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A Cellulose-based Hydrogel Composite with Antibacterial Activities Dressing on Wound Healing

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DECLARATION

I declare that this thesis entitled "A Cellulose-based Hydrogel Composite with Antibacterial Activities Dressing on Wound Healing" is the result of my own research except as cited in the references. The thesis has not been accepted for any agreement and is not concurrently submitted in the candidature of any degree.

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Date :_________________________

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Supervisor's Name :_________________________

Stamp :_________________________

Date :_________________________

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ABSTRACT

In this study, an improved approach for the production of hydrogel and its characterization of the hydrogel. The purpose of this research is to extract the cellulose from the corncob waste using the alkaline treatment method. Besides, the prepared cellulose-based hydrogel was incorporated with tannic acid, and the characterization of swelling, tensile strength, and antibacterial properties was performed on the cellulose-based hydrogel. The cellulose was used to form the cellulose-based hydrogel cross-link with the gelatin (GEL). Thus, the extraction of cellulose using 95% ethanol in a liquid pre-treatment, 5% acetic acid was used for the bleaching process to remove lignin, and 5% potassium hydroxide (KOH) was used to treat the cellulose extract from the corncob. Two different percentages of GEL, 40% GEL, and 50% GEL were used to prepare the cellulose-based hydrogel for wound healing. Therefore, to increase the chemical properties of the sample, Fourier transform infrared analysis (FTIR) was used on cellulose powder and cellulose-based hydrogel to identify the chemical reaction that occurred in the sample, and an antibacterial assay was performed using two different strains of bacteria, *E. coli* and *S. aureus*, to enhance the used of tannic acid as an antibacterial agent in biofilm with a different spectrum of bacteria. Swelling analysis was used to increase the mechanical properties of cellulose-based hydrogel with different percentages of cross-linker used, and tensile strength was performed to increase the physical properties of cellulose-based hydrogel itself. Thus, the results indicated that 40% of extracted cellulose was successfully produced in 30 g of dried corncob. From the all characterizations of the cellulose-based hydrogel, the cellulose-based hydrogel sample that cross-links with 40% GEL can be correlated to the better properties of swelling and other characterizations show the same properties of each sample.

ABSTRAK

Dalam kajian ini, untuk meningkatkan pernghasilan hidrogel dan pencirian hidrogel tersebut. Tujuan penyelidikan ini adalah untuk mengekstrak selulosa daripada sisa tongkol jagung menggunakan kaedah rawatan alkali. Selain itu, hidrogel berasakan selulosa yang dihasilkan dengan campuran asid tanik, akan diuji pencirian terhadap pembengkakan, kekuatan tegangan, dan sifat antibakteria. Selulosa digunakan bagi menghasilkan hidrogel daripada pautan silang bersama gelatin (GEL). Oleh itu, pengekstrakan selulosa menggunakan 95% etanol dalam pra-rawatan cecair, 5% asid asetik digunakan untuk proses pelunturan untuk mengeluarkan lignin, dan 5% kalium hidrosida (KOH) digunakan untuk merawat ekstrak selulosa daripada tongkol jagung. GEL yang berbeza peritus, 40% GEL dan 50% GEL telah digunakan untuk menyediakan hidrogel berasaskan selulsosa untuk penyembuhan luka. Oleh itu, untuk meningkatkan sifat kimia sampel, analisis inframerah transformasi fourier (FTIR) digunakan pada serbuk selulosa dan hodrigel berasaskan selulose untuk mengenal pasti tindak balas kimia yang berlaku di dalam sampel, dan ujian antibakteria dilakukan menggunakan dia bacteria yang berbeza strain eperti *E. coli* dan *S. aureus*, untuk meningkatkan penggunaan asid tanik sebagai antibakteria dalam biofilm dengan speltrum bakteria yang berbeza. Analisis pembengkakan digunakan untuk meningkatkan sifat mekanikal hidrogel berasaskan selulosa dengan perbezaan peratus penghubung silang yang digunakan, dan kekuatan tegangan dilakukan untuk menguji sifat fizikal hidrogel berasakan selulosa itu sendiri. Oleh itu, keputusan menunjukkan bahawa 40% selulosa berjaya diekstrak daripada 30 g serbuk tongkol jagung yang kering. Daripada semua pencirian hidrogel berasaskan selulosa, sampel hidrogel hidrogel berasakan selulosa yang memaut silang dengan 40% GEL boleh dikaitkan dengan sifat pembengkakan yang lebih baik dan pencirian lain menunjukkan sifat yang sama bagi setiap sampel.

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LIST OF ABBREVIATIONS

CNCs	Cellulose nanocrystals
FTIR	Fourier Transform Infrared Spectroscopy
FTIR-ATR	Fourier Transform Infrared Spectroscopy-Attenuated Total Reflectance
ROS	Reactive Oxygen Species
KOH	Potassium hydroxide
NaOH	Sodium hydroxide
GEL	Gelatine
TA	Tannic acid
ZOI	Zone of inhibition

LIST OF SYMBOLS

°C	Degree Celsius
g	Gram
Kg	Kilogram
kHz	kilohertz
L	Litre
Mins	Minutes
mL	Millilitre
inch	Inches
mm/min	Millimetres/minute
rpm	Revolutions per minute
v/v	Volume/Volume
w/v	Weight/Volume
W	Watt
cm^{-1}	Centimeter
cm^-1	Wavelength
µg/ml	Micrograms per milliliter
µm	Micrometre
mmHg	Milimetres of mercury
%	Percent
wt%	Percentage by weight
W_s	Weight of swollen
W_d	Weight of dry film sample

CHAPTER 1

INTRODUCTION

1.1 Background of Study

A hydrogel is a three-dimensional network composed of chemically, physically, or polymerization crosslinked hydrophilic polymer chains (Zainal et al., 2021). The network of a hydrogel has the ability to expand and trap a significant amount of water thanks to surface tension and capillary pressures. The availability of a functional group, the composition of the water, and the density of the crosslinking network in a hydrogel are the three factors that determine whether or not the hydrogel is capable of absorbing water. In addition, hydrogels can be used in a variety of physical configurations, including but not limited to: solid moulded forms (soft contact lenses), pressed powder matrices (pills or capsules for oral ingesting), microparticles (as bio-adhesive carriers or wound treatments), coatings (on implants or catheters), membranes or sheets (as a reservoir in a transdermal drug delivery patch), and encapsulated solids (Shoukat et al., 2020). In contrast, hydrogels are adaptable materials with unique properties that have applications as intelligent materials in fields as diverse as drug delivery, tissue engineering, wound healing, agriculture, textiles, and industry (Ali et al., 2022).

Traditional wound dressings, such as gauze, cotton, bandages, and other similar items, are intended to prevent bacteria and other pathogens from entering an open wound. On the other hand, they are unable to keep the wound bed suitably moist. As a replacement, hydrogels such a kind of modern dressing that have been develop recently and attracted wide attention which not only cover the wound, but also accelerate the haling of skin wounds (Qi et al., 2022).

The most important property of a hydrogel for use as a medical dressing is its antimicrobial properties (Liu et al., 2022). In addition, to prevent infections in wounds and stopping the transfer of germs, it should be able to destroy bacteria or inhibit their growth. It has been demonstrated that direct contact with a human wound and the application of an antibacterial hydrogel bandage successfully isolate microorganisms and maintain oxygen to the wound site, thereby preventing the in vitro infection of pathogenic microorganisms and promoting a more rapid healing process (Liu et al., 2022). The resistance of chronic wound biofilms to conventional antibiotics emphasizes the need for novel antibacterial wound-healing strategies.

Since it was how simple it was to design hydrogels and incorporate bioactive components into them, these materials have various uses as wound dressings. Hydrogel properties, such as composition, sensitivity to wound stimuli, etc., can be modified to deliver a wide range of mediators, such as those designed to kill bacteria and prevent infection, as well as those that combat inflammation and free radical damage, both of which are major obstacles in the treatment of chronic wounds. (Fan et al., 2021). As an alternative to antibiotics, research is being conducted on compounds that have proven to be effective against microorganisms, inflammation, oxidative stress, and wound healing but do not contain antibiotics. As antibacterial wound dressings, antibiotics, nanoparticles, cationic chemical compounds, and other biomaterials have been developed.

Tannic acid has been studied for its antibacterial properties and has been found to be effective against several bacterial strains (Jailani et al., 2022). Tannins are representatives of the polyphenol chemical family; tannic acid is a subset of tannins. This chemical can be found in its natural state in bark, fruits, foliage, and seeds. Tannic acid's low stability, poor bioavailability, and light sensitivity might limit its biological performance at the wound site; nonetheless, the promising features that make it a potentially efficient alternative to commercial antibiotics speak to the rising desire for new wound care treatments. Tannic acid's low stability, poor bioavailability, and light sensitivity could limit its biological performance at the wound site (Guimaraes et al., 2023).

The use of corn cob waste had a beneficial by-product to extract the cellulose. Among agriculture residue, corn cob is an essential residue of corn that contain nutrient-dense and is difficult to recycle as fertilizer or feed (Choi et al., 2022). In the preceding decade, the global output of maize grain increased by 40%, bringing the total quantity of maize grain produced to over 1 billion tonnes. Between 47% and 50% of the materials that are discarded are maize plant components such as stems, leaves, cobs, and stalks. In addition, the content of cellulose is rich in the corncob, if the cellulose of the corncob can be effectively separated, purified, and fully utilized, it will bring additional economic value and ecological benefits (Chen et al., 2020). Cellulose is a compound that has a hydrophilic character because of the presence of hydroxyl groups in each 2-polymer unit and corncob contains cellulose at about 44.9% (Isa et al., 2020).

Natural polymers are biocompatible and biodegradable, making them ideal for the production of biomedical hydrogels. Cellulose is nature's most prevalent polysaccharide, and it is found in plant cell walls. It is a strong and stiff material that provides structural support to plants. Cellulose has emerged as a compelling sustainable, non-toxic, and renewable material for water-based gels (Curvello et al., 2019). Due to the presence of lignin, hemicellulose, pectin, and ash in the cell wall, isolating extremely pure cellulose has been a topic of study for decades. The effectiveness of cellulose extraction in producing cellulose with low concentrations of lignin and hemicellulose depends on the extraction method and the chemical composition of plants. In order to obtain pure cellulose, it is necessary to remove lignin and hemicellulose, which are both non-cellulose components of plant materials. Either by utilizing enzymes or by treating the source material with a chemical mixture that contains sodium hydroxide and sodium sulphite, this can be accomplished.

1.2 Problem Statement

In the majority of cases, medical-grade adhesives are utilized in adhesive dressings. Before use, the adhesive substance is polymerized. However, currently, more than 300 million tonnes of polymers are produced annually, and their manufacturing processes generate a large deal of post-industrial waste that is difficult to recycle mechanically due to the complexity of its macro-contaminant separation phase. (Branco, 2020). Adhesive dressings have polymer backing, sticky layer, and protecting liners. Due to their different materials, certain parts may not be recyclable. Separating and recovering components becomes more diligently and expensive.

The benefits and qualities of hydrogels made from cellulose are vastly different. Wound dressing derived from chemicals are adaptable, but they may also be hazardous to the environment. In contrast, cellulose is deriving from the plant cell walls, thus cellulose-based hydrogels are preferable for use in biomedical, because it biodegrades and renews without harming the environment. Cellulose-based hydrogels are environmentally friendly and easy to break down their life cycle.

In addition, corn is known as the Queen of Cereals which is the second most significant cereal commodity in the world by acreage. Global corn production has reached 114.60 million metric tons and has been aimed to increase corn production by the year 2022 (Gandam et al., 2022). Thus, after the harvest, corncobs are another form of abundant and readily available organic refuse that contains high amounts of polysaccharides, cellulose, and xylan. Besides the low cost or even free agricultural waste, putting them to productive use will prevent them from being discarded or dumped in landfills, thereby contributing to a global reduction in waste.

1.3 Objectives

In this research, there are three objectives that need to be achieved. The objectives are:

- To extract the cellulose from the corn cob waste using alkaline treatment.
- To prepare cellulose-based hydrogel incorporating tannic acid.
- To characterize the swelling, tensile strength, and antibacterial properties of the cellulose-based hydrogel.

1.4 Scope of Study

The scope of the study to includes the preparation of cellulose hydrogel and the extraction of cellulose from the corncob waste by using alkaline treatment. The cellulose is incorporated with gelatine from bovine skin to produce hydrogel to create a sustainable and environmentally beneficial. Cellulose-based hydrogel loaded with tannic acid will be characterized based on swelling, tensile strength, and antibacterial properties.

1.5 Significant of Study

The present study is significant in several ways. Firstly, the extraction of cellulose from corncob waste has been identified as a promising alternative to reduce the volume of waste and stabilize the environment ecosystem. Hydrogel is one of the modern materials that potentially to replace adhesive material in biomedical applications for wound healing due to its biocompatible and non-toxic. The preparation of cellulose-based hydrogel that uses gelatine as a cross-linker has good characteristics in terms of swelling and tensile strength. Furthermore, the incorporation of cellulose-based hydrogel with tannic acid increases the antibacterial properties. Therefore, this study aims to produce cellulose-based hydrogel by characterizing swelling and tensile strength, besides having antibacterial properties for wound healing applications.

CHAPTER 2

LITERATURE REVIEW

2.1 Cellulose-based Hydrogel

Cellulose is one of the most predominant organic compounds because it is present in so many biomaterials (Popa et al., 2022). These biomaterials embrace trees, vegetation, fruits, vegetables, and biowaste, among a few more. Now, "green" and "renewable" must be incorporated into the methodologies of research initiatives that seek to create and carry out environmentally friendly products produced from natural raw materials. Bio-based materials are those derived from agricultural and edible industry refuse. Thus, bio-based materials evolve into innovative materials with applications in a variety of renewable resource and environmental problem sectors. Cellulose is the most prevalent organic molecule and polysaccharide on Earth; it is a linear, naturally occurring polymer that is made up of repeating glucose units ($C_6H_{10}O_5$). Cellulose is the most important component of plant cell walls (Seddiqi et al., 2021). It is odourless and tasteless, and it does not dissolve in water or the majority of organic solvents.

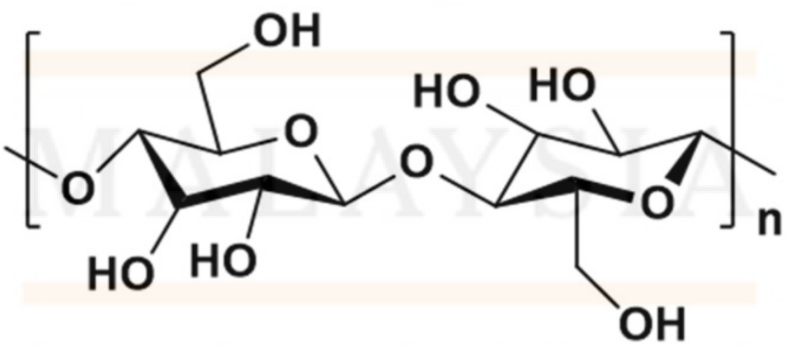

Figure 2.1: Chemical structure of cellulose

(Source: Chen at al., 2022)

Hydrogels are highly water-absorbent materials capable of retaining large amounts of water. As for the material, hydrogel can be produced either from natural or synthetic polymers (Zainal et al., 2021). Polymer hydrogels derived from natural sources are increasingly utilized because they are biodegradable, safer for use around living tissue, and less expensive (Klein & Poverenov, 2020). Since natural-based polymeric hydrogels require more complex processes and have a more limited spectrum of viable applications, their production is more difficult (Bae et al., 2022). However, natural polymers are preferred because they are more biocompatible and biodegrade more rapidly.

Synthetic polymers are preferable to natural polymers as a starting material for the production of synthetic polymer hydrogels because their chemical and physical properties can be altered to a greater extent. Synthetic polymers can have either a lengthy chain structure or a high molecular weight. Even so, synthetic hydrogels are more challenging to manufacture and have fewer potential applications, besides synthetic polymer hydrogels have lower biological activity than natural hydrogels (Bashier et al., 2020).

In medicine and healthcare, biomedical applications embrace a vast array of tools, materials, and methods that enhance the quality of an individual's life. Thus, because of their ability to absorb and retain large quantities of water, cellulose-based hydrogels make excellent wound dressings. These hydrogels can provide a moist environment, which promotes wound healing, inhibits infection, and stimulates tissue regeneration. They also have many kinds of advantages such as easy formulation to deliver hydrophilic and hydrophobic drugs, high drug loading capacity, convenient administration, sustained drug release capacity, and target specificity thereby avoiding the need for surgery (Tian et al., 2021) which makes them useful for drug delivery. Additionally, they can be used as a platform for advanced tissue engineering and regenerative medicine (Chen et al., 2022).

2.2 Tannic Acid

In recent years, there has been an advancement toward using natural substances as the fundamentals of material science. This tendency is exemplified by the expression "from nature to nature". Biopolymers have numerous applications in the field of biological research; however, the way to improve the properties of biopolymers as a drug delivery is under investigation (Gheorghiță et al., 2021). Incorporating organic and inorganic additives into biopolymers has the potential to improve their performance. Tannic acid is an interconnected molecule consisting of a gallic acid or glucose the supporting structure esterified with numerous galloyl groups. The inert polyphenol tannic acid has recently attracted a great deal of interest in biomedical research due to its unique biochemical properties (Bhattacharyya et al., 2022).

Abundant hydroxyl groups and a high affinity for forming hydrogen bonds with proteins and other biomolecules render tannic acid useful in a wide variety of circumstances. In biomedical applications, tannic acid has been shown to reduce inflammation as an antioxidant, act as an antibiotic in common pathogenic bacteria, and induce apoptosis in several cancer types (Baldwin & Booth, 2022). In sufficient amounts, tannic acid may be used to treat neurological disorders, repair severe burns, and ameliorate gastrointestinal issues such as haemorrhoids and diarrhoea.

In the research from Wu et al. in the journal of Regenerative Biomaterials (2022), tannic could help reduce inflammation early on and maintain a chronically low level. Hydrogels can be formed, their mechanical strength can be enhanced, and they can be imbued with anti-inflammatory, antioxidant, and antibacterial properties due to TA's ability to cross-link with a wide variety of polymer hydrogels through their hydrogen-bonding interactions.

Tannic acid has been reported to present activity against both Gram-positive and Gram-negative bacteria, such as Staphylococcus aureus, Escherichia coli, Streptococcus pyogenes, Enterococcus faecalis, Pseudomonas aeruginosa, Yersinia enterocolitica, and Listeria innocua (Kaczmarek, 2020). Even though the precise mechanism by which tannic acid exerts its antibacterial properties has not been determined, it is likely to involve the chelation of iron and the inhibition of peptidoglycan synthesis. Tannic acid has also been demonstrated to be effective at preventing biofilm formation (Conde, 2023).

2.3 Incorporation of Tannic Acid into Cellulose-based Hydrogel

Tannic acid is not effective on its own for wound treatment (Hafeji & Danckwerts, 2020). Given that it is so readily accessible, direct application of tannic acid to incisions necessitates careful consideration of the acid's concentration and purity. Attempt to utilize tannic acid in its undiluted or purified form, it can experience certain unpleasant adverse effects. When administered topically, tannic acid has the potential to aid in wound healing and make the epidermis more elastic (Setiawati et al., 2023). This may not only be irritating, but it may also interfere with the body's normal healing process by delaying wound healing or causing excessive dryness.

The possibility for the antibacterial, antioxidant, and metal ion chelation properties of tannic acid to be transferred into a cellulose-based hydrogel. Because of cellulose-based hydrogel biocompatible, and high water-absorption capacity, tannic acid can be incorporated into a cellulose hydrogel via a variety of techniques, including physical merging, chemical cross-linking, and electrostatic interactions, among others. Depending on the application and the desired characteristics of the hydrogel, a variety of concentrations, solvents, and crosslinking procedures can be applied to the hydrogel. Tannic acid can be used to construct a hybrid hydrogel dressing by simple copolymerization (Yang et al., 2022). However, tannic acid may be readily incorporated into cellulose hydrogel for use in wound healing by means of an effortless and efficient procedure. By continuously stirring, gradually incorporate the tannic acid solution into the cellulose hydrogel. The mixture must be vigorously stirred to ensure that the tannic acid is uniformly distributed throughout the hydrogel.

In the aftermath of producing the gel and incorporating tannic acid, the resultant cellulose hydrogel can be applied to lesions to expedite healing. Even though this material appears capable of accelerating wound healing, additional research and testing are necessary to determine its maximum potential. In some issues, increasing the amount of tannic acid in the hydrogel result in a decrease in the water vapor permeation rate (Wekwejt et al., 2022). The incorporation of tannic acid into the cellulose hydrogel is proportional to the concentration of the hydrogel. As the concentration of tannic acid rises, the matrix with which it interacts expands, which may affect the rate at which it is incorporated.

2.4 Antibacterial Activity of Tannic Acid Incorporated Cellulose-based Hydrogel

The cellulose-based hydrogel containing tannic acid is effective against microorganisms. In order to determine whether or not the hydrogel inhibits the growth of bacteria, it is frequently tested against a wide range of bacterial strains. Biofilm-associated infections are at a high rate of recurrence and biofilms show formidable resistance to current antibiotics, making them a growing challenge in the biomedical field (Zhang et al., 2020). In some case, the disk diffusion assay is being used to assess the antibacterial properties of tannic acid in cellulose hydrogel (Leite et al., 2021).

Tannins have been shown to be detrimental to the bacterial cell wall. Although tannic acid has demonstrated antibacterial activity and the ability to inhibit biofilm formation. These have been reported in a research study based on the results of the time-kill assay, binding ability assay, lysozyme susceptibility assay, and the transmission electron microscope, we tentatively speculated that peptidoglycan might be the target of the process that tannic acid destroys the integrity of cell wall, moreover, tannic acid could reduce the biofilm formation at optimize concentrations (Siddiqui et al., 2019). This can disrupt vital biological processes, leading to the eventual demise of the bacterium.

According to Onyeogaziri and Papaneophytou (2019), a time-kill assay is a test that evaluates the growth or mortality of bacteria over time following exposure to varying antimicrobial agent concentrations. This examination is conducted over a period of time. The bacteria for this test are prepared by diluting the bacterial culture so that the test can be conducted with a known number of bacteria, adding the antimicrobial agent in varying concentrations, plating the bacterial culture to determine the number of viable bacteria, counting the colonies to determine the rate at which the bacteria grow and die over time, and analysing the data to determine the minimum effective concentration of the antimicrobial agent. All of these stages are required in order to ascertain the optimal antimicrobial agent concentration.

Binding ability assay can disclose protein-molecule interactions. These techniques can be utilised to investigate ligand-receptor interactions, protein-protein interactions, and binding kinetics. Enantiomer-specific properties include specific binding ability, the ability of a protein to bind to a nucleic acid aptamer in its free state but not in its target-bound folded state, the difference in diffusion rates between the targeted molecule and the aptamer/target complex, and the ability of two enantiomers to replace a pre-binding redox probe, resulting in different dual signals for the two enantiomers (Ebrahimi et al., 2021).

Assays for lysozyme susceptibility can measure the sensitivity of microbes to the enzyme. Lysozyme ruptures bacterial cell walls by cleaving the peptidoglycan layer (He et al., 2020). The lysis of microorganisms is measured by exposing them to lysozyme. Changing the bacterium's peptidoglycan coating is one method to increase or decrease its sensitivity to lysozyme.

Tannic acid can induce the generation of reactive oxygen species (ROS) within bacterial cells, such as superoxide radicals and hydrogen peroxide, which cause oxidative stress and damage to bacterial components, including DNA, proteins, and lipids (Mhlanga et al., 2019). It is possible for microorganisms to cease functioning when subjected to an inordinate amount of oxidative stress.

In the study by Wang et al. (2020), the content of silver nanoparticle present in the gel could change the surface charge and affects its swelling behaviour, besides this gel exhibited good antimicrobial activity on typical gram-positive and gram-negative bacteria, implying the potential applications of these silver nanocomposite hydrogels as antibacterial agents in related areas. Some bacteria may respond similarly to the antibacterial effects of tannic acid hydrogels, but the antibacterial activity of silver-based hydrogels is often higher than that of tannic acid-based hydrogels. Conversely, cytotoxicity or other adverse reactions in living tissues may occur if nanoparticles are present in excessive quantities or if the wrong types of nanoparticles are utilized.

2.5 Characteristics of Hydrogel

In order to build hydrogels that have the necessary performance and structure, it is crucial to first determine and then describe the network properties of the hydrogel. The approaches that are often used to investigate hydrogels are only a tiny portion of the numerous that are now accessible. A simple approach for testing whether or not a system contains hydrogel may be accomplished by dispersing the polymer in water using a cylindrical vial and then witnessing the development of material that is insoluble (Azeera et al., 2019).

Hydrogels are a type of polymeric material that, when exposed to water, expands and retains a high-water content without decomposing. Thus, one of the most crucial technical aspects of hydrogels is their swelling rate. By taking measurements of the free absorbency capacity at predetermined intervals, one may calculate the swelling rate of the hydrogel sample by obtaining a profile of the swelling capacity versus the passage of time for the material (Zhang et al., 2020). It is common practice to assess free-absorbency capacity using either the tea-bag method, the sieve method, or the filtration method, with the selection of methodology typically based on the quantity of the sample and the required precision. The maximum swelling ratios for cellulose hydrogels by the freezing method and the heating method were 2420.93% and 2467.72%, respectively achieved by using cellulose concentration of 2.5 w/v % and 4 % (freezing method) (Chin et. al., 2021).

Hydrogels, which are typically flexible and highly water-absorbent, can vary in tensile strength based on factors such as their chemical composition and degree of cross-linking. On the other hand, a study from Kang and Yun (2022) reported that double-network hydrogel films based on cellulose derivatives and k-carrageenan exhibited an increase in tensile strength by 305% compared with single-network hydrogels. It is possible to draw the conclusion that the tensile strength of cellulose hydrogel may be significantly increased by cross-linking it with other materials such as bacterial cellulose, gelatine, CNCs, or carrageenan. This can be concluded from the previous sentence.

Additionally, with the use of Fourier Transform Infrared Spectroscopy (FTIR), it may be possible to gain a deeper comprehension of the chemical interactions and bonding that take place inside the hydrogel network. The FTIR spectrum of cellulose typically exhibits characteristic peaks related to cellulose's chemical structure, such as O-H stretching vibrations around 3300 cm^-1, C-H stretching vibrations around 2900 cm^-1, and C-O-C stretching vibrations around 1000-1150 cm^-1 (Zope et al., 2022). Amide bond peaks, also known as C=O stretching and N-H bending vibrations, can sometimes be detected in the FTIR spectra of gelatin. These peaks often appear between 1550 and 1650 cm-1 and 1650 and 1700 cm-1, respectively. Tannic acid is a phenolic compound that exhibits peaks associated with aromatic C=C stretching vibrations around 1600-1700 cm^-1 and hydroxyl (O-H) stretching vibrations around 3200-3600 cm^-1 in its FTIR spectrum (Mukhaaifi, 2020).

CHAPTER 3

MATERIALS AND METHOD

3.1 Materials

In this study, 5 kg of fresh corncob waste was used as the raw material. The corncob waste was collected from Berkat Agro Sdn. Bhd., Sungai Petani, Kedah.

3.2 Apparatus and Equipment

The apparatus used in this study were 1000 mL conical flask, 1000 mL beaker, Whatman No. 4 filter paper, Buchner funnel (90 mm diameter), Stirrer, Pyrex glass petri plates, Wire loop and Ruler.

Equipment used in this study were Immersion blender, Filtration vacuum pump (685 mmHg), Orbital shaker, Hot-air oven, Fourier Transform Infrared Spectroscopy (FTIR), Desiccator, Autoclave, and Texture analyzer (TA XT plus, Stable Microsystem, Godalming, UK).

3.3 Chemical

Chemicals used in this study were 99% toluene, 95% ethanol, 90% ethanol, 1.4% sodium chlorite ($NaClO_2$), 5% acetic acid, 5% potassium hydroxide (KOH), 14% NaOH, Tannic acid, Gelatine, Phosphate-buffered, Nutrient agar, Nutrient broth, and Distilled water.

3.4 Preparation of Raw Materials

3.4.1 Preparation of Dried Corncob Powder

The preparation of the dried corncob powder required multiple steps. 5 kg corncob was collected and cleaned with distilled water in order to eliminate dust and dirt particles that had stuck to it. After the sample is washed, it was divided into pieces ranging from 3 to 5 cm in length and placed in a hot air oven set to a temperature of 60ºC for a period of 48 h. After the sample dries, it was crushed into a powder using an immersion blender to produce 50 g of samples.

Figure 3.1: Dried corncob that was cut into a piece and crushed into a powder

3.5 Methods for the Production of Cellulose

3.5.1 Preparation of Cellulose

From the Figure 3.1 which 30 g corncob samples that was cut into a piece and crushed into a powder were dissolved in 150 mL of 95% ethanol using 1000mL of the conical flask, and left for shaking in a closed cabinet of the bench-top temperature-controlled orbital shaker at 150 rpm and 25°C for 20 h.

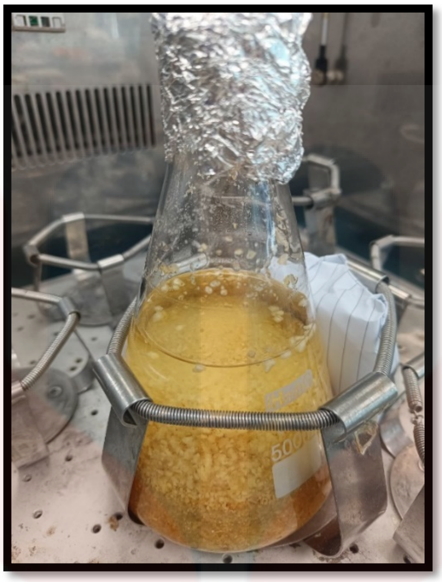

Figure 3.2: Pre-treatment process of dry corncob using 1.4% $NaClO_2$

Based on Figure 3.2, the pre-treatment of dry corncob using 1.4% $NaClO_2$, the pre-treatment sample was collected after the shaking process and was filtered through Whatman No. 4 filter paper using a Buchner funnel (90 mm diameter) equipped with a filtration vacuum pump (685 mmHg). After the removal of the solvent by filtration, the sample was washed with absolute ethanol and filtered again before drying for 1 h at 100ºC, dried fibers was mixed with 1.4% (w/v) sodium chlorite ($NaClO_2$) to bleach the sample fibers. The pH of all samples was adjusted to 4 by using 5% (v/v) acetic acid solution followed by heating at 70ºC with continuous stirring using an overhead stirrer at 500 rpm for 5 h.

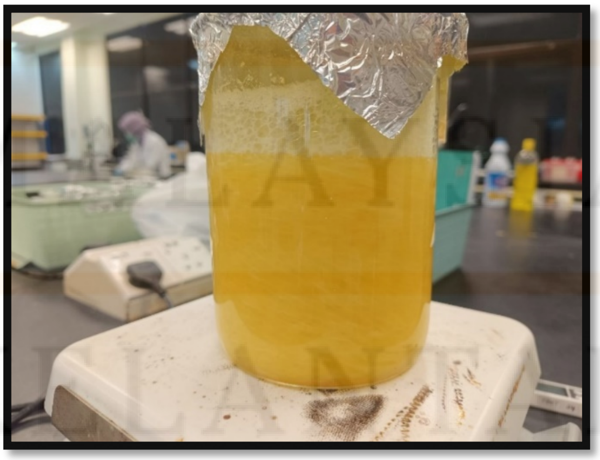

Figure 3.3: Bleaching process using 5% acetic acid to remove the lignin

From the Figure 3.3, the bleaching process using 5% acetic acid to remove the lignin, the sample fibers were filtered and washed with distilled water until a neutral pH was maintained and dried in the hot-air oven at 100°C for 16 h. After bleaching, dried fibers was soaked in 600 mL of 5% (w/v) potassium hydroxide (KOH) using a 1000 mL beaker and stirring was continued at 500 rpm under room temperature for 24 h prior to heating at 90°C for 2 h for the extraction of cellulose.

Figure 3.4: Extraction of cellulose using 5% KOH

The KOH-treated sample was washed with distilled water until a neutral pH was attained, followed by drying in a hot-air oven at 100°C for 20 h to obtain cellulose. The yield of cellulose was determined by using formulation between dried corncob and cellulose powder obtained.

Figure 3.5: KOH-treated sample that has been washed with distilled water

$$\textbf{\textit{Extraction yield}}\ (\%) = \frac{\textbf{\textit{Dry mass of cellulose powder obtained}}\ (g)}{\textbf{\textit{Dry mass of corncob}}\ (g)} \times \mathbf{100}$$

Table 3.1: The percentage (%) of extraction yield from 30 g of dry corncob

Dry corncob (g)	Cellulose powder (g)	Extraction yield (%)
30	12	40

Figure 3.6: Sample of extracted cellulose after drying in hot air oven at 100ºC

3.5.2 Analysing cellulose yield

To determine the functional group present in plants, such as lignin, cellulose, and hemicellulose. Fourier Transform Infrared Spectroscopy (FTIR) analysis was performed on approximately 500 mg of the fine powder that included the extractives. Reflectance spectra were obtained by placing the sample powder in a position that was directly in front of the light source. The FTIR spectra were acquired using a Perkin-Elmer FTIR at the temperature of the surrounding air, with a resolution of 4 cm^{-1} and a wavenumber range of 4000 to 400 cm^{-1}. Each spectrum was obtained by averaging the results of four separate scans, and the speed of each scan was 0.2 $cm^{-1}s^{-1}$. It was determined by interpreting the spectrum to which functional group the extractives belonged.

3.6 Production of Cellulose-based Hydrogel

3.6.1 Preparation of Hydrogel

4% of cellulose were prepared with 6 g of urea solution that have been dissolve in 3.5 g of NaOH and 50 mL distilled water at 0ºC and was stirred for 2 min. Then 0.1 g tannic acid was added and reacted at room temperature for 12 h. Crosslinking will accomplish with gelatine (GEL), 50 mL of distilled water solution was mixed with GEL at 40ºC until the GEL was completely dissolved to produce 40% and 50% of GEL. After 1 h at 40ºC, the cellulose-TA solution was added to the GEL solution and thoroughly mixed. These solutions were vacuum-pumped to eliminate bubbles. The resulting gel solutions were distributed evenly among several Pyrex glass petri plates with 5-inch diameters and allowed to air dry at normal room temperature for 2 days. Dried films then were peeled from the petri plates and stored in a desiccator for further use.

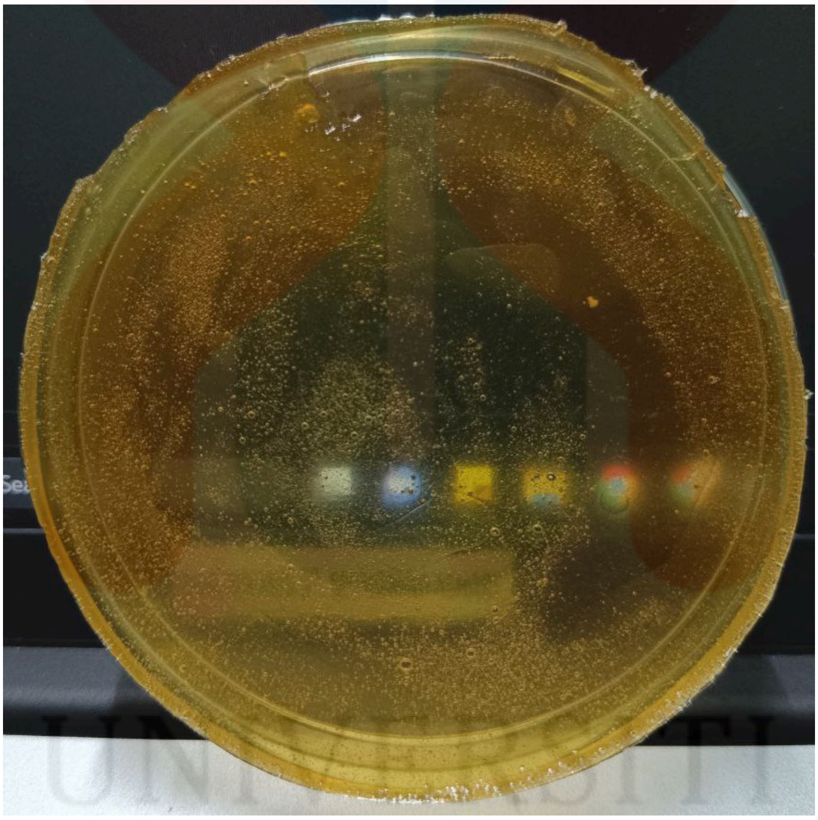

Figure 3.7: Cellulose-based hydrogel with 40% GEL

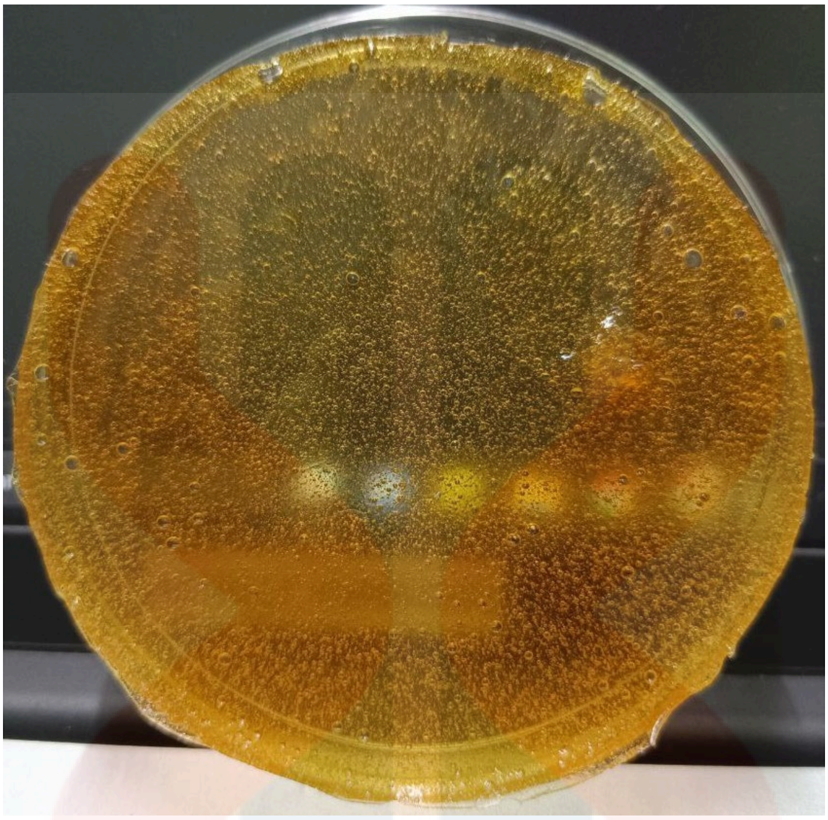

Figure 3.8: Cellulose-based hydrogel with 50% GEL

3.7 In-vitro Antibacterial Assay

3.7.1 Preparation of agar Medium

28 g of nutritional agar were suspended in 1 litre of distilled water. For the preparation of the medium, distilled water was used. The composition remains in this form until all components have dissolved. The medium is then sterilized in an autoclave for 15 minutes at 121°C. After the petri dish has been sanitized, place nutrient agar on a level surface, and let it for 30 minutes to allow the medium to be solidify.

3.7.2 Preparation Suspension Culture of Bacteria

Two kinds of different gram-staining bacteria were used in this study, *Staphylococcus aureus* as a gram-positive bacteria and *Escherichia coli* as a gram-negative bacterium. Approximately one colony of bacteria was dissolved in nutrient broth (13 g of nutrient broth were dissolved in 1 litre of distilled water, and was sterile by autoclaving at 121°C for 15 minutes before use). A single colony bacterium was transferred using the wire loop that has been sterilized with 90% ethanol and heat for at least 30 seconds to avoid crosscontamination. The bacteria culture was incubated overnight in an orbital shaker at 37ºC with 150 rpm.

3.7.3 Disk Diffusion Method

Antibacterial activity was determined by the agar well-diffusion method. In this method, two samples were prepared. The first sample is the cellulose hydrogel with tannic acid, and the second sample is the cellulose hydrogel without the addition of tannic acid. Thus, approximately 1 cm in diameter of the test hydrogels was added to the plate and the plate was kept in an incubator at 30ºC. After 48 h, the zones of inhibition (ZOI) were measured by using a standard ruler.

3.8 Characterisation of Hydrogel

3.8.1 Swelling Test

The dried hydrogel films were cut into square-shaped specimens (2 cm x 2 cm). The samples were weighed and immersed in 250 mL of distilled water, at 25ºC. After 8 h, the film samples were weighed after blotting with tissue paper to remove the surface water.

$$\boldsymbol{Swelling}\ (\%) = (\boldsymbol{Ws} - \boldsymbol{Wd})/\boldsymbol{Wd} \times \mathbf{100}$$

Equation 3.1: Swelling percentage (%)

where W_d is the initial weight of the dry film samples and W_s is the weight of swollen film samples. The experiment was performed in triplicate.

3.8.2 Tensile Strength

The samples of hydrogel films were analyzed for mechanical properties (tensile strength (in N/mm^2) and elongation at break (%)) by texture analyzer (Universal Testing Machine (UTM)) with 5 kg of the loaded cell. Film of size 1 cm^2 was cut and clutched between the clamps followed by separation at the rate of 5 mm/min longitudinally (Chopra et al., 2022). The experiment was performed in triplicate.

3.8.3 Chemical Composition in Hydrogel

In general, it is advised to use hydrogel particulates with a size distribution that promotes accurate and representative analysis. To ensure uniform dispersion and interaction with infrared radiation, standard procedure dictates that the particle size should be sufficiently small in micrometer. The Fourier Transform Infrared Spectrophotometer (FTIR) with Attenuated Total Reflectance (FTIR-ATR) accessory to obtain infrared spectra of the samples. 10 µm of the sample were analyzed with the spectra in the range of 400-4000 cm-1, using 64 scans, and a resolution of 4 cm^{-1}.

CHAPTER 4

RESULTS AND DISCUSSION

The characterization of all samples that are extracted cellulose and cellulose-based hydrogel with 40% GEL and 50% GEL. The cellulose was obtained by the extraction of dried corncob using the alkaline treatment method, and the cellulose-based hydrogel was cross-link with GEL to improve their mechanical properties. All samples were analyzed by FTIR to discover the specific functional group in the molecule of the sample, and the characterization of cellulose-based hydrogel were analyzed for efficacy in vitro antibacterial assay, swelling analysis, and tensile strength analysis. The characterization of in vitro antibacterial assay is to evaluate the properties of tannic acid in the hydrogel as effectiveness compound substance against bacterial to be used in medical application for wound healing. The analysis of swelling due to the characterization of hydrogel itself that excel at absorbing water which for medical application the potential for effective absorption of wound exudate can promote wound healing and infection prevention. The tensile strength analysis was to the measure the physical properties of hydrogel to adapt with different environment.

4.1 FTIR Analysis

In order to gain details investigation of the samples, identification on chemical composition and structural features all the sample of extracted cellulose and the form cellulose-based hydrogel using FTIR analysis. FTIR works by passing the sample through infrared light and observing the resulting absorption patterns, which can reveal the chemical properties of the samples. The FTIR machine used was a Nicolet iS50 model, located at University of Malaysia Kelantan.

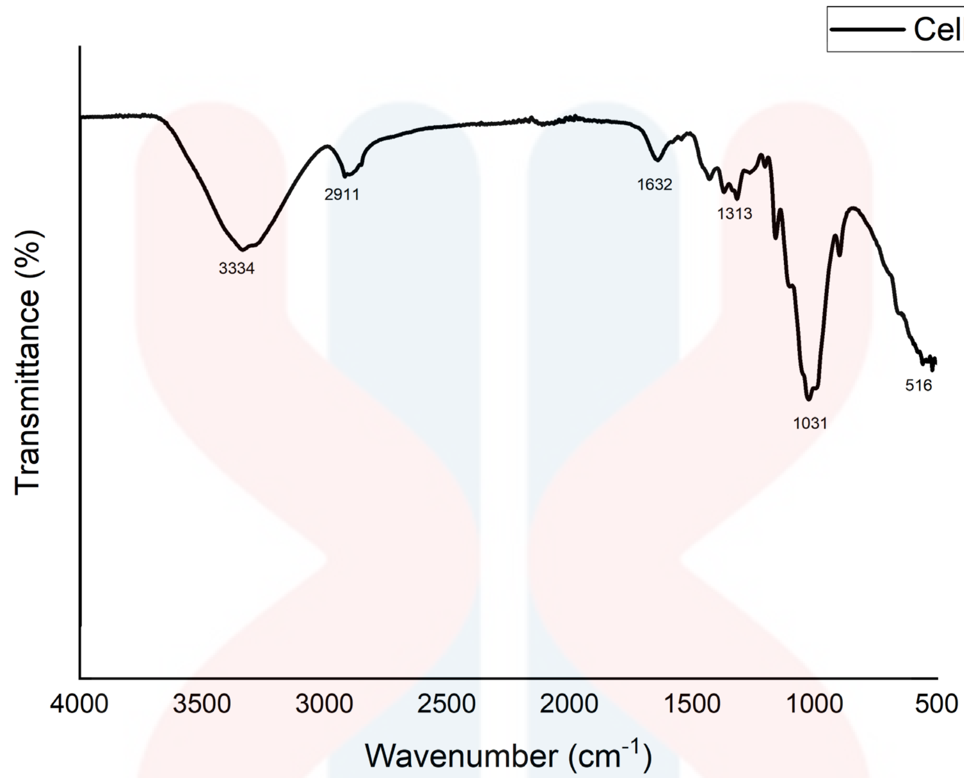

Figure 4.1: FTIR spectra of the functional group of aliphatic hydrocarbons at peak 2911 cm^{-1} using wavelength range 400-4000 cm^{-1}

As shown in Figure 4.1 which FTIR spectra of the functional group of aliphatic hydrocarbons at peak 2911 cm^{-1} using wavelength range 400-4000 cm^{-1}, the absorption at 2911 cm^{-1} in the extracted cellulose samples are indicative of increased C-H stretching vibrations and O-H bending of absorbed water. These peak shows the present of aliphatic hydrocarbons that have being discharged. Plus, peak 2911 cm^{-1} provides evidence of a polysaccharide consisting of glucose units joined by β-1,4-glycosidic linkages, as hypothesised by Eddy et al. (2019). Furthermore, Chen et al. (2020) in the journal of Catalysis Science & Technology conclude that cellulose is often-used as a biopolymer and is formed by β-1,4-glycosidic bonds connecting anhydro-glucose units.

Based on Table 4.1, which the group frequency of absorption bands of extracted cellulose sample using FTIR wavelength range 400-4000 cm^{-1}, a frequency of 1313 cm^{-1} signifies the presence of a C-O stretching vibration in cellulose. The frequency resonance is caused by the glycosidic link between glucose units in the cellulose polymer chain. The intensity of this prominent peak was further amplified in the sample containing extracted cellulose at peak 1031 cm^{-1}. The spectra of the samples exhibited a prominent peak at a frequency of 1031 cm-1 shows the stretching vibrations of the C-O in the changes of cellulose structure. An antisymmetrical phase ring found in cellulose molecules, were identified as the source of this peak (Ashurov et al., 2021).

Table 4.1: Group frequency of absorption bands of extracted cellulose sample using FTIR wavelength range 400-4000 cm^{-1}

Group frequency wavenumber, cm^{-1}	Origin	Assignment
~1031	C-O	C-O stretching vibration in changes of cellulose structure
~1313	C-O	C-O stretching vibration in cellulose
~1632	O-H	Bending vibrations of water molecules
~2911	C-H	C-H stretching vibration in aliphatic groups
~3334	O-H	Free and hydrogen bonded OH stretching

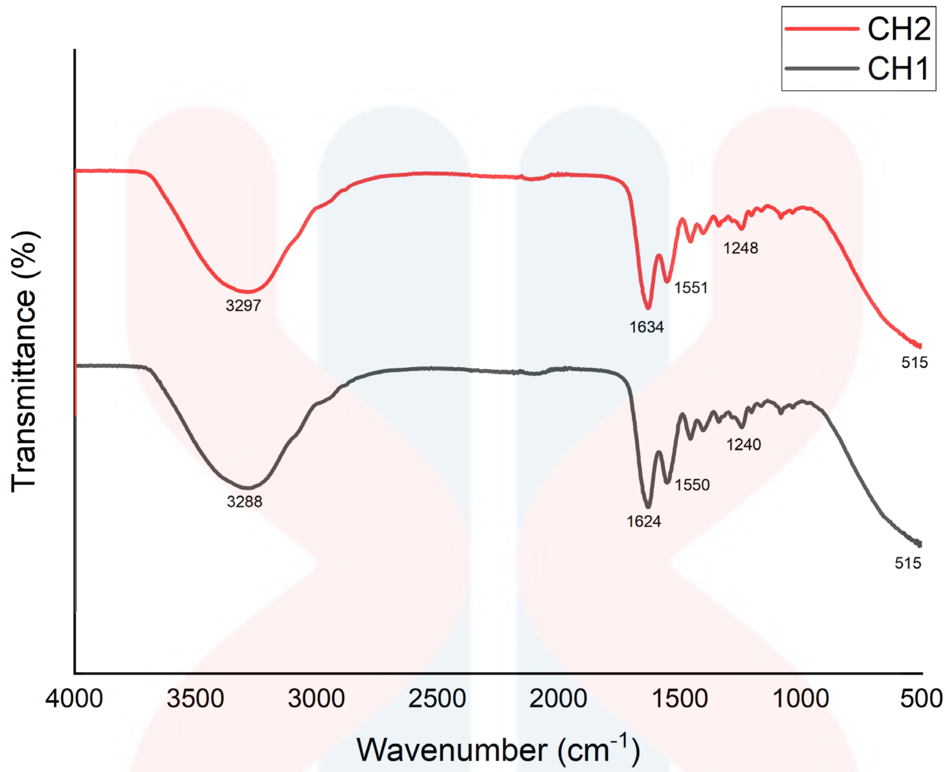

Figure 4.2: FTIR spectra of cellulose-based hydrogel of (CH1) cellulose-based hydrogel with 50% GEL, (CH2) cellulose-based hydrogel with 40% GEL using wavelength range 400-4000 cm^{-1}

Based on **Figure 4.2** consists of FTIR spectra of cellulose-based hydrogel of (CH1) cellulose-based hydrogel with 50% GEL, (CH2) cellulose-based hydrogel with 40% GEL using wavelength range 400-4000 cm^{-1}, all spectra samples were almost identical which indicated no changes in the functional group. The absorption bond at ~515, ~1240, ~1550, ~1624, and ~3288 cm^{-1} shows the same spectrum of cellulose-based hydrogel that have been treated with different GEL %.

Moreover, all of the sample's cellulose-based hydrogel showed a broad of bands in the region 3350 to 3180 cm^{-1} that indicate correspond to N-H stretching and aliphatic C-H stretching. To facilitate the dispersion or dissolution of cellulose, a reaction occurs between the all samples of cellulose-based hydrogel which the present of amide functional groups present in the extracted cellulose and cellulose-based hydrogel.

The interaction between amide groups and cellulose molecules facilitates the formation of gel, ultimately resulting in the formation of a three-dimensional network. In advance, interactions may enhance the swelling characteristics of cellulose hydrogel

(Khalifah et al., 2022). By that, the nature characteristic of hydrogel is possessing the capacity to absorb substantial volumes of water. Thus, this characteristic suggests that the presence of functional groups of amides have been successfully form the hydrogel with better mechanical properties.

Table 4.2: Group frequency of absorption bands of cellulose-based hydrogel samples using FTIR wavelength range 400-4000 cm^{-1}

Group frequency wavenumber, cm^{-1}	Origin	Assignment
~1248	C-O	C-O stretching vibrations from cellulose
~1551	N-H	N-H bending, C-N stretching vibration in gelatine
~1634	C=O	C=O stretching vibration
~3297	O-H	Hydrogen bonded OH stretching within cellulose and gelatine

4.2 In Vitro Antibacterial Assay

In vitro antibacterial assay used to evaluate the effectiveness of cellulose-based hydrogel in inhibiting the growth of bacteria. Thus, in vitro antibacterial assay was performed using disk diffusion method using the different strain of bacteria, by representative *E. coli* and *S. aureus*. The purpose of this experiment was to determine whether tannic acid's antibacterial activity remained unchanged following its addition to the films. Thus, **Figure 4.3** consist of disk diffusion antibacterial on cellulose-based hydrogel using (I) *E. coli* and (II) *S. aureus* shows the results of the efficacy of cellulose-based hydrogel as antibacterial properties.

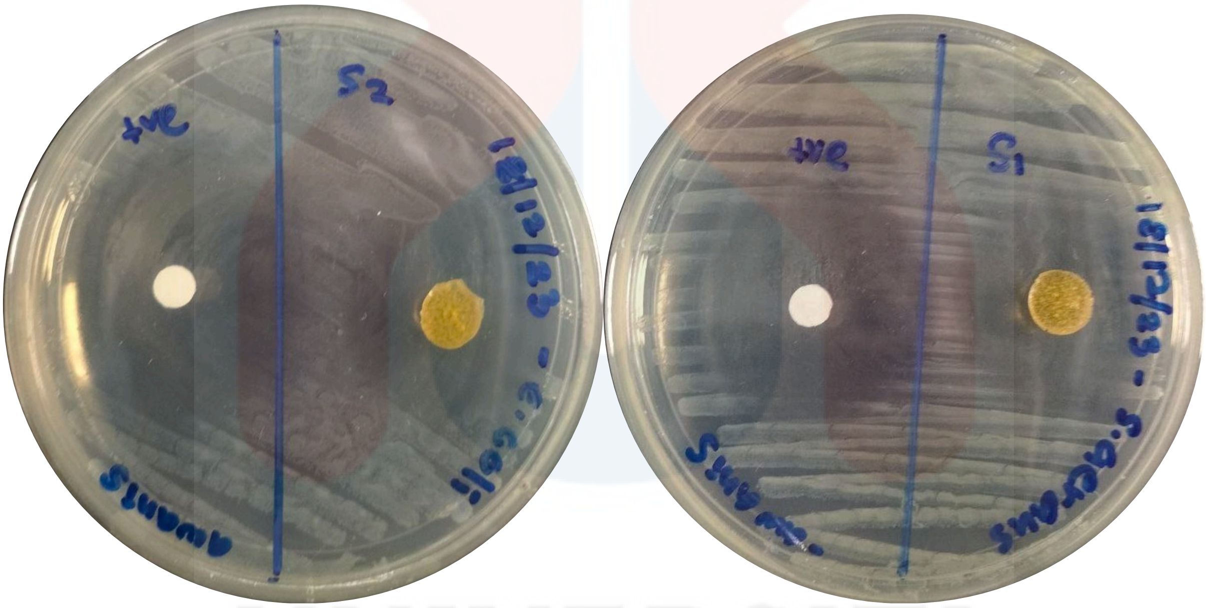

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Figure 4.3: Disk diffusion antibacterial on cellulose-based hydrogel using (I) *E. coli* and (II) *S. aureus*

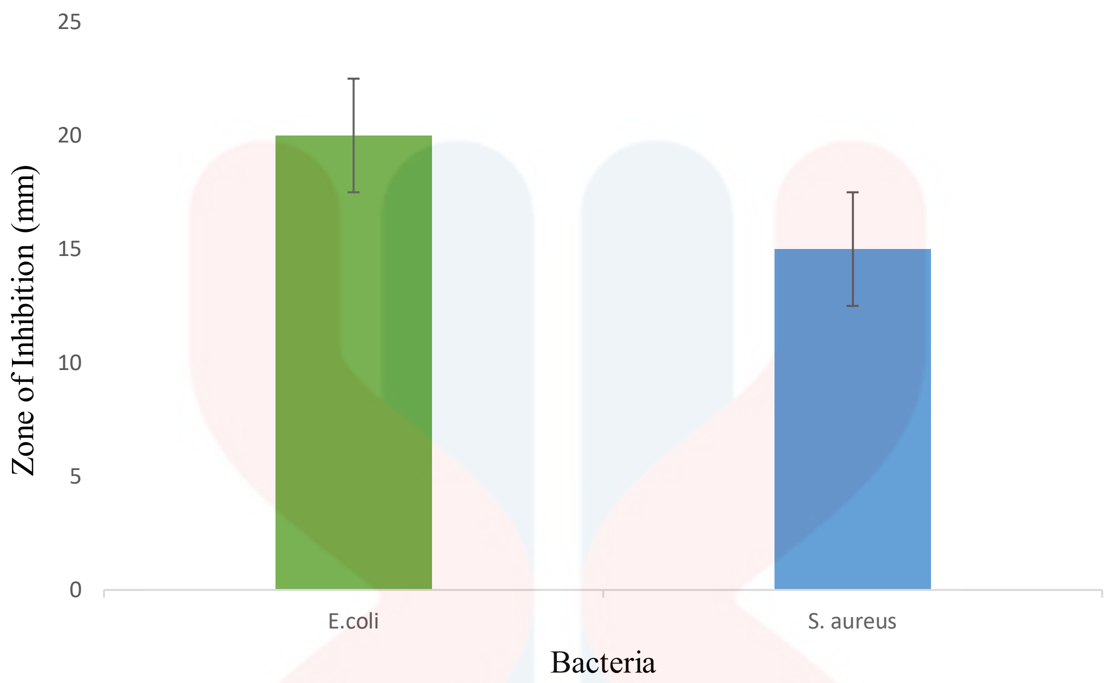

Figure 4.4: Zone of inhibition (ZOI) of antibacterial assay on cellulose-based hydrogel

As illustrated in **Figure 4.3** consist of disk diffusion antibacterial on cellulose-based hydrogel using (I) *E. coli* and (II) *S. aureus*, the presence of distinct inhibitory zones encircling the hydrogels served as evidence for the incorporation of tannic acid towards the cellulose-based hydrogel can prevent the growth of bacteria. In the research of Colloids and Surface Biointerfaces (2021), tannic acid has shown promise as an antibacterial agent in hydrogel systems designed for wound healing. Hydrogels containing tannic acid have been proven in several studies to successfully fight bacterial infections and enhance wound healing. Nevertheless, the impact on the film sample remained to a significant degree and the agar medium exhibited a clear indication of an inhibitory zone when the tannic acid was in the form of a film.

To determine the width of the inhibitory zone (*W*), the formula $W=Dz - Ds$ was applied. In this instance, the diameter of the outer zone is denoted by *Dz*, whereas the diameter of the hydrogel sample is represented by *Ds*. As illustrated in **Figure 4.4** consists of zone of inhibition (ZOI) of antibacterial assay on cellulose-based hydrogel, the hydrogel samples exhibited an average inhibitory zone width of approximately ±15.0 mm against *S. aureus* and ±20.0 mm against *E. coli*. The results indicated that the antibacterial properties of the hydrogel exhibited greater efficacy against *E. coli* in comparison to *S. aureus*.

Notwithstanding this, the hydrogel coating retains its remarkable antibacterial properties even subsequent to the introduction of tannic acid.

S. aureus is one of the most prevalent wound-infecting microorganisms (Feng et al., 2021). Furthermore, *E. coli* is often utilised in the evaluation of hydrogels designed to promote wound healing. It is possible to ascertain the superior antimicrobial properties of two distinct bacterial species by combining them against specific bacterial strains.

4.3 Swelling Analysis

Swelling analysis helps determine the water absorption capacity of hydrogel which increasing the water capacity leads to the increasing the physical properties of the hydrogel. The swelling properties of hydrogels is influenced by a multitude of factors. These types of factors include the quantity of crosslinking, the composition of the hydrogels, and the existence of functional groups (Vo et al., 2022).

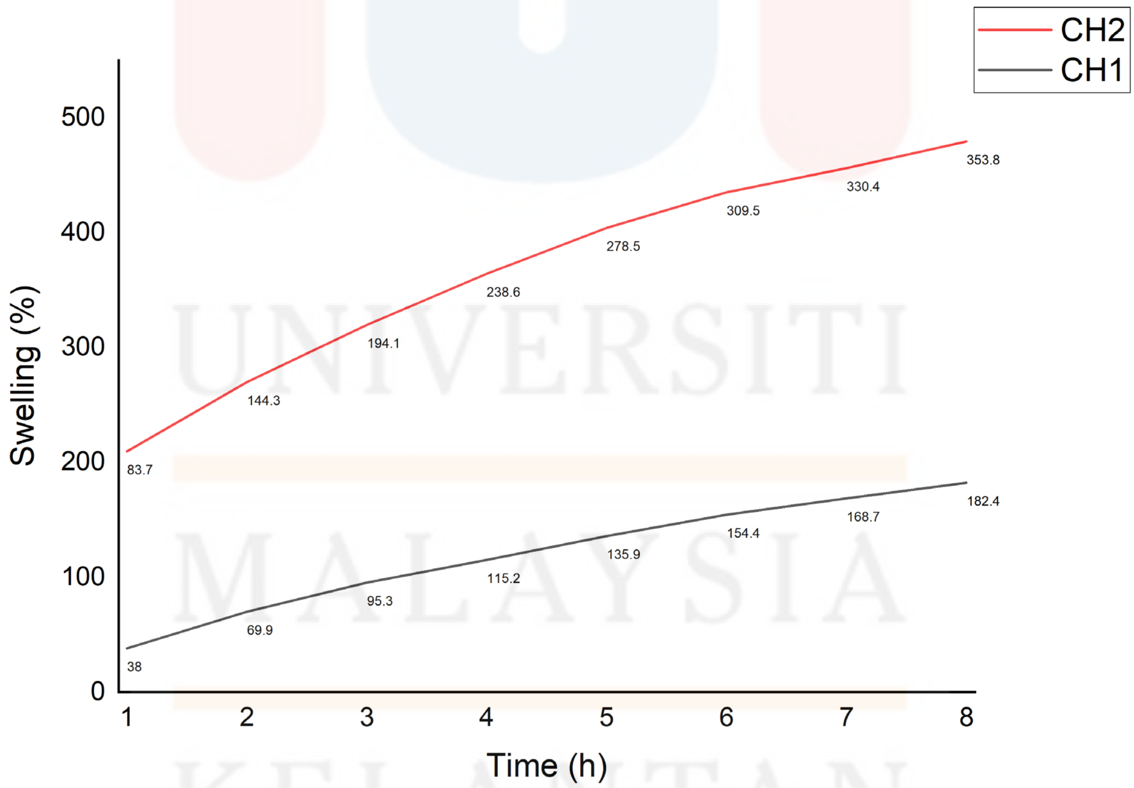

Figure 4.5: Swelling behaviour of cellulose-based hydrogel of which (CH1) is cellulose-based hydrogel with 50% GEL and (CH2) is cellulose-based hydrogel with 40% GEL

To ascertain the percentages of swelling exhibited by the hydrogel fabricated from cellulose-based hydrogel with different percentage of GEL, gravimetric method was used to observed the water absorption value. As shows in Figure 4.5 consists of swelling behaviour of cellulose-based hydrogel of which (CH1) is cellulose-based hydrogel with 50% GEL and (CH2) is cellulose-based hydrogel with 40% GEL, tabsorption of water by hydrogels containing 40% GEL was found to be greater than that of those containing 50% GEL. For example, according to Enawgaw et al. (2021), after 60 minutes, the swelling of corncob cellulose hydrogel coorperate with AMPS reached 168.8 g, up from 47.4 g initially.

Rosiska et al. (2022) conducted research which demonstrated that augmenting the concentration of gelatin molecules in hydrogel results in a increasing augmentation of cross-links. This phenomenon holds promise for enhancing the stability of the material. In contrast, the swelling characteristics and mechanical strengths of hydrogels manufactured via alternative techniques may differ when gelatin is employed as a cross-linking agent. When the polymer content was minimal, the swelling capacity of gelatine networks with structures increased significantly. The influence of gelatin quantity and degree of cross-linking on the water absorption and swelling capacity of gels has been demonstrated, as stated by Skopińska-Wiśniewska et al. (2021).

In the journal reported by Vo et al. (2022), because hydrogels are capable of forming hydrogen bonds with water molecules, they are capable of absorbing solutions containing water. By depending on the concentration, this polymer can absorb 500 to 800 times its weight in purified water. It has the capacity to absorb 300 times its own weight in water at the surface. It demonstrates an absorption capacity ranging from 50 to 60 times its own weight in a 0.9% sodium chloride solution.

4.4 Tensile Strength Analysis

In order to enhance comprehension of the response of cellulose hydrogels to tensile stresses, tensile strength analysis is a fundamental analysis to measure the physical properties of cellulose-based hydrogel. The term "tensile strength" refers to the amount of tensile tension that a material can withstand prior to failure. Thus, the tensile analysis was conducted using Universal Testing Machine (UTM), that located at the University of Malaysia Kelantan. Two different samples were test which representing of different percentage of gelatin.

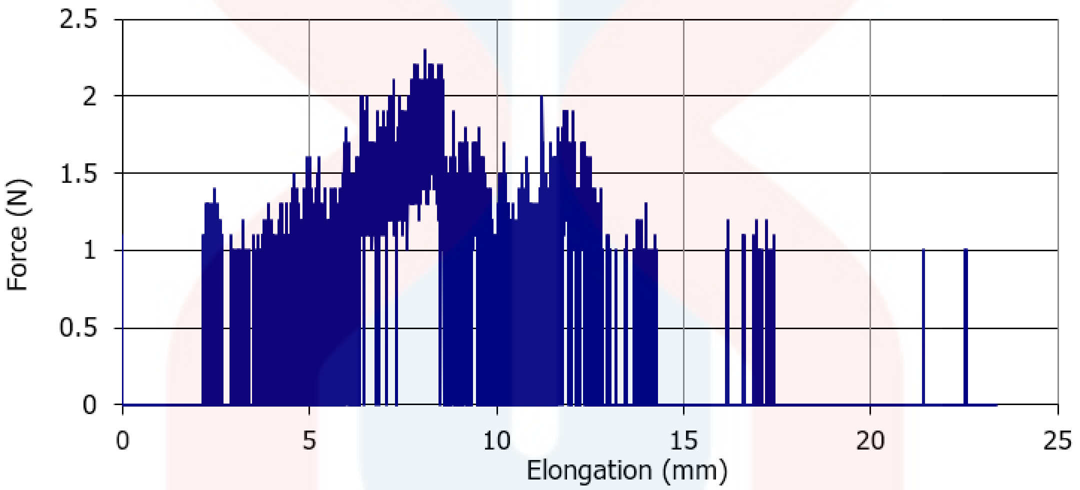

Figure 4.6: Tensile strength graph of cellulose-based hydrogel with 40% GEL

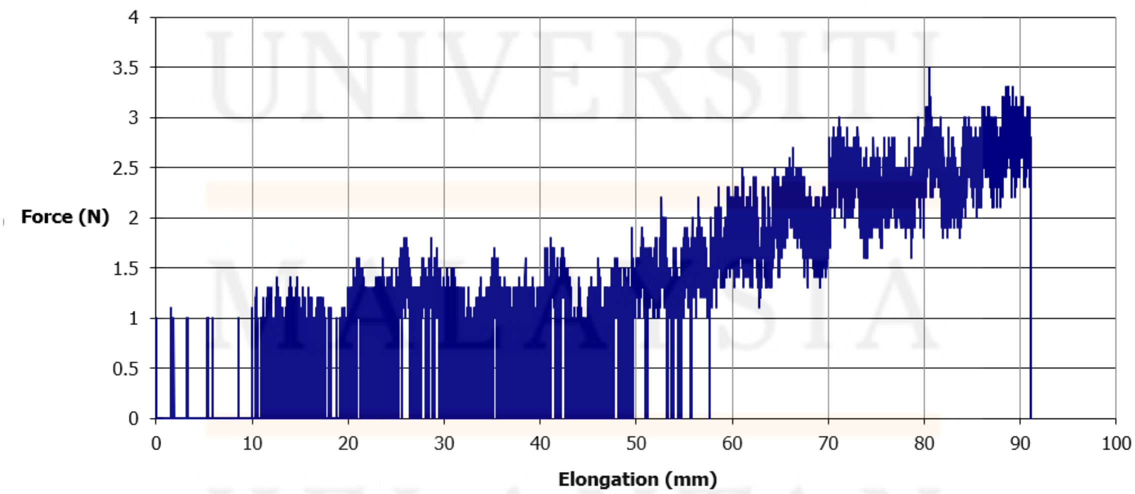

Figure 4.7: Tensile strength graph of cellulose-based hydrogel with 50% GEL

By analysing Figures 4.6 consist of tensile strength graph of cellulose-based hydrogel with 40% GEL and 4.7 consist of tensile strength graph of cellulose-based hydrogel with 50% GEL, it becomes evident that the graph's reading display lacks accuracy. The tensile strength of hydrogels is significant influenced by factors including their composition and crosslinking density. Typically, mechanical properties and resistance to stress improve as tensile strength increases. Notwithstanding, the graph consistently illustrates a progressive amplification of peak values. At the outset, the sample exhibited a minimum length variation of ±60mm. By following tensile strength analysis, the ultimate length of the sample was found to be within ±120mm with an elongation rate of 5mm/min. Comprehending the tensile graph of hydrogel containing varying percentages of GEL proves difficult due to the material's exceptional sensitivity and stretchability, which result from the comparatively high-water content.

When dealing with hydrogels exhibiting notable tensile characteristics, such as strain sensors, obtaining and comprehending the outcomes of tensile testing can prove to be a challenging endeavour. For example, a study conducted by Ma et al. (2023) investigated that it is challenging to obtain results from tensile strength analysis with hydrogels that high-water content.

The flexibility of cellulose-based hydrogel is highly recommended for medical applications, specifically those necessitating the repair of wounds. This could be attributed to the material's stretchability. Furthermore, it has been reported by Ma et al. (2023) that certain hydrogels possess the ability to adhere to lesion surfaces and sustain contact with the healing tissue. This enables them to facilitate tissue regeneration while ensuring continued contact with the wound's surface. According to the research conducted by Ma et al. (2023), biocompatible hydrogels exhibit a certain level of flexibility that enables them to endure interactions with living tissues without inducing an immune response. However, it is possible for hydrogels to demonstrate tensile characteristics, including resilience and stretchability (Lu et al., 2020).

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

Chemical Extraction Process

The chemical extraction process achieved success following the alkaline treatment which dried corncob was pre-treatment with 95% ethanol solution, the removal of lignin by bleaching with 4% $NaClO_2$, and the final extraction using a 5% solution of KOH. The sequential execution aimed to eliminate the lignin. Thus, based on Table 3.1 the percentage (%) of extraction yield from 30g of dry corncob, around 40% of the extraction yield successfully produced from 30 g of dried corncob.

Cellulose Identification

Based on the FTIR analysis, spectra of the hydrogel samples exhibited a striking resemblance to each other as well as to the spectra of the other samples. Furthermore, further analysis using FTIR revealed a little discrepancy in the peaks of the absorption bands corresponding to the O-H and C-H bonds, as well as the C-O stretching vibration and other vibrations. The extracted cellulose exhibited distinctive peaks at observed at 2911 cm^{-1} and 1031 cm^{-1}, confirming its cellulose composition. The C-O stretching vibrations at 1031 cm^{-1} provided insights into cellulose molecule's chemical surrounding.

Hydrogel Analysis

The formation of cellulose-based hydrogel is successfully produced by the ratio 1:2 of cellulose-gelatin. The hydrogel was described by analysing its chemical properties with FTIR analysis, biological properties with in vitro antibacterial assay, physical properties with swelling analysis and mechanical properties with tensile strength analysis. Based on FTIR analysis, the reading of FTIR spectra exhibited similarity among hydrogel samples and showed variations in O-H, C-H, and C-O stretching vibrations.

Swelling Characteristics

From the swelling analysis result, the results indicated that the hydrogel formulation containing 40% GEL showed highest expansion after eight hours. This finding suggests that the water absorption capacity of hydrogels derived from cellulose is influenced by the overall quantity of GEL present in the hydrogels. It would appear that water absorption influenced by the cellulose-to-GEL ratio with increasing GEL percentage.

Tensile Strength and Mechanical Characteristics

The results obtained from the analysis of the material's tensile strength suggest that the value depicted on the graph is inaccurate and nor achieve the objective. Tensile strength measurements questioned due to hydrogel's composition (90% water) and inherent instability. Despite challenges, hydrogel shows potential for medical applications, particularly wound healing.

Antibacterial Activity

Based on the effective in vitro antibacterial assay activity, hydrogels containing tannic acid exhibited effective antibacterial activity against gram-staining bacteria (*S. aureus* and *E. coli*). This discovery suggests that potential for impeding bacterial growth and possessing antibacterial properties.

Recommendation

For the recommendation for this study, further investigation recommended to enhance hydrogel's tensile strength through material modification. This action would be taken to enhance the characterization characteristics of the hydrogel. It could potentially be employed to explore cross-linking agents or technique to augment the hydrogel network's structure. Furthermore, it is suggested feasibility of fabricating hydrogels with controlled expansion based on environmental factors for application in tissue engineering, medication delivery, and wound healing.

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APPENDICES

Figure A.1: Tensile strength analysis using universal testing machine (UTM)

Figure A.2: FTIR machine (Nicolet iS50 model) to measure composition in extracted cellulose and cellulose-based hydrogel