RESEARCH AND APPLICATION OF ANTIMICROBIAL PACKAGING MATERIALS BASED ON ADVANCED PHYTOCHEMICAL AND NANOCELLULOSE

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Abstract. The increasing demand for sustainable and safe packaging solutions has accelerated the development of advanced antimicrobial materials derived from natural resources. This research comprehensively investigates the design, synthesis, and evaluation of phytochemical-nanocellulose hybrid composites for enhanced antimicrobial packaging performance. Utilizing bioactive phytochemicals extracted from medicinal herbs, combined with nanocellulose as a high-strength, biodegradable matrix, we fabricated a series of hybrid materials with optimized physicochemical properties. Characterizations techniques, including Fourier-transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), and mechanical testing, confirmed the successful integration and uniform dispersion of active compounds within the nanocellulose matrix. The antimicrobial efficacy, tested against a spectrum of pathogenic bacteria, fungi, and viruses, demonstrated up to 99.9% suppression of microbial activity. Furthermore, the hybrid materials exhibited superior barrier properties, mechanical robustness, and environmental stability, indicating their suitability for food packaging and biomedical applications. Our findings highlight the potential of phytochemical-nanocellulose hybrids as eco-friendly, high-performance alternatives to conventional synthetic antimicrobial materials. This work lays the groundwork for

ISSN 3030-3907

further optimization and scalable production, contributing to advancing sustainable packaging technologies with broad applicability in food preservation, healthcare, and environmental sectors.

Keywords. Phytochemical-nanocellulose hybrids, Antimicrobial packaging, Natural bioactive compounds, Sustainable materials, Food preservation, Biomedical applications, Microbial inhibition, Barrier properties, Eco-friendly packaging, Scalable fabrication

Annotatsiya. Barqaror va xavfsiz qadoqlash echimlariga bo'lgan talabning ortishi tabiiy resurslardan olingan ilg'or mikroblarga qarshi materiallarning rivojlanishini tezlashtirdi. Ushbu tadqiqot antimikrobiyal qadoqlash samaradorligini oshirish uchun fitokimyoviy-nanotsellyuloza gibrid kompozitlarini loyihalash, sintez qilish va baholashni har tomonlama o'rganadi. Dorivor o'simliklardan olingan bioaktiv fitokimyoviy moddalarni yuqori quvvatli, biologik parchalanadigan matritsa sifatida nanotsellyuloza bilan birlashtirib, biz optimallashtirilgan fizik-kimyoviy xususiyatlarga ega bir qator gibrid materiallarni ishlab chiqdik. Xarakterlash texnikasi, shu jumladan Furye-transform infraqizil spektroskopiya (FTIR), skanerlash elektron mikroskopi (SEM) va mexanik sinovlar nanotsellyuloza matritsasi ichida faol birikmalarning muvaffaqiyatli integratsiyasi va bir xil tarqalishini tasdiqladi. Patogen bakteriyalar, zamburug'lar va viruslar spektriga qarshi sinovdan o'tgan mikroblarga qarshi samaradorlik mikrob faolligini 99,9% gacha bostirilishini ko'rsatdi. Bundan tashqari, gibrid materiallar yuqori to'siq xususiyatlarini, mexanik mustahkamlikni va ekologik barqarorlikni namoyish etdi, bu ularning oziq-ovqat mahsulotlarini qadoqlash va biotibbiyot dasturlariga yaroqliligini ko'rsatdi. Bizning topilmalarimiz an'anaviy sintetik antimikrobiyal materiallarga ekologik toza, yuqori samarali alternativ sifatida fitokimyoviy-nanotsellyuloza duragaylarining imkoniyatlarini ta'kidlaydi. Ushbu ish oziq-ovqat mahsulotlarini saqlash, sog'liqni saqlash va atrof-muhit sohalarida keng qo'llaniladigan barqaror qadoqlash texnologiyalarini rivojlantirishga hissa qo'shib, yanada optimallashtirish va kengaytiriladigan ishlab chiqarish uchun zamin yaratadi.

Kalit so'zlar. Fitokimyoviy-nanotsellyuloza duragaylari, mikroblarga qarshi qadoqlash, tabiiy bioaktiv birikmalar, barqaror materiallar, oziq-ovqat mahsulotlarini saqlash, biotibbiyot ilovalari, mikrobial inhibisyon, to'siq xususiyatlari, ekologik toza qadoqlash, kengaytiriladigan ishlab chiqarish

Аннотация. Растущий спрос на экологически чистые и безопасные упаковочные решения привел к ускорению разработки современных антимикробных материалов, получаемых из природных ресурсов. В этом исследовании всесторонне изучаются разработка, синтез и оценка гибридных композитов фитохимик-наноцеллюлоза для повышения антимикробных

ISSN 3030-3907

характеристик упаковки. Используя биоактивные фитохимические вещества, извлеченные из лекарственных трав, в сочетании с наноцеллюлозой в качестве высокопрочной, биоразлагаемой матрицы, мы изготовили серию гибридных материалов с оптимизированными физико-химическими свойствами. Методы определения характеристик, включая инфракрасную спектроскопию с преобразованием Фурье (FTIR), сканирующую электронную микроскопию (SEM) и механические испытания, подтвердили успешную интеграцию и равномерное распределение активных соединений в наноцеллюлозной матрице. Антимикробная эффективность, протестированная против целого спектра патогенных бактерий, грибков и вирусов, продемонстрировала подавление микробной активности на 99,9%. Кроме того, гибридные материалы продемонстрировали превосходные барьерные свойства, механическую прочность и экологическую стабильность, что указывает на их пригодность для упаковки пищевых продуктов и биомедицинских применений. Наши результаты подчеркивают потенциал гибридов фитохимии и наноцеллюлозы в качестве экологически чистых и высокоэффективных альтернатив традиционным синтетическим антимикробным материалам. Эта работа закладывает основу для дальнейшей оптимизации и масштабирования производства, способствуя развитию экологически чистых упаковочных технологий, которые широко применяются в сфере консервирования пищевых продуктов, здравоохранения и охраны окружающей среды.

Ключевые слова. Фитохимические гибриды с наноцеллюлозой, Антимикробная упаковка, Натуральные биоактивные соединения, Экологически чистые материалы, Консервирование пищевых продуктов, Биомедицинское применение, Подавление микробов, Барьерные свойства, Экологичная упаковка, Масштабируемое производство

Introduction

Research Background

The COVID-19 pandemic has profoundly heightened global awareness of food safety and hygiene, making the development of robust and effective hygienic packaging solutions more urgent than ever. Throughout this crisis, antimicrobial and sterilization technologies for packaging have proved to be essential tools in preventing the spread of infectious diseases, highlighting the critical importance of antimicrobial packaging materials. Natural-derived compounds and environmentally friendly materials with proven microbial inhibition capabilities have emerged as cutting-edge solutions, capable of meeting the rapidly evolving demands of the post-pandemic market.

ISSN 3030-3907

With the increasing environmental and ecological problems caused by petroleum-based packaging materials, the focus has gradually shifted to natural resources for the preparation of functional food packaging materials [1]. Nanocellulose, a structural polysaccharide that has attracted significant interest recently, is known for its renewability, inherent biocompatibility, biodegradability, abundance in resources, and environmentally friendly nature. Derived from cellulosic biomass, nanocellulose can be disintegrated into cellulose nanofibrils (CNF) or cellulose nanocrystals (CNC), depending on their sensitivity to hydrolysis at the axial spacing of disordered domains. These promising green nanomaterials offer a sustainable alternative for advanced packaging applications [2]. In recent years, the pressing need to innovate in packaging technologies has intensified, driven by growing concerns over food safety, environmental sustainability, and the imperative to extend the shelf life of perishable goods. Antimicrobial packaging is no longer merely an advancement; it has become a necessity for actively suppressing microbial growth, preventing spoilage, and ensuring food safety with measurable efficacy.

This research is dedicated to advancing this vital field by designing and applying antimicrobial materials that harness the synergistic potential of advanced phytochemicals—naturally occurring bioactive compounds with strong antimicrobial properties—and nanocellulose, a sustainable, high-performance nanomaterial derived from cellulose. The integration of these natural and nanotechnological components offers a groundbreaking approach: significantly enhancing the antimicrobial efficacy, safety, and functionality of packaging solutions.

Consumers' demand for safe ingredients and concerns over the harmful effects of synthetic additives have prompted food manufacturers to seek safer alternative solutions. Also, consumers have increasingly preferred natural over synthetic products in recent years. As a result, naturally derived antioxidant and antimicrobial compounds are becoming essential in active packaging materials since they are regarded as providing a lesser danger to consumers and simultaneously increasing the food shelf-life. Smart food packaging containing phytochemicals, found in many plants, vegetables, fruits, and herbs, emerged as an alternative solution against the nocive nature of synthetic additives. Plant extracts can be added to packaging products and incorporated into films, giving them high antibacterial and antioxidant capacity. The volatile compounds might affect the willingness of consumers to buy products, and other sensory effects could be potentially present, such as color change. Therefore, in each territory of the world market for the food industry, it is necessary to deal with a variety of external and internal factors; therefore, for consumers of all levels, phytochemicals and

ISSN 3030-3907

Bioactive compounds can be applied as an additional element included in food packaging [3].

Designing and manufacturing functional bioactive ingredients and pharmaceuticals have grown worldwide. Consumers demand for safe ingredients and concerns over harmful synthetic additives have prompted food manufacturers to seek safer and sustainable alternative solutions. In recent years the preference by consumers to natural bioactive agents over synthetic compounds increased exponentially, and consequently, naturally derived phytochemicals and bioactive compounds, with antimicrobial and antioxidant properties, becoming essential in food packaging field.

This paper aims to provide a comprehensive review of recent breakthroughs in the synthesis, characterization, and practical applications of phytochemical- and nanocellulose-based antimicrobial packaging materials, emphasizing their crucial role in safeguarding food safety and promoting environmental sustainability in a world shaped by pandemic challenges and increasing ecological awareness.

Research Objectives

Antimicrobial agents can be applied to foods systems through two ways i) by direct addition to the food products, and ii) by an indirect system incorporating the antimicrobial compounds into the packaging materials [4]. The direct addition of the antimicrobial compounds into the foods and food products can reduce the effectiveness of the antimicrobial activity of the compounds due to the interaction between the compounds and food components [5,6]**.** Indirect contact can solve this issue because the compounds diffuse from the packaging material into the food surface to protect the food from unwanted spoilage and pathogenic bacteria. The efficiency of the indirect system depends on the release kinetics of compounds from the packaging material and relies on the properties of compounds incorporated into the packaging material [7], and the nature of the base matrix used in the packaging formulation.

The primary goal of this research is to design, synthesize, and thoroughly evaluate innovative antimicrobial packaging materials that incorporate advanced phytochemicals with proven antimicrobial properties and nanocellulose, a high-performance nanomaterial derived from cellulose known for its safety for human contact. This study specifically aims to:

1. Investigate the optimal methods for extracting, modifying, and incorporating bioactive phytochemicals to maximize their antimicrobial effectiveness within packaging matrices.
2. Develop nanocellulose-based nanocomposites loaded with these phytochemicals, ensuring uniform dispersion and strong interfacial interactions.

ISSN 3030-3907

3. Characterize the physicochemical, mechanical, and antimicrobial properties of the resulting materials to determine their suitability for practical packaging applications.
4. Assess the safety of the developed materials for human use, ensuring they pose no harm and are suitable for direct contact with food products.
5. Explore the potential applications of these materials in preserving various perishable food items, with a focus on extending shelf life and enhancing food safety.
6. Provide insights into the synergistic effects of combining natural phytochemicals with nanocellulose, emphasizing their advantages over conventional packaging materials.

Furthermore, to facilitate commercialization, this research aims to develop scalable, cost-effective manufacturing processes and evaluate the environmental and safety compliance of the materials to ensure they meet regulatory standards. A strategic plan for market entry, including collaboration with industry partners and pilot testing in real-world settings, will be formulated to accelerate the transition from laboratory research to commercial application.

This comprehensive approach intends to contribute significantly to the development of eco-friendly, effective, and safe packaging solutions that are critically needed in response to heightened hygiene concerns, environmental challenges, and the growing demand for sustainable food packaging in a post-pandemic world.

Material & Methods

1.Selection of medicinal plants with antimicrobial properties

In nature, there are plenty of medicinal herbs and plants which contain active compounds decisive for their diverse prophylactic/ therapeutic behavior against many human diseases. A thorough analysis of these Phyto-compounds for antimicrobial activities has considered greater importance in the context of recent days [8]**.** Plants exhibit powerful antibacterial properties through various compounds that exist in nature, and these components are used in food preservation, medicine, and household hygiene products. Since they are eco-friendly and biologically safe, they are receiving great attention in the development of sustainable antibacterial materials. Phytochemicals are secondary metabolites produced by plants, and the term is generally used to describe non-essential nutrients that may affect health. Phytochemicals are the physiologically active substances that plants produce to protect themselves from ultraviolet rays, pests, and microorganisms for their survival. Phytochemicals help plants resist bacteria, fungi, viruses, and insects. They also play a role in human health, acting as antioxidants and protecting cells from damage.

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The medicinal plants contain many important bioactive constituents such as terpenoids, essential oils, polyphenols, and flavonoids. These compounds demonstrated numerous biological activities, such as sedative, analgesic, antibacterial, and anti-inflammatory activities [9].

The antimicrobial properties of plants are expressed by various natural components. Representative antibacterial components and their properties are as follows:

Flavonoids

They have natural antioxidant and antibacterial effects and are abundant in leaves, flowers, and stems. Flavonoids are polyphenolic compounds with a basic structure of two phenyl rings connected by a three-carbon chain (C6-C3-C6). The specific structure of a flavonoid determines its unique properties and functions. Examples: Quercetin, Rutin, Catechin

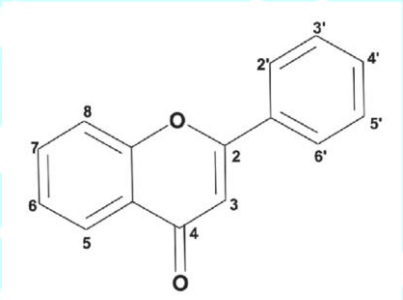


Figure 1. Basic skeleton or structure of flavonoids

Phenolics

They have powerful antimicrobial effects and act as a defense mechanism for plants. Phenolics consist of a benzene ring with one or more hydroxyl (-OH) groups attached directly to it. Many phenolics are secondary metabolites of plants, playing a role in their defense against stress and pathogens. They are naturally occurring molecules found in plants, fungi, and bacteria, and are a diverse group of bioactive compounds with a wide range of biological activities, including antioxidant, antimicrobial, and anti-inflammatory properties [10]. Phenolics are abundant in fruits, vegetables, tea, coffee, and other plant-based foods. Examples: Tannins, Catechol, Phenolic acids

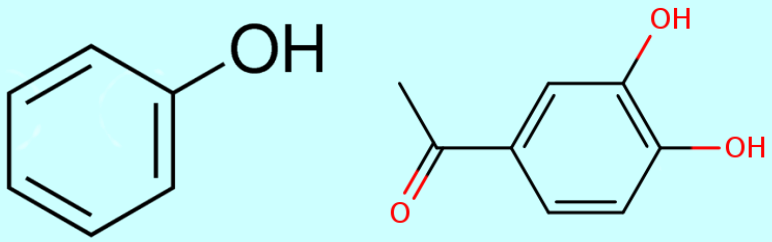


Figure 2. Basic skeleton or structure of Phenolics and Phenols in Plants, Natural Phenolic Compounds

Alkaloids

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They have strong physiological activity and antibacterial effects. Examples: Morphine, Nicotine, Solanine. Alkaloids are a diverse group of naturally occurring organic compounds, primarily found in plants, that contain at least one nitrogen atom. They are known for their wide range of pharmacological effects, including analgesic, anticancer, and antiarrhythmic properties, and are used in various applications in modern medicine. Many alkaloids, such as morphine, quinine, and vincristine, have important medicinal applications, including pain relief, malaria treatment, and cancer therapy [11].

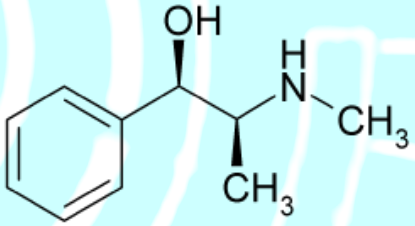
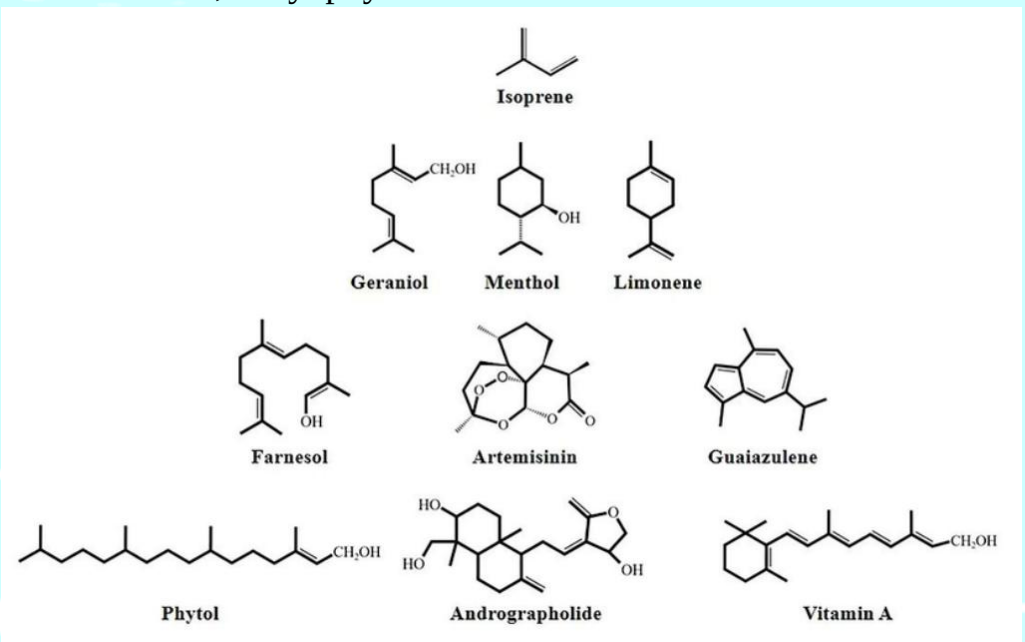


Figure 3. Basic skeleton or structure of Alkaloids

Fragrance derivatives and Terpenes [12]

Various compounds with antibacterial and anti-inflammatory properties. Terpenes are the foundation of many fragrance derivatives, playing a crucial role in the natural and synthetic fragrance industries. They are naturally occurring compounds found in plants and are responsible for the distinctive scents and flavors of various substances, like essential oils and plant extracts. Terpenes are composed of isoprene units, and their derivatives are widely used in the fragrance industry to create new semi-synthetic fragrances. Terpenes are a class of hydrocarbons, and their oxygenated derivatives are found widely in plants, microorganisms, marine organisms, fungi, and insects. There are many kinds of terpene compounds, and their isomers exist in various forms. These can be divided into monoterpenes (limonene), sesquiterpenes (element), diterpenes (camphene), and polystyrenes Examples: Limonene, Caryophyllene.



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Figure 4. Basic skeleton or structure of Fragrance derivatives and Terpenes, terpenoids

Glycosides

Many contain ingredients with antibacterial properties. Glycosides are a class of organic compounds where a sugar molecule (aglycone) is linked to a non-sugar molecule (aglycone) through a glycosidic bond. These compounds are widely found in plants and play various roles, including energy storage and defense mechanisms. Many glycosides, like cyanogenic glycosides and saponins, act as defense mechanisms against herbivores. Some glycosides have medicinal properties, like cardiac glycosides found in foxglove (Digitalis) and used to treat heart conditions. Examples: Spirulina, Saponins [13].

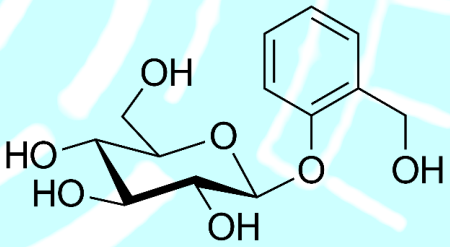


Figure 5. Basic skeleton or structure of Glycosides

Other naturally derived ingredients

Saponins: Antibacterial, anti-inflammatory and immune-enhancing effects.

Antibacterial peptides [14]: Naturally produced in some plants and exhibit antibacterial activity. Antibacterial peptides, also known as antimicrobial peptides (AMPs), are short chains of amino acids that naturally exhibit antimicrobial activity. They are a crucial part of the innate immune system and are found in a wide range of organisms, from bacteria and fungi to plants and animals. AMPs primarily target bacterial membranes, disrupting them and leading to cell death. AMPs disrupt bacterial cell membranes by forming pores, disrupting the membrane's integrity, or interacting with intracellular components.

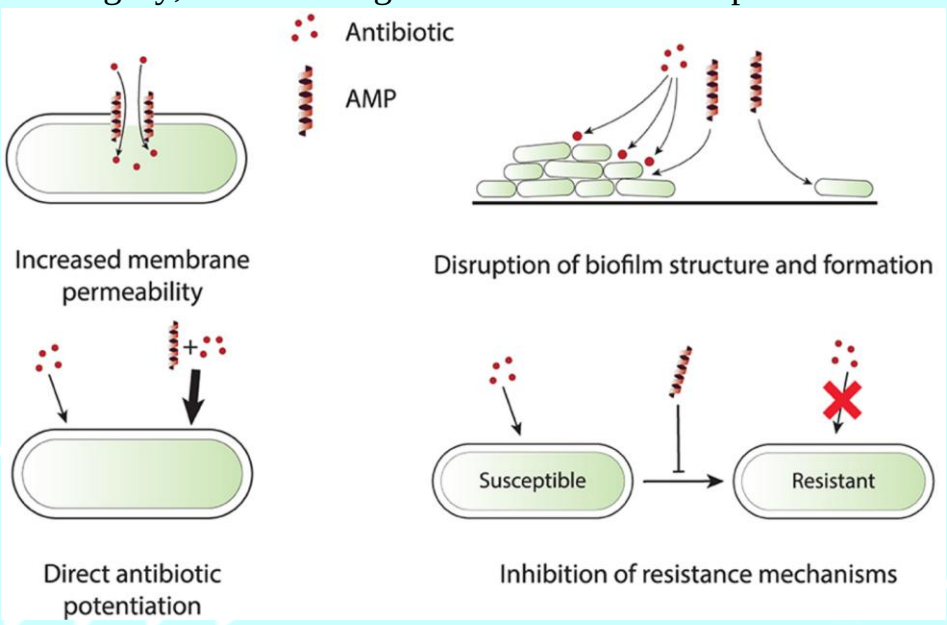


Figure 6. Antimicrobial Peptides: Future of Antibiotic Solutions

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2.Selection of medicinal plants with antimicrobial properties

2-1. Literature Review and Data Analysis

- Investigate existing research, scientific papers, traditional medicine texts, and literature related to the medicinal and antibacterial properties of plants to identify potential candidates.
- Include plants based on regional medicinal uses and ethnobotanical knowledge.

2-2. Plant Collection and Preprocessing

- Harvest the selected plant parts (leaves, roots, stems, fruits, etc.) in fresh or dried form.
- Standardize collection methods, timing, and location to ensure consistency of samples.

2-3. Extraction of Bioactive Compounds

- Use solvents (water, ethanol, methanol, ethyl acetate, etc.) to extract phytochemicals.
- Standardize extraction conditions such as temperature, duration, and solvent volume for reproducibility.

2-4. Evaluation of Antibacterial Activity

- Disc Diffusion Method: Apply extracts onto discs placed on agar plates inoculated with bacteria to observe inhibition zones.
- Minimum Inhibitory Concentration (MIC): Determine the lowest concentration of extract that inhibits bacterial growth.
- Broth Microdilution and Agar Well Diffusion: Test various concentrations to assess bacterial growth suppression.

2-5. Selection of Potent Plants

- Identify plants or plant parts showing the lowest MIC values or widest inhibition zones.
- Repeat experiments to confirm reliability and consistency of results.

2-6. Further Studies

- Isolate and characterize active compounds.
- Investigate mechanisms of antibacterial action.

Evaluate potential for commercialization.

These plants have been traditionally used and scientifically studied for their capacity to inhibit or kill pathogenic bacteria. The choice of plant depends on the specific bacteria targeted, traditional use, availability, and further research findings.

Common Medicinal Plants with Antibacterial Properties:

· **Tea Tree** (Melaleuca alternifolia) – Known for its potent antimicrobial terpinen-4-ol.

· **Garlic** (Allium sativum) – Allicin exhibits broad-spectrum antibacterial activity.

· **Turmeric** (Curcuma longa) – Curcumin has shown antibacterial effects.

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· **Neem** (Azadirachta indica) – Contains azadirachtin and nimbin with antimicrobial activity.

· **Cinnamon** (Cinnamomum zeylanicum) – Cinnamaldehyde provides antimicrobial properties.

· **Lemon** (Citrus limon) – Rich in vitamin C and citrus oils with antimicrobial activity. Lemon peel contains antibacterial substances that inhibit microbial growth. Antibacterial effect against norovirus

· **Tulsi/Basil** (Ocimum sanctum) – Contains eugenol and other phenolic compounds with antibacterial properties.

· **Mulberry** (Morus alba L.) – Inhibit bacterial growth and prevent pathogenic intestinal bacteria.

· **Rosemary** (Rosmarinus officinalis L.) – Methicillin-resistant Staphylococcus aureus (MRSA) is highly resistant to these bacteria.

· **Cockleburs** (Xanthium strumarium) – it is effective against typhoid bacteria, dysentery bacteria, and Staphylococcus aureus.

· **Milk thistle** (Silybum marianum) – against to six food-borne pathogens (Bacillus cereus, Bacillus subtilis, Staphylococcus aureus, Listeria monocytogenes, Salmonella enterica and Vibrio vulnificus)

· **Grapefruit** (Citrus × paradisi) – Grapefruit seed extract (GSE), grapefruit peel extracts, and grapefruit peel essential oil (EO) can all be used to inhibit E. coli, S. epidermidis, and S. aureus

· **Multiflora rose** (*Rosa multiflora*) – Antibacterial effect against food poisoning bacteria

3.Method for extracting antimicrobial substances from plants.

Method for antimicrobial screening and characterization can be tailored to specific plant species and the antimicrobial compounds of interest. This can be achieved through several strategies, including optimizing extraction and isolation techniques, selecting appropriate test organisms, and adapting antimicrobial assays. Extracting antimicrobial substances from plants typically involves several key steps:

3.1 Plant Material Preparation:

· Collect appropriate plant parts (leaves, stem, roots, etc.).

· Wash thoroughly to remove dirt and contaminants.

· Dry the plant material (air drying or oven drying at low temperature).

· Grind into a fine powder to increase surface area.

· Weigh a specific amount of dried, powdered medicinal plant material (e.g., 10 g).

· Store in a dry, cool place until extraction.

3-2. Extraction Process:

· Choose an appropriate solvent (e.g., methanol, ethanol, acetone, water) depending on the polarity of target compounds.

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· Choose a solvent suitable for extracting antimicrobial peptides, typically aqueous-based solvents like water or aqueous ethanol (e.g., 80% ethanol).

· Maceration: Soak the powdered plant material in solvent for a specific period (hours to days) with occasional shaking.

· Alternatively, use techniques like Soxhlet extraction, ultrasonic-assisted extraction, or cold percolation for improved yield.

· Add enough solvent to fully immerse the plant material (usually a 1:10 to 1:20 weight-to-volume ratio).

3.3 Filtration and Concentration:

· Filter the extract to remove plant debris.

· Concentrate the filtrate by rotary evaporation or lyophilization to obtain a crude extract.

3.4 Isolation and Purification:

· Use chromatographic techniques (e.g., column chromatography, TLC, HPLC) to isolate specific antimicrobial compounds.

3.5 Testing for Antimicrobial Activity:

· Evaluate the extract or isolated compounds using assays such as disk diffusion, MIC (Minimum Inhibitory Concentration), or broth dilution against target bacteria or fungi.

This general method can be tailored based on the plant species and the specific antimicrobial compounds of interest.

In this study, we chose the following processing method. Ultrasonic-assisted extraction (UAE) is a modern, efficient technique used to isolate antimicrobial substances, including antimicrobial peptides, from medicinal plants. It utilizes high-frequency sound waves to create cavitation bubbles in the solvent, which enhances cell disruption and improves the release of bioactive compounds. This method offers advantages such as reduced extraction time, lower solvent use, and higher yields compared to traditional extraction methods.

Procedure overview:

Ultrasonic-Assisted Extraction:

Place the mixture into an ultrasonic bath or insert ultrasonic probe.

Set the ultrasonic device to appropriate parameters: Frequency: Typically 20-40 kHz.

Duration: Usually 15-30 minutes.

Temperature control: Maintain at approximately 25-40°C, using an ice bath, if necessary, to prevent heat degradation.

Turn on the ultrasonic device and sonicate the mixture, ensuring even exposure.

· Preparation: Dry and grind plant material.

· Extraction: Mix plant powder with suitable solvent (water, ethanol, methanol).

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• Ultrasound application: Subject the mixture to ultrasonic waves using an ultrasonic bath or probe for a specific duration, often at controlled temperature.

• Filtration: Remove plant debris.

• Concentration and purification: Use techniques such as rotary evaporation or chromatography to isolate antimicrobial peptides.

• This approach is promising for obtaining potent antimicrobial compounds, including peptides, from medicinal plants, contributing to the development of new antibiotic solutions.

How it works:

Cavitation:

Ultrasonic waves create tiny bubbles in the liquid, which implode rapidly, generating intense localized energy. This energy disrupts cell walls, making the extraction process more efficient.

Increased Mass Transfer:

The mechanical effects of ultrasound improve the penetration of solvents into plant tissues, enhancing the transfer of bioactive compounds from the cells into the solvent.

Non-Thermal and Efficient:

This method is known for its non-thermal nature, preserving the integrity of bioactive compounds. It is also efficient, producing better yields and reducing extraction time compared to traditional methods.

Hielscher Ultrasonics from Germany was used in this study. The probe-type ultrasonicator UIP1000hdT is very flexible and adaptable. The flexibility of the UIP1000hdT is made possible by various manifold accessories such as sonotrodes, boosters and flow cells. The UIP1000hdT can typically handle throughputs of 0.5 to 4.0 L/min when used for the sonication of liquids in flow mode. A typical throughput of about 1 to 5 m³ per day can be handled.



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Figure 7. Sonicator UIP1000hdT for batch and inline processing by Hielscher Ultrasonics

Post-Extraction Handling:

· After sonication, allow the mixture to cool if heated.

· Centrifuge the extract at 3000-5000 rpm for 10-15 minutes to pellet plant debris.

· Carefully decant or filter the supernatant through Whatman filter paper or a vacuum filter.

Concentration:

A concentrated extract is desired, use a rotary evaporator at a reduced pressure and temperature (around 40°C) to remove solvents.

For aqueous extracts, lyophilization (freeze-drying) can be used.

Purification:

Subject the crude extract to chromatographic techniques such as:

Column chromatography to separate peptides.

High-performance liquid chromatography (HPLC) for further purification.

Antimicrobial Activity Assay:

Test the extract or purified peptides for antimicrobial activity using disc diffusion, MIC, or broth dilution methods against target pathogens.

4.Method for combining antimicrobial substances with nanocellulose from plants.

Combining antimicrobial substances with nanocellulose involves several methodological approaches depending on the desired application (e.g., packaging, wound dressing, film formation). Here are common methods for compounding these materials:

4-1. In Situ Incorporation

Process: Mix the antimicrobial agent directly into the nanocellulose suspension during its preparation.

Steps:

Prepare nanocellulose suspension via mechanical fibrillation or chemical methods.

Add the antimicrobial compound (e.g., essential oils, peptides, silver nanoparticles) into the nanocellulose suspension.

Homogenize using stirring, ultrasonication, or ultrahigh shear mixing to ensure uniform dispersion.

- Cast or dry the mixture into films or sheets as needed.

4-2. Physical Blending

Process: Add the antimicrobial substance to pre-formed nanocellulose films or gels.

Steps:

Prepare nanocellulose films or hydrogels via casting or freeze-drying.

Soak or administer the antimicrobial agent onto or into the nanocellulose matrix.

Allow for absorption or diffusion; optionally, crosslink to enhance stability.

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4-3. Chemical Grafting or Crosslinking

Process: Covalently attach antimicrobial molecules to nanocellulose fibers.

Steps:

Activate nanocellulose surface groups (e.g., hydroxyl groups) using chemical agents.

Graft antimicrobial compounds via chemical reactions (e.g., esterification, amination).

Repeat washing and drying steps to remove unreacted agents.

4-4. Nano-embedding (Nanoparticle Incorporation)

Process: Synthesize antimicrobial nanoparticles (e.g., silver, zinc oxide) within or on nanocellulose.

Steps:

Mix nanocellulose suspension with metal salts.

Reduce metal salts in situ using chemical reducers (e.g., sodium borohydride).

Wash and purify the nanocellulose-nanoparticle composite.

4-5. Solution Casting and Film Formation

Disperse nanocellulose in a solvent with antimicrobial substance.

Cast onto a flat surface and dry to form films with embedded antimicrobial activity.

Results and Discussion

1.Modification of nanocellulose for antimicrobial materials

Nanocellulose alone is not an antibacterial agent and cannot prevent viral, bacterial, and fungal infections. Nanocellulose was used in combination with various phytochemical antibacterial agents. It was confirmed that nanocellulose-based plant-based antibacterial agents can be synthesized by surface modification with biocides and are effective against bacterial infections.

Meanwhile, surface functionalization can be performed through various strategies (e.g., oxidation, esterification, and etherification) involving chemical reactions of hydroxyl groups. Through this, nanocellulose can have various functional groups including aldehyde groups and quaternary ammonium groups, which gives it bacteriostatic and biocompatible properties.

Alvarado et al. [15] and Li et al. [16] reported studies on the antibacterial properties of other cellulose binders including metal/metal oxide nanoparticles (e.g., Au, Ag, Cu, CuO, MgO, ZnO, TiO_2), chitosan, silanes, and chlorine.

Nanocellulose can be modified to create antimicrobial materials by introducing functional groups with antibacterial properties through various methods like oxidation, esterification, or etherification. These modifications can enhance the material's effectiveness against a range of microorganisms, including bacteria, fungi, and viruses.

The modification methods used in this study are as follows:

· Surface modification: Modifying the outer layer of nanocellulose to improve interaction with biological systems, reduce side effects, and enhance antibacterial activity.

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- Chemical grafting: Chemically attaching functional groups such as amino or aminosilane groups to the surface of nanocellulose to mimic the antibacterial properties of other materials.
- Oxidation: Oxidizing the hydroxyl groups of nanocellulose to generate aldehyde groups, which are then reacted with other molecules to impart antibacterial properties.
- Esterification/etherification: Introducing ester or ether functional groups that can enhance the antibacterial properties of nanocellulose.
- Addition of antibacterial agents: Adding phytochemical antibacterial agents such as antibiotics to the nanocellulose matrix.
- Combination with other materials: Combining nanocellulose with nanomaterials or phytochemical antibacterial polymers to achieve synergistic effects.

The key function of these materials relies on their surface properties such as adhesion, biocompatibility, non-toxicity biomimicry and microbial retention/extinction. Particularly, the antimicrobial properties are of utmost significance in preventing/reducing spreading of infectious diseases and combating the continuous emergence of new microbes with drug resistance. Onthe other hand, antimicrobial properties are not only needed to reduce the risk posed to human health by the contamination of medical tools, implants and other healthcare products by bacterial colonization forming biofilm, but also to enhance the performance of many industrial processes including food processing and packing, marine transport, and water treatment.

2.Antibacterial Test Result

Antibacterial test results, also known as antimicrobial susceptibility testing, determine which antibiotics are effective against a specific bacterial infection. The results are typically categorized as susceptible, intermediate, or resistant, indicating how well the antibiotic can kill or inhibit the bacteria's growth. In this study, the results of antibacterial tests were analyzed for a total of six types of bacteria. An antimicrobial coating agent synthesized based on nanocellulose was created and antimicrobial testing was performed.

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Table 1. Antibacterial Test Results

Test Organism	Test Result (Unit)	Control	Sample
Staphylococcus aureus	After 24 hours (CFU)	3.8×10^4	<10
ATCC 6538	Percent Reduction (%)	-	99.9
Escherichia coli	After 24 hours (CFU)	2.9×10^5	<10
ATCC 8739	Percent Reduction (%)	-	99.9
Pseudomonas aeruginosa	After 24 hours (CFU)	1.7×10^7	<10
ATCC 10145	Percent Reduction (%)	-	99.9
Salmonella Typhimurium	After 24 hours (CFU)	2.9×10^6	<10
ATCC 14028	Percent Reduction (%)	-	99.9
Staphylococcus aureus MRSA	After 24 hours (CFU)	6.1×10^5	<10
NCCP 14752 (*MRSA)	Percent Reduction (%)	-	99.9
Vibrio parahaemolyticus	After 24 hours (CFU)	1.1×10^4	<10
ATCC 17802	Percent Reduction (%)	-	99.9

MRSA: Methicillin resistance Staphylococcus aureus

Test method : In vitro method
Sample material : Liquid
Sample info. : Concentration – Undiluted
Test condition : Reaction time – 24 hrs, Temperature – Room temperature (22 ± 2) ℃
Control – Saline solution (0.9% NaCl) + NB(10%)
Evaluation : Percent Reduction (%) = {(Ct - St)/Ct} X 100
(Ct = CFU of control after the specified contact time, St = CFU of sample after the specified contact time)

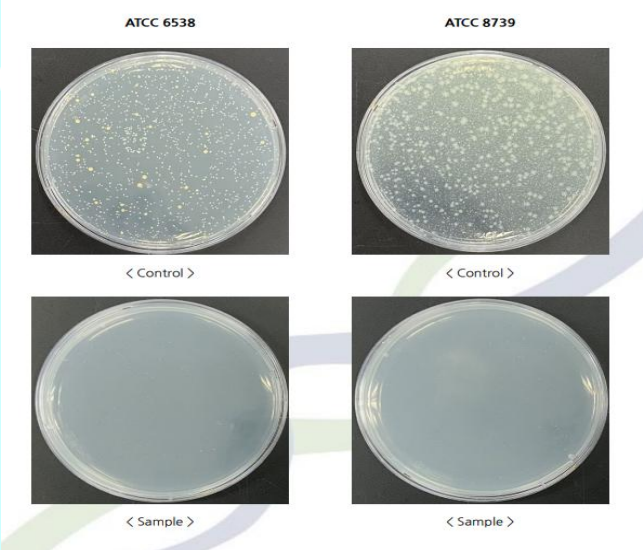


Figure 8. Antibacterial Test Result of ATCC 6538 (*Staphylococcus aureus*) and ATCC 8739 (*Escherichia coli*)

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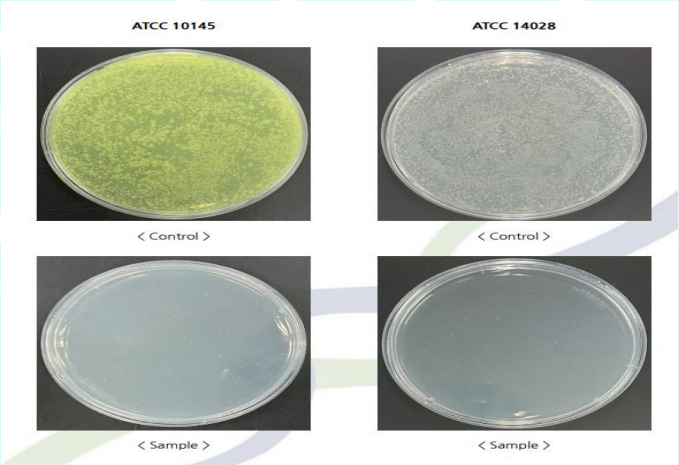


Figure 9. Antibacterial Test Result of ATCC 10145(*Pseudomonas aeruginosa*) and ATCC 14028(*Salmonella Typhimurium*)

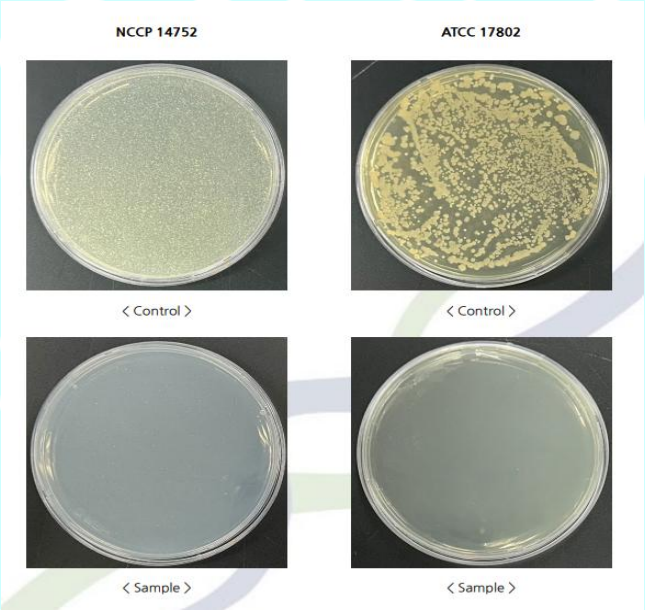


Figure 10. Antibacterial Test Result of NCCP 14752(*Staphylococcus aureus* MRSA) and ATCC ATCC 17802 (*Vibrio parahaemolyticus*)

3.Antifungal Test Result

An antifungal test result determines a fungus's sensitivity to specific antifungal medications, helping diagnose fungal infections and guide treatment decisions. The test measures the lowest concentration of an antifungal agent that inhibits fungal growth, known as the minimum inhibitory concentration (MIC). Fungal infections can be challenging to diagnose, and antifungal testing helps confirm the presence and type of fungus. The results of the test guide the choice of the most effective antifungal medication, ensuring that the drug will be effective against the specific fungus.

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Table 2. Antifungal Test Results

- Test Result

Test Organism	Test Result	Control	Sample
Mixed five fungi	Observed growth	3	1

Test mold : ATCC 9642 (*Aspergillus brasiliensis*) ATCC 9645 (*Trichoderma virens*)
ATCC 11797 (*Penicillium funiculosum*) ATCC 15233 (*Aureobasidium pullulans*)
ATCC 6205 (*Chaetomium globosum*)
Sample info. : #Material - a liquid phase Size - (50 X 50) mm
Test condition : Incubation condition & temperature - (25 ± 1) ℃, 2 weeks
Control - Qualitative Filter Paper (Whatman) - Grade 1, Φ55 mm
Evaluation : Observed growth 1 = No growth Observed growth 2 = Microscopic growth
Observed growth 3 = Macroscopic growth
: The data was provided by the client



Figure 11. Antibacterial Test Result of ATCC 9642 (*Aspergillus brasiliensis*), ATCC 11797 (*Penicillium funiculosum*), ATCC 6205 (*Chaetomium globosum*), ATCC 9645 (*Trichoderma virens*) and ATCC 15233 (*Aureobasidium pullulans*)

4.Cytotoxicity Test Result

Cytotoxicity test is a test to confirm the concentration of a sample that is not toxic to cells in vitro. This is a test method to find the maximum concentration that does not affect cell viability when cells treated with continuously diluted samples are cultured for a certain time.

It is a test of the adverse effects of natural or artificial chemicals on living organisms and is performed to ensure biological safety as we are exposed to more and more chemicals both in the environment and in the products we use. The test is mainly performed to ensure the biological safety of medical devices. The test

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content is a cytotoxicity test according to the international standard ISO 10993-5 and is based on the MTT assay. The test results were determined to be free of cytotoxicity and a cell viability of 94% or more using MRC5, a basic cell line of human lung cell tissue.

It can also be used to determine the concentration of a substance of interest through serial dilution or to prepare samples for further testing. From the previous experimental information, even in the case of real-time PCR, a standard curve must be obtained for absolute quantification, and serial dilution is performed to obtain this curve.

This is used as a necessary method in that the solution can be diluted step by step to check whether the concentration of the sample to be viewed is within the detection range.

We performed a human cytotoxicity test. As a result, more than 94% of viable cells were confirmed. This meets the cytotoxicity safety standard of more than 70% cell viability requirement and was shown to be non-cytotoxic.

Table 4. Cytotoxicity Test Results

- **Test Result**

〈Table1〉 Cell viability according to each concentration treatment (Means±SD)

Treatment concentration (%)	0	0.05	0.1	0.25
Cell viability(%)	100	94.54 ± 0.85	95.58 ± 2.70	94.57 ± 0.45
Toxicity	-	None	None	None

Cell line : MRC5
Sample material : Liquid
Test condition : Sample was diluted by concentration and treated with cells for 24 hours.
Evaluation : Three independent test.
Set control to 100% and check whether the relative cell viability is reduced.
Toxicity is evaluated as having cytotoxicity when the cell viability is reduced by 30% or more based on ISO 10993-5: 2009.

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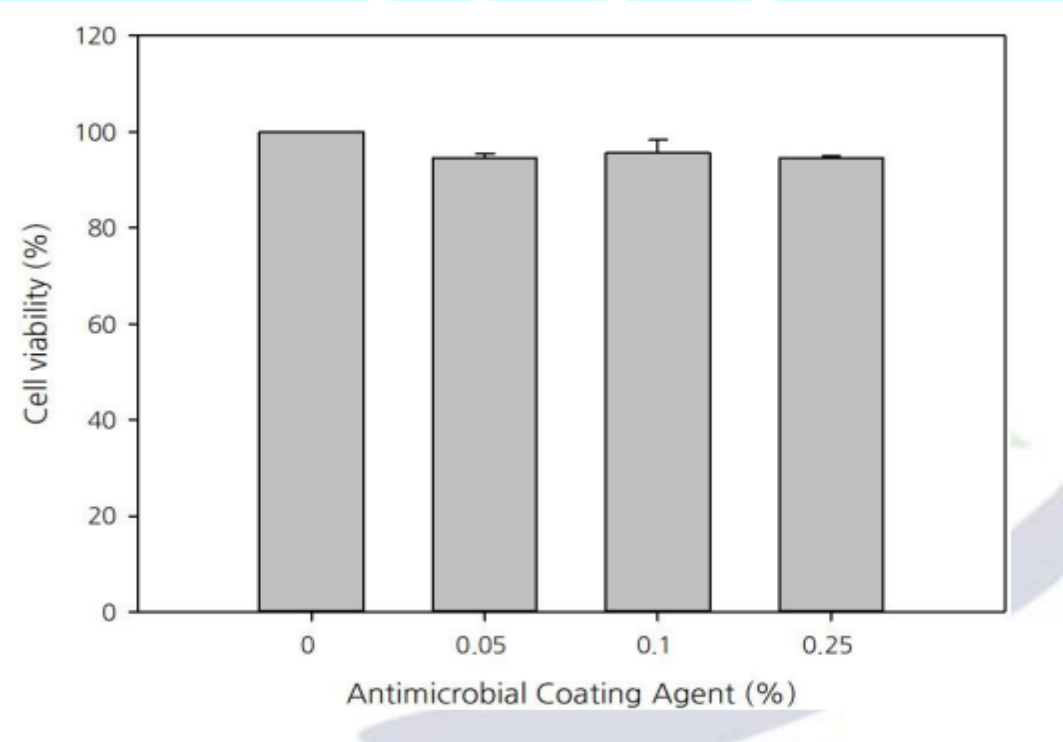


Figure 12. Cytotoxicity test result

Conclusion

This study demonstrates that nanocellulose can be effectively modified to develop antimicrobial materials with broad-spectrum activity against bacteria, fungi, and viruses. The various chemical surface modifications, including oxidation, esterification, etherification, and grafting with antibacterial agents such as phytochemicals and metal/metal oxide nanoparticles, significantly enhance the antimicrobial properties of nanocellulose. These modifications not only improve interaction with microbial pathogens but also contribute to the materials' biocompatibility and safety, as evidenced by the cytotoxicity tests showing cell viability above 94%.

The antimicrobial coating synthesized using nanocellulose exhibited promising antibacterial and antifungal activities, effectively inhibiting the growth of selected bacterial and fungal strains. The results of susceptibility testing confirmed its potential application in healthcare, food packaging, water treatment, and industrial sanitation. Importantly, the cytotoxicity evaluations confirmed that these modified nanocellulose materials are safe for biological applications, with minimal adverse effects on human cells.

Overall, the findings suggest that functionalized nanocellulose can serve as a versatile, eco-friendly platform for the development of advanced antimicrobial materials. Future research should focus on optimizing different modification strategies, investigating long-term stability and efficacy, and exploring scalable manufacturing processes for commercial applications. This approach holds

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significant potential for reducing microbial contamination and combating drug-resistant microorganisms across various fields.

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