o mezclas para darle actividad antimicrobial. El objetivo de esta investigación fue probar fibras de diferentes plantas para encontrar propiedades antimicrobiales basado en sus propiedades físicas, químicas y morfológicas. Para este propósito, las fibras de plantas fueron caracterizadas para conocer su estructura y composición usando técnicas comunes como difracción de rayos x (XRD), espectroscopia de infrarrojos por transformada de Fourier (FT-IR) y microscopia electrónica de barrido (SEM). Pruebas antibacteriales y de inhibición con *Escherichia coli* fueron realizadas en Yachay Tech. Los resultados indicaron que dos de las siete fibras estudiadas mostraron propiedades inhibitorias, esta investigación podría tener un significativo impacto en aplicaciones biomédicas.

Palabras Clave:

Propiedades antimicrobiales, fibras de plantas, celulosa

Abstract

Nowadays, in the world of materials, there is an increasing demand for biopolymers that replace their synthetic pairs. One alternative is plant fibers, which are largely used by its availability, biocompatibility and degradability characteristic. However, antimicrobial properties of plant fibers have not been reported, usually, this biopolymer is modified with antibacterial agents, metals or blends to give it antimicrobial activity. The objective of this research was to test fibers from several plants to find antimicrobial properties based on physical, chemical and morphological characteristics. For this purpose, the plant fibers were characterized in order to know their structure and composition using common techniques such as X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FT-IR) and scanning electron microscopy (SEM). Antimicrobial and inhibitory tests were performed against *Escherichia coli* in Yachay Tech. The results indicate two of seven fiber studied shown inhibitory properties, this research could have a significant impact on biomedical applications.

Keywords:

Antimicrobial properties, plant fibers, cellulose

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INTRODUCTION

The emerge the new products with antibacterial properties are strongly needed to fight against bacterial infection due to the ability of these microorganisms rapidly colonize surfaces, migrate from the skin to a surgical wound through the incisional route despite all of the asepsis methods applied before, during and after surgery(1). Another important bacterial characteristic is the capacity of mutating and becoming antimicrobial resistant as a protective measure, therefore current antibiotics cannot effectively fight against common bacterial infections that in the past years were controlled. According to the World Health Organization (WHO), infectious diseases are becoming untreatable with standard antibiotics prescriptions, as a result, this leads to lethal consequences to patients as well an increment of permanence in hospitals and in medical cost(2).

In addition, the Centers for Diseases Control and Prevention (CDC) reported in 2013 two million people were contaminated by antibiotic resistance bacteria, as a consequence 23000 died in EEUU, whereas WHO reported 500000 deaths produced by drug-resistant each year(3,4). Moreover, antimicrobial resistance not only affects human health but also animals, food and environment resulting from the misuse and overuse of antibiotics, such as prescription in cases where they are not needed as in virus infections, or used without prescriptions and not taking them in the required dose and time. Besides, antibiotics are adjuvants in surgical procedures, organ transplant, cancer therapy, joint replacements, among others, that is the reason why is important combat and provide new solutions against antibiotic-resistant bacteria and avoids coming back to a pre-antibiotic era where common diseases could kill millions of people(3,5).

In Ecuador and Latin America, the situation is not different from the rest of the world, bacterial infections are acquired mainly by two sources through the community and nosocomial infections(6). According to the National Institute of Public Health Research (INSPI) during 2015, the resistant bacteria more frequently reported in the Ecuadorian health system were *Staphylococcus aureus* of community**,** hospital, and intensive care unit origin**;** *Enterococcus faecalis*, *Enterococcus faecium***,** *Acinetobacter baummanii*-complex, *Pseudomonas aeruginosa, Escherichia coli,* and *Klebsiella pneumoniae*(7).

Equally important to mention that most of these bacteria are associated with surgical procedures, as in the case of *Staphylococcus aureus* has been found in the placement of implants, grafts, and prostheses(8)**.** In addition, *Enterococcus faecalis, Acinetobacter baummanii, and Escherichia coli* were reported in surgical site infection in an orthopedic and traumatology procedures in a hospital in Guayaquil(9)**,** likewise, they have the capacity to colonize blood vessel and urinary catheters, pacemaker electrodes, neuro implants, cardiac valves, among other short and long term biomedical devices(1).

At the present day, there are several proposal or alternatives to battle against to bacteria, some of them include a therapeutic non-compound substitution of the antibiotic for systematic or invasive infections, which may be applied orally and parenteral. Some of these therapies consist in: the use of antibodies in order to inactivate the pathogens; wild-type or engineering bacteriophages that target and kill bacteria, lysins to destroy their cell wall, and vaccines to prevent bacterial infection and avoid the use of antibiotics. The inconvenience with these alternatives are still in preclinical and clinical phases, hence it will take many years and the investment of millions of dollar to be available in the market, and only in few cases may be accessible for 2025 (10).

Different approaches have biomedical devices and implants which seek to avoid the bacterial attachment on their surfaces and the formation of biofilm. The sources of contamination of those devices usually are planktonic bacteria that may be found circulating in the vascular system(11) likewise pathogenic bacteria from the patient or medical personnel skin that may travel from the incision wound to the device placement site during the surgery or in postoperative care(1). The bacterial mechanism of attachment begins with the attraction of the bacteria to the biomedical device surface by hydrophobic and electrostatic interaction, temperature, van der Wall and hydrodynamic forces(12), as well as, host-derivate proteins such as collagen, fibronectin, fibrinogen that allow the binding of the bacteria(8). The attachment is produced by the adhesins proteins secreted from the bacteria, as a result, stimulate the excretion of extracellular polysaccharide substances also called slime layer which favors bacterial aggregation, and the creation of the biofilm that is three-dimensional polymeric microenvironment that provides protection against antibiotic drugs and the host immune system, and nutrients which are essential for their continuous proliferation (1,8,12).

Basically, antibacterial or antimicrobial surfaces have two important characteristics, the first one is a bactericidal effect that directly causes bacterial death and the second one is

an anti-biofouling effect that prevents bacteria adsorption(12). Surface coating and surface modification are the main strategies to provide these two features to biomedical devices, and they can be achieved through physical, mechanical and chemical methods. In surface coating, diversity of antimicrobial agent is loaded on the surface of the device and then released them over time. Antimicrobial agents frequently used in the biomedical applications are silver, hydroxyapatite, poly-ammonium compounds, antibiotics and also bioinspired such as antimicrobial peptides, anti-quorum-sensing molecules, essential oils, bacteriolytic enzymes. Despite their promising antibacterial power, toxicity, effectiveness, bacterial resistant and releasing time still need to be investigated to fulfill all of the safety requirements of antibacterial coating surfaces need to achieve. (13,14)

In a surface modification, the properties of the surfaces are modified and improved by chemical or physical means, in this specific case to give them the antimicrobial feature. The most used chemical techniques for this purpose are functionalization, polymerization, and derivatization. In these surface treatment methods, antibacterial agents are adsorbed or immobilization on the surface with the help of polymeric molecules, chains of functional groups, hydrophobic molecules or nanoparticles, besides they can be antibiotic agents by themselves. The agents are immobilized by covalent bonding or atom radical transfer, and examples of these are covalent bonding and hydrophobic polycations of quaternary ammonium salts, single-walled carbon nanotubes, and alkylated polyethyleneimines among others. (13)

The goal of a physical method of surface modification is to mimic structures and topographies observed in nature that possess the ability to avoid bacterial attachment or even may kill bacteria if they got to stick on the surfaces, together with superhydrophobicity and self-cleaning features(12,15). The natural models so far studied are plant leaves (lotus, taro, and rice), animal skin (shark and gecko) and insect wings (cicada and dragonfly)(12,13,16). Cicada wings show an array of nanopillars structure which has a bactericidal effect against Pseudomonas aeruginosa, this kind of topography allows the cell attachment but also cause the mechanical disruption of the cell membrane, therefore, cell death(15). Bio-inspired micro and nanopatterns have been mimic in material like black silicon and in polyurethane catheter taking the topographical structure of cicada wing and shark skin respectively. (12)

PROBLEM STATEMENT

The study of unexplored antimicrobial natural biopolymers is a promising area where is required deeper research, one of the advantages of this type of biomaterial that may not show an unfavorable effect on mammalian cells(15), equally important is that they may not need antibiotics or chemical treatments, therefore, can offer an alternative to short and long term medical devices infection and antibacterial resistance. However, there are still pending issues to be unrevealed, such as the exploration for new models in nature as well as the better comprehension of the effects of the geometry, dimension, interspacing, effectiveness over time and how these influence in the bacterial attachment, biofilm-forming or death and the interaction with mammalian cells.(13,15)

Cellulose is the most abundant biopolymer found in nature, and it has been used in a diversity of products in health care, textile and food industries by the reason of its biocompatibility, biodegradability, and renewability, also non-toxic effects and low cost. (17–19) In addition, cellulose has been employed as an emulsifier, stabilizer, dispersion agent, thickener and gelling agent(20) which make it suitable for the preparation a variety of hygiene, food packaging, fabrics, wound care dressing products.(17,20)

Furthermore, cellulose can be derivatized and functionalized(21) in order to improve its properties, for this reason in many research to add the antimicrobial characteristic cellulose has been modified with natural polymer (sodium alginate, chitosan)(18,20), synthetics polymers(PVA,PAA)(17), nanoparticles (silver, gold)(22) and natural derived substances (cinnamon oil, curcumin)(18,19) to develop aerogels, films, paper sheets, biocomposites, and hydrogels. However, some of these blends could have toxic effects, and provoke a severe immune response(22), hence it is important to further research in alternative non-chemical mechanisms such as antibacterial surface structures.

To my knowledge in current literature, antibacterial properties of plant fibers based on their physical, chemical and topographical structure has never been reported, therefore I hypnotized that it is possible to find antibacterial properties in plant fibers based on their physical, chemical and surface morphology without chemically modifying their composition due to the enormous flora diversity that exists in our country that has not been explored and studied yet.

GENERAL AND SPECIFIC OBJECTIVES

The main objective of this research is to test fibers from several plants to find bactericidal or bacteriostatic effect based on the physical, chemical and morphological characteristics.

Specific Objectives

1. Extraction of cellulose fibers from chosen plants.
2. Characterization of the fibers by X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FT-IR) and Scanning electron microscopy (SEM) in order to know their structure and composition.
3. Testing of fibers by the agar dilution method against gram-negative bacteria *Escherichia coli* to find a bactericidal effect.
4. Measurement of optical density at 600nm in bacterial cultures with cellulose fibers to determine if there is a bacterial growth inhibition.

MATERIALS AND METHODS

Five cellulose microfibers and two fibers were extracted from seven different natural sources (23). The cellulose fibers were labeled as F1, F20, T1, T2, T3, CB and CC.

Fiber characterization

The physical and chemical properties of the extracted cellulose fibers were obtained by XRD, FT-IR and SEM techniques that were performed at ESPE University. Fourier transform infrared spectroscopy is a non-destructive and fast technique that allows the chemical identification of compounds. FT-IR spectra show the presence or absence of different functional groups that determine the qualitative and quantitative components of the structure of cellulose fibers in the infrared region(24). Every spectrum portrays the frequencies of vibration of the bonds the atoms that are represented by adsorptions peaks as a unique fingerprint of material(25). FT-IR spectra were recorded using Spectrum Spotlight 200 FT-IR instrument (Perkin Elmer, USA), the wavelength range was between 4000 to 600 cm^{-1} with a total number of scans of 36 and a 4 cm^{-1} wavelength resolution.

X-ray diffraction is another non-destructive technique that is commonly used to determine the crystalline structure of the materials as well as physical and chemical properties. The atomic position within the lattice planes defines the peak intensities represented by x-ray diffraction patterns that permit to identify crystallinity, grain size, crystal orientation and defects, phases and other structural specification.(26,27) X-ray diffraction measurements of the seven cellulose fibers were obtained using an EMPYREAN diffractometer (PANalytical, NL) in a Bragg-Brentano configuration at 40kV and 45A and monochromatic X Rays of Cu K-α wavelength (λ = 1.541 Å) using a Ni filter.

The crystallinity index (CrI) was calculated with the equation for each cellulose fiber according to the method described by Segal *et al* (1959).

$$CrI(\%) = \frac{I_{002} - I_{am}}{I_{002}} x100\%$$

Where I_{002} is the maximum intensity of the 002 lattice diffraction peak and I_{am} is the intensity shown by the amorphous part of the cellulose samples.(28)

In scanning electron microscope (SEM), the images are obtained by signals emitted when samples interact with the electron beam in order to study the surface morphology and topography of a material. (29) MIRA 3 (TESCAN, CZ) field emission scanning electron microscope (FEG- SEM) was used to examine the morphology and surface structure of the cellulose fibers.

Antimicrobial surface activity

The seven types of cellulose fibers were tested by agar dilution method with two different *Escherichia coli* strains, TG1 and ACTT 25922, 400µl of 24 hours bacterial culture were spread in a petri dish with dry agar medium. Cellulose fibers (20 mg) were added in 1ml of distilled water, and 10-fold serial dilution was made, 3 µl of each cellulose dilution was put in 1 to 4 numbered spaces inside the plate, number 5 space correspond to hydrated cellulose fibers, in addition as positive control was used 1 µl of ampicillin and then incubated at 37 °C for 24 hours.

Bacterial growth curve

Optical density at 600 nm (OD 600) of the seven cellulose fiber in an *E. coli* TG1 culture was measured using UV-Vis spectrophotometer (NanoDrop 2000, Thermo Fisher Scientific Co,. Ltd,. MA, USA) every 30 minutes by 12 hours. First, 100µl of E. coli TG1 was inoculated in 2ml Luria Bertani (LB) Broth and incubated by 12 hours, 180rpm, 37°C. Then, solutions of each of seven cellulose fibers 2% w/v in LB medium (1.65 ml) and bacterial culture (0.35 ml) were prepared, as well as, negative control and positive control with 2 µl of ampicillin, and incubated at the same initial conditions for 12 hours. A 10 µl aliquot of each solution was sampled and measured three times every 30 minutes, 24 measurements in total. Moreover, cellulose fiber solutions at same concentration with LB medium were prepared, incubated and measured in order to quantify the interference of cellulose in the final OD measurement, it should be noted that these measurements were taken at the end of 12 hours.

RESULTS AND DISCUSSION

Fibers characterization

The Fourier transform infrared (FTIR) spectra of the five microfiber F1, F20, T1, T2 and T3, and two fibers CB and CC are shown in fig 1. Every spectrum of the seven cellulose fibers exhibits common characteristics as it is indicated in fig 2. Two principal absorptions bands arise at the region of 3650 – 2800 cm^{-1} and 1650 -600 cm^{-1}. The first absorption band placed in the wave number range of 3650 -2800 cm^{-1} represent hydrogen-bonded OH and CH stretching which are a feature of polysaccharides, the second band located from ~ 1650 to 900 cm^{-1} is the typical fingerprint of cellulose. The peak observed at ~ 1615 cm^{-1} corresponds OH bending of water absorbed from cellulose and the absorption band at ~ 1155 cm^{-1} is attributed to COC glycoside ester bond. The bands around ~1420 cm^{-1}, ~1340 cm^{-1}, ~1315 cm^{-1}, ~1275 cm^{-1}, and ~1030 cm^{-1} refer to CH_2 symmetric bending, CH_2 stretching vibration, -OH bending, CH bending and CC ring breathing. In addition, the absorption around ~1430 cm^{-1}

and ~896 cm^{-1} are used to study the crystalline structure and amorphous region of cellulose respectively.(24,30)

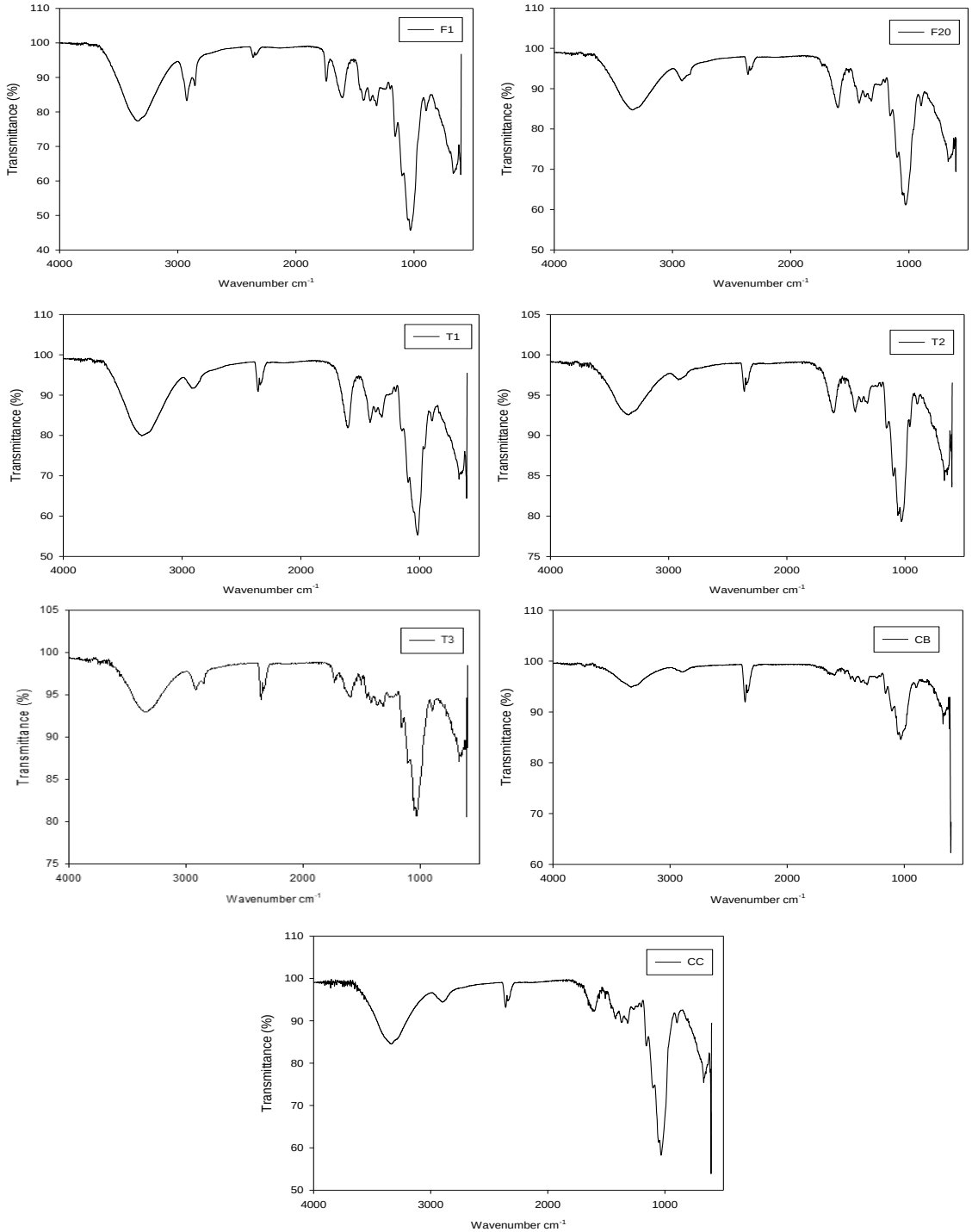

Figure 1 FT-IR characterization of seven cellulose fibers. First row from right to left F1 and F20, second row T1, and T2, third row T3, and CB, and fourth row CC fiber.

The FT-IR results indicate that the chemical process of cellulose extraction from the seven natural sources was successfully achieved. Cellulose is the principal component of the samples, with the exception of F1 microfiber that shown minor contamination in the band at ~1740 cm^{-1} that indicates lignin carboxylic acid or hemicellulose acetyl and ester groups.(23)

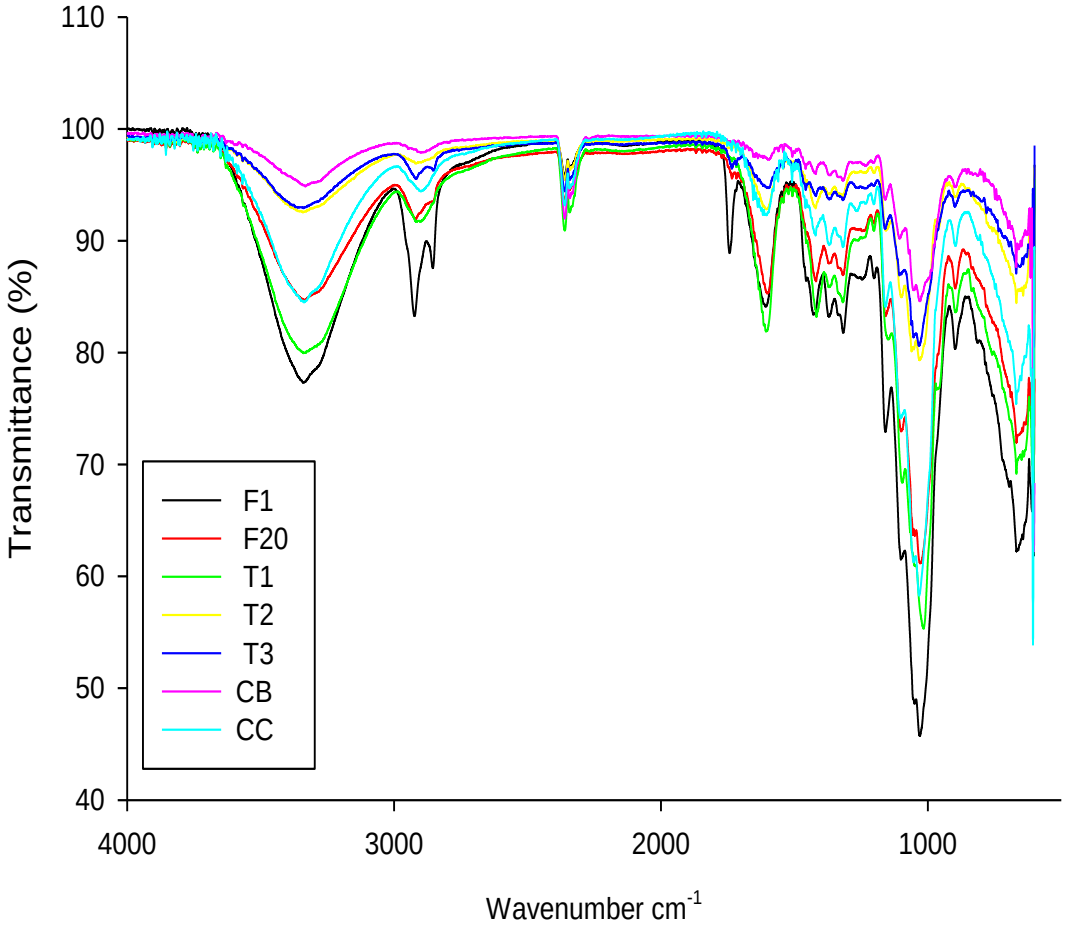

Figure 2 FT-IR spectra of the seven cellulose fibers

X-ray diffraction patterns of the seven cellulose samples are shown in fig 3 and fig 4. According to the literature, the crystalline structure of the cellulose is because of Van der Wall forces and bonding interaction with neighboring molecules, unlike hemicellulose and lignin that have a natural amorphous structure. (23)

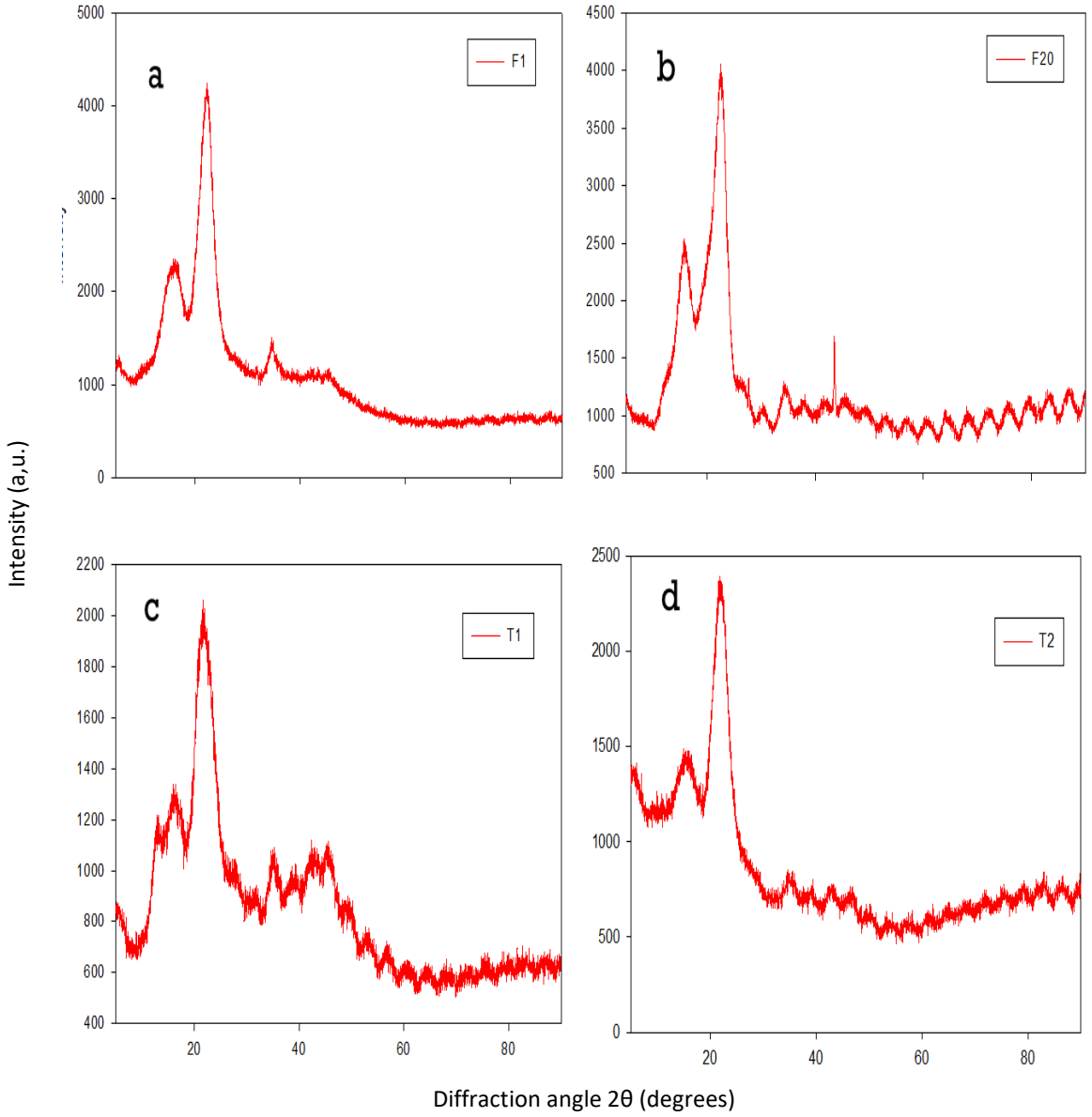

Figure 3 X-ray diffraction patterns of a. F1, b. F20, c.T1, d. T2

The patterns of seven samples shown the presence of the typical cellulose diffraction peaks around 2ϑ= 18°, 22° and 35° which are the result of the entire chemical process applied during the fiber extraction and has an impact in the crystallinity index. Two major and well-defined diffraction peaks are taking in account for the calculation of this index, the peak at 2ϑ= ~ 22| ascribed to the crystalline structure of the cellulose, and 2ϑ= ~ 18 assigned to the amorphous region of the samples. (31)

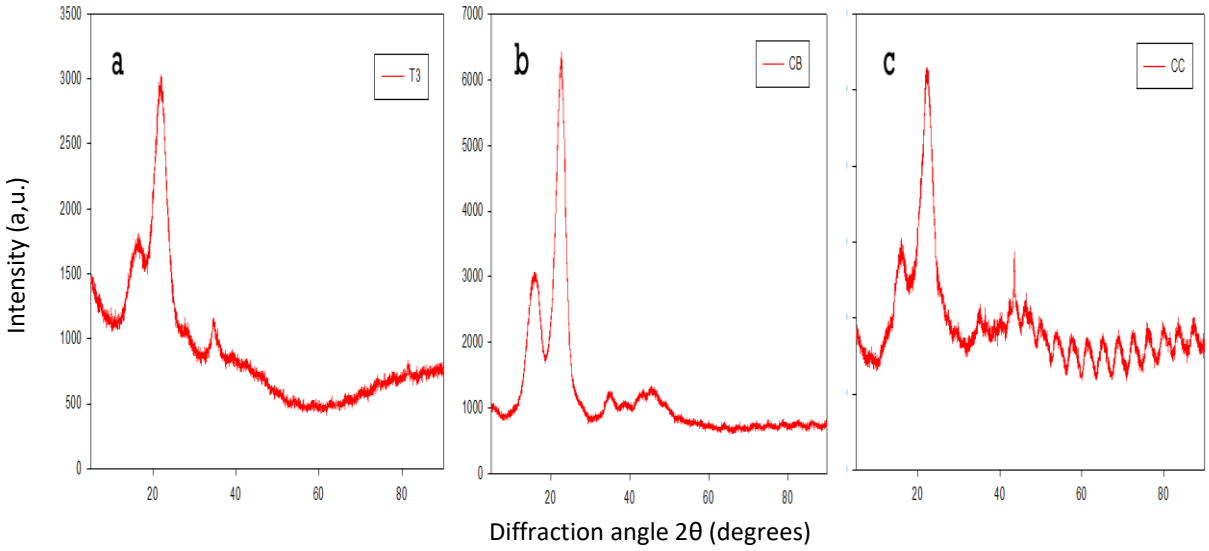

Figure 4 X-ray diffraction patterns of a. T3, b. CB and c. CC fibers

In table 1 are presented the crystallinity index of the seven cellulose samples, where CB fiber has the highest degree of crystallinity 73.7%, whereas T1 has the lowest 50.2%. F1, F20, T2, T3, and CC have a degree of crystallinity mean of 55.08%. The extraction of seven cellulose samples followed the same chemical protocol, therefore the differences in the X-ray diffraction patterns and crystallinity index could be due to the origin of plant fibers. (32)

FIBERS	CRYSTALLINITY INDEX
F1	60.0
F20	56.9
T1	50.2
T2	52.8
T3	50.3
CB	73.7
CC	55.4

Table 1 Crystallinity index of the seven cellulose fibers

The morphology and topography of the seven cellulose fibers were analyzed by scanning electron micrographs displayed in fig 5. F1, CB and CC samples present tubular microstructures, CC fiber also present small porous segment. On the other hand, F20, T1, T2, and T3 exhibit flake-like structures. Moreover, SEM images highlight the singularity of sizes, porosity, and shapes that possess every cellulose fiber, considering further their distinctive chemical and physical properties make every one of them a unique natural combination.

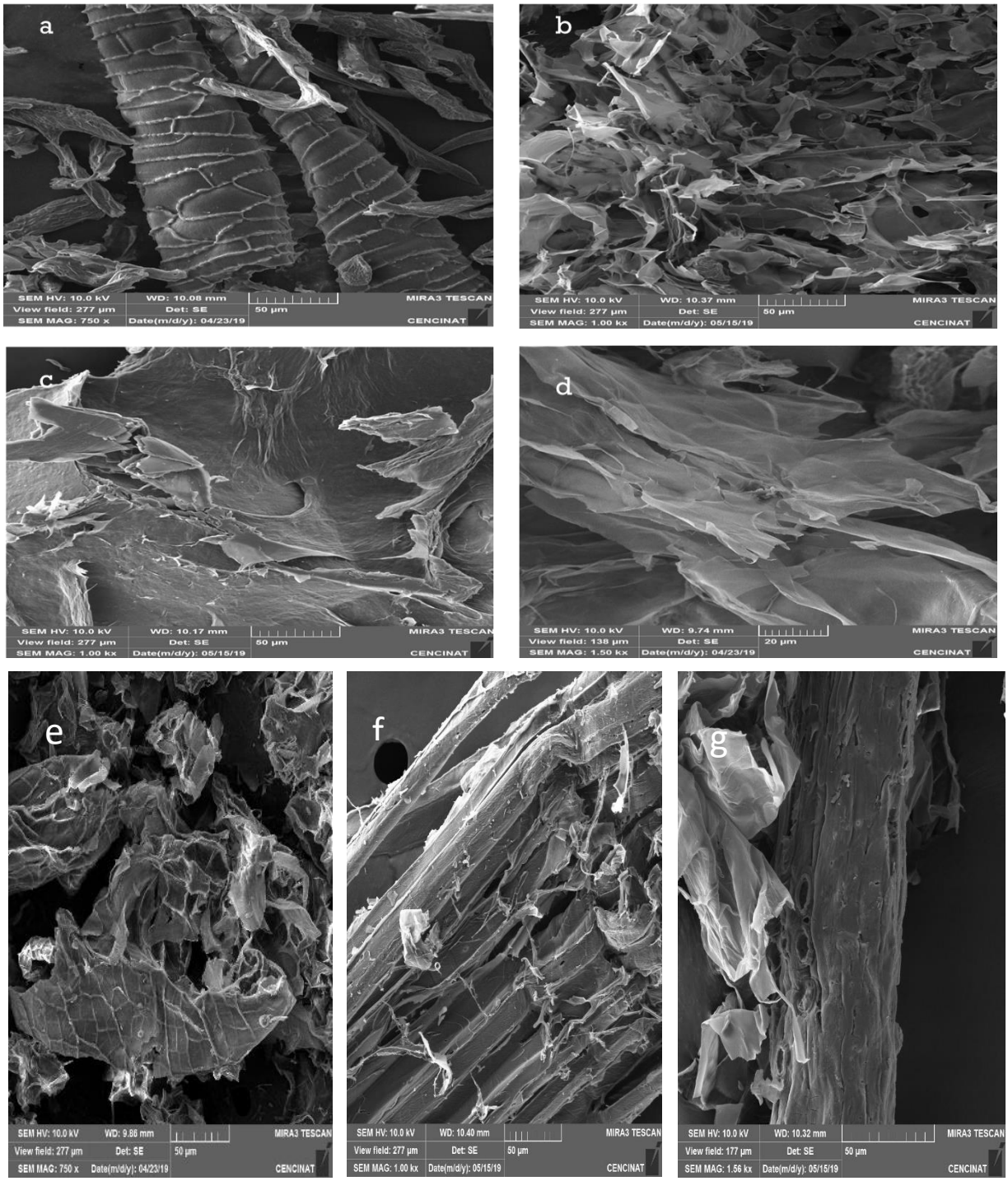

Figure 5 SEM micrographs of a. F1, b. F20, c. T1, d. T2, e. T3, f. CB and g. CC fibers

Antimicrobial activity

Antimicrobial effect of the seven cellulose fibers was tested against two strains of *Escherichia coli* TG1 Fig. 6 and ATCC 25922 Fig. 7 by agar dilution method. This type of essay is frequently used to test both bactericidal and bacteriostatic effects of antimicrobial products and also to determine the minimal inhibitory concentration(MIC) in which microorganisms are susceptible to an inhibitor agent(33). In this qualitative test, the area around the sample that not present bacterial growth is considered positive for antimicrobial activity.

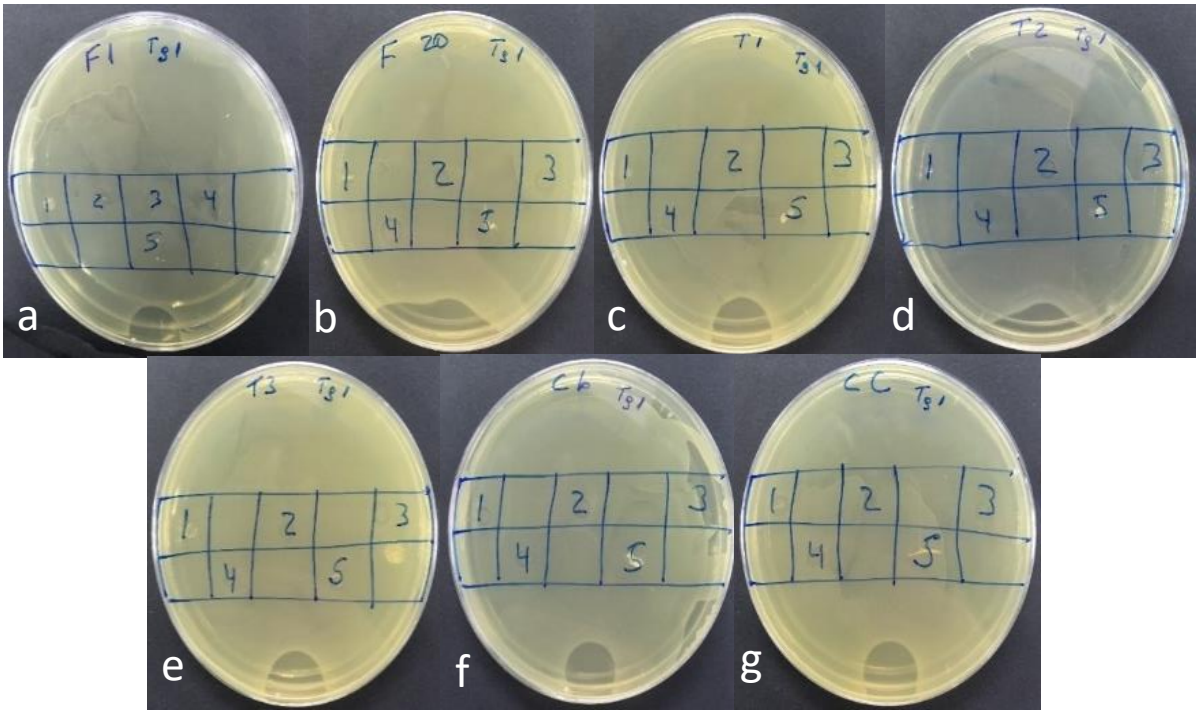

Figure 6 Agar dilution test against E. coli TG1 a. F1, b. F20, c. T1, d. T2, e. T3, f. CB and g. CC fibers

For the this test, four different concentration of cellulose samples in distilled water were placed in numbered spaces within the plate, in space number one was placed 20mg/ml, number two 2mg/ml, number three 2 x 10^{-1} mg/ml, number four 2 x 10^{-2} mg/ml, and in number 5 a small number of hydrated cellulose fibers, then incubated at 37 °C for 24 hours. The results in both *Escherichia coli* strain tests did not present an antibacterial activity in any of the concentration of the seven samples compared with antibiotic control that the mark is clearly noted at the bottom of every petri dish; therefore, bacterial growth can be seen around where the dissolutions were settled.

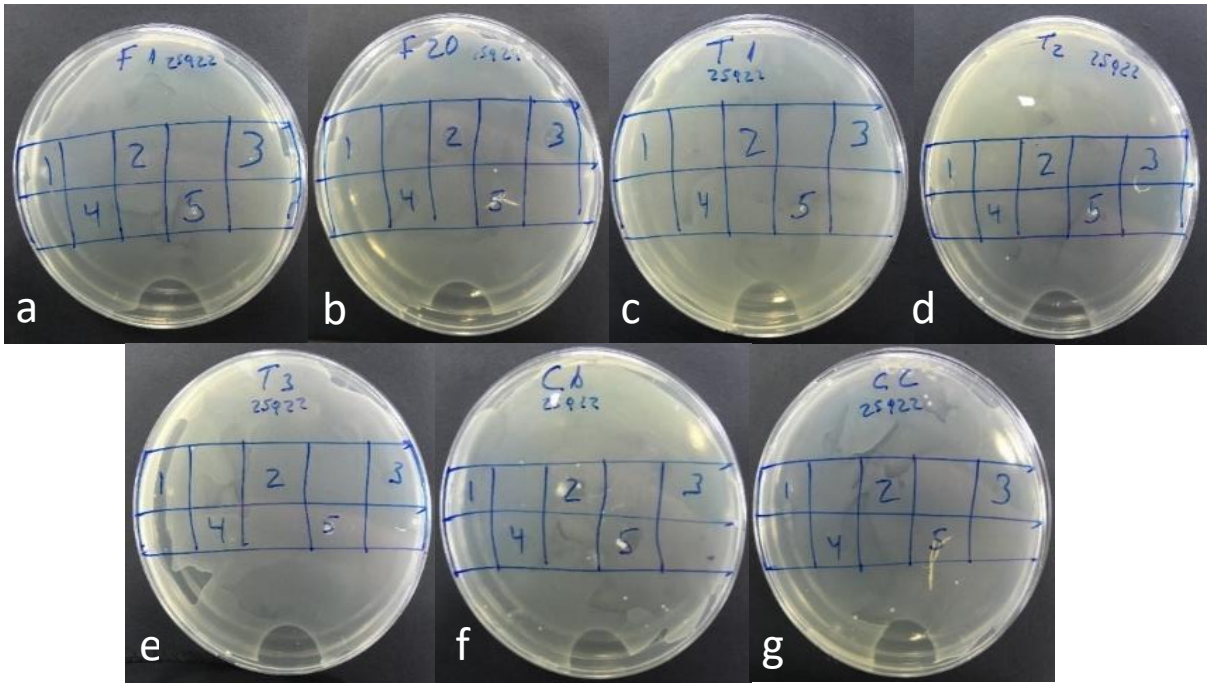

Figure 7 Agar dilution test against E. coli ATCC 25922 a. F1, b. F20, c. T1, d. T2, e. T3, f. CB and g. CC fibers

The minimal inhibitory concentration could be affected by factors such as characteristics of the microorganism, temperature, size of the inoculum, and low water solubility of the compounds as cellulose. (34)

Bacterial growth inhibition

Bacterial growth curves were performed by measured optical density at 600nm of bacterial suspension (O.D. = 0.05) with each cellulose fiber in a concentration of 20mg/ml every 30 minutes over 12 hours in order to study the growth rate of *Escherichia coli* TG1 shake cultures in the presence of each cellulose fiber and LB medium. The plot was built with the mean of three measurements taken every 30 minutes with a total of 24 measurements and each cellulose bacterial growth curve was compared against *E. coli* cultures with (positive control) and without (negative control) ampicillin as is shown in fig 8. F1, F20, T1, T2, and T3 fibers cultures (Fig. 8 a, b) present a notably higher level of bacterial growth rate compared with both controls, nevertheless a different behavior was observed with CB and CC fibers (Fig. 8 c) that show a lower bacterial growth rate than *E. coli* culture and the decrease tends to have a higher range as culture time increases.

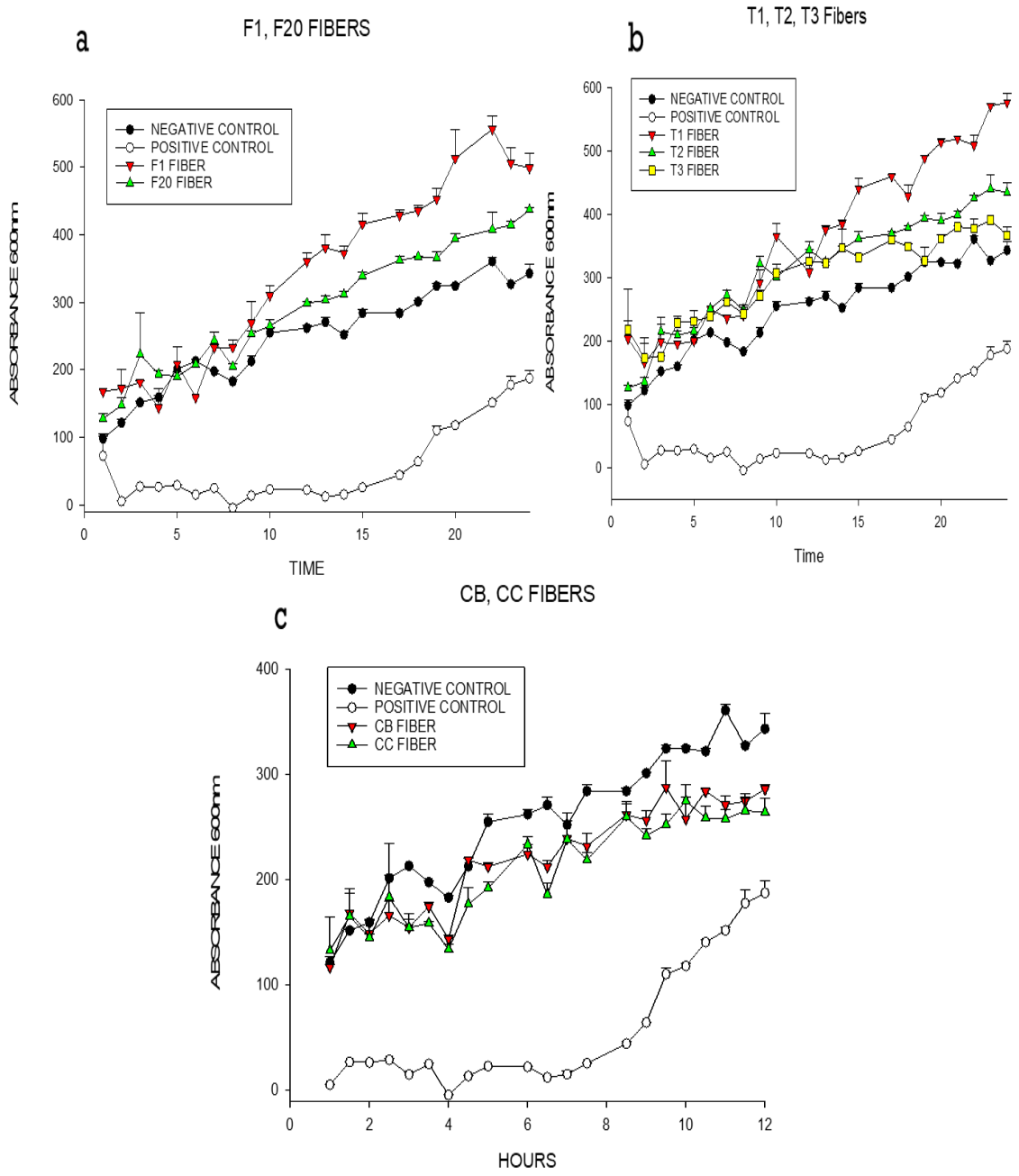

Figure 8 Bacterial growth curves a. F1 and F20; b. T1, T2, and T3; c. CB and CC

In the graphs below are shown the bacterial growth behavior of the cultures with every fiber, however, the measurements are affected by the optical density of cellulose, for this reason the same concentration of each cellulose fibers was diluted in LB medium and taken the optical density in order to remove the cellulose background. In Fig 9 the 24th final reading of the optical density of seven cellulose bacterial cultures and two controls are represented in dark red bars and in dark yellow bars are the last measurement without

cellulose interference. F1, F20, and CB showed a ~ 50% decrease of the optical density, whereas that T1 and T3 displayed less 5% of interference of cellulose background.

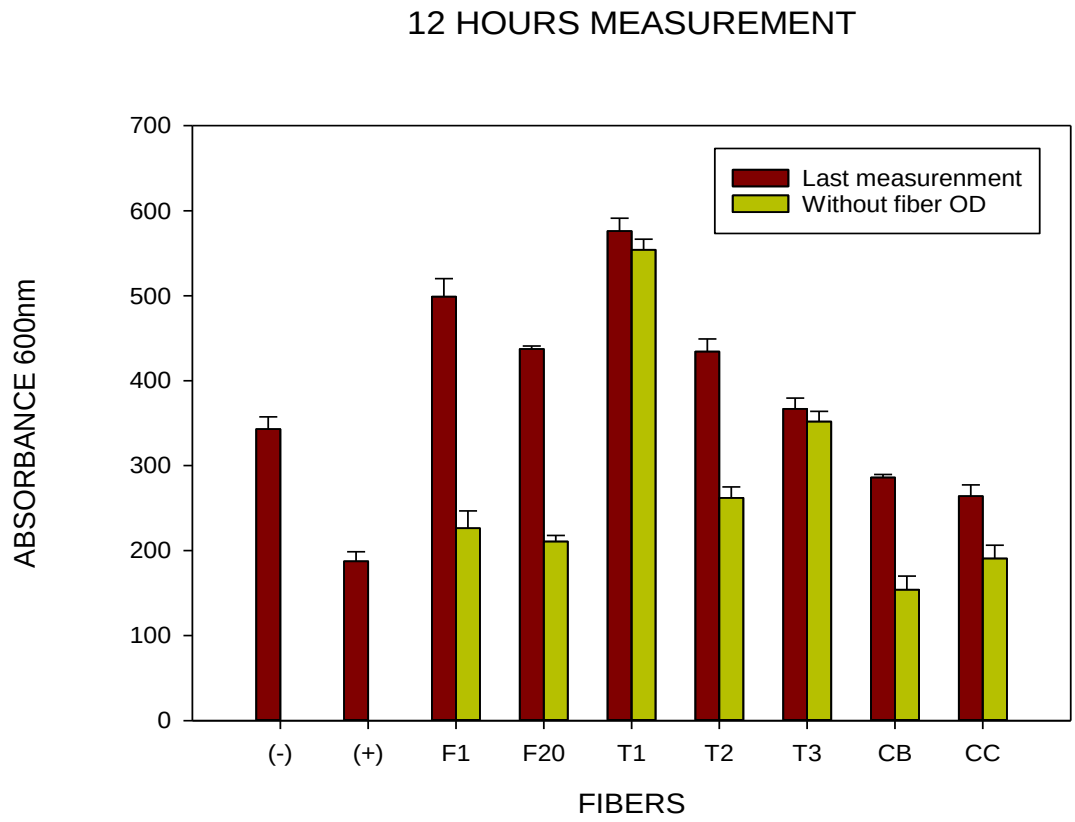

Figure 9 Barr chart of final optical density measurement of fibers cultures (dark red) and measurements without cellulose background (dark yellow)

The last measurements of the seven bacterial cultures with the plant fibers without the interference of cellulose are displayed in Fig 10. The graph confirms that CB and CC fiber have an inhibition bacterial growth rate similar to antibiotic control. F1, F20, and T2 present a lower bacterial growth rate than E. coli culture, and just T1 and T3 show a higher rate than

E. coli culture and antibiotic control. The mechanisms of how cellulose fibers affect the bacterial growth rate should be elucidated in posterior research.

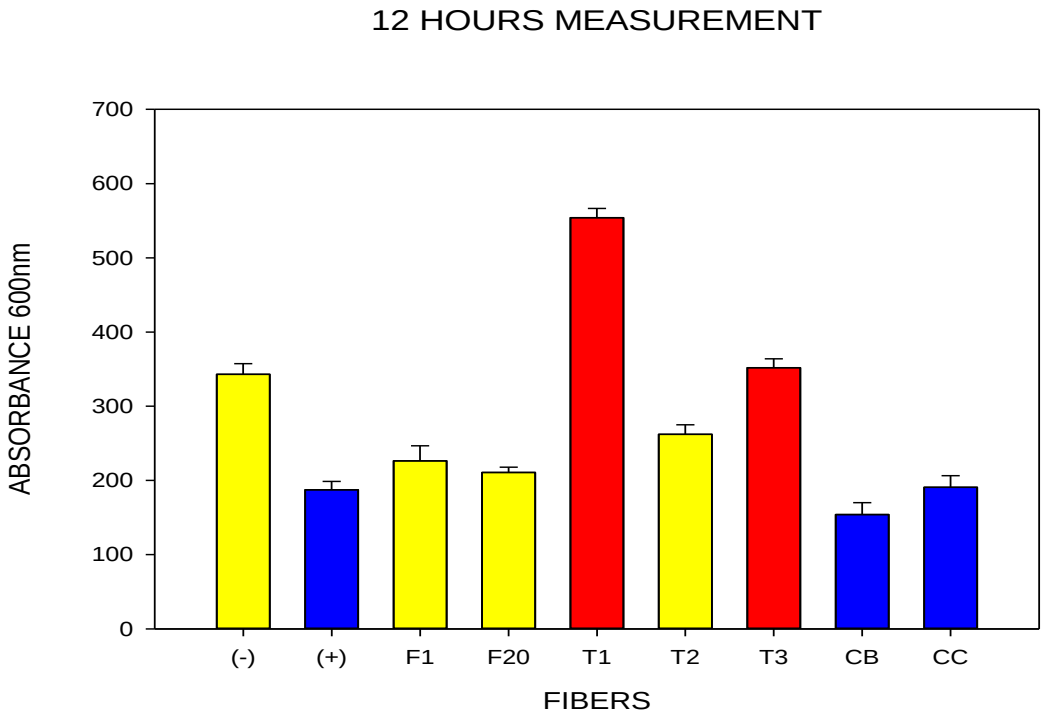

Figure 10 Barr Chart of Final optical density measurement without cellulose interference

Antimicrobial activity of plants usually is associated to their secondary metabolites and chemical components such as phenols, flavonoids and triterpenoids in aqueous and organic extracts and essential oils.(35) Nonetheless, antibacterial activity of cellulose and other types of plant fibers have not been reported, commonly cellulose-based materials are modified with antibacterial agents in order to improve them because of the removal of the antimicrobial compounds of the plants in the cellulose extraction process. One possible explanation of the decrease of the bacterial growth rate in the cellulose cultures may be due to the morphology of the surface of the fiber as it is indicated in Fig. 5f and 5g, CB and CC fibers display unique nanostructures. In current studies have been proven that a diversity of nanostructures for example lamellar structures, nanospikes, nanopores, nanopillars, nanopatterned array among others with diameters from 10 to 200 nm, and heights from 150 nm to 10 µn have antibacterial power against *P. aeruginosa, E. coli, E. faecalis*, aside from prevention of attachment and biofilm formation.(36)

CONCLUSION

The findings in the present research indicate that seven cellulose fibers studied (F1, F20, T1, T2, T3, CB, and CC) exhibit unique physical, chemical and morphological characteristics that may give them antibacterial properties. The fibers were tested against two *Escherichia coli* strain, any of them presented positive result for antibacterial activity. However, measurements of optical density at 600nm in bacterial cultures with cellulose fibers showed that F1, F20, T2, CB, and CC showed a decrease in the bacterial growth rate over 12 hours. Even more, CB and CC present a similar inhibition rate than antibiotic control. The results suggest is possible to find antibacterial properties in plant fibers based on their physical, chemical and surface morphology without chemically modifying their composition and could be used in biomedical applications.

However, for future works is recommended to test the cellulose fibers against fungi, other gram-negative and gram-positive bacteria types due to different characteristics that they exhibit for instance cell wall thickness and rigidity. Moreover, further research is needed to identify the bacterial killing mechanism of CB and CC cellulose fibers as well as their effect on mammalian cells.

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