ISTANBUL TECHNICAL UNIVERSITY ★ GRADUATE SCHOOL

3D PRINTED ANTIBACTERIAL HERBAL LOADED ALGINATE-PECTIN MEDICAL PATCH: FABRICATION AND CHARACTERIZATION

M.Sc. THESIS

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ISTANBUL TEKNİK ÜNİVERSİTESİ ★ LİSANSÜSTÜ EĞİTİM ENSTİTÜSÜ

3D BASKI YARDIMIYLA ANTİBAKTERİYEL BİTKİSEL EKSTRAKT YÜKLÜ ALJİNAT-PEKTİN MEDİKAL YAMA: ÜRETİMİ VE KARAKTERİZASYONU

YÜKSEK LİSANS TEZİ

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Ghazaleh DINI, a M.Sc. student of ITU Graduate School student ID 513201032, successfully defended the thesis entitled "3D Printed Antibacterial Herbal Loaded Alginate-Pectin Medical Patch:Fabrication and Characterization", which she prepared after fulfilling the requirements specified in the associated legislations, before the jury whose signatures are below.

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Date of Submission : 21 June 2023
Date of Defense : 07 July 2023

To my family that always believed in me and support me,

FOREWORD

It was a great pleasure that Inwrite this foreword for my thesis. As a student of genetics during my bachelor’s degree and now pursuing a master’s degree in nano science and nano engineering, I have a keen interest in fields of technology, material science in combination with medical research. This thesis represents the culmination of my efforts to integrate my knowledge in these diverse areas of study.

The field of genetics has seen remarkable advancements in recent years, and its application in various fields, including medicine, agriculture, and biotechnology, has been significant. As a student of genetics, I have been exposed to various aspects of genetics and also microbiology. With my interest in technology and material science, I have also explored the use of advanced materials in microbial research and their potential applications this is why I added antimicrobial activities to my thesis.

In my pursuit of a master's degree in nano engineering, I have gained a deep understanding of the design and development of nanomaterials and their applications in diverse fields. This knowledge has been a valuable asset in my research and has enabled me to explore the use of nanomaterials in biomedical research.

This thesis represents an integration of my knowledge in genetics, microbiology, technology, material science, and medical research. The research presented here provides valuable insights into the potential of nanomaterials in biomedical, and the results obtained have significant implications for the development of therapeutic tools and techniques.

It is my sincere hope that this thesis will inspire further research in the field of genetics and nanomaterials and contribute to the development of new technologies that benefit society.

Finally, I would like to thank my parents for raising me and supporting my decisions and also my advisor Dr. Birgül BENLİ for her patience and all things that she taught me and also, I would like to thank the Research Fund of Istanbul Technical University (BAP) for financially supporting the project: MYL-2022-44167.

July 2023

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(Nano Engineer)

TABLE OF CONTENTS

Page

ABBREVIATIONS

CNCs	**:** Cellulose Nanocrystals
CNFs	**:** Cellulose Nanofibrils
PPP	**:** Pomegranate Peel Powder
AgNPs	**:** Silver Nanoparticles
3Dp	**:** 3D printing
FDM	**:** Fused Deposition Modeling
ABS	**:** Acrylonitrile Butadiene Styrene
PET	**:** Polyethylene Terephthalate
HNTs	**:** Halloysite Nanotubes
FTIR	**:** Fourier-Transform Infrared Spectroscopy
SEM	**:** Scanning Electron Microscopy
SLA	**:** Stereolithography
MAE	**:** Microwave-Assisted Extraction
SA	**:** Sodium Alginat

LIST OF TABLES

Page

LIST OF FIGURES

Page

3D Printed Antibacterial Herbal Loaded Alginate-Pectin Medical Patch: Fabrication and Characterization

SUMMARY

This thesis focuses on a comprehensive and multi-disciplinary approach to the development of an effective and sustainable wound dressing. The thesis consists of three main parts, each layer building upon the previous to create a final product that is both innovative and practical.

In the first part, the necessary materials such as pectin was synthesized in order to serve as the base layer of the wound dressing. This involves careful selection and testing of materials to ensure that it meets the necessary requirements for strength, flexibility, and biocompatibility.

In the second part of the thesis, the antibacterial agents from herbal extracts were prepared. These natural agents offer several advantages over synthetic alternatives, including reduced toxicity and improved biocompatibility. Also, natural and biocompatible nanoclay as a carrier for these agents were used to controlled release, with the assistance of synthesized carboxymethylcellulose (CMC) which was extracted from plant cellulose, expected effects were improved, and targeted delivery to the wound area.

In the third and final part of the thesis, advanced 3D printing technology was used in two ways. Firstly, a skeleton was designed for the prepared hydrogels using 3D printing. This skeleton provides structure and support for the hydrogel while allowing for air flow and moisture control. Secondly, the polymers (pectin:alginate/gelatin and nanoclay loaded ones themselves were 3D printed using Fused Deposition Modeling (FDM). We also integrated the hydrogel and clay material to form more hydrophilic film patch that is both strong and flexible.

The final product is an antibacterial wound dressing that combines advanced materials and technologies to promote healing and prevent infection. This innovative approach has the potential to revolutionize wound care and improve patient outcomes.

As it has been demonstrated in literature, wound healing is a complex and intricate process that involves multiple stages and requires the right type of dressings to prevent infection and promote the regrowth of tissue. The ideal dressing should have several key properties, including the ability to keep the wound moist, allow air to flow through, protect the wound from external contaminants, absorb excess fluid, and be easy to remove without causing additional damage to the wound.

Hydrogels are an excellent choice for wound dressings due to their unique physical and chemical properties. These materials are highly absorbent and can retain large amounts of water, which helps to keep the wound moist and promote healing. A hydrophilic wound dressing is necessary for rapid therapeutic effect and absorption of exudate. This characteristic also helps to maintain a moist environment, promote high blood absorption, and enhance erosion capability and it was measured with contact angles. They are also permeable to gases, allowing air to flow through and providing oxygen to the wound. Additionally, hydrogels can be formulated to have antimicrobial properties, which can help to fight against bacteria and prevent infection.

In this thesis, pectin was synthesized from pomegranate and grapefruit peels using citric acid extraction and cellulose was synthesized from pomegranate peels, making the study sustainable and environmentally friendly. Also, the fallen fruits from the grapefruit trees in the faculty garden were collected and utilized in the study, emphasizing their significant contribution to the fruit industry and agricultural research. Additionally, the effect of grapefruit juice instead of citric acid was alternatively used during the process of pectin extraction with the aim of achieving zero waste.

Malva sylvestris and *Cichorium intybus L*, two local Turkish herbs, were used as antibacterial agents. The extraction of these herbs was done using microwave-assisted extraction with ethanol and membrane-assisted purification methods. Additionally, dialysis tubes which are kind of membrane, were used during the purification of antibacterial herbal extracts and the resulted extracts were compared with traditional extraction methods. The synthesized extracts were analyzed using optical spectroscopy techniques such as UV-Vis Absorption Spectroscopy. Herbal extracts were loaded into Halloysite nanotubes (HNTs) and injected into the hydrogel pores in order to help with air flow in the hydrogel area. Glycerol also used as a plasticizer. The morphology of the prepared nanoclay tubes were analyzed using Laser Diffraction Particle Size Analyzer, Fourier-Transform Infrared (FTIR) Spectroscopy and Atomic Force Microscopy (AFM).

The process of extracting materials from plants and fruit wastes was also carried out using microwave-assisted synthesis methods, which are low-cost and time-efficient. The effectiveness of *Malva sylvestris* and *Cichorium intybus L* against bacteria was examined using the disc diffusion method. Their antibacterial activities were tested against *E. coli* and *S. aurous* that are effective two common types of wound bacteria to create pectin/alginate-based hydrogel wound dressings. Disc diffusion tests confirmed the antibacterial activity of the chosen plant extracts against common wound bacteria.

To improve the effect of nanotubes such as high water absorbency and elongation as fillers of reinforced hydrogels composites, CMC synthesized from extracted cellulose. Ash content tests and moisture level measurements of the synthesized pectin and cellulose confirmed their high purity. FTIR analysis confirmed the presence of herbal extract and glycerol in the synthesized hydrogels.

In the next stage of the thesis, biopolymers and HNTs were combined to form a composite that was used to formulate a hydrogel that could be used inside a 3D printed skeleton. To design an effective antibacterial patch, 3D FDM printer were used. A patch-shaped skeleton with several pins were designed using 3D printing technology to make the hydrogel porous masks. The pectin/alginate based composites were poured on top of a cross-linker solution to form the hydrogel on the mask surface. Then, the pectin/alginate based hydrogels were cross-linked with calcium chloride ($CaCl_2$).

In the final stage of the thesis, formulated layer from cellulose and HNTs composite were prepared. These nanoclays were designed to act as carriers for herbal extracts. The effectiveness of these hydrogels in preventing bacterial growth was also tested against common wound bacteria, *E coli* and *S. aurous* and the results were successful. Additionally, the ability of these hydrogels to absorb water was evaluated using a gelatin hydration test. The behavior of the hydrogels was also examined in detail.

In conclusion, the results showed that hydrogels containing either *Malva sylvestris* or *Cichorium intybus L* plant extract have great potential as antibacterial patches for wound dressing. However, further in vivo studies are necessary before any clinical application can be made.

3D Baskı Yardımıyla Antibakteriyel Bitkisel Ekstrakt Yüklü Aljinat-Pektin Medikal Yama: Üretimi ve Karakterizasyonu

ÖZET

Bu tez çalışması ile etkili ve sürdürülebilir bir medikal yama geliştirmesi hedeflenmiştir. Bu doğrultuda, kapsamlı ve çok disiplinli bir yaklaşımla, üç ana bölümden oluşmaktadır. Her bir bölümde farklı malzemeler sentezlenerek farklı kompozitler hazırlanmış, ardından bir arada kullanılarak hem yenilikçi ve hem de pratik bir ürün olan medikal yama 3B baskı yardımıyla hazırlanmış ve antibakteriyel özellikleri incelenmiştir.

İlk bölümde, medikal yamanın taban katmanı olarak kullanılacak malzemeler sentezlenmiştir. Bu amaçla bitkisel polimerlerden pektin ve selüloz gibi doğal polimerler seçilerek, laboratuvar ortamında güç, esneklik ve biyouyumluluk gibi gerekli gereksinimleri karşılayacak şekilde yeşil sentezle hazırlanmıştır.

İkinci bölümde, bitkilerden antibakteriyel ajanların hazırlanmasına ağırlık verilmiştir. Bu bitkisel kaynaklı doğal ajanlar, sentetik alternatiflerine göre daha az toksisite ve daha iyi biyouyumluluk gibi birçok avantaja sahiptir. Ayrıca, bu ajanların kontrollü salınımı ve yara yerine hedeflenmiş teslimatı için nanokil taşıyıcılar hazırlanmıştır. Bu amaçla doğal Halloysit nanotüpleri (HNTs) taşıyıcı olarak hazırlanan ekstraktlarla doldurulmuştur.

Çalışmanın üçüncü bölümünde çeşitli oranlarda aljinat ilavesinin hidrogel üzerine etkileri incelenmiş, sentezlenmiş selülozun yardımıyla, hidrojel ve kil malzemesini bir arada entegre ederek hem güçlü hem de esnek olan bir film yaması oluşturulmuştur. Tez çalışmasının son bölümünde ise hidrojellerin basımına geçilmiş, bu kısımda iki yöntem uygulanmıştır: İskelet kalıplama ve doğrudan basım. Gelişmiş 3B baskı teknolojisi kullanarak bir iskelet yapı oluşumuna yönelik maske tasarlanmıştır. Bu iskelet, hidrojel için yapı ve destek sağlarken, aynı zamanda hava akışına ve nem kontrolüne izin vereceği düşünülmüştür. Diğer yöntemde ise hazırlanan hidrojeller kişiye özel yamaya imkan verecek tasarımla 3B yazıcıda bastırılmıştır.

Tez çalışmasının sonunda, antibakteriyel etki sayesinde iyileşmeyi teşvik eden ve enfeksiyonu önleyebilecek gelişmiş malzemeleri ve teknolojileri birleştiren antibakteriyel bir medikal yama hazırlanmıştır. 3B yazıcıyla yaraya özel tasarıma gidebilecek yenilikçi yaklaşım sayesinde, yara bakımını devrim niteliğinde değiştirme ve hasta sonuçlarını iyileştirme potansiyeline sahiptir.

Yara iyileşmesi, birden fazla aşamayı içeren karmaşık ve detaylı bir süreçtir. Bu süreçte, enfeksiyonu önlemek ve dokunun yeniden büyümesini teşvik etmek için doğru türde yara örtüleri kullanmak çok önemlidir. İdeal bir yara örtüsü, yaranın nemli kalmasını sağlama, hava akışına izin verme, yarayı dış etkenlerden koruma, fazla sıvıyı emme ve yaranın zarar görmesini önlemek için kolayca çıkarılabilme gibi bazı önemli özelliklere sahip olmalıdır. Bu özellikler, yaranın hızlı ve sağlıklı bir şekilde iyileşmesine yardımcı olur.

Hidrojeller, yara örtüleri için son yıllarda dikkat çeken mükemmel bir seçenektir çünkü benzersiz fiziksel ve kimyasal özelliklere sahiptirler. Bu malzemeler yüksek derecede emici olup büyük miktarda suyu tutabilirler, bu da yaranın nemli kalmasına ve iyileşmeyi teşvik etmeye yardımcı olur. Ayrıca, hidrojeller gaz geçirgenidir, bu da

havanın akmasına ve yaraya oksijen sağlanmasına olanak tanır. Ek olarak, hidrojeller antimikrobiyal özelliklere sahip olacak şekilde formüle edilebilirler, bu da bakterilere karşı savaşmaya ve enfeksiyonu önlemeye yardımcı olabilir. Bu özellikler sayesinde, hidrojeller yara iyileşmesini hızlandırabilir ve sağlıklı bir iyileşme sürecine katkıda bulunabilir.

Bu çalışmada, *E. coli* ve *S. aureus* gibi yaygın yara bakterilerine karşı etkili olan pektin/alginate dayalı hidrojel yara örtüleri oluşturulmuştur. Hidrojelin poroz olması için 3D baskı teknolojisi kullanılarak 25mm X 25mm ve 50mm X 50mm boyutlarında toplam 16 ve 25 pin içeren özel şekilli bir iskelet tasarlandı.

Tasarlanan bu iskelet kalıp olarak kullanılmıştır. Bu kalıba, 50:50 oranında pektin/alginate polimeri eklendi, hidrojel yapıya stabilite ve dayanıklılık kazandırmak için kalsiyum klorür ($CaCl_2$) ile temasa getirilerek çapraz bağlanması sağlanmıştır. Bu teknik, kişiye özel yara iyileşmesini hızlandırmak ve enfeksiyonu önlemek için etkili bir yöntem olabilir.

Yerel bitkisel antibakteriyel ajanlar olarak Türkiye'deki *Malva sylvestris* ve *Cichorium intybus L* bitkilerinden elde edilen bitkisel özütler kullanılmıştır. Yüksek kaliteli bitkisel özütler elde etmek için mikrodalga destekli ekstraksiyon yöntemleri ve membran destekli saflaştırma yöntemleri kullanılmıştır. Saflaştırma işlemleri sırasında diyaliz membranı kullanıldığından daha saf malzeme ele geçmiştir. Diyaliz membranı kullanımı, bitkisel ekstraktların zamanla bozunmasının da önüne geçmekte olduğu tesbit edilmiştir.

Malva sylvestris ve *Cichorium intybus L*'nin bakterilere karşı etkinliği disk diffüzyon yöntemi kullanılarak incelenmiştir. Bu yöntem, bitki özütlerinin antibakteriyel etkinliğini değerlendirmek için kullanılır.

HNT nanotüpleri doğal malzemelerden hazırlanmış, ardından bitkisel özütlerin taşıyıcısı olarak kullanılmıştır. Nanokiller hidrojel alanında hava akışına yardımcı olurken, ortama katılan gliserol plastikleştirici olarak kullanılmıştır. Hazırlanan nanokiller, bitkisel özütler için taşıyıcılar olarak tasarlanmıştır.

Bu hidrojellerin bakteri büyümesini önlemedeki etkinliği, yaygın yara bakterilerine karşı, *E. coli* ve *S. aurous*'a karşı test edilmiş ve sonuçlar başarılı olmuştur. Ayrıca, bu hidrojellerin suyu emme yeteneği jelatin hidrasyon testi kullanılarak değerlendirilmiştir. Hidrojellerin davranışı da ayrıntılı olarak incelenmiştir. Hidrofilik bir yara örtüsü, hızlı terapötik etki ve eksüdat emilimi için gereklidir. Bu özellik aynı zamanda nemli bir ortamı korumaya, yüksek kan emilimini teşvik etmeye ve erozyon kabiliyetini artırmaya yardımcı olur ve temas açılarıyla ölçülmüştür.

Ayrıca, tez kapsamında çevreci biyopolimerler sürdürülebilir malzemeler olarak seçilen nar ve greyfurt kabuklarından pektin, selüloz ise nar kabuğundan sentezlenmesi, bu çalışmayı sürdürülebilir ve çevre dostu yapar. Ayrıca, pektin ekstraksiyon sürecinde sitrik asit yerine sıfır atık hedefine ulaşma amacıyla greyfurt suyu kullanılmış, pektin üretimi mümkün olmasına rağmen verimi düşük olduğu görülmüştür.

Sentezlenen hidrojeller, Fourier-dönüşümlü kızılötesi (FTIR) spektroskopisi ve Atomik Kuvvet Mikroskobu (AFM) gibi gelişmiş analitik teknikler kullanılarak analiz edilmiştir. Bu teknikler sayesinde, hidrojellerin yapısı, özellikleri ve performansı detaylı bir şekilde incelenmiştir. Hidrojellerin özellikleri jelatin testleri aracılığıyla belirlenmiştir. Bu testler sayesinde, hidrojellerin mekanik özellikleri, şişme kapasitesi ve biyouyumluluğu gibi önemli parametreler değerlendirilebilir.

Disk diffüzyon testleri ile seçilen bitki özütlerinin yaygın yara bakterilerine karşı antibakteriyel etkinliği göstereceği doğrulamıştır. Sentezlenen pektin ve selülozun kül içeriği testleri ve nem düzeyi ölçümleri, yüksek saflıkta oldukları görülmüştür.

Tez çalışmasını son aşamasında, selülozdadn elde edilen CMC ve nanokil tabakası içeren hidrojellerden meydana gelen hidrojel kompozit hazırlanmıştır. 3D baskıya uygun hidrojel haline getirilmesinde jelatinden yararlanılmıştır.

Sonuç olarak, *Malva sylvestris* veya *Cichorium intybus L* bitki özütü içeren hidrojellerin, yaraların tedavisinde antibakteriyel yamalar olarak büyük potansiyele sahip olduğunu göstermektedir. Bununla birlikte, herhangi bir klinik uygulama öncesinde sitotoksite ve in vivo çalışma gerekmektedir. Bu çalışmalar sayesinde, bu hidrojellerin güvenliği ve etkinliği daha iyi değerlendirilebilir.

1. INTRODUCTION – 3D PRINTED ANTIBACTERIAL MEDICAL PATCH

After the appearance of Three-dimensional printing (3Dp) in the early 1980s, when the first 3D printer was designed by Hull for stereolithography (SLA) (Hull, 1993), this method rapidly spread around the world. 3Dp, or additive manufacturing, is known as a method of layer-by-layer deposition, in other words, the addition of material to build a 3D object with the assistance of a digital model with complex structures (Bracaglia et al., 2017). On the other hand, the emergence of additive manufacturing has facilitated the fabrication of customized therapeutic systems by allowing precise control over the manufacturing process. This technology enables the creation of intricate three-dimensional structures that can be tailored to meet the specific needs of individual patients (Cui et al., 2022). Unlike traditional mass production methods, additive manufacturing offers the flexibility to design personalized implants, scaffolds, and drug delivery systems with enhanced functionalities (Zhang et al., 2018). In addition to this, 3D printing can be used to manufacture antibacterial film patches that can be used in different fields like wound dressings (Milojević et al., 2021).

Furthermore, to obtain the desired object with proper 3D architectures, a suitable material must be chosen. Several materials are being used in this process, like; metals, composites, plastics, resins, bio-inks, and wood (Ahmad et al., 2019). Bioprinting is a method of additive manufacturing that involves the processing of living cells and biomaterials like hydrogels in a manner that progresses layer by layer (Ramadan & Zourob, 2021). Since its discovery in the1960s, synthesized hydrogels have been utilized in biological applications with acceleration (Wichterle & Lím, 1960). Hydrogels are suitable materials owing to both their physical and chemical properties. These materials have been developed for their antibacterial activities and angiogenesis (Ghobril & Grinstaff, 2015). However, a single material bio-ink doesn't have the required criteria from the points of physical (printability) and bioactivity. Lots of attempts have been made to overcome this issue. For instance, using multiple components like nanomaterials and polymers (Valot et al., 2019). One of these materials is alginate (Axpe & Oyen, 2016). Alginate or alginic acid sodium salt is used for the fabrication of scaffolds in different applications like tissue engineering (Leu Alexa et al., 2021). In recent years hydrogels containing alginate have been used in 3D printing (Amr et al., 2021; Erkoc et al., 2020). In particular, nanomaterials can effectively incorporate with polymers of hydrogels owing to their large surface areas and often demonstrate biological functionality; hence, various composite hydrogels integrated into nanomaterials have been prepared in order to improve the physical and biological properties of hydrogels as bioinks to create accurate objects. In addition to these materials, nano clays were mostly utilized during the compounding process in order to develop injectable biomaterials among fillers that are inorganic (Alexa et al., 2021; Cidonio et al., 2020; Jin et al., 2018). Hydrogels with clay-based nanocomposites have been known for their potential in antibacterial activities and wound healing. Accordingly, these materials can accelerate wound closure and promote the recovery of injured tissues. Because of different issues, these outcomes were enhanced by adding clay nanoparticles to the hydrogel structure. To name some of these properties, first, they have a large surface area. Additionally, they have ion exchange capacity, and finally, they can be loaded against microbes (Rezanejad Gatabi et al., 2022). Cellulose is considered an attractive macromolecule biopolymer for many applications. One of the most common materials on earth, cellulose has recently attracted a lot of attention from technological applications since it is biocompatible, renewable, sustainable, and eco-friendly (Piantanida et al., 2019; C. Wang et al., 2019; Zhou et al., 2019). Cellulose is known as an ideal material for biosensing and film patch designing due to its fascinating properties, like; flexible surface chemistry (Vignesh et al., 2022), biocompatibility (Bahloul et al., 2021), renewability (Trache, 2018), biodegradability (Mateo et al., 2021). The incorporation and blending of the before-mentioned components and cellulose within the bionanocomposite hydrogel are known to lead to the development of a hydrogel with remarkable rheological properties, including shear-thinning and self-healing characteristics. Moreover, the hydrogel exhibited biocompatibility and controlled mechanical and transport properties while also demonstrating sustainability through its constituent components (Alizadehgiashi et al., 2021). As antibacterial material, *Cichorium intybus L.* and *Malva sylvestris* was used due to the fact that, these herbs known to be effective against bacteria present in wounds (Keleş et al., n.d.). In this study, most synthesizes are carried out with the assistance of a microwave. The reason is that, in contrast to conventional extraction methods like Soxhlet, microwave-assisted extraction (MAE) offers targeted and selective heating within a closed system, minimizing heat loss to the environment. This unique heating mechanism significantly reduces extraction time, typically less than 30 minutes. The elevated temperature during MAE leads to enhanced dehydration of cellulose and decreased mechanical strength, facilitating easier access of solvents to compounds within the cell. Additionally, microwave heating induces changes in plant tissue that result in a substantial increase in the yield of extractable pectin. The migration of dissolved ions further promotes solvent penetration and facilitates the release of chemicals from the matrix (Mandal et al., 2007).

This thesis aims to design and develop an innovative antibacterial film patch using 3D printing technology and composite materials derived from cellulose, pectin and alginate as well as some gelling agents such as gelatin, and glycerol. To be able to enhanced antibacterial activity achieved through the incorporation of herbal extracts loaded onto Halloysite clay nanotubes. By harnessing the potential of 3D printing technology and incorporating composite materials wastes, this research aims to create a highly versatile and effective solution. The addition of herbal extracts loaded onto nanoclay further enhances the antibacterial properties of the composite, providing a natural and sustainable alternative to synthetic antibacterial agents. Through meticulous experimentation and characterization, this study seeks to contribute to the production of the organic-based 3D-printed patch as antibacterial dressing with the assistance of antibacterial herb extractions.

2. LITERATURE REVIEW

2.1 Development of Antibacterial Herb Extract

Antibacterial activity plays a crucial role in maintaining the health and well-being of living organisms. In the face of injury or damage, the body relies on effective antibacterial mechanisms to prevent infections and facilitate the restoration of damaged tissue. This mechanism involves several cellular and molecular processes that work together to combat bacterial pathogens (Kirsner and Eaglstein 1993). For instance, in wounds if the process goes as intended, it prevents infections, minimizes blood loss, and enhances tissue regeneration (Menke et al. 2007). By appearing antibiotic resisted bacteria, an urgent need for new antibacterial agents occurred. According to the World Health Organization (WHO), antibiotic resistance is one of the top ten worldwide public health hazards to humanity (Tacconelli 2017). As can be understood from the name, they are bacteria that have been developed to resist the antibiotics that are commonly utilized to cure bacterial infections. This issue occurs subsequently after misuse and overuses of antibiotics. Failure of the antibacterial activity and also wound-healing process results in chronic wounds, morbidity and mortality afterward (Lazarus et al. 1994). Development and launching of new antibiotics are slow, and effective antibiotics have become limited (Spellberg 2014). Different materials have been used as antibacterial agents, like Ag nanoparticles (AgNPS) (Dini and Benli 2023), herbal extracts (Keleş et al. n.d.) and etc. To name some of these Herbs which are native herbs of Türkiye; *Cichorium intybus L.* and *Malva sylvestris*. Even though the concentrations of active constituents in plant extracts may be lower, they can still be a better source of antimicrobial compounds compared to synthetic drugs (Cox and Balick 1994). Firstly, the extract of *Malva sylvestris* has been found to have high antibacterial effects against human pathogen bacteria strains such as *E. Coli*, *Staphylococcus aureus*, *Streptococcus agalactiae,* and *Enterococcus faecalis*. Among all extracts tested, the ethanol extract of *Malva sylvestris* showed the best antibacterial activity against these bacteria. Studies have also shown that the extract of *M. sylvestris* can increase the formation of well-organized collagen bands, increase the number of fibroblasts, and reduce the number of inflammatory cells in tissue. These findings suggest that the extract of *M. sylvestris* can effectively possesses significant antibacterial activity (ed Stems and Roots 2016).

Studies have shown that the ethanolic extract of *Malva sylvestris* has greater antibacterial effects on gram-positive bacteria than on gram-negative bacteria. Given its antibacterial effect on *S. aureus*, the extract can be used to improve the performance of antibiotics or even replace them by evaluating its effects inside the body (Yousefi 2023).

In addition, *Cichorium intybus*, also known as *Hindibag* in Turkish, is one of six species in the genus *Cichorium* that is mostly found in Europe and Asia. While it is popularly used as a coffee substitute, it also has a wide range of medicinal uses for treating various conditions including wounds and diabetes (Street et al., 2013). In Türkiye, an ointment made from *Hindibag* leaves was used as antibacterial agent to promote wound healing (Sezik et al., 2001). Additionally, it has been found that extracts of *C. intybus* have antimicrobial activity against several pathogenic microorganisms, including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Candida albicans*, *Salmonella typhi*, and *Micrococcus luteus* (shaikh et al., 2016; Siddhan & Kumari, 2006).

Additionally, by using selective dialysis with dialysis tubing, it is possible to remove nonspecific components from an alcoholic extraction of herbs, as the extraction mainly consists of small chemicals. Also, selective dialysis with dialysis tubing enables the isolation and refinement of constituents within an extract according to their molecular weight. This technique facilitates the elimination of non-target components, thereby yielding purer and more precise fractions suitable for subsequent analysis (Huiying et al., 2010).

In this regard, the anti-inflammatory properties of safe and non-toxic plant extracts are achieved by inhibiting the production of cytokines/chemokines and blocking the nitric oxide pathway. In terms of antibacterial activity, plant compounds act by interfering with the synthesis of essential bacterial proteins, cell walls, and cell membranes. Additionally, these molecules can inhibit bacterial DNA replication and other critical metabolic pathways (Agarwal et al., 2021).

2.2 Composite Patch: Sustainable and Green Extraction

2.2.1 Cellulose

First material of patch composite is cellulose. The cell walls of natural plant fibers are made up of cellulose networks that are a mixture of lignin, cellulose, and hemicellulose. The cellulose is firmly surrounded by hemicellulose via hydrogen bonds and physically fills the gaps (Dilamian & Noroozi, 2019). Firstly, cellulose is an important element of plant tissue that has a linear structure. It is a compound of D-anhydroglucopyranose units (AGUs) connected by β –glycosidic bonds which is shown in Figure 1 (Das et al., 2022). These bio-based materials have lots of benefits, like being eco-friendly and biodegradable, which makes them appealing. Cellulose has been used for more than 150 years. In plants, it is known as the cell wall, and it acts as an important element in reinforcement. Cellulose can be produced from plants and also alternative non-plant sources like bacteria or tunicate as the only cellulose-producing animal like it is shown in Figure 2.1. However, the function of cellulose in animals and bacterial cells is different (Gauss et al., 2021; Hasanin, 2022). Cellulose is produced as lignocelluloses which are in the non-pure form of cellulose, so purification methods come next, and the purification process of cellulose can be either chemically or biological. In fact, the biological methods include enzymes so that the process would be more eco-friendly in comparison with chemical ways. Hence, its productivity is low (Hasanin, 2022).

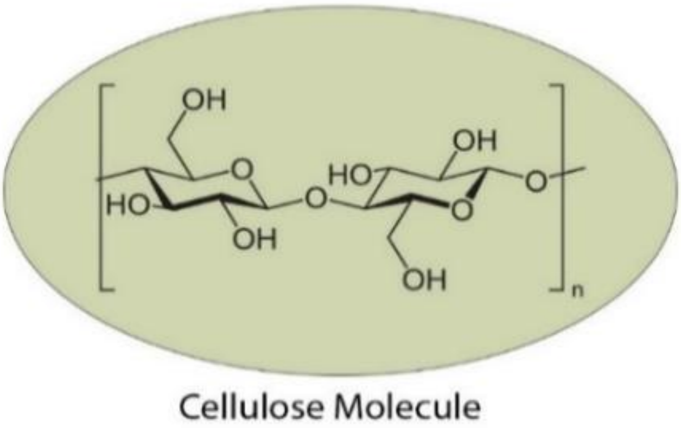


Figure 2.1 Cellulose structure

2.2.2 Types of cellulose by source

2.2.2.1 Plant cellulose

Cellulose plays an important role in the structure of plant cells. The plant cell wall is reinforced by cellulose fibrils. In addition to these, plant cellulose is the most abundant organic substance in nature, which is produced by green plants with the assistance of sunlight, carbon dioxide, and water (Mateo et al., 2021). According to the literature, plant-derived cellulose is produced from mechanical and chemical extraction from different parts of the plant (Hasanin, 2022; Gabrielli & Frasconi, 2022). On the other hand chemical methods that are non-ecofriendly, have high productivity (Hasanin, 2022).

2.2.2.2 Bacterial Cellulose

Additionally, another source of cellulose is bacterial cellulose (BCN). The quality of cellulose extracted from bacterial and animal (tunicate) sources is better. This cellulose is highly pure (Hasanin, 2022; Meftahi et al., 2021). Production of bacterial cellulose is assembling glucose together (down to top process). The nanocellulose isolated from bacterial cellulose is possible by deconstruction of its cellulose fiber packaging (Mateo et al., 2021). Cellulose is produced by bacteria like; *Acetobacter* and *Agrobacterium*. This bacteria can produce cellulose when there is a high concentration of glucose in the medium (Hasanin, 2022). BCN has a unique characteristic that allows it to be classified as a hydrogel. It is crystal clear from the name that they can absorb water and can store high amounts of water. In addition, the mechanical structure of BNC is high. They are expected to be used in biological scaffolds used as drug delivery and sensors (Al-Ahmed & Inamuddin, 2022). The synthesis of BCN proceeds through bottom-up approaches. In this regard, glucose monomers are taken up by bacteria and converted to a chain named cellulose inside the cell (Vignesh et al., 2022).

2.2.2.3 Animal Cellulose

The only example of animal cellulose is in a sea animal named tunicate. Tunicate is the only cellulose-producing animal. This sea animal has a tunic. The unique leather-like epidermis of the animal contains highly pure cellulose (Dunlop et al., 2020).

Accordingly, its body is covered with a cuticle, and under this cuticle is a layer of cells that contain tunicin (Kononova et al., 2022). Tunicate tissue has cellulose nanofibrils inside its structure and can potentially be used as a source of nanocrystalline cellulose (Dunlop, 2018). The summary of cellulose sources is shown in Figure 2.2.

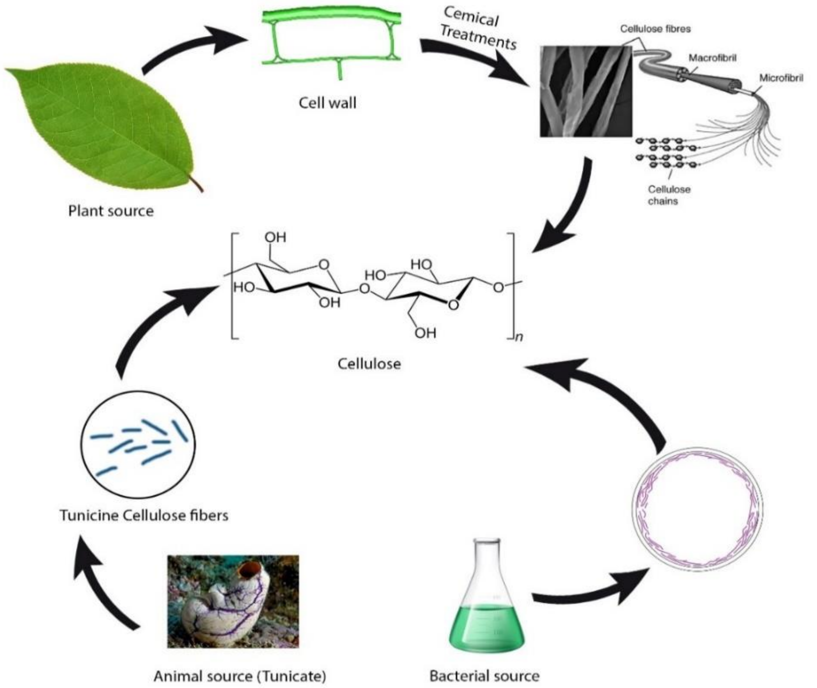


Figure 2.2 Cellulose and its sources.

In this study, we used plant-based cellulose. Because incorporating plant cellulose with hydrogels is known for the development of materials with remarkable physical and rheological properties. Furthermore, the hydrogel demonstrated compatibility with biological systems and exhibited precise control over its mechanical and transport properties. Additionally, the hydrogel showcased sustainability through the use of its constituent components, emphasizing its environmentally friendly nature (Alizadehgiashi et al., 2021). The application of cellulose material has increased significantly, and it has been replaced with synthesized and fuel-based toxic materials. Cellulose alone or engineered with other materials to create a sustainable material for biomedical approaches such as drug delivery. This drug delivery system is the release of a chemical drug in response to an external factor (Al-Ahmed & Inamuddin, 2022). For example, pomegranate peel is a source of cellulose that is being used in different types of industries, including biomedical.

2.2.3 Hydrogels

Hydrogels, first synthesized in the 1960s, have revolutionized biological applications with their remarkable properties (Wichterle & Lím, 1960). These materials possess advantageous physical and chemical properties, making them highly suitable for wound dressing applications due to their antibacterial properties and ability to stimulate angiogenesis which makes them suitable for candidates in advanced wound care (Ghobril & Grinstaff, 2015). This technology has encouraged researchers to publish massive numbers of papers over the past 60 years. Interestingly, most of these papers belong to the last five years! Hydrogels are a kind of polymer that contains a significant amount of water; as much as 99% of their weight can be water which makes them suitable to be used in the human body (Peppas & Berner, 1980). In this study we synthesized pectin and used alginate because hydrogels are implantable, injectable, and sprayable biomaterials (Correa et al., 2021). After appearing the first synthesized hydrogel, researchers attend to start engineering it to be used as a drug carrier and delivering drugs in a controlled manner (am Ende et al., 1995; Kim et al., 1992). This sustained release of drugs reduces needed doses that are required for treatments (Tam et al., 2016). On the other hand, hydrogel has favorable mechanical properties to be used in interaction with the human body and tissues, and as the concentration of polymer increases, the hardness decreases. The earlier-mentioned properties enhance the utility of this biocompatible material in regenerative medicine. Additionally, hydrogels can provide water to the wound site and keep it moist for cell movement, which aids in quicker wound recovery (Yoshii et al., 1999). Natural polymer-based films that are biodegradable have found extensive use in various biomedical applications, including wound dressing, drug delivery, and tissue engineering scaffolds (Heimbuck et al., 2019). Hydrogel dressings that possess adhesive properties can bond damaged tissues together and serve as a barrier against bacterial infections and therefore aiding in the wound healing process (Liang et al., 2019). These nanocomposite films made from natural materials like chitosan, can provide biochemical signals that aid in cell adhesion, proliferation, and differentiation with the ability of sustained drug releases and therefore make the composite antibacterial (Zhang et al., 2021). Hydrogels can be categorized into different groups depending on their characteristics, properties and polymeric composition. They can be either synthetic or organic which organic one is the aim of this study. Some kinds of organ hydrogels are : pectin, alginate, gelatin and chitosan (Sánchez-González et al., 2021).

2.2.3.1 Pectin

Pectin (PEC) is a linear anionic polysaccharide that is other than cellulose, derived from plant cell walls. It is composed of α-1,4-linked galacturonic acid residues (shown in Figure 2.3) and due to its several beneficial properties such as anti-inflammatory effects, gelling ability, and low toxicity it is a suitable polymer material for constructing hydrogels with good biocompatibility (Eliyahu et al., 2021; Neufeld & Bianco-Peled, 2017; Tentor et al., 2017). This polymer is primarily used in the food industry as a thickening and gelling agent. However, it is also extensively researched for its potential use in drug delivery systems within the pharmaceutical field (Hiorth et al., 2005). The gelling ability of pectin incorporated with other components known to be used to enhance resistance to environmental factors and extend the shelf life of a product (Assifaoui & Chambin, 2020). The ratio of esterified carboxyl groups to total carboxyl groups is referred to as the degree of esterification (DE). The degree of esterification plays a significant role in the gel-forming ability of pectin. Pectin is classified into two groups based on its degree of esterification: high methoxyl (HM) pectin and low methoxyl (LM) pectin. Pectin that is esterified by more than 50% is called "high methoxyl pectin", while pectin esterified by less than 50% is referred to as "low methoxyl pectin (Abid et al., 2017; Benli, 1994).

Microwave and ultrasonic-assisted extractions have been used for over twenty years to extract pectin. Microwaves are a form of non-ionizing radiation with frequencies between 300 MHz and 300 GHz. When microwaves come into contact with polar compounds in cells, they generate heat through ionic conduction and dipole rotation. Ultrasonic-assisted extraction is another method for isolating pectin from various sources. In this process, ultrasonic waves (16 Hz-16 kHz) cause cavitation bubbles to collapse near cell walls, allowing solvents to enter the cells and enhancing mass transfer. Pectin has been extracted from pomegranate and grapefruit peels using both traditional and microwave-assisted heating methods. Both methods can yield high amounts of pectin, but the microwave-assisted method can reduce the extraction time from 1.5 hours to just 4 minutes (Li et al., 2012).

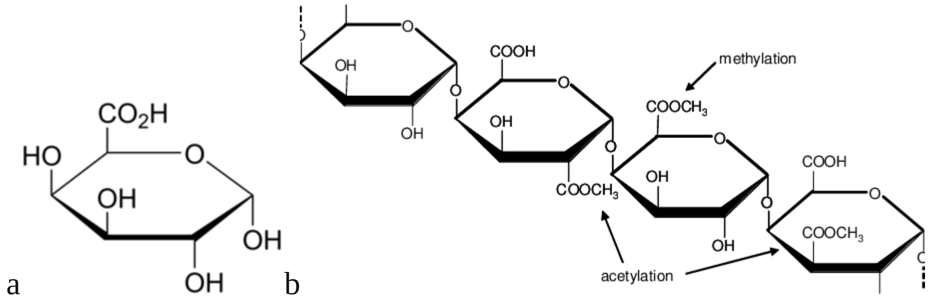


Figure 2.3 The basic chemical structure of galacturonic acid unit (a) and pectin (b).

2.2.3.2 Alginate

Since single-material bioinks do not have the required properties to be used in 3Dp, utilizing multiple components appeared in the development of bioinks. One of these materials is sodium alginate (SA) (Axpe & Oyen, 2016). Alginates are linear polysaccharides made up of 1,4 linked residues of β-d-mannuronic acid (M) and its C5-epimer α-l-guluronic acid (G), which are unbranched (Figure 2.4) (Andersen et al., 2015). Alginate is a biopolymer material extracted from seafood (Wang et al., 2021). Although alginate is a biocompatible and biodegradable material with shear thinning behavior, it is unstable in physical conditions. Moreover, increasing the concentration of utilized alginate won't improve its proliferation potential or cellular viability (Anon n.d.-a; Erkoc et al., 2020, p. 33; Yang et al., 2018). The viscosity of hydrogels containing alginate is important to overcome the challenge of printability and stability of printed objects. Therefore, several components like polyvinyl alcohol (PVA) have been incorporated to it to overcome this issue (Bendtsen et al., 2017). On the other hand, because of their viscosity, they are not utilized as single components in bioinks (Hatami & Ganji, 2014; Hentati et al., 2020; Rezende et al., 2009). As a matter of fact, in order to enhance their accuracy, modifications and additional components are needed. Different approaches like pre-crosslinking, mixing it with other polymers, adding inorganic materials, or mixing these have been made in order to improve stability and printability, and also mechanical strength (Chung et al., 2013; Mallakpour et al., 2021). Sodium alginate is known to be more hydrophilic than cellulose. When combined in a cross-linking hydrogel, the semi-stiff chains of cellulose and the –COOH groups in sodium alginate create a microporous structure. These hydrogels have excellent mechanical strength (Chang et al., 2009).

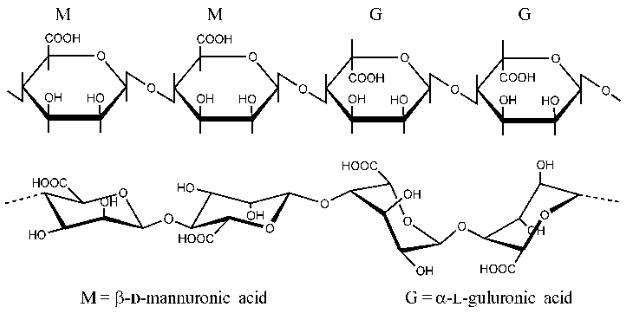


Figure 2.4 Schematic representation of sodium alginate.

2.2.3.3 Gelatin

Gelatin is a form of collagen with basic chemical structure shown in Figure 2.5. Gelatin has been altered and is frequently used in biomedicine because of its outstanding properties, such as its ability to be biocompatible, biodegradable, non-immunogenic, and to promote cell adhesion and proliferation (Wang et al., 2021). Gelatin can make a composite material more pliable, and by increasing the amount of gelatin in the material, its durability can be improved (Samp et al., 2017).

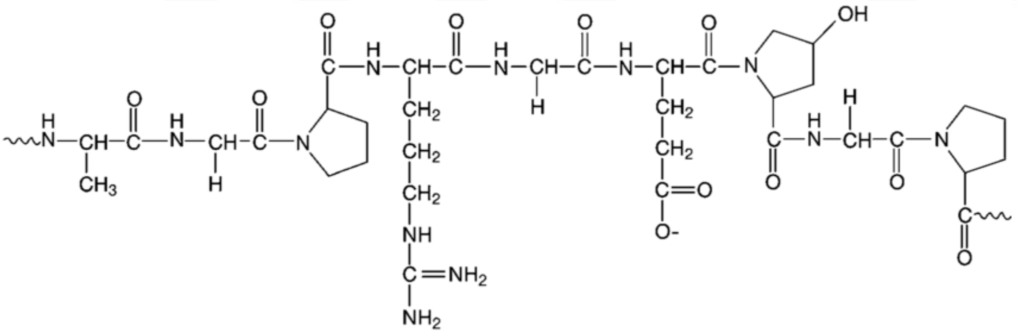


Figure 2.5 Schematic representation of gelatin.

2.2.4 Nanoclay

Integration of different nanomaterials with hydrogels is particularly advantageous due to the large surface area and inherent biological functionality of nanomaterials. Nanoclays, also known as NC, are very small polar nanomaterials with a thickness of approximately 1 nm and a width of 30 nm. They consist of nanoparticles that contain various elements found in body, such as sodium, silicates, calcium, iron, zinc, magnesium, and aluminum. These nanoclays have the unique ability to effectively interact and disperse within the networks of polymeric hydrogels due to their electric charge. It is worth noting that nanoclays show promise as synthetic materials that can serve as an alternative to silica nanoparticles and other ceramic materials commonly used in the field of bio-sector. This is due to their diverse mineral composition and their capacity to combine with other substances (Mahdavinia & Shokri, 2017). Nanoclays, specifically, have been extensively employed as inorganic fillers during the compounding process to create injectable biomaterials with improved properties (Alexa et al., 2021; Cidonio et al., 2020; Jin et al., 2018). Additionally, in order to control and modify the physical and chemical characteristics of polymeric systems, like flow behavior, stiffness, swelling, decomposition, and function of the produced materials, nano-clay has been added to improve of these systems. When nano-clay is added to polymers, the solution may develop an internal structure that allows for shear-thinning qualities and enhanced recoverability without damaging the molecular structure. One of the most popular hydrogels printed with 3D printing is the addition of nano-clay to polymeric solutions (PEG–alginate) that mimic the properties of soft tissue (Hong et al., 2015). In recent years, lots of studies have focused on the incorporation of nano-clays in the 3D printing process. Significantly by evaluation of these studies, we can formulate suitable clay-alginate composite for utilization in the 3D printing process of composite scaffolds. Accordingly, in a study, the mineral clay with a general formula [(Si12Mg8O30(OH)4(OH2)4·8H2O] has a 2:1 layered silicate. The specific structure of the mineral clay provides a selective interaction with alginate. The mechanism of interactions is that carboxylate and hydroxyl groups of alginate and hydroxyl groups of clay structure have a high likelihood of interacting. According to the study, although the addition of nanoclay had a small effect on absorptions of alginate, it had significant modifications on the viscosity of the composite. The mechanical properties of the alginate-clay composite were improved. So the rheological and mechanical behavior of alginate can improve by adding clay to the structure of the composite (Leu Alexa et al., 2021).

For example, halloysite clay nanotubes (HNTs) can be used in regenerative medicine due to the fact that they are biocompatible, show different properties of ceramic composites, and therefore act as nanofillers. HNTs have been shown to enhance adhesion, release loaded active agents in a sustained manner, and improve cell growth.

Halloysite, with the chemical formula (Al2Si2O5(OH)4_nH2O), can be easily absorbed by cells and removed by macrophages in the body. HNTs have a tubular structure and a large surface area. Studies on the nanotoxicity effects of HNTs have shown that they are biocompatible at doses of 25 mg/ml or less (Same et al., 2022). In this regard, the potential of clay minerals to adsorb and release various substances, including water, suggests their potential utility in the management of infected wounds (Hamilton et al., 2019).

Since the practical application of a single component sodium alginate (SA) hydrogel is limited due to its high rate of degradation in cell culture and weak mechanical properties it has been combined with various other components to create composite scaffolds with improved properties. In order to address the limitations of single component SA hydrogels, various components have been combined to create different composite scaffolds. One such material is HNTs, which have been incorporated into the alginate matrix with the goal of improving adsorption efficiency, mechanical strength, and other physical properties (Huang et al., 2017). Halloysite (shown in Figure 2.6) is a tubular mineral that can be processed using techniques similar to those used for other clays. Its unique elongated rod-like shape, with an axis ratio of 20:1, and inner lumen allow it to contain different functional chemicals for sustained release over hours, days, or even months. Additionally, halloysite is easier to disperse into single particles than other clays such as kaolin, montmorillonite, or bentonite because its nanotubes do not stack together in the same way as platy particles (Lvov et al., 2016).

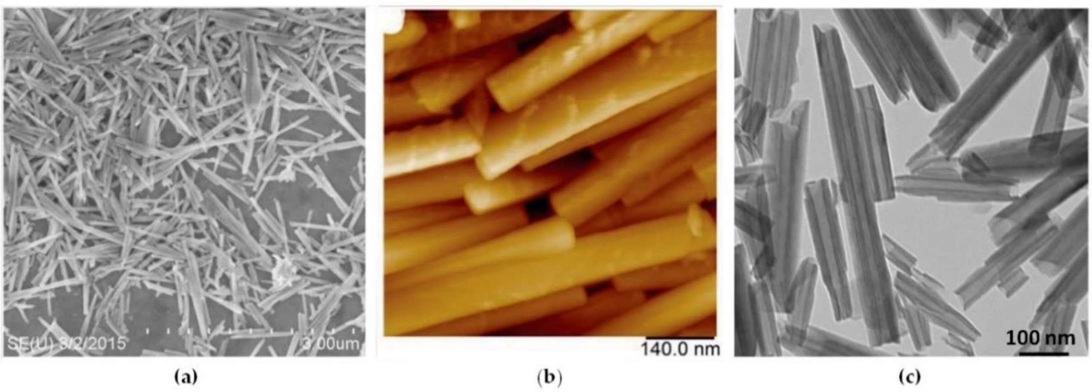


Figure 2.6 (a) Scanning electron microscopy (SEM), (b) atomic force microscopy (AFM), (c) transmission electron microscopy (TEM) images of halloysite nanotubes (Lvov et al., 2016).

2.3 3D Printing in Biomedical Applications

3Dp or additive manufacturing is a method of layer-by-layer deposition of materials. There are several popular methods of 3D printing, including Selective Laser Sintering (SLS), Stereo Lithography (SLA), and Fused Deposition Modeling (FDM) (Yun et al., 2016). According to Dermanaki Farahani & Dubé, there are different kinds of polymers that can be used in 3Dp based on the material and method that are being used. To name some; thermoplastics, photopolymers, and thermosets. Thermoplastics are one of the most used polymers used in 3D printing, which can be melted and reformed several times (Dermanaki Farahani & Dubé, 2018). Fused Deposition Modeling (FDM) is a process that involves the transition of thermoplastic filaments from a solid to a liquid state. The filament is fed through a heated nozzle, usually at temperatures between 100-300°C. Once the molten plastic is deposited in the desired location, it cools and solidifies quickly. Photopolymers are also used in 3Dp. These materials solidify by UV light or other sources of radiation. In this study we used FDM without UV light. In addition, Stereo Lithography (SLA) is a 3D printing process that uses a liquid photopolymer resin. It has the benefits of being accurate and allowing for flexible design, but it also has some drawbacks. These include limited material options, high material costs, and lower strength of the printed objects (Layani et al., 2018). As a matter of fact, photopolymers help to create detailed and high-resolution parts. In fact, they don't have the same mechanical property as thermoplastics. Thermosets are the third. These are cured by heat or chemical reactions and cannot be melted after formation, unlike thermoplastics. The favorable characteristics of thermosets are that they can create objects with high strength and resistance to heat and chemicals (Ligon et al., 2017).

Bioprinting is a method of additive manufacturing that requires special biomaterial named bio-inks, and bioprinting owes its successful structure and bioactivity to it (Gungor-Ozkerim et al., 2018; Lee et al., 2020; Mobaraki et al., 2020). As an example, Figure 2.7 shows a schematic application of plants as bio-inks in 3D printing.

The development of bio-inks is essential for different applications since there are different aspects to be considered. Some of the properties that should be optimized are:

- Viscosity: The bio-ink should be fluid during and solid after dispensing.

- Stabilization: The resolution of the printed objects depends on the process of solidifying bio-inks.
- Biocompatibility: Natural bio-inks can't endure harsh conditions. On the other hand, although synthetic bio-inks are applicable to large production capacities, they do not provide cell adhesion sites (Ramadan & Zourob, 2021).

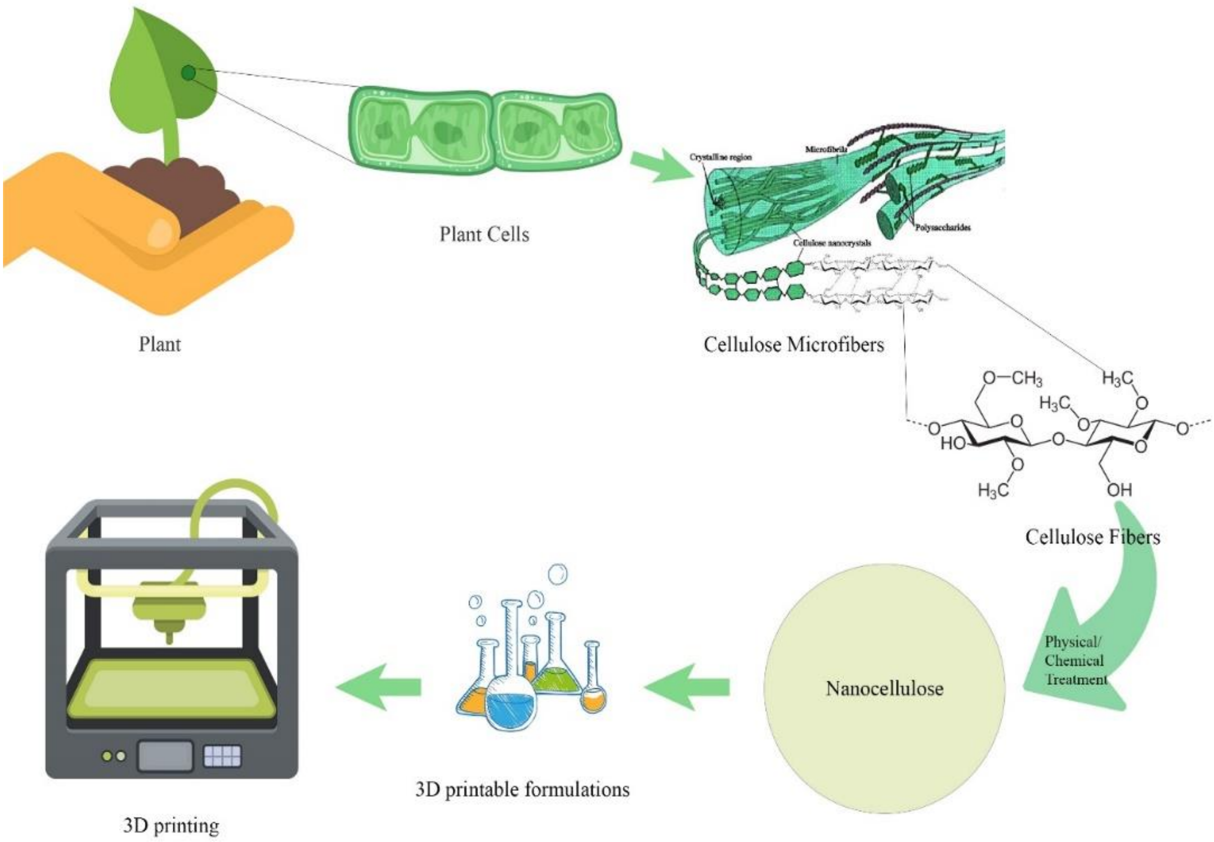


Figure 2.7 Schematic representation of plant-based bio-inks.

2.4 3D Printed Wound Dressings Patterns in Literature:

The development of 3D printed antibacterial hydrogels has gained significant attention in recent years due to their potential applications in wound healing and tissue engineering. Several studies have investigated the use of different materials, patterns, and designs to create effective 3D printed hydrogels with enhanced antibacterial properties. For instance, in a study the designed a cubic mesh scaffold which is shown in Figure 2.8 (Long et al., 2019).

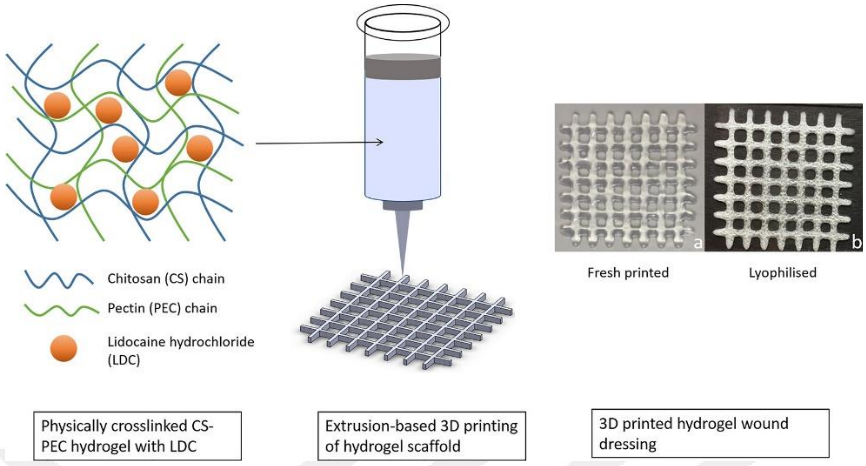


Figure 2.8 Cubic mesh scaffold shaped hydrogel (Long et al., 2019).

In another study shown in Figure 2.9, 3D porous PLA was designed that could control changes of hydrogel size with assistance of superpours after swelling (Wu & Hong, 2019).

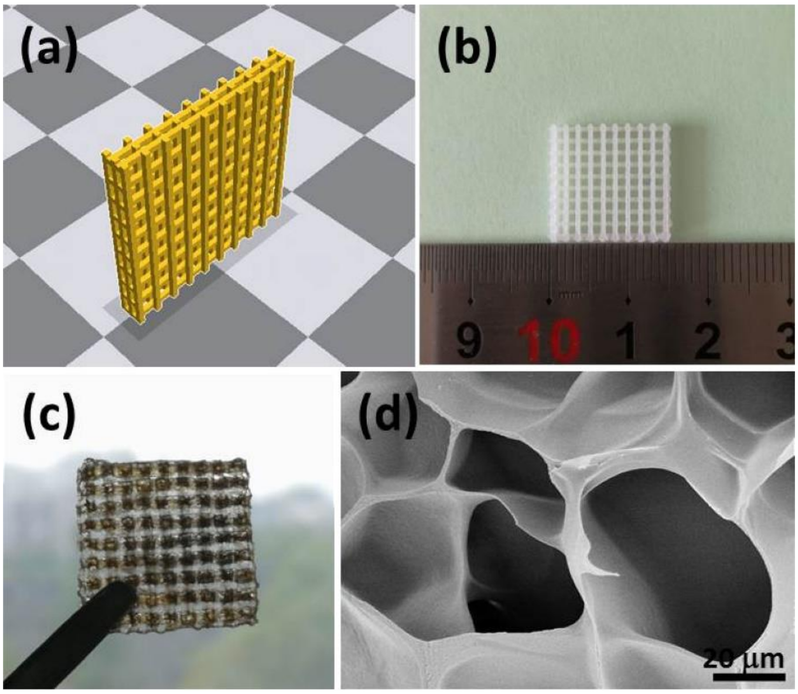


Figure 2.9 A designed prototype and a printed PLA template (15 x 15 x 3 mm) (Wu & Hong, 2019).

2.5. Importance of Antibacterial Patch

Wound healing is an essential process for the survival and well-being of living organisms. It is a natural process in which the body repairs damaged tissue and restores it to its original condition after injury. This restoration involves several cellular and molecular processes that work together to repair wounds (Kirsner & Eaglstein, 1993). If the wound healing process goes as intended, it prevents infections, minimizes blood loss, and enhances tissue regeneration (Menke et al., 2007). By appearing antibiotic resisted bacteria, an urgent need for new wound healers occurred. According to the World Health Organization (WHO), antibiotic resistance is one of the top ten worldwide public health hazards to humanity (Tacconelli, 2017). As can be understood from the name, they are bacteria that have been developed to resist the antibiotics that are commonly utilized to cure bacterial infections. This issue occurs subsequently after misuse and overuses of antibiotics. Failure of the wound-healing process results in chronic wounds, morbidity and mortality afterward (Lazarus et al., 1994). Development and launching of new antibiotics are slow, and effective antibiotics have become limited (Spellberg, 2014). Different materials have been used as antibacterial agents, like Ag nanoparticles (AgNPS) (Dini & Benli, 2023) and herbal extracts (Keleş et al., n.d.) and some of these Herbs; *Cichorium intybus L.* and *Malva sylvestris.*

Even though the concentrations of active constituents in plant extracts may be lower, they can still be a better source of antimicrobial compounds compared to synthetic drugs (Cox & Balick, 1994). *Malva sylvestris* is one of the Türkiye' native plants. The extract of *Malva sylvestris* has been found to have high antibacterial effects against human pathogen bacteria strains such as *Staphylococcus aureus*, *Streptococcus agalactiae*, and *Enterococcus faecalis*. Studies have also shown that the extract of *M. sylvestris* can increase the formation of well-organized collagen bands, increase the number of fibroblasts, and reduce the number of inflammatory cells in tissue. These findings suggest that the extract of M. sylvestris can effectively stimulate wound contraction (ed Stems & Roots, 2016). Studies have shown that the ethanolic extract of *Malva sylvestris* has greater antibacterial effects on gram-positive bacteria than on gram-negative bacteria. Given its antibacterial effect on *S. aureus* in the laboratory, the extract can be used to improve the performance of antibiotics or even replace them by evaluating its effects inside the body (Yousefi, 2023).

3 MATERIALS AND METODS

3.1 Materials

Pomegranate were purchased from local shops and plants (*Cichorium intybus L. and Malva sylvestris)* purchased from a botanical store in Istanbul, Türkiye. Grapefruits were gathered from fallen fruits of a tree of Istanbul Technical University, Ayazaga Campus for their peels and juice. All the chemicals (Ethanol, citric acid, hydrogen peroxide, sodium hydroxide, calcium chloride, alginic acid, glycerol, nutrient broth, agar-agar) were purchased from Sigma-Aldrich. Dialysis membrane with width of 24 mm and diameter of 15 mm and their closures were purchased from Repligen.

3.2 Methods

3.2.1 Preparation and microwave-assisted extraction of plant materials

To extract bioactive compounds, a microwave extraction method utilizing ethanol was employed. Ethanol is a commonly used and safe solvent, capable of extracting both lipophilic and hydrophilic compounds, while yielding a non-toxic and safe final product. Dried leaves and flowers of plants were used to prepare extracts. *Cichorium intybus L. and Malva sylvestris* were cleaned and washed with distilled water, oven-dried at 60 °C for 24 h, and ground with mechanical blender before extraction (Hashemifesharaki et al., 2020). After that, 6 gr of powder was added to 200 ml ethanol 99.6% (v/v) and microwaved (at constant 500W using a typical microwave oven) subsequently. During the process, a glass plate was used to cover extractors for preventing too much evaporations (Sample 1, 2 from Figure 3.1). High purified extracts were obtained by dialysis tubes. The membrane was cut in 10 cm pieces and soaked in a large volume of deionized water for 30 minutes at room temperature to remove sodium azide. On top of that, 6 gr of the plant material was added to 200 ml ethanol 70% (v/v) and microwaved for 5 and 10 minutes and after filtration and centrifuge, extracts were poured inside tubes and left for one week inside ethanol. Therefore, purified extracts were kept for further analysis (Wang et al., 2010). In this regard, 10 different extracts from 2 herbs were processed (Figure 7). Samples are shown in Table 3.1.

Table 3. 1. Herbal Extracts

Sample	Herbal name	Extraction Time (mins)	Applied Method	Storage Temperature (°C)
Sample 1	*Malva sylvestris*	15	Microwave	4
Sample 2	*Cichorium intybus*	15	Microwave	4
Sample 3	*Malva sylvestris*	5	Microwave	24
Sample 4	*Malva sylvestris*	10	Microwave	*24*
Sample 5	*Cichorium intybus*	5	Microwave	*24*
Sample 6	*Cichorium intybus*	10	Microwave	*24*
Sample 7	*Malva sylvestris*	5	Microwave Dialysis-tube	*24*
Sample 8	*Malva sylvestris*	10	Microwave Dialysis-tube	*24*
Sample 9	*Cichorium intybus*	5	Microwave Dialysis-tube	*24*
Sample 10	*Cichorium intybus*	10	Microwave Dialysis-tube	*24*

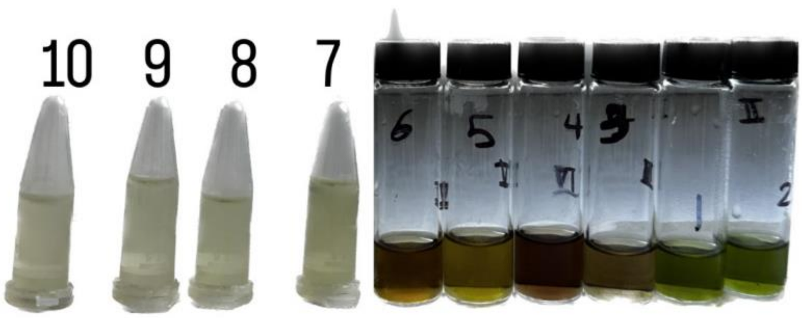


Figure 3.1 Herbal Extracts obtained from microwave extraction method.

3.2.2 Microwave-assisted cellulose extraction from pomegranate peels

Cellulose extraction was carried out from pomegranate and grapefruit peel but since the cellulose obtained from grapefruit was inconsiderable, Therefore, only pomegranate is used for cellulose synthesis. The pomegranate peels were oven dried at 50 °C for 48 h and finely ground into powder using a mechanical blender. After sifting, the pomegranate peel powder (PPP) was saved for later usage.

The synthesize process divided to 4 steps; pretreatment (Figure 3.2), acid base treatments (Figure 3.3) and bleaching. To synthesize nanocellulose from pomegranate peels, first 20 gr of PPP was added to 100 ml ethyl alcohol (Ethanol) at 60% (v/v). The sample was stirred at room temperature for 24 h.

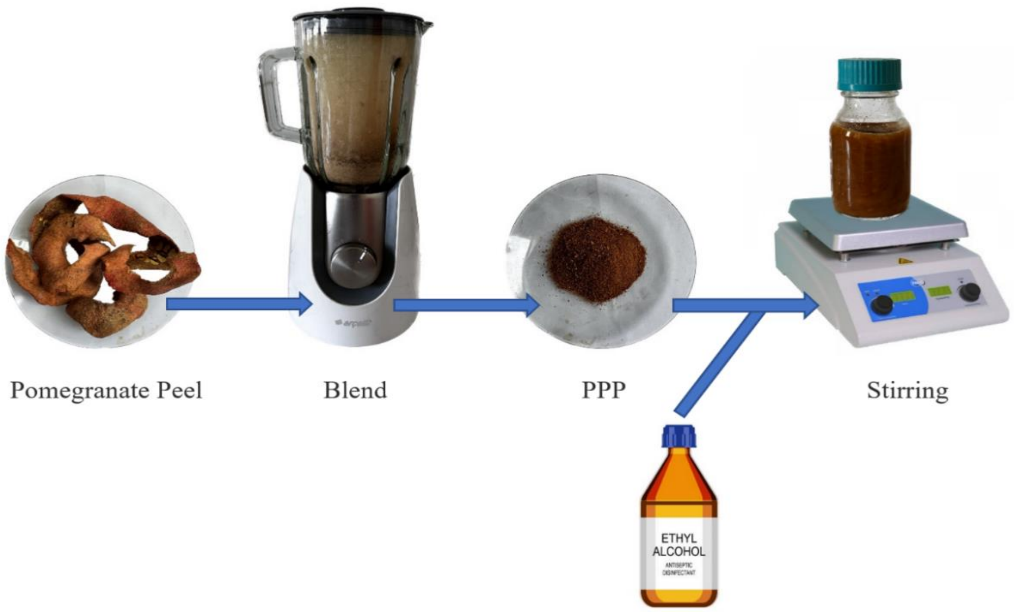


Figure 3.2 Pretreatment process applied to the peels.

Additionally, the solid residue was filtered after 24 h, and washed thoroughly several times until the pH reached neutral. In order to keep the synthesis green, we used citric acid. Subsequently, the residue was blended with 100 ml of distilled water, and then 500 ml of 1N citric acid was added to the residue as shown in Figure 3.3. The pH of the acid was 1.68. The mixed solution was adjusted at pH 2.0 (by adding acid drop by drop) and stirred at 80 °C for 10 mins in the microwave to remove the pectin. Since the pH of the sample was 4.4, we added extra 35 ml of citric acid to adjust to pH 2. The solution containing pectin was saved for further pectin extraction process.

Figure 3.3 Acid treatment of extracted residue.

The total microwave duration was 10 mins. Every time the pH was high, we adjusted it to pH 2 and added it to water if the evaporation was high to avoid the mixture from being higher than 80°C. The residue was collected after filtration and washed with distilled water until the pH reached neutral. And obtained extracts were microwave dried as shown in Figure 3.4. During the bleaching process, the acid-treated powder was delignified with H_2O_2 (1.4% w/v). The total amount of H_2O_2 that was added to the sample was 15 ml. Subsequently, the solution was microwaved for 2 mins and 800 watts.

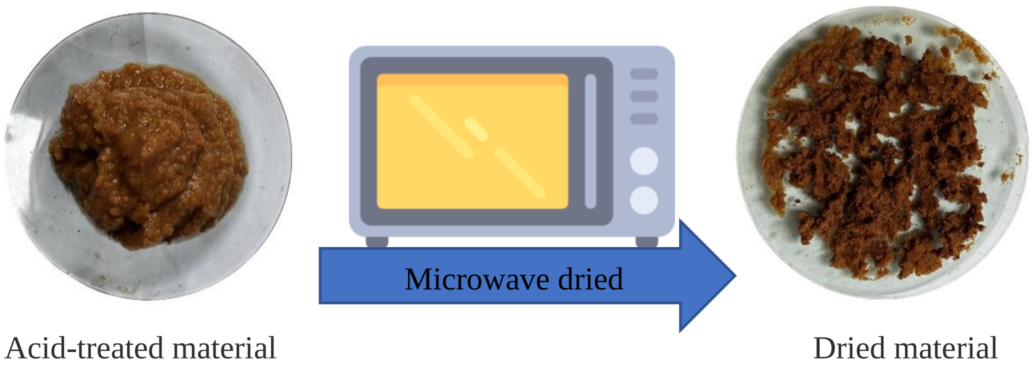


Figure 3.4 Microwave-assisted drying process.

After filtration, the residue was washed with distilled water until the pH reached neutral. At this time, the mass was 90 gr. In alkali treatment, the delignified residue was added to NaOH (10%, w/v) (10 gr of NaOH was added to 100 ml of water) solution at 80 °C for 2 mins. The residue was washed after filtration until the pH reached neutral. Then the residue was stored in the oven for further analysis. Process of pectin and cellulose extraction from fruit peel is summarized in Figure 3.5.

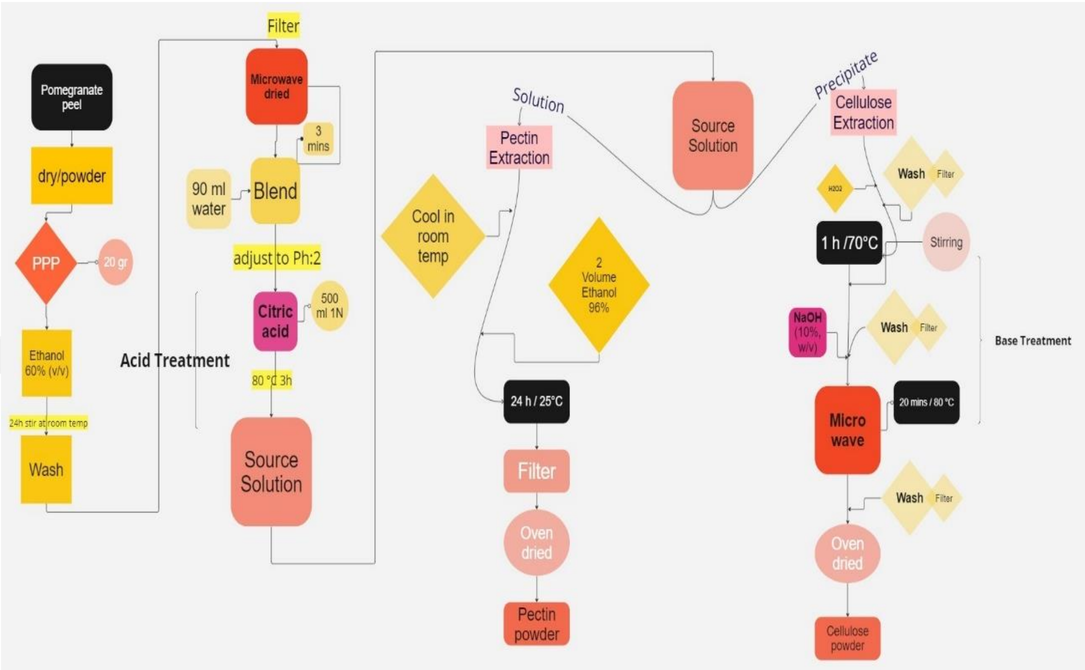


Figure 3.5 Summary of the steps for the extraction of cellulose and pectin process.

3.2.3 Pectin extraction

Pectin was extracted from both pomegranate peel and grapefruit peel. Pomegranate peel pectin was extracted with citric acid 1N. According to above mentioned process, the solution containing pectin was cooled and in the next step, two volumes of 96% w/w ethanol were added to it. The same steps were adjusted to grapefruit peel powder. Subsequently, the resulting materials were filtered through cheesecloth. Finally, obtained pectin was oven dried at 45 °C to a constant weight and saved for further analysis (Abid et al., 2017). The summery of pectin extraction process is shown in Figure 3.6.

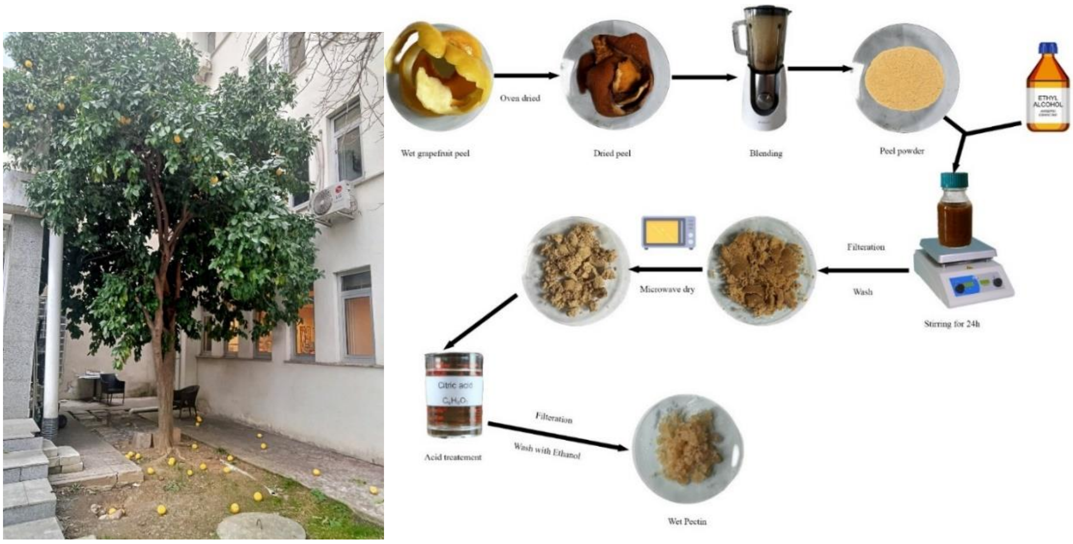


Figure 3.6 Summary of pectin extraction from grapefruit peels.

3.2.4 Antibacterial activity

To test the antibacterial activity of the extracts, the disk diffusion method was used against *E. coli*, and *S. aurous*. Round filter papers with 10 mm diameters were sterilized by exposing them to ultraviolet light for 60 min. Then, they were impregnated with the extracts and placed on Tryptone Soya Agar plates that had been inoculated with about 1×10^5 CFU/plate of the test bacteria. The plates were incubated at 37 °C for 24 h, and the diameters of the inhibition zones around the papers were measured to evaluate the antibacterial effect of the samples.

3.2.5 Design and production of 3D printed wound dressings

FDM 3D printer was used to produce film patches skeleton. Firstly, the porous scaffold was designed using Autocad software and converted to G codes for 3D printing the model is shown in Figure 14. The porous hydrogel dressing was created with large, regular pores 1 mm diameter created by piles of skeleton. The configuration was designed to be square with dimensions of 25 mm x 25 mm x 5 mm with 25 and 16 piles in it and 50 mm x 50 mm x 5 mm with 25 piles. The skeleton is shown in Figure 3.7.

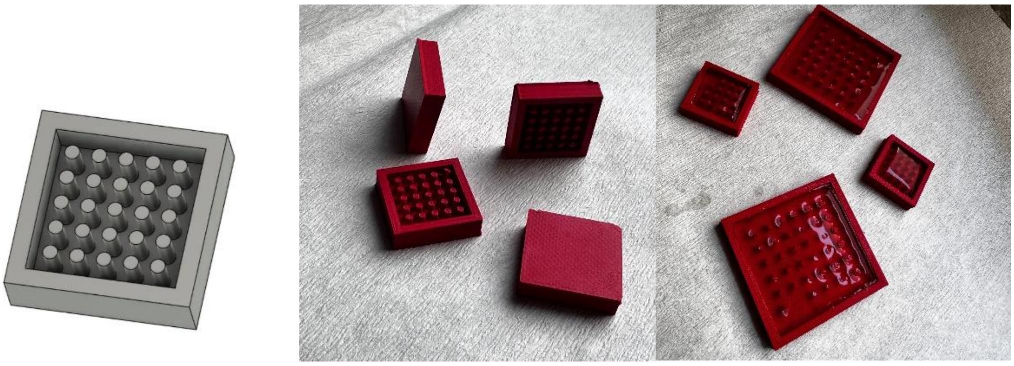


Figure 3.7 Designed skeleton model and resulted 3D printed skeletons.

Secondly advanced 3D printing technology was used to design and create double layered hydrogel with FDM. The process is shown in Figure 3.8. The materials and ratio used in the 3D printed hydrogel were the same as the formulation used for the hydrogel that was poured into the skeleton. This ensured consistency in the properties of the hydrogel, regardless of whether it was 3D printed or poured into the skeleton. The 3D printing process allowed for precise control over the shape and structure of the hydrogel, enabling the creation of a double-layered hydrogel with FDM.

Figure 3.8 Process of 3D printing for hydrogels

3.2.6 Preparation of gels for 3D printed skeleton

Gel solutions were used to prepare composite materials using the solvent casting method. Sodium alginate (SA) films with a 2% weight-to-weight (w/w) ratio were prepared, along with SA composite films blended with pectin (SA-PC) in equal mass fractions (1:1). The gels for casting the films were created by dissolving the polymers in distilled water with glycerol while stirring constantly at 1000 rpm for 2-3 hours at 40 °C. These well-mixed gels were then subjected to sonication for at least 60 min and left to settle at room temperature to remove any remaining air bubbles. Subsequently, the gels were poured into a 3D printed skeleton shown in Figure 3.8. To cross-link the films, they were immersed in $CaCl_2$ for 24 h, washed with ethanol and then dried in an oven at 45°C for 48 hs. Subsequently porous skeleton was obtained to be loaded with herbs.

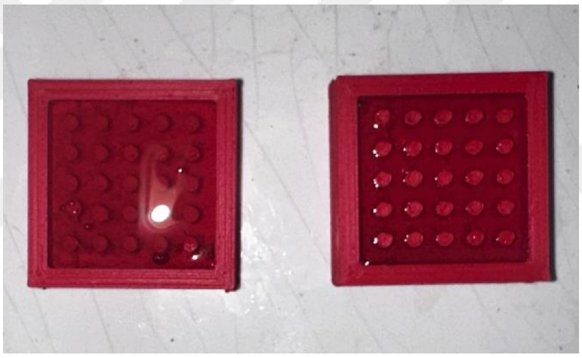


Figure 3.9 Gel poured skeleton.

3.3 Characterization Analysis of Film Patch

After manufacturing, characterization tests were conducted to understand and observe the characteristic properties.

3.3.1 Hydration of films

Matthews et al. (2005) provides details on our experiment to examine the hydration or fluid uptake capacity of films in a wound environment utilizing gelatin plates as well as a non-animal wound model. We prepared the needed 4 wt.% of gelatin solution by dissolving 4 g of gelatin in 100 ml sterile distilled water and mixing it at approximately 1 h at 45 °C. The gel started to form when roughly four milliliters were poured into each 6 cm diameter Petri dish left at room temperature for 24 h. Once they hardened into gels. We placed circular films measuring 15 mm across on top of some plates while leaving others blank before starting incubation around 37 °C setting. After studying their dimensional changes over time via periodic measurements until completion. Their respective expansion ratios were calculated by applying;

$$\% \text{ E} = \frac{Dt-D0}{D0} * 100$$

E refers to expansion ratios. D_t illustrates the diameter after expansion while D_0 represents the diameter before any changes occur.

3.3.2 Morphological analysis of film patches

Initially, for the observation and measuring surface topography, Atomic Force Microscopy (AFM) was used. SEM (EVA MA10) was used to observe morphological features such as pore size.

3.3.3 Chemical analysis of film patches

Fourier Transform Infrared Spectroscopy (FTIR, JASCO-4000) was used to determine the chemical composition of the wound dressings.

3.3.4 Hydrophilicity of scaffolds

The hydrophilicity of the scaffold was assessed by measuring the contact angles of water and herbal extract droplets on the surface of the films using a contact angle analyzer. The contact angle of a liquid drop on the surface of the film was measured by the sessile drop method. Prior to measurement, all samples were dried at 60°C for 24 hours and films measuring 1 cm x 2.5 cm were attached to glass slides to improve their extension. Droplets of water and herbal extract (5.0 µl) were carefully placed on the surface of the films and their dynamic changes were recorded. Two measurements were taken for each film.

3.3.5 Extraction Yield Calculation

The extraction yield of pectin is calculated by dividing the dry weight of the pectin powder by the dry weight of the raw material. (W Sample). Table 4.1 shows yield of pectin with microwave assisted extraction method in literature.

$$\text{Yield}(\%) = \frac{W_{MRPs}}{W_{Sample}} \times 100$$

Table 4. 1. Yield of extraction, moisture and ash content of pectin

Material	Pectin Yield (%)	Ash content (%)	Moisture (%)	Est (%)	Source
Pomegranate	23.87	4.0	16.5	52.85	(Pereira et al., 2016)
Grapefruit	27.81	5.04	17.5	61.5	Bagherian et al., 2011

3.3.6 Esterification Degree Determination

Degree of esterification were determined by the titration method (Pasandide et al., 2018). To determine the degree of esterification, 50 mg of pectin is weighed and soaked in 2 ml of ethanol. It is then mixed in 100 mL of distilled water at 50ºC until completely dissolved. Afterwards, 5 drops of phenolphthalein are added, and it is titrated with 0.1N NaOH. The consumption is recorded as V1. Then, 20 ml of 0.5N HCl is added. After adding phenolphthalein, it is titrated with 0.1N NaOH until pink color appeared. The consumption is recorded as V2. The degree of esterification is calculated according to the equation below, as seen in Table 4.2.

$$\%\ \text{Degree of esterification} = \frac{V2}{V1+V2} * 100$$

3.3.7 Moisture levels and ash contents

1 gr of the sample was dried until it reached still weight at 145°C for moisture level analysis and 1 gr of the sample was burnt at 600 °C for 3 hours, for ash content analysis.

3.4 Spectrophotometric Analysis of Herbal Extracts

The absorbance spectra of the herbal extracts were measured using a UV-Vis spectrophotometer. The extracts were prepared by microwave assisted extraction method and dialysis tube purification and diluted to appropriate concentrations for analysis. The spectrophotometer was set to ethanol 60% as baseline and the absorbance spectra were recorded over a wavelength range of 200 - 650 nm.

4 RESULTS AND DISCUSSIONS

The aim of this study is synthesizing antibacterial gel patch with assistance of 3D printing that can be used as wound dressing in prospective applications since it can provide moisture and can be removed easily without any damage. The results of this experiment are presented below.

The total extraction yield ranged from 23.5 to 24 wt%, which is similar to maximum yield of 24 wt% reported by Moorthy et al (Moorthy et al., 2015). The extraction yield of synthesized pectin is shown in Table 4.2.

Table 4. 2 Yield of synthesized pectin

Source fruit	Sample (gr)	Extracted powder (gr)	Yield%
Pomegranate	4.8	20	23.5 - 24
Grapefruit	4.312	20	21.56

The degree of esterification values of pectin extracted from the various samples were found to be 52.85%, 61.5% and 60-75% for the pomegranate pectin, grapefruit pectin and commercial pectin samples, respectively (Table 4. 3). The degree of esterification for pomegranate peel was found to range from 47% to 71%, indicating that the extracted pectins were predominantly high methoxyl pectins. Lower values were obtained when extraction was performed at higher temperatures and times, and at lower pH values. To extract pectin with high yield and purity while maintaining a relatively high degree of esterification, a region of extraction for high methoxyl pectins was defined, which produced a degree of esterification of at least 54% (to ensure safety). Our study was able to achieve this.

Also in literature moisture level of pomegranate peel pectin was 84.72 ± 1.60 % (Abid et al., 2017; Güzel & Akpınar, 2019).

Table 4. 2. Summary of moisture level, ash content and esterification measurement of pectin and cellulose.

Material	Moisture level	Ash content	V1	V2	% Est
Pomegranate Pectin	6.2	4.38	16.5	18.5	52.85
Grapefruit Pectin	2.4	5.04	17.5	28	61.5
Apple Pectin	7	9	-	-	60-75
Cellulose	55	31.89	-	-	-

Glycerol can have an effect on the properties of hydrogels. Glycerol improves the elasticity, flexibility, and moisture sensitivity of hydrogels without affecting the gelation mechanism, by reducing the interactions between adjacent chains. Figure 4.1 displays images of hydrogel samples containing pectin from various sources and alginate in a 1:1 ratio, as well as sample containing pure alginate.

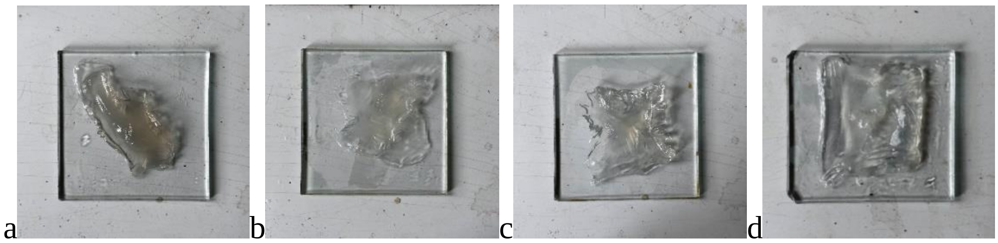


Figure 4.1 a. Pectin with grapefruit juice/Alginate, b. Grapefruit Pectin/Alginate, c. Pomegranate Pectin/Alginate, d. Alginate

In order to determine effect of glycerol on hydrogel performance, two different hydrogel formulations containing and not containing glycerol as plasticizer were embraced. Hydrogels containing glycerol had less size decreasing during drying process and also it is mechanically stable (Hardman et al., 2022). (Shown in Figure 4.2) The results indicated that the alginate/glycerol film (Figure 4.2 a) exhibited superior mechanical strength compared to the pure alginate film (Figure 4.2 c). In contrast, the pectin/alginate film (Figure 4.2 b) demonstrated reduced flexibility compared to the pectin/alginate/glycerol film (Figure 4.2 d), with breakages and tearing observed during examination.

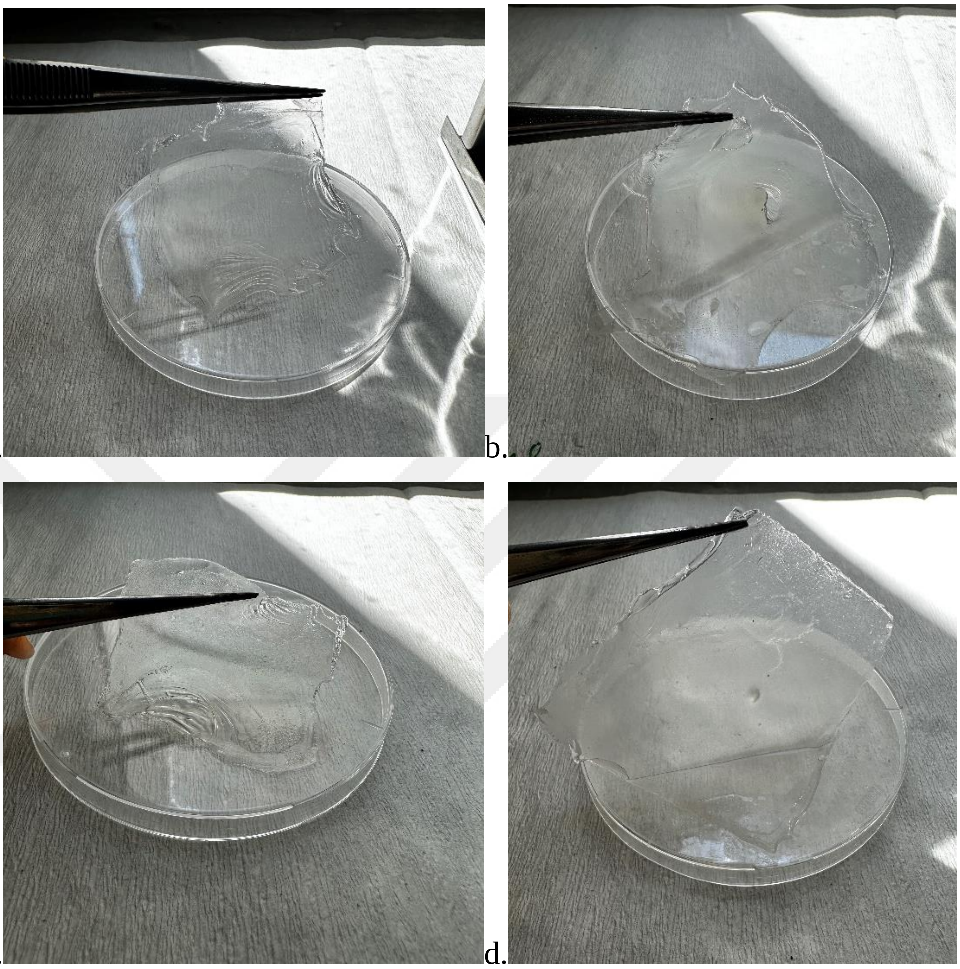


Figure 4.2 a. Alginate glycerol composite, b. alginate pectin composite, c. alginate , d. alginate pectin glycerol composite film.

In this regard we formulate alginate/pectin/ glycerol (1:1:1). In order to synthesize porous gel with 3D printed skeleton three models were designed. 25mm x 25mm x5mm with 25 and 16 pipes and 50mm x 50 mm x 5mm with 36 pipes. Accordingly, 25mm x 25mm x 5mm with 16 pipes and 50mm x 50 mm x 5mm with 36 pipes were successful due to the fact that the hydrogel could easily be removed from it. Additionally, it was observed that as $CaCl_2$ amount increases, stiffness of the hydrogel increases and in order to obtain our best result the least consantration of $CaCl_2$ is

10 % of $CaCl_2$. Figure 4.3 shows the physical appearances of porous crosslinked hydrogels containing alginate/pectin and glycerol.

The presence of large, regular pores in the porous hydrogel dressing allows it to expand inward during swelling. As shown in first line of Figure 4.3, the pores in the porous hydrogel dressing are almost completely filled by the swollen hydrogel after swelling. In contrast, the non-porous hydrogel can only expand outward during swelling due to the absence of large pores. As a result, porous hydrogel dressings with large pores are preferred for wound management.

Figure 4.3 Photos of the swollen solid and porous hydrogels.

After the hydrogel was prepared using both the skeleton and 3D printing methods, it was allowed to dry to obtain a dry hydrogel. The drying process involved removing excess water from the hydrogel, resulting in a solid, yet flexible material. The dry hydrogel obtained from both methods had similar properties and could be used for various applications.

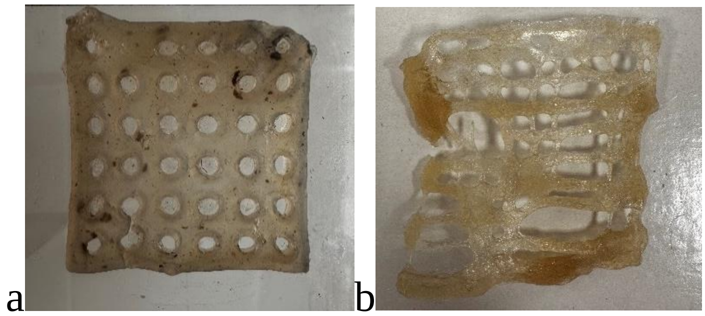


Figure 4.4 a. Dried hydrogel from the skeleton method and b. dried hydrogel from 3D printing.

A hydrophilic wound dressing is necessary for rapid therapeutic effect and absorption of exudate. This characteristic also helps to maintain a moist environment, promote high blood absorption, and enhance erosion capability (Kus & Ruiz, 2020). The composition of a scaffold can be tailored to influence its hydrophilicity, which in turn affects the adhesion and proliferation of cells which are key factors in wound dressing and antibacterial activities (Nosrati et al., 2021). A contact angle of less than 30° indicates that the surface is highly hydrophilic, while a contact angle greater than 90° suggests that the surface being evaluated is hydrophobic (Fu & Zhang, 2018; Law, 2015)

To simulate the surface of the scaffolds, hybrid materials were cast into films. The results, shown in Figure 4.5 - 4.7 and Table 4.3, indicate that the contact angles on glass indicate moderate wetting properties of the herb extract, with a slight difference between the left and right sides.

The contact angles on the alginate film suggest improved wetting properties compared to the glass substrate. The herb extract shows good spreading ability on the alginate film.

The contact angles after 20 seconds on the alginate pectin film indicate very good wetting properties of the herb extract. The liquid spreads almost completely on the film, indicating high surface affinity.

The contact angles on the alginate glycerol film show good wetting properties of the herb extract, similar to the alginate film. The liquid spreads easily and uniformly on the film.

The contact angles on the alginate pectin glycerol film indicate good wetting properties of the herb extract. The liquid spreads well and evenly on the film, similar to the alginate film.

Overall, the contact angle results suggest that the herb extract has varying wetting properties on different substrates and films. The alginate pectin film exhibits some uneven wetting behavior, while the alginate and alginate glycerol films show good and consistent wetting properties.

Our results indicate that the addition of plasticizer to biocomposites resulted in a decrease in contact angle compared to biocomposites without plasticizer.

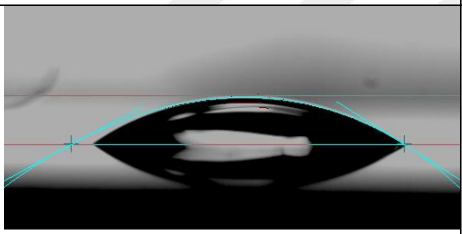
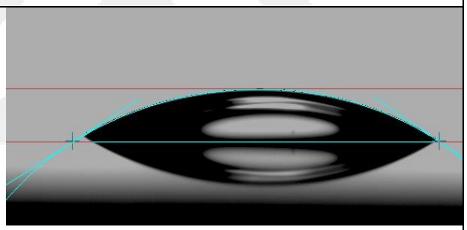
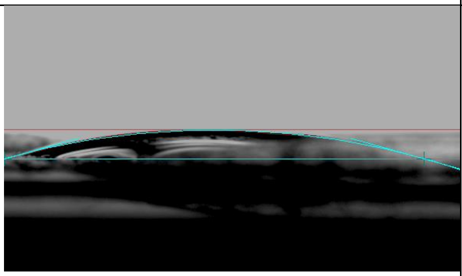
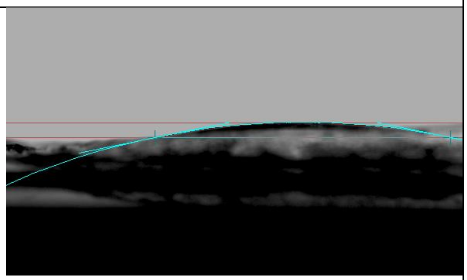
	Malva sylvestris	*Cichorium intybus*
Glass	 CA left: 24.7° CA right: 28.9°	 CA left: 27.1° CA right: 28.4°
Alg	 CA left: 15.4° CA right: 15.4°	 CA left: 15.4° CA right: 15.4°

Figure 4.5 Contact angles of herbal extracts onto glass and alginate film.

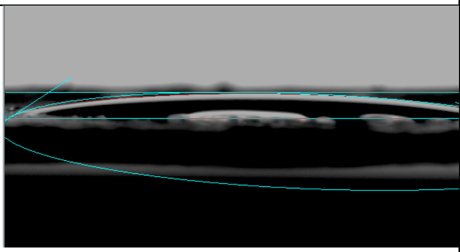
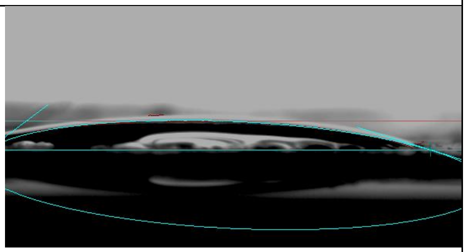
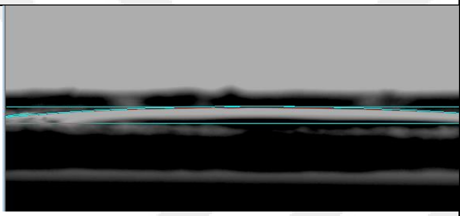
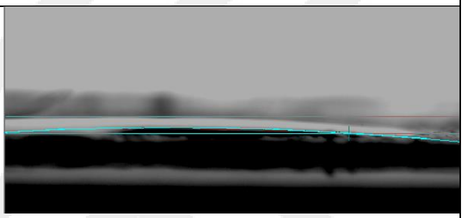
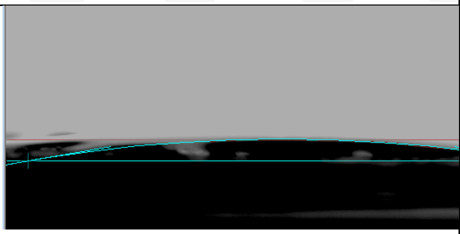
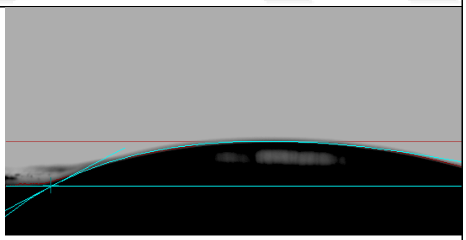
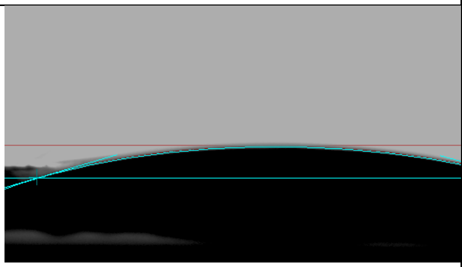
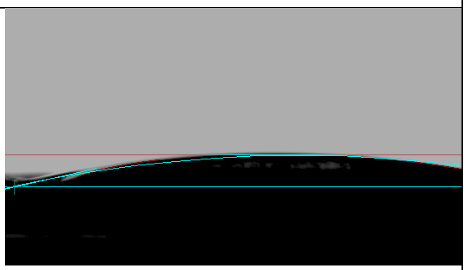
	Malva sylvestris	*Cichorium intybus*
Alg-P	 CA left: 32.6° CA right: 11.8°	 CA left: 36.9° CA right: 17.2°
Alg-P (after 10 sec)	 CA left: 7.5° CA right: 6.5°	 CA left: 3.4° CA right: 3.4°
Alg-Gly	 CA left: 9.5° CA right: 9.5°	 CA left: 27.7° CA right: 14.5°
Alg-Gly-P	 CA left: 16.5° CA right: 15.6°	 CA left: 12.5° CA right: 12.5°

Figure 4.6 The behavior of a herbal extract droplet on the surface of the scaffold after 20 seconds

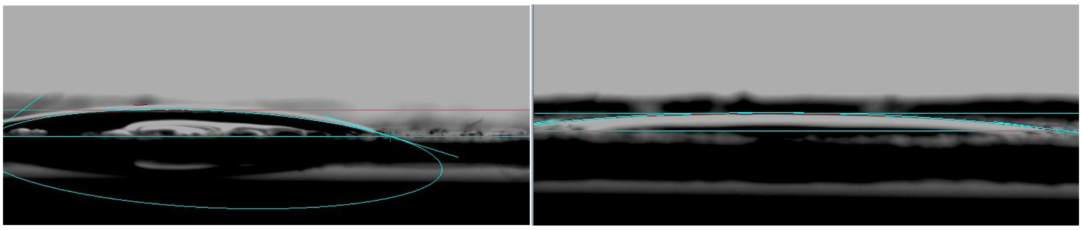


Figure 4.7 The behavior of a herbal extract droplet on the surface of the scaffold after 20 seconds.

The hydrophilicity of cellulose bio composites is due to the presence of microfibers on the surface of cellulose that can absorb water. As a result, no contact angle was observed with water and within 20 seconds, water was absorbed. However, when other liquids were used, a contact angle was observed. (Shown in Figure 4.8) addition of cellulose improves hydrophilicity of composite.

Figure 4.8 The rough surface of cellulose which absorbs water, keeps other types of liquids.

The optical properties of biosynthesized nanoparticles were evaluated using UV-Visible spectrophotometry at room temperature. The results are presented in Figure 4.9 below.

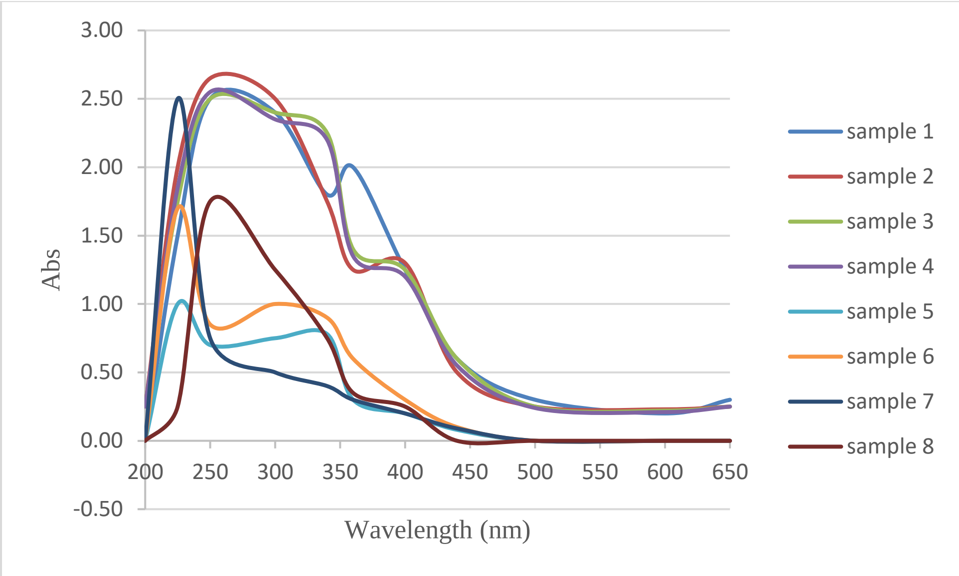


Figure 4.9 Spectrophotometric absorbance peaks of several herbal extracts.

Subsequently, herbal extracts were absorbed to nanoclays and injected to pours of hydrogel. As it can be seen in Figure 4.10, *Cichorium intybus L.* and *Malva sylvestris* showed significant antibacterial activity against *E. coli* and *S. Aureus*. In similar studies, the hydrogels containing *Milefolii flos* and *Calendulae flos* extracts did not exhibit any antimicrobial activity. Accordingly, none of the hydrogel formulations were effective against *Escherichia coli*, except for the one containing a blend of extracts, which showed an inhibition zone of 8 mm (Gavan et al., 2022). This shows successful effect of *Cichorium intybus L.* and *Malva sylvestris* against these bacteria. There have been few studies that have incorporated *Cichorium intybus L.* and *Malva sylvestris* extracts into a hydrogel.

a. b.

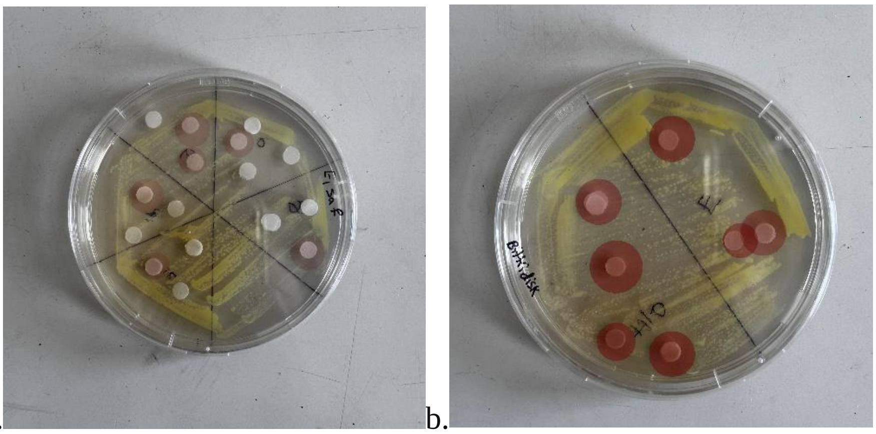


Figure 4.10 Disk diffusing method analysisseen in petri belongs to a. *E. Coli* and b. *S. Aureus.*

Accordingly, the herbal extracts had better prevention effect on *S. Aureus* and the duration of microwave during extraction of herbal extract did not affect quality of extracts.

Second part of study involves the extraction of cellulose pectin from pomegranate peels and grapefruit peels using microwave-assisted extraction and acid and base testaments in order to formulate hydrogel. The peel of pomegranate and grapefruit was stored and was used for extraction of cellulose and pectin with the microwave-assisted method, with acid and base treatments. Apparently, cellulose extraction from pomegranate peel was unsuccessful, and after acid treatment, there was no rough material that could be gathered. But pomegranate peel is a good source for cellulose extraction. Microwave-assisted process decreased the whole duration of experiments and sped them up. This was effective in cellulose extraction, pectin extraction, and also antibacterial herb extractions. On the other hand, because of utilizing citric acid in acid treatments, the experiment was green, and it did not have the toxicity of normal procedures. Additionally, extracts from dried leaves and flowers of *Cichorium intybus L*. and *Malva sylvestris* were effective against *E. coli*. In this regard, it can be used as an antibacterial agent in patches. The study found that hydrogels with *Malva sylvestris* or *Cichorium intybus L* plant extract have high potential as antibacterial patches for wound treatment.

5. CONCLUSION

In conclusion, this study aimed to create an antibacterial gel patch using 3D printing technology for potential use as a wound dressing. The results showed that the gel patch was successfully synthesized and several important findings were highlighted.

1. This study aimed to create an antibacterial gel patch using 3D printing technology that could be used as a wound dressing due to its ability to provide moisture and be easily removed without causing damage. The gel patch was made by combining pectin, alginate, and glycerol in equal parts.

2. Pectin and cellulose were extracted from sources such as pomegranate and grapefruit peels. The extraction yield, degree of esterification, moisture level, and ash content of the extracted pectin were measured. The results showed that the extraction yield of pectin was between 23.5% and 24%, consistent with previous studies. The degree of esterification indicated that the extracted pectins were mainly high methoxyl pectins.

3. The study investigated the addition of glycerol as a plasticizer to the hydrogel formulation. Hydrogels containing glycerol showed improved elasticity, flexibility, and moisture sensitivity. They also had enhanced mechanical stability and reduced size decrease during the drying process.

4. Porous gels with 3D printed skeletons were successfully created using the alginate/pectin/glycerol formulation in equal parts. The stiffness of the hydrogel increased as the concentration of $CaCl_2$ increased, with the best result obtained at a concentration of 10% $CaCl_2$.

5. The study examined the hydrophilicity of the scaffold materials and found that adding herbal extracts, cellulose and HNTs to the composite improved their hydrophilicity. This property is important for rapid therapeutic effect, absorption of exudate, and maintenance of a moist environment. Contact angle measurements showed that the hydrophilicity of the scaffold materials was enhanced.

6. The antibacterial activity of the synthesised porous gel patch was evaluated using herbal extracts. herbal extracts of *Cichorium intybus L.* and *Malva sylvestris* was obtained by microwave assisted extraction method and purifued

by dialysis tubes. these herbs showed significant antibacterial activity against *E. coli* and *S. aureus*. Hydrogels containing these extracts were effective in preventing *S. aureus*, highlighting their potential as antibacterial patches for wound treatment.

7. 3D printing technology was used to design and manufacture a patch-shaped material from organic materials for use as a wound dressing patch.
8. Further in vivo studies are needed to validate the efficacy and safety of the gel patch. These studies will provide valuable insights into the performance of the gel patch in real-world clinical settings and pave the way for its potential use as an advanced wound dressing.

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