“Formulation of hydrogel using shellac and curcumin for wound healing”

Project report submitted in partial fulfillment of the requirement

For the degree of Bachelors of Technology in

Biotechnology

by

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and

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under the supervision of

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to

Department of Biotechnology and Bioinformatics

Jaypee University of Information and Technology,

Waknaghat, 173234, Himachal Pradesh

CERTIFICATE

This is to certify that the work titled “Formulation of hydrogel using shellac and curcumin for wound healing”, submitted by Bhavini Sharma (201808) and Wakeeta Sharma (201810) in partial fulfillment for the award of degree of B. Tech in Biotechnology at Jaypee University of Information Technology, Solan has been carried out under my supervision. This work has not been submitted partially or wholly to any other University or Institute for the award of this or any other degree or diploma.

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Date: 29th May, 2024

CANDIDATE'S DECLARATION

It is hereby declared that the work presented in this report entitle "Formulation of hydrogel using shellac and curcumin for wound healing" in partial fulfilment of the requirements for the award of the degree of Bachelor of Technology in Biotechnology submitted in the Department of Biotechnology and Bioinformatics, Jaypee University of Information Technology, Waknaghat is an authentic record of our own work carried out over a period from AUGUST 2023 to May 2024 under the supervision of Dr.Ashok Kumar Nadda (Associate Professor), Department of Biotechnology and Bioinformatics, JUIT. The matter embodied in the report has not been submitted for the award of any other degree or diploma. This is to certify that the above statement made is true to the best of our knowledge.

Bhavini Sharma (201808)

Wakeeta Sharma (201810)

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It was really helpful to us, and we learned many interesting facts regarding the growth and development of Hydrogels from shellac and curcumin. We were also enlightened to the various processes of characterizing the hydrogel in terms of its physio-chemical Properties and other processes like Soxhlet extraction and TLC .

The lab staff, for their dedication to academic excellence and for providing a stimulating environment conducive to learning and research. Their passion for their respective fields of study has inspired us and played a significant role in the development of our knowledge and research skills.

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CHAPTER 1 INTRODUCTION

The female lac insect Kerria lacca, which has a six-month life cycle, secretes and deposits shellac on trees in the forests of Thailand and India. Chemically, it is mostly composed of natural waxes such as aleuritic acid and shellolic acid.

The source organism of shellac is shown in **(Fig. 1)**

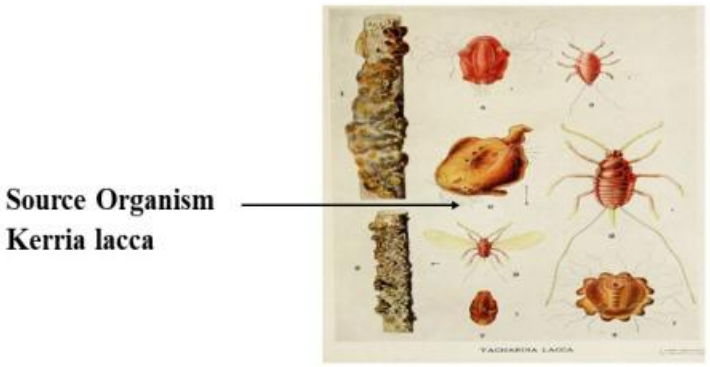

Fig.1 Female bug Kerria lacca [1]

After being processed and sold as dry flakes, it becomes liquid shellac that can be used as a wood finish, culinary glaze, or brush-on colorant **[1].** Shellac can be used as a high-gloss varnish, tannin and odor blocker, sanding sealer, stain, and long-lasting natural primer. Shellac has long been utilized in electrical applications because of its potent insulating properties and capacity to keep moisture out. Since it replaced oil and wax finishes in the 19th century, shellac has been a popular wood finish in the western world. Warm colors ranging from various tones of brown, yellow, orange, and red are available in shellac **[1].** These colors span from extremely pale blonde to extremely dark brown. Color is influenced by the lac bug's habitat, the sap of the tree it lives on, and the harvest season. Throughout the 20th century, wood paneling and cabinetry were frequently stained and protected with "orange shellac," the most common kind of shellac. The shellac chemical structure is displayed in **(Fig. 2B).**

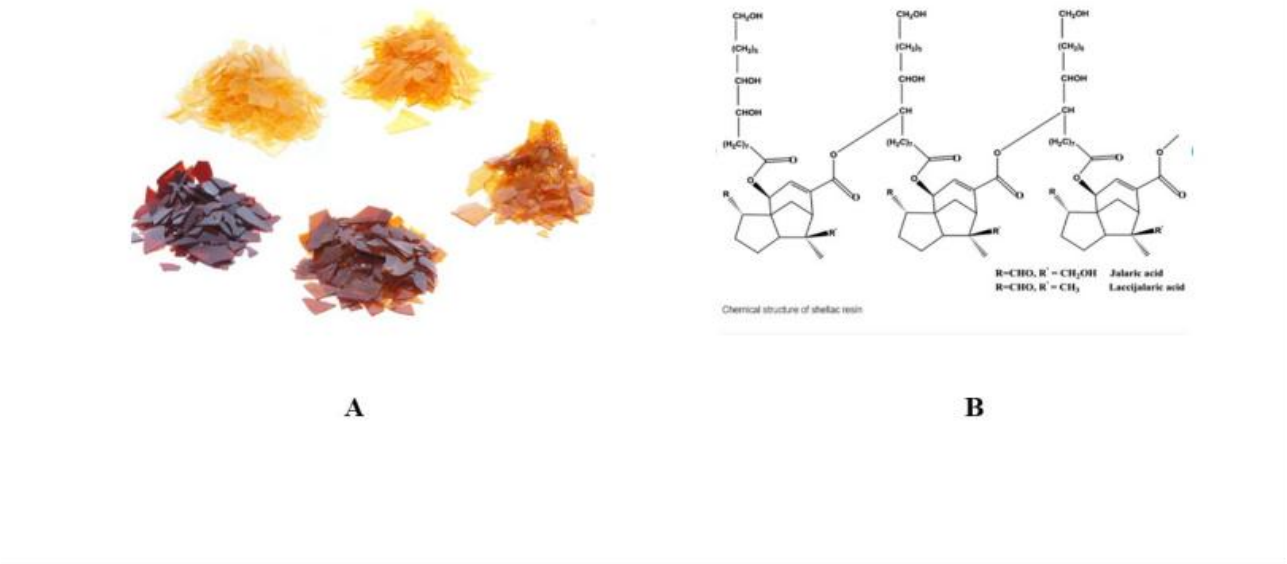

Fig. 2 ; A = Different colours of shellac flakes

B = Chemical structure of shellac [2]

In the past, shellac was widely available wherever paints and varnishes were sold. However, in terms of decorative wood finishing for homes, such as kitchen cabinets, hardwood flooring, and plank paneling, polyurethane has virtually completely replaced it. It is more resilient to chemicals and abrasions and costs less. But, if the user want to color the wood, they must apply these alternative treatments over a stain. Clear or blonde shellac can be applied over a stain as a protective coating without altering the final product's color. Polished paste wax put over multiple layers of shellac, or "wax over shellac" finishes for hardwood floors are often commended for their beautiful yet delicate look **[1].** Fine acoustic stringed instruments are still French polished with shellac by luthiers, but in many workshops, particularly in high-volume production settings, synthetic plastic lacquers and varnishes have taken its place.

Shellac is a naturally occurring, synthetic-polymer-like bio adhesive polymer. As a result, it can be regarded as a natural kind of plastic. With a melting point of 75 °C, it falls into the thermoplastic category and is utilized to bind wood flour, enabling the mixture to be molded under pressure and heat. Plastic has mostly replaced shellac since it is more difficult to apply and more prone to scratches than most lacquers and varnishes. For example, Urushi lacquer is significantly superior in terms of chemical and mechanical resistance, while Shellac is much softer. However, shellac that has been damaged can simply be touched up with more shellac **[2]**. Shellac is soluble in alkaline solutions of ammonia, sodium borate, sodium carbonate, and sodium hydroxide in addition to a variety of organic solvents. When applied as a coating, shellac that has been dissolved in alcohol produces a surface that is hard and durable. Shellac flakes having different colour are shown in **(Fig. 2A).**

The precise makeup of the complex mixture of aliphatic and alicyclic hydroxy acids and associated polymers that emerges after mild hydrolysis of shellac varies depending on the source of the shellac and the season in which it was collected. Aleuritic acid is a significant aliphatic component, whereas shellolic acid has a significant alicyclic component. Shellac resists UV rays and doesn't oxidize over time. Although shellac has been used before written records date back 3,000 years, they do exist **[2]**. The Mahabharata, an ancient Indian epic text, claims that dried shellac was used to construct an entire castle. It was rarely utilized as a dyeing substance over the duration of trade with the East Indies. Merrifield claims that the usage of shellac as an artist's pigment dates back to 1220 in Spain. Venetians were the first to adopt large furniture items decorated with paint or varnish.
There are multiple mentions of painted or varnished cassone from the thirteenth century, usually dowry cassone that were intentionally made beautiful as part of dynastic weddings [3]. Varnish's precise definition is unknown, but it seems to have been a spirit varnish made from gum benjamin or mastic, which were both traded over the Mediterranean. There came a time when shellac was also used. In this historical period, "varnisher" was acknowledged as a distinct profession from both artist and carpenter. Shellac can also be used for sealing wax. The different formulations and the historical context of the introduction of shellac into early beeswax preparations are examined in Woods' The Nature and Treatment of Wax and Shellac Seals **[3].**

- Role of the lac bug:
    1. Swarms of a tiny red colored bug named Kerria lacca feed on trees, primarily in India and Thailand which are thereby known as lac trees. These insects eat, propagate and secrete the resin on the trees which they feed on.
    2. These insects suck the tree sap until they die and produce a minimum number of 1000 eggs before dying. As the sap comes in contact with air the secretion forms a hard covering around the swarm so as to protect its eggs from predators.

- The process of refining:
    1. The branches of the trees having the lac bug's excretions or the hard covering (known as sticklac), are collected and with help of mallets break off the crusty coating as depicted in **(Fig 3)**.

Fig. 3 Female bug secreting waxes which are converted to shellac [1]

    2. The sticklac is then refined at refining centers to remove all sort of impurities in form of twigs, insect remains, leaves etc.
    3. Now the pure shellac is transferred into large jars and a force is applied onto the sample so as to make a sheet like structure and allowed to dry **[3]**

- Heating process:

  The sheet formed are known as seedlac which are further melted over steam grids. The molten lac is put under hydraulic pressure. The further filtered shellac is dropped over a steam heated kettle from where the liquid is dropped over roller which again makes fine sheets of the sample and allowed to dry. They are then broken into flakes like structure.

- Solvent making:

The solvent used for making seedlac solution is Ethyl alcohol as it is insoluble in water. This solution is further used to carry out experiments. Pharmaceutical glaze is used to tablets and candies as a glazing agent. Because shellac has an acidic quality, it can tolerate stomach acids, which makes it suited for timed enteric or colonic release. Shellac "wax" is applied to citrus fruit to increase its shelf life. It also serves to replenish the natural wax that is lost from the apple during cleaning.

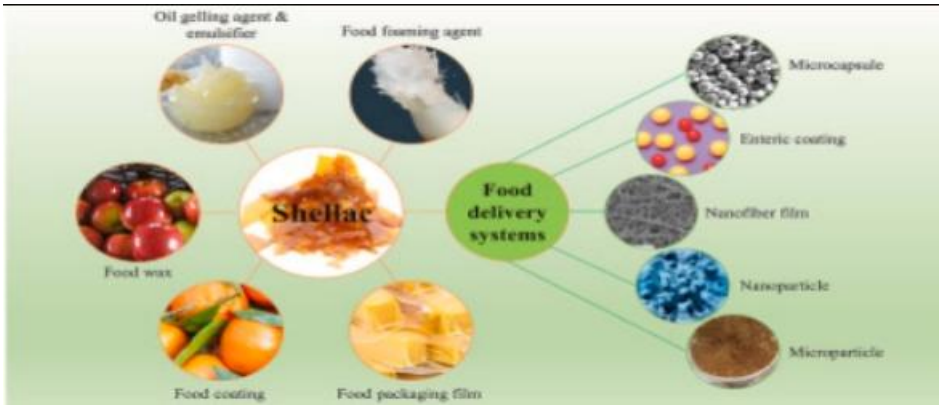

Fig. 4 Application of shellac [3]

Shellac is often the base for "all-purpose" primers since it absorbs stains and smells. Shellac works well as a barrier against water vapor penetration, but it is not very effective against abrasives and many common solvents. Primers based on shellac are effective sealers for addressing odors associated with fire damage. The northeastern region of Thailand has historically colored cotton and, more especially, silk textiles with shellac [3]. It yields a spectrum of warm hues, from pale yellow to deep orange-reds and dark ochre. Shellac-colored organically dyed silk fabric is widely available in the rural northeast, especially in Ban Khwao District, Chaiyaphum Province. In Thai, the word "khrang" describes both the liquid and the bug.

Curcumin is a brilliant yellow chemical produced by the Curcuma longa plant type. The primary curcuminoid present in turmeric is a member of the Zingiberaceae family of ginger plants, as depicted in Figure 5A. It is sold as a herbal supplement, food coloring, flavoring in cooking, and cosmetic ingredient. From a chemical perspective, curcumin is a

diarylheptanoid that is a member of the phenolic pigment class called curcuminoids, which are responsible for the distinctive yellow color of turmeric. Clinical or laboratory studies have not supported the possible medicinal uses of curcumin. It is hard to study because of its volatility and low bioavailability. It is improbable to produce meaningful leads for medicinal research [3].

Fig. 5 ; A Image of curcumin , B Chemical structure of curcumin [2]

When scientists first isolated a "yellow coloring-matter" from the turmeric rhizomes in 1815, they gave it the name curcumin. It was later discovered to be a blend of resin and turmeric oil. According to Milobedzka and Lampe's research from 1910, curcumin has the chemical composition diferuloylmethane. The same team succeeded in synthesizing the chemical later in 1913 **[4]**. Although curcumin has historically been utilized in Ayurvedic medicine, its potential for therapeutic benefits when taken orally has not been established. Curcumin's potential medical applications have not been validated by laboratory or clinical research. Due to its instability and low bioavailability, it is challenging to research. It is unlikely to generate insightful leads for pharmaceutical research. Curcumin has one unsaturated diketone moiety, two aromatic O-methoxyphenolic groups, and a seven carbon linker. The phenolic aromatic ring systems are connected by two unsaturated carbonyl groups, as shown in (Fig. 5B). It is a diketone tautomer that can be found in organic solvents in the enolic form and in water in the keto form. The diketones readily deprotonate to generate stable enols and enolates. The unsaturated carbonyl group is introduced nucleophilically and serves as a great Michael acceptor [4]. Because curcumin is hydrophobic, it is not particularly soluble in water.

However, it easily dissolves in organic solutions. Curcumin is a complexometric indicator of boron. It forms the crimson compound rosocyanine when combined with boric acid. Curcumin's synthesis pathway is unclear. Peter J. Roughley and Donald A. Whiting hypothesized two pathways for the synthesis of curcumin in 1973. Cinnamic acid and five malonyl-CoA molecules engage in a chain extension process in the first mechanism, which results in arylization into a curcuminoid. The second method entails the coupling of two cinnamate molecules via malonyl-CoA. Both begin with cinnamic acid, which is produced from the amino acid phenylalanine. Cinnamic acid is an uncommon precursor in plant biosynthesis, as opposed to the more frequent p-coumaric acid. Cinnamic acid is only the building block for a few number of known compounds, like anigorufone and pinosylvin **[4]**

- It treats the inflammatory conditions, metabolic syndrome, arthritis, anxiety
- It also helps in management of muscle soreness caused by exercising, thus enhancing recovery and performance.
- It is used as a therapeutic drug for cancer treatment and neurodegenerative diseases.
- It is also used to cure liver related issues and digestive disorders. Not only this it also treats several skin ailments.

The objectives of the study are as follows

- To extract curcumin sample from the given Turmeric sample using soxhlet
- To check the purity of the extracted curcumin sample using TLC
- To formulate a hydrogel using shellac and curcumin for wound healing
- To charecterize the formed hydrogel for its physio - chemical and antimicrobial properties.

CHAPTER 2 LITERATURE SURVEY

2.1 Introduction to hydrogel

Networks of three-dimensionally cross-linked water-soluble polymers are called hydrogels. Almost any water-soluble polymer can be used to create hydrogels, which come in a variety of bulk physical and chemical compositions. Hydrogels can also be prepared into a wide range of physical forms, such as coatings, films, microparticles, and slabs. For this reason, hydrogels are widely used in clinical practice and experimental medicine for a range of applications, including cellular immobilization, biomolecule or cell separation, tissue engineering and regenerative medicine, diagnostics, and barrier materials to regulate biological adhesions **[5].** Particular interest in the use of hydrogels in drug delivery applications has been generated by their distinct physical characteristics. By adjusting the density of cross-links in the gel matrix and the hydrogels' affinity for the aqueous environment in which they expand, it is easy to tailor the highly porous structure of hydrogels. Furthermore, because of their porosity, drugs can be incorporated into the gel matrix and released at a rate determined by how well the small or macromolecule diffuses throughout the gel network. **[5]** The main benefit of hydrogels for drug delivery may be pharmacokinetic, meaning that they can create a depot formulation from which drugs slowly elute and maintain a high local concentration of drug in the surrounding tissues for a prolonged amount of time, even though they can also be used for systemic delivery. The fact that hydrogels are successfully used in the peritoneum and other in vivo locations indicates that they are also typically very biocompatible. Biocompatibility is enhanced by hydrogels' high water content and their compositional, mechanical, and physiochemical resemblance to the natural extracellular matrix **[6].** Enzymatic, hydrolytic, or environmental pathways can be used to create hydrogels for biodegradability or dissolution; however, depending on the location and time scale of the drug delivery device, degradation may not always be desirable. Hydrogels are pliable materials that conform to the surface they are applied to. In the latter scenario, the muco- or bioadhesive properties of hydrogels may be advantageous when applying them to non-horizontal surfaces or when immobilizing them at the application site. Drugs are usually externally injected into hydrogels before the hydrogel-drug combination is put into the body when hydrogels are utilized for drug delivery.

2.2 How does hydrogel cross link?

UV photopolymerization and chemical cross-linking are two cross-linking techniques that can be used [6]. These cross-linking methods are only beneficial if all toxic substances can be removed prior to hydrogel implantation, which may be difficult to accomplish without also releasing the medication that is included into the hydrogel. The main disadvantage of these techniques is that the premade material must be implanted, since bulk hydrogels are usually too elastic and have specified dimensions to be extruded using a needle. On rare occasions, creating nano- or microparticles out of the premade gel can address the latter problem. For some uses, hydrogels can also be created in vivo, or in situ, however there are dangers involved with being near UV light or cross-linked substances **[7].** Linear polymers that are not cross-linked may be used as an alternative for medication delivery. The viscosity and drug release rate of a linear polymer matrix are generally negatively associated. It may be difficult or impossible to dissolve the target polymer to a high enough concentration in order to regulate the rate of medication release to the required degree. Because of the high yield stress of the resulting material, injection might not be possible even if it were viable. Furthermore, unless they are cross-linked in some way, the water-soluble polymer chains stretch and eventually dissolve in the aqueous in vivo environment—sometimes in as little as a few minutes or hours for very hydrophilic polymers. These reasons have led to a great deal of interest in formulations that combine the benefits of gel in situ within the body (offering longer drug release profiles) with the advantages of linear polymer solutions outside the body (facilitating straightforward injection) **[7].** Physical and chemical cross-linking techniques have been applied to achieve in situ gelation. Physical cross-linking of polymer chains can be achieved through a variety of environmental stimuli (pH, temperature, ionic strength, etc.) and physicochemical interactions (charge condensation, hydrogen bonding, stereocomplexation, hydrophobic interactions, supramolecular chemistry, etc.) **[8].** It is believed that wound healing is a dynamic process, and that the need for particular dressings to function better as the healing process progresses. Warm, humid surroundings are known to facilitate speedy wound healing; therefore, modern wound dressing materials should be manufactured in a way that may provide these benefits. Fluid balance is important because significant evaporative water loss during skin burning lowers body temperature and raises metabolic rate. Hydrogel is a polymeric material that expands significantly in an aqueous

media but does not dissolve in water at physiological temperatures or pH levels. It is one of the most promising types of polymers being employed for a number of biomedical purposes **[8].** A lot of research has been done on hydrogels as possible materials for wound dressings. These three-dimensional materials can facilitate moist wound healing with good fluid balance because of their transparency, which makes it simple to monitor the healing process. Pectin, one of the primary components of citrus wastes, gels well **[9].** Chemically, pectin is composed of poly α1-4—galacturonic acids with different degrees of methylation of the carboxylic acid residue.

2.2.1 Cross linking hydrogel using gelatin and pectin

Pectin has long been the subject of research investigations as a potential drug carrier for colon-specific medicine administration **[10].** To increase its mechanical and thermal stability, pectin films were combined with other polymers due to their low thermal stability and poor characteristics. Gelatin, which is made up of prolonged polypeptide chains with a triple helical superstructure, is regarded as denatured collagen. Since it comes from animals, collagen contains antigenicity. The primary protein present in connective tissue, cartilage, bone, and skin is collagen. It also makes up the majority of the extracellular matrices in animals. On the other hand, due to its denatured state, gelatin has comparatively little antigenicity. Furthermore, gelatin is far less expensive than collagen. Gelatin-based biomaterials have been used recently for tissue engineering scaffolds, bone grafts, and artificial skin **[11]**. It is extensively utilized in controlled release systems and wound dressing products . However, the fact that gelatin dissolves in aqueous solutions is its primary drawback when it comes to use in tissue engineering and drug delivery systems. Crosslinking is a technique to get around this restriction. Pectin-based films and pectin-gelatin microglobules have already been the subject of a small number of publications **[12]**. The creation and analysis of pectin/gelatin hydrogel membranes crosslinked with gluataraldehyde are presented in this paper.

2.3 Types of Chemically cross-linked hydrogels

2.3.1 From radical Polymerization

By radical polymerizing low molecular weight monomers with crosslinking agents present, chemically crosslinked gels can be produced. An extensively researched and well-known hydrogel system is poly(2-hydroxyethyl methacrylate). The first description of this hydrogel was given by scientists. It is created by polymerizing HEMA with the right crosslinking agent. Comparable techniques have been used to construct a wide variety of hydrogel systems [13]. The amount of crosslinker used can change the hydrogel's characteristics, such as swelling. For example, methacrylic acid or N-isopropylacrylamide can be added to create stimuli-sensitive polymers [13].In addition to the radical polymerization of vinyl-monomer combinations, water-soluble polymers modified with polymerizable groups can also be radical polymerized to form chemically crosslinked hydrogels. Hydrogels have been made using a variety of water-soluble polymers, including natural, semi-synthetic, and synthetic ones. In particular, the basis for (biodegradable) hydrogels is dextran. Most of the D-glucopyranose residues in bacterial polysaccharide dextran are joined by α-1,6 links. As such, research has been done on the use of dextran to transport drugs, proteins, and imaging agents. Furthermore, because the colon contains dextranase, dextran-based gels are being investigated as a colon delivery technique [14]. In Edman et al.'s seminal work on polymerizable dextran, glycidylacrylate was reacted with dissolved dextran in water. A hydrogel was formed by reacting an initiator system consisting of ammonium peroxydisulfate and N,N,N′N′-tetramethylene-diamine with an aqueous solution of acryldextran containing N,N,-methylenebisacrylamide. By employing an emulsion polymerization technique, enzymes were ensnared in polyacryldextran microspheres while keeping almost all of their function. Water-soluble polymers other than dextran, such as albumin, (hydroxyethyl)starch, poly-aspartamide, poly(vinyl alcohol) [14], and hyaluronic acid, were also derivatized with (meth)acrylic groups using essentially the same method as Edman et al. Because the reaction is carried out in an aqueous solution, glycocidyl(meth)acrylate hydrolyzes both before and after the reaction with the watersoluble polymer, producing a very low degree of substitution that is difficult to control. Consequently, an alternative method has been developed to produce methacrylated dextran [14]. By using a suitable aprotic solvent (DMSO), we dissolve the dextran and then catalyze the glycidylmethacrylate (GMA) derivatization process with 4-(N,N-dimethylamino)pyridine. It was demonstrated that GMA was nearly quantitatively integrated and that the degree of substitution could be fully controlled. After enzymatic degradation, the products were thoroughly examined using mass spectroscopy and NMR [16]. This analysis revealed that, in the specific conditions used, the reaction between GMA and

dextran was a transesterification that resulted in a dextran derivative with the methacrylate group attached directly to the dextran chain. Other compounds like sucrose and inulin that contain methacrylate groups could potentially be derivatized using the synthesized technique. Water-soluble polymers can have (meth)acrylate groups added to them using methacrylic anhydride, (meth)acryloyl chloride, or the reaction of dextran with sodium acrylate and bromoacetyl bromide. A polymerizable dextran derivative was created via the reaction of dextran and maleic anhydride. When exposed to UV light, the vinyl groups of these dextran derivatives can polymerize and form a hydrogel. The gels remained intact in a physiological environment. However, because the network comprised carboxylic acid groups, they did exhibit a notable pH-dependent swelling tendency. Methacrylate esters are extremely resistant to hydrolysis, although methacrylate groups connected to water-soluble polymers are sensitive to it under physiological conditions after polymerization [16]. This suggests that these polymer gels can only degrade physiologically when they are hydrolyzed by an enzyme that is compatible with the major chains of the polymer. Gels consisting of dextran, starch, and albumin have demonstrated this [16].

2.3.2 *By reaction of complementary groups*

Water-soluble polymers' solubility is ascribed to the presence of functional groups, mainly OH, COOH, and NH2, which promote the formation of hydrogels. The production of Schiff bases or the reaction of functional groups with complementary reactivity, like amine-carboxylic acid or isocyanate-OH/NH2, can lead to the formation of polymer chains.

2.3.3 *By aldehyde reaction*

Water-soluble polymers with hydroxyl groups, like poly(vinyl alcohol), can be crosslinked with glutaraldehyde [16]. Crosslinking cannot occur unless extreme requirements are met (high temperature, low pH, and methanol added as a quencher). Conversely, under mild conditions, the same reagent can be used to cross link amine-containing polymers, forming Schiff bases in the process. This has been specifically studied for the production of polysaccharides containing amines and crosslinked proteins. Alternatives to glutaraldehyde have been developed since it is a hazardous chemical that inhibits cell growth even at low concentrations. There have been reports of crosslinking

gelatin with polyaldehydes made by partially oxidizing dextran **[16]**. These gels were designed to be applied to wounds, and in order to promote healing, epidermal growth factor (EGF) was added. As the duration of storage increased, the rate of EGF release reduced, which was explained by the continuous processes of the hydrogel matrix's physical and chemical crosslinking. After prolonged storage, only a fraction of the protein was released, most likely due to Schiff base formation between the groups in the protein and the aldehyde groups in the oxidized dextran. The biocompatibility of dextran (dialdehyde)The hydrolysis of the carbonate and lactate ester, respectively, is the process of degradation for dex-HEMA and dex-(lactate)2-HEMA [19]. Following both in vitro and in vivo examination, the crosslinked hydrogels were found to be satisfactory. Poly(aldehyde guluronate) is made by oxidizing partially depolymerized alginate with periodate and can be crosslinked with adipic acid dihydrazide to make a hydrogel.

2.3.4 *By using enzymes*

An interesting method for enzymatic PEG-based hydrogel synthesis was reported by Sperinde et al. Glutaminyl groups were introduced to a tetrahydroxy PEG (PEG-Qa) by means of their methodology. To create PEG networks, transglutaminase was subsequently added to aqueous solutions of PEG-Qa and poly(lysine-co-phenylalanine). This enzyme joins the α-carboxamide group of PEG-Qa with the ε-amine group of lysine to catalyze the process that forms an amide link between the polymers. The properties of the gel could be changed by varying the lysine copolymer to PEG-Qa ratio [19]. Under the correct conditions, gels with an equilibrium water content of 90% were produced. In recent work, poly(lysine-co-phenylalanine) was substituted with lysine end-functionalized PEG, and hydrogels were produced by adding transglutaminase to an aqueous solution containing macromers modified by peptides. The ratio of the reactants, the concentration of the enzyme, and the shape and composition of the macromers all affected the gelation kinetics.Usually, the gelation periods ranged from five to thirty minutes. These systems are ideal for in-situ gelling since the gel formation takes place in extremely mild circumstances and the gelation kinetics may be precisely regulated. Enzymes that require Ca^{2+} are called transglutaminases. Westhaus et al. created a triggered gelling system based on this. When heated to 37 °C, a mixture of fibrinogen, Ca^{2+}- dependent transglutaminase, and Ca-loaded liposomes quickly gelled, but remained fluid at ambient temperature **[20]**. The heat treatment caused the liposomes to become unstable, releasing Ca-ions into the surrounding fluid and concurrently activating the

enzyme . Natural products for wound healing are prefered because they are safe and have the potential to have fewer negative impacts on healthy cells, natural goods like plants are more advantageous for enhancing therapy outcomes. Turmeric is one herbal plant that is frequently linked to anticancer medications **[21]**. Turmeric is widely used by scientists, as well as both the food and medical sectors. The ginger family includes the rhizomatous herbaceous perennial plant Curcuma longa. The medicinal properties of curcumin, which is found in turmeric, have long been known. Thin layer chromatography (TLC) is one chromatographic technique for sorting mixtures One chromatographic method for separating mixtures is thin layer chromatography (TLC). The basic method of performing thin-layer chromatography is to thinly coat a glass, plastic, or aluminum foil sheet with cellulose (ink paper), silica gel, or aluminum oxide as an adsorbent. This adsorbent layer is known as the stationary phase [22]. After the sample has been dappled onto the plate, capillary action pulls the solvent, or mixture of solvents, onto the plate (known as the mobile phase). Because different analytes ascend the TLC plate at different rates, separation is achieved. Other names for HPTLC are Planar Chromatography, Flatbed Chromatography, and High-Pressure Thin Layer Chromatography. Both qualitative and quantitative analysis can be required for assignments; powerful analytical methods such as HPTLC can be used. The stationary phase for TLC was Silica gel, Mobile phase was Chloroform, the solvent used was Methanol. The TLC method is inexpensive and simple to use, which are two of its many benefits. Long separation durations and poor resolution in the case of turmeric analysis are two drawbacks of the TLC approach, though **[22]**. With good sensitivity, HPTLC can test samples in small quantities up to the nanogram level. Additionally, because the process is automated, HPTLC also has a minimal amount of human error. However, there are drawbacks to HPTLC as well. These include the need for big, roomy instruments, dust-free environments, controlled temperature settings, and knowledgeable operators who possess the necessary technical skills. **[23]**

2.4 Process of wound healing

A wound is a physical or thermal skin defect or fracture that results from underlying medical or physiological disorders. These abnormalities can cause the area to become physiologically unstable and even disrupt regular bodily functions. Wound healing, one of the most complex and dynamic biological processes, is the result of multiple biological substances and stem cells working together to assist wound repair on all fronts **[24]**. Any disruption in these elements will result in persistent wound healing. Chronic wounds typically heal very slowly,

and in some cases, they may never fully heal because of underlying physiological disorders like diabetes, which are thought to be the largest obstacle to wound healing. Due to their high rates of morbidity, death, and recurrence, diabetic chronic wounds are, as we all know, the leading cause of non-traumatic limb amputation globally and inflict great harm to society. Reactive oxygen species (ROS) can promote angiogenesis and cell migration, which can help with normal wound healing. However, high ROS levels may hinder or even block wound healing, especially in chronic wounds, according to a number of studies [25]. Extended inflammatory responses leading to a significant build-up of reactive oxygen species (ROS) that surpasses the antioxidant capacity of cells are characteristic of chronic wounds. By doing this, the wound's transition from the inflammatory to the proliferative phases is stopped. It ultimately resulted in persistent wound healing by maintaining a vicious cycle of protracted inflammation at the wound site. Thus, preserving the redox balance in cells—that is, achieving antioxidant status—should help prevent aberrant cell division and immunological response disorders. Antioxidation has been shown to enhance wound healing, as predicted, particularly for chronic wound repair. As a result, adding antioxidant function has proven to be a useful strategy for quickening the healing of chronic wounds.

2.4.1 Hydrogel as a wound healer

Due to its superior biocompatibility, strong permeability, 3D structure, and capacity to create a moist environment for wound healing—a feature that standard dressings cannot match—hydrogel is regarded as an outstanding choice for a dressing **[26]**. To improve the treatment environment for chronic wounds, many hydrogel dressings with antioxidant qualities have been created with the intention of accelerating the healing process. Chronic wounds with high ROS can be rapidly healed by lowering oxidative stress, promoting the wound microenvironment, and accelerating wound healing with the application of the antioxidant hydrogel. Antioxidant hydrogels offer a new approach to treating chronic wounds that may speed up their healing, and this approach is one that merits our consideration. The goal of this review was to give a theoretical framework for the healing of chronic wounds by thoroughly introducing the function and effects of antioxidant hydrogels in wound care. The process of wound healing is dynamic and systematic. Acute and chronic wounds can be distinguished based on the type of wound that heals. Commonly referred to as "normal wounds," acute wounds are typically the result of chemical, mechanical, or burn traumas. The slightest scar can indicate full healing of the wounds. An average recovery takes eight to twelve weeks. As

is well known, the epidermis, dermis, and subcutaneous layers comprise healthy skin. The uppermost layer is called the epidermis **[26]**. The epidermis's strong impermeability allows it to function as a barrier to stop damaging stimuli from the outside world in addition to its ability to regulate water loss. 2) The dermis supports a variety of circulatory systems and gives skin elasticity and physical support. It is made up of extracellular matrix, fibroblasts, elastin, and glycosaminoglycan. Adipose tissue, which is abundant in the subdermal layer and has good vascularization, helps regulate skin temperature and mechanical characteristics. Our comprehension of wound healing is aided by knowing the makeup of normal skin. The reaction to normal tissue damage is a sequence of intricate processes called functional recovery that happens in a timely and organized manner. Hemostasis is the initial step after injury and bleeding. The wound site will coagulate due to the coagulation factors in the exudates, providing mechanical support for the injured tissue. To create room for the formation of granulation tissue, lymphocytes, macrophages, monocytes, and inflammatory cells remove debris from wound beds during the inflammatory stage. In the proliferative stage, granulation cells gradually cover the exposed wound surface as fibroblasts and epithelial cells replace lost or damaged tissues **[27].** Maturity, or remodeling, is the last stage that includes the development of new epithelium in addition to connective tissue formation. It is important to note that these phases more often overlap than are clearly and rigorously separated by time period. Furthermore, the length of the transition overlap is often determined by the maturation and differentiation of various cells involved in wound healing, such as macrophages and fibroblasts. The primary categorization of modern dressings is based on the materials they generate, which are often films or hydrogels **[28]**. Hydrogel is currently considered the best material for healing chronic wounds; in addition to sealing wounds to prevent fluid loss and accumulation of purulent substances, it may also prevent crust formation from promoting bacterial development and infection. Ever since the hydrogel was discovered for the use for wound dressing, researchers have been attempting to improve its physiochemical properties. **[29]**. In addition, it has been proposed that dressings for wounds should facilitate leukocyte migration, inhibit infection, eliminate surplus exudates, encourage gas exchange, and even act as a barrier against heat shock and recurrence of damage **[30]**. Functional hydrogels, which were primarily made of materials with good biocompatibility and biodegradability, were given multiple functions. Research indicates that multifunctional hydrogels with good biocompatibility are the best choice for biomedical and pharmaceutical applications because they can achieve multistage and multifunctional combination therapy.

The rapid improvement of antioxidant research and the substantial hazards associated with reactive oxygen species (ROS) in chronic wound repair have led to a great deal of interest in the combination of antioxidant function with hydrogel. Consequently, a new era in wound care has been brought about by the development of many hydrogel dressings with antioxidant properties **[30].**

CHAPTER 3 MATERIALS AND METHODS

3.1Material Required :

Shellac flakes, turmeric sample, HCl, Glutaraldehyde, Pipettes and Tips of 100 and 200 micro-liter, Pectin, Gelatin, Distilled water, Measuring Cylinder.

3.2 Procedure:

3.2.1 To extract Curcumin from turmeric sample :

The method used to extract curcumin was the soxhlet extraction method which was proceeded as follows **[7]**:

(1) 20 g of turmeric sample was measured

(2) Turmeric was wrapped in a blotting paper and put into soxhlet's extractor

(3) A condensor was placed at top of the extractor, in which ethanol (solvent) was added.

(4) A Round bottom Flask was attached to the bottom of extractor to collect the extracted curcumin. as shown in **(Fig. 9)**

(5) 2 rubber tubes were attached each at inlet and outlet of stream , the other end of inlet stream was attached to tap for allowing continuous flow of water.

(6) The heating mantle under the RBF was kept at 40 degrees .

(7) This process performed in cycles and each cycle took around 2.5 hours to complete .

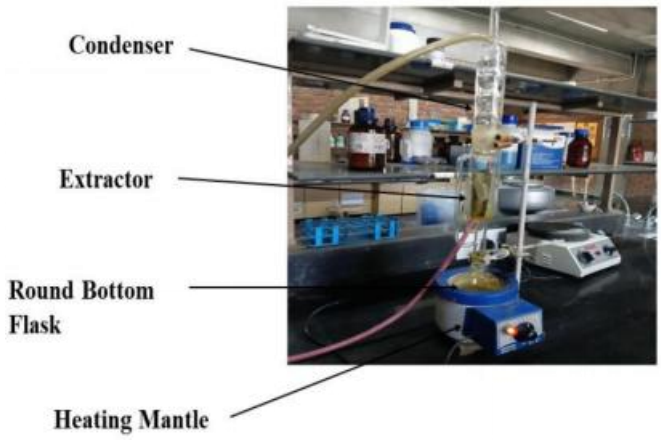

Fig. 6 Curcumin extracted with help of soxhlet

3.2.2 To check the purity of the extracted curcumin sample [15]:

TLC was performed for purity test of our extracted curcumin sample.

(1) TLC was performed using silica gel plate which was used as stationary phase .

(2) Sample of chloroform (9.5 ml) and methanol (0.5 ml) mixture was used as mobile phase .

(3) A reference dot of our extracted curcumin was made with pencil on the plate .

(4) The plate was then immersed in a flask containing the mobile phase .

(5) Different components travelled through the TLC plate at different rates where separation is

(6) The plate was taken out after the mobile completely travels through it .

(7) The plate was immediately shook in a ionized Bromine chamber .

(8) Different bands were observed in sequence on the gel plate .

(9) The observed bands were compared with the TLC performed on the standard curcumin sample made of pure curcumin .

10) TLC of our extracted sample showed same bands as our standard sample . Hence we concluded that our sample had no impurities .

3.2.3 To form the hydrogel from shellac and curcumin sample for wound healing [17]:

(1) 5 g of pectin solution was measured and put into a conical flask. To this 50mL of distilled water was added and then heated to let pectin completely mix into the water.

(2) Similarly, 4g, 5g and 6g of Gelatin was measured and put into another conical flask to which 50 mL of distilled water each was added and then heated to let the gelatin powder dissolve completely.

(3) Both, the gelatin and pectin solutions were mixed together to form 3 different solutions.

(4) Now 3 separate beakers were taken to which 0.2mL of HCl and 1mL of glutaraldehyde each was added. This solution is known as glutaraldehyde reagent which worked as a cross-linking agent for the hydrogel.

(5) The glutaraldehyde reagent was then added to all 3 gelatin-pectin solutions.

(6) The dispersion was then allowed to stir at 400 RPM speed for 1 hour to form a uniform mixture.

(7) Finally, the solutions were poured into 3 different petri plates.

(8) To the same petri plates, a solution of shellac and curcumin containing an equal amount of both that is 5g shellac and 5mL of curcumin solution was added.

(9) At last the solutions in the petri plates were allowed to self-bind for 3 days.

***NOTE: It was observed that the hydrogel best formed at 6g concentration as in Fig 10.**

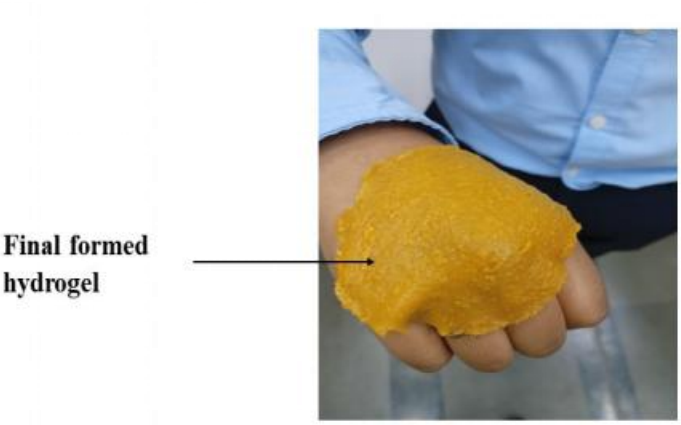

Fig. 7 Hydrogel before freeze thaw cycles

3.2.4 To Characterize the formed hydrogel for its physio-chemical properties [18]:

4 different tests were performed to check the physiochemical properties of the formed hydrogel.

(1) Solubility Test :

Sample of hydrogel is measured according to 1% concentration (0.2g) and immersed into the distilled water of 20 mL. for 48 hours. After 48 hours, The gel was dried in a microwave and then weighed. The weight came out to be 0.1g.

(2) Swelling Test:

Sample was taken in an amount of 0.09 g and immersed into water in a volumetric vial. The hydrogel remained suspended into the water for 48 hours. After this, the solution was centrifuged at 6000 RPM for 10 minutes. The water was removed after centrifugation and the hydrogel was weighed. The weight came out to be 0.372g.

(3) Spreadibility Test:

For this, 1g of sample was put between two petri plates. Over these petri plates, weight of 200g was put to allow the hydrogel to spread. Then, the diameter of the was measured using a scale.

(4) pH Test:

The pH of the hydrogel was measured with a litmus paper. It came out to be 6 g that is slightly acidic.

CHAPTER 4 RESULTS AND DISCUSSIONS

4.1 Extraction of curcumin by soxhlet apparatus:

Curcumin was extracted from turmeric sample by using soxhlet apparatus with the principle of solvent reflux and siphon's principle. The yield of extracted curcumin was 9% **(Fig. 8A)** which was much higher than Foozie Sahne et. al. where the yield was 6.9% The yield for same process by Naresh D Joshi et. al. was 4.09%. A confirmatory test with Sulphuric acid was applied to check the presence of curcumin. When reacted with sulphuric acid, curcumin gives a colour change that is yellow solution changes to red **(Fig. 8B)**. The colour change occurs due to the presence of various pigments present in the curcumin that react with alteration in pH upon adding acid.

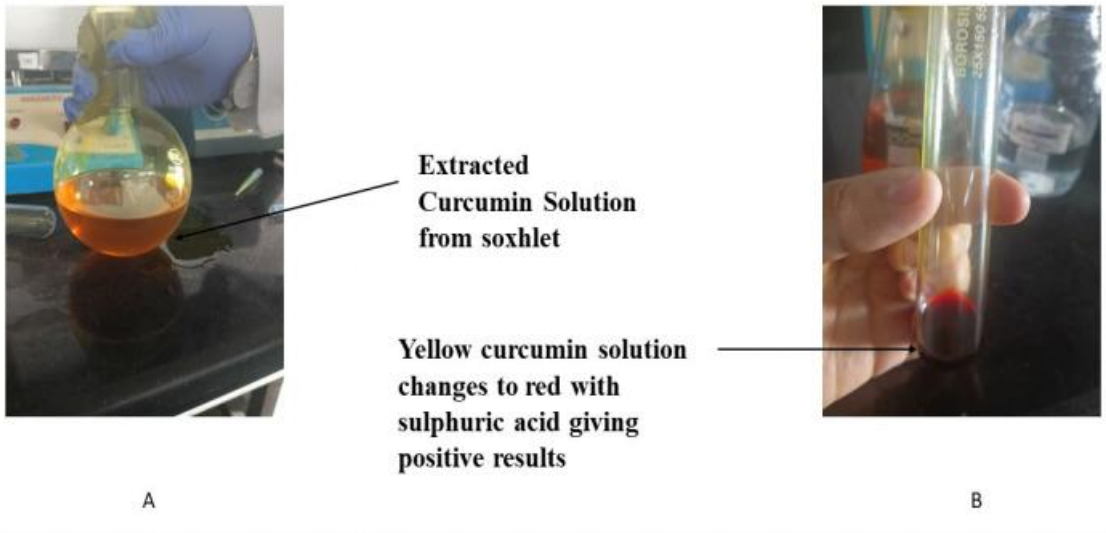

Fig. 8 A = Curcumin soltion extracted from turmeric sample using soxhlet

B = Postive result for presence of curcumin

4.2 Checking the purity of the extracted curcumin sample:

One significant secondary metabolite that can be extracted from C. longa (turmeric) is curcumin . The optimum TLC condition, that is temperature of 22℃, migration distance of solvent to be 100 mm was established and a pre-concentrated plate developed in chloroform methanol gave a good separation for Curcumin, Demethoxycurcumin (DMC) and bis-demethoxycurcumin (BDMC) **(Fig. 9)** . Our experiment showed no other impurity present in the extracted sample as both the test sample and standard sample of curcumin produced similar bands. A similar experiment performed by W E Hennik et. al. for hydrogel was conducted **[17]** and resulted in formation of 3 bands only. With the mobile phase containing 97:3 V/V of chloroform and methanol, the current work established and tested precise analytical procedures with broad applicability to identify and quantify concentration in certain plant species like curcumin over here.

Fig. 9 TLC of curcumin sample; A = Standard sample of curcumin, B = Test sample of curcumin

4.3 Hydrogel formation from shellac and curcumin

A hydrogel for wound healing was formed from shellac and curcumin sample for wound healing. The hydrogel was successfully formed and had yellow colour due to presence of cucumin and shellac having anti-microbial properties . The antimicrobial properties were tested on the hydrogel with various conc. of Gelatin **(Fig 10 A and B)**. The formed hydrogel was optimized for microbial growth and observed that hydrogel with 8mL each of shellac and curcumin solution had no microbial growth hence proving that the formed hydrogel with loaded shellac and curcumin successfully prevented microbial growth over it.

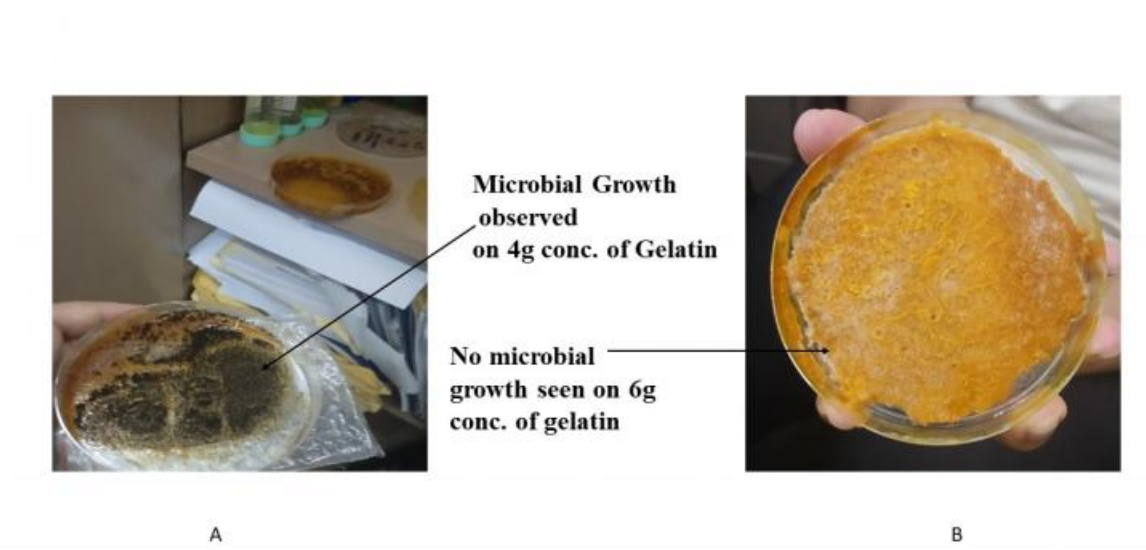

Fig. 10 A = Microbial growth at 4g conc. of gelatin

B = No microbial growth observed at 6g conc. of gelatin

4.4 Charecterization of the hydrogel

After the formation of the hydrogel, it was characterized using various methods for checking its physio-chemical parameters.

4.4.1 Solubility test

The insoluble portion of a dried sample is measured by the below mentioned formula to determine the hydrogel content of a given material after performing the solubility test **(Fig. 11).**

Gel Fraction = [Wd/Wi]*100

;Wi = Weight of the dried sample before putting it into water.

Wd = Weight of the dried insoluable part of the sample after immersed into water.

The soluble part of the hydrogel was calculated to be 50% . In a similarly performed experiment by Nicholas martin et. al., the solubility of the hydrogel came out to be 60% **[29]** . Hence comparing the solubility of our's hydrogel, we can say that it had lesser solubility thereby making it a better alternative. The improvement in solubility would have occurred due to usage of drugs like shellac and curcumin with stronger hydrophobic groups and the strong cross linking bonds between the functional groups.

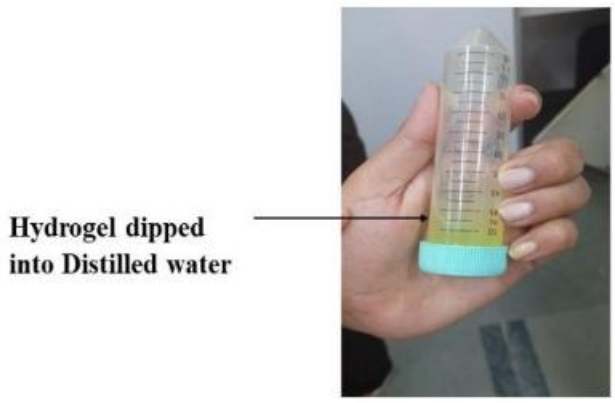

Fig. 11 Test for checking the solubility of the hydrogel

4.4.2 Test for swelling

Measuring hydrogel's swelling was the second test for charecterization **(Fig 12).** The swelling of the formed hydrogel was calculated according to the below mentioned formula.

$$= \frac{Ws - Wd}{Wd}$$

; Ws = Weight of the swollen hydrogel.

Wd = Weight of dry hydrogel.

The swelling came to be 3.2 cm.

The ideal swelling capacity for the hydrogel is considered to be between 3-7 cm **[5]**. The method used by us was by dissolving hydrogel sample into water for letting it to swell whereas method used previously by K. Zhang. et. al. **[17]** was Tea bag method and Filtration method, where the swelling resulted to be 5 cm. The variation in results would have occurred due following factors like change in the method used to determine the solubility, the inter molecular spaces between hydrogel's molecules and also how strong hydrophobic or hydrophyllic compound has been used for the hydrogel's construction.

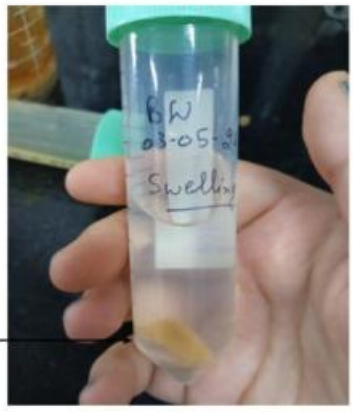

Fig. 12 Test for checking the swelling of the hydrogel

4.4.3 Test for spreadibility

A commonly used method for testing spreadibility is “Parallel plate method” **(Fig. 13)** The process was continued for 1 minute. Later, the change in sample’s diameter was measured with a scale and hence the spreadibility was calculated.

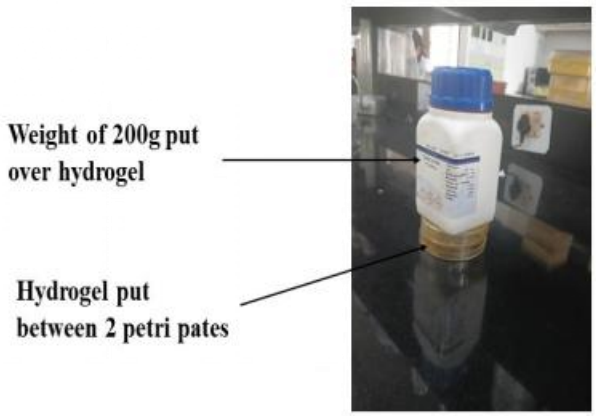

Fig. 13 Test for checking the spreadibility

5-7 cm being the ideal range for the spreadibility test, the spreadibility of our hydrogel came out to be 5.2 cm that is well in the ideal range . N. A. Peppas et. al. , conducted a similar experiment on spreadibility, the results came out to be a bit varying that is 6 cm **[13]** . The difference in results might be due to using different measuring methods like slip and drag method. Also another factor which might have varied the results would be the ionic strength, the pH difference or the temperature.

4.4.4 pH of the solution

The pH of the solution was measured using Litmus paper shown in **(Fig. 14)** The paper was dipped into the hydrogel solution and then checked for its pH. The pH came out to be acidic

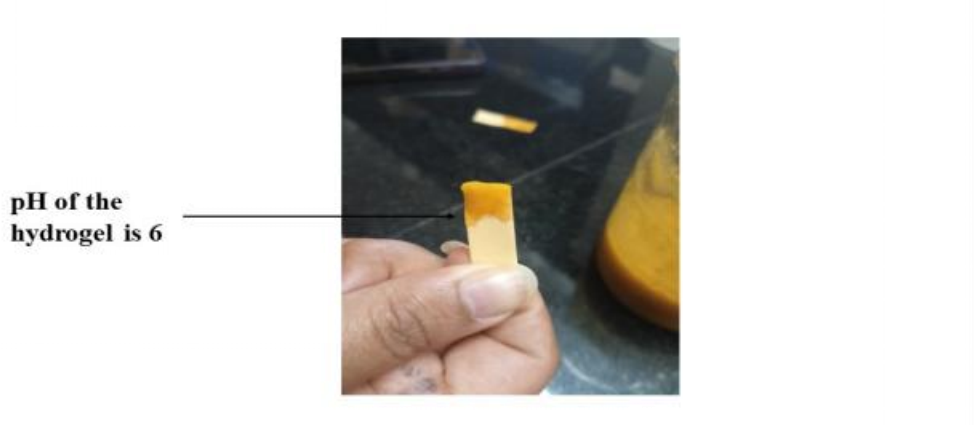

Fig. 14 Test for finding pH of the hydrogel

For the pH test, a simple Litmus paper was used to measure the pH as the hydrogel solution before attaining its 3-D structure was quite thick to use the pH Meter for measuring pH. A similar experiment conducted by Su J, Li J et.al. **[18]** showed that the pH of their hydrogel was 7 whereas our's hydrogel's pH came out to be 6. that is acidic. This was due to the presence of shellac that is a weak acid as well as a very minute amount(0.2 μL) of diluted HCl.

CHAPTER 5 CONCLUSIONS

To conclude from the above experiments, we can say that shellac and cucumin can be a good choice for the formation of hydrogel having various good properties like being anti oxidant, anti- bacterial and anti -inflammatory. The extracted curcumin sample from soxhlet, was free from any other impurity . Clear 3 bands indicating the presence of curcuminoids in the curcumin sample were visible. Another indicating test for curcumin was done by adding sulfuric acid which reacted with curcumin and gave blood red colour. Solution casting method was used to form Pectin/gelatin based hydrogel membranes. Various tests for its physio chemical tests were performed . The solubility test showed the % solubility of the hydrogel. The swelling test showed the capacity of the hydrogel to hold water by swelling up. Next, the hydrogel was tested for its spreadibility that is how much area would it stretch upon putting weight over it. Lastly, the hydrogel was tested for its pH with help of a litmus paper. We can say that our hydrogel from shellac and curcumin was successfully formed and had properties of antimicrobial and anti inflamatory properties. The presence of shellac in the hydrogel helps to give it its structure and also has antioxidant activity. We also added gelatin and pectin. Pectin was found out to be a better cross linking agent for our hydrogel and gelatin was added to fascilitate polymerizatrion. It was also observed that adding HCl and gluteraldehyde both having properties of toxicicity and irritant did not harm the skin as they were added in a very less amount. The solution of HCl and gluteraldehyde worked as a disinfective in the hydrogel.

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