STUDY ON THE ANTIBACTERIAL EFFECT OF ALGINATE FILM MODIFIED WITH CENTELLA ASIATICA EXTRACT

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This is to certify that the dissertation entitled **"STUDY ON THE ANTIBACTERIAL EFFECT OF ALGINATE FILM MODIFIED WITH CENTELLA ACIATICA EXTRACT"** Submitted by **Ms. Athira S, Ms. Devika B and Ms. Devikrishna S**, *in BSc Chemistry is an authentic work carried out under my supervision and guidance during the period of 2019-2022.*

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DECLARATION

I hereby declare that the thesis entitled **"STUDY ON THE ANTIBACTERIAL EFFECT OF ALGINATE FILM MODIFIED WITH CENTELLA ACIATICA EXTRACT"** submitted to University of Kerala Thiruvananthapuram for the partial fulfilment of the requirements for the award of graduation In Bachelor of Chemistry is a bonafide record of work done by me under the supervision of **Dr. Deepa Thomas**, Assistant Professor of P.G & Research Department of Chemistry, Bishop Moore College, Mavelikara during the academic year 2019-2022 and no part of this record has formed on the basis of power of any degree, diploma or other similar titles of any university.

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ABSTRACT

Alginate and Centella asiatica are natural materials having immense applications in biomedical field. These materials are widely used because of their biocompatibility, low cost, availability, non-toxic nature etc. In this work, hydrogel films composed of alginate, *Centella asiatica* extract, honey, crosslinked using calcium chloride is prepared. The surface wettability of film is detected. The developed film exhibits antibacterial properties against *Escherichia coli* and *Staphylococcus aureus*. Alginate film modified with *Centella asiatica* extract and honey shows antibacterial property and hydrophilic character so we suggest that it has application in wound healing.

Key words: Alginate, *Centella asiatica,* wound healing, biocompatibility, non-toxic

CONTENTS

Chapter-1

INTRODUCTION

1.1 ALGINATE

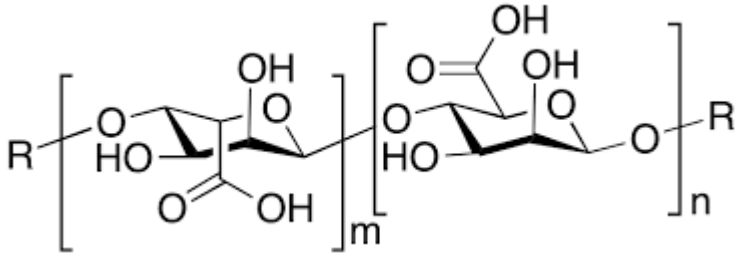
Alginates are natural colloidal polysaccharide group, which are water soluble, biodegradable, nontoxic and non-irritant in nature [1]. These are salts of alginic acid.

Alginic acid, also called algin, is a naturally occurring, edible polysaccharide found in brown algae. It is hydrophilic and forms a viscous gum when hydrated. Sodium alginate is a cell wall component of marine brown algae, and contains approximately 30 to 60% alginic acid. The conversion of alginic acid to sodium alginate allows its solubility in water, which assists its extraction [2].

Commercially available alginate is typically extracted from brown algae (Phaeophycean), including Laminaria japonica, Ascophyllum nodosum and Macrocystis pyrifera, by treatment with aqueous alkali solutions, typically with NaOH [3]. The extract is filtered and either sodium or calcium chloride is added to the filtrate in order to precipitate alginate. This alginate salt can be transformed into alginic acid by treatment with dilute HCl. After further purification and conversion, water-soluble sodium alginate powder is produced [4].

1.2 COMPOSITION OF ALGINATE

Alginate are linear or unbranched binary polymers made up of 1, 4 linkages between β-d-Mannuronic (M) and α-l-Guluronic (G) acids [2]. Brown algae are composed primarily of alginates, a carbohydrate polymer found in the form of an insoluble combination of potassium, sodium, magnesium and calcium salts of alginic acid that are structural components of brown seaweed cell walls [5].



α-l-guluronic acid β-d-mannuronic acid

Fig. 1 *Structure of Alginic acid*

The composition of alginates i.e., G:M ratio varies based on the source [6]. They can also be tailored or resized in many varieties by varying molecular weight, cation content, particle form,

volume fraction and G:M ratio. On a dry weight basis, the alginate contents are 22-30% for Ascophyllum nodosum and 25-44% for Laminaria digitata.

Alginate is a powder material that contains sodium alginate, calcium sulphate, trisodium phosphate, diatomaceous earth, zinc oxide and potassium titanium fluoride. When mixed with water, it makes a smooth gel like consistency that sets firmly enough to mold.

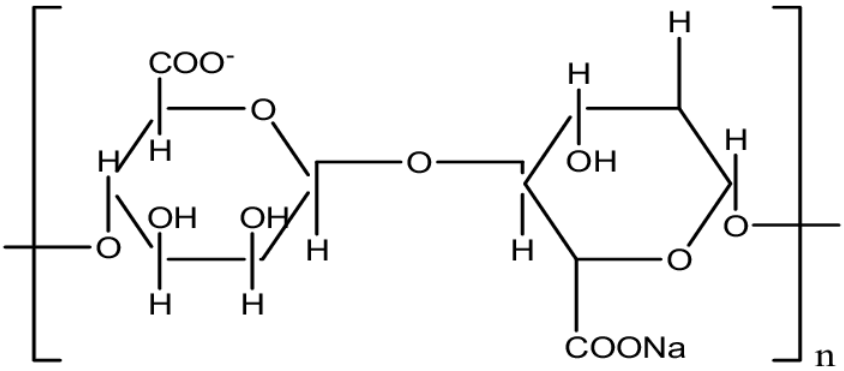


Fig. 2 *Structure of sodium alginate*

1.3 PHYSIO-CHEMICAL PROPERTIES

Alginates have the ability to form uniform, transparent, water insoluble and thermo-irreversible gels at room temperature, by cross-linking with di-valent or tri-valent ions. They have several properties such as film-forming ability, pH responsiveness and gelling, hydrophilicity, biocompatibility, bio-degradability, non-toxic, processability and ionic crosslinking. The biological function of alginate is structure supporting materials in brown algae.

Alginates due to their acidic nature make them a favourable biopolymeric biodegradable product in biomedical applications. Because of high acid content, alginates form gels due to the presence of Guluronic acid monomer in alginates within a short period especially in the presence of calcium ions. This property of gelling allows the alginate to possess multiple applications such as encapsulation of varied fragments or even cells interior of the alginate matrix with very low side effects [7].

Bacterial biosynthesis may provide alginate with more defined chemical structures and physical properties that can be obtained from seaweed-derived alginate. Bacteria such as Pseudomonas and Azotobacter can develop bacterial alginate as an exopolysaccharide. These

bacterial alginate producers may be able to produce alginates with particular monomer formulations and might be capable of producing `tailor made' bacterial alginate using genetic and protein engineering [8].

The ability of aqueous alginate solutions to form gels when treated with divalent ions or trivalent ions has been extensively explored for the fabrication of carriers for sustained or controlled delivery of therapeutic agents. This is due to intramolecular bonding and ionic interactions that exist within the carboxylic acid groups on the polymer matrix and the cations present [9,10]. The calcium or any other divalent or trivalent ions will interact with the G monomer present in the alginate structure to crosslink with another molecule, and the structure is identical to the egg box model [11].

The alginates with divalent or trivalent cations are not soluble in water because the alginates contain a terminal carboxylic ion, so these cations bond to this and yield an insoluble product. As a consequence, the alternative is the absorption of as much as 200-300 times their weight in water, thus swelling to a hydrogel of paste-like consistency. However, alginates with monovalent cations are soluble in hot and cold water. Alginates have a wide range of solubilities due to their different molecular weights. Alginates derived from *Ascophyllum,* for example, have aqueous solubility in the range of 22-30% weight percent, whereas those of two Laminaria groups are 17-33% weight percent and 25-44% weight percent, respectively [12,13].

Fig. 3 *Sodium alginate powder*

1.4 APPLICATIONS OF ALGINATE

Alginates are particularly known for their applications in pharmaceutical industries for controlled drug delivery [14], wound healing [15], dermatology [16], and scaffolds [17] because of their properties such as natural disintegration, gel formation, biocompatibility and non-toxicity. Alginates are natural gum that has an advantage over synthetic polymers because they form hydrogels, are less expensive and are readily accessible. Alginate gels may be orally administered into the body in a minimally invasive manner, enabling a wide range of pharmaceutical applications.

Alginate gels are promising biomaterials for tissue engineering and cell transplantation, to replace organs in patients that have lost or failed organs or tissues. In this approach, hydrogels are used to deliver cells to the desired site, provide a space for new tissue formation and control the structure and function of the engineered tissue [18,19]. Bacterial alginate may provide alginate with more defined chemical structures and physical properties that can be obtained from seaweed-derived alginate [20]. It is used for protection from the environment and the synthesis of biofilms in order to adhere to surface.

Alginates have various applications such as food manufacturing, pharmaceuticals and in textiles and cosmetics, particularly as an emulsifier and is also used in dentistry to make mold. More recently, alginate have been studied extensively due to its tissue compatibility and use in tissue engineering, including the regeneration of skin tissue, cartilage, bone, pancreas, liver, muscles and nerves, in addition to being used in the encapsulation of cells for the controlled release of drugs.

Due to their gelling and non-toxic properties, as well as their abundance in nature, the cosmetics and health care industries have shown a great deal of interest in biodegradable polymers in general and alginates particularly over the last few decades. Alginate wound dressings maintain a physiologically moist microenvironment, minimize bacterial wound healing. Drug molecules, from small chemical drugs to macromolecular proteins, can be released from alginate gels in a controlled manner, depending on the cross-linker types and cross-linking methods.

1.5 CENTELLA ASIATICA

Centella asiatica, commonly known as Gotu kok, kodavan, Indian pennywort and Asiatica pennywort, which is a clonal, perennial herbaceous creeper belonging to the family Umbellifere (Apiceae) is found throughout India growing in moist places upto an altitude of 1800 m. It is found in most tropical and subtropical countries growing in swampy areas, including parts of India, Pakistan, Sri Lanka, Madagascar, South Africa, South Pacific and Eastern Europe. About 20 species related to C. *asiatica* grow in most parts of the tropic or wet pantropical areas such as rice paddies and also in rocky, higher elevations [21]. It is a tasteless, odourless plant that thrives in and around water.

It has small fan-shaped green leaves with white or light purple-to-pink or white flowers and it bears small oval fruit. The whole plant is used for medicinal purpose [22]. It is widely used as a blood purifier as well as for treating high blood pressure, for memory enhancement and promoting longevity. In Ayurveda, *C.asiatica* is one of the main herbs for revitalizing the nerves and brain cells [23,24].

The primary active constituents of C. *asiatica* are saponins (also called triterpenoids), which include asiaticosides, in which a trisaccharide moiety is linked to the aglycone Asiatic acid, Madecassoside and Madasiatic acid [25]. These triterpene saponins and their sapogenins are mainly responsible for the wound healing and vascular effects by inhibiting the production of collagen at the wound site.

1.6 APPLICATION OF CENTELLA ASIATICA

Centella asiatica showed antimicrobial activity against all pathogenic bacteria tested except B.subtilis [26]. In Southeast Asia, it is traditionally used for the treatment of a wide variety of disorders such as skin diseases, rheumatism, inflammation, syphilis, mental illness, epilepsy, hysteria, dehydration and diarrhea.

It is widely used as a blood purifier as well as for treating high blood pressure, for memory enhancement and promoting longevity. In ayurveda, C. *asiatica* is one of the main herbs for revitalizing the nerves and brain cells [23,24]. Eastern healers relied on *asiatica* to treat emotional disorders, such as depression, that were thought to be rooted in physical problems. In the western medicine, during the middle of the twentieth century, C. *asiatica* and its alcohol extracts reported

to have shown positive results in the treatment of leprosy. It has been considered as brain tonic due to its wide beneficial neuroprotective activity. It shows effects such as anti-inflammatory, antiproliferative, anticancer, antioxidant, antiulcer, wound healing, etc. [27].

Chapter-2

AIM AND OBJECTIVE

AIMS AND OBJECTIVES

Preparation of Antimicrobial Alginate-Centella asiatica extract film.

- Preparation of alginate film.
- Preparation of the extract of C. *asiatica.*
- Preparation of alginate film modified with the C. *asiatica* extract.
- Antibacterial evaluation of alginate film modified with C. *asiatica* extract.

SCOPE OF THE STUDY

Alginates are established among the most versatile biopolymer, used in the wide range of application. The conventional use of alginates as excipient in drug products generally depends on the thickening, gel forming and stabilizing properties. The main industrial application of alginate is linked to its stabilizing, viscosifying, gelling properties and its ability to retain water. Alginate films are widely used in the technical applications like engineering, biomedical engineering or cell encapsulation etc. Biodegradable alginate films can be used in wound healing and the sustained release of drug to the specific target site. Due to the high affinity for chelation with polyvalent metal cations in particular with divalent metal ions the alginate readily formed the coordination biopolymer cross-linked metal Alginate hydrogel. The aim of the present study is to develop a film based on alginate, honey and *Centella asiatica* extract which is proposed for wound healing application. Alginate-*Centella asiatica* extract film show antibacterial and hydrophilic property so it can be used in wound healing purpose. The materials used in this study are biodegradable, biocompatible, nontoxic etc.

Chapter-3

LITERATURE REVIEW

- **Natalia C Homem, et al.** studied the antibacterial assessment of sodium alginate films loaded with Propolis extract (PE). This extract has been applied as antibacterial against the most prevalent bacteria found in infected wounds. Moreover, PE can induce tissue regeneration. The goal of this work was to produce PE-loaded biocompatible polymeric films, composed of sodium alginate (SA) / gelatin (GN), via the solvent casting / phase inversion technique, followed by cross-linking with CaCl2 at 2 wt.% in dH2O. SA/GN films were fabricated and functionalized with PE before and after confirmed via FTIR spectroscopy. The antibacterial activity of the films was evaluated via agar diffusion and killing time-kinetics. Results showed that PE loaded SA/GN films were capable of efficiently inhibit the growth of specific bacteria which is found in wounds like Staphylococcus aureus and Pseudomonas aeruginosa and thus SA/GN/PE films can be considered as potential delivery platforms of PE for applications in wound healing [20].
- **Angel Serrano Aroca, et al.** studied the antiviral properties of alginate-based biomaterials as promising antiviral agents against SARS-Cov-2. Alginate is a biodegradable, renewable, biocompatible, water soluble and antiviral polymer with many potential biomedical applications. In this regard, this shows 17 types of viruses that have been tested in contact with alginate and its related biomaterials. This biopolymer has the capacity to bind to RAV envelopes. The anionic polysaccharides such as alginates could increase the negative charge of the viral envelope glycosylated G protein and the ionic receptor sites of eukaryotic cells. Furthermore, SA exhibited a strong inhibitory effect against TMV (Tobacco Mosaic Virus). When an alginate was added to the inoculum mixture, the quantity of lesions observed on Xanthi tobacco leaves was significantly reduced and the inhibition effect improved as the alginate concentration rise. All these results shows that the positive sense single stranded RNA viruses like SARS-Cov-2 render alginate-based materials highly promising in the COVID-19 pandemic. It is mainly attributed to viral aggregation and viral inhibition through interaction of alginate-based materials with components of the viral envelope [21].
- **Dong Su Cha, et al.** were prepared sodium alginate and K-Carrageenan-based antimicrobial films to examine their antimicrobial effect and properties. Antimicrobial agents: lysozyme, nisin, grape fruit seed extract (GFSE) and ethylenediamine tetra acetic acid (EDTA) were incorporated into films, both alone and in combination. Sodium alginate-based films exhibited larger inhibitory zones compared to K-Carrageenan-based films even within similar

combinations and levels of antimicrobial agents. GFSE-EDTA in both sodium alginate and K-Carrageenan-based films showed inhibitory effect against all indicator organisms. Tensile strength values were weaker and elongations before breakage is less frequent for films with antimicrobial compounds compared to that of films without the antimicrobial compounds [22].

- **Lokender Kumar, et al.** conducted study antimicrobial biopolymer formation from sodium alginate and algae extract using aminoglycosides. Antimicrobial polymers provide a biodegradable, sustainable, safe and cheap approach to drug delivery and wound dressing to control bacterial infection and improve wound healing. Here, they report a one-step method of making antimicrobial alginate polymer from sodium alginate and aqueous extract of Wakame using antibiotic aminoglycosides. Thin layer chromatography of commercially available sodium alginate and Wakame extract showed similar oligosaccharide profiles. Screening of six aminoglycosides showed that kanamycin disulphate and neomycin sulphate produces the highest amount of biopolymer, however, kanamycin disulphate produces the most malleably and form fitting biopolymer. Slow release of antibiotics and the resulting zone of inhibition against E. coli DH5α were observed by agar well diffusion assay. Inexpensive method of production and slow release of antibiotics will enable diverse applications of antimicrobial alginate biopolymer reported in this paper [23].
- **Tugce Senturk Parreidt, et al.** prepared alginate based edible films and coatings for food packaging applications. Alginate-based edible coatings and films attract interest for improving qualities and extending shelf-life of fruits, vegetables, meat, poultry, sea food and cheese by reducing dehydration (as sacrificial moisturising agent), controlling respiration, enhancing product appearance, etc. In this cited paper they review the most recent essential information about alginate based edible coatings. Emphasis will be placed on active ingredients incorporated into alginate-based formulations, edible coatings/film application methods, research and development studies of coated food products and mass transfer and barrier characteristics of the alginate-based films [24].

Chapter-4

MATERIALS AND METHODS

4.1 MATERIALS

Materials needs for the preparation are:

Sodium alginate, extract of *Centella asiatica,* honey, calcium chloride, deionised water.

4.2 METHODS

4.2.1 Preparation of alginate film:

3g of sodium alginate is diluted with 100 ml of deionised water. It is stirred continuously to get a homogenous solution. Polyethylene glycol is added into deionised water and ultrasonicated for 10 minutes. This solution is added inti alginate solution dropwise with continuous stirring. The principle behind this method is the solvent casting method. To the above solution of 1% of 15/10 ml CaCl2 is added dropwise with continuous stirring. It is then brought to a homogenous stirrer and again ultrasonicated to about 20 minutes. The resulting solution is then transferred to a pan and heated in an oven at room temperature (about 40°C) for 1 day. The film thus obtained can be used for further study.

4.2.2 Preparation of the extract of *Centella asiatica*

Plant leaves were collected from their natural sources and grinded to a paste with a little amount of deionised water. The paste is then diluted with about 100 ml of deionised water. The solution is stirred continuously to get a uniform concentration throughout. It is then filtered through a muslin cloth or sieving vessel to get extract only. For more accuracy, the solution can be centrifuged for about 40 minutes and the supernated solution or extract can be filtered by using the above process. The pure extract devoid of leaf particles thus obtained can be stored in air tight bottles for further studies. To get more concentrated extract, the amount of water or organic solvent which are added in the initial step can be reduced.

4.2.3 Preparation of Alginate film modified with the extract

About 3g of sodium alginate powder is dissolved in 100ml of deionized water and stirred continuously for about 15 minutes. Then about 10ml of the extract of C. *asiatica* is measured in a measuring jar and added carefully to the above solution with stirring. All through the process stirring is very essential to get rid of the solid particle formation in the solution. It is then

transferred to a mechanical stirrer and continue the process of stirring for about 20 minutes. During this time, 5ml of honey is added drop-wise to this beaker. It is added as a plasticizer to improve the binding nature and also to increase the viscous property of the solution. Meanwhile a few grams of 1% CaCl2 is dissolved in 10ml of deionized water in a small beaker. When the CaCl2 particles are completely soluble, it is added to the solution in the stirrer using a dropper very slowly by taking about 1 hr. After 1 hr, we get a viscous liquid with very low mobility. It is then fed to a homogenous stirrer and stirred for half an hour. Now we are left with a solution with uniform concentration throughout. For more accurate homogenous solution, the beaker is then transferred to an ultrasonicator. If any minute solid particles were present, they can be removed in this step. Sonication uses sound waves via an ultrasonic bath or ultrasonic probe to agitate particles in the solution. It converts an electrical signal into a physical vibration to break substances apart. These disruptions can mix solutions, accelerate the dissolution of a solid into a liquid form. After 20 minutes, it is removed from the sonicator and the solution is transferred to a Teflon pan. It is trapped 3-4 times to get the uniformity throughout. The pan is then placed in a microwave oven for 24 hours. Thus, we get the film of sodium alginate modified with the extract of C. *asiatica*.

4.2.4 Antibacterial Studies

Antibacterial activity – (Zone of inhibition) Well diffusion method.

To test the antagonist activity of films, it was dissolved in DMSO tested against both gram-negative and positive strain was detected using well-diffusion assay, for selected entero-pathogens purchased from Ramachandra Medical college are as follows: Escherichia coli, Staphylococcus aureus as indicator strains. Overnight bacterial culture in MRS broth was centrifuged for 20 min at 4°C and 10,000 g. Supernatants were filtered through a 0.22 mm filter (Millipore, France) to remove residual cells. The supernatant placed in 4 mm diameter wells punched using German punch borer was tested for antagonist activity against the above-mentioned pathogens which were plated into Muller Hinton agar (Becton, Dickinson and Company, USA). The final concentration applied was (0.0004) mg/ml. The volume of extract used in the well was 100µL.

Growth curve profiling.

To examine the bacterial growth curve in presence of the film, the test organisms (Escherichia coli and Staphylococcus aureus) were grown in Nutrient broth (M002, HI Media, Mumbai, India). Subsequently, 2 × 108 CFU/ml of test organisms were added to the above broth as inoculum and all the flasks were put on shaker incubator (180 rpm) and incubated at 37◦ C. The control group was maintained without sample. The bacterial growth was indexed by measuring optical density at every 1 h (up to 24 h) at 600 nm using a spectrophotometer (Shimadzu UV-Spectrophotometer UV-1601).

Antibacterial assay

The bacteriosedal or bacteriostatic activity of the sample was tested against pathogens including Escherichia coli and Staphylococcus aureus by micro-broth dilution assay, following the guidelines of the Clinical and Laboratory Standards Institute (CLSI) using Muller-Hinton broth (HI Media, Mumbai, India). Sample was serially diluted to obtain the final concentration ranges from 0.02 and 0.004 mg/mL in a sterile cuvette. 1L suspension of inoculum whose density was adjusted to that of 0.1 McFarland standards by 10 times diluted media (approximately 1 to 2 × 108 CFU/ml) were added and incubated at 37 ◦C for 24 h. The MIC values were read at 600 nm in a Bio-Photometer (Eppendorf Spectrophotometer UV/VIS) (Volex cuvettes, India) and values were calculated.

4.2.5 Surface film wettability measurement

Wettability is an important factor that influence several properties of the biomaterials, such as the water absorption, in vitro and in vivo degradation and cell interaction. The water contact angle was determined to evaluate the surface films wettability, as well the influence of *Centella asiatica* extract content in its properties. The static contact angle (&) measurements were performed through the equipment OCA 20 (Data Physics) using water (10 µl of volume drop and 2 µl/s of velocity). For each condition, ten measurements were performed using the sessile drop method.

Chapter-5

RESULT AND DISCUSSION

Alginate and *Centella asiatica* are very useful natural materials that have tremendous benefits in the biomedical field especially for drug delivery applications and its wound healing properties. A wound dressing material can successfully be prepared from alginate, a natural polymer capable of forming into hydrogels, and the extract of the plant *Centella asiatica* which has commonly been used in traditional medicine to heal wounds. Alginate was selected as the matrix for the film preparation due to its high blending properties with *Centella asiatica* and honey. Also, its availability, low cost, biocompatibility and non-toxic nature to human tissues helped best to achieve the goal.

Alginate- *Centella asiatica* film was prepared by stirring a mixture of sufficient amount of alginate, *Centella asiatica* extract, honey and drop wise added $CaCl_2$ on a magnetic stirrer for 1 hour. It is then fed to a homogenous stirrer for half an hour to increase the mobility of viscous liquid. For more accurate homogenous solution, the beaker is then transferred to a ultrasonicator. Sonication uses sound waves via an ultrasonic probe to agitate particles in the solution. The solution is poured on the pan and dried in the oven to get the film.

The therapeutic properties of *Centella asiatica* like antiseptic, anti-inflammatory and antibacterial properties enhanced the wound healing properties and many limitations of the alginate. Honey in the film promotes autolytic debridement, stimulates growth of wound tissue and stimulates anti-inflammatory activities thus accelerates the wound healing process. In addition of honey improves the outcome of the wound healing by reducing the incidence and excessive scar formation.

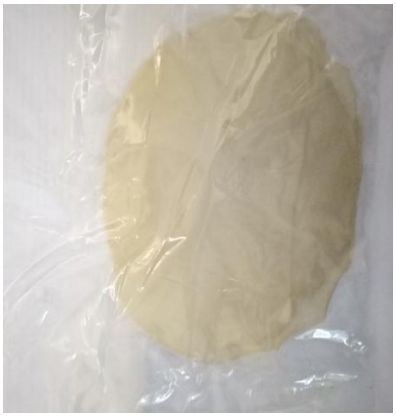


Fig.4 *Alginate film*

5.1 ANTIBACTERIAL STUDIES

The antibacterial properties of the Alginate film modified with *Centella asiatica* extract are studied against indicator organisms like *E-coli* (gram negative bacteria) and *S.aureus* (gram positive bacteria) by plate assay and the formation of a clear zone was obtained as shown in the Figure below. The antibacterial activity was effective against both gram negative bacteria and gram-positive bacteria. From the results it is evident that the *Centella asiatica* extract in the alginate matrix can effectively prevent the growth of microbes in wound healing process. The developed film is effective against *E.Coli* and *S. aureus.*

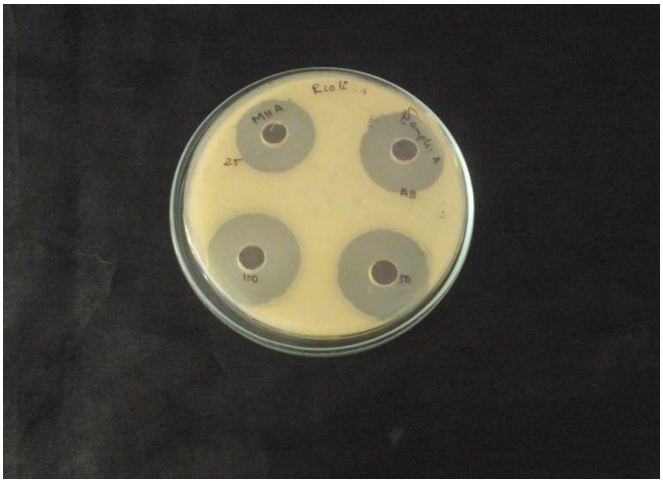


Fig. 5

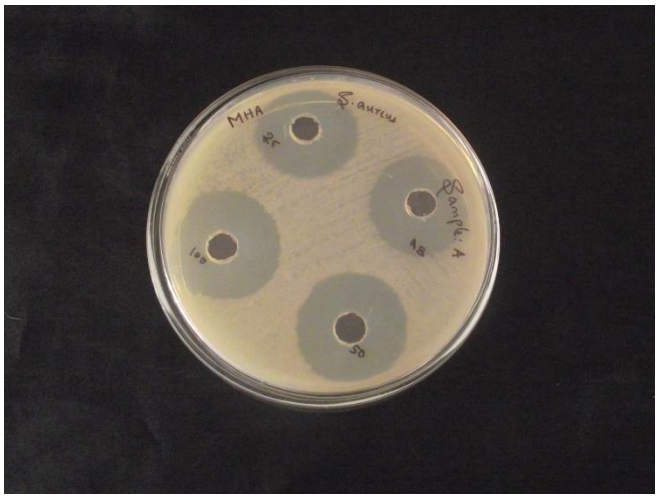


Fig.6

Antibacterial activity of Alginate- *Centella asiatica* extract film against

Fig. (5) *E-Coli*.

Fig. (6) *S.aureus*.

5.2 SURFACE FILM WETTABILITY

Wettability is dependent on many properties of biomaterials such as water absorption, cell interactions, in-vivo and in-vitro degradations etc. The obtained water contact angle gives the hydrophilicity of thin films and its values are given in the table. The values support the affinity of alginate film towards water. A small reduction in the contact angle was observed in the case of alginate film modified with *Centella asiatica* extract. But the addition of *Centella asiatica* extract does not make any drastic changes in the hydrophilicity of the films so it can be used in wound dressings.

Sample	Contact angle (º)
ALG	32.66±2.67
ALG + Extract	28.89±2.41

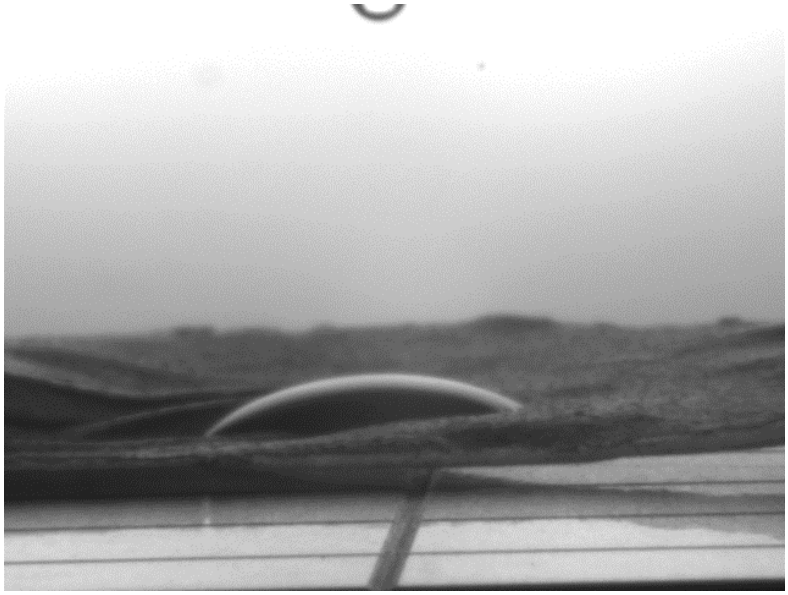


Fig (7)

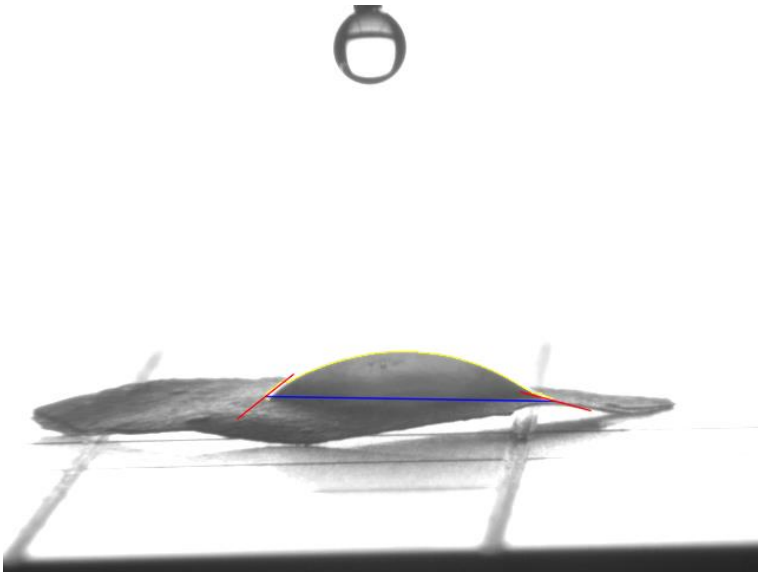


Fig (8)

Image of contact angle measurement; ***Fig (7).*** Alginate base film.

Fig (8). Alginate- *Centella asiatica* extract film.

Chapter 6

CONCLUSION

CONCLUSION

Effective wound healing using natural materials is acquiring immense attention in the current status. In this study thin films composed of alginate, *Centella asiatica* extract, and honey crosslinked using $CaCl_2$ is successfully prepared by solvent casting method. The crosslinking using Ca to the film improved its mechanical properties. The hydrophilicity of the film is clear in the contact angle measurement. The extract of traditional plant *Centella asiatica* used in the film had enhanced antibacterial resistance for the efficient wound healing process. Honey in the film promotes autolytic debridement, stimulates growth of wound tissue and stimulates anti-inflammatory activities thus accelerates the wound healing process. In addition of honey improves the outcome of the wound healing by reducing the incidence and excessive scar formation. Due to the antibacterial and hydrophilic nature of the developed film it can be proposed for wound healing application and further studies may require.

REFERENCE

1. Md Saquib Hasnain, et al. Alginates: Sources, Structure and Properties, Alginates in Drug Delivery, 2020.
2. L.A. Loureiro dos Santos. Natural Polymeric Biomaterials: Processing and Properties, Materials Science and Materials Engineering, 2017.
3. Clark DE, Green HC. Alginic acid and process of making same. 2036922 US Patent. 1936.
4. Rinaudo M. Main properties and current application of some polysaccharides as biomaterials, 2008.
5. S.Giridhar Reddy. Alginates-A Seaweed Product: Its Properties and Applications, 2021
6. Domb AJ, Wiseman DM. Handbook of Biodegradable Polymers. Boca Raton, CRC Press, 1998.
7. Klock G, et al. Biocompatibility of mannuronic acid-rich alginates,1997.
8. Lain Hay D, et al. Bacterial biosynthesis of alginates, Journal of chemical tech and Biotech, 2010.
9. Miyazaki S, et al. Oral sustained delivery of theophylline using in-situ gelation of sodium alginate, 2000.
10. Katchalsky A, et al. Counter-ion fixation in alginates, 1961.
11. Grant G T, et al. Biological interactions between polysaccharides and divalent cations: The egg-box model,1973.
12. Das M K, Senapati P C. Furosemide-loaded Alginate microspheres prepared by ionic cross-linking technique: Morphology and release characteristics, 2008.
13. da Silva T L, et al. Alginates and Sericin: Environmental and Pharmaceutical Applications. In Biological Activities and Applications of Marine Polysaccharides, 2017.
14. Hanne Hjorth Tonnesen, Jan Karlsen. Drug Development and Industrial Pharmacy, Volume 28, 2002.
15. Alginate dressings, Hand book of medical textiles, 2011.
16. Maedeh Bahadoran, et al. Scientific Reports, 2020.

17. Maryam Farokhi, et al. International Journal of Polymeric Materials and Polymeric Biomaterials. Alginate Based Scaffolds for Cartilage Tissue Engineering: A Review, 2020.
18. Langer R, Vacanti J P. Tissue engineering. Science. 1993.
19. Lee K Y, Mooney D J. Hydrogels for tissue engineering 2001.
20. Remminghorst U, et al. Bacterial alginates: from biosynthesis to applications 2006.
21. Bown D. Encyclopaedia of Herbs and their Uses, 1995.
22. Singh P, Singh J S. Recruitment and competitive interaction between ramets and seedlings in a perennial medicinal herb, *Centella asiatica*, 2002.
23. PDR for herbal medicine. 1st edition, 1999.
24. Hagemann R C, et al. Gotu Kola, In, The Lawrence Review of Natural Products: facts and comparisons, Facts and Comparisons Division, 1996.
25. Singh B, Rastogi R P, A reinvestigation of the triterpenes of *Centella asiatica*, 1969.
26. Siti Mudaliana. Antimicrobial activity of *Centella asiatica* and *Gigantochloa apus*, 2021.
27. Tarun Belwal, et al. Nonvitamin and Nonmineral Nutritional Supplements, 2019.
28. Natalia C Homem, et al. Antibacterial assessment of sodium alginate/gelatin film loaded with propolis extract, 2021.
29. Angel Serrano Aroca, et al. Antiviral properties of alginate-based biomaterials: promising antiviral agents against SARS-Cov-2, 2021.
30. Dong Su Cha, et al. Anti-microbial films based on Sodium alginate and K-Carrageenan, 2002.
31. Lokender Kumar, et al. Anti-microbial biopolymer formation from sodium alginate and algae extract using aminoglycosides, 2019.
32. Tugce Senturk Parreidt, et al. Alginate based edible films and coatings for food packaging applications, 2018.