Synthesis and characterization of PVA/Starch hydrogel membranes incorporating essential oils aimed to be used in wound dressing applications

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Abstract

Wound care has come through various trials and errors with primitive cultures applying old age techniques and knowledge. Recent research has shown that the moist environment promotes wound healing than the dry. In the present research, hydrogel membranes were fabricated by esterification of polyvinyl alcohol (PVA) with starch and glutaraldehyde as a cross-linker. The essential oils (clove oil, Oregano oil and tea tree oil) have been incorporated in PVA/Starch based hydrogel membranes. The aim was to achieve optimized anti-bacterial activity and mechanical strength. The anti-bacterial testing was performed using the disc diffusion method. The maximum antibacterial activity for fabricated hydrogels was attained by addition of 0.1 mL clove oil in PVA/Starch hydrogel was 39±0.57 mm and 37±0.29 mm for MRSA and E.Coli, respectively. The FTIR results presented the occurrence of -OH group in hydrogel membrane. The SEM results showed around dense nature of membranes with having an antibacterial agent in it or not. Mechanical examination of hydrogel membranes presented suitable tensile strength of 19.36 MPa for 0.1 mL Clove oil. Furthermore, water vapour transmission rate (WVTR) and moisture retention capability (MRC) for 0.1 mL clove oil was 36.22 g/m^2h and 95.50%, respectively. The experimental conclusion nominated that fabricated hydrogel articulates good antibacterial, mechanical and physical properties that it could be used in wound dressing applications. The best results were obtained for clove oil using 0.1 mL as an antibacterial agent.

Keywords:

Hydrogel; Polyvinyl alcohol (PVA); Starch; clove oil; Tea tree oil; Oregano oil; Antibacterial activity.

1 Introduction

Over the past few decades, membrane technology has gained the significant importance in finding its applications in desalination, food processing and medical fields. But since last few years, the hydrogel membranes have gained importance due to their feasibility in healing the burn wounds [1]. Though, in scenario of wound dressing application, the life span of membrane is not an issue because it is used for a limited period of time[2]. Despite the largest human body organ, the skin is the least well-known organ. The reason is that its function is easily defined and measured rather than the circulatory or renal systems. According to the World Health Organization (WHO), approximately 180,000 deaths occur due to burns annually. Most of these casualties occur in underdeveloped regions and two-third in African and Asian countries. Burning is the foremost reason of disabilities [3]. The scald injuries are incontrovertibly amongst the maximum problematic ones to handle with, where extensive liquid loss and tissue damages weaken numerous momentous functions[4]. Each year, millions of people are exposed to burns caused by flames, hot water, boiling oil and accidents and these end in major disabilities or even death [4]. The wound healing process is defined through four steps; Homeostasis, inflammatory response, proliferation and remodeling [5].

In the past, dry wound was considered optimal for the wound dressings. This was the major drawback of these types of dressings. Because the recent research has shown that wounds needs the moist environment. It helps in mimicking the skin functioning. That’s why hydrogels are introduced for heal wounding. Hydrogels are polymeric 3-D cross-linked arrangements that have high moisture retaining capabilities. This moisture absorption is caused by the presence of carboxyl, hydroxyl and hydrophilic compounds present in them[6]. They tend to swell when

exposed to various fluids. The moisture absorbing ability makes them a potential candidate for wound dressing applications. Exudates from the wound are collected inside the hydrogel membrane as a gel, providing suppressing and conserving effect to the wound and hinders bacterial infections [7]. This effect also helps in reducing the unpleasant odor caused by the exudates present at the wound site. Hydrogel membranes can be easily removed from the wound surface without rupturing, due to hydrophilic groups present in them[8]. Hydrogel membranes strength must be greater than the strength of human skin (11.5 MPa) which makes them further a suitable candidate[9]. The strength of the hydrogel membrane depends primarily on the polymer used. Since they are hydrophilic, it can help in hydrating dried wounds and does not leave any debris behind [10].

Hydrogels from natural polymers such as polysaccharides are getting some prominence over the last few years[11]. But the polysaccharides cannot be used alone because of low mechanical and physical properties such as moisture retention, gel fraction, water vapour transition rate, etc. Hence, polysaccharides are blended with some synthetic polymers (poly (vinyl alcohol), poly (methyl methacrylate), polyurethane etc). The natural polymers have many advantages over synthetic i.e., ecofriendly, non-toxic, biodegradable and absorbent. Starch is a glucose-based natural polymer, which is considered as the best option due to common availability, biodegradability, biocompatibility and low cost. It partially dissolves in water and can be easily modified i.e. physically or chemically. However, starch cannot be used as a base polymer alone because it cannot form a stable hydrogel. Mechanical strength has a good impact on the hydrogels but using starch alone it loses the strength. Hence, it is mixed with other polymers to overcome this issue. Polyvinyl alcohol (PVA) is one of the most widely applied and important polymers used

in hydrogels [12]. This is attributed to its solubility in water, biocompatibility, non-toxicity, biodegradability, low cost, ease of formulation as a hydrogel and non-carcinogenic properties. Glutaraldehyde acts as a cross linker to chemically link PVA Starch. Secondly, glycerine is used as a plasticizers to enhance its strength.

The antibacterial characteristics of essential oils extracted from herbs have been implied heuristically for centuries. The innovative potential of essential oils towards anti-microbial composites is highly appreciated, particularly while resisting against bacterial strains [13]. Medicinal and edible herbs like turmeric, oregano, rosemary, ginger, basil, clove, garlic and nutmeg have been effectively utilized either individually or in conjunction with other conservational procedures [14]. From food packaging to preservation and dentistry to medicine, more research data is being collected on the antibacterial properties of numerous essential oils. These all perplexes makes them the best candidate in advancing their research in wound dressing applications [15]. Three different essential oils have been used in this research work.

Clove oil has been widely studied for its biological characteristics including antioxidant, antifungal, antibacterial and insecticidal properties [16]. Chami et al. used clove oil on the yeast model due to its anti-bacterial properties[17]. It has also been found operative against listeriosis and salmonellosis causing bacteria [18]. Strong antimicrobial and biological characteristics of clove oil are due to the presence of large amounts of eugenol in it [19]. Tea tree oil can be utilized in the number of medicinal ways together with keeping skin healthy and hair strong [20]. The presence of terpinen-4-ol in tea tree oil makes it a better candidate for fighting bacteria and fungi[21]. These antibacterial properties make this essential oil an esteemed natural cure in

handling skin conditions and to stimulate wound healing [22]. Besides averting septicity in wounds, tea tree oil also boosts wound healing. Nano emulsions of tea tree oil were used. It showed good anti-bacterial and anti-fungal phenomena with no adverse effects[23].

Oregano oil is a herbal oil that is extracted from the oregano plant and is extensively reported to have therapeutic characteristics [22]. P.E. Simitzis et al. studied the effect of dietary oregano essential oil supplementation on lamb meat characteristics was investigated. No difference in weight was observed after supplementation of oil while its strong anti-oxidant effects enhances its long term frozen storage[24]. These three essential oils have been selected because of their anti-bacterial properties. Moreover, they have also been used in the packaging and in pharmaceutical industry but have never been incorporated in PVA/starch based hydrogel membranes. Dilutions of oils or in the form of gel for skin treatment had already been used and available in the market but incorporation in hydrogel membranes were done for the first time.

In this scenario, hydrogel membranes for wound dressing were fabricated through solution casting technique. The key interest is to promote the moist wound environment through enhanced anti-bacterial activity. Hydrogel membranes containing polyvinyl alcohol (PVA) and starch incorporated with essential oils were prepared. Morphology and molecular interaction among various polymers used in the hydrogel membranes were investigated by using Scanning Electron Microscopy (SEM) and Fourier Transform Infrared Spectroscopy (FTIR). Atomic Force Microscopy(AFM) was used to find the roughness. Anti-bacterial testing was performed using the disc diffusion method. The physical and mechanical characteristics of the membrane were also considered to check their usefulness in practical application.

2 Experimental

2.1 Materials

Poly Vinyl Alcohol (Degree of Polymerization=1500), glycerin and starch were supplied from Dae-Jung, Korea. Clove oil, tree tea oil and oregano oil of 100% purity was provided by plant therapy, Inc. Glutaraldehyde (50% aqueous solution) was purchased from Sigma Aldrich whereas ethanol of 99.7% purity from BDH Laboratory Supplies. Analytical grade Hydrochloric Acid (HCL) (37% purity) was purchased from Lab scan Asia Co. Distill water was used in the overall research.

2.2 Preparation of hydrogel membrane

The hydrogel membranes are prepared by solution casting method. 10% (w/v) of the PVA solution was prepared in water. The blend was heated at 70 ˚C for 2 hours with continuous stirring until the solution became transparent. After that, 7% (w/v) of the starch solution was prepared, and heating was carried out for 15 minutes at 100 ˚C with continuous stirring to get a homogenized solution. Essential oils were added in varying concentrations to the starch solution and then it was cooled after the addition of essential oils. These starch/clove oil, starch/tea tree oil and starch/oregano oil solutions were then added to the PVA solution along with the cross-linking agent. The cross-linking agent was prepared by using 10 mL ethanol, 0.5 mL glutaraldehyde and 0.05 mL diluted HCl. Furthermore, PVA/Starch/Essential oil solution and crosslinking agents were mixed; 2 mL glycerin was added to the polymer/cross-linker mixture as a plasticizer. All the solutions were introduced to sonication for the preparation of homogenization solutions. Coldwater was used during the sonication process to control the temperature. The solution casting method was applied for the fabrication of membranes. After overnight drying, the hydrogel membranes were extracted

from the petri dish. Hydrogels were then stored in cool, dry and air free bags to avoid contamination. Table 1 shows the composition of hydrogels prepared.

2.3 Membrane characterization

2.3.1 Scanning electron microscopy

SEM (JSM-64900) was employed to investigate the surface morphology of the hydrogel membrane. It also gives information about structure i.e., porous or dense membrane. The acceleration voltage of 10 kV was utilized and membranes were coating with a thin conductive layer of platinum/palladium.

2.3.2 Fourier transform infrared spectroscopy

The dry and impurity-free samples of hydrogel membranes were directly exposed to FTIR. The spectra were noted in the array of 450 cm^{-1} to 4000 cm^{-1}. In the case of the hydrogel membrane, the samples were placed right in the FTIR machine for processing. But pellets are made for the powered samples.

2.3.3 X-Ray diffraction

XRD analysis was conducted to determine phase identification and crystalline nature of prepared hydrogel membranes. The XRD was performed using XR D8 advanced (Bruker Germany). The membranes were used directly for the XRD analysis. The current and voltage of X-ray basis were 40 mA and 40 kV mA, respectively. The scanning of sample was done at step size of 0.04 while the step time is 0.5s/step and 2θ ranges from 10° to 70. The wavelength of CuKα radioactivity was 1.540 Å.

2.3.4 Atomic force microscopy:

Surface roughness of the membrane is an important parameter to check the functioning in respect to biocompatibility. Atomic Force Microscopy, JOEL (JSPM-5200) was used to investigate topography; porosity and roughness. The 3-D micrographs images were taken. The AFM tip or cantilever in a raster scanning motion contacts sample surface. In this contact mode the repulsive forces between the tip and sample were converted into spatial variation of an image. The scanning area was approximately in 10 µm x 10 µm and all the roughness parameter was determined by using AFM software. In the data "Ra", "R_t" and "RMS" shows mean roughness and root mean square roughness, respectively.

2.3.5 Hydrophilicity:

Tantec Contact Angle meter was used to study hydrophobicity and hydrophilicity of membrane. Single water droplet was allowed at dosing rate of 0.1µL/s, with a constant dosing rate of 0.2µL/s using a micro syringe. The membranes were cut into thin strips and the sessile drop method was used for static angle. On an average thrice times the angle was measured. During the experiment the relative humidity and temperature were at ambient conditions.

2.3.6 Water vapor transmission rate measurement

To determine WVTR, 10 mL distilled water was poured into media glass bottles of 29.5mm mouth diameter. These bottles were covered with hydrogel membranes, wrapped through Teflon tape and then were weighed. These bottles were located at 40 ^{o}C inside an oven for 1 day[9]. After 1 day, they were weighed again and WVTR (g/m^{2}h) was evaluated using Eq. (1) [25]:

$$WVTR = (W_i - W_t)/ (A \times 24) \times 10^6 \quad (1)$$

Where, A is the area of the round opening of the bottle, W_i is the mass of bottle before heating and W_t is the weight of bottle after heating.

As the temperature of human body is 37.2 ^oC so the testing was done at 40 ^oC because the practical application of our research work is human skin. That's why the temperature was kept near to it in both of the cases i.e water vapor transmission rate and moisture retention capability [26].

2.3.7 Moisture retention capability

Prepared hydrogel membranes were cut into equal pieces and weighed. These samples were then placed inside an oven for 6 hours at 40 ^oC. Later, they were removed from the oven and weighed again. Eq. (2) was used to determine the moisture retention capability[9]:

$$\text{Moisture retention capability (\%)}: (W_{6hrs} / W_i) \times 100 \quad (2)$$

Where, W_i is the initial weight and W_{6hrs} is the weight in g after 6 hours of heating at 40 ^oC.

2.3.8 Gel fraction

The fabricated hydrogel membranes were cut into identical pieces and dried in the oven to get their weight constant. After attaining constant weight, they were assessed and put in de-ionized water aimed at 96 hours. Afterwards, the hydrogel membrane samples were again dried in a vacuum oven until a constant weight is reached. Gel fraction was determined using Eq. (3) [27]:

$$\text{Gel fraction (\%)} = (W_t/W_i) \times 100 \quad (3)$$

Where, W_i is the initial weight before immersing in distilled water and W_t was the final weight after drying.

2.3.9 Hydrogel membrane porosity

The hydrogel membranes were immersed into ethanol until they got saturated. Ethanol is used to wet the sample and immerse into it. Hydrogel membranes were assessed earlier and later having absorption in ethanol. Eq. (4) was used for calculation of porosity[28]:

$$\varphi = (W2-W1)/(\rho V2-\rho V1) \times 100 \quad (4)$$

Where, $W1$ and $W2$ specify the weight of samples earlier and later having absorption in ethanol, respectively. $V1$ is the volume of ethanol before absorption, $V2$ is the volume of ethanol after absorption and ρ *is* density is the density of alcohol at room temperature.

2.3.10 Swelling behavior measurement

The capacity of the hydrogel membrane to absorb fluids that are surfaced by the wound is called its swelling behavior. The swelling behavior of prepared hydrogel membrane was examined contrary toH_2O (water), 0.9 % $MgCl_2$(Magnesium Chloride), 0.9 % NaCl(Sodium Chloride) and blood. A 0.9 % $MgCl_2$, and 0.9 % NaCl solution is said to be isotonic: when blood cells reside in such a medium, the intracellular and extracellular fluids are in osmotic equilibrium across the cell membrane, and there is no net influx or efflux of water. To determine the swelling behavior of prepared hydrogel membranes, they were identically cut and balanced to be equal in size and weight. These samples were then immersed into water, $MgCl_2$, NaCl, and blood solutions for 1 day and cleaned through filter papers and weighed again. The swelling percentage was determined using the Eq. (5) [29]:

$$\text{Swelling (\%)} = [(W_s - W_d)/W_d] \times 100 \quad (5)$$

Where, W_s is weight of the swelled sample and W_d is weight of dry sample.

2.3.11 Tensile testing

The prepared hydrogel membrane was primed for tensile testing by following the standing operating procedures as described in "SOP - Tensile testing of electrospun nanofiber membrane"[30]. Samples that were equal in thickness and had no surface defects were selected and were attached to the holding clamps of the universal testing machine. With a constant strain rate of 10 N/mm^2, the clamps were allowed to move in opposite directions and the stress over hydrogel membrane samples was recorded.

2.3.12 Anti-bacterial activity measurement

Disc diffusion method was used to measure the anti-bacterial activity of fabricated hydrogel membranes. Two bacterial strains have been used for this purpose i.e Gram Negative *Escherichia Coli* and Gram-Positive *Staphylococcus aureus*. The bacteria were cultivated in a test tube having broth in it, then it was sited in a shaky water bath at a temperature of 37 ℃. The agar media was arranged by dissolving 11.5 g nutrient agar in 500 ml distilled water. This agar solution was then consistently decanted into the petri dishes and was left for few minutes to freeze it. The bacteria was spread on these petri dishes with the help of spreader. The fabricated hydrogel membranes were cut into 6 mm disks and were positioned over the agar plates. Later, they were sited into an incubator for 24 hours at 37 ℃. Then, the plates were taken out and region of inhibition were measured by Vernier calipers. The negative control was a PVA/St membrane without essential oil while the positive control was gentamicin. All the apparatus was autoclaved which includes petri dishes, forceps, pipette, spreader, LB broth and agar media. The purpose of doing autoclave was to avoid contamination.

2.3.13 Statistical Analysis

ANOVA two factor without replication was performed on all analysis to calculate the statistical significant and non-significant. In table “ss”, “df” and ”ms” represent sum of squares, degree of freedom and mean square, respectively. The ANOVA table of gravimetric has provided as significant value of less than 0.05 (p=0.05). Anti-bacterial test has provided us higher value in the case of oregano oil. Hence, they have no effect on the E-coli and MRSA. Hence, this proves the effect of essential oils as anti-bacterial agent when incorporated in hydrogels. Moreover, it also depending on their concentration levels.

3 Results and discussion

3.1 Morphological Analysis

The results of SEM show that the morphology of the hydrogel membrane was dense, and there were no pores even at advanced magnifications. The dense surface of the hydrogel membrane restricts any bacteria passage and approach to the wound. As oils are hydrophobic in nature and immiscible in water, their particles are seen on the surface through SEM images. Fig 1 shows the SEM of a pristine hydrogel membrane. Smooth and homogenous surface of hydrogel films are obtained because of the absence of essential oils. On the other hand, fig 2(a,b,c) shows the SEM images of oregano oil with concentrations of 0.1,0.2 and 0.3 mL, respectively. The following similar observations were obtained from the results as in literature [31]. The surface becomes rough with an increase in essential oil concentration. The essential oil becomes immiscible in water above 0.3mL concentration due to its hydrophobic nature. The micro porosities range from few microns to macro voids were distributed evenly on the surface. The roughness causes issue in the biocompatibility of the membrane as more wound healing cells such as fibroblasts and

keratinocytes will adhere to the surface. When we go into the chemistry of oregano oil, it has carvacrol component present in it. The structure of carvacrol shows that it has the phenolic group, while three methyl groups and one hydroxyl group is attached to it. When the concentration of oil in hydrogel membranes increases from 0.1mL to 0.3mL, agglomeration starts to take place. This is due to the presence of three methyl groups. They are not soluble in polar solvents[32]. Fig 2 (d,e,f) shows the hydrogel membrane with concentration of clove oil 0.1, 0.2, and 0.3mL respectively. 0.1 mL clove oil has shown best results as no pore formation takes place and all the oil particles are trapped within the 3-D structure of the membrane. Whereas, the formation of macro voids represents the rapid evaporation of the oil. These results are also validated by anti-bacterial testing. It is also observed from the SEM that the insoluble particles of oil become visible with the increase in essential oil concentrations. But as the concentration of clove oil is increased above 0.3mL, pores start to generate because of their immiscibility in water. This is because at higher concentrations hydroxyl group becomes less efficient and phenolic group becomes dominant which is not suitable for polar solvents[33].

Fig 2 (g,h,i) shows the result for tea tree oil concentrations. Similar, results are obtained within oil as well. Pores started to generate above 0.3 mL as the concentration of oil is increased in the hydrogel membrane. This is because of the presence of phenolic group present in it because it is not soluble[34]. From the SEM images of all hydrogel membranes, it has been observed that the clove oil and tea tree oil has more smooth surfaces than the oregano oil. This is because of the presence of hydroxyl group attached to them while oregano oil has three methyl groups which are not soluble resulting in rough surface. But when the concentration of oil is increased in tea tree and oregano oil, the phenolic group becomes more dominant that’s why pores started to appear.

3.2 Fourier transform infrared spectroscopy

The FTIR results designate the occurrence of hydroxyl groups which are accountable for water holding capacity in hydrogel membranes. Fig 3(a) shows the FTIR of the hydrogel membrane have a similar peak around 3300 cm^{-1} which represent the hydroxyl group present in PVA, Glycerin and Starch. This peak is present in all formulations. Clove oil consists of eugenol, eugenyl acetate and caryophyllene. There is no change observed in the spectra with increase in concentration of clove oil. First of all, the broad peak at 3000-3500 cm^{-1} is observed. This can be ascribed to the presence of OH group. It broadens due to the presence of the carboxyl group due to acetate group[35]. Moreover, the peaks between 1600-1800 cm-1 is due to the presence of C=O and C=C double bonds found in all the three major components of clove oil[36]. Also, the Csp2-H bond is signified in the wavenumber range greater than 3000 cm^{-1} as seen in all spectra[37]. The presence of hydroxyl group handles its water holding capacity. The stronger the peak of the hydrogel membrane, the higher will be the tendency to absorb water and exudates coming from the wound. The main difference occurring in the functional group peaks is due to the transmittance difference in the various concentrations. The intensity of the essential oil in hydrogel membranes dependent directly on the concentration, because of presence of anti-oxidants.

Fig 3(b) shows the FTIR spectra of tea tree oil. The main components of tea tree oil are alpha and gamma Terpinene along with Cymene and Cineole. In these spectra, the broad peak between 2000 to 3000 cm^{-1} can be attributed to the presence of the abundant and interconnected Csp2-H bonds as well as the Csp3-H bonds[38]. Other than that, the only peak to be mentioned are the prominent peaks between 1600-1800 cm^{-1}, which is due to the presence of C=C double bonds[39]. These are the only bonds found in the components making up tea tree oil.

Fig 3(c) shows the FTIR spectra of oregano oil. In the case of oregano essential oil, the major components are carvacrol, beta-fenchyl alcohol, thymol and gamma-terpinene[40]. The main component functional groups are O-H, C=O and C=C as well as Csp2-H and Csp3-H. O-H as well as the presence of C=O bond is proven by the excessively broad peak at 2500-3500 cm^{-1}. This same region also contains the peak for Csp3-H at less than 3000 cm^{-1}, and Csp2-H at a value slightly greater than 3000 cm^{-1}. The peaks between 1600-1800 cm^{-1} signify the presence of both C=O and C=C bonds[41]. The sharp and broad peak further validates the presence of a wide array of both C=O and C=C double bonds.

3.3 X-Ray diffraction

XRD spectrum tells us about the nature of material crystallinity. Fig 4 shows the XRD spectrum of PVA/Starch hydrogel membrane with essential oils. For PVA/Starch hydrogel membrane with essential oils, single peak was detected at 19.8°, (101) plane of PVA and no other sharp peak can be seen in the pattern. The amorphous nature of PVA/St membrane was hence proved by XRD analysis. This typical diffraction peak confirms the presence of hydrogen bonds between hydroxyl groups present in PVA[42]. From the XRD spectrum, it has been observed that essential oils do not show any peaks. This is due to the absence of crystallinity in them[12]. Varying the essential oil concentration does not affect the lamellar structure[43].

3.4 Atomic Force Microscopy

The PVA/Starch formulated membranes were studied under “tapping mode” and results are presented in Fig 5. The dark regions repent depression whereas the lighter region represents height in 3-D images of membranes topography[44]. The results of pristine membranes showed smoothest surface among all the formulated membranes. However, the smoothness started to decrease after the incorporation of essential oils. It can be ascribed to the immiscibility of essential

oils at higher concentrations in membranes thus forming pores at micro and Nano level, which formed heighted structures [45]. The tea tree oil has the lowest roughness as compared to clove oil and oregano oil due to high hydroxyl group which forms a uniform structure as compared to other two oils.

3.5 Hydrophilicity

For material contacting the human skin, a balance between the hydrophobic and hydrophilic is important [46]. All formulated membranes have contact angle less than 90 as shown in table 2 and Fig 6, thus making them hydrophilic in nature. The presence of hydroxyl group, carboxyl group and phenolic groups form hydrogen bonding and Van der Waals's forces creates physical bonding with water which lowers the contact angle[47]. Table 2. represents the behavior of essential oils incorporated in hydrogel membranes. At lower concentration, hydrogen bonding is produced. However, as the concentration increases the phenolic group overshadows the hydroxyl group of PVA. This results in higher contact angle. The essential oils showed hydrophilic behavior at lower concentrations. However, membranes become hydrophobic at higher concentrations of essential oils. For hydrogels, hydrophilicity is an important factor because it produces a crosslinking between the membranes and anti-bacterial agent.

3.6 Water vapor transmission rate (WVTR)

Hydrogel membranes sustain the humid environment underneath the wound, thus curtailing the fluid loss. Water loss without any dressing film as reported in the literature for second degree burn skin wound is 178.55±4.5 g/m^2h and that of third degree is reported as 143.2±4.5 g/m^2h [48][49]. Water loss in the body occurs through two processes: sweat glands and diffusion take place through the body at low relative humidity regions.70% of the inner milieu of the body is water and 20 % of it is retained in the skin. The epidermis and dermis of the layer have sweat glands in which there

384 is 70% of moisture. The second degree burns mostly affect the dermis of the skin whereas in the
385 case of third-degree muscles loss takes place. Without any dressing, major water loss happens in
386 a second degree [50]. The higher WVTR indicates that the wound will dry up quickly while if the
387 WVTR is low it will slow the process of healing and will increase the bacterial infections [51].
388
389 Fig 7(a) shows the graph for WVTR of hydrogel membranes using essential oils. The hydrogels
390 prepared without essential oil has the WVTR of 61.25±3.06 g/m^{2}h. By adding essential oils, it was
391 observed that the clove essential oil provides the best WVTR results of 36.22±1.81 g/m^{2}h with
392 only 0.1mL. The WVTR decreases due to the increment of clove oil. This is because of the
393 presence of hydroxyl group in clove oil. Therefore, it is evident that clove oil induced hydrogel
394 membranes can preserve the wound environment and consequently results in minimal loss of fluid
395 from the wound. However, a tea tree showed WVTR of 45.63±2.28 g/m^{2}h and oregano oil of
396 51.15±2.55 g/m^{2}h has shown the best results in their respective composition. This can be attributed
397 to the fewer pore formation on the membrane surface morphology which causes more water
398 retention. The essential oils are immiscible in water and leached out of the membrane through the
399 drying process which resulted in the generation of pores and macro-voids. Hence, clove oil shows
400 the best result then the tea tree and oregano oil. Clove oil has hydroxyl group present with the
401 phenolic group. However, oregano and tea tree oil has methyl and phenolic groups present which
402 are not soluble in polar solvents. Therefore, their WVTR value is less than the clove oil. However,
403 with the increasing concentration of clove oil the phenolic group becomes more dominant which
404 also becomes insoluble. The water content plays a characteristic role in polymeric network
405 formation. In gel system, water is present in three different structures: bulk water in which
406 polymers are dissolved and present within the matrix, interfacial water has a certain cage like

geometry and hydrated water. The presence of water introduces the biocompatibility in the hydrogel as mimicking the function of water in the human cells [52].

3.6.1 Moisture retention capability (MRC)

The measure of moisture that is preserved inside the hydrogel membrane is termed as its moisture retention capability. The WVTR of any hydrogel membrane is inversely proportional to MRC i.e. higher the amount of vapour loss, lower will be the tendency of the membrane to hold moisture, which makes it difficult for the wound to get a healing environment. It is reported in the literature that lack of moisture at the wound's surface will halt cellular migration, decrease oxygenation of the blood and vastly delay the wound treatment process[53]. Furthermore, many advantages of moist wound treatment over dry wound treatment have been reported. The moist environment helps in mimicking of skin functioning and tissue regeneration. Therefore, it can be inferred that hydrogels that have a large tendency to retain moisture content hold a better chance to be used as wound dressings.

It can be seen in fig 7(b), the highest MRC value was obtained at 0.1 mL of clove oil with 95.50±0.48%, while that of neat hydrogel was 90%. The MRC values of tea tree and oregano oil was 93 and 92%, respectively. As discussed in the WVTR, the decrease in the values of other than clove oil owes the occurrence of methyl and phenolic groups present in them. These results are in line with the WVTR results and confirm that lower the WVTR, higher will be the corresponding MRC. The results showed that essential oils especially clove oil is the best candidate for wound dressing applications. But with the increase in concentration of clove oil, the value of MRC decreased. This is ascribed to the dominance of phenolic group present in it. It provides more than 90% moisture prevention within the wound when use in small concentrations. Due to

hydrophobicity of the oils as the concentration increases the retention decreases or *vice versa*. Thus, it can maintain the healing environment under the dressing and prompts the healing procedure [54]. Consequently, the MRC is reduced which entails the non-suitability of higher concentrations of essential oils.

3.6.2 Hydrogel membrane porosity

Porosity is more likely to depend on the fabrication process of membrane, relatively than the compositions[55] Porosity of hydrogel membranes have been calibrated by using the alcohol displacement method [56]. The porosity of neat hydrogel membranes is 54 %. Porosities of hydrogels using essential oils has been observed in fig 7(c). It has been observed that the porosity is enhanced as the concentration of the oil is increased in the hydrogel membrane. Hydrogels used as wound dressing allow the permeation of gases such as oxygen, water vapors to maintain the moisture in the dressing and small protein molecules while retaining the microorganisms [57]. The percentage of porosity for 0.1 mL clove oil is 38%. However, as the concentration is increased it goes up to 62%. The porosity of tea tree oil was observed 43% for 0.1mL concentration and 67% for 0.3mL. Furthermore, porosity of oregano oil is 41% at 0.1 mL concentration and 59% for 0.3mL concentration. The porosity in the range of (30-40)% is considered good for heal wounding[58]. The size and the surface of membranes containing pores tells us how much it uptakes water. The proportion of the water must be less than 40–60 % to get transparent hydrogels. Whereas, if the content increased to 80%, phase separation occurs and the macroporous network is obtained [59]. The porosity of the membrane is highly dependent on the water content presented during the synthesis of a membrane in the polymer solution. It can also be observed from SEM images that increasing the concentration of essential oils enhances porosity as the profile pattern

represents. Moreover, when the concentration of oil was increased more than 0.3mL these pores become macro voids.

3.6.3 Gel fraction

Polar, naturally hydrophilic and synthetic polymers are crosslinked through physical or chemical processes forming a 3-D network and bonding a large number of water molecules (up to 100g/g or higher)[60]. The gelation percentage is done to evaluate how water is affected by temperature parameters. Gel fraction (GF) test is performed to check how much the cross linker is effective. The crystallinity of network and extent of crosslinking determines the gel fraction values. Also, it influences the flexibility and strength of the films. Secondly, the burn patients are treated in cold environments so that there wound does not disintegrate and deteriorate more. The gelation percentage of neat PVA/Starch hydrogel membrane was found to be 78.24±0.39%. this was the maximum value attained. This high value can be attributed which indicates the PVA and starch cross-linking has taken place to form a 3-D network [43].

Fig 7(d) shows the graph of gel fraction with essential oils. With the increment of essential oils, the gel fraction starts to decrease. This is because with the increasing concentration the cross linking becomes poor[27]. The gel fraction of 0.1mL clove oil is 64%. However, 0.3mL clove oil is 59%. Tea tree oil and oregano oil has gel fraction concentrations in the range of 61-56 % and 58-53 %, respectively[61]. Higher gel fraction is suitable for hydrogel membranes. It tells the extent of cross linking taken place between materials used for the fabrication of hydrogel. It has been observed 0.1mL clove oil gives the best results. As discussed earlier, this is attributed to the presence of hydroxyl group [62]. While, the oregano and tea tree oil has methyl and phenolic group present. This is not soluble in polar solvents.

3.6.4 Swelling Behavior

A hydrogel to be used as a wound dressing should absorb fluids that surfaced over the wound [37]. Swelling is considered a chief and significant criterion for evaluating how a membrane will behave with the wound. Wound exudates are absorbed by the membrane; it prevents wound maceration and the healing process is achieved. The degree of swelling can be affected by pH, temperature, nature of the chemicals, wound environment and degree of crosslinking[51]. Swelling is controlled by hydrogen bonding in the water molecules [63]. Starch and PVA have the affinity to absorb moisture from the environment and hold it together, owing to the existence of –OH radicals within their polymeric chains[64]. Starch maintains equilibrium by absorbing and desorbing water molecules from the atmosphere. On the other hand, the ability of cross-linked polymer to absorb moisture decreases since cross-linking causes the formation of compact 3D structures and consequently the amount of –OH radicals decreases [65].

The swelling behaviors of prepared hydrogel, membranes were tested against water, blood, $MgCl_2$ solutions, and NaCl solutions as shown in Fig 8. These solutions are used for testing because of the presence of their excess amount in human body. It could be seen that the swelling behavior of hydrogels is highest at 0.1 mL concentration of essential oils. The 0.1 mL clove oil within the polymer matrix has given the 135, 176, 121 and 118% swelling in all the fluids with respect to other essential oils. The swelling capacity is moderately good for tea tree and oregano oil. Their swelling behavior is also higher than 100%. Thus, implying their use in wound dressing. As the concentration of essential oil is increased the swelling behavior decreases. This is due to the interaction of oil with the cross-linking sites of PVA/Starch co-polymer [66]. Secondly, as the crosslinking density decreases due to the presence of oils swelling percentage reduced due to

498 decrease in the entanglement of polymeric chains. This interaction causes an increase in the
499 amount of free –OH radicals, thus promoting moisture absorbing capacity and increasing the
500 swelling ability [67]. The propensity of PVA/Starch hydrogel films to swell decreased with the
501 increase in essential oil concentration. This can be accredited to the precipitation of essential oils
502 over the hydrogels at higher concentrations and eventually, voids are created within the membrane
503 [68]. The decrease in swelling behavior percentage can also be explained by the analogous increase
504 in the gel fraction percentage which implies the increase in the rigidity of cross-link due to the
505 addition of essential oils.
506
507 The swelling behavior is highest against blood which makes them suitable for wound dressing
508 applications. The maximum value of welling is achieved with blood. This may be attributed to the
509 circumstance that crosslinking strengthen the molecular spacing between the chains and weaken
510 the hydrogen bonding[69]. Furthermore, a supplementary osmotic pressure is formed owed to the
511 ionic nature of blood that increases the electro-neutrality effect and origins swelling. Moreover,
512 blood has the property to form clots[70]. Because of development of clots, extra capacity is formed
513 inside the system, that allows more fluid to penetrate inside. With the increase in amount of
514 essential oils, the structure was disrupt, and the macromolecular chains were extended
515 straightforwardly[71].
516
517 The second highest swelling behavior is seen against water and then the salt solutions. The
518 hydrophilic groups have a greater influence in swelling. It is started when water is exposed to the
519 membrane and due to the concentration gradient, it starts moving into the polymer matrix. The -
520 OH group present on the essential oils attract the water and forms weak Van der Waals bonding.

This mechanism is taking place at the macro-molecule level. The reduction in the crystallinity of PVA is also attributed to high swelling as already seen in XRD graphs. For the case of a salt solution, it is of the minimum value. The electrostatic repulsion due to the presence of the ionic charge on the salts induced the swelling, the stops the accumulation of polymeric chains and tend to increase the matrix[72]. As the ionic strength is improved, the osmotic pressure change arises between the polymer matrix and solution. This deferred the water penetration into the matrix triggering swelling volume transition[73]. pH is another vital factor that rules the swelling behavior of hydrogel membranes. The pH of 0.9% $MgCl_2$(Magnesium Chloride), 0.9% NaCl(Sodium Chloride),H_2O(distilled water) and blood is 6,7,7 and 7.45 respectively. While for native skin the pH is in the range of 4.7-5.45 and that for injured skin is 7.15-8.93. Therefore, it is quite evident from the results that increase in pH enhances the degree of swelling. Consequently, swelling ability also upsurges.

3.6.5 Tensile Testing

Many natural and synthetic polymers have been developed for the treatment of wounds, but low mechanical properties and weak water absorption capacity limited their application in tensile testing. The mechanical properties of all formulated hydrogel are investigated by measuring tensile behaviour. Mechanical strength defines the integrity of the hydrogel membrane during the handling and dressing on the patient's body. The mechanical properties such as breaking stress (MPa) and percentage elongation (%) at the break of all formulated membranes were investigated by using a Universal tensile machine (UTM). The hydrogel membranes should have sufficient strength to engage these frictional stresses without breakage. Neat hydrogel membrane showed good tensile strength i.e. 26.5 MPa. During the wound healing process, the hydrogels withstand the frictional stresses while attachment on the skin and absorb the moisture without any rupture.

The tensile strength of the human skin is up to 11.5 MPa. The strength of the hydrogel should be higher than the skin as mentioned above [9].

The 0.1 mL clove has shown better result in all the specimens. With the incorporation of essential oils, the tensile strength underway deterioration. As observed from porosity and gel fraction, the cross linking reduces with the increase in essential oils. Hence, tensile strength starts to decrease. Their reduction in values was due to a decrease in chain length and mobility of the polymer chain due to the incorporation of essential oils [74]. Incorporation of oil decreases the breaking stress. The reduction in value was due to a reduction in polymer content[75]. The essential oils are not been entirely soluble in water so it gives the weak point for breakage [68]. Clove oil 0.1 mL gives the maximum breaking stress i.e. 19.55 MPa. However, tea tree and oregano oil has maximum breaking strength stress of 18.7 and 14.5 MPa, respectively as shown in Fig. 9. This can be qualified to presence of essential oils as mentioned above in water vapor transmission rate. Similar trend is observed in the percentage elongation results. As the concentration of oils was increased, its pore size decreased. Therefore, the membrane becomes more brittle.

3.6.6 Anti-Bacterial Activity

Anti-bacterial activity is of key importance in the hydrogel membrane essential for the healing of wounds. Almost all anti-bacterial agents (essential oils) showed good results when incorporated with hydrogels. However, the best result was obtained from clove oil with 0.1mL concentration in PVA/Starch hydrogel membrane. The neat hydrogel membrane in which there was no anti-bacterial agent present did not show any anti-bacterial activity. Clove oil incorporated in polymer hydrogel give excellent antibacterial activity. Clove oil showed good resistance against gram positive bacteria (MRSA) and gram negative bacteria (E.coli) i.e., *Staphylococcus aureus* and

DH5-ALPHA, respectively. For MRSA, 0.1 mL clove oil shows the best results and its antibacterial activity was in 39±0.57mm while that of 0.3mL is 34±0.42mm while that for E. coli is in the range of (37–31 mm) from lower to higher concentration. It has been observed from results with increasing concentration the inhibition zone decreases, and it gives the less anti-bacterial activity. The larger zones of inhibition were achieved for gram negative bacteria than that of gram positive and this is due to their thick cell walls as shown in Fig 10. The key component in the clove oil is eugenol which is around 80–90% [76]. This is insoluble in water [77]. Hence, with an increase in the concentration of clove oil, its solubility decreases. Therefore, it becomes less homogeneous and the anti-bacterial activity decreases [78]**.** Fig 11 (a) shows the anti-bacterial activity against clove oil with all concentrations in which 0.1mL showed the best results.

The use of tea tree oil with hydrogel membranes shows good anti-bacterial activity. But the tea tree oil shows less inhibition zones than clove oil. The main constituent of the tea tree oil is terpinen-4-ol which show resistance against both gram positive (MRSA) and gram-negative bacteria (E.coli). The inhibition zones in MRSA are in the range of (35–26 mm) while that for E.coli is (32–19 mm). The 0.1 mL Tea Tree oil showed good resistance against both gram positive (MRSA) and gram-negative bacteria (E.coli). The inhibition zone is 35± 0.36and 32±0.42 mm, respectively. But as the concentration increases the inhibition zones started to deplete. This is because of the poor dispersion of oil with increasing concentration. Fig 11 (b) shows the result for both gram positive (MRSA) and gram negative bacteria (E.coli)[79]. Oregano oil showed less resistance against both gram positive (MRSA) and gram-negative bacteria (E.coli) than the other essential oils. The major constituents of oregano oil is carvacrol which has the highest phenolic content and it shows resistance against the bacteria [80].

The inhibition zones for gram positive (MRSA) and gram-negative bacteria (E.coli) are in the range of (33–34 mm) and (30–31 mm), respectively. All the essential oils show almost similar result which means that they are miscible. Fig 11 (c) shows the inhibition zones for both bacteria [81]**.** The formulated hydrogels are hydrophilic and have a cross-linking property that imparts excellent biocompatibility. They exhibit soft material nature, which encourages the uptake of water. Therefore, it forms hydrated solid materials, like cells in the body. Due to the composition and presence of functional groups in active compounds and synergistic interactions of the hydrogel, they give more anti-bacterial activity as compared to original essential oils. Clove oil shows the best result at 0.1 mL which is due to its composition. It comprises 90% of eugenol which is very much resistant against bacteria. The antiseptic compound in tea tree oil is tripenin-4-ol and in oregano oil is carvacrol but they comprise 60% and 30% of the compound, respectively [82].

4 Conclusions

The existing investigation designates the effective fabrication of PVA and Starch membranes crosslinked with glutaraldehyde. The investigation stayed focused on the development and characterization of antibacterial polymeric wound dressings. PVA and Starch entailed the polymeric matrix, and essential oils (clove oil, tea tree oil, oregano oil) as antibacterial agent. Nine samples with three different combinations were tested. The antibacterial efficacy was inspected against *Escherichia Coli* and *Staphylococcus aureus.* The diameter of inhibition zone was less against E-coli, which disclosed their greater contest to anti-biotic effects in 0.1 ml clove oil. The inhibition zone measured was 39±0.57mm for *Staphylococcus aureus* and for *Escherichia Coli* is 37±0.29mm. The hydrogels have exposed exceptional swelling capabilities against water, blood, $MgCl_2$ solutions and NaCl solutions. The results evidenced the ability of the prepared hydrogels to provide moist environment by meaningfully reducing the transmission of moisture from wound

bed. FTIR, SEM and XRD characterization techniques were applied to check the morphology as well as the 3-D crosslinking taking place in the membrane. Increasing the oil concentration starts to generate pores and became immiscible. XRD proves the semi crystalline nature of membranes, while FT-IR spectra showed that PVA and Starch are properly cross-linked with essential oils providing amine, hydroxyl and ether groups. The mechanical strength was decreased by the addition of essential oils but it was greater than the human skin. The 0.1 ml clove oil has shown best result from oregano oil and tree tea oil. Proceeding the basis of above outcomes, it can be claimed that the hydrogels with 0.1 ml clove oil have the outstanding results to be used as wound dressings operative for burn wounds.

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List of Tables

Table 1. Composition of hydrogel membranes.

Sample No	PVA:Starch (gm)	Cross-Linker	Oregano Oil (mL)	Tea Tree Oil (mL)	Clove Oil (mL)	Glycerin (mL)
1	5: 3.5	Ethanol/GA/HCL	0.1	-	-	2
2	5: 3.5	Ethanol/GA/HCL	0.2	-	-	2
3	5: 3.5	Ethanol/GA/HCL	0.3	-	-	2
4	5: 3.5	Ethanol/GA/HCL	-	0.1	-	2
5	5: 3.5	Ethanol/GA/HCL	-	0.2	-	2
6	5: 3.5	Ethanol/GA/HCL	-	0.3	-	2
7	5: 3.5	Ethanol/GA/HCL	-	-	0.1	2
8	5: 3.5	Ethanol/GA/HCL	-	-	0.2	2
9	5: 3.5	Ethanol/GA/HCL	-	-	0.3	2

Table 2. Contact angle measurement of hydrogel membranes with essential oils at different concentrations

Sr.No.	Classification	Essential Oil ml	Contact Angle	Standard Deviation
1.	PVA/Starch	--	51.1	0.11
2.	PVA/Starch/Clove oil	0.1	52.45	0.11
3.	PVA/Starch/Clove oil	0.2	55.85	0.23
4.	PVA/Starch/ Clove oil	0.3	71.7	0.522
5.	PVA/Starch/ Tea Tree oil	0.1	59.05	0.6
6.	PVA/Starch/ Tea Tree oil	0.2	63.8	0.98
7.	PVA/Starch/ Tea Tree oil	0.3	73.05	0.65
8.	PVA/Starch/ Oregano oil	0.1	60.65	0.87
9.	PVA/Starch/ Oregano oil	0.2	66.45	0.355
10.	PVA/Starch/ Oregano oil	0.3	80.4	0.22

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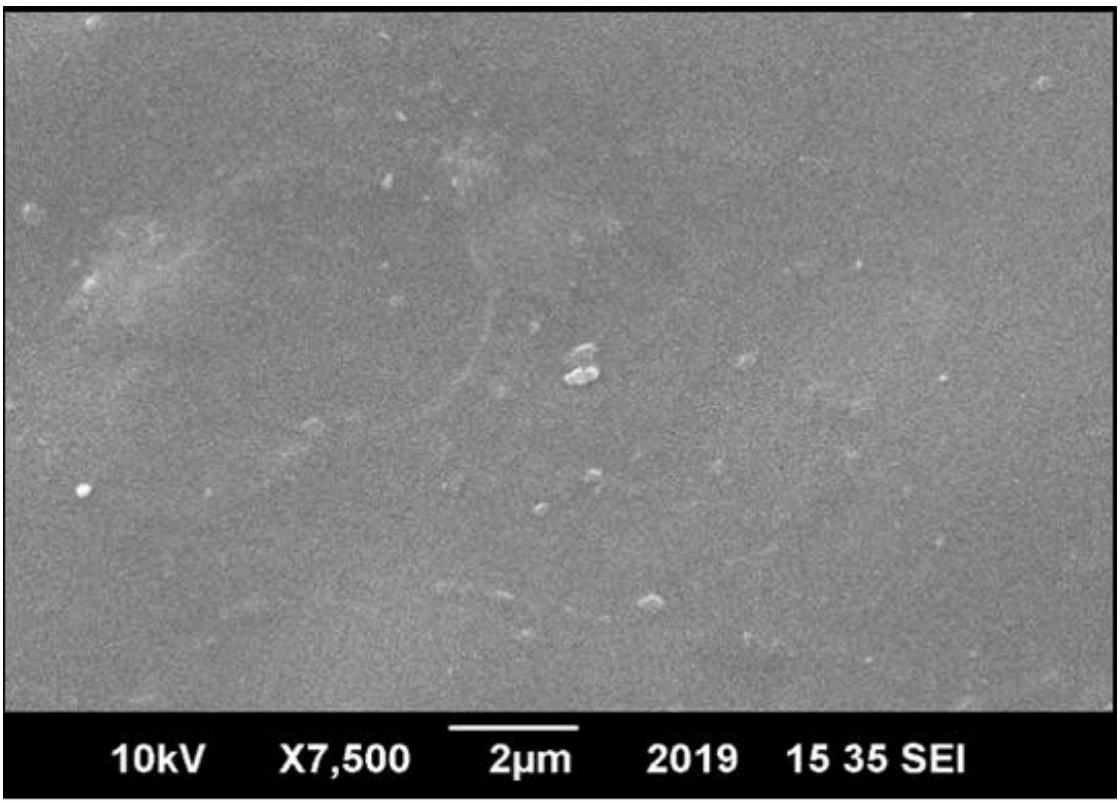

Fig. 1. Surface morphology of pristine hydrogel membrane.

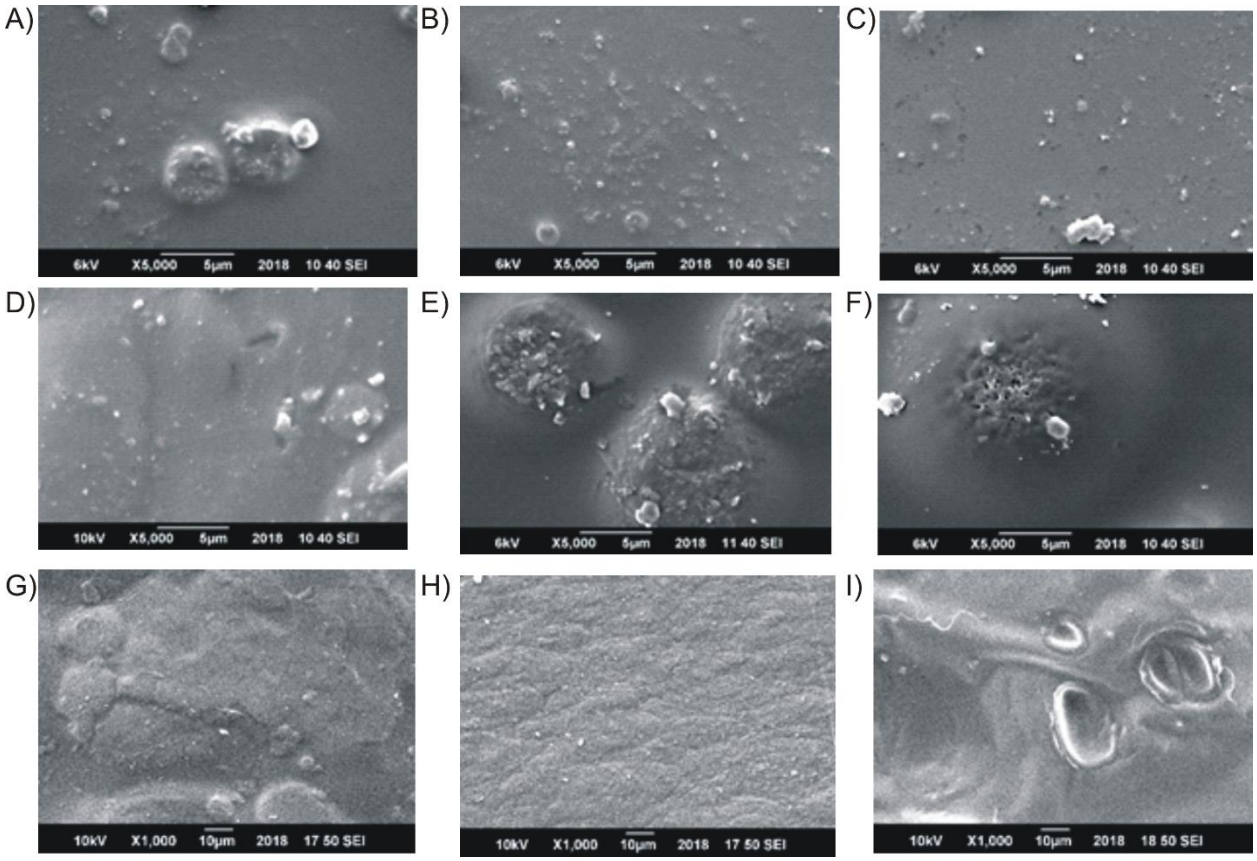

Fig. 2. SEM Images of hydrogel membranes using essentials oils; (a) 0.1 mL tea tree oil, (b) 0.2 mL tea tree oil, (c) 0.3 mL tea tree oil, (d) 0.1 mL clove oil, (e) 0.2 mL clove oil, (f) 0.3 mL clove oil, (g) 0.1 mL oregano oil, (h) 0.2 mL oregano oil, (i) 0.3 mL oregano oil.

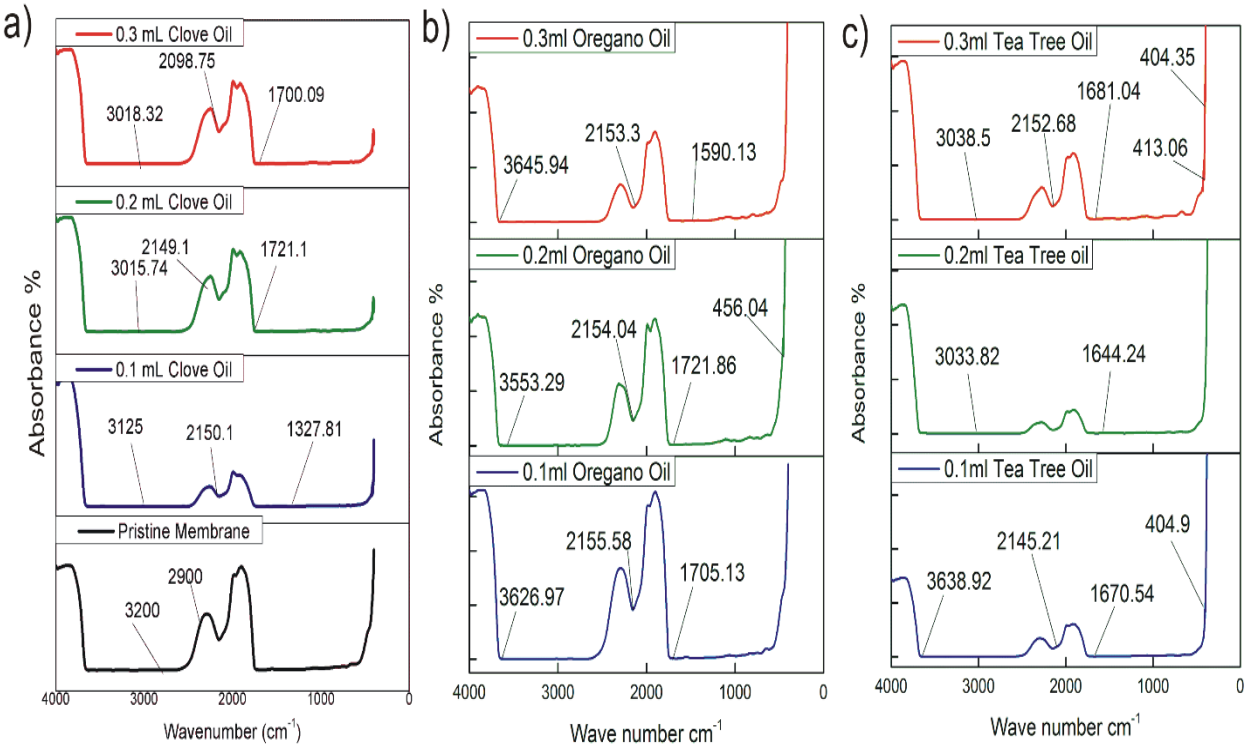

Fig. 3. FTIR spectra of hydrogels with essential oils at different concentrations.

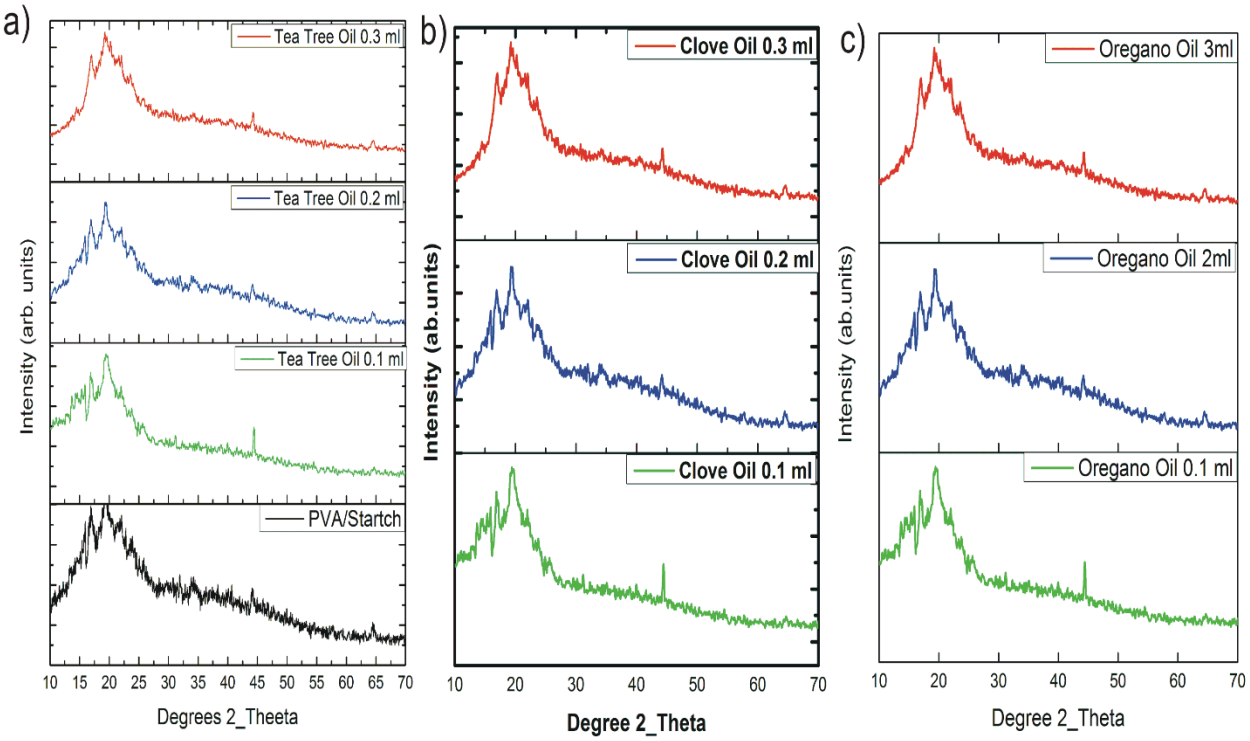

Fig. 4. XRD spectrum of hydrogels with essential oils at different concentrations.

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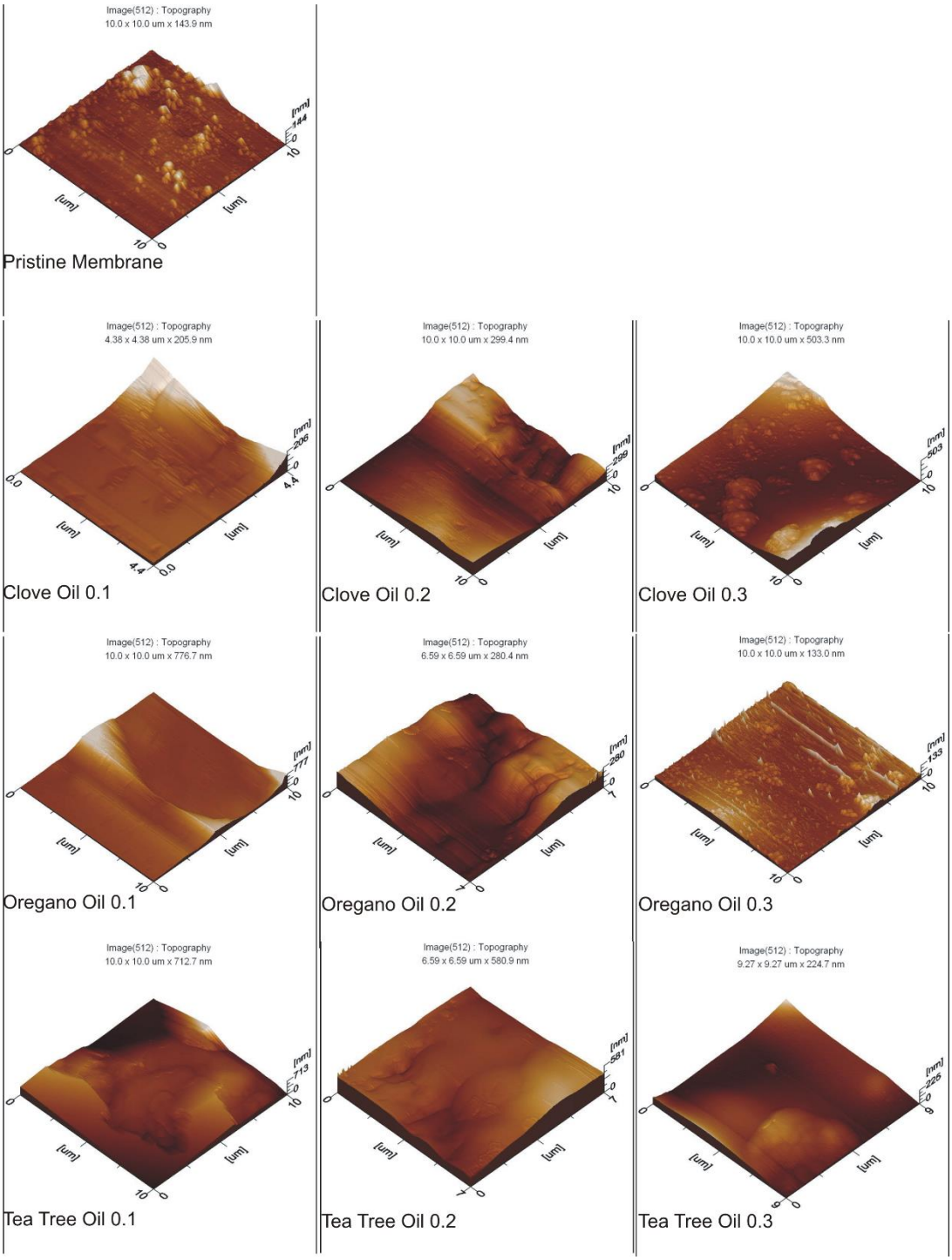

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877 **Fig. 5.** AFM analysis of hydrogel membranes with essential oils at different concentrations.

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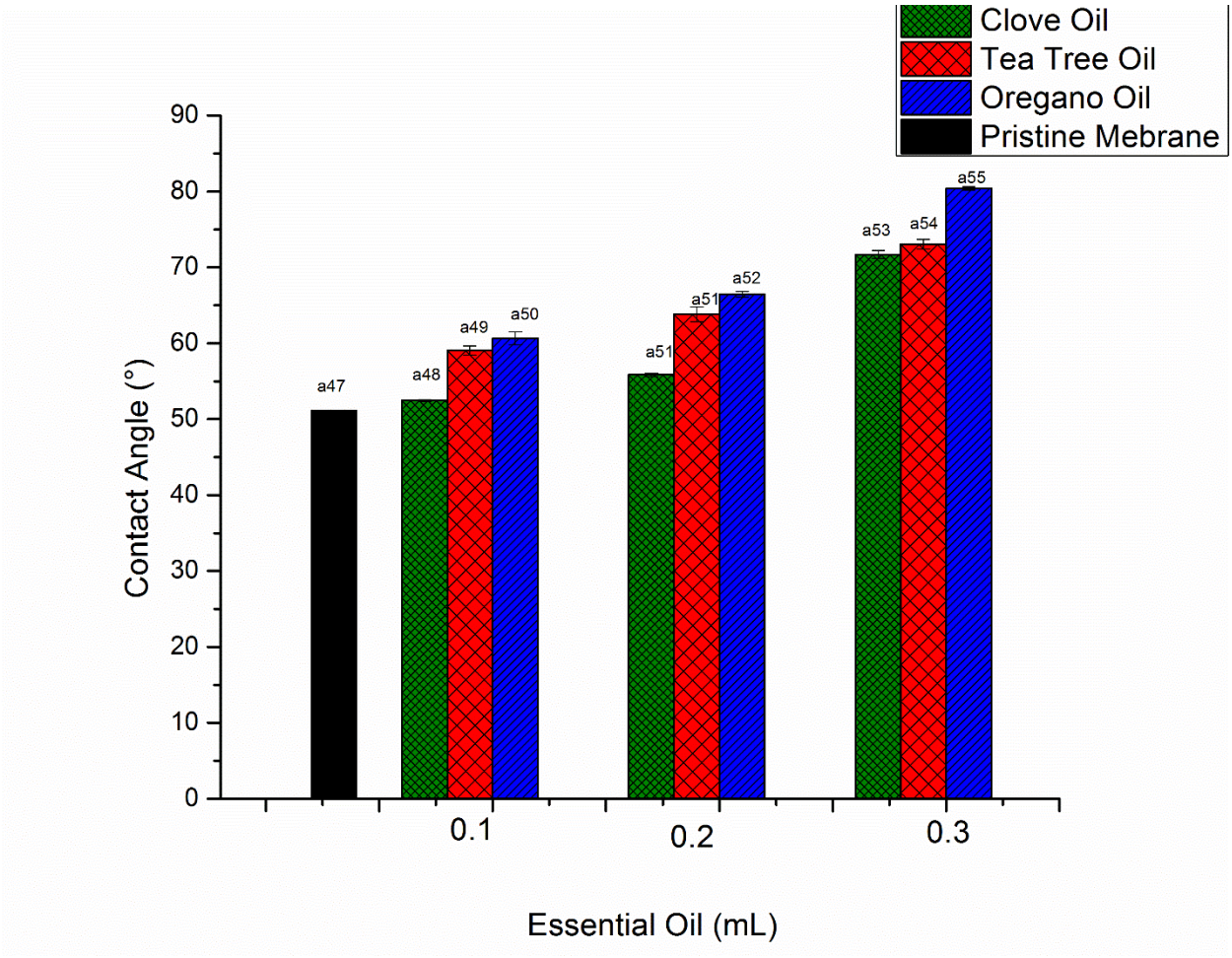


Fig. 6. Contact angle measurement of hydrogel membranes with essential oils at different concentrations.

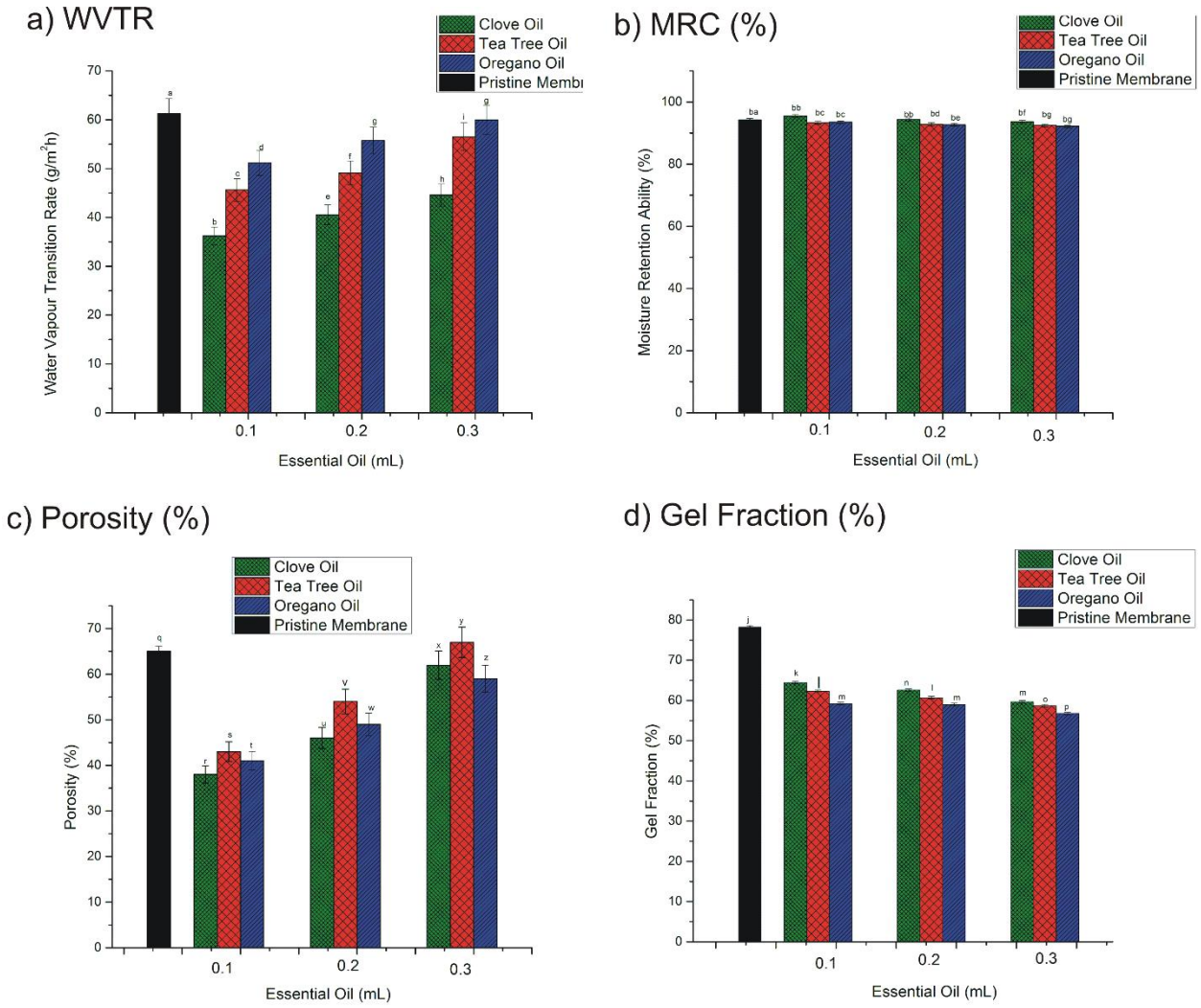
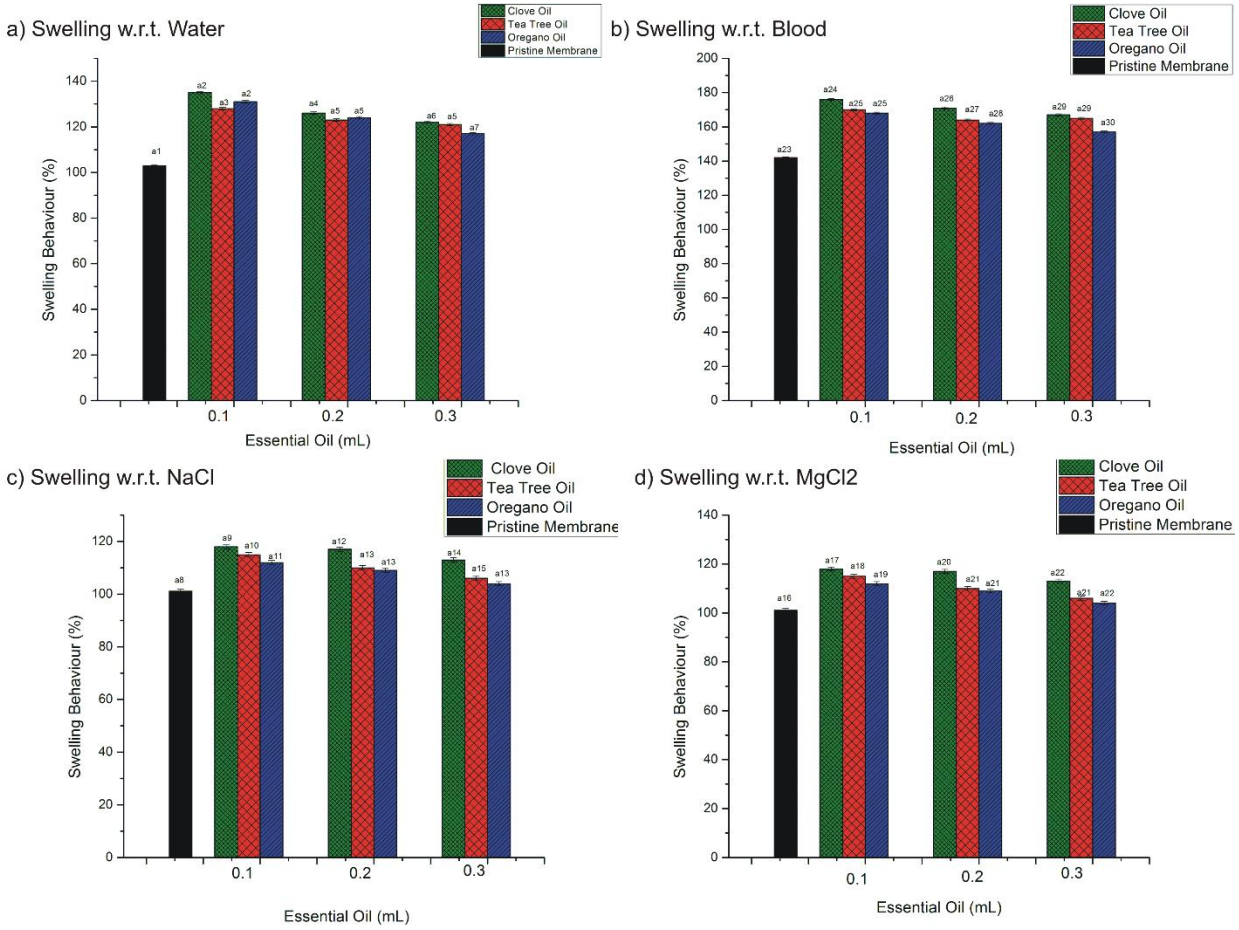
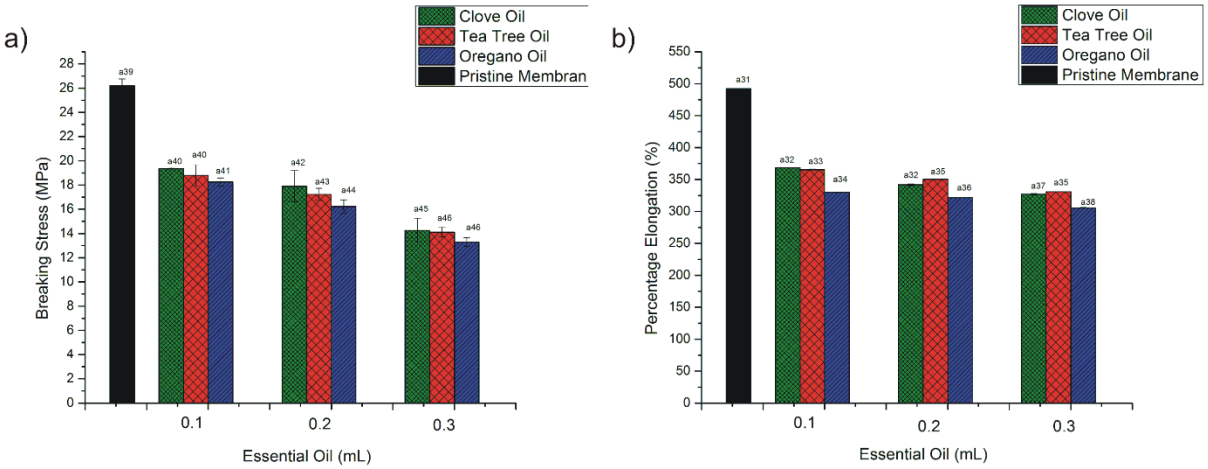


Fig. 7. Physical testing of hydrogels; (a) WVTR of PVA/starch/essential oil hydrogel membranes, (b) MRC of PVA/starch/essential oil hydrogel membranes, (c) Porosity of PVA/starch/essential oil Hydrogel Membranes, (d) Gel Fraction of PVA/starch/essential oil hydrogel membranes.



Bars with same alphabets are not significantly different ($p>0.05$) from each other.

Fig. 8. Swelling behavior of PVA/starch/essential oil hydrogel membranes against water, blood NaCl and $MgCl_2$.



Bars with same alphabets are not significantly different (p>0.05) from each other.

Fig. 9. Breaking stress and percentage elongation of hydrogels with essential oils.

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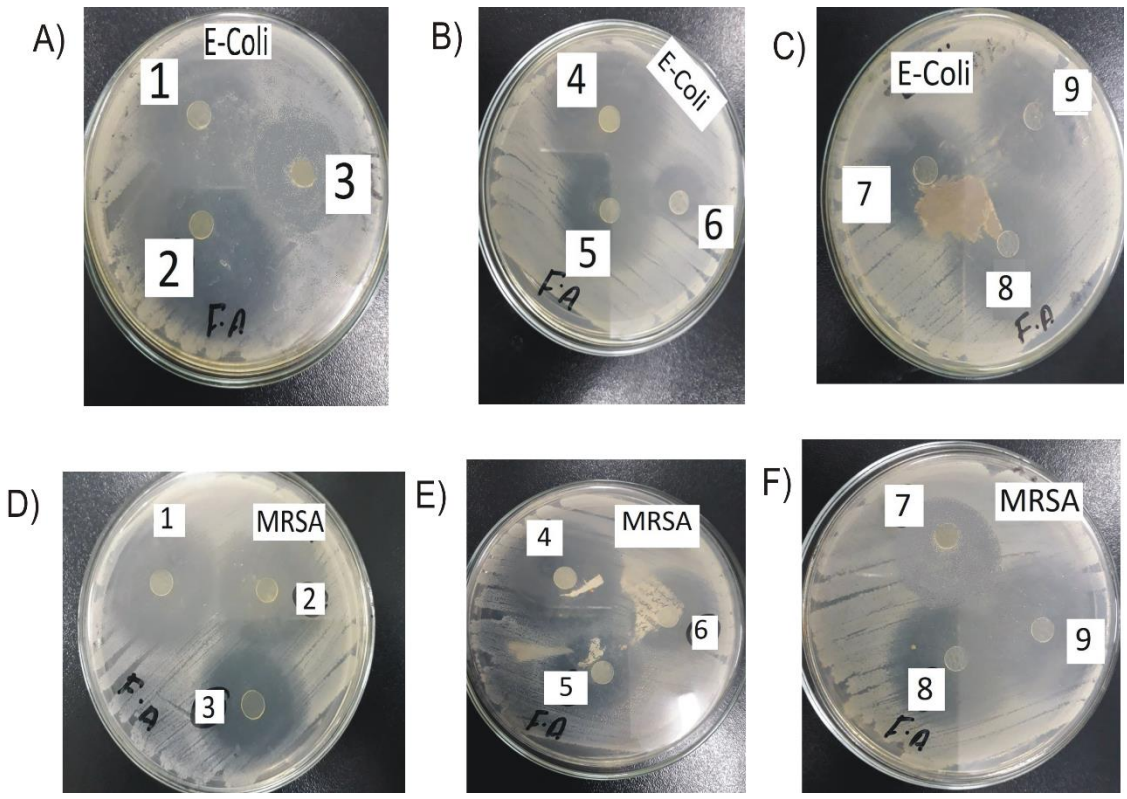

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911 **Fig. 10.** Inhibition zones of hydrogels; (a) E. coli with clove oil, (b) E. coli with tea tree oil, (c) E.
912 coli with oregano oil, (d) MRSA with clove oil, (e) MRSA with tea tree oil, (f) MRSA with oregano
913 oil.

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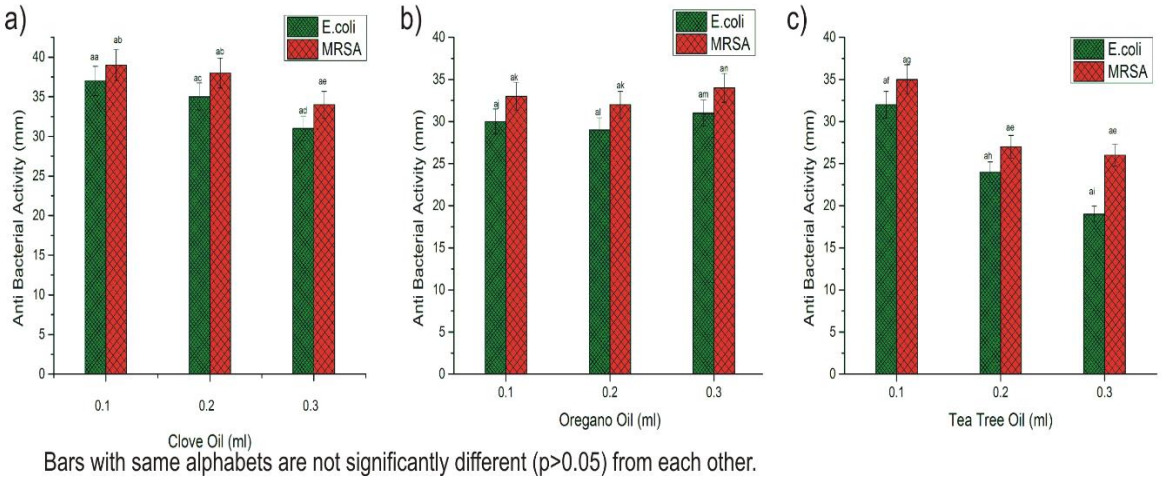

Fig. 11. Anti-bacterial activity with essential oils a clove oil, b tea tree oil c oregano oil.