Antimicrobial Activity of Piper Nigrum and Cinnamomum Verum in PVA-Starch Hydrogel Matrix Membrane

By

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Antimicrobial Activity of Piper Nigrum and Cinnamomum Verum in PVA-Starch Hydrogel Matrix Membrane

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This thesis is submitted as a partial fulfillment of the requirements for the degree of MS in

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FORM TH-4

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3	CHE-843	Separation processes in CHE	CORE	3	B+
4	MSE-871	Polymer Engineering	ELECTIVE	3	B
5	CHE-814	Product Technology	ELECTIVE	3	A
6	CHE-816	Molecular Nanotechnology	ELECTIVE	3	A
7	EME-981	Advance Fuel Technology (Elective)	IN PROGRESS	3	A
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Dedicated to Muhammad Iqbal Qazi and Jamshida Yasmeen (My parents)

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Hafsa Iqbal

Abstract

A series of preeminent poly vinyl alcohol (PVA)/starch hydrogel membranes was prepared with varying contents (0, 0.3, 0.5, 0.7, 1, 1.5g) of *piper nigrum* and *cinnamomum verum* by solution casting method. The comparison was made between the pure PVA/starch hydrogel and natural antimicrobial agents assembled hydrogel membrane matrix. Glutaraldehyde was added as a cross linker in ethanol/HCL dispersion to form acetal bridges. Glycerin was selected as a plasticizer to increase the ductility of hydrogel membranes. The concentration of natural antimicrobial agents incorporated in hydrogel has significant effect on the physical properties as compared to pure hydrogel, determined by swelling behavior, gel fraction, moisture retention and water vapor transmission rate. The prepared matrix was characterized by scanning electron microscope (SEM) and Fourier Transform Infrared Radiation (FTIR). The tensile strength shown by incorporated membranes was 9.8 MPa which had sufficient strength to be used as a wound dressing. Antimicrobial activity was investigated against *Escherichia coli* and methicillin-resistant *staphylococcus aureus*. Hydrogel membrane with 1.5g of natural antimicrobial agents showed highest inhibitory activity and further characterization had been performed on 1.5g assembled natural antibacterial agent's membrane. It was further tested against fungus strains i.e., *Aspergillus Flavus, Aspergillus Oryzae and Aspergillus Subolivaeus*. The prepared membrane could be used as a wound dressing to provide a cushioning effect on the skin.

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List of Abbreviations

PVA	Polyvinyl alcohol
SEM	Scanning Electron microscopy
FTIR	Fourier Transform Infrared Radiation
MBA	Methylene bis acrylamide
PMMA	Poly N, N-dimethyl-acrylamide
HES	Hydroxyl ethyl starch
SBF	Simulated body fluid
E. coli	Escherichia coli
MRSA	Methicillin resistant staphylococcus aureus
SAXS	Synchrotron small angle X ray scattering
NP	Nanoparticles

Chapter 1: Introduction

1.1 Introduction

The external surface of the human body is composed of the largest organ i.e. Skin, which is very gentle and composed of different layers. The outer layer (dead cell layer) provides the major protection against the environment is called the epidermis, it is made up of the protein material called the keratin. The middle layer consists of living cells and contains blood vessels that provide the support and structure to the human body. These are the layers that provide the protection and have absorbance of any shock. In case of any wound, the skin regenerates itself by replacing the cells beneath the layers. The process of repairing skin is a natural process. "Three healing gestures" is one of the late medical words in 2000BC describes the science of healing of wound. It includes three things, wash the injury happened, making bandaging and applying the bandage. Nowadays there is quite a great advancement on the healing bandaging in this era. As the late famous scientists Joseph Lister and Louis Pasteur concluded that a bacteria enters the wound by the outside source .This provides the courage to wash injuries and wounds rapidly such as antiseptic technique to kill bacteria on the surface to avoid any infection[1].

Any injury results in damaging or loss of human tissues that may results in to some disability or becomes life threatening. In such cases, wound membrane has a great importance which acts as a lining that avoids loss of any liquid and kills entrance of any pathogens into the damage area[2].Injuries can be classified into two categories, chronic and acute injuries. Acute injuries can be caused by traumas that can be healed up to 9 to 11 weeks. That type of injuries can be caused some stabbing actions or some incautious or reckless actions. While the chronic injuries refers to that are caused by particular illness or inherited disease such as blast-ocytoma or polygenic disease[3]. Injury healing is impulsive and self-going process, consists of three main healing stages , inflammation, tissue development and tissue restructuring[4].

For a long time humans used cloth, flax, honey and vegetable /animal fibers for wound bandaging. on the popular era is looking towards the synthetic bio gradable polymers to take its place by increasing its performance[3]. Bandaging can be categorized in followings types.

Regular dressings are the Passive products that acts as a protective layer on the injury so that the beneath wound reconstruct itself. While the second one is the interactive dressings that are made up of polymer films such as PVA, hydrogels, polymeric films which happen to be penetrable for oxygen, sorbs moisture and great barrier to bacteria. And third one is bioactive dressings composed of active agents such as collagen and chitosan[1].

1.2. Membrane

Membrane is defined as semi permeable barricade between two stages. Perhaps one constituent of a mixture moves faster through the film than the other constituent, separation can be accomplished. The essential properties for membranes make them well suited for major industrial growth. As they are easy in process, with very low energy consumption makes them exceptional potential for energetic features and environmental impinging. Inorganic membranes and polymeric membranes are widely used for many applications such as drinking water purification, desalination and macromolecule separations[5].

1.2.1 History

Osmosis term was produced by Abbe Nolet in 1748 to explain the phenomena of water passing through a diaphragm. In the early twentieth century, membranes were not used as commercial material but were used as to formulate chemical or theoretical theories in laboratory. The membranes prepared by Traube and Pfeffer, then utilized by Vant Hoff in 1887. They examined the membranes by solution osmotic weight for a limit law to explain the ideal diluter solution behaviors. This work paved the way for invention Vant Hoff equation. Almost in the same era the Maxwell along with other scientists worked on selective semi permeable membrane and came with the invention of kinetic theory of gases.

During the development phases, the scientists worked on the quality of diaphragm and its available sources such as cattle's gutor hound, animal bladders and frankfurter casings. Later on the reproducible membranes of nitrocellulose were made and recommended. Bechold in 1907 upgraded the nitrocellulose membranes by performing bubble test to update the pore length[6].

In medical field the purpose of membranes is for use as artificial kidneys, as the scientific approach to the knowledge of the world. In 1945 in Netherlands the W J Kolf explained the artificial kidney. After 20 years later, on large scale the technology was fully extracted and

move towards developments .The artificial kidney membranes have important role in life saving procedure. The development of device such as membrane flesh oxygenator was served more than 80000 people urgently with artificial kidney replacement procedure and also helped in open heart surgery. Another application for membranes is the dope travail systems. The Alex Zaffaroni made these devices in 1966. The Alza Andits take the business to a new level by using membrane techniques in the pharmaceutical trading and drug delivery[6]. During the World War II the membrane technology got their significance in the field off drinking water.

1.3 Hydrogels History

In 1960 Wicherle and Limin developed the understanding for using hydrogels in the biological field. After that the usefulness of hydrogels in medical field was also observed and acknowledged. The number of research paper related to hydrogels information was around one hundred till the 1974. The research related to hydrogels and nano technology has been grown and more than one thousand papers has been published in year 2000[7].

In 1984, hydrogels are hydrophilic three dimensional structure mainly consist of colloidal gel of inorganic salts as polymeric material joined together by physical or chemical means as shown by the Figure 1.

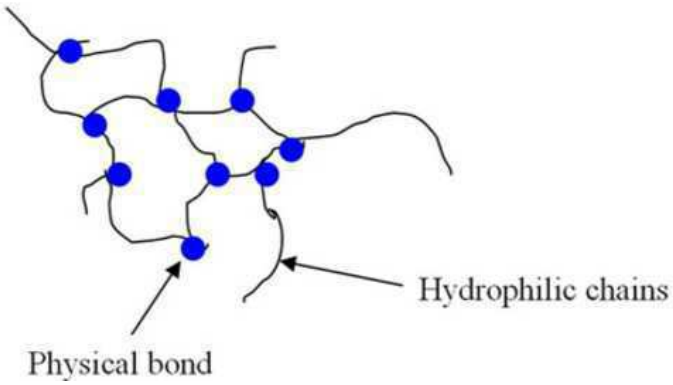


Figure 1. Hydrogel structure

Biomedical plant material, PVA was used as a first hydrogel material formed along with other foaming agents and formaldehyde cross linked compounds. PVA invented in 1949 and its trade name for market placing is 'Ivalon'[8]. In the starts of 1950s, it was regarded as

fascinating compound for use in biomedical application that includes vascular prostheses, skin replacement, embolic material, for regenerative bone scaffolds and replacement of articular cartilage. As PVA sponges can be modeled to serve as scaffold static compound for specific material properties to provide some specific shape. For contact with blood PVA and heparin PVA composites are used as shunt in platelets in case of chronic canine arteries-venous [9]. PVA can also be used in hydrogels for the inhibiting clotting of blood contacting by reduction in adsorption of platelets and amount of proteins. In artificial kidneys, PVA hydrogels were prepared with heparin and used as arterivenous shunts (AV) in bodies for coating on implants for blood contacting[10].

1.3.1 Hydrogels

Hydrogels are cross-linked linear polymers that have three dimensional system[11]. Due to their high water sorption content they can absorb moisture from wounds and have great applications in tissue engineering, bandaging , drug delivery , nosology , clinical medicine, revival of skin tissues and cellular restringing[11, 12]. Greater advantages of hydrogels are non cyto toxicity, flabbiness and non-adhesion. For treatment of wounds and to recuperate burns they have astonishing properties like, they can take up noxious and untangle other germs, conserves little wetness on the injury [13].

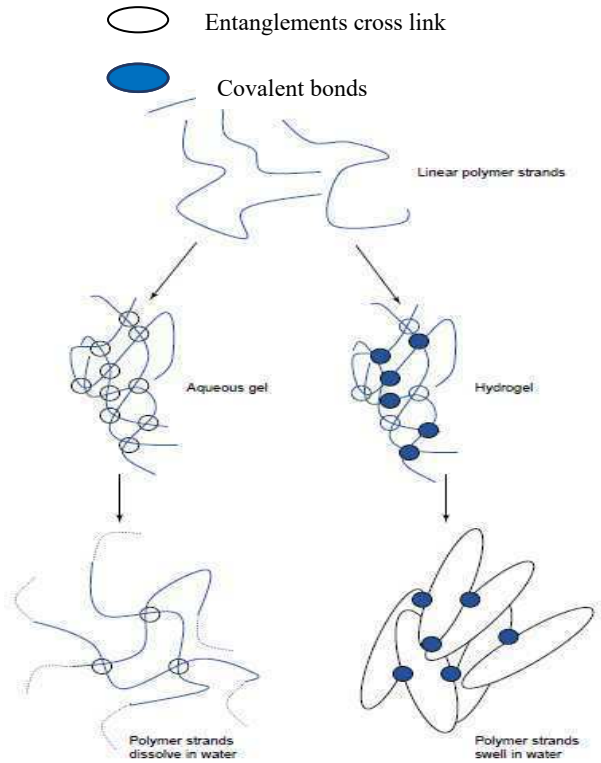


Figure 2. Formation of Hydrogel[14]

Hydrogel and gel both are physically different but chemically same. Hydrogels are basically hydrophilic and have capacity to take-up more amount of water while maintaining its 3-dimensional structure as compared to simple gel, which are stiff and unable to keep their structure intact under stress as shown in Figure 2. Hydrogel is more cross linked as compared to gel. Gel is semi solid and dissolves in water while the hydrogel is a substance that's undergoes swelling in water.

1.3.1.1 Distribution of hydrogels

Distribution of Hydrogels is categorized into,

Source

Natural (proteins, gelatin or collagen) or synthetic (PVA, polymers using chemical methods to form) as shown in Figure 3.

1.3.1.2 Polymeric composition

Polymeric composition categorized into:

Homo-polymeric Hydrogels:

Depends on the technique for polymerization or how the single component monomer joins to form cross linked network.

Co-polymeric Hydrogels

It has two hydrophilic substances combines in rare formation to form polymer structure e.g. alternating, random or block.

Multi-polymer Hydrogels

It's formed by two or more synthetic or natural substances to form scheme of cross linked polymers or one of them is not a cross linking polymer.

1.3.1.3 Configuration

Physical natural of hydrogel can be crystalline, amorphous or semi crystalline, followed by the chemical (permanent) or physical (transient) crosslink joint. These configurations arise due to intermolecular interactions such as ion-ion, hydrogen water bonds, and entanglements[15].

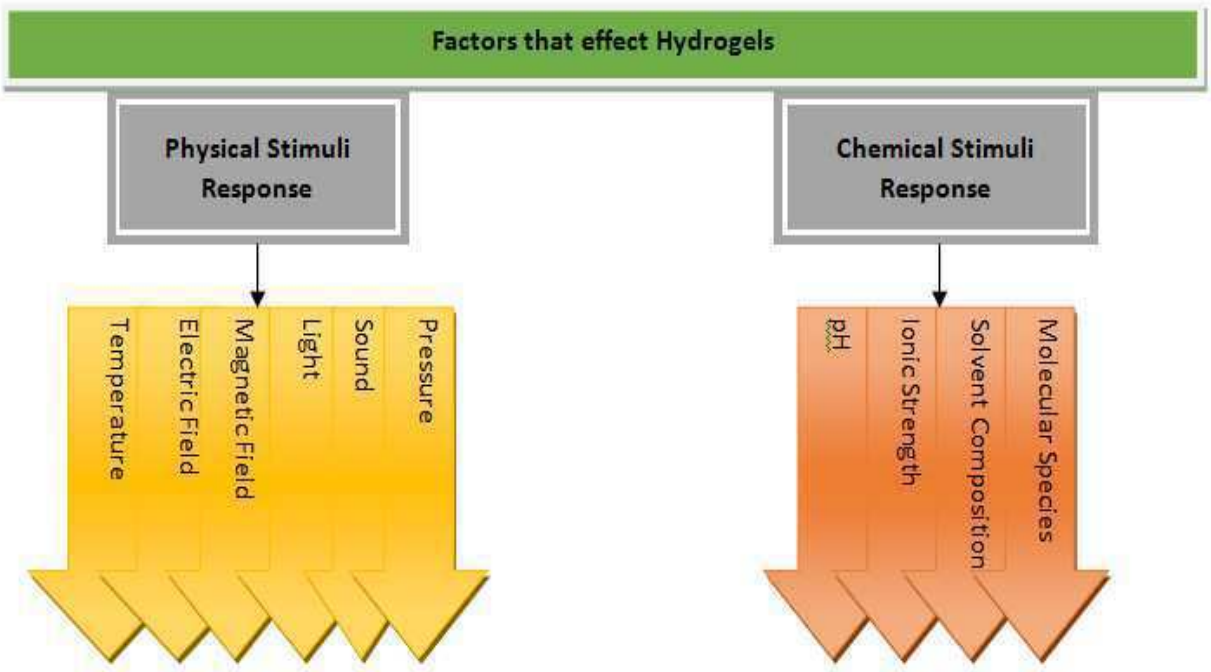


Figure 2. Factors that affect Hydrogels

1.4 Applications

Following are the applications of PVA

- Wound dressing
- Artificial skin[16]
- Contact lens[17]
- Artificial pancreas[18]
- Drug Delivery
- Lacquers
- Resins
- Food packaging[19]
- Paper coating
- Textile sizing[20]

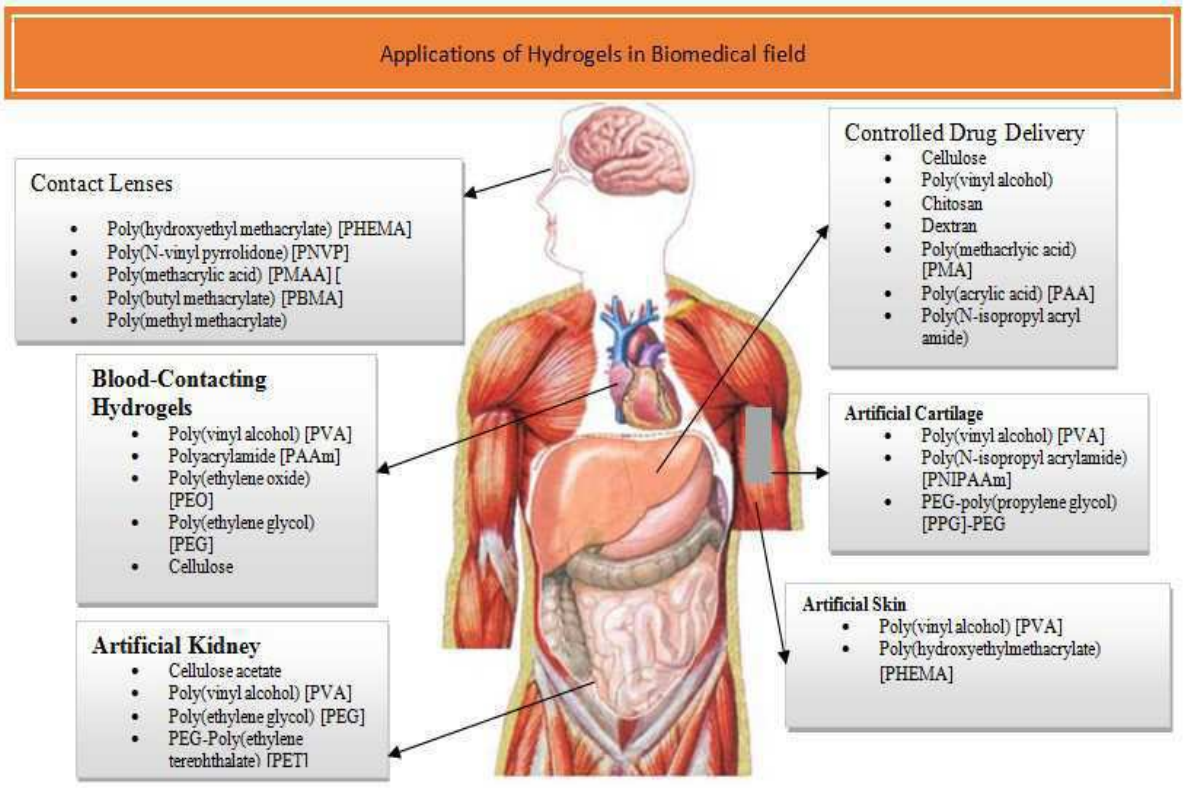


Figure 3. Applications of Hydrogel[10]

1.5 Motivation behind research

Polyvinyl alcohol is a biodegradable polymer and starch is an abundant substance. They have been utilized as a substrate and filler respectively. Glutaraldehyde has been added as cross linking agent and PVA structure modifier. In this research natural antimicrobial agents has have been utilized instead of Ag NPs i.e. very famous antimicrobial agent. We have tried to overcome the issue related to the seepage of Ag NPs via skin pores having 50µ diameter[21] because Ag NPs may affects the healing process. The prepared wound dressing would have less toxicity and more reliability.

Chapter 2: Literature Review

This chapter includes the literature related to the research done by using polyvinyl alcohol for biomedical applications, the composites prepared with PVA and the agents added up into it till now.

2.1 Polyvinyl Alcohol with Sodium Alginate hydrogel

Fabricated by free radical polymerization formed the hydrogel composite in presence of monomer of acryl amide with silver nano particles on PVA substrate and sodium alginate (Na-Alg). In this the cross linking agent was methylene bis acrylamide (MBA). Silver nano particles were synthesized through green route in which sodium borohyride is the reducing agent. According to this study the amount of Na-alg increased, the silver nps size also increased. The density of the hydrogel increased, but while increasing the cross linking amount the silver loading decreases. Its analysis showed the stability of Ag NPs added. TEM explains the morphology and presence of uniform Ag NPs over the substrate[22].

2.2 PVA with natural fillers

PVA was blended with chitosan, starch and gelatin as natural fillers by bath immersion coagulation method after freeze-thawing technique. For cell attachment purposes as its hydrophilicity hinders the capacity of PVA. There physical properties are measured against protein solution and cell proliferation which were higher in gelatin/PVA hydrogel[23].

2.3 PVA PMMA blends

By Free radical polymerization the Networks of PVA and PMMA poly (N, N-dimethyl acrylamide) hydrogels were prepared. Silver NPs were assembled on the hydrogel where the Ag+ may form complex compound via forming weak bonds with oxygen and nitrogen atom. Studies showed, the spherical structure of Ag NPs with average size between 10 to 20 nm[24].

2.4 PVA Chitosan Glycerol blends

By freeze thawing method, the composite of PVA with chitosan and glycerol was prepared for wound dressing. The wounds were healed after 11 days which showed the great epidermal layer was grown and prove that its resistant toxicity up to L929 mouse fibroblasts.

In the comparison between the gauze and hydrogels healing capability, the composite showed good wound dressing[25].

2.5 Effect of Plasticizer

In thermoplastic PVA/ starch blends, glycerol and urea mixture was used as a plasticizer. They formed strong and stable bonds with water in hydrogels as compared to use a single plasticizer. SEM confirmed the continuous phase morphology of PVA/starch. The mechanical properties of glycerol and urea (20wt % and 10wt %) mixture gave rheological properties gave better as compared to single plasticizer[26].

2.6 PVA with PVP (poly vinyl pyrrolidone)

Silver NPs have been employed as antibacterial agent but they have potential to seep into the skin causing irritation; The procedure followed was Franz diffusion cell method. 70g/cm2 Ag NPs were dispersed and coated with PVP poly (vinyl pyrrolidone) on the skin for 24 hours. Comparison was made by silver concentration absorption on damaged skin and on intact skin. The Ag NPs absorbed were very low but somehow damaged skin showed more permeation[27].

2.7 Composite hydrogels

Hydrogels with silver nanoparticles have been utilized for wound dressing. Ag NPs in hydrogels. Ag NPs in 2-acrylamido-2-methylpropane sulfonic acid sodium salt hydrogels. The Ag NPs were prepared by ultraviolet radiation and its concentration into the hydrogel matrix showed indirect cytotoxicity test, which was negative for all cell lines. The silver released from the hydrogel in 72 hrs was 70-81%. This sulfonic sodium salt hydrogels (5mM) prepared was tested against gram-positive S. aureus and MRSA as well as gram-negative P. aeruginosa showed great antibacterial activity The physical properties including the water vapor transmission rate , swelling and gel fraction were measured. The WVTR showed the ranges from 94.0 to 107.0g makes the hydrogel more suitable for human skin due to control of less drawing of body fluid loss and provides the moist environment[13].

2.8 Chitosan with alginate

Another wound dressing of chitosan/chitin with fucoidan and alignate (4:2:20:40) complex hydrogel have been prepared. Dressing was tested on rats which showed the wound treated in 7 days after applying mitomycin solution in saline for 10 minutes after the composite dressing was placed and compared to simple calcium alginate fiber[28].

2.9 PVA-HES blend

PVA has great importance because of its wide range of biomedical applications. Another composite with PVA–HES (hydroxyl ethyl starch) was prepared in different ratios in ampicillin via freeze thawing method and by physiochemical properties such as gel fraction, elongation, protein absorption, morphology and swelling were exmined. In increased concentration of HES the gel fraction and elongation were decreased. This composite is biodegradable because it showed degradation in phosphate buffer saline when it was dipped for two days. As the addition of HES into the PVA structure influenced the mechanical and thermal properties as they showed more values in swelling behavior and more elastic. But its introduction lowers the overall thermal stability. Incorporation of ampicillin makes it more suitable for wound dressing because it controls its release[29].

2.10 PVA-CM chitosan blend

Another composite including carboxy methylated chitosan in PVA prepared at room temperature by electron beam irradiation. The best blend was 3wt% of CM-chitosan against *E coli.* Both the CM chitosan and PVA are cross linked under irradiation swelling behavior was investigated in which the swelling decreased but increased in case of addition of CM-chitosan. While the gel fraction decreased with the degradation of gels and due to increase of CM chitosan content[30].

2.11 PVA-PVP

The properties of synthesized PVA/PVP were tailored for biomedical application by varying the amounts and gave repeated freeze thawing cycles. With 10% concentration of PVP it showed changes in structure and mean pore size of the hydrogel but with further addition of PVA, the pore size increases in PVP hydrogel but its crystalline nature decreases. During the FT cycles the polymer mass decreases up to 5% with increase in FT cycles and manages

to improves its cross linking ability of hydrogels and its deformation also increases with increase in quantity of PVP[31].

2.12 PVA/PVP with Silver Nano particles

Silver NPs were fabricated in PVA/ PVP substrate and the hydrogel was prepared by freeze thawing method. The average particle size range of Ag NPs was 20 to 100 nm and have composition up to 0.1 to 1.0wt% in the hydrogel membrane. Three dimensional and mono dispersity of Ag NPs have been confirmed via SEM. Hydrogels membrane was tested against *Escherichia Coli* and *Staphylococcus aureus* to check its antibacterial activity[32].

2.13 PVA/PVP-HA

The composite PVA/PVP and hydroxyl apatite were prepared by freeze thawing technique and comparison between the simple PVA/PVP and PVA/PVP/HA has been done. Denser structure and high rate of dehydration is more prominent in PVA/PVP/HA composite hydrogel. Incorporation of HA into the PVA/PVA hydrogel induces high diffusion activation energy and the molecular attachment is enhanced because HA has crystalline structure and has been confirmed via XRD pattern. As HA was filled the voids in PVA/PVP it reduces the water content in hydrogel. If stress is applied on PVA/PVP/HA it has more stability and strength as compare to plain hydrogel[33].

2.14 PVA/ Chitosan-Dextran

PVA was incorporated by different amounts of chitosan and dextran to check their effect on hydrogel properties by freeze thawing method that leads to formation of cross linking sites with large capacity to swell. Presence of dextran showed the crystallization process in PVA polymer and is responsible for uniform structure while chitosan exhibits less uniform structure[34].

2.15 Antibacterial Agents

Antibacterial activity of Cinnamonum verum has been checked against *Escherichia coli* and *staphylococcus aureus* bacteria. Cinnamon showed excellent inhibition and it gave 500g/ml minimum inhibition concentrations (MIC) against the seven other strains. Main components of cinnamon are cinnamaldehyde and eugenol. Cinnamaldehyde ratio is 50-70%.

Cinnamaldehyde has hydroxyl group with benzene ring[35]. Cinnamon shows higher antibacterial activity[36].

Another antibacterial agent studied was black pepper (piper nigrum L) along with coriander and aniseed against 176 bacterial strains. In this study, piper nigrum showed the highest antibacterial activity around 75% as compared to aniseed and coriander. Piperin compound found in black pepper because it gets absorbed readily through the intestine[37].

2.16 Fungus Strains

Genus named as Aspergillus which is from Ascomycota division and Eurotiomycetes class, consists of hundreds of species in different climates all over the world. In 1729 Aspergillus family was first catalogued by Pier Antonio Micheli which stated that the Aspergillus have asexual spore forming structure, which is common in its all species.

Aspergillus Oryzae has been used in Chinese and other fermented sauces such as soya sauce , vinegars and in making alcoholic beverages[38] .

2.17 PVA-glutaraldehyde Cross linker

Glutaraldehyde has been added as a cross linker in PVA hydrogel with varying concentrations and was check by FTIR (Fourier Transform Infrared radiation) analysis. Investigations were made by the FTIR and SAXS (synchrotron small angle X-ray scattering technique. By the reaction of GA and OH present in hydrogels represent the acetal bridges formed. Crystalline nature of the PVA hydrogel shows vibration band at 1141/cm. SAXS shows its semi crystalline structure with cross linker[39]. Addition of GA into the PVA hydrogels modifies the structure by formation of acetal bridges and covalent bonds thus reducing the flexibility[40].

Chapter 3: Materials and Methods

3.1 Materials

3.1.1 Poly Vinyl Alcohol (PVA)

PVA emerged as artificially synthesized polymer worldwide in the 20thcentury [41].PVA is hydrophilic, biodegradable, water soluble none ionic poly hydroxyl synthetic polymer. Because of its non cyto toxicity and biomedical application it is extensively use. It has great bio compatibility[42].It's not soluble in organic solvents

To prepare PVA, polyvinyl acetate monomer is polymerized with some alcohol in presence of an alkaline catalyst normally sodium hydroxide the acetate groups formed are removed without causing any disruption in the polymer chain.

PVA is categorized as thermoplastic polymer and due to the presence of hydroxyl functional groups on the carbon atoms its degradability is increased. PVA decomposes biologically are form the petroleum based synthetic materials that degrades naturally under the landfill and composting conditions[41].

PVA is an atactic material and also used as a sizing agent and also added in adhesives and emulsifiers because it's the starting material and gives the desired strength to the compound. Applications include medical surgical threads, lacquers and food packaging materials.

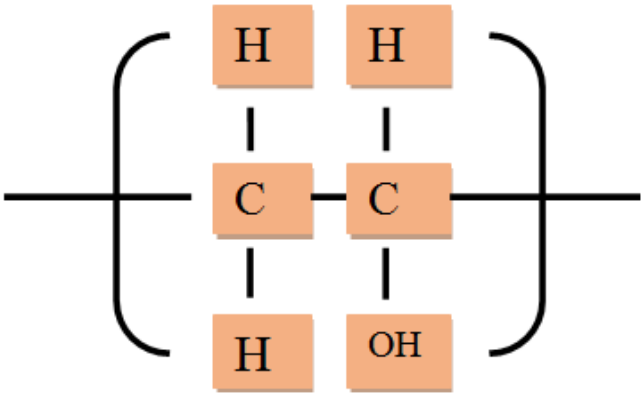


Figure 4. PVA structure

3.1.2 Starch

Starch is natural abundant occurring polysaccharides and its very much biodegradable and non-toxic[43].

In this glucose units are joined by the glycosidic bonds to form the large number of the polymeric carbohydrate. Mainly these are two polymer which makes starch as biopolymer as the amylopectin have chains up to 30 glucose molecules regarded as highly branched polymer attached to the 20-30 units of glucose . Plants store energy as the form of polysaccharide. Widely present in rice, maize, wheat and potatoes[44].

From plant source it contains mostly70% of amyl pectin, 30% amylase and less proteins and lipids. In different quantities of starch prepared by nature or purified amylopectin and amylase the resulting physical gels and dispersion are different and have great effect on the properties during the physical gelatin. Crystallization, phase separation and aggregation[45].

Starch is renewable and cheap but it lacks water resistibility, strength and thermal stability. So it has been blended with PLA poly lactic acid and poly vinyl alcohol .Starch forms biodegradable or edible films[44].

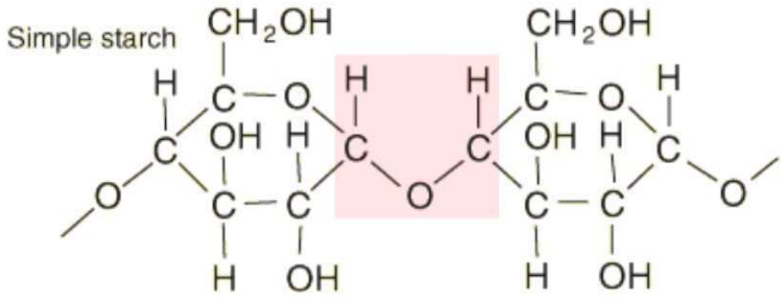


Figure 5. Starch structure

3.1.3 Glutaraldehyde

Glutaraldehyde is oily organic compound. It's basically an aldehyde and undergoes cross linking with proteins. It gives oligomers on partially polymerizes. It has pungent smell. and for health care its 0.1 to 0.5 ppm Concentrations are safer to use.

Glutaraldehyde as a cross linking agent provides the fixation of cellular network and the un-solubilization of proteins. Due to its cross linking with the proteins it is used as to crosslink the carboxypeptidase crystals and for enzymatic activity[46].

Wide applications include hardener in X ray film processing, cleaning agent, water treatment, tanning, agriculture and cosmetics.

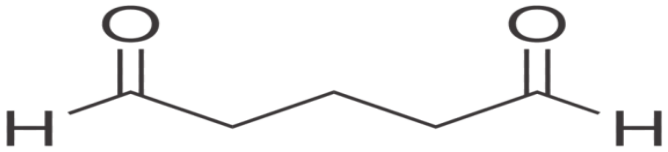


Figure 6. Glutaraldehyde Structure

3.1.4 Black Pepper:

The world most traded spice. Peperine ([1-[5-[1,3-benzodioxol-5-yl]-1-oxo-2, 4, pentadienyl piperidine), is an alkaloid present that is responsible for causing antibacterial activity used as an insecticide and in medical applications. It has increases the biological availability of different modified drugs. Piperine may form non polar complexes with drugs and absorbed very rapidly through the intestinal barrier and increases the permeability between the barrier[37].

Black pepper has been used to treat obesity, fever, colic, diarrhea, asthma and chronic indigestion.

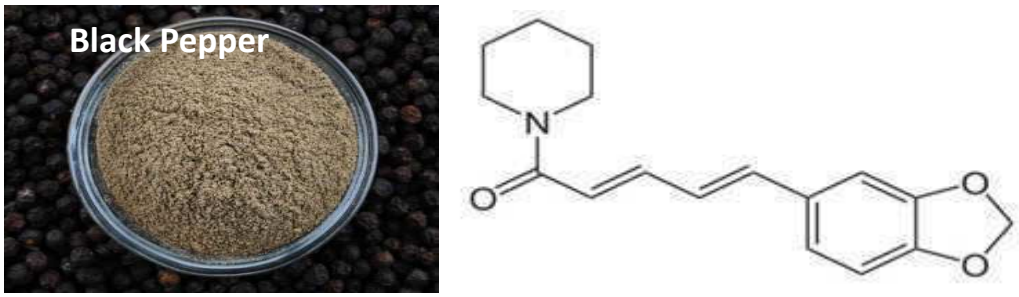


Figure 7. Black Pepper (Peperine Structure)

3.1.5 Cinnamon

Mid brown colored spice referred to as cinnamon obtained from the several trees .i.e. from the inner bark of *Cinnamomum*. Cinnamon refers to its many derivatives and all of them have antibacterial activity. Cinnamaldehyde is an organic component responsible for antibacterial activity with chemical formula $C_6H_5CH=CHCHO$. Cinnamaldehyde and coumarin are the main constituents present in inside the bark. It contains up to 50% cinnamaldehyde[35]. Other natural plants as used antibacterial agents are coriander, aniseed, thyme and clove[36, 47].

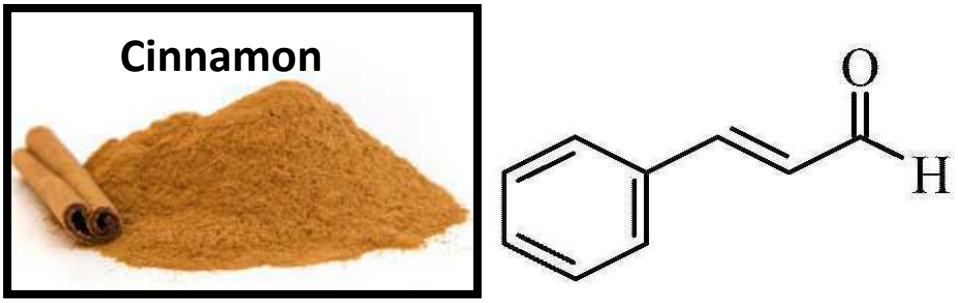


Figure 8. Cinnamaldehyde structure

3.1.6 Glycerin

Glycerin is used as a plasticizer. In polymer industry, plasticizers are non-volatile and low molecular weight substances. Their main function is to increase the process ability of polymers and enhances the flexibility by lowering the glass transition temperature (Tg). It also helps to lessen the tension of deformity, density, viscosity and electric charge of polymer. It play the role to increase its resistance to fracture[48].

3.1.7 Bacterial Strains:

Escherichia Coli (E. coli):

Escherichia is anaerobic gram negative bacteria, which do not retain violet color. They have very thin wavy like peptidoglycan cell-wall (Figure 10) and has outer membrane present around it. It is normally present in the digestive tracts of human body or animals. They have more activity towards the antibiotic materials. *E. coli* has many strains some of them are quite dangerous and can cause food borne diseases .i.e. diarrhea, urinary tract infections and

kidney failure. *E. coli* can be spread by unwashed hands, uncooked food, feaces, contaminated water and also from improperly boiled raw milk[49, 50].

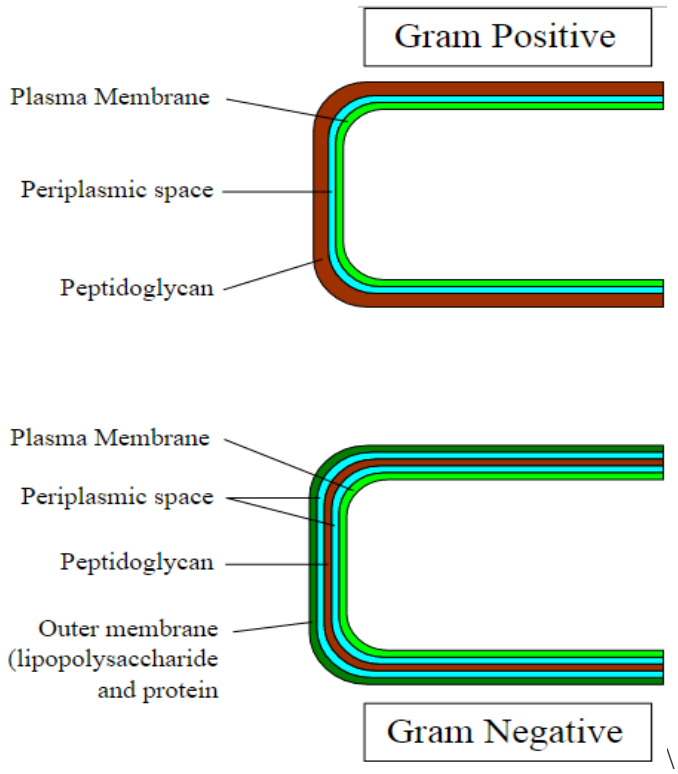


Figure 9. Cell wall differences between gram positive and gram negative bacteria

Methicillin-resistant Staphylococcus aureus (MRSA)

MRSA is a gram positive bacterium which retains purple color. It is composed of multi layered thick peptidoglycan cell wall and lacks of outer membrane. It is less resistant to antibiotics and can be inhibited by dyes. It is quite difficult for doctors to treat it and known as 'super bug'. It is present at hospitals, and people normally carry it but do not get affected and also present in human noses. MRSA is resistant to penicillin, methicillin after the outbreak of MRSA diseases in 1961[51-53].

3.2 Methods

Following were the methods used for hydrogel preparation and characterization during this research work:

3.2.1 Preparation of hydrogel membrane

Polymeric hydrogel membranes were prepared using the method applied by Kunal Pal et al (2006)[54] with more refinement. 5% w/v aqueous solution of PVA was prepared and heated for 2 hours at constant stirring at 70°C until the appearance of transparent solution. Separate solution of 3.5%w/v starch was prepared in 25ml distilled with constant stirring for 20 minutes at 100°C. Different concentrations of cinnamon and black pepper were added into the starch solution i.e. 0gm, 0.1gm, 0.3gm, 0.5gm, 0.7gm, 1gm, 1.5gm. Both solutions were mixed. Again separately cross linking solution was prepared by taking 5ml of ethanol with 0.05 ml of glutaraldehyde and 0.025ml of HCL and added into the hydrogel mixture. After some passage of time 1ml of glycerin[48] was added as a plasticizer. Hydrogel solution was sonicated by probe sonicator for 2 hours to make it bubble free and, homogenous and care should be taken that the sonication water temperature do not exceeds. By using solution casting method the mixture was poured into the plastic petri dishes and let it dry overnight. The dried hydrogel membranes were removed from the dishes and secured in air tight plastic pouches, the same procedure was followed for the preparation of hydrogel membrane without cinnamon and black pepper.

3.2.2 Antibacterial Activity

Antibacterial activity was evaluated by disk diffusion method and Agar well disk diffusion method. This method was developed in 1940. This method was selected on basis of its low cost, and the simplicity to evaluate results and also provides ability to test more microbes[55].

Preparation of Culture Medium

Nutrient Agar (OXOID Ltd, England) was prepared by dissolving 28g of agar in 1L of distilled water. It was stirred for a while and autoclaved at 121°C for 2 hours to sterilize in order to avoid contamination. Under the laminar flow cabinet before solidification agar was poured into the autoclaved petri plates. Plates were covered with their lids, sealedand placed into incubator at 37°C for 18-24 hours in order to check contamination.

Preparation of LB broth

LB broth (Merck Damstadt Germany) for bacterial culture was prepared by taking 25g of broth powder in 1L of distilled water. Swirl to mix and autoclaved at 121°C for 2 hours.

Saline solution

It was prepared by making 0.9% solution of NaCl (aq) and autoclaved.

Growth of bacterial cultures

In two separate test tubes, 10 ml of broth solution was added and 1ml of DH5-α (*E. coli* strain) was added in one test tube and MRSA in another test tube. Covered by aluminum foil and placed in incubator at 37 °C with continuous shaking for 24 hours. After 24 hours the solutions became cloudy and they were diluted with saline solution.

Sample preparation

Antibacterial activity was checked by disc diffusion method and Agar disc well diffusion method[55]. Membrane was punctured by sterile puncture machine to make 6mm discs.

Test Plates preparation

Incubated agar plates were placed into the Laminar flow Cabinet. Bacterial solution 80µL was spread over the agar plate. The agar medium was inoculated by using a sterilized tip or by forceps to create a hole of 7or 8mm on the agar surface. Disks were placed into the wells. Plates were covered by their lids. Sealed them in plastic wrap properly and were placed in incubator at 37° C for 24 hours[56]. After 24 hours results were collected by measuring the inhibition zone diameter with a scale and by taking an average of the readings.

3.2.3 Antifungal Activity

Materials and methods for antifungal activity are mentioned in the following section.

Potato Dextrose Agar (PDA)

In order to check antifungal activity the PDA agar was used, 9.25g was dissolved in water and autoclaved at 121°C for 2 hours along with petri dishes to avoid contamination. The PDA solution was poured on the Petri dishes under the laminar flow cabinet and wait until the agar got solidified. After the solidification, plates were covered in plastic wrap and put them in incubator for 24 hours at 37°C to observe any contamination. If the plates remained un-contaminated then they were used for further experimentation.

Test Plates Preparation

Under the laminar flow cabinet, 30 µL of Fungi glycerol stocks streaked on the plates with the help of the glass spreader. The membrane 6.mm disc was prepared with help of a punch machine were placed on the plate with help of tweezers. Plates were wrapped in polythene film and were placed in an incubator at 28.5°C for 3 days.

3.2.4 Water vapor transmission rate

It is the measure of hydrogel moisture permeability. The sample contained 10ml De-ionized water with 29.5 mm bottle diameter. The hydrogel is co-fixed on the mouth of the bottle sealed with help of para-film tape. It was placed in an oven at 40°C for 24 hours after 24 hours. The bottles were taken out and the water vapor transmission rate was calculated by using this formula[13].

WVTR: $\frac{w_i - w_t}{A \times 24} \times 10^6\, gm^{-2}h^{-1}$

Where, A is the area of the round bottle (mm),

W_i and W_t are the weight of bottle before being placed in the oven and after being removed from the oven, respectively.

3.2.5 Moisture Retention capability measurement:

For the measurement of hydrogel moisture permeability, hydrogel membrane was sliced into the equal pieces and weighted at room temperature. They were placed in oven at 40°C for 5 hours and they were taken out of the oven and weighed again. Moisture retention was calculated by using following formula.

$$Rh(\%) = \frac{w_t}{w_i} \times 100\%$$

W_i is the initial weight and W_t is the final weight after taking out from the oven.

3.2.6 Swelling Behavior measurement:

It is the measure of the hydrogel to absorb, extrude and fluids coming out of the wound. Charge concentration, ionization degree, hydrophilicity and cross linking density influence the properties of the swelling hydrogel[24] swelling behavior was investigated against simulated body fluid (SBF). Hydrogel membrane was equally squared and placed in the oven to obtained constant weight. Then the samples were immersed in SBF solution for 24 hours. The membranes were taken out and the excess water on the film was absorbed by the filter paper and again weighed[13]. The swelling ratio was determined by the following equation,

$$\text{Swelling Ratio} = \frac{w_s - w_d}{w_d} \times 100\%$$

W_d is the constant weight while the W_s is the weight of the swollen hydrogel.

Simulated body fluid (SBF):

SBF (simulated body fluid) solution have the ion concentration nearly same as of the human blood plasma. It was kept under moderate conditions to attain neutral pH. It contained calcium phosphates ions forming a meta-stable solution.

Preparation:

- Laboratory beaker and plastic bottles were repeatedly wash with dilute hydrochloric acid, ion exchange water and sterilizing agents and then dried in oven. Immersed in 1M HCL for several hours and washed with ultra pure water and finally dried in oven.
- Taking 750mL of De-ionized water in 1000mL beaker. Temperature was maintained up to 36.5^{o}C with constant stirring in water bath. Chemicals were added to above beaker in the list one by one after of the former reagent completely dissolved.

Table 1. Chemicals for SBF

Sr.No	Chemicals	Quantity(gm)
1	NaCL	7.996
2	$NaHCO_3$	0.350
3	KCL	0224
4	$K_2HPO_4.3H_2O$	0.228
5	$MgCL_2.H_2O$	0.305
6	$CaCL_2$	0.278
7	Na_2SO_4	0.071
8	$(CH_2OH)_3CNH_2$	6.057

- The pH of the solution was checked after addition of the chemicals. The pH was evaluated at the temperature 36.5ºC. If the pH was towards basic 1M HCL solution was added drop wise and until it maintained at pH at 7.5. The total volume of 1000mL was adjusted by addition of De-ionized water.
- Store the SBF prepared solution was stored at 5-10º C in polyethene bottle.

3.2.7 Gel Fraction

To determine gel fraction the hydrogel was slit in the equal pieces, weighted and kept in vacuum oven at 40°C to maintain constant weight. Then the weighed hydrogels were then immersed in water for 4 days at room temperature. Gel fraction was measured by the following formula,

Gel fraction (%) = $\frac{w_e}{w_o} \times 100\%$

W_e is the weight of the hydrogel after the extraction and W_o is the constant weight.

3.2.8 Tensile Strength

Tensile strength is resistance of the material to fracture during applied tension. Strength has prime very importance for hydrogel membrane to be used as wound membrane. Ductility, stiffness and toughness are the main keys for a material to be having best strength. Hydrogel membrane is tested on UTM (universal tensile machine) at constant strain rate 10N/m2.

Sample Preparation:

Tensile machine specimen was prepared by taking 20mm width of the membrane measured by vernier caliper like a dumb-bell shaped sample. Sample width was 0.136mm. Sample selected should not have any surface defect or notches at the edges. Samples were prepared according to ASTM D 638 – 14 standards. The samples were loaded into the clamps of the machine which holds the membrane edges smoothly machine was turned on by putting all the values into needed for tensile test[57]. The two clamps in opposite directions starts moving at constant rate with constant speed. The strain was measured till the membrane fractures.

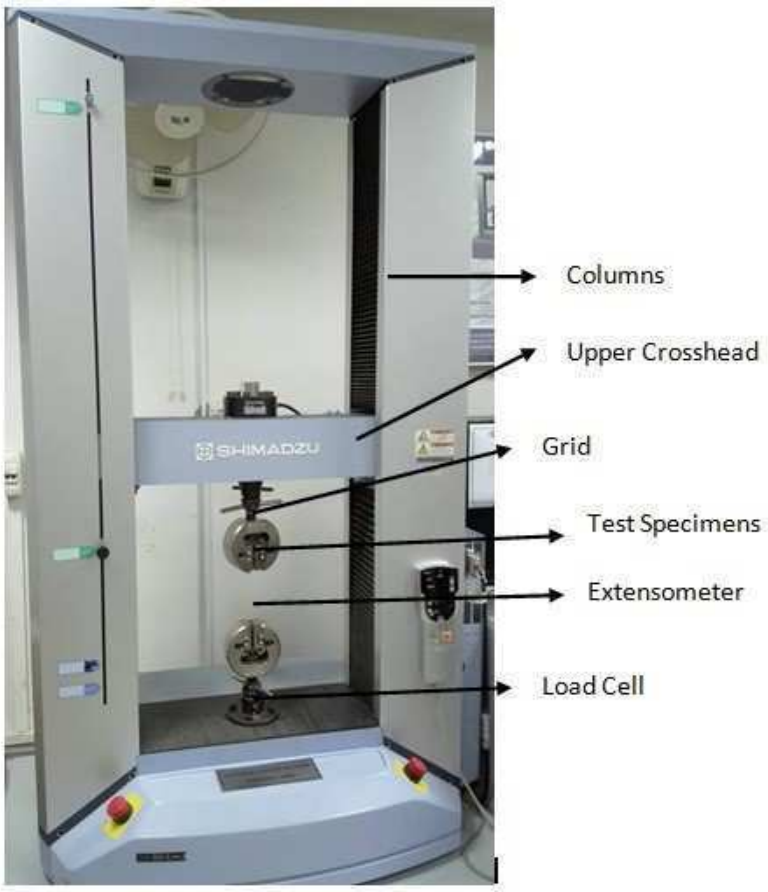


Figure 10. Universal Testing machine

3.2.9 Fourier Transform Infrared:

Technique used to get an infrared spectrum of gas, liquid or solid of absorption or emission. It provides the data about the functional groups by stretching, twisting, bending or vibrations .The basic principle it works upon the interactions of electromagnetic radiation interaction with compounds that have induced dipole and vibration states excitation. The molecule present in a compound should be IR active that have dipole moment changes during vibrations. As the IR inactive molecules are H2, O2, N2 .FT-IR (Perkin Elmer, spectrum 100) was available to evaluate the polymeric hydrogel membrane and it works in the range of 450 /cm to 4000 /cm .The hydrogel membrane was directly placed into the FTIR machine. The spectrum formed on the computer under 10 minutes. All analysis was taken place at room temperature[40].

3.2.10 Scanning electron microscopy:

It's an analytical tool that provides information about the morphology and topography of the prepared hydrogel. It has resolution up to 4nm and up to 10-15000 magnification. It has basic principle in which the tiny electron beam examined and scanned the whole surface on which the back scattered electrons are detected. The working and assembly is shown in the Figure 11. The hydrogel membranes were examined under JEOL (JSM-6490A). It provides information about the membrane whether it's dense or not. The solid hydrogel sample was coated by gold or carbon with help of sputter coater. The voltage of 5-20KV is used to examine the sample.

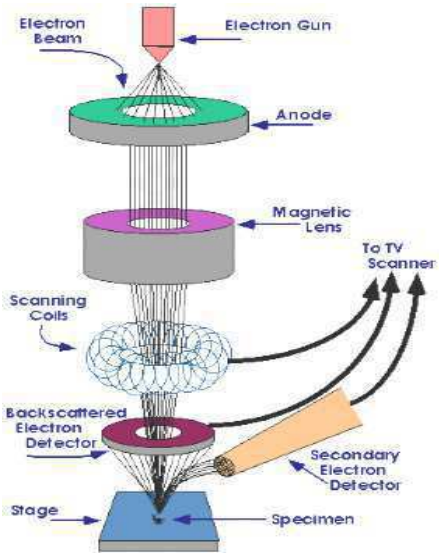


Figure 11. Assembly and working of SEM

Chapter 4 : Results and discussion

4.1 Antimicrobial activity

The hydrogel prepared in this research have been tasted against microbes like bacteria and fungi in order to utilize them for wound dressing.

4.1.1 Antibacterial Activity

For wound dressing application, the antibacterial activity is most important criteria to meet standards of application. The best concentration is selected on the basis of its cyto-toxicity and antibacterial activity. Hydrogels with different amount of cinnamon and black pepper prepared by adding 0, 0.1, 0.3, 0.5, 0.7, 1, 1.5gm all of them were tested against E. coli and MRSA. It can be observed from the figure 13, 15 shows that the neat hydrogel is inactive towards both types of bacteria which confirm that the antibacterial activity is due to the presence of natural antimicrobial agents. As the concentration of the natural antimicrobial agents increased the activity also increased maximum bactericidal activity was observed with 1.5g of black pepper and cinnamon against *E. coli* and MRSA but overall activity is less against *E. coli* because its gram negative bacteria which can be confirmed by the small inhibition zones in case of *E. coli* and MRSA in figure 13 and 15.

Escherichia Coli (DH5-Alpha)

The antibacterial activity of PVA hydrogel containing black pepper and cinnamon is as follows:

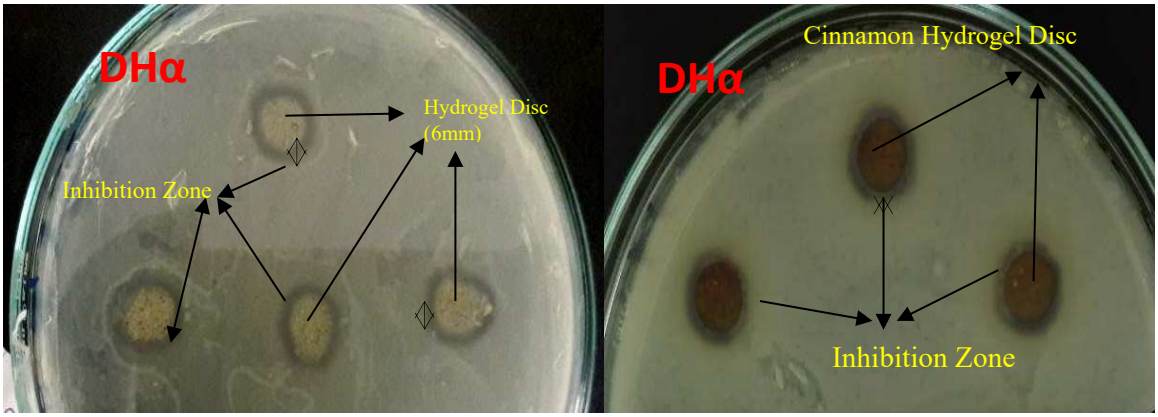


Figure 12. Antibacterial test plates for black pepper and cinnamon hydrogel membranes

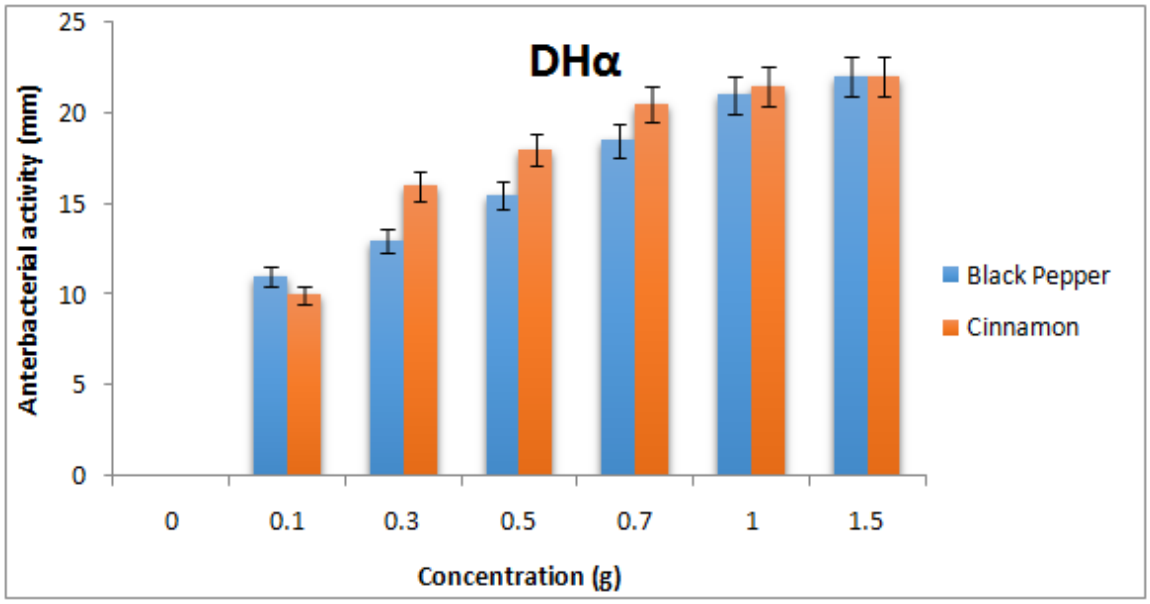


Figure 13. Graph for hydrogels against E. coli Bacteria

MRSA

The antibacterial activity of PVA hydrogel containing black pepper and cinnamon is as follows:

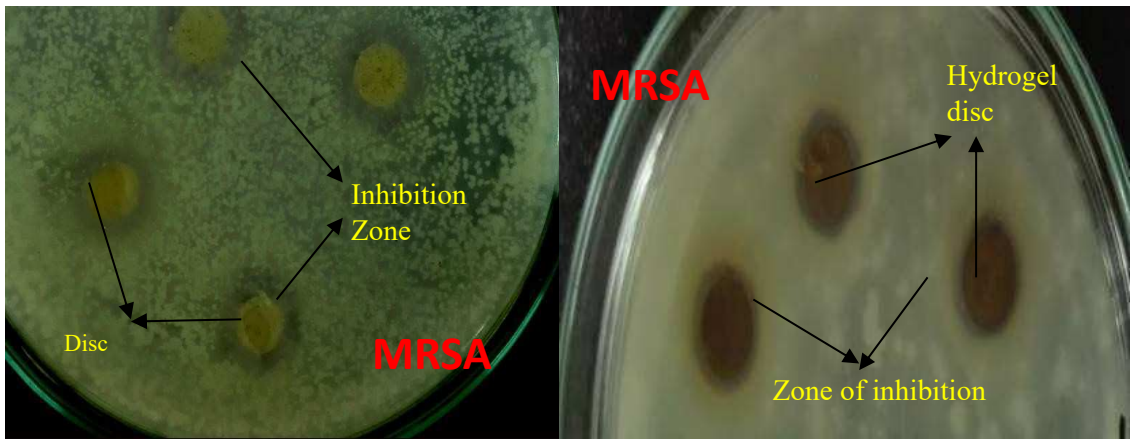


Figure 14. Antibacterial test plates for black pepper and cinnamon hydrogel membranes

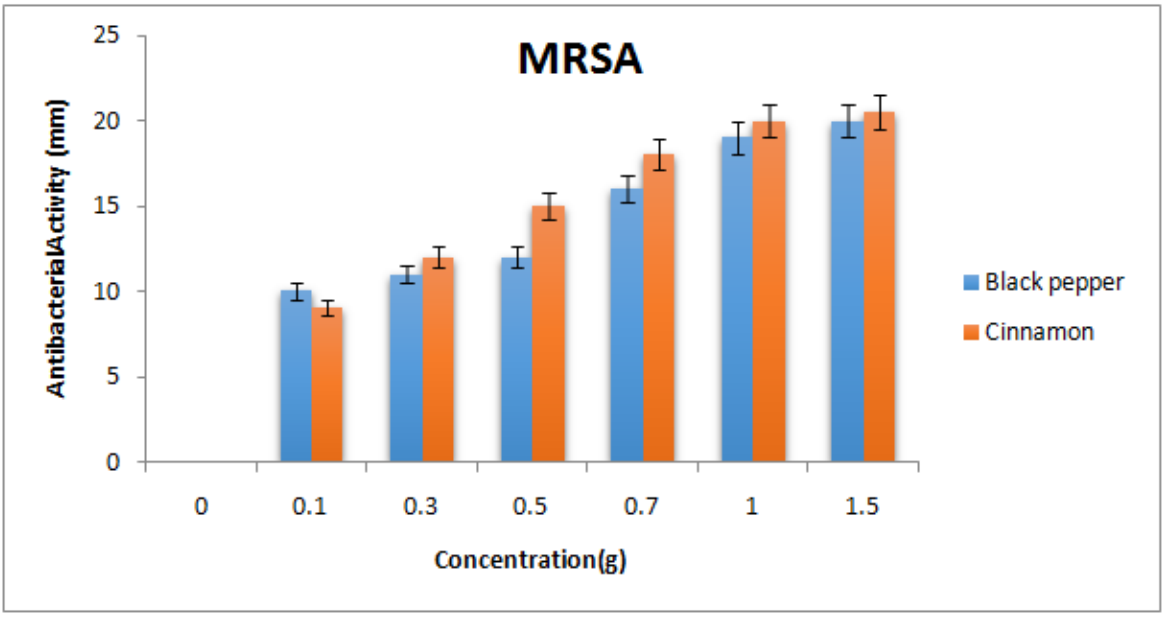


Figure 15. Graph for hydrogels against MRSA Bacteria

The highest value of inhibition zone was obtained .i.e. 20mm in MRSA and 1.9mm in *E. coli* in black pepper. Increase in concentration of cinnamon results in increase of antibacterial activity increases. The inhibition zones of the 1.5gm and 1gm are almost comparable. Similar trend was observed for hydrogel containing black pepper. So the incorporation of 1.5gm is the most suitable amount. As the gram negative is more resistant to the antibacterial activity so its inhibition zone is lesser than the gram positive.

Mechanism:

Furthermore, gram-positive bacteria are less resistant than gram-negative bacteria. The differences between the cell wall structures, gram-positive cell wall of bacteria consist of 90-95% of peptidoglycan where the other molecules such as proteins, are joined. Hydrophobic compounds can penetrate through the cell wall of the gram-positive bacteria and can act on the cell wall and deeper inside of the cytoplasm while the PVA hydrogel is hydrophilic the bacterial structures may disrupt and results in the degradation of the cell wall by the actions of the membranes. Different compounds present in the natural antibacterial agents have different effects on the structure of the bacteria. The gram negative cell wall is more complicated, the peptidoglycan layer is much thinner than the gram positive cell wall. The mechanism of antibacterial activity by black pepper and cinnamon depends upon the composition added. Both will have different mechanism in gram positive and gram negative

bacteria. The functional groups present on the compounds have different activity. Cimmaldehyde has the capability to alter the lipid profile of the cell membrane .the phenols and mono-terpenes present in the natural compounds exhibit antifungal and antibacterial activity. The active agents in black pepper and cinnamon cause the leakage in the cell wall which leads to the cellular cytoplasmic contents, the cells after some time starts to disintegrate and collapsed showing the activity of the agents against bacteria's. This activity shows the more effect on the gram-positive bacteria rather than gram-negative bacteria due to the major cell wall differences[58, 59].

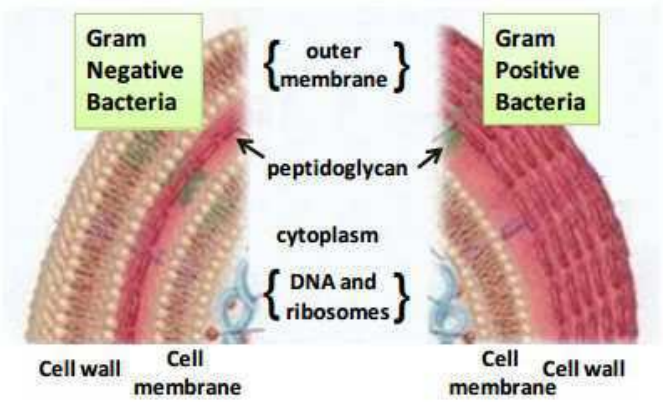


Figure 16. Gram-negative bacteria and Gram-positive bacteria structure [57]

*Further tests have been performed on 1.5g cinnamon and black pepper incorporated membrane.

4.1.2 Antifungal Activity:

Antifungal Activity was checked against three fungal strains from Aspergillus family, Aspergillus flavus, Aspergillus oryzae and Aspergillus subolivaceus. In which the aspergillus flavus was resistant to antifungal activity while the other two were sensitive.

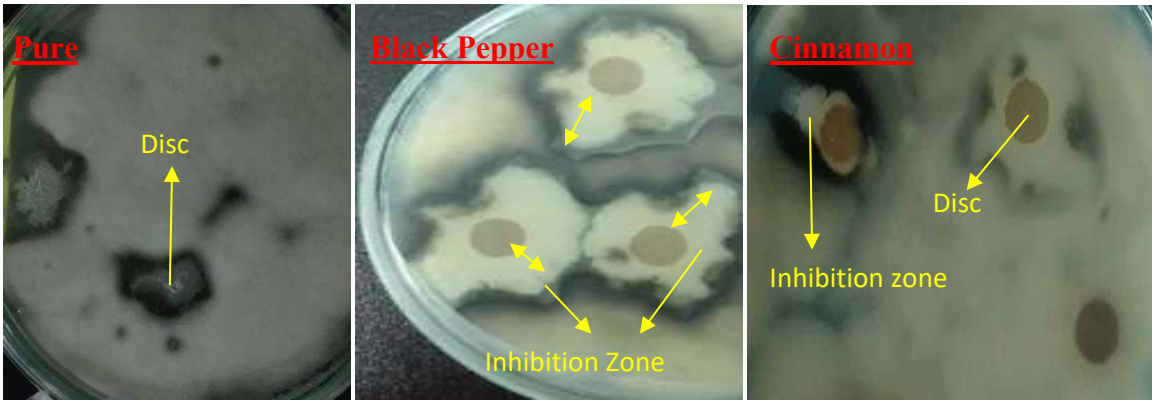


Figure 18. Antifungal test plates for hydrogel membrane (pure (1), black pepper (2) and cinnamon (3) against Aspergillus Subolivaceus fungus

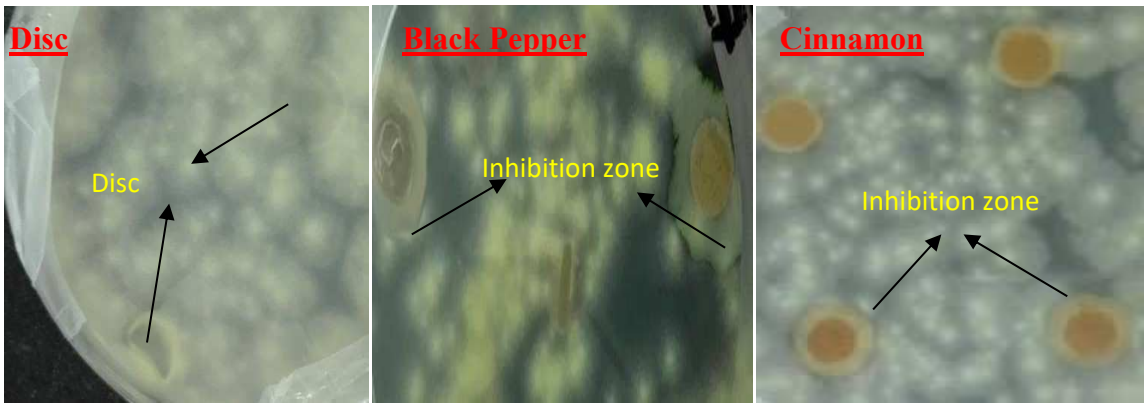


Figure 19. Antifungal test plates for hydrogel membrane (pure, black pepper and cinnamon) against Aspergillus Oryzae fungus

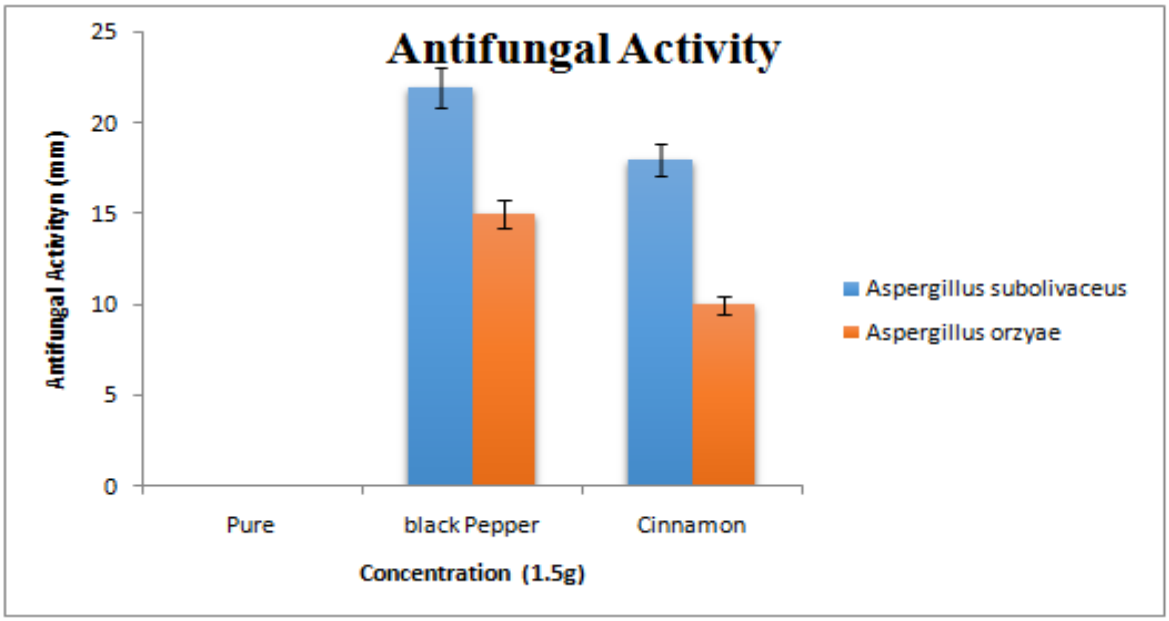


Figure 20. Graph for hydrogels against Fungus strains

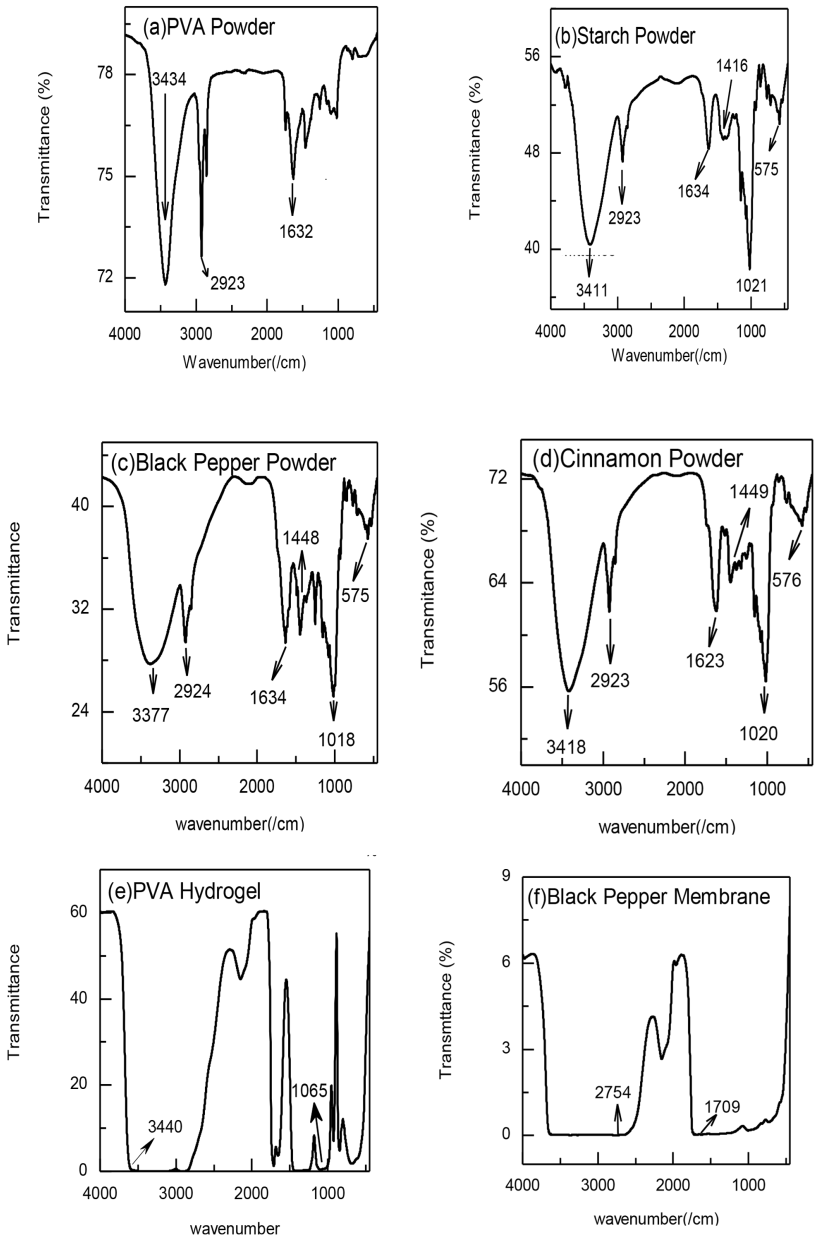
It can be observed from Figure 21, pure membrane didn't show any antifungal activity. While the hydrogels incorporated with black pepper and cinnamon have significant results. In case of Aspergillus subolivaeous fungi black pepper and cinnamon has great activity and shows inhibition zone up to 22mm and 17mm respectively. While Aspergillus orzyae shows 15mm inhibition zone in black pepper and 10mm in cinnamon. So black pepper incorporated hydrogels have greater antifungal activity.

4.2 Fourier transform infrared Spectroscopy

The dominant peaks in FTIR spectra of pure PVA powder shows the peaks are due to acetate groups and hydroxyl groups. OH stretching shows the intra-molecular and intermolecular hydrogen bonds in the region 3434-3000 cm^{-1}. The band at 2900-3000cm^{-1} refers to its stretching and the vibrational bands of C-H bond formed in Figure 18 (a). Starch, cinnamon and black pepper at 3411, 3377, 3418 cm^{-1} show the hydroxyl peaks stretching. Sp^3 hybridized carbon showed at 2923-2940 cm^{-1} in PVA, starch, black pepper and cinnamon. The 1620-1640cm^{-1} shows the C=O in powdered material such as PVA, starch cinnamon and black pepper. In starch 1020cm^{-1} peak corresponds to C-O and C-C bonds present. The bands at 3377cm^{-1} in black pepper correspond to N-H bonds in the range of 3500-2800cm^{-1} and 1400-600cm^{-1} in fingerprint region shows the C-N bond. In cinnamon 3418cm^{-1} represents the hydroxyl components O-H and C-H bonds and 1800-1600cm^{-1} shows the

presence of C=O, C=C stretching and in fingerprint region 1400-600cm^{-1} confirms the presence of C-O bond in Figure (b, c, d).

The neat hydrogel and hydrogel containing the natural antibacterial agents shows the presence of hydroxyl groups at 3440, 3213cm^{-1} for hydrogel moisture absorption. As shown in Figure 18 (e, f, g) a large dip of hydroxyl groups is shown in both of the FTIR graph of black pepper and cinnamon. The added glutaraldehyde for cross linking process have peaks at 1690cm^{-1} neat and 1721.19cm^{-1} natural antibacterial agents membrane showed the C=O of aldehyde group in glutaraldehyde .



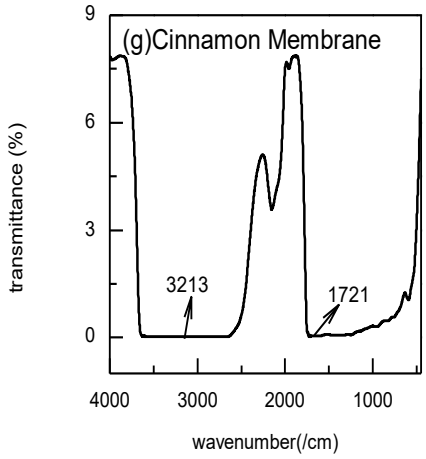


Figure 21. FTIR spectra for (a) PVA, (b) Starch, (c) Black pepper, (d) Cinnamon powders (e) Hydrogel membrane,(f) black pepper membrane and (g) Cinnamon membrane

4.3 Moisture retention capability of hydrogel membrane

The amount of the water given up to an atmosphere is the measure of moisture retention. More vapors evaporated from the hydrogel shows the more loss of moisture from the injury. For wound recovery it requires environment to recover rapidly absorbing extrudes coming out from the wound.

Table 2. Moisture Retention

Sr. No	**Membrane (PVA)**	**Rh (%)**
1	Pure	96.89 ± 0.64
2	1.5 BP	96.65 ± 0.71
3	1.5 CM	96.65 ± 0.62

These values show a very good retention capability. Pure hydrogel slightly shows higher value because it has no other particles inside to stop the moisture from leaving while the hydrogel membrane with cinnamon and black pepper shows less value because presence of particles in the hydrogel structure restrict the evaporation of vapors.

4.4 Water vapor transmission rate of hydrogel membrane

To overcome the loss of body fluid that occurs due to exudation an evaporation hydrogel is prepared that maintains the moist environment around the wound and lessens the loss of body fluid WVTR value of 53gm^2/h is considered good in hydrogels[60].

Table 2. WVTR

Sr. No	Membrane	WVTRH (g/m^{2}h)
1	Pure	74.16
2	1.5 BP	72.4
3	1.5 C	73.19

These high values shows the rapid drying of the injury that means it increases the metabolism rate and lessens the body temperature by loss of the moisture via evaporation or exudation. The less values for cinnamon and black pepper hydrogel shows the presence of particles in the neat hydrogel and presence of hydroxyl and carboxylic groups which prevents the moisture loss from the wound.

4.5 Swelling Behavior:

Swelling behavior of hydrogel membrane was investigated in simulated body fluid (SBF) of pH 7.40. As hydrogel had hydrophilic nature but they do not get dissolve into the solution because of the added cross linking agent probably. Swelling increases the separation between the polymer chains[24]. Absorption of water is shown in Table No 4, by black pepper hydrogel membrane could be due to the presence carbonyl group of peprine, –OH groups in polysaccharides and PVA. Cinnamon incorporated showed higher than black pepper swelling rate, because of aldehyde group of cinnamon. Due to absence of –OH groups in black pepper and cinnamon hydrogel lacks the swelling ratio as compare to pure hydrogel [54, 61]. But still the observed values were greater than 100% which helps the dressing to maintain moist environment to provide ease in healing of the wound.

Table 3. Swelling Behavior

Sr.No	Membrane	Swelling rate
1	Pure	140 ± 0.43
2	Black pepper	122 ± 0.55
3	Cinnamon	132 ± 0.12

4.7 Scanning Electron Microscopy:

SEM micrographs shown in Figure 22, 23 which shows the morphology of the PVA hydrogel and agents incorporated in hydrogel membranes. At higher magnification the structure was dense and no pores were observed. It is beneficial because no pathogens can penetrate through the space and infect the wound. Starch and agents added were partially soluble in water. The particles are clearly seen in the SEM micrographs.

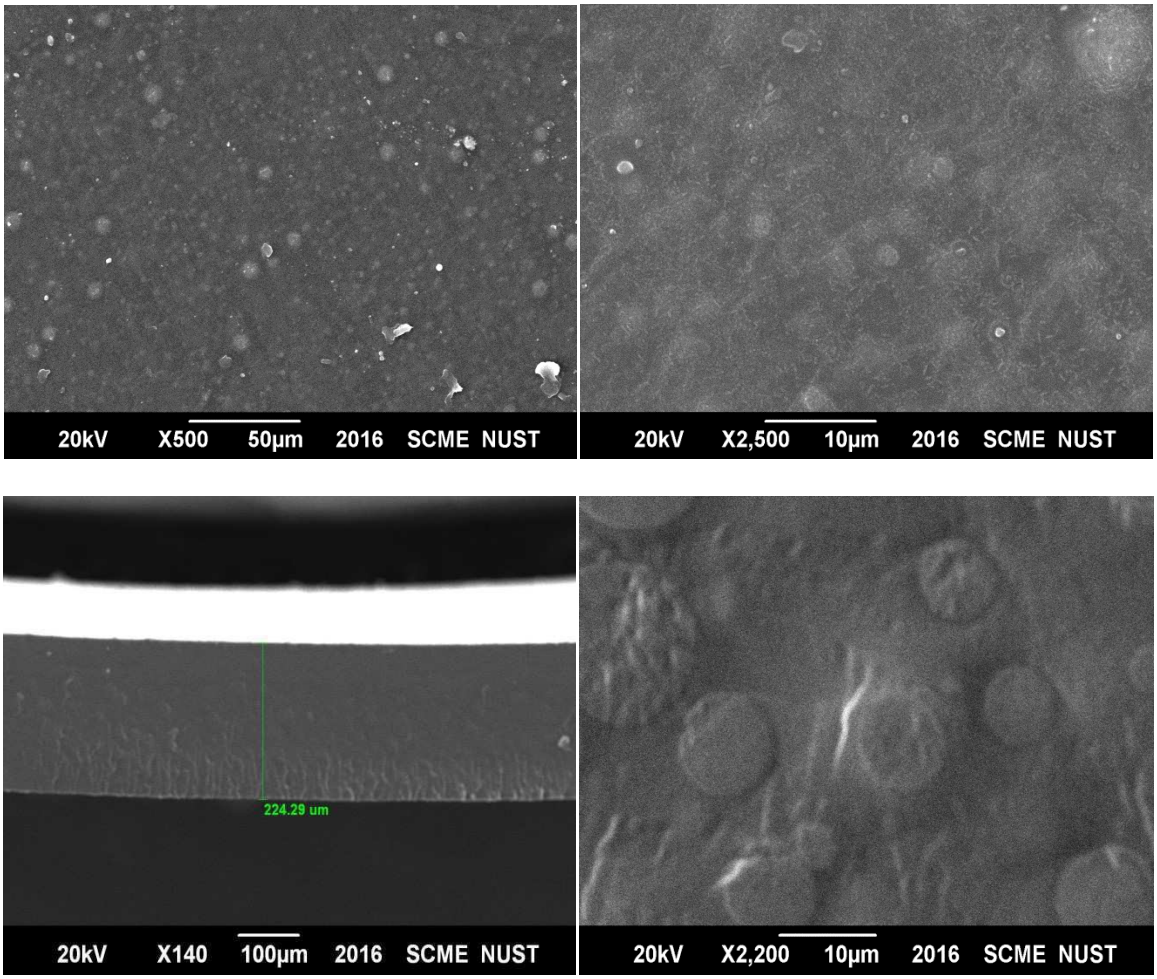


Figure 23. SEM Images of Neat PVA hydrogel

Figure 22 shows the morphology of the neat PVA hydrogel. No pores are present which confirms the smooth uniform surface of the hydrogel. Starch is seen incorporated into the surface and small particles over the smooth dense surface having the cross sectional area width of 224.29nm and confirms the presence of solidified particles of unsolvable starch.

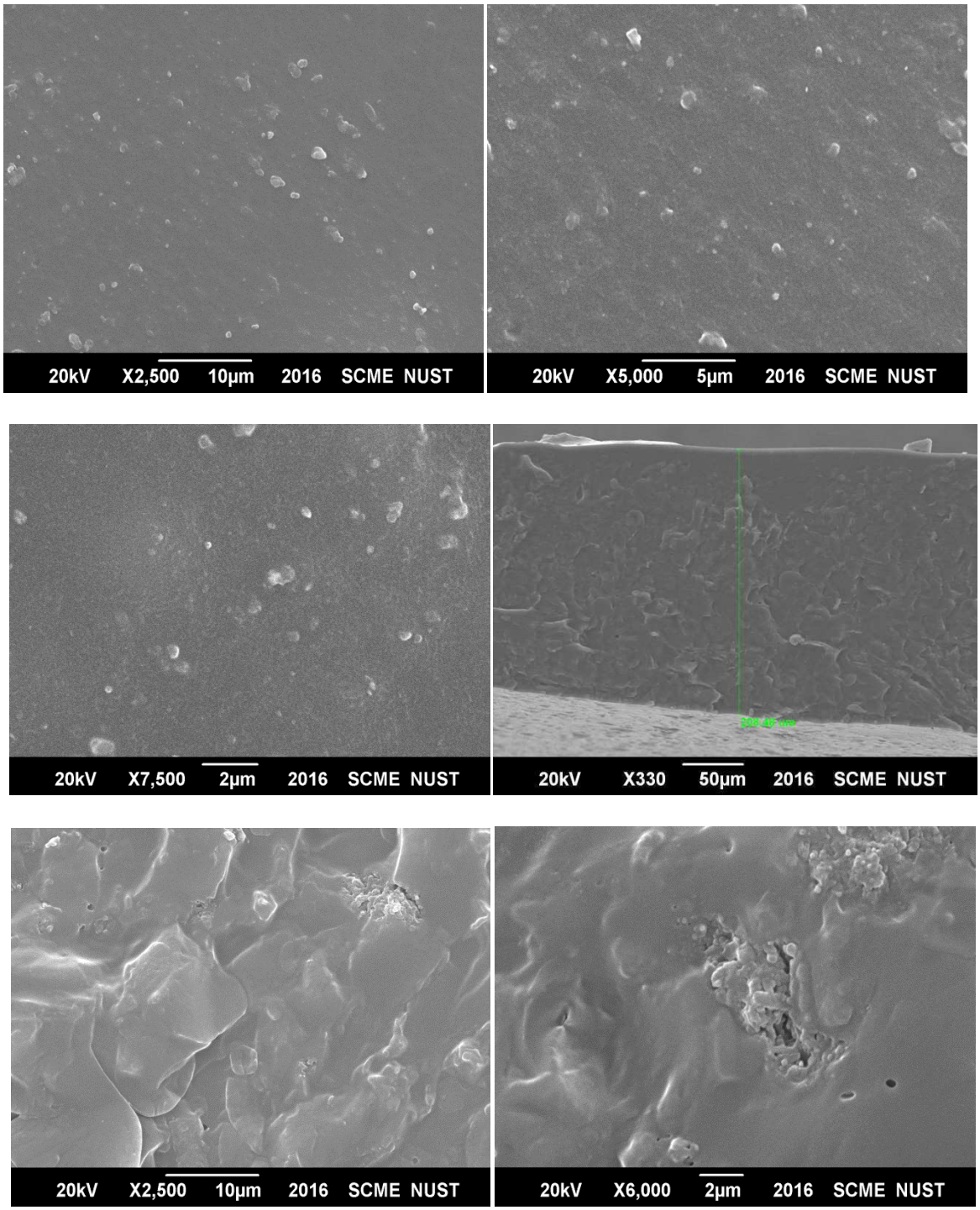


Figure 24. SEM images of Cinnamon Embedded in PVA Hydrogel membrane

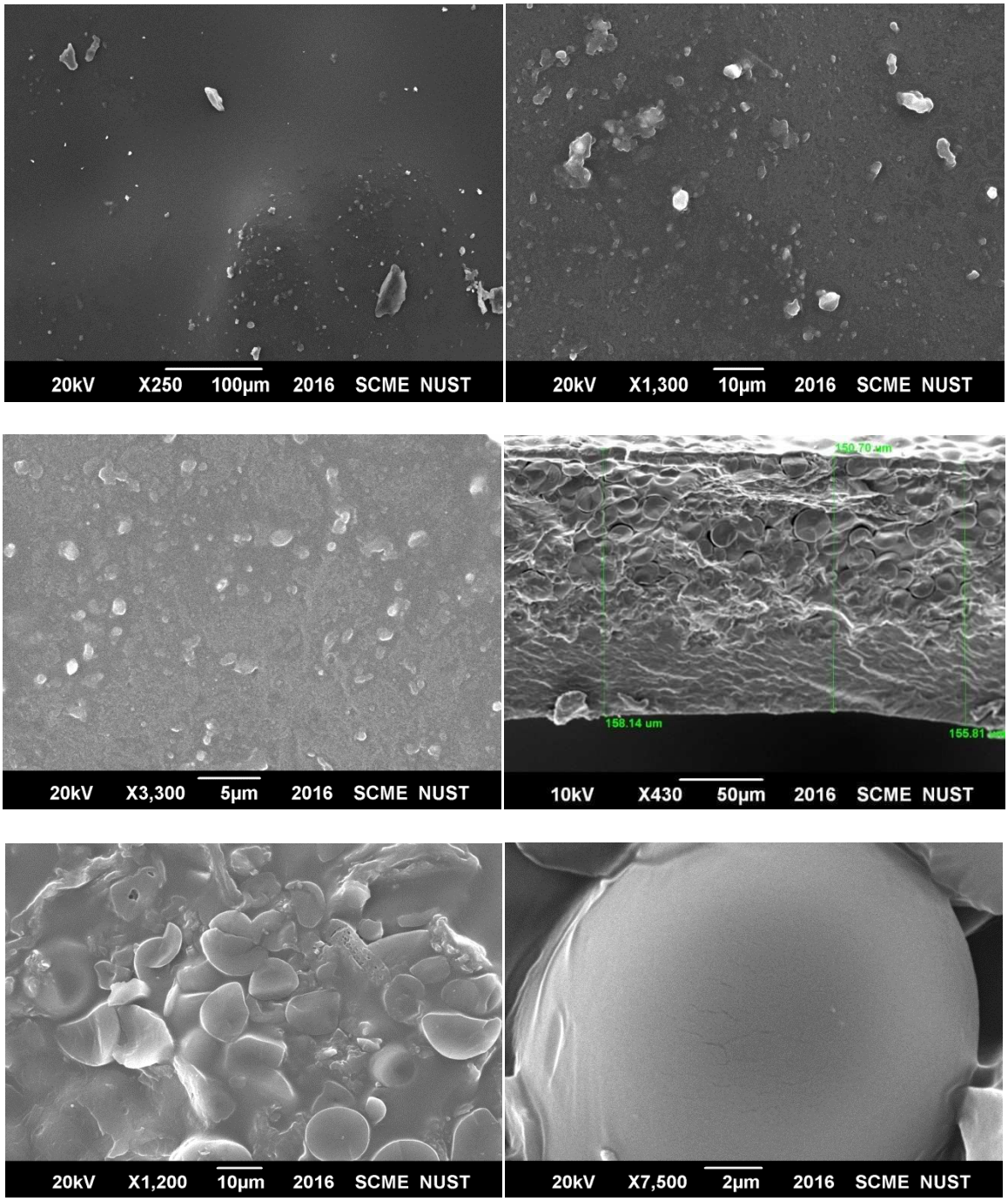


Figure 25. SEM images of black pepper embedded in PVA hydrogel Membrane

The incorporation of micro sized natural antimicrobial agents in PVA-starch surface is confirmed from Figure 23, 24 which also confirms the equal distribution of particles over the surface, the dense nature of membrane is responsible for agglomeration of the particles. The cross sectional area of the membrane with black pepper and cinnamon is 208.48nm and

190.69nm respectively. SEM micrographs shows the rough surface of the hydrogel membranes

4.8 Gel Fraction:

Gel fraction is checked for the cross linking of the starch and PVA. The hydrogel in water remains insoluble then it has perfect cross linking and if the hydrogel gets dissolved in water shows improper cross linking. Following are the results in Table No 5, Addition of glutaraldehyde forms more entangled structure. Induced interactions occur between the polymeric bonds and the functional groups. Black pepper and cinnamon can also get dissolve in water due to per long immersion, causing leaching to give antibacterial activity[62].

Table 4. Gel fraction

Sr. No	Membrane	Gel Fraction
1	Pure	56.1 ± 0.98
2	Black pepper	42.65 ± 0.63
3	Cinnamon	46.23 ± 0.56

4.9 Tensile Strength:

In order to evaluate the mechanical properties of the hydrogels for wound application, tensile testing is very important. The tensile strength turns to be 19.5MPa for neat PVA hydrogel while 9.5MPa and 9.38MPa for black pepper and cinnamon respectively, which is less than the failure strength of human skin (34MPa) [54, 63]. The prepared could be used as wound dressing to provide cushioning effect on the skin. It will absorb the additional frictional stresses comes in day to day activities, without breaking provides the desired safety to the injury.

Table 5 Tensile Strength

Sr. No	Antibacterial Agents	UTS (N/mm^2)	Strain (%)	Force (N)
1	Pure	19.5 ± 4.10	783.7 ± 0.67	24.7 ± 0.9
2	Black pepper	9.5 ± 0.784	614.89 ± 0.92	12 ± 0.2
3	Cinnamon	9.38 ±0.337	320.9 ± 0.334	11.9± 0.71

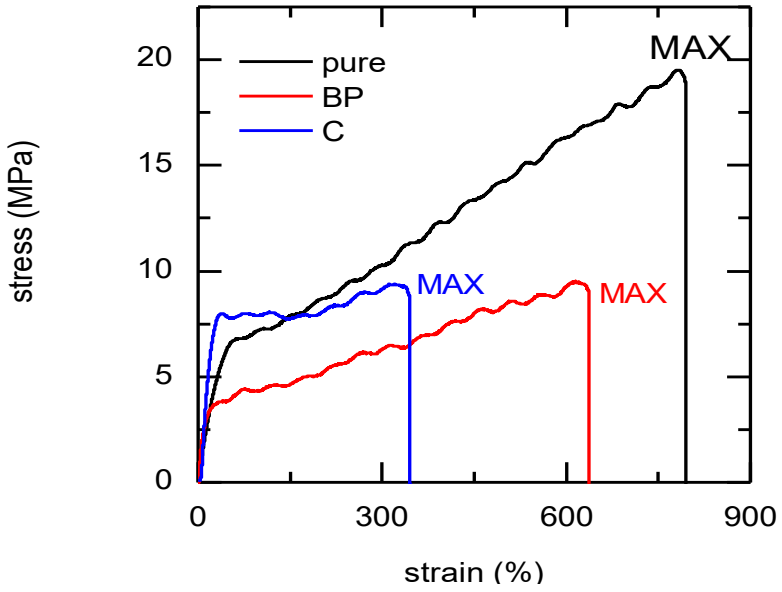


Figure 26. Ultimate Tensile Strength (UTS) Graph for pure, black pepper and cinnamon hydrogel membranes.

As the results showed the value of pure hydrogel is higher than the incorporated membranes. Values can be affected by various factors such as temperature, pressure, branching, chain length and cross linking. The cross linking effect is higher in the pure hydrogel it has smooth and plain morphology but the hydrogels with added agents lowers the values because it limits the chain mobility of the polymer substrate. Best solution for the wound dressing problem.

Conclusion:

Usage of all the biodegradable materials such as PVA, starch along with natural antimicrobial agents to increase the therapeutic effect has made it very suitable for wound dressing. However, hydrogel with 1.5g content of black pepper and cinnamon hydrogel showed the best antibacterial activity results.

Morphological studies explained the effective incorporation of macro sized particles over the surface as well as in the interior of the poly vinyl alcohol substrate. An FTIR spectrum of the hydrogel membranes affirms the presence of –OH groups, which were mainly responsible for the hydrogel water holding capacity. Presence of aldehyde peak in hydrogel membranes confirms the presence of cross linking agent glutaraldehyde as it does not let the hydrogel dissolve in any solution. By the addition of natural antimicrobial agents increases the physical properties such as moisture retention, WVTR but decreases the mechanical strength. Antibacterial activity showed that gram negative bacteria were more resistant to the antimicrobial agents so inhibition zones were lesser than the gram positive bacteria. Antifungal activity of M1 membrane showed highest activity against Aspergillus subolivaceus while M2 had intermediate activity against Aspergillus orzyae. Hence, the prepared hydrogel can be served for various biomedical applications as well as for the wound dressing application.

Future Aspects:

Nowadays, the available dressings in the market have great problems such as they don't stick to the skin properly and have weak mechanical properties and their frequent use may dry the wound but also don't provide proper protection to the wound. They may cause more traumas and have no activity against pathogens.

Addition of silver into the dressing may cause further issues such as using silver is suitable in limited quantity for the healing process. Silver NPs may seep though into the skin causing irritation or some disease.

To overcome these problems we have developed this hydrogel successfully, consisting of polyvinyl alcohol with natural antibacterial agents to provide better natural healing of the injury. Combination of natural antibacterial agents can be used to make the hydrogel dressing more suitable for use.

Antimicrobial activity along with the toxicity of the hydrogel can be performed to increase its use in the wound dressing application.

Natural occurring polymers which are biodegradable such as cellulose, poly lactic acid, chitin etc. can be used further for biomedical applications.

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