Antimicrobial Properties of Plant Fibers

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Healthcare-associated infections (HAI), or nosocomial infections, are a global health and economic problem in developed and developing countries, particularly for immunocompromised patients in their intensive care units (ICUs) and surgical site hospital areas. Recurrent pathogens in HAIs prevail over antibiotic-resistant bacteria, such as methicillin-resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa*. For this reason, natural antibacterial mechanisms are a viable alternative for HAI treatment. Natural fibers can inhibit bacterial growth, which can be considered a great advantage in these applications.

plant fibers antimicrobial properties biomedical applications

1. Introduction

Healthcare-associated infections (HAI), or nosocomial infections, include contaminations acquired by patients in the hospital, but symptoms usually appear after surgical procedures or during the recovery [1]. They represent a serious public health problem, with a high impact on the mortality rate and quality of life, thereby becoming a worldwide concern and priority. HAIs are associated with medical devices and present a significant economic burden on the public health system in developed and developing countries. HAI rates in ICUs in high-income countries are 5–10%, which is 2–10 times higher in low- and middle-income countries [2][3].

In Europe, according to data from The Healthcare-Associated Infections Surveillance Network, in its 2017 Epidemiological Annual Report, from 2014 to 2017, a statistically significant increasing trend of HAIs in Surgical Site Infection (SSI) procedures was observed. In 2017, 8.3% (11,787) of patients who stayed in intensive care units (ICU) for more than two days had at least one HAI [4]. This problem becomes even more relevant in low- and middle-income countries, as they face greater barriers and additional risk factors due to the lack of human resources, lack of medical supplies and disinfection, inefficiency in infection control, little training, and hospital staff continuous training [5]. Among Device-Associated Healthcare-Associated Infections (DA–HAI), it is common to find bloodstream and urinary tract infections associated with catheters, ventilator-associated pneumonia, and surgical site infections (SSI) due to sutures or implants [6]. The incidence of DA–HAI depends on different factors, such as frequency and duration of device use, infection control practices in the hospital, and immune status of patients [7]. Specifically, HAIs are derived from four factors: the patient, a foreign material (e.g., implants), the infectious agent, and the environment. The recurrent pathogens in HAIs are saprophytic and commensal microorganisms with the potential to become opportunistic pathogens commonly found on the skin, the oral and nasopharyngeal cavities, lungs, the vagina, the large intestine, or colon. The pathogens can spread and develop under suitable conditions

[7], where the patients eventually enter in contact with contaminated surfaces or objects (fomites). In the 1980s, HAIs were mainly caused by Gram-negative bacteria such as *Escherichia coli* and *Klebsiella pneumoniae* but antibiotic resistance and the increased use of plastic medical devices have increased bacterial infections recently. According to the Centers for Disease Control and Prevention (CDC), carbapenem-resistant *Enterobacteriaceae* (CRE), methicillin-resistant *Staphylococcus aureus* (MRSA), extended-spectrum ß-lactamases-producing *Enterobacterales* (ESBL-E), vancomycin-resistant *Enterococci* (VRE), multidrug-resistant *Pseudomonas aeruginosa* (MDRPA), and multidrug-resistant *Acinetobacter* species (MDRAs) are considered infectious agents [8].

Currently, several mechanisms have been investigated to eradicate the incidence of DA-HAI caused by multidrug-resistant pathogens (MDR). Many of the developed methods are to incorporate antibacterial properties or encapsulate antibiotics in biomedical devices, as well as to prevent the adhesion of bacteria on them. Among the most common mechanisms are polymer coatings, nanoparticle deposition, and encapsulation within the material [9]. The first seeks to make devices with polymer films, which are synthesized as an anti-infective, antimicrobial, and biocompatible coating on a substrate [10]. The second includes nanoparticles in a multilayer coating on the surface of the devices. The last includes the layer-by-layer technique assemblies and modifies the surface to encapsulate drugs, thus giving an antibiotic property to a substrate [9]. These mechanisms provide a possible solution to the proliferation of bacteria in medical devices but could also represent a risk to patients. The principal reason is their synthetic origin components, nanomaterials, and polymers with non-biodegradable characteristics. They can also produce inflammatory responses or cell death. Therefore, natural alternatives such as plant fibers are an option to eliminate possible side effects [11].

2. Pathogens in Biomedical Devices

Microbial infection is a prevalent issue among biomedical devices, both during routine procedures and surgical interventions. This problem is currently increased by the frequent use of catheters, surgical equipment, sutures, or implants needed to treat several medical conditions [12]. In fact, bacteria are the most common type of microorganism causing worldwide morbidity due to acute and chronic infections [13]. Furthermore, there is an alarming growth rate of infections because of MDR bacteria generated by the overuse of antibiotics and other factors that facilitate their development, such as persistent colonization in the facilities and biofilm mode of growth, among others [14]. The bacteria frequently related to biomedical devices are *E. coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus viridans*, *Enterococcus faecalis*, *Proteus mirabilis*, and *Pseudomonas aeruginosa* [15].

Nevertheless, there is a difference in the risk of bacterial infections between developed countries and undeveloped ones. The antibiotic resistance process is a major concern in low- and middle-income countries (LMICs). Due to a variety of available antibiotics in drugstores and poor sale regulation systems, the spread of MDR bacteria is a significant problem for these countries [16]. Contrary to high-income countries, where the regulations for antibiotic sale are strict, LMICs are more vulnerable to the appearance of more aggressive and diverse MDR bacteria [17]. The following section will specifically discuss *E. coli*, *P. aeruginosa*, *S. aureus*, and *S. epidermidis*, which are the most common bacteria found in biomedical device infections, as well as briefly review viral and fungi infections.

2.1. Escherichia coli

E. coli is the most common Gram-negative microorganism isolated from SSIs, being associated with severe morbidity and mortality rates [18][19]. In addition to SSIs, *E. coli* biofilm formation on biomedical devices is responsible for some infections in patients due to their frequent use. These acquired infections usually occur in the bladder and urinary tract [20]. Even though humans have *E. coli* as a commensal bacterium in their gastrointestinal tracts, and they help to regulate metabolism, there are other harmful strains, so-called *E. coli* pathotypes, responsible for numerous and severe infections, more exactly enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAEC), enterohemorrhagic *E. coli* (EHEC), enteroinvasive *E. coli* (EIEC), and diffusely adherent *E. coli* (DAEC) [21]. Because *E. coli* is a Gram-negative bacterium, it is resistant to several antibiotics, which represent a high infection risk in the case of pathogenic strains and new emerging clones [22]. Moreover, specific *E. coli* strains are associated with infection of materials such as shunts, urethral and intravascular catheters, and prosthetic grafts and joints [23]. To overcome this issue, several studies are providing new methodologies to avoid *E. coli* biofilm formation. Therefore, it is wise to focus on new natural fibers as biomedical materials capable of inhibiting bacterial adherence and proliferation, so as it prevents infections [24].

2.2. Pseudomonas aeruginosa

Pseudomonas aeruginosa is a Gram-negative bacterium and can cause infections in both immunocompromised and immunocompetent hosts. Due to its multiple antibiotic resistance and extreme versatility against immune responses and clinical treatments, this bacterium is an organism that can hardly be treated in contemporary clinical practice [25].

P. aeruginosa infections are commonly found in immunocompromised patients who used invasive devices such as endotracheal tubes or indwelling catheters, because this microorganism can form biofilms in these devices [26]. Therefore, several mechanisms of some *Pseudomonas* species have been studied to characterize their intrinsic resistance to multiple antibiotics, their efflux systems, and their antibiotic-inactivating enzymes [27]. The ability of *Pseudomonas* to develop biofilms is their main mechanism of virulence, which causes ineffective clinical treatment in the hosts and resistance against their immune responses [25][27]. Therefore, this capacity is critical for patients suffering from cystic fibrosis, who acquire this infection mainly in health centers.

It is well known that doctors should avoid prescribing antibiotics unless necessary to prevent the emergence of resistant strains of *Pseudomonas*. Therefore, the prevention of *Pseudomonas* infections, especially in the hospital setting, avoids huge rates of nosocomial infection among patients [25][26]. However, due to the adaptable nature of the strains, the best approach is to prevent the initial adhesion and the colonization of this bacterium in medical devices [28]. To achieve this purpose, the use of natural fibers in biomedical materials is again proposed as a viable tactic for inhibiting bacterial adherence and spreading in health centers.

2.3. Staphylococcus aureus

Staphylococcus aureus is a Gram-positive and aerobic bacterium that can adapt to various environments, causing a series of infections and diseases [29]. This bacterium is present in approximately 30% of the healthy human population, either in their skin or nasopharyngeal membranes, being part of the normal microbiota. This bacterium does not cause infections as long as the immune system is reinforced [30]. Depending on the *S. aureus* strains involved and the site of infection, certain strains, such as methicillin-resistant *Staphylococcus aureus* (MRSA), are considered primary pathogens, which cause invasive infections or toxin-mediated diseases [31]. Nonetheless, if it crosses into the bloodstream and somehow gets into internal tissues, *S. aureus* can cause significant health problems, from mild skin infections to severe life-threatening systemic diseases [31]. In fact, *S. aureus* is the primary cause of skin and soft-tissue infections (e.g., cellulitis, impetigo, furuncles, folliculitis, and carbuncles) because the primary way of transmission of this microorganism is by direct contact, such as skin-to-skin, or from contact with contaminated objects [32]. Consequently, despite being a widespread bacterium across the population, under certain conditions and the location of the infection, *S. aureus* can produce health issues, ranging from soft to severe clinical conditions, such as meningitis, endocarditis and urinary tract infections, septic arthritis, pulmonary infections, prosthetic device infections, gastroenteritis, and toxic shock syndrome [33]. Additionally, researchers have used natural cellulose fibers of cotton, which, after functionalization and enhanced hygroscopicity, exhibited bacterial contact inhibition and diffusion inhibition when tested against *S. aureus* [34]. Thus, because *S. aureus* is a prevalent bacterium in wound infections, more research is necessary to find natural alternatives for avoiding its proliferation in wounds.

2.4. Staphylococcus epidermidis

The human skin is densely colonized by several different bacteria, archaea, viruses, and fungi [35]. However, *Staphylococcus epidermidis* is a common symbiont bacterium found in healthy human skin. Nevertheless, even though most humans carry the *S. epidermidis* bacteria without presenting infection symptoms, it is the principal reason for nosocomial infection related to invasive procedures [36]. Depending on the context, *S. epidermidis* can help or damage the human skin barrier, being frequently associated with the invasion of the skin or other human barriers via catheter/medical/prosthetic devices [37]. Then, this bacterium can produce biofilms that help to protect them from host defense or antimicrobials [38]. Therefore, despite the widespread presence of *S. epidermidis* on human skin and the evidence suggesting a mutual benefit relationship between skin and bacteria, this microorganism is also the principal reason for human skin infections, being one of the most common nosocomial infections with infection rates as high as those of *S. aureus* [38].

Therefore, *S. epidermidis* is known as the principal nosocomial pathogen related to biomaterial-associated biofilm infections [39]. The main problem is that the bloodstream eventually becomes infected after the sudden release of bacteria from biofilms into surrounding tissues. That is why *S. epidermidis* is present in 22% of the patients with bloodstream infections in intensive care units [40]. Additionally, because of its vast presence across the human skin, staphylococcal biofilm formation is related to a delay in the natural process of re-epithelialization and healing of chronic wounds [41]. Thus, biofilm formation is also one of the main factors for the evolution of infection by *Staphylococcus* species and there are new biomaterials with specific characteristics to solve this issue [39]. The

prevention of biofilm formation is essential to avoid infections with *S. epidermidis* when using biomedical devices and during the wound healing process.

2.5. Viruses

Antiviral compounds added or contained in natural fibers are valuable in the development of hygienic fabrics for infectious diseases. Among the added compounds are metal nanoparticles, carbon nanotubes, metal oxides and heterostructures with a high degree of efficacy against bacteria, mold, and viruses [42]. In addition, antiviral textiles can inhibit the spread of virus infection and effectively reduce the risk of cross-infection and reinfection. Antiviral materials can inactivate viruses or reduce the surface area of pathogen adhesion [43][44].

Several studies have been conducted on the antiviral efficacy of modified fibers, especially those that were coated with nanoparticles, because it has been observed that these inorganic compounds provide stability and robustness for antimicrobial and antiviral textile nano finishes, at high temperature and pressure, due to their physicochemical characteristics and high coverage of the surface area [42]. For example, Afzal et al. [42] treated a fabric with zinc oxide nanoparticles, suggesting that its antiviral activity resulted from the release of Zn^{2+} ions and other reactive oxygen species that damaged host cell proteins, membranes, and nucleic acids by diffusing into them, causing virus inactivation and cell death. This treated fabric was effective against the herpes virus, influenza, dengue, and hepatitis C with long-lasting antiviral activity, even after 30 wash cycles, suggesting that this fabric could be used in medical equipment to prevent viral transfer. Galante et al. [45] applied a reactive silver ink fabric and a low-surface-energy PDMS polymer to provide the fiber with superhydrophobicity and durable antiviral properties against herpes. This link improved antiviral efficacy and durability compared to silver nanoparticles by having better adhesion and coverage of reactive ionic silver in microfibers. Likewise, Iyigundogdu et al. [46] developed functionalized cotton fibers for antiviral properties against adenovirus type 5 and poliovirus type 1 with positive results. Even functionalized natural fibers have been used for the effective elimination of viruses (MS2) in water as an integral solution with environmental benefits.

2.6. Fungi

Fungi are more complicated microorganisms than viruses and bacteria due to their cell eucaryotic structure also being able to be found as yeast and mold that often live in soil and generally are not pathogenic in most healthy people. In fact, most fungi are commensals and certain genera are part of the human microbiota, such as *Candida* spp. [47]. Nonetheless, many fungi can cause hospital-related infections with high mortality rates in patients with compromised immune systems [48][49]. Generally, for human infections, the most common fungi and yeasts are *Candida* and *Aspergillus* spp. [50], as these fungi can spread quickly and damage many organs.

Several plant fibers by themselves or associated with nanoparticles already demonstrated antifungal activity and inhibition of the initial adhesion of opportunistic fungi pathogens' adhesion [51][52]. Alkan et al. [51] reported different degrees of antifungal activity against *Candida albicans* DSMZ 1386 with silk material separately dyed with madder (*Rubia tinctorium* L.) and gallnut (*Quercus infectoria* Olivier). Arenas-Chávez et al. [52] showed a relevant antifungal

activity against *C. albicans* and *Aspergillus niger* through functionalized fabrics, more exactly, cotton natural fiber with nanocomposites based on silver nanoparticles and carboxymethyl chitosan (a natural material derived from the shells of sea crustaceans). Moreover, Okla et al. [53] were able to demonstrate the antifungal activities of the various parts of *Avicennia marina* (a mangrove plant) against *Aspergillus fumigatus* and *C. albicans*. However, little is still known about the applicability of different plant fibers or their several parts by themselves or combined with other antifungal agents (such as metal nanoparticles or other types of natural materials) against the diversity of opportunistic fungi pathogens.

2.7. Biofilms

Bacterial biofilms are linked to all nosocomial infections that are mostly associated with devices, which represents a challenge for modern practice. Bacterial cells can coexist in two different forms, in a planktonic state as floating free cells and in a sessile state as cells in biofilms attached to a surface [41]. In this second state, cells demonstrate a phenotypic change with the expression of an exopolysaccharide substance (EPS), commonly known as “silt” production. This expression begins immediately after bacterial adhesion and initial colonization of the surface, leading to the production of a protective barrier of bacteria against the human immune system and therapeutic agents such as antibiotics [54]. Therefore, biofilms are well-defined as complex communities of antibiotic-resistant mono- or multispecies bacteria that reside within an exopolysaccharide matrix after irreversible binding and colonization on a biotic or abiotic surface [55]. Therefore, the medical context is the main source of chronic infections and device-related nosocomial infections.

Both Gram-positive and Gram-negative bacteria can form biofilms in medical devices [56]. The most common are *E. coli*, *P. aeruginosa*, *S. aureus,* and *S. epidermidis*, which are most often found in hospitalized patients, as described in the subsections above. According to the National Institute of Health, these bacterial biofilms are responsible for up to 80% of the total number of microbial infections [13], which include cystic fibrosis, meningitis, chronic wounds that do not heal, endocarditis, and catheters, among others. Although medical personnel have made a continuous effort to maintain a sterile environment in health centers, they are still contaminated by these pathogenic bacteria, making it extremely difficult to eradicate them from surfaces due to their high tolerance against antibiotics and detergents [57]. In addition, biofilms are able to resist host immune responses even when treated with larger or combined antibacterial therapies that exhibit certain biofilm cells called persistent cells, which are inactive cells with low metabolism that can be activated after treatment is over [58]. Therefore, an important approach to address this problem is to prevent the development of biofilms through plant-based fibers as new antimicrobial materials, modification of the surface of the device, and even with local administration of drugs.

3. Comparison between Natural and Synthetic Fibers

The focus in the development and research of natural fibers with potential biomedical application is based on characteristics such as lower production cost, renewable, cost-effective, lightweight, and biodegradability [59]. The production of natural fibers is environmentally friendly, contributing to the new generation of sustainable materials and waste reduction [60]. Natural fibers are shown to be a viable biomaterial to replace synthetic fibers due to their

composition, sustainable potential, and biological function for biomedical applications [61]. The advantages and drawbacks of natural and synthetic fibers are presented in **Table 1**:

Table 1. Natural fibers vs. synthetic fibers.

Characteristic	Natural Fibers	Synthetic Fibers	Refs
Source	It is produced from plants, animals, and minerals	It is manufactured from petroleum-based chemicals.	[60]
Density	It makes the composites lighter because the density is between 1.2 and 1.6 g/cm^3	It has limited application for composites application by their density (glass fiber = 2.4 g/cm^3, carbon fiber = 1.9/cm^3)	[62] [63]
Production	Relatively aligned, long and discontinuous fibers	Well-aligned continuous fibers	[62]
Principal compounds	The presence of cellulose, lignin, hemicellulose, and pectin	Formed by joining chemical monomers into polymers	[64]
Mechanical properties	High specific properties related to elastic modulus and strength, but drawbacks such as hydrophilic character and low thermal stability	High thermal stability, high elasticity and durability	[61]
Nature	Hydrophilic	Hydrophobic	[65]
Environmental	It is renewable and recyclable	High durability and cost	[66]

4. Antimicrobial Mechanism in the Vegetable Fibers

The current methods to fight bacterial infections in biomedical devices and implants seek to inhibit biofilm formation by reducing bacterial adhesion on their surfaces or killing bacteria [67]. Predominantly, plant fibers are modified to exhibit two essential characteristics. The first characteristic is a bactericidal effect that causes bacterial death by adding a bioactive molecule [68] to cause cytoplasmic membrane disruption, changes in membrane conductivity, protein synthesis inhibition, and nucleic acid inhibition [69]. The second feature is an anti-biofouling effect that prevents bacterial adhesion to the surface of the fiber [70]. However, the antibacterial effect of plant fibers can be found naturally without any modification, as it was observed in brown-colored cotton fibers due to pigments with tannins content [71]. The fiber extraction process could influence the natural antibacterial properties of plant fibers, considering the removal of carbohydrate and inorganic salts that benefit bacterial growth, changes in pH, and the addition of secondary metabolites that enhance antibacterial function [72]. Antimicrobial agents frequently used in plant fibers are classified as organic and inorganic [68]. Organic agents are natural biopolymers and biomolecules such as chitosan, phenols, alginate, and bioinspired formulations (e.g., antimicrobial peptides, anti-quorum-sensing

molecules, and bacteriolytic enzymes) [69]. The most used inorganic agents are metallic nanoparticles, for instance, silver and copper nanoparticles, hydroxyapatite, poly ammonium compounds, antibiotics, and synthetic polymers [70][71]. Surface coating and surface modification are the main strategies to provide antibacterial features for plant fibers. Surface treatments can be achieved by physical, mechanical, and chemical methods. For example, in surface coating, a diversity of antimicrobial agents is loaded onto the device surface and then released over time. The most used surface modification techniques include polymerization and derivatization. Antibacterial agents are adsorbed or immobilized on the surface with polymeric molecules, functional groups, hydrophobic molecules, or nanoparticles. They are immobilized by covalent bonding or radical atom transfer. Examples of these are covalent bonding and hydrophobic polycations of quaternary ammonium salts, single-walled carbon nanotubes, and alkylated polyethyleneimine [73].

In addition, plants already have several bioactive mechanisms to fight against bacterial infections and protect themselves. Those mechanisms can directly affect microorganisms through cytoplasmic membrane disruption, changes in membrane conductivity, and clotting cellular content [74]; or they can indirectly stimulate the release of CD4+ and CD8+ lymphocytes by positive regulation of IL-7 for microbe removal [75]. The antibacterial activity of plants is associated with phytochemicals compounds such as sugars, polypeptides, lectins, quinones, simple phenols and phenolic acids, flavones and flavonoids, terpenoids, tannins, coumarins, alkaloids, cannabinoids, and essential oils. Their chemical structure and hydrophobic and hydrosoluble characteristics have antiseptic action in some cases or can lead to enzyme inactivation, proteins, adhesin bindings, and substrate deprivation to cause bacterial death [76].

Phenolic compounds, such as thymol and carvacrol, extracted from thyme (*Thymus vulgaris*) and oregano (*Origanum vulgare*) have shown effects against *Listeria monocytogenes, S. aureus*, and *E. coli*. Their action is focused on the increment of bacterial cytoplasmic membrane permeability, allowing the release of lipopolysaccharides, and losing their functions as an enzyme matrix, energy transducer, and bacteria's protective armor [74]. Serrulate-type diterpenoids extracted from *Eremophila neglecta*, *E. serrulata*, *E. sturtii*, and *E. dutonii* have antibacterial activities against some Gram-positive strains, especially methicillin *S. aureus*, which leads to biomedical devices infections. Serrulatanes' compounds are used as potential coats for biomedical device surfaces avoiding biofilm formation. Serrulatanes' diterpenoids have been tested against *S. epidermidis* and have shown 99% effectiveness in the prevention of bacterial colonization [67].

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