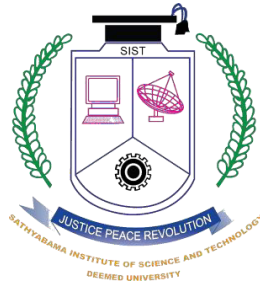


AN ALTERNATIVE FOR CHEMICAL SANITIZER BIO-SANITIZO BALLS

Submitted in partial fulfillment of the requirements for the award of Bachelor of
Technology degree in Biomedical engineering

By

Nisitha S (38240020)



**DEPARTMENT OF BIOMEDICAL ENGINEERING
SCHOOL OF BIO AND CHEMICAL ENGINEERING**

SATHYABAMA

**INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)**

Accredited with Grade "A" by NAAC

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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of **Nisitha S (38240020)** who carried out the project entitled "**An alternative for chemical sanitizer_ Bio sanitizo balls**" under our supervision from **September 2021 to March 2022**.

Dr. T. SUDHAKAR, M.Sc., Ph.D.,
Guide

Dr. T. SUDHAKAR, M.Sc., Ph.D.,
Head of the Department

Submitted for Viva-voce Examination held on **02.05.2022**

Internal Examiner

Dr. BETHANNEY JANNEY J

External Examiner

DECLARATION

I Nisitha S (38240020) hereby declare that the Project Report entitled “**An alternative for chemical sanitizer_Bio sanitizo balls**” done by us under the guidance of Dr. **T. Sudhakar**, MSC., Ph.D., **Department** of Biomedical Engineering is submitted in partial fulfillment of the requirements for the award of Bachelor of Technology degree in Biomedical Engineering.

DATE: 23/04/2022

Nisitha S

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SIGNATURE OF THE CANDIDATE

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ABSTRACT:

Hand sanitizers prevent the hands from becoming a vector of cross-infection and it renders the hands socially clean in order to continue the delivery of health care. The outbreak of covid -19 made individuals use hand sanitizers worldwide. But, currently, available hand sanitizers are useful in killing germs, it is chemical-based, toxic, inflammable, and overuse it can lead to cracked skin, redness, cracking, and can also cause blindness or damage if it enters into our eyes. To overcome that, I possess a new idea of bio sanitizers based on essential oil extract. These essential oils are concentrated natural extracts from plants, fruits, and seed extracts like citrus essential oil, cinnamon oil, and a few oil blends to make them competent. These essential oils can be captured in alginate beads using immobilization techniques (encapsulating the oil in inert structure)/dripping technique (dispersion of oil into biopolymer and droplet extrusion into the cross-linker). This will be effective with minimal or no side effects, non-toxic, chemical-free, safe for hands, fragrance, and re-usable. I named it bio-sanitizoballs, which can be safe for Environment and easily degradable.

Keywords: *Bio-sanitizers, essential oils, non-toxic, Biodegradable, re-usable, disinfectant*

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LIST OF ABBREVIATION:

1. ABHS	Alcohol Based Hand Sanitizer
2. NABHS	Non-Alcohol Based Hand Sanitizer
3. COVID 19	Coronavirus disease 2019
4. LG	Lemon Grass
5. SA	Sodium Alginate
6. NE	Nano Emulsion
7. EO	Essential Oil
8. BC	Benzalkonium Chloride
9. WHO	World Health Organization
10. EDTA	Ethylene diamine tetra acetic acid
11. CFE	Cell Free Extract
12. SARS	Severe Acute Respiratory Syndrome
13. SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2

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CHAPTER-1

INTRODUCTION

Airborne microbes are biological airborne contaminants (also known as bio aerosols) such as bacteria, viruses, or fungus, as well as airborne poisons that are spread through the air from one victim to the next. Humans, pets, plants, plumbing systems, heating, ventilation, air-conditioning systems, mould, dust resuspension, and the external environment are all key sources of airborne microorganisms. There are numerous types of microscopic organisms that could be present in the air, and it is hard to list them all. The most popular ones can be divided into four groups. i.e., Bacteria, viruses, fungus, and allergens.

Bacteria are single-celled organisms that come in a variety of shapes and sizes. Some of these are potentially harmful to your health. In certain situations, these organisms can float around in your air for weeks. You'll inhale them, but they'll also find their way into your meals, onto your skin, and anywhere else you can think of. While some bacteria are harmless, some can cause serious diseases, so it's important to keep their numbers to a minimum. Pathogenic bacteria tiny enough to be expelled from an infected person through coughing, sneezing, laughing, and close human contact or aerosolization of the germ produces airborne infections. The most common airborne bacteria are Gram-negative bacteria: *Escherichia coli*, *Pantoea* (*Enterobacter*) agglomerans, *Pseudomonas* spp, *Acinetobacter* spp, and *Mycobacterium tuberculosis*, *Neisseria gonorrhoea*, *Enterococcus faecalis*, and Gram-positive bacteria: *Staphylococcus aureus*, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Streptococcus agalactiae*, *Corynebacterium* spp, *Listeria monocytogenes*, *Bacillus* spp.

Viruses are microscopic particles that exist almost everywhere on Earth. COVID-19 is the virus on everyone's mind right now. That virus, as dangerous as it is, isn't the only one to be concerned about. Humans are known to be infected by about 200 different viruses. For varied durations of time, they may dwell in the air and on surfaces. A virus inhaled through the air can induce a respiratory infection such as a cold or the flu. The common airborne viruses are Rhinovirus, influenza, paramyxovirus, SARS, variola virus, and hantavirus.

There are millions of species of fungi. They live in the dirt, on plants, on household surfaces, and on your skin. Mold is well-known for its risks, but it's just one of many species of fungi that can pollute your air. Fungi are found in 144,000 different species, albeit not all of them are harmful to humans. The reproductive spores of fungus that grow on surfaces will break off and move into the air. These spores can lodge on surfaces and start new fungus colonies. Worse, you can breathe in the spores, which can make you sick if they get into your lungs. This can cause respiratory infections such as pneumonia, particularly in patients who already have respiratory problems. The common airborne fungus are *Aspergillus*, *Candida albicans*, *Cladosporium*, *Penicillium*, nonsporulating fungi.

Mites are spider-like organisms that are very small. Allergens are released mostly through the faeces of these tiny creatures. Those allergens, unfortunately, become airborne and enter your body in a variety of ways. They may cause allergic reactions such as asthma, sneezing, and coughing.

Hands can easily get contaminated by airborne microorganisms so, hand hygiene is very important. Hand sanitizers Prevent the hands from becoming a vector of cross-infection and it renders the hands socially clean in order to continue the delivery of health care. The outbreak of covid -19 made individuals uses hand sanitizers worldwide. Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Globally, the pandemic remains the top priority. Given the current ineffectiveness of many tactics for preventing viral propagation, the lack of focused treatments, and the daily increase in cases, disinfection is a crucial available measure to limit COVID-19 spread and directly combat SARS-CoV-2. Disinfectants are chemical agents that inactivate or destroy microorganisms, whereas sanitizers are liquid, gel, or foam forms that are used to reduce the number of the microorganisms present and to clean hands. SARS-CoV-2 is a virus that can be disinfected. The SARS-CoV-2 virus is resistant to a wide range of disinfectants. To inactivate SARS-cov 2, lipid solvents such as ethanol (> 75 percent), formaldehyde (> 0.7 percent), isopropanol (> 70 percent), povidone-iodine (> 0.23 percent), sodium hypochlorite (> 0.21 percent), or hydrogen peroxide (H₂O₂; > 0.5 percent) might be utilized. In order to prevent COVID-19 transmission and infection waves, disinfection of environments such as offices, healthcare settings, public transportation, markets, restaurants, and auditoriums is required, taking into account viral presence, persistence, stability, viability, and environmental influence on

viral persistence. WHO recommends only preventive steps and a healthy lifestyle with a strong immune system to combat COVID-19 and keep safe. Alcohol-based hand sanitizers, which are mostly made up of ethanol, isopropyl alcohols, and hydrogen peroxides in various ratios, are recommended by the WHO for frequent hand hygiene. When these preparations are utilized incorrectly, they can be harmful to human health and the environment. When released via evaporation, these compounds are known to be poisonous and detrimental to the environment.

There are two types of sanitizers Alcohol-based and Non- alcoholic sanitizer. Most of the effective sanitizers are alcohol-based hand sanitizer (ABHS), "An alcohol-containing preparation (liquid, gel, or foam) designed to inactivate germs and/or temporarily limit their growth when applied to the hands"; and contain chemical components such as 62-95% of alcohol like ethanol and isopropanol, and hydrogen peroxide. The Center for Disease Control and Prevention recommends formulations containing 80% (percent volume/volume) ethanol or 75% isopropyl alcohol; however, generally speaking, sanitizers containing 60 to 95% alcohol are acceptable. It will work by denaturing the protein and lipid membrane of microorganisms and it also has the ability to inactivate the viruses, hydrogen peroxide inactivates the microorganisms by contaminating the spores in the bulk solution. Mechanism of ABHS on bacteria and viruses the alcohol molecule n-propanol is the most widely utilized in biocides. The specific mechanism of alcohol's antimicrobial activity is unknown with certainty; nevertheless, it may be related to membrane damage, inhibition, or uncoupling of mRNA and protein synthesis via effects on ribosomes and RNA polymerase, or associated with protein denaturation. Its optimal bactericidal efficiency is achieved at concentrations between 60% and 90% for activity against bacteria.

The viral envelope, if present, which is generated from host lipid envelopes, the protein capsid, which stores and shields the genetic material, and the genetic material itself are the primary viral targets of alcohol-based hand sanitizers.

Non-alcoholic-based hand sanitizer(NABHS) contains antiseptic agents such as chlorhexidine, benzalkonium chloride, chloxynol, triclosan, iodine, iodophores, and quaternary ammonium compounds. Disinfectants with benzalkonium chloride are generally less irritating than those with alcohol and its mode of action are Benzalkonium chloride (BC), the key ingredient in NABHS, is generally ineffective against nonenveloped viruses, similar to alcohol-based hand sanitizers, yet a study indicating its efficiency against the nonenveloped human coxsackievirus suggests

exceptions exist. Despite this, it appears that the lipid envelopes of bacteria and viruses are important components for BC's efficacy. BC's cationic "head group" is gradually adsorbed to the negatively charged phosphate heads of phospholipids in the lipid bilayer, causing its concentration to rise. Furthermore, by infiltrating the barrier and disturbing its physical and biological properties, the alkyl chain "tail" component of BC further perturbs and disrupts the membrane bilayer. Protein function is then disrupted, resulting in the solubilization of bilayer materials into BC/phospholipid micelles as a result of the aforementioned impacts. Intercellular targets are also disrupted by BC. It's also crucial to make sure you're purchasing an effective and safe hand sanitizer, as non-approved goods may include allergens, cause rashes, or cause other reactions like itching. In addition to ensuring that the hand sanitizer is safe, inspect the bottle or dispenser it comes in for signs of tampering, as there may be a danger of cross-contamination. Though it is non-alcoholic, it is made up of chemicals so, it also has a harmful effect on humans.

Hand sanitizers can damage the skin by denaturation of stratum corneum proteins, alternation of intercellular lipids, Irritant contact dermatitis. n- propanol and isopropanol have a high irritant property and ethanol has the least irritant property. Adverse effects of alcohol-based hand sanitizer are dry and cracked skin, skin irritation, antibiotic resistance, weakens the immune system, flammable, it is poisoning if a person swallows (alcohol poisoning). The US, National poison Data System reports 65000 cases of ingestion of ABHS between 2011 and 2014. Children are likely used to swallowing colorful and scented things and there are 85000 cases were reported between 2011-2015. ABHS cause eye blindness if it gets into our eyes, skin disorders (irritation, eczema, dermatitis, etc.).

Ingestion of a low quantity of hydrogen peroxide (3 percent solution) is known to cause modest gastrointestinal tract irritation, as well as portal vein embolism, mild mucosal irritation, and vomiting in a few cases. Isopropyl alcohol consumption, whether by accident or on purpose, causes severe respiratory and central nervous system depression. Methanol toxicity was shown to be oral and cutaneous in toxicological evaluations released by Public Health England, with the recommendation that it not be used in hand hygiene products.

Ethanol poisoning also causes respiratory depression, which can lead to respiratory arrest, hypothermia, cardiac dysrhythmias, and cardiac arrest, as well as hypoglycemia, ketoacidosis, and hypotension.

To overcome the adverse effect of alcohol-based sanitizer bio-sanitizers are developed. Bio sanitizers are prepared from natural products without adding alcohol. Basically, these bio sanitizers are made up of glycerine, plant extract, glycol, Citricidal, Waxy Corn Starch, Xanthan, Pectin, Soy Bean Oil, Petrolatum, Deionized Water, water, calcium hydroxide, sodium hydroxide, propylene glycol, carbopol, etc., Herbal disinfections are now being promoted in the industrial sector as an alternative and environmentally acceptable method of disinfectant. Many researchers from a variety of fields are seeking for new antimicrobials to use in the production of phytochemicals. Disinfection, antiseptics, toothpaste, and chemotherapy could all benefit from these compounds. Natural saponifiers are well-known as an excellent source for disinfectant formulation. Because of the antibacterial capabilities of saponin-containing plants, it is classified as both an addition and a component.

Some of the Herbal hand sanitizer and its formula used for the development of hand sanitizer disinfectants are Bacteriostatic Hand sanitizer which contains Eucalyptus, Sadabahar (*Catharanthus roseus*), and other chemicals like glycerine it shows effective against microbes. Herbal hand gel contains Beluntas Leaf (*Pluchea indica* L) and glycerine and it shows effectiveness against *S. aureus*. Water-based herbal hand sanitizer gel containing *Azadirachta indica*, *Ocimum sanctum*, and Citrus limon extracts Carbapol 9400, EDTA, Glycerine, Perfume it acts against *Salmonella*, *E. coli*, *S. aureus*, *Albicans*.

Using biological components such as essential oil and biopolymer, I created a bio sanitizer in the form of balls for this project. No one has yet built a ball sanitizer like the one I created here. Essential oils are chosen for their global availability and antibacterial properties. These essential oils are combined with biopolymer material and blended together. To prevent vaporization, it is then enclosed as microbeads. Finally, the results of the characterization investigation are presented below.

CHAPTER-2

LITERATURE SURVEY

Stevan cucic, *et al.* (2019) Bacteriophages as sanitizers: using lytic phage to control and eradicate listeria monocytogenes biofilm, he used Listex P100 phage to degrade a 24-h L. Monocytogenes biofilm was investigated at 25°C in static culture, and the outcome is Effective to control mono-species biofilms.

Prathistha verma, (2020) A natural antimicrobial from bacillus subtilis as a biosanitizer for resilient membrane biofilms. The bio sanitizer is produced by freeze the cell-free extract (CFE) for 12 hours and dried it. The result is B. Subtilis was observed to exhibit zones of inhibition

Sudhakar bhandare, *et al.* (2021) bacteriophages as bio-sanitizers in food production and healthcare settings. Bacteriophages prevent bacterial recontamination and the advantage of using bacteriophage as bio sanitizer is low toxicity, are environmentally friendly, are not corrosive, and do not have any harmful or offensive odors.

Dyani primasari sukamdi, *et al.* (2020) Formula optimization of antimicrobial hand sanitizer with lemongrass essential oil, advances in health sciences research, this antimicrobial hand sanitizer was produced by adding Alcohol 96% was put in a container, then added aqua dest, glycerin, and essential oils. The whole materials were stirred until they were mixed. The outcome is Hand sanitizer with lemongrass essential oil had the same bacterial inhibitory effect as the market product.

Bo sun, *et al.* (2019) antibacterial cellulose based biomedical hand sanitizer. The silver nanoparticle is Synthesized by reducing AgNO_3 with sodium borohydride in the Nano cellulose gel-medium. The beeswax was then added into the above medium to prepare Nano cellulose/ag/beeswax antimicrobial gel for coating the paper. The innovative alcohol-free hand sanitizer formulated with nanocellulose, affords a long-lasting antibacterial effect without skin dehydration.

Siva surini, *et al.* (2018) formulation and effectiveness of a hand sanitizer gel produced using salam bark extract. The gel base was optimized by preparing three formulations containing carbomer and triethanolamine at ratios of 0.25%:0.5%, 0.5%:1%, and 0.5%:2%. The best gel formulation was mixed with salam bark extract. The gel base was optimized by preparing three formulations containing carbomer and triethanolamine at ratios of 0.25%:0.5%, 0.5%:1%, and 0.5%:2%. The best gel formulation was mixed with salam bark extract.

Nattaya lourith, *et al.* (2020) formulation and efficacy evaluation of the safe and efficient moisturizing snow mushroom hand sanitizer. The stable base ABHRS was developed. The preferred bases were incorporated with various concentrations of snow mushroom extract. The snow mushroom gel significantly ($P < .05$)

Nanik Wijayati, (2020) Formulation of Hand Sanitizer Gel of A-Pinene Isolated from Turpentine Oil and its Antibacterial Activity. Antibacterial activity assay using the disc diffusion method showed turpentine oil. Turpentine oil and α -pinene in hand sanitizer gel formulations exhibit antibacterial activity against *S. Aureus* and *E. Coli*.

J Environ, *et al.* (2021) formulation and evaluation of alcohol-free hand sanitizer gels to prevent the spread of infections during pandemics. Extraction of trembesi leave and stevia leaves are used maceration method with ethanol 70% and macerated for 24 hours. Hand sanitizer formulation with extracts of stevia leaf extract is brownish-green, and color density increases with the addition of extract. Antibacterial and antifungal potentials to control the fungal and mycotoxin contaminations in food grains.

Setareh gholamian, *et al.* (2021) Formation and characterization of calcium alginate hydrogel beads filled with cumin seeds essential oil. After cleaning and grinding, 150 g cumin seeds were immersed in distilled water (250 ml) in a 1000 ml round bottom flask. The cumin essential oil was obtained through hydro-distillation via the Clevenger type apparatus for 4 h. The nano emulsion for cumin essential oil is prepared using low energy emulsification and high energy emulsification. Using impregnation, they had encapsulated the cumin oil into the alginate. They have identify the effects of sodium alginate and CaCl_2 concentrations, hardening time, methods of encapsulation, and emulsion fabrication on the loading capacity of hydrogel beads. Running a fewer number of experiments compared to the full factorial design, the most effective variables and their working ranges were obtained. According to FFD, the best methods

for encapsulation and emulsion fabrication were filled bead preparation and stirring, respectively.

CHAPTER 3

AIM AND SCOPE

3.1. AIM:

- The aim is to create a bio-sanitizer from an essential oil blend, which is an alternative for alcohol-based hand sanitizer.
- This bio-sanitizer is biocompatible, biodegradable, non-toxic, and effective against microbes.

3.2. SCOPE:

- Hand hygiene is very important in this modern world as well as the product should be safe to use.
- Here essential oil blend is created and it is encapsulated into the sodium alginate to protect the oil from vaporization.
- The main scope is to overcome the harmful effect of alcohol-based hand sanitizer.

CHAPTER 4

MATERIALS AND METHODS

4.1.MATERLS:

1. Essential oils (lemongrass EO, basil EO, cinnamon EO)
2. Sodium alginate
3. Calcium chloride
4. Magnetic stirrer
5. Ultrasonicator
6. Spatula
7. Filler
8. Beaker

ESSENTIAL OIL:

Essential oils are concentrated Natural extracts from plants and it is volatile lipophilic components that are used in, nutrition, agriculture, food, cosmetics, and pharmaceuticals because these oils are oxidizable, has antimicrobial properties, and may readily lose their properties. There are a variety of plants that may contain active chemicals. Hundreds of these plant oils have been transformed into essential oil products by manufacturers. Each essential oil has its own distinct aroma due to its unique aromatic components. The following are some of the most popular essential oils: lavender, peppermint, tea tree, lemon, sweet orange, eucalyptus, orange, chamomile, ylang-ylang, basil, cinnamon, rosemary, clove, oregano, etc.

Uses:

- The cosmetic and makeup industry uses essential oils to create perfumes, add fragrance to creams and body washes.
- It is used in pharmaceuticals.
- Aromatherapists believe that breathing in essential oils might allow them to enter the lungs and bloodstream, where some of the potentially helpful compounds may benefit the body.

- As well as inhaling them, adding essential oils to a carrier oil and massaging them into the skin may also deliver the active compounds to the body.
- People should never apply essential oils directly to the skin without diluting them, unless under the direct guidance of a healthcare professional.
- We can preserve the foods by adding the essential oil.

Lemongrass essential oil:

Lemongrass (*Cymbopogon citratus*) essential oil has good antimicrobial properties and it was purchased from (). The active phytochemicals present in lemongrass essential oil are Myrcene, citral, citronellal, Geranyl Acetate, Nerol, Geraniol, and limonene. The major component which has antimicrobial properties is citral (65-85%). citral is a mixture of 2 isomeric acyclic monoterpene aldehydes known as geraniol and neral.



Fig 4.1: Lemon grass essential oil

- Myrcene= Anti-inflammatory, Analgesic, Anti-biotic, Anti-mutagenic,
- Citral= Anti-viral, Anti-septic, Anti-oxidant
- Citronellal= Anti-fungal, Anti-viral, Anti-microbial
- Geranyl Acetate= Anti-fungal, Anti-microbial, Anti-inflammatory
- Nerol= Anti-oxidant, Sedative, Anti-inflammatory, Anti-depressant
- Geraniol= Anti-oxidant, Anti-bacterial, Anti-septic, Analgesic
- Neral= Apoptotic, Anti-nociceptive, Anti-inflammatory
- Limonene= Anti-oxidant, Digestive, Appetite-suppressant, Detoxicant

The stalks of the lemongrass are much smell like lemon. Topical regions such as Africa, Asia, Southeast Asia, Australia, and Oceania are native of lemongrass. It has been used for medicinal, cosmetic, and culinary purposes. In India, China and

Thailand it has been used as a flavouring agent in beverages, desserts, and other culinary preparations for its ability to promote better digestion, boost circulation and immunity, treat infections, and relieve irregularities in the menstrual cycle. Due to its ability to reduce fever, it is also called fever grass in some cultures.

Basil essential oil:

Basil (*Ocimum basilicum*) essential oil is a rich source of EO and The major phytochemicals present in lemongrass essential oil is linalool. Basil Essential Oil is made from the leaves of the Basil herb, *Ocimum basilicum*. The Latin word *basilius* and the Greek word *basilikón phutón*, which both mean "royal plant," are used to name this plant.



Fig 4.2. Basil Essential Oil

Sweet Basil Essential Oil is said to stimulate, clarify, calm, reinforce, energize, and raise the mind in aromatherapy. It is also supposed to repel insects, kill bacteria that cause odors, reduce headaches, and treat respiratory and digestive problems.

Sweet Basil Essential Oil is said to nourish, repair, balance, relax, smooth, exfoliate, and brighten the skin when used topically. The active components of basil are methyl cinnamate (70.1%), linalool (17.5%), β -elements (2.6%) and camphor (1.52%).

Cinnamon essential oil:

Cinnamon (*cinnamomum cassia*) is a very strong essential oil and it has antibacterial, antifungal, antiviral, and antioxidant properties. The major active phytochemical is cinnamaldehyde and the main chemical constituents of Cinnamon Bark Essential Oil and Cinnamon Leaf Essential Oils, albeit in varying amounts, are Cinnamaldehyde, Cinnamyl Acetate, Eugenol, and Eugenol Acetate.



Fig 4.3. Cinnamon Essential Oil

Cinnamaldehyde is known to:

- Be responsible for Cinnamon's characteristic warming and comforting scent
- Exhibit anti-fungal, anti-bacterial, and anti-microbial properties

Cinnamyl acetate is known to:

- Be a fragrance agent
- Have the sweet, peppery, balsamic, spicy, and floral scent that is characteristic of Cinnamon
- Be commonly used as a fixative in manufactured perfumes
- Repel and prevent insect infestations
- Enhance circulation, thereby allowing the body and hair to receive the required amounts of oxygen, vitamins, and minerals to sustain the health of each

Eugenol is known to:

- Soothe ulcers and related pain
- Address gastric pain
- Reduce the chances of developing sores
- Exhibit anti-septic, anti-inflammatory, and analgesic properties
- Eliminate bacteria
- Prevent the growth of many fungi

Eugenol acetate is known to:

- Exhibit anti-oxidant properties
- Have a sweet, fruity, balsamic scent that is reminiscent of Cloves

Medicinal: Anti-parasitic, Analgesic, Anti-Inflammatory, Anti-viral, Immune- Boosting, Anti-bacterial, Anti-fungal, Anti-microbial, Astringent, Anti-septic, Anti- spasmodic, Carminative, Emmenagogue, Stomachic, Tonic, Vermifuge, Anti- Rheumatic, Detoxifying, Immunostimulant, Antioxidant.

Extractions are used to collect a plant's active botanical elements, which serve as the "vital force" of the plant. They are essentially a liquid version of a plant. Younger plants produce more oil than older plants, but because the lighter fractions of the oil continue to evaporate, older plants produce more resinous and darker oils.

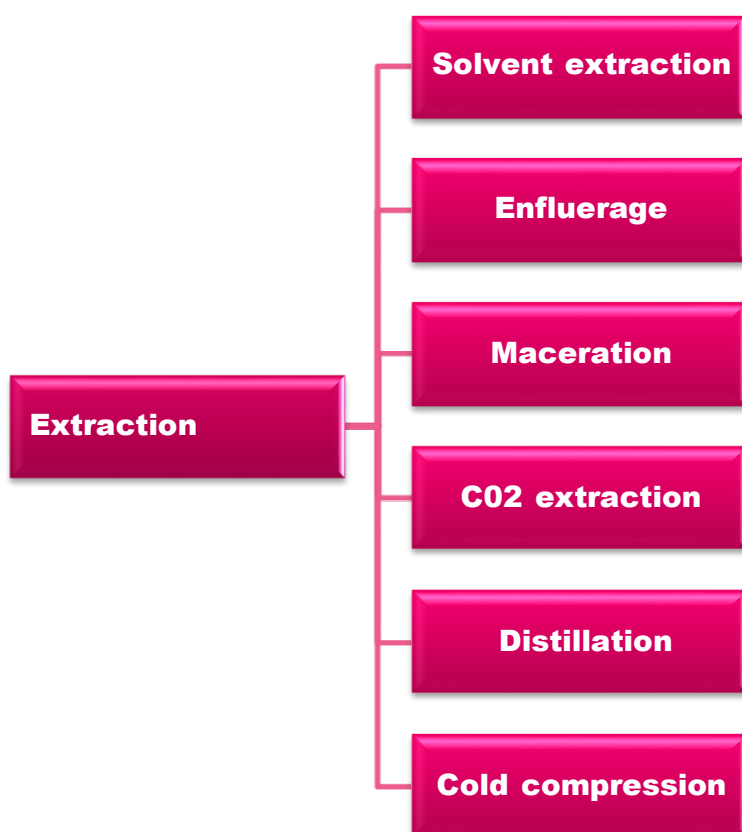


Fig 4.4. Types of extraction

The common method for extraction of essential oil is the steam distillation method. Distillation methods necessitate taking into account the high efficiency at low cost in

unit time as well as its suitability for industry and bioavailability. Some volatile oils cannot be distilled without decomposition and thus are usually obtained by expression (lemon oil, orange oil) or by other mechanical means (cold pressing).

Alginate:

Alginate is a naturally occurring anionic (negatively charged) polymer typically obtained from the cell wall of brown seaweed((Phaeophyceae), also called alginic acid, and has been extensively investigated and used for many biomedical applications, due to its biocompatibility, low toxicity, relatively low cost, and mild gelation by addition of divalent cations such as Ca^{2+} . Alginate hydrogels can be prepared by various cross-linking methods, and their structural similarity to extracellular matrices of living tissues allows wide applications in wound healing, delivery of bioactive agents such as small chemical drugs and proteins, and cell transplantation.

Alginate is typically used in the form of a hydrogel in biomedicine, including wound healing, drug delivery, and tissue engineering applications. Hydrogels are three - dimensionally cross-linked networks composed of hydrophilic polymers with high water content.



Fig 4.5. Sodium alginate

Alginate is used in the food industry as a thickening agent, gelling agent, emulsifier, stabilizer, texture - improver. Nowadays, alginate is added to numerous kinds of food, such as ice cream, jelly, acid milk drinks, dressings, instant noodles, beer, etc.

Calcium chloride:

Calcium chloride is an inorganic compound and its chemical formula is CaCl_2 . The properties are it is white in color, crystalline solid, highly soluble in water. It is produced by neutralizing the hydrochloric acid with calcium hydroxide or directly from limestone, but a large amount is also produced as a by-product of the Solvay process. When we add calcium chloride into the alginate the calcium ions in the solution cross-linked the polymers in the alginate, attaching them to each other at many points. This cross-linking created a flexible, soft solid – a gel bead (hydrogel).



Fig 4.6. Calcium chloride

4.2. METHODS:

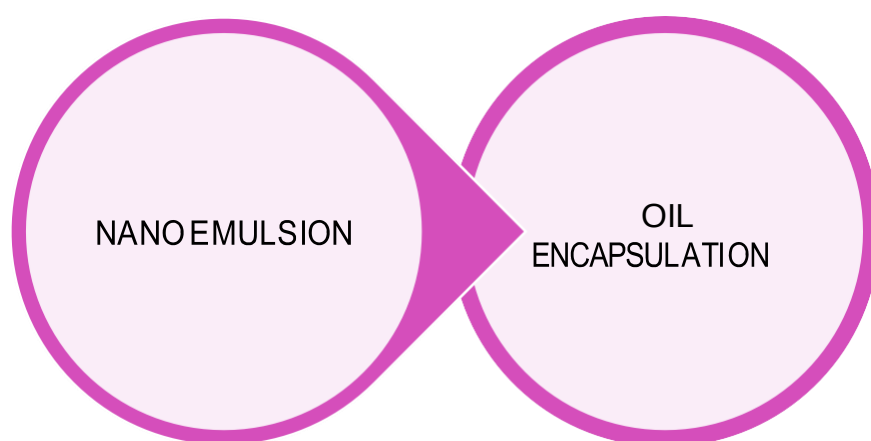


Fig 4.7. Methodology

4.2.1. BLOCK DIAGRAM AND EXPLANATION

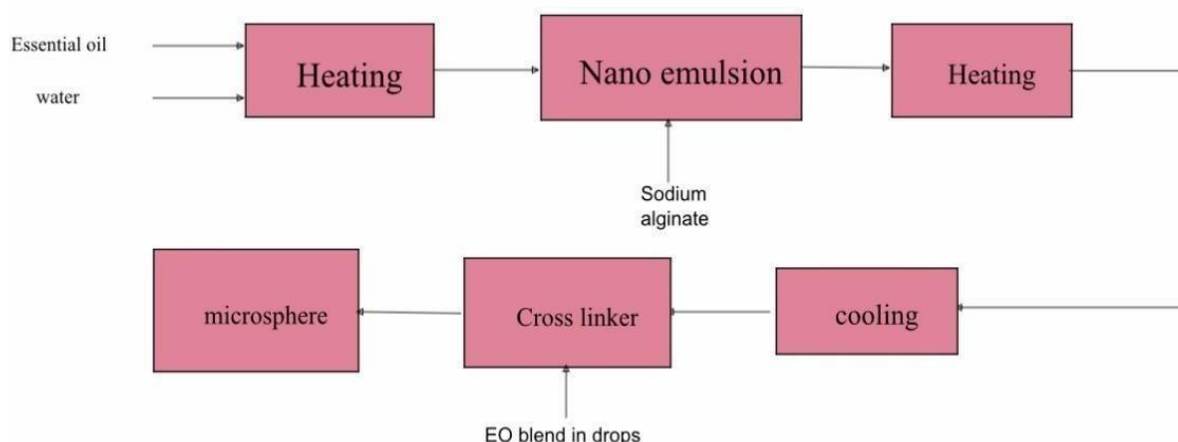


Fig 4.8. Block diagram

Add essential oil and the required amount of water into the beaker and heat until it forms a milky substance (Nano emulsion). Take another beaker and add sodium alginate and water, heat it until a homogenous mixture is formed. Mix the Nano emulsion and homogenous mixture together by heating. Let it cool till it reaches room temperature.

Add the suspension into the calcium chloride drop by drop. Leave it for a minute. Then we can see the bead formation with oil in it.

4.2.2. WORKFLOW:

Nano emulsion:

Nano emulsions are isotropic, homogeneous, translucent, and optically clear systems. In which two non-miscible liquids are mixed together to form a single system. Normally there are two types of Nano emulsion, they are oil in water and water in the oil system.

In OW oily system dispersed in an aqueous system.

In W/O aqueous system is dispersed in an oily system.

Both systems are used for the formation of droplets or oily phases of nonmetric sizes.

Nano emulsions are synthesized by two methods they are low -energy methods and high-energy methods. The low energy method uses internal physical properties to form Nanoparticles and the low energy techniques are phase transition, self-emulsification, and

phase inversion temperature. High energy methods utilize the mechanical devices such as Ultrasonicator, microfluidiser and high energy homogenizer which produce disruptive force to break up the water and oil phases to form a Nano emulsion. The size of the Nano particle is depending upon the instrument used, temperature and time. In this project I have used high energy sonication technique and low energy magnetic stirrer to synthesis the Nano emulsion. These technique is suitable for small scale production.

Advantage:

- Nano emulsions are nontoxic and non-irritant so it can be used for skin and cosmetic products.
- Nano emulsions are isotropic, homogeneous and optically clear substance
- It is kinetically stable the micro emulsions
- It improves the bioavailability
- Nano emulsions have higher loading capacity than micro emulsion for lipophilic active components.

Dis-advantage:

- It is thermodynamically unstable the micro emulsions

NANOEMULSION FORMATION:

Trial 1: -

NE was synthesized using ultra sonication technique and magnetic stirrer. Here ,1ml of essential oil such as lemon grass, basil and cinnamon was taken in 1:1:1 ratio and mixed it with 20 ml of double distilled water. It was heated for 15 min at 60 degrees Celsius using magnetic stirrer. Again heated it for 120 min at 60 degrees Celsius using Ultrasonicator. Perfect emulsion was formed with small oil layer.

Trial 2: -

NE was synthesized using ultra sonicate and magnetic stirrer. Here ,0.5ml of essential oil such as lemon grass, basil and cinnamon was taken in 1:1:1 ratio and mixed it with 20 ml of double distilled water. Heated it for 1 hr. at 65 degrees Celsius using Ultrasonicator. Perfect emulsion was formed with small oil droplets

Trial 3: -

NE was synthesized using ultra sonication technique. Here ,4 drops of essential oil such as lemon grass, basil and cinnamon was taken in 1:1:1 ratio and mixed it with 20ml of double distilled water. Heated it for 1 hr. at 65 degrees Celsius using

Ultrasonicator. Perfect emulsion was formed.



Trial 1 Nano emulsion



Trial 2 Nano emulsion



Trial 3 Nano emulsion

Fig 4.9. Nano emulsion

GELATION TECHNIQUE:

The process of formation of three dimensional network by adding a cross-linker is known as gelation technique. When gelation occurs, a dilute solution is converted in to a gel.

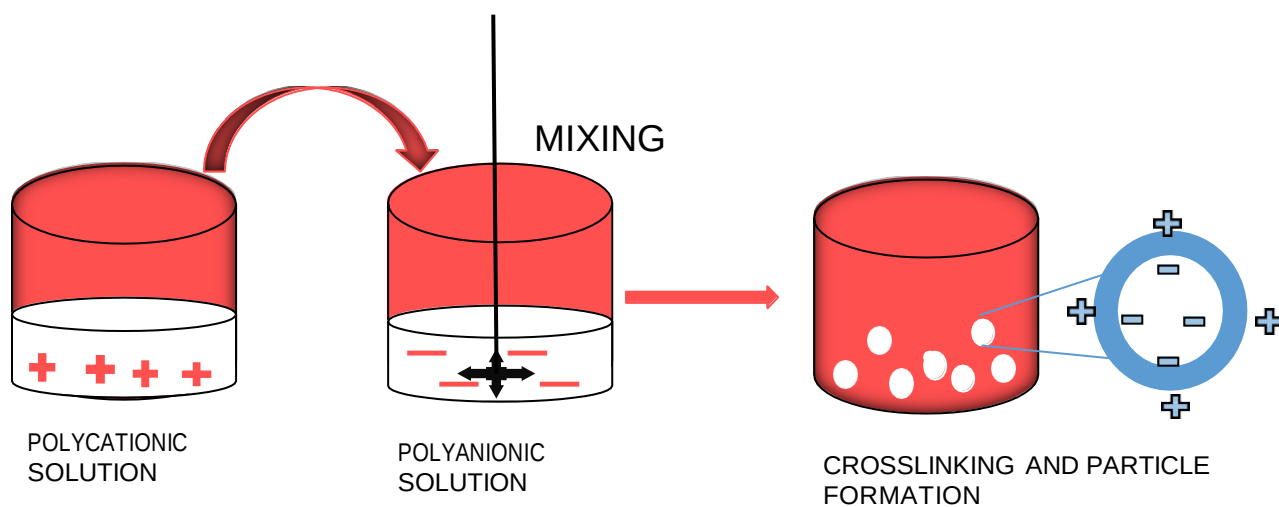


Fig 4.10. Schematic diagram of gelation process

GELATION FORMATION:

Ionic gelation method:

When Polyanionic solution (more negatively charged molecules) such as alginate, pectin and carboxymethyl cellulose is added with polycationic solution (more positively charged molecules) such as aqueous calcium chloride, glutaraldehyde (cross linker). The Polyanionic particles get dissociate with aqueous solution and the polymers get charged and it get precipitate to form a gel.

Example: When alginate (negatively charged) is dissolved in aqueous calcium chloride (positively charged), the calcium displaces the sodium from the alginate, it holds the long alginate molecule together and forms a calcium alginate beads.

OIL ENCAPSULATION TECHNOLOGY:

Oil encapsulation is the protection of oils inside the capsule. The important role of oil encapsulation technology is to protect the properties against moisture or oxygen, prevention of chemical reactions between 2 species, modification of density, colour and shape. Due to these advantages, this technology has more applications in pharmaceutical, food, cosmetics, and perfume formation.

Different types of oil encapsulation techniques are there such as extrusion dipping technique, jet cutting and vibrating get technique, spray-drying techniques, dispersion-cross-linking and dispersion-coacervation technique and droplets (milli)- microfluidic techniques.

In my project I used extrusion technique (external gelation mechanism) for oil encapsulation. The main advantage is oil encapsulation is easy to form and no sophisticated equipment's are required (low cost), and formation of core-shell capsules with high oil loading.

NANOEMULSION (EO BLEND) ENCAPSULATION AND BEADS FORMATION:

TRIAL 1: -

4% of sodium alginate was added to the 20 ml of double-distilled water and heated it for 30 minutes to form a homogenous mixture. Then 5ml of essential oil was added into the homogenous mixture and heated it for 15 min. Then this suspension was then taken into a pillar and added drop by drop into 2% CaCl₂ solution. Kept it for 30 min (hardening time). Very hard beads are produced hard to break.



Fig 4.11. Trial 1 Micro beads

TRIAL 2: -

2% of sodium alginate was added to the 20 ml of double-distilled water and heated it for 30 minutes to form a homogenous mixture. Then 10ml of essential oil was added into the homogenous mixture and heated for 15 min. Then this suspension was then taken into a pillar and added drop by drop into 1% CaCl₂ solution. Kept it for 10 min (hardening time). Slightly hard beads were produced, when pressed to produce uneven pieces.



Fig 4.12. Trial 2 Micro beads

TRIAL 3: -

0.5% of sodium alginate was added in to the 20 ml of double-distilled water and heated it for 30 minutes to form a homogenous mixture. Then 20ml of essential oil was added into the homogenous mixture and heated it for 15 min. Then this suspension was then taken into a pillar and added drop by drop into 0.5% CaCl_2 solution. Kept it for 2 min (hardening time). Evenly breaking beads are formed. Easy to break.



Fig 4.13. Trial 3 Micro beads

SCHEMATIC DIAGRAM:

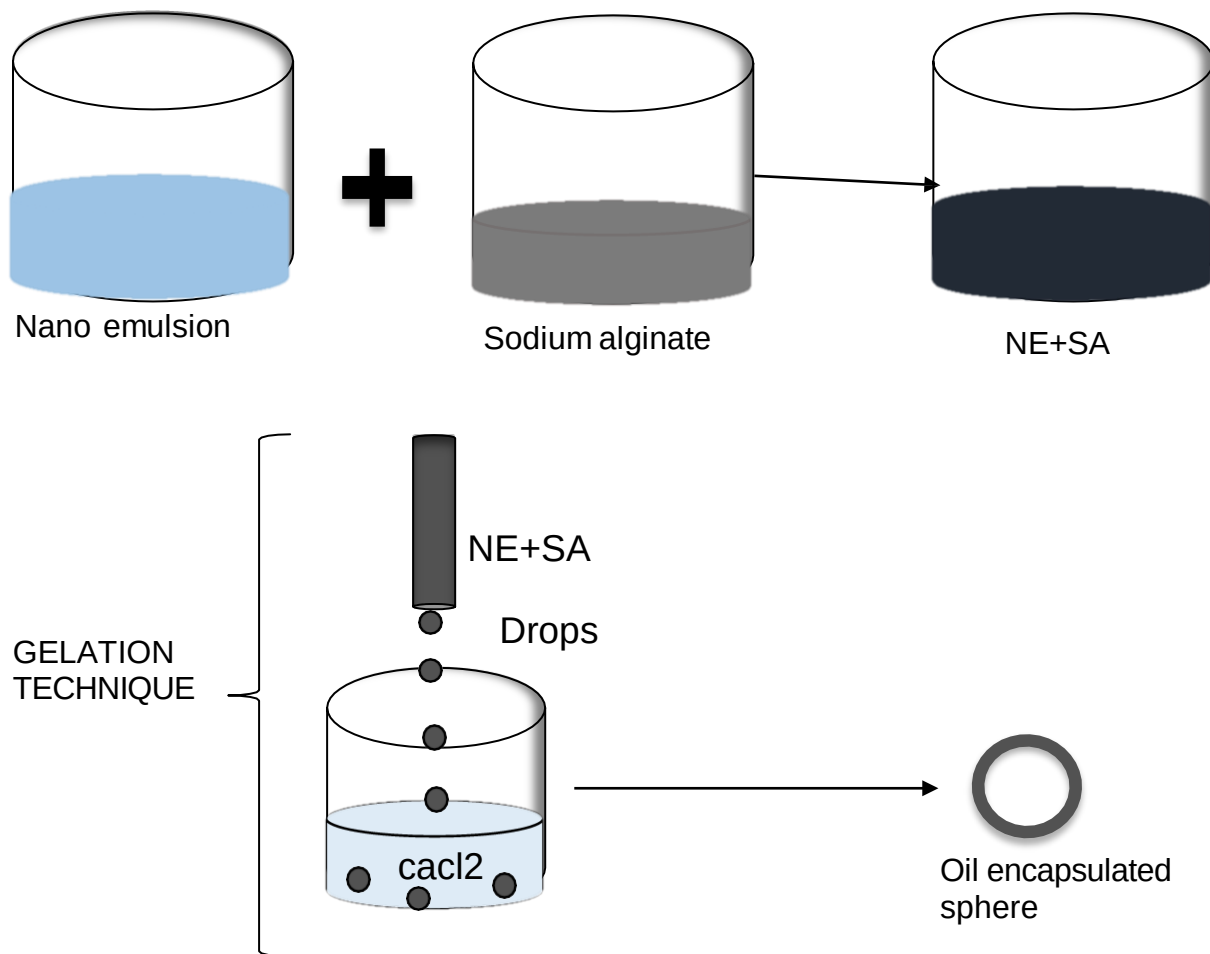


Fig 4.14. The above schematic diagram states the formation of bio- sanitizo balls

4.2.3. CHARACTERIZATION STUDY:

FTIR:

FTIR (Fourier Transform Infrared spectrometer) is used to identify the unknown composition of solids, liquids, and gases. The most important application is identifying the unknown materials. We can also identify the surface contamination via FTIR analysis. The results of the tests can be used to determine the molecular composition and structure of a material. The range of wavelengths in the infrared region that is absorbed by a material is measured via FTIR analysis. To evaluate the material's molecular composition and structure, the sample's absorbance of infrared light energy at various wavelengths is measured. As long as a standard curve with known concentrations of the component of interest can be constructed, materials can be measured using the FTIR materials characterization approach.

An interferometer, a simple device that produces an optical signal with all of the IR frequencies recorded in it, is used to identify samples. The signal can be immediately measured. The signal is then decoded using the Fourier transformation, a mathematical technique. The spectral information is then mapped using a computer - generated technique. The resulting graph is the spectrum, which is subsequently identified by comparing it to reference libraries.

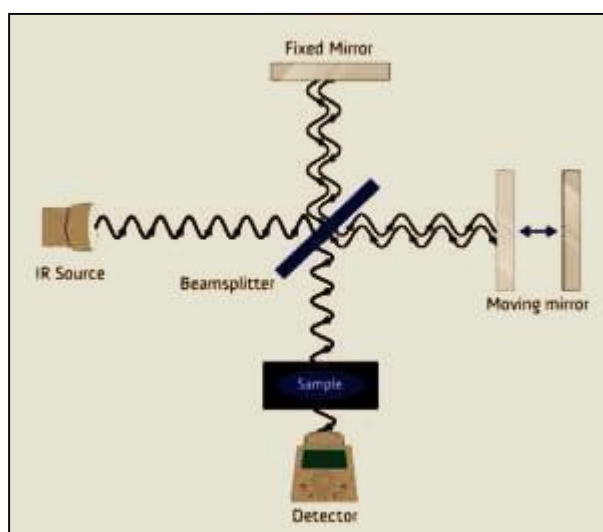


Fig 4.15. FTIR

The testing process first places the sample in an FTIR spectrometer. The spectrometer sends infrared light beams at the sample and detects how much of the beam and at what frequencies the sample absorbs. A thin slice of the material must be removed or the sample must be thin enough for the infrared light to pass through. On some samples, reflectance techniques can be employed without causing damage to the sample. Residues, stains, or coatings on a very flat reflecting surface, or slightly malleable materials thin enough to fit under the microscope utilizing the attenuated total reflectance attachment to the microscope, are samples favorable to reflectance.

Thousands of spectra are stored in the reference database, allowing samples to be identified.

SEM:

SEM (Scanning Electron Microscope) analysis, or scanning electron microscopy, provides high-resolution imaging that can be used to evaluate various materials for surface fractures, flaws, contaminants, or corrosion. The incident electron beam sweeps across the sample surface and interacts with the sample, generating backscattered and secondary electrons that are used to build an image of the sample. Electrons are such little particles that they behave like photons in light. In the electron source, electrons are emitted from a filament and collimated into a beam. A beam of electrons travels through the specimen before being magnified by a series of lenses. The picture is caused by electron scattering by atoms in the specimen.

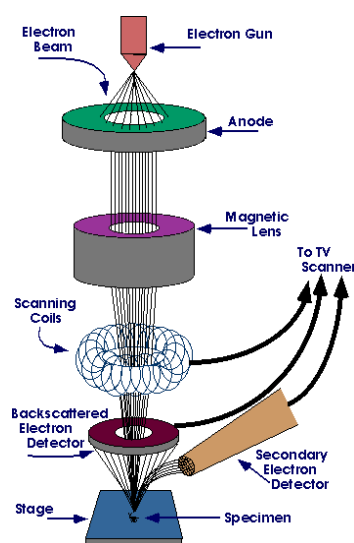


Fig 4.16. SEM

DEGRADATION STUDY:

Degradation tests reveal the molecule's chemical stability, which aids in the development of stable formulations with appropriate storage conditions. Alginate is degraded by a group of enzymes known as alginate lyases or alginate depolymerases in nature.

I took both wet and dry sanitizo balls for the degradation study. I immersed wet balls in distilled water and dry balls were kept in an airtight container. Both were observed for several months and their weight was noted.

PH test:

A PH-meter was used to measure the PH of 0.5 milliliters of formula, and the findings were recorded. The purpose of this test was to see if the preparation could be used at the PH of human skin (4,5-7,0), as it was later used on hands.

INVERTED MICROSCOPIC IMAGE:

Inverted microscopic image is used to see the morphology of Nano emulsion. An inverted microscope has the light source pointing down onto the stage, and the sample is viewed from below. The transmitted light source and the condenser are situated on top of the stage in an inverted microscope, pointing down toward the stage. The sample at the bottom of the stage, pointing up.



Fig 4.17. Inverted microscope

ANTIMICROBIAL ACTIVITY:

Antimicrobial activity refers to all active principles (agents) that stop bacteria from growing, prevent the formation of microbial colonies, and sometimes kill microorganisms. The antimicrobial testing was done by using the agar well diffusion method. The antibacterial activity of plants or microbial extracts is commonly assessed using the agar well diffusion method. The agar plate surface is inoculated by spreading a volume of microbial inoculum over the entire agar surface, similar to the approach employed in the disk-diffusion method. Then, using a sterile cork borer or tip, a hole with a diameter of 6 to 8 mm is punched aseptically, and a volume (20–100 L) of the antimicrobial agent or extract solution at the necessary concentration is put into the well. The agar plates are then incubated at the appropriate conditions for the test microorganism. The antimicrobial ingredient diffuses across the agar medium and prevents the bacteria strain from growing.

CHAPTER 5

RESULT AND DISCUSSION

MICROSCOPIC IMAGE OF BEADS:

Fig 5.1. shows the microscopic picture of oil encapsulated alginate beads prepared using external gelation technique which looks spherical shape it is correlated with Ca-alginate beads formed using the 127 mm/165 mm micro nozzle under a microscope Stefan haeberle *et al.* (2008). Bead micro picture taken using optical microscope with 0.05 M calcium carbonate and citrate, they look spherical in shape (Bryshila Lupo *et al.* (2014)). The size of the beads was also influenced by the inner diameter of the syringe needle. The dripping process is used to make calcium alginate beads. After drying, the size of SA beads was drastically altered Zhang Z-H *et al.* (2011)



Fig 5.1. Microscopic image of beads

FTIR:

FTIR absorption spectrum shows the presence of materials I have used such as sodium alginate, calcium chloride, lemongrass essential oil, cinnamon essential oil, and basil essential oil. With its characteristic peak, I analyzed the presence of each material via its functional group. Free hydroxyl (OH stretch) and N-H stretch at wavelength 3500-4000 cm^{-1} shows the presence of sodium alginate. The sodium alginate characteristic band appeared at 3400 cm^{-1} , O-H stretching and stretching vibration band at 1595 cm^{-1} for C=O and 1408 cm^{-1} C-C groups Hamzeh Alkhatib *et al.*, 2020. The band at 1500 cm^{-1} shows a symmetrical stretching bond between Ca-Cl so it's a calcium chloride spectrum and the broad peak at 3400 cm^{-1} shows a O-H stretching vibration so it's because of residual water. C-Cl stretch at 400-750 cm^{-1} indicates the presence of calcium chloride. C=O stretch unsaturated aldehyde at 1750-

1900 shows the presence of lemongrass. The peaks at 1630.17 and 1465.85 cm^{-1} shows an asymmetric and symmetric stretching of COO^- vibration, respectively (Najafi_Soulari *et al.*, 2016). $=\text{C-H}$ (alkene), O-H stretch (alcohol) at 4000, 800-1000 cm^{-1} indicates the presence of Basil. The highest bands appeared at 2926.96 cm^{-1} ($-\text{CH}_2$ symmetrical stretching), 2855.69 cm^{-1} ($-\text{CH}_2$ asymmetrical stretching), 2718.32 cm^{-1} (CH stretching of aldehyde), 1745.28 cm^{-1} ($\text{C}=\text{O}$ stretching), 1377.57 cm^{-1} ($-\text{CH}_3$ symmetric bending), and 1158.65 cm^{-1} (aromatic CCH) (Amiri *et al.*, 2020; Rohman & Ariani, 2013; Rohman, Wibowo, Sudjadi, Lukitaningsih, & Rosman, 2015). Peaks at 1300-1500 cm^{-1} replying the aromatic ring (C-C stretch) indicates the presence of cinnamon. For the alginate control study, peaks at 3271, 2932, 1300, 1077 and 1025 cm^{-1} were observed, corresponding to bonds: O-H, C-H, C-C-H and/or O-C-H, C-O-C of sodium alginate, respectively. Similar results were reported by Lawrie *et al.* (07) and Aguirre & Santagapita (2016).

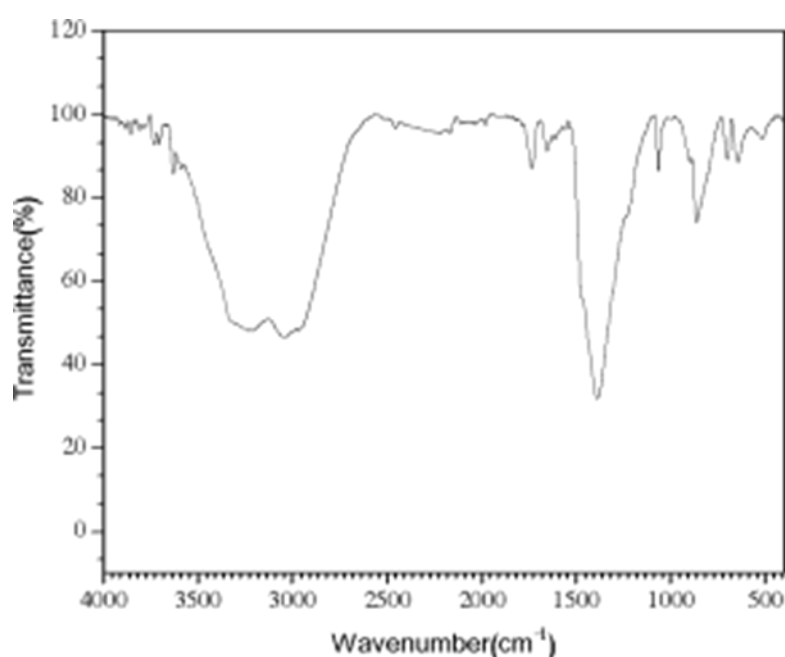


Fig 5.2. Shows the FTIR spectrum of sodium alginate and Nano emulsion suspension

SEM:

Scanning Electron Microscope images show the surface of microbeads. The cross-sectional morphologies and surface morphology examination done by SEM. The smooth surface and homogenous distribution of the essential oil bio-sanitizer beads as shown in Fig 5.3.

In this mechanism, calcium ions are diffused inside the alginate droplet to start crosslinking where they fill the spaces between the polymer chains Hamzeh Alkhatib *et, al*, 2020. During the hydrogel preparation, Na^+ ions are replaced by Ca^{2+} ions of the CaCl_2 , released into the hardening medium, and formed sodium chloride Natalia Riquelme *et, al*, 2021. The SEM images were taken in 2.00kx, 5.00kx, and 500x. At 500x we can see the sodium chloride binding and equal distribution of calcium alginate hydrogel. At 2.00kx and 5.00kx we can see the further magnified surface image.

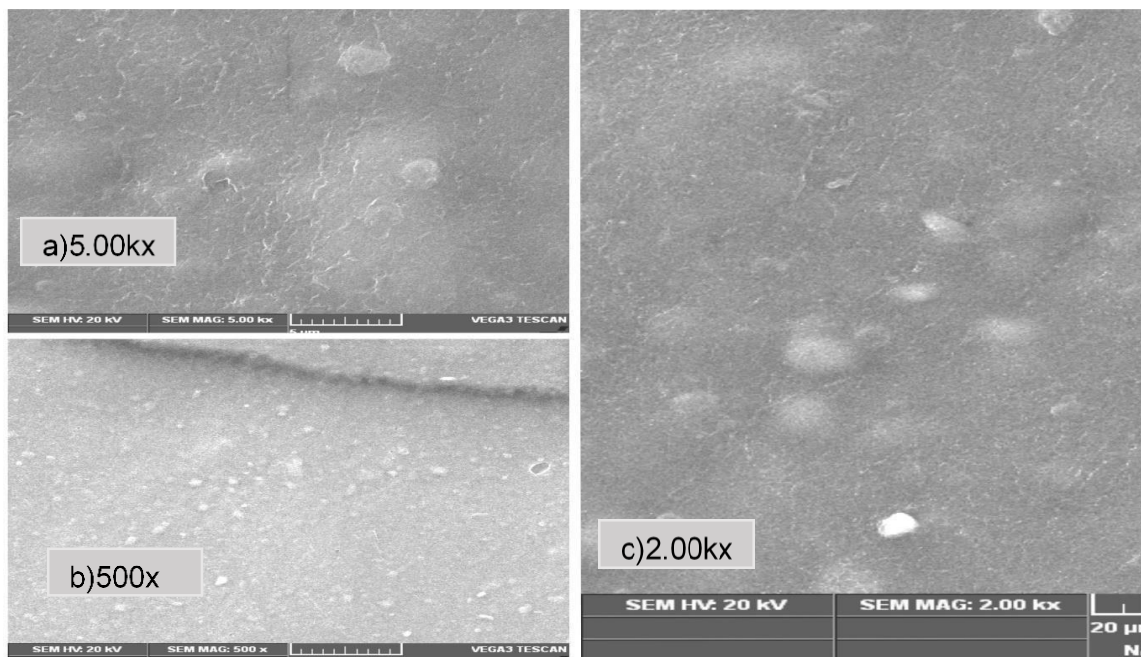


Fig 5.3. Shows the Microstructure of beads

DEGRADATION STUDY:

Degradation study was done by taking the weight for dry and wet balls. It shows that dry balls degraded faster than wet balls and wet balls get swollen and degraded slowly. The values are shown below.

ONE DRY BALL WEIGHT	ONE WET BALL WEIGHT
0.059	0.047
0.057	0.053
0.052	0.046
0.024	0.048

Table 5.1. Degradation study

Bio-sanitizer balls should be reabsorbed with an appropriate degradation rate. The choice of a biomaterials is thus of great importance with regard to tissue engineering (Iudovica Parisi *et al* 2018). Degradability of the bio-sanitizer balls material is a concerned property for use-case of the sanitizer, biomaterials because it's a crucial for sanitizing purposes. The degradation study was performed according to the ISO 10993-14 "Biological evaluation of medical Device-Part 14". Table shows the swelling ratio of the Nano-emulsion balls in double distilled water in different time intervals. Here the samples were subjected in double distilled water with pH of 5-7 in different time interval.

PH test:

The first test was a PH reading with a PH meter. The Nano emulsion had a PH of 6.0, indicating that it was compatible with the skin's PH range. DYANI PRIMASARI SUKAMDI, *et al.* (2020), in the existing paper they have used 96% alcohol and essential oils, but in my project, I haven't used alcohol or other chemicals and technology I utilized is easy and it takes 2 hours to develop a whole sanitizer. In the existing paper, they have done an antibacterial test for E. coli alone. But my sanitizer has the ability to disintegrate bacteria, viruses, and fungus. Currently available alcohol-

based sanitizer has more harmful side-effects whereas this bio sanitizer balls are eco-friendly, which means it is totally made up of biomaterials, and it is biodegradable so it won't pollute the environment and the PH is the same as the PH of our skin, it doesn't cause any irritation to the skin. In order to live a healthy life hand hygiene is very important because, hands are the vector for cross infection. So, this bio sanitizer protects us from these infectious microbes with no side effects. The risk of skin irritation could be reduced if the pH of the preparation was in line with the pH range of the skin, indicating that it was compatible with the skin's PH range. The risk of skin irritation could be reduced if the pH of the preparation was in line with the pH range of the skin.

INVERTED MICROSCOPIC IMAGE:

In fig 6.3. We can see the nanoparticles of Nano emulsion clearly. It is equally dispersed so it confirms that the Nano emulsion of lemon grass, basil, and cinnamon was formed perfectly. Microscopic image showed that the thyme essential oil nanoemulsion was spherical in shape and moderately mono or didispersed and was in the range of 26.6 – 45.3 nm (Fig.4). The droplet size was correlated well with the results obtained from droplet size analysis using the dynamic light scattering (Abd-El salam and Khokhlov, 2015). Microscopic image has been described as an appropriate tool to characterize nanoemulsions (Klang, Matsko, Valenta, & Hofer, 2011) and nanomaterials in foods (Blasco & Picó, 2011; Dudkiewicz *et al.*, 2011), since it is able to provide enough resolution to see nanosized structures which could not be detected by classical microscopy techniques.

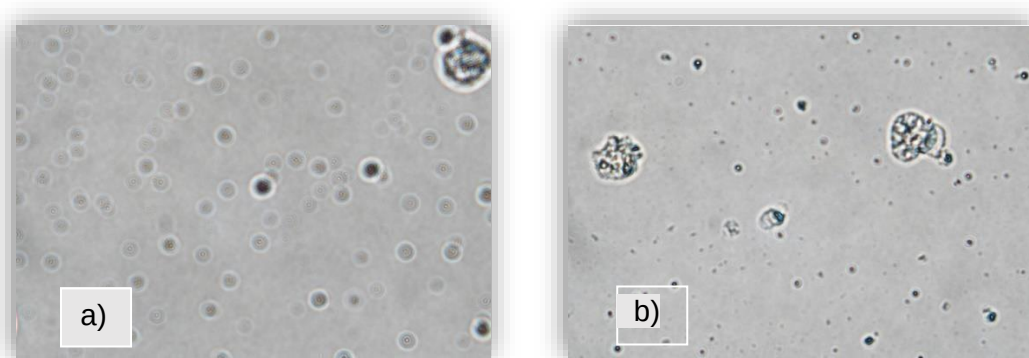


Fig 5.4. Morphological analysis of Nano emulsion with inverted microscope

ANTIMICROBIAL EFFECT:

The overall inhibition of essential oil blended with sodium alginate (Bio- sanitizo balls) against E.coli, Candida, and staphylococcus showed 60% activity which is correlated with the SFPE films had the highest levels of inhibition (60 percent of colony growth inhibition), which was attributed to the antimicrobial agent being protected by the encapsulating agent, boosting the antimicrobial activity of these films (Natalia riquelme *et al.* (2017)). Films containing 3% of basil essential oil microcapsules were able to lower the total starting population of E. coli 32 by 3 log and that of S. saprophyticus 3S by 2 log, Amor G *et al.* (2021). Lemongrass essential oil inhibited all three gramme positive pathogens, Staphylococcus aureus, Bacillus subtilis, and Bacillus cereus, with inhibition zones of 32.0 ± 0.75 , 48.0 ± 1.05 and 21.0 ± 0.64 mm, respectively Shendurse AM *et al.* (2021).



FIG 5.5. Antimicrobial effect

CHAPTER 6

CONCLUSION

An alternative for chemical based sanitizer i.e., bio sanitizer was developed using oil dripping technique, essential oils are encapsulated to protect its properties and its presence is confirmed using FTIR spectrum and microstructure was seen by SEM images. Dripping technique is the easy and cost effective (doesn't required sophisticated equipment's) technique for oil encapsulation. Hand sanitizer showed to be effective in limiting bacteria growth. However, soap and water showed not be as effective as hand sanitizer. Essential oils like cinnamon, lemongrass, basil oil used in micro beads act as barrier for microorganisms. To overcome the adverse effect of alcohol based hand sanitizer_ bio sanitizer is the best and most effective alternative one.

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