ESSENTIAL OIL (EO) BLEND LOADED SOLID LIPID NANOPARTICLES (SLNS) AS NOVEL DEODORANT.

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2022

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List of Abbreviations

BAP	**Blood agar plates**
DPPH	**2,2-Diphenyl-1- picrylhydrazyl**
EO	**Essential oil**
FTIR	**Fourier transforms infrared spectroscopy**
LDCs	**Lipid drug conjugates**
NLCs	**Nanostructured lipid carriers**
SEM	**Scanning Electron microscopy**
SLNs	**Solid lipid nanoparticles**
SPR	**Surface plasmon resonance**
TSA	**Tryptic soy agar**
TSB	**Tryptic soy broth**
UV	**Ultraviolet**
VIS	**Visible**

Abstract

Human odors have been of great concern to society. The generation of malodor on the skin surface of modern humans is caused by the biotransformation of naturally secreted non-odorous precursor molecules into volatile odorants by members of the commensal microbiome. Culture-based microbiological studies revealed that the axillary microbiota consists primarily of Gram-positive bacteria of the genera *Staphylococcus*, *Corynebacterium*, and *Propionibacterium*. Currently there is need to study the selective suppression of the growth of microorganisms involved in diseases and unpleasant odors may result in the establishment of a good symbiotic relationship between microorganisms and humans. As the growth of axillary microbiota which causes odor can be controlled by several ways including hygiene and antimicrobial skin friendly essential oils. Given the limitations in currently available products, a deodorant/antiperspirant formulation that effectively prevents body odor without producing skin sensitivity or perceived undesirable consequences is required. Our work is the deodorant production which is made from all naturally extracted products which includes the lipid which sources from whale fish and is completely alcohol and toxins free. This deodorant is fabricated using lecithin, solid lipid nanoparticles, natural essential oils, and fragrance which are incorporated in a gel. The gel phase contains triethanolamine, TEA and Carbopol-940. TEA is used in safer limits for the polymerization of Carbopol-940 to form a clear gel. The essential oils used possess antibacterial properties which are effective against sweat bacteria. The deodorant is aluminium and parabens free as they contribute to cancer development and hormonal imbalance.

Key Words: *Essential Oils, SLNs, Human axillary malodor, Human sweat, Axillary microflora, EO based deodorant, Al free deodorant, Paraben free deodorant, Alcohol free deodorant.*

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CHAPTER 1: INTRODUCTION

1.1 Objectives

The study is based on the antibacterial deodorant formulation which is comprising of essential oil (EO) blend, loaded in solid lipid nanoparticles, SLNs. The deodorant possesses the ability to kill the microbes present in human axilla which are responsible for malodor. This research study is divided into two sections. The first section is comprised of synthesis of essential oil blend loaded solid lipid nanoparticles, SLNs and its characterization. The essential oil blend was used as therapeutic agent due to their antibacterial and skin-friendly properties. Hot homogenization method was used for synthesis of SLNs. Differential properties of nanoparticle formulations are evaluated using a range of characterization approaches, allowing for an in-vitro investigation of antibacterial properties of SLNs loaded with essential oil blend.

The second section includes the study of microbiology of human axilla and the management of microbiota present in this region of the body. This was carried out by incorporating the SLNs into a gel, forming alcohol and all toxic elements free deodorant. The incorporation of SLNs into the gel was regarded as an important step as it's a promising commercial product which possesses no toxins, totally alcohol free and also free of any toxic elements which are harmful for the body.

1.2 Essential oils

Essential oils (EO) are extracted from plant material through steam distillation. Even though they are obtained from natural sources, the chemical composition of all oils is still undetermined (Chiasson, et al., 2001). Essential oils undergo "post treatment" during the manufacturing process, which causes the change in color, removal of certain concentrations of oil or specific chemicals (Li et al., 2014). The final composition of individual oil varies with the production process, harvest

year and country of production (De Groot and Schmidt, 2016). Oil quality is not always correlated with its purity. The processes of adulteration, ageing (which produces hydroperoxides from autooxidation), and contamination reduce the quality of essential oils (mixing additional products to essential oils). Most essential oils have between 100 and 500 different parts. Terpenes, B-caryophyllene, limonene, and linalool are the most prevalent constituents. (Sindle and Martin, 2021).

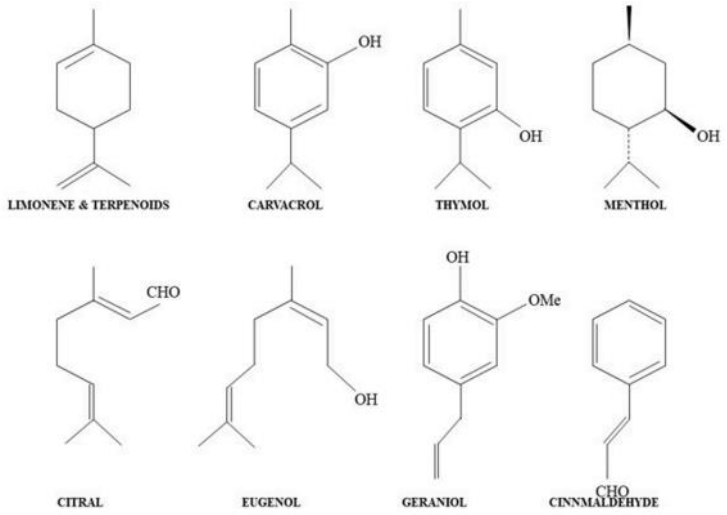


Figure 1.1: Chemical structures of essential oils.

1.3 Essential oils in therapeutics

Essential oils and their components are commonly found in therapeutics, that are highly suggested by physicians for their antipruritic, cough suppressant/decongestant and analgesic properties. Menthol, an integrant of peppermint oil, is usually present in cough-suppressant pain- and itch-relief products. Camphor is a terpene obtained from camphor trees, is frequently present in combination with menthol to give similar beneficial results. Eucalyptus oil, extracted from eucalyptus trees, is in formulations that act as a cough suppressant/decongestant. The doctors

utilize these products as a less dangerous alternative to topical and systemic medications. To achieve added benefits, these products are often employed in conjunction with additional therapies. Inspite of the fact that these plant- derived components contain their allergenic tendency, they are added in significantly low concentrations as compared with the direct application of oils to the skin and are therefore considered reliable alternatives (Raskin et al., 2002).

Essential oils are encapsulated in micro or nanometric systems to ensure stability and protection from chemical degradation (de Matos et al., 2019). It also enhances the efficacy and bioavailability of formulations due to controlled release of bioactive compounds and improved cellular absorption. Modified nanostructured systems have been introduced intending topical administration to handle the drawbacks in administrative routes, such as mucoadhesive systems that caused delayed delivery of drugs through skin or mucosal tissue layers (Guilherme et al., 2017). Another strategy employed to strengthen the stability of essential oils includes the molecular encapsulation through complexation with cyclodextrins. It helps evade volatilization and enhance transfusion of bioactive molecules (Deluzio, T. G. 2014)). In recent years, the interest in the use of natural products and nanotechnological approaches have triggered search and development of drug delivery systems.

1.4 Nanoparticles

Nano, a Latin word, means dwarf. Nanoparticles can be defined as objects ranging in size from 1-100 nm that due to their size may differ from the bulk material. Nanotechnology offers a size of one thousand millionth of a particular unit (i.e., 1 nm = 10 −9 m). It is an emerging field of science that deals with synthesizing and developing various nanomaterials. It has now acquired the status of allied science, due to its common use in other fields of science such as engineering, physics,

and electronics. Recently, the integration of nanotechnology in pharmaceutical and medical sciences proved a success for drug delivery system and therapeutic purposes (Busquets et al., 2017).

1.5 Solid lipid nanoparticles

Aqueous colloidal dispersions with a lattice of solid biodegradable lipids are known as solid lipid nanoparticles (SLNs). They are used as a carrier system for an effective corrective dynamic and water dissolvable medication. Colloidal particles that range in size from 10-1000nm are known as nanoparticles. These particles are incorporated from manufactured characteristic polymers to enhance medication conveyance and reduce lethality (Lingayat,et al., 2017). They are used as a substitute for liposomes as medication carrier. SLN put forth interesting properties, such as huge surface zone, little size, communication of stages at the interface and high medication stacking (Rupenagunta et al., 2011). SLNs integrate favorable circumstances and ensures a strategic distance from the down sides of a few colloidal carriers of its class, such as controlled release, physical stability, reduced degradability, excellent tolerability of SLN formulation for different routes (dermal, oral, parenteral, visual, rectal, pulmonary). SLNs are considered a potential colloidal transport system which is distinguishable from oil in water emulsion for parenteral nourishment (Reddy and Shariff, 2013).

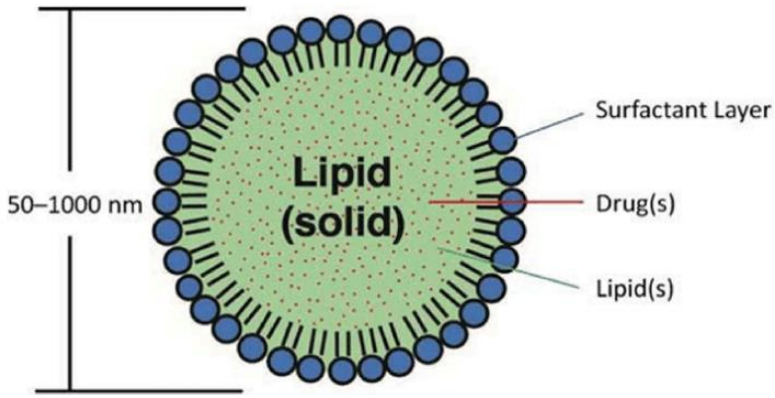


Figure 1.2: Structure of SLNs.

1.6 Skin friendly and toxins free deodorant

The body's physiological response of sweating helps in body thermoregulation. Although this is a necessary bodily function, it can make a person feel uneasy in social situations, particularly when it results in unpleasant odors or excessive perspiration due to hyperhidrosis. Numerous chemical components seen in human odor are produced by certain bacteria that inhabit the armpits when they break down sweat secretions. Deodorants are products that are applied to the skin to hide or eliminate unpleasant odors by using perfumes or by killing bacteria using antiseptics. For example, triclosan, a broad-spectrum antibacterial that is incredibly common in deodorant compositions, has raise concerns over its safety and mainly its environmental toxicity. Aluminum salts are the most often utilised active ingredients in antiperspirant compositions, as was already mentioned above. Numerous research have looked into the relationship between breast cancer growth and the regular use of goods containing aluminium salts, but some experts contend that the data is still insufficient to draw any strong conclusions. The commonly used antiperspirants and deodorant actives have limitations relating to human or environmental safety and have been on the market for years. Additionally, there are concerns regarding how these substances will affect the bacteria in the

underarm area. Therefore, there is a lot of demand in this industry to develop new materials and actives that can control sweat and eliminate unpleasant odours without endangering the environment or human health (Arora et al., 2022).

CHAPTER 2: LITERATURE REVIEW

2.1 Nanocarriers

Structures that are between 1 and 100 nm in size in at least one dimension are known as nanoparticles. A particle that is several hundred nanometers in size is designated with the prefix ‘nano’. When compared to larger molecules, cells can perform the optimum physicochemical and biological qualities more easily, which makes their usage as delivery vehicles for bioactive chemicals already on the market successful (Shi et al., 2020). The examples of nanoparticles are liposomes, dendrimers, polymers which are tested in systems of drug delivery (Cheng et al., 2022).

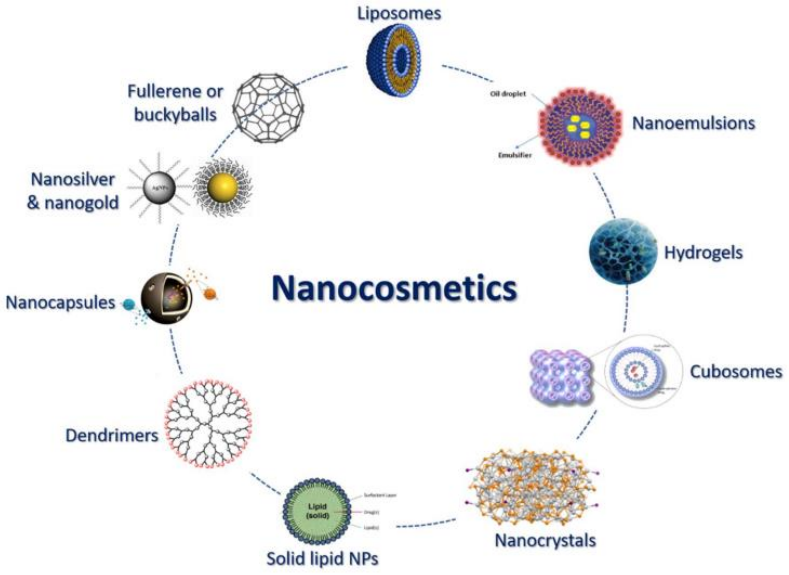


Figure 2.1: Various types of nano systems used in cosmetics.

2.2 Nanocarriers used for medical applications

For their application in the medical field, nanocarriers must be biocompatible and nontoxic. Biocompatible means that they can integer with a biological system without evoking any negative effects. The size, shape, amount, and surface chemistry determine the undesired effects of

nanoparticles. The toxicity of nanoparticles is affected by many factors which makes their estimation difficult and thus each new DDS formulation is toxicologically (Sala et al., 2018). It is generalized that nanoparticles which are small have greater surface area and are more reactive as well as toxic. The in vivo applications of nanoparticles have a hydrodynamic diameter of 10-100 nm which shows its optimal pharmacokinetic characteristics. The large size nanoparticles are quickly removed from the bloodstream while smaller nanoparticles are subjected to tissue extravasations and renal clearance (Amiri et al., 2019).

2.3 Nanoparticles based on solid lipids

The proposed systems fall into several categories, including solid lipid nanoparticles (SLN), nanostructured lipid carriers (NLC), and lipid drug conjugates (LDC), all of which are based on solid liquid matrixes. Lipids are solid at normal temperature. For cutaneous, peroral, parenteral ocular, pulmonary, and rectal distribution, they have been determined to be appropriate. SLN are particles made of waxes, complicated glyceride mixes, or highly purified triglycerides that have been stabilised by a variety of surfactants. A few of SLN's many qualities include good physical stability, regulated drug release, and good tolerability. It also protects integrated pharmaceuticals from degrading. Some drawbacks of these nanoparticles have also been noted, including poor loading capacity, drug ejection after crystallisation, and dispersions with a disproportionately high-water content. (Montoto et al., 2020).

2.4 Role of EO blend loaded SLNs in cosmetic applications

SLNs are popular unconventional systems used in cosmetics and have several advantages. The toxicity of SLNs is low due to their composition of several physiological and biodegradable lipids. These are submicron colloidal carriers which range in size from 50 to 1000 nm and are made of

physiological lipid dispersed in water or an aqueous surfactant solution. SLNs are lipoidal carriers used as emulsions and liposomes. Due to the small size of SLNs, they come in close contact with stratum and increase their penetration through skin. Skin hydration is increased due to occlusive properties of these nanoparticles. Due to its contents, such as complex glyceride combinations, waxes, and refined triglycerides, liquid lipids have replaced solid lipids. These liquid lipids are further stabilized by certain polymers or surfactants. (Najafi-taher et al., 2018).

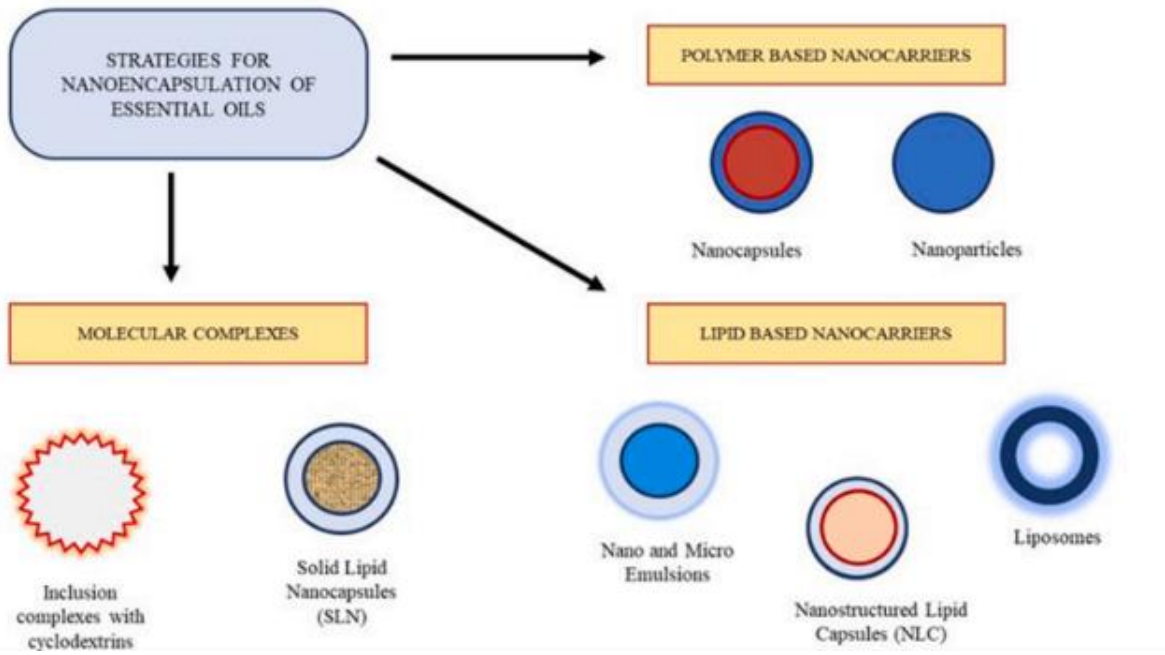


Figure 2.2: Nanocarriers loaded with essential oils.

The Precipitation method and the High-pressure homogenization method are two major principles that undergo in the preparation of these nanoparticles. Their great UV resistant property makes them well known in sunscreen products and they are considered ideal as day creams due to their occlusive properties which are helpful in glow, plumpness, and skin hydration (González-Pedro et al., 2019).

The body odor of adults is an axillary odor which is a particular scent of adults. This is the dominant odor among the odors originating from other body regions. Its main source is apocrine sweat which is sterile and odorless on its appearance. When resident microorganisms interact with apocrine sweat the pungent odor is generated. There is generally a dispute among the type of bacteria responsible for liberating odorous substances. First, it was believed that gram positive and gram-negative bacteria are responsible for producing the malodor characteristic. However, only gram-positive organisms had the capability of odor. However, it is not known whether there are quantitative or qualitative differences in the axillary microflora of individuals having different degrees of body odor (Wang et al., 2020).

2.5 Axillary odor

Axillary odor is a distinctive malodorous scent of adults, popularly called "body odor." This odor originates in various body regions and is considered a dominant note. Apocrine sweat is the ultimate source of axillary odor. It is odorless and sterile when it appears on the surface. The resident microbes interact with the apocrine sweat to create a pungent odor. The type of bacteria which liberate the odiferous substances is not clearly known (Fredrich et al., 2013). Initially the scientists believed that it was gram negative and gram-positive bacteria that produce the malodor. This capability is only the characteristic of gram-positive bacteria (Selwyn, 2018). The ambiguity still exists whether the qualitative or quantitative differences in the axillary microflora of individuals with different degrees of body odor.

2.6 Axillary microbiology

In the axilla region (underarm), a permanent and large population of microorganisms grows on secretions from apocrine, eccrine, and sebaceous glands. It is inferred through the traditional

culture-based microbiological studies that it contains resident microbiota of gram-positive bacteria of genera including *Propionibacterium*, *Micrococcus*, *Corynebacterium*, and *Staphylococcus* (Shin et al., 2017). Since the 1950s the fact is evident that it's the resident microorganisms that convert the odorless natural secretions into volatile odorous molecules in axillary skin and other bodily regions (Semkova et al., 2015). (Commons and Lim, 2009) explained the relationship between intensified malodor and microbial numbers, reinforced the opinion regarding *Corynebacterium* being primary cause of underarm odor. The population of *Corynebacterium* was initially produced through culturing on selective medium but now they are probed through genetic techniques of 16SrRNA gene sequencing. By using culture-independent metagenomics tools and pyrosequencing, the individual species that were discovered by genetic and biological studies are now identified. As an outcome, data generation has considerably enhanced both depth and breadth (Jung et al., 2014).

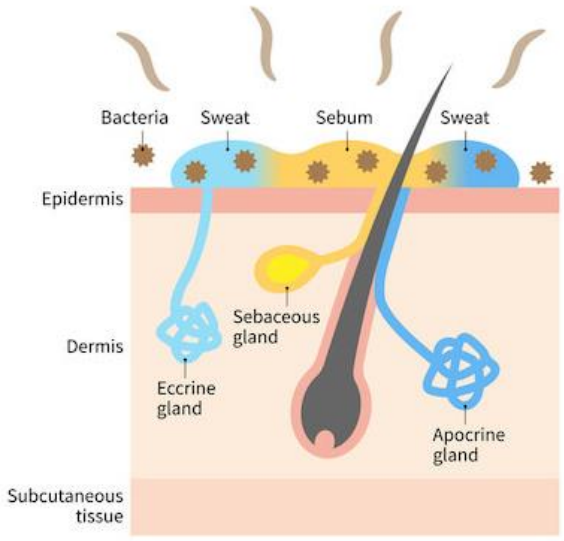


Figure 2.3: Bacterial action on sweat in human axilla.

2.6.1 Axillary cosmetics

Our society is conscious about personal hygiene and allergic to bad body odors. Underarm cosmetics help control malodor formation and sweating. These products raise the quality of life of people and enhance social confidence. Antiperspirants and deodorants are consumed by 90% of the US community which makes up sales of over $1B/year. Deodorants are applied on the human body to counteract the malodor produced in axillae, feet, and other sites of body as well as to create pleasantness on skin. Antiperspirants are the subgroup of deodorants; they block the sweat glands to prevent sweating and improve the odor. The US Food and Drug Administration classified it as "over-the-counter drug" and allowed use after extensive testing procedure. Deodorants are described as cosmetics that help mask the body odor and make skin smell better. Antiperspirants are often used as deodorants, but deodorants do not act as antiperspirants. European legislation characterizes both as cosmetics (Arora et al., 2022).

Propionibacterium, *Micrococcus*, *Corynebacterium*, and *Staphylococcus* are some of the microbes colonizing the axillary region. They secrete different enzymes to convert the non- odorous sweat into malodor. Aerobic lipophilic *Corynebacterium spp.* (*Actinobacteria phylum)* are responsible for a strong axillary malodor, while *Staphylococcus spp. (Firmicutes phylum)* and other axillary species only reveal low levels of odor. Researchers have also confirmed the presence of steroid derivatives, sulfonyl alkanols and short volatile branched-chain fatty acids in human sweat. *Corynebacterium spp.* role in all three processes is also evident (Chen et al., 2015).

2.7 Antibacterial deodorants

To decrease the skin bacterial community, deodorants and antiperspirants are mixed with antimicrobials. Benzalkonium chloride, Triclosan, metal salts and Propylene glycol are commonly

used in deodorants and antiperspirants and have antifungal and antimicrobial properties. Eugenol, geraniol and cinnamaldehyde are some of the flavoring agents that have antimicrobial properties. They also eliminate the abundance of microbes, but complete removal of microbial colonies has not been observed until now. Some people blame the use of such deodorants for decreased pleasant axillary odor (Wanigasekara et al., 2022). The effectiveness of the ingredients is also under study to determine whether they cause targeted effect or have a broad- spectrum effect on microbiotas. This study indicates the impact of discontinued use of deodorants and resumed use. Main objective of this study was to determine whether the usage could affect the dynamics, diversity, and autochthonous microbial community structure (Ermenlieva et al., 2020).

2.8 Essential oil

An essential oil is a liquid having strong aromatic properties and is distilled from different parts of plant such as roots, leaves, stems and flowers. For instance, it is obtained from flowers in roses while in basil it is extracted from leaves and so on. Essential oils are mostly used in aromatherapy which is an alternative to medicine extracted from plants to support health and wellbeing of individuals. These are basically compounds obtained from plants. The scent and flavor of the plant is captured in the essence of oil (Baser and Franz, 2010). The characteristic essence of each plant is given to it by its unique properties of aroma. The distillation or cold pressing methods are used to obtain essential oils. First, the aromatic chemicals are extracted from plants which are then combined with career oil and a product is then created which is ready to use. The method of oil extraction is important because oils obtained from chemical processes are not thought of as pure essential oils while are considered synthetic ones (Brenner, 1993).

The oils are sourced from over 3,000 plants using several technologies of which about 300 plants are commercially valuable. Different chemical compounds which are naturally available in plants made these aromatic compounds. Alcohol, hydrocarbons, phenol, aldehydes, esters, and ketones are examples of some major components. Despite this, hundreds of organic constituents including hormones, vitamins and other natural elements are also present in them. The concentration of these oils is 75-100 times more than oil extracted from dried herbs. Further, the aromatic properties of essential oils are involved in different functions of plants which includes insect attraction or repulsion due to odor of flowers or few may be involved in the process due to metabolism of plants (Burt, 2014). The oils obtained from leaves, wood and roots may provide protection against parasites of plants or depredation of animals as well as hormones involved in utilization of plants as an antibacterial agent. These oils are different from perfumes or fragrance oils as they are extracted from true plants. Artificial substances are present in perfume oils, and they are fragrances created unnaturally and have no therapeutic benefits like essential oils. The cost of essential oils is very high, but they are very effective as only a few oil drops are sufficient to get the desired result (Carson and Riley, 1995).

There are three major uses of essential oils in soaps, perfumes, detergents, and cosmetics as odorants, in baking goods, meat, candies, etc. as flavors and in dental products as pharmaceuticals. Synthetic oils can be easily bought from markets which are cheaper as compared to pure oils. Pure essential oils are different than synthetic ones. The aromatic chemicals obtained from coal tar are blended to produce synthetic oils. These oils may mimic the scent of pure oils, but each oil's aromatic benefit is decided by its intricate chemical makeup. Crafts, potpourri, soap, and perfume all include an approximate natural scent, making synthetic oils inappropriate for aromatherapy. That is why the production cost of these artificial products is mainly reduced (Edris, 2007).

2.8.1 The Parts of Plants Yielding Essential Oils

The name of the oil usually comes from the plant name, which was its origin, for instance, bergamot oil and rose oil. These oils are known as essential due to their representation of flavors and odors. The oxygenated compound is usually the main compound on which odors and flavors are dependent. Some oils are derivatives of benzene while the majority are terpenoids (Knobloch et al., 1986).

2.9 Importance of the EOs in cosmetics

A variety of substances of different chemical compositions in different concentrations make essential oils. The flavor, fragrance and biological properties is determined by the compound present in highest concentration. Since ancient times, oil has been considered important for health, beauty, and wellness. The risks of side effects of artificial ingredients present in essential oils for human health is high in recent days. That is why the consumption of natural compounds for human health and beauty is increasing and essential oils have remarkable use in cosmetics industry (Bilia et al., 2014). A wide range of products is produced by cosmetic companies which includes skin care, make-up, hair care, perfumes etc. Several innovations are constantly made in cosmetics industry and the products for consumers are improving due to which the popularity of cosmeceutical products is increasing. Different cosmetics categories are mutated due to essential oils (Binic et al., 2013). The name of an essential oil can be the common name of plant which was its source. Only the common name shows that this oil can be extracted from different species of that plant. The essential oils had been used in cosmetics in the past due to their fragrance. Despite fragrance, other essential oils have many other properties which were also a matter of interest in the past. EOs are basically the constituents mixed with other natural products containing fragrance.

The odor of a product is improved by the addition of essential oil in it. The most popular ingredients of aroma in the industry of cosmetics are the oils extracted from flowers like rose, tuberose etc (Joshi and Pawar, 2015). Other than flowers essential oils which are used in cosmetics industry for aroma are patchouli (*Pogostemon cablin*), citronella (*Cymbopogon winterianus*), sandalwood (*Santalum album*), etc. Essential oils have a wide range of anti-microbial properties due to which they can be used as a preservative in cosmetics. These properties are usually found in oils obtained from oregano, clove, coriander, cinnamon, thyme, mint, rosemary, mus-tard and sage (Kauul et al., 2018).

Anti-microbial efficiency of some essential oils is reported which contains collagen hydrolysate. *Staphylococcus aureus* and *Escherichia coli* was used to test the anti-microbial efficiency of formulations of cosmetics.it was concluded that 2% of *T. vulgaris* or *O. onites* show highest activity against microbes and both these are efficient against Staphylococcus *aureus* and *Escherichia coli*. No microbial activity was observed in control formulation according to expectations. It seems that the reasonable concentrations of essential oils play an anti-microbial role in cosmetic industry (Dorman AND Deans, 2000). The same efficiency is observed in oral care products containing essential oils. mouthwashes containing Eos are efficient against plaque bacteria (Edris, 2007). Synthetic chemicals can be replaced by the natural Eos as synthetic ones are dangerous for human health. EOs are also present as cooling agents in cosmetics e.g., mint and oil extracted from eucalyptus has a long-lasting feeling of refreshing for both skin and mouth (Isman, et al., 1990).

The shelf life of cosmetics is increased by using antioxidants in them. The skin is protected against free radicals e.g., discoloration through bio-active ingredients present in cosmetics (Marques et al.,

2009). Eos are considered an ideal source of natural antioxidants such as EOs from *Clausena anisate* and *Eucalyptus camaldulensis* species, rosemary and Egyptian corn silk are examples (Rao and Panday, 2007). In hair care, EOs are well known for their shining and conditioning effects and enhancing scalp beauty. Healthy hair growth is improved, and dandruff is controlled by EOs (Regnault-Roger, 1997). The pharmacological properties of Eos made their wide use in cosmeceutical products. An example is use of Geranium oil in cleansing, acne etc. some EOs are anti-inflammatory such as *Agathosma betulina* and *Eriocephalus punctulatus* EOs. These Eos are important in treatment and prevention of skin problems. Due to presence of ursolic acid EOs promote circulation of blood in both scalp and skin. These different characteristics and bioactivities of Eos make them attractive to cosmetics industry. The EOs have a physicochemical nature due to which its several cosmetic benefits are not yet unveiled. However, these limitations can be overcome by the process of microencapsulation (Zellner et al., 2010).

2.10 Essential Oil encapsulation

In cosmetic industry, essential oils are considered valuable ingredients. The components of EOs are volatile and labile and chemical interactions can change the process of sensory perception. Microencapsulation can effectively minimize these physical and chemical effects. The EOs are protected from the external medium due to this technique (Mateeva, Karov, 1983). The process of microencapsulation must consider the microparticle development and ultimate destination's mechanism. A variety of substances and materials are encapsulated. The resultant nano particles decide most physical and chemical properties. Several factors such as stability of core, physical and chemical properties of core and its release characters should be considered. The different

active material is microencapsulated through many techniques. The method of microencapsulation should be simple, fast, and effective for its implementation on an industrial scale (Burt, 2004).

2.11 Tea tree essential oil

Tea tree oil, an essential oil, is produced by steam distilling Melaleuca alternifolia, a native Australian shrub. It is also available in several other countries. The constituents are terpinen-4-ol at a minimum content and 8-cineole at maximum. The main component of this oil is Terpinen-4-ol having strong properties of anti-microbes. The antioxidant activity of tea tree oil shows a broad spectrum of anti-microbial activity against several infections affecting both mucosa and skin where it is also used in soaps and lotions. The research has suggested using this oil to treat acne problems and chronic gingivitis. This essential oil has anti-skin cancer properties and speeds up the healing process for injuries. However, the oral intake of oil can cause serious health issues. It should be used along with other drugs for treating bacterial or fungal infections, however, no evidence of drug interactions is known to date (Yadav et al., 2017).

Figure 2.4: Tea tree.

2.12 Sandalwood essential oil

Inflammation, infection, and hyperplasia usually characterize several skin conditions and diseases. Treatments which are safe and effective and can be used long term are required. The new active

ingredients in dermatology such as medicines obtained traditionally having biological action are studied. *Santalum album* tree is used to distill an essential oil called as Sandalwood album oil (SAO) or East Indian sandalwood oil (EISO). The tree is one of the most beneficial trees in the world and the products obtained from the tree are used worldwide. The oil obtained from the tree has a variety of applications in the field of medicine and has many benefits for health due to its anti-microbial and anti-inflammatory activity. This oil has been used in clinics to treat acne, eczema, common warts, and *molluscum contagiosum.* Further, it is also beneficial in treating skin cancer. The oil has many novel therapies in dermatology as it is favorable due to its safety profile, ease of tropical use and pharmaceutical grade (Subasinghe et al., 2013).

Figure 2.5: Bark of *Santalum album* tree.

2.13 Essential oil blend

Due to increasing demand of consumers, there is a dire need to obtain new active products to manufacture more safe and eco-friendly products where botanical extract is an unlimited source of new products. The odorous characteristics of essential oils in manufacturing perfumes and fragrances make their use very common in toiletries and cosmetics and antimicrobial properties of these oils make them suitable for use both as active constituents and as preservatives. There are

several benefits of essential oils due to their individual specific components and therefore the use of their potential applications in cosmetics requires formulators to seek suitable EOs or EOCs mixtures to get desired benefits in final products (Newbold et al., 2004).

CHAPTER 3: METHODOLOGY

3.1 Synthesis of SLNs

SLNs were prepared by using the solid lipid as liquid phase. Firstly, the spermaceti wax was melted at 55°C. In the melted wax i.e., 20ml, 5% of hot aqueous Tween-80 solution was added. 5% of surfactant solution was prepared prior to the melting of the wax. The temperature was maintained at 55°C as the temperature must be maintained above the melting temperature of the wax throughout the process. 1% lecithin solution made in ethanol was added into the solution. EO blend was made by mixing the tea tree essential oil and sandalwood oil in 1:1. 1% EO blend was then added into the solution. The solution was then subjected to hot homogenization for 10 min at 10,000 rpm. The temperature was maintained at 55°C. The lipid droplets will be recrystallized forming a semisolid consistency in gel form (Lippacher et al., 2001) (Suksuwan et al., 2021).

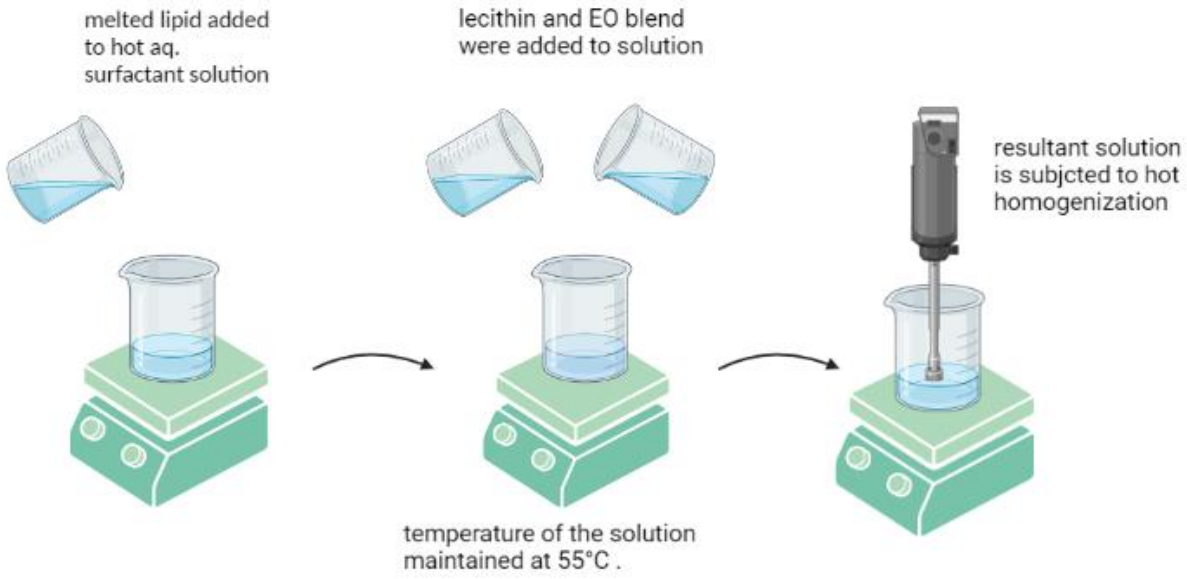


Figure 3.1: Syntesis of EO blend SLNs.

3.2 Incorporation of SLNs into gel

The gel-based deodorant was produced by using a thickening agent. Carbopol 940 was used as thickening agent. The formulation included 0.75% of thickening agent, 1.5% of glycerol was used as humectant, 0.125% of EO blend loaded SLNs were added, 0.05% of vitamin E was added as skin conditioner, 0.05% of fragrance was added, and then the final volume of the solution was raised by using distilled water.

3.3 Characterization of solid lipid nanoparticles, SLNs

3.3.1 Ultraviolet Visible (UV-Vis) Spectroscopy Analysis

UV-Vis absorption spectroscopy is the most frequently used technique in both industrial as well as medical field. When a beam of light passes through the sample it measures the absorption capacity of it through the reflection of light beams. The light beam is divided into two halves, one passes through the reference cuvette (solvent only) and the other passes through the sample cuvette. The results are observed and calculated at specific wavelength, in the estimated range. The resultant spectrum describes the absorbance made at certain wavelength; it is plotted on graph having absorbance against wavelength. Lambda max is the absorbance peak at a given wavelength. The mechanism used by UV-Vis spectrophotometer is based on principle of Beer Lambert Law that states A=EcL or E= A/cL, where A = absorbance, c= concentration of sample, L = length of light path through cuvette in cm and E = molar absorptivity. Through this procedure the electronic transition of molecules is measured. Absorbance of sample is proportional to molar concentration of the sample. Thus, the molar absorptivity/ absorption value is used to compare different compounds.

Figure 3.2: UV spectrophotometer and its working principle.

3.3.2 Fourier transforms infrared spectroscopy (FTIR) Analysis

Fourier transform infrared spectroscopy (FTIR) is used to observe the formation, detection, and chemical comparison between silymarin gold nanoparticles and pure silymarin. The spectrum was obtained ranging from 500-4000 cm by using attenuated reflectance technique. The KBr crystals are used and samples of dried silymarin gold nanoparticles are placed over them. The spectrum is obtained in transmittance mode. FTIR is one of the most used characterization techniques that helps to detect the functional groups in pure compounds such as for comparative analysis or mixtures. Spectrum of photoconductivity, absorption, emission, or Raman scattering of gas, liquid or solid molecules can be detected with FTIR. It is used for the surface analysis and characterization of nanoparticles. The surface of nanoparticles contains different absorbents which cause peaks in FTIR spectrum in comparison with samples having no absorbents. It easily detects changes in the surfaces. It has a complicated mechanical and electrical system that sense minimal changes on the surface of samples. It helps analyze a wide range of materials such as pastes, bulks, fiber, films, or powders. Both quantitative and qualitative analysis can be done by determining the size of the peaks. The frequency of absorption is made based on vibrionic coupling, shape of the surface and atomic mass.

3.3.3 Scanning Electron Microscopy

Scanning Electron microscopy (SEM) is a technique used for imaging the samples to the Nano scale. SEM use beam of electrons to scan the surface of the sample and image. When the beam of electrons interacts with the atoms and molecules in the sample, they give rise to different signals. These signals provide information about morphology, topography, and composition of the sample. Scanning is done by the raster scan pattern. The position of the beam of electron and the detected signals produces a highly refined image. The SEM can check more than 1 Nanometer resolution. High vacuum is used for imaging in case of conventional SEM, while wet conditions and low vacuum is used in environmental SEM. Specified instruments are used when imaging must be performed at high temperature. The beam of electrons hit the sample molecules and it causes the sample to emit secondary electrons. The secondary electrons are detected depending upon the topography of sample. The detector then made the image of the sample. Before passing the sample through this process it must be checked whether the sample will withstand the vacuum condition and high energy beams or not. The sample is made according to the conditions of the experiment such as it should be small enough to fit the specimen stage. A stub is used for mounting and staging the sample. Some SEMs provide 360-degree rotations for proper imaging of samples.

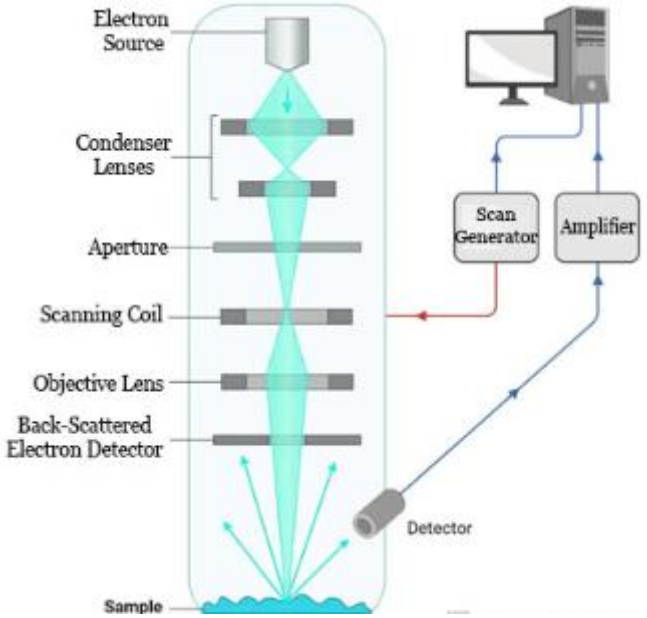


Figure 3.3: SEM working principle.

3.3.4 DPPH Assay

DPPH assay is famous for testing the antioxidant capacity of natural products. It is a frequently used method as it is sensitive and simple. DPPH (originally known as 2,2-Diphenyl-1-picrylhydrazyl) is available commercially and contains stable organic nitrogen radical. The reaction causes a change in color of DPPH solution from purple to yellow, which indicates the absorption of hydrogen taken from an antioxidant. Since DPPH shows strong absorption at 517 nm the antioxidant effect can be easily evaluated by using the decrease of UV absorption at 517 nm.

3.3.5 Test sample preparation

For the free radical testing of SLNs and the prepared gel DPPH assay was carried out. Solution of DPPH was prepared in methanol of 0.1mM concentration. 2.4ml of prepared DPPH solution was

mixed in 1.6ml of extracts. The solution was mixed thoroughly and was kept at room temperature, rt for 30 min in the dark. The color change was observed from purple to yellow depending on the presence of free radicals. The absorbance of the resultant solutions was observed at 517nm.

3.3.6Viscosity testing

The internal resistance of a fluid is described by the viscosity. Viscosity is mainly carried out on the fluids in engineering systems but to study the rheological properties of nanofluids, viscosity testing is carried out. The rheological analysis is important as they are further used to determine the further processing of the fluid for processing or storing.

3.4 Antibacterial activity

3.4.1 Sample collection

3.4.1.1 Subjects

A total of fifty volunteers were selected aged between 20-50 years. These included twenty-five male and twenty-five female subjects. All the volunteers were in good health. They refrained from taking any medications, including antibiotics, one week prior to the sample collection. They were also asked not to use any kind of deodorants or antiperspirants 6 hours prior to sample collection.

3.4.1.2 Sampling

To study the axillary microflora, sweat samples were collected using sterile cotton swabs. The swabs were rubbed for 30s over 2cm diameter skin area. The swabs were then pooled in sterile phosphate buffered saline (PBS) and stored at 4°C for not more than 24h.

3.4.2 Non-selective growth

The samples were spread on tryptic soy agar (TSA) for non-selective growth. TSA plates were prepared by taking 40g of tryptic soy agar media and mixing it in 1L of distilled water. Autoclave the media at 121°C at 15psi for 15min. The plates were prepared by pouring 20ml of sterile media aseptically. 50µl of the sample was spreaded using pasture pipettes on TSA plates. The plates were incubated for 24-48h at 37°C.

3.4.3 Selective growth

For selective growth, blood agar plates (BAP) were used. The colonies from TSA plates were picked and each individual colony was spread on BAP using an inoculating loop. The reason for using BAP is that it allows us to morphologically identify bacterial growth with distinct characteristics. The BAP were incubated for 24-48h at 37°C. After incubation, the bacterial strains were identified and were stored at -80°C in glycerol stocks.

3.4.4 Antibacterial activity

To study the antibacterial activity, TSA was used as it allows the growth of axillary microflora. The antibacterial activity was carried out using agar well diffusion method. The TSA plates were prepared as mentioned above and wells were created using pipette tips. Bacterial inoculums were prepared as mentioned above and 50µl of inoculum was spreaded on TSA plates using pasture pipettes. Then 50µl of test samples were loaded into the wells and the plates were incubated for 24-48h at 37°C. Observe the zones after incubation.

CHAPTER 4: RESULTS

This study included the isolation of sweat microflora which are responsible for the malodor and the antibacterial effect of EO blend loaded SLNs based deodorant formulation on these bacteria.

4.1 Synthesis of SLNs

The SLNs produced by hot homogenization method were milky white in color with semi-solid consistency. The developed nanoparticle solution was aromatic due to the natural fragrance of spermaceti wax. The produced SLNs were semi solid at room temperature and were very feasible to be used for incorporation into the gel.

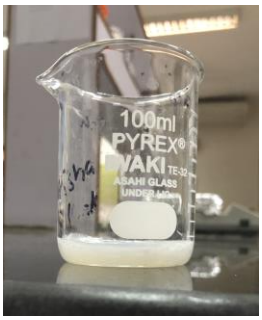


Figure 4.1: Synthesized SLNs.

4.2 Gel formulation

The deodorant gel prepared was clear and thick. The consistency is good enough to resist skin friction. The resultant product was free of alcohol, toxic elements which are proved to be contributing to skin and other health problems. The clear consistency makes it reasonable to be used for commercial scale product.

Figure 4.2: Deodorant formulation.

4.3 Characterization of SLNs

4.3.1 Ultraviolet Visible Spectroscopy

UV-VIS absorption spectroscopy of EO blend loaded SLNs exhibited the surface plasmon resonance (SPR) primarily at 350 nm, EO blend at nm, Sandalwood essential oil at 339 nm, Gel at 430 nm, Lecithin at 350 nm and surfactant at 419 nm. The peaks Tea tree essential oil at 345 nm and Wax at 300 nm.

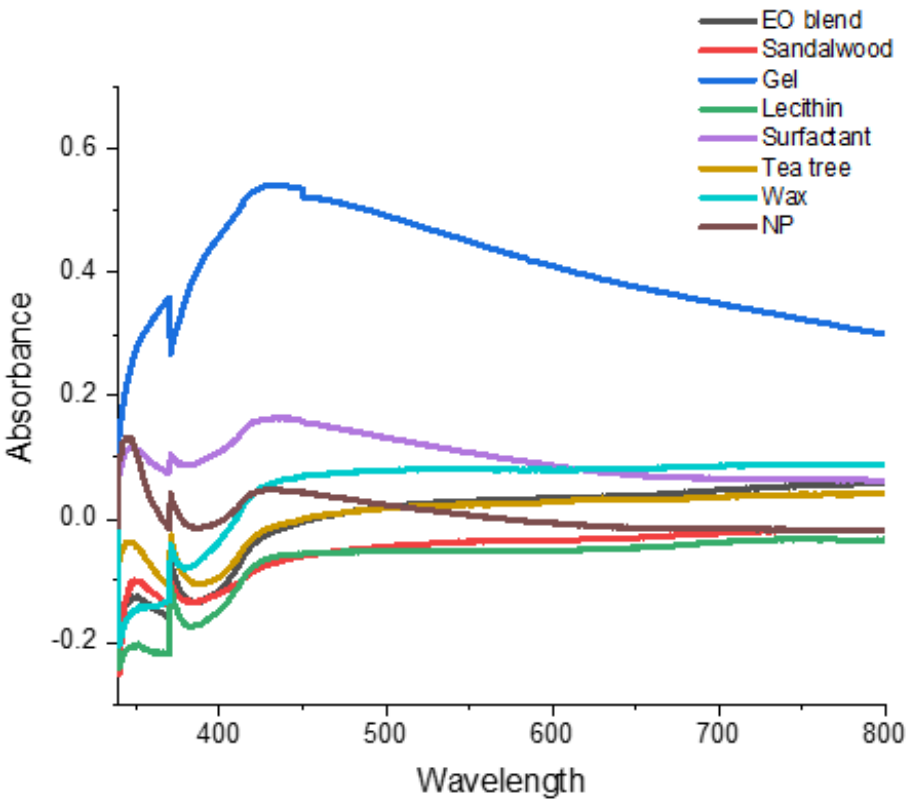


Figure 4.3: Ultraviolet spectrum of SLNs and components.

4.3.2 Fourier transform infrared spectroscopy (FTIR) analysis

The FTIR spectrum of SLNs and spermaceti wax indicated bands or peaks at 2850/cm (CH stretch, Alkanes) and 1750/cm (RCOOR, Ester). The lecithin spectrum indicated peaks at 1620/cm (C=C, unsaturated compounds) and 1120/cm (polysaccharides). Tween 80 spectrum indicated the peaks at 2855/cm (-CH2), 3436/cm (hydroxyl stretching) and 1740/cm (C=O, ester group). Numerous peaks are observed from < 1600/cm in the case of tea tree essential oil which indicates the presence of terpinene-4-ol. Sandalwood essential oil indicated peaks at 3300/cm (OH, polyphenols) and 1740/cm (C=O, ester group). The EO blend indicated the peaks at 3350/cm (-OH, bonded groups), 2950/cm (CH stretch, Alkanes) 1750/cm (C=O, ester group).

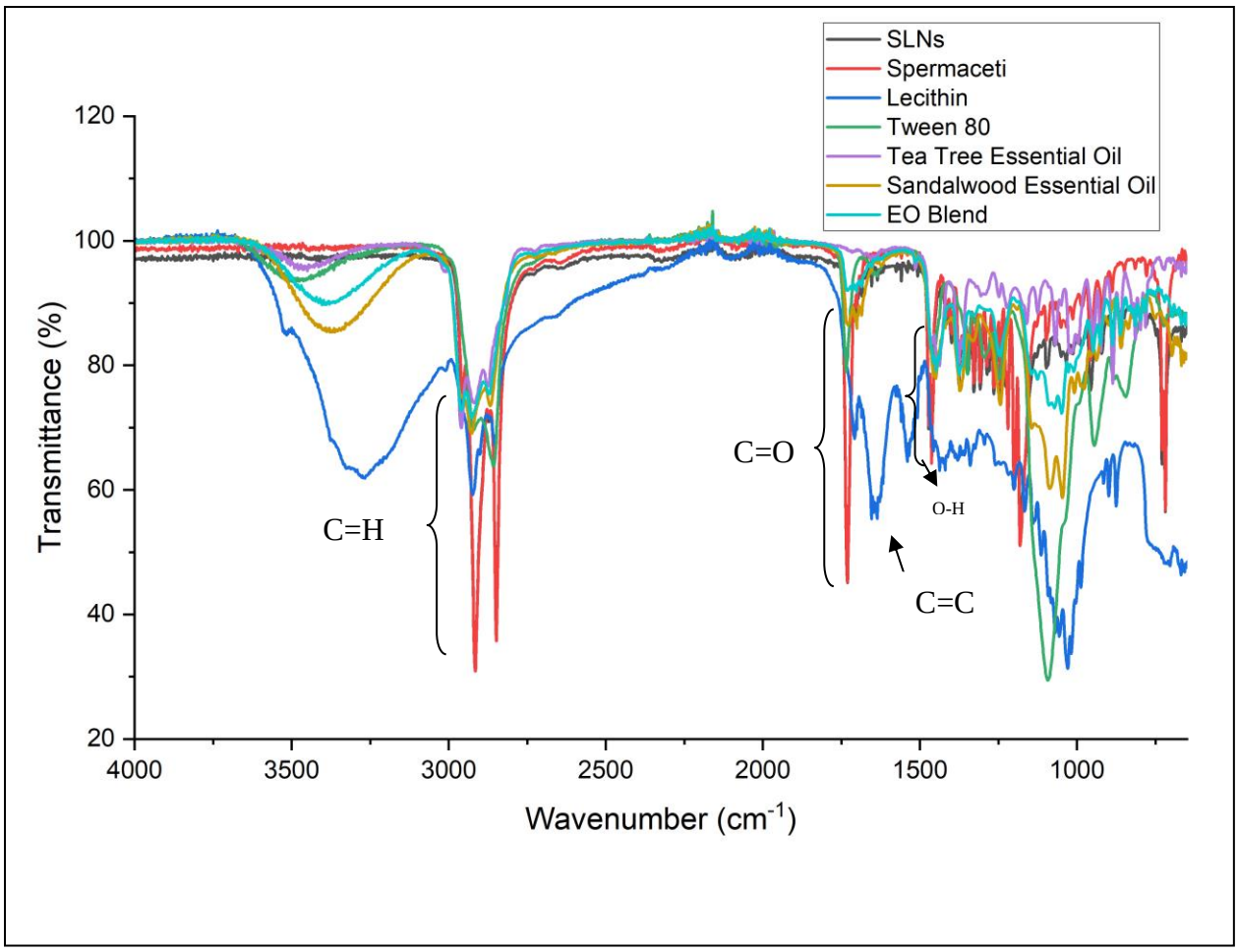


Figure 4.4: FTIR spectrum of SLNs and components.

4.3.3 Particle size

The scanning electron microscopy provides the tool for the determination of nanoparticles size. A scanning picture revealed that the EO blend loaded SLNs were in spherical form and had an average size of 35 nm.

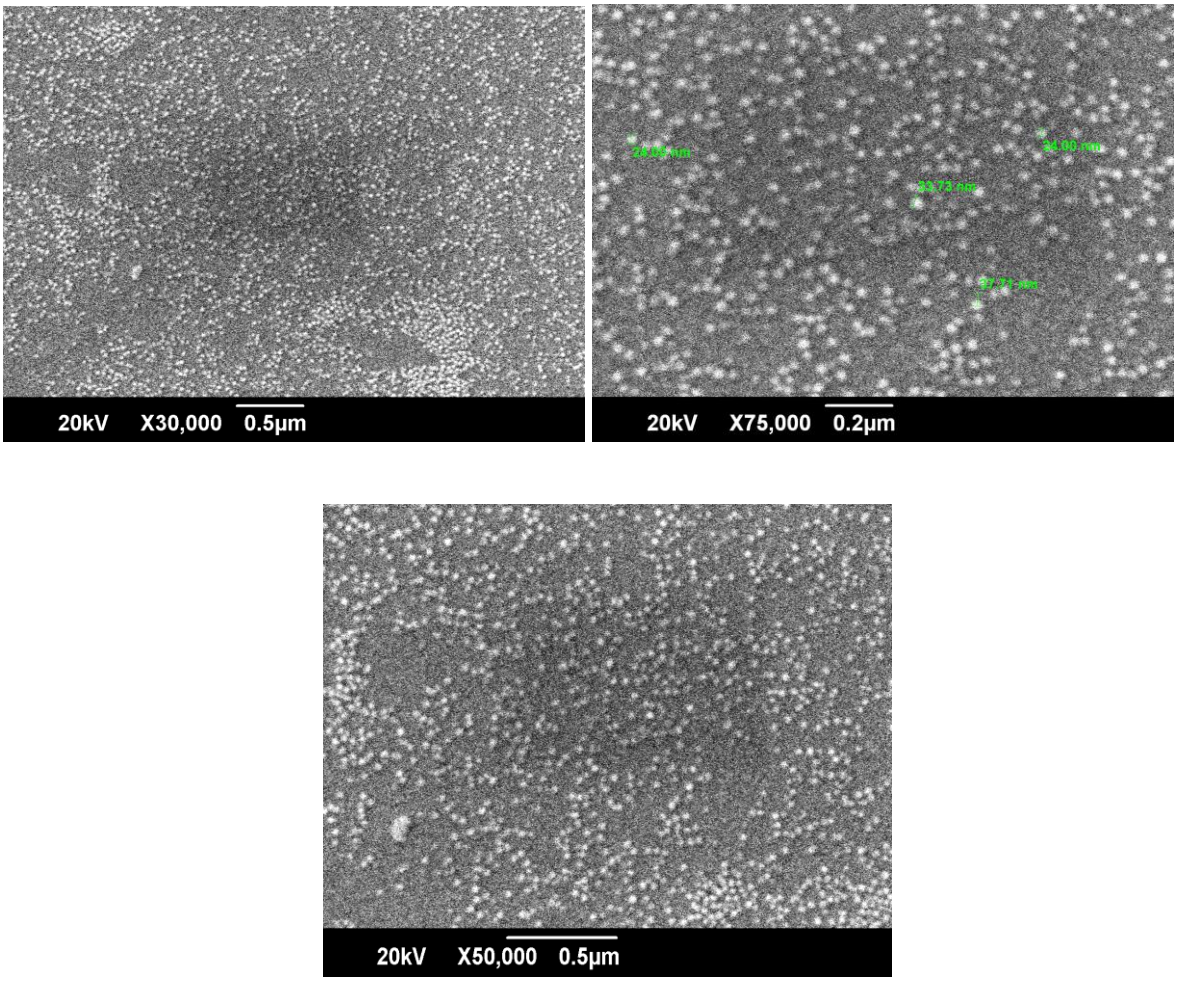


Figure 4.5: SEM images of EO blend loaded SLNs.

4.3.4 Antioxidant Scavenging Activity of EO blend loaded SLNs and SLNs loaded gel formulation

DPPH assay was carried out for this investigation on EO blend loaded SLNs and the gel formulation including the components used in the SLNs production.

Spermaceti wax showed antioxidant activity up to 2% and Lecithin up to 5% on average. SLNs showed 59% of antioxidant activity on average, Surfactant Tween 80 showed 1%, EO blend showed the highest percentage of antioxidant activity up to 80% and the SLNs loaded gel showed 52% on average.

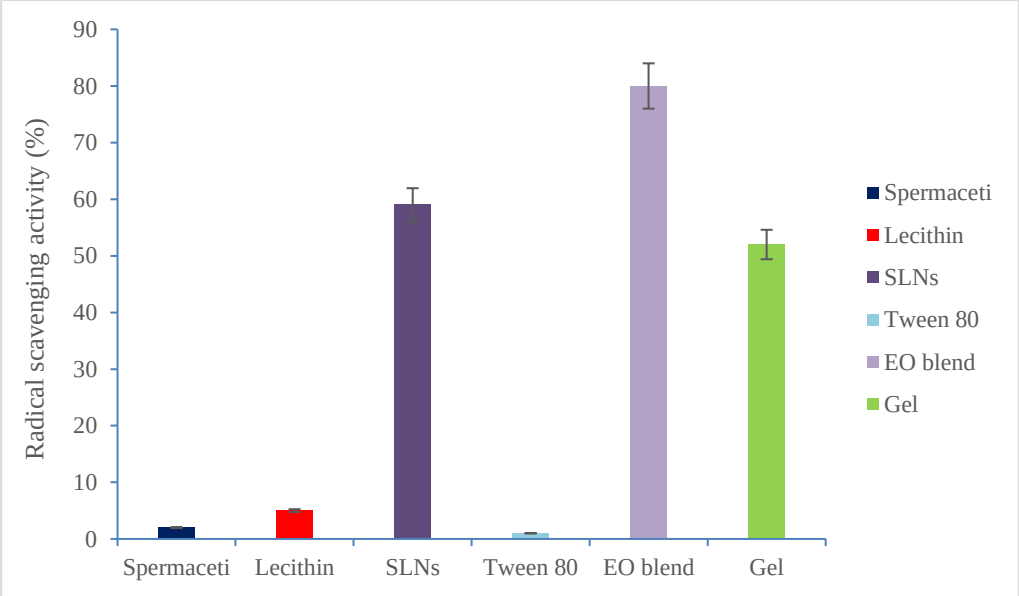


Figure 4.6: DPPH activity of test samples.

4.3.5 Viscosity

The incorporation of nanoparticles tends to stabilize the fluids more effectively than the microparticles. The viscosity testing showed that SLNs and the gel has 12,480 cP and 14,460 cP viscosity respectively at 37°C.

	Viscosity (cP)	Accerelation (± cP)	Temperature (°C)
SLNs	12,480	213.3	37
Gel	14,460	160	37

Table 4.1: Viscosity and acceleration of SLNs and gel.

4.4 Antibacterial testing

4.4.1 Non-selective growth

The non-selective growth was carried out on TSA plates. The colonies were further picked and spread on BAP.

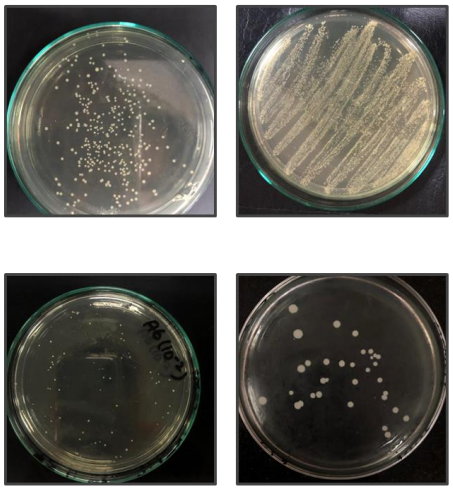
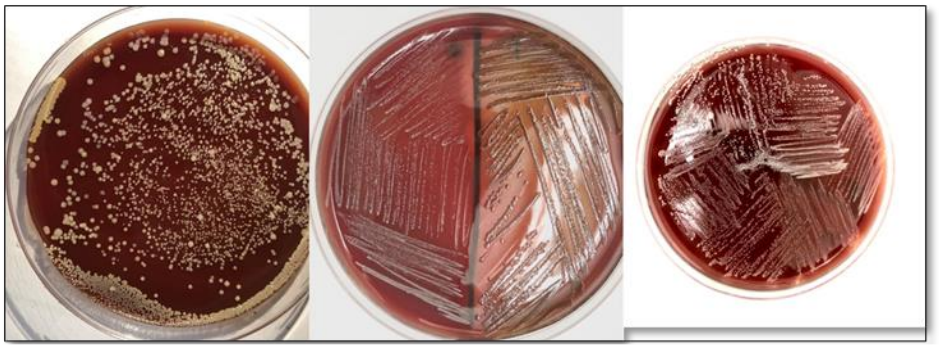


Figure 4.7: Non-selective growth of human axillary microflora.

4.4.2 Selective growth

Selective growth was carried out on BAP. The bacteria on BAP grows with different characteristic morphology. The bacteria were identified as White-yellow, grey, grey growth with ß-Hemolysis and white colonies represent *Micrococcus* (1), *Corynebacterium* (2), *Staphylococcus aureus* (3) and *Propionibacterium* (4), respectively.



1 2 3 4

Figure 4.8: Selective growth of human axillary microflora.

4.4.3 Agar well diffusion assay

The antimicrobial activity test was carried out using this method. The wells were created on the TSA plates and after the incubation of plates loaded with the test samples, the zones were observed. The standard was used 1 µg/ml Ciprofloxin and the diameter was 20 mm, the Tea Tree essential oil developed zones of 14 mm, 12 mm, 12 mm and 9 mm against *micrococcus, Corynebacterium, staphylococcus aureus* and *Propionibacterium* respectively. The sandalwood essential oil developed zones of 4 mm, 4 mm, 5 mm and 3 mm against *micrococcus, Corynebacterium, staphylococcus aureus* and *Propionibacterium* respectively. The EO blend showed the highest antibacterial activity against *micrococcus, Corynebacterium, staphylococcus aureus* and

Propionibacterium with 15 mm, 16 mm, 15 mm, and 11 mm diameter zones respectively. The SLNs developed zones of diameter 12 mm, 10mm, 9 mm and 9 mm against *micrococcus, Corynebacterium, staphylococcus aureus* and *Propionibacterium* respectively. Gel also developed the zones against isolated sweat microflora with zones of diameter 10 mm, 7 mm, 9 mm and 8 mm against *micrococcus, Corynebacterium, staphylococcus aureus* and *Propionibacterium* respectively.

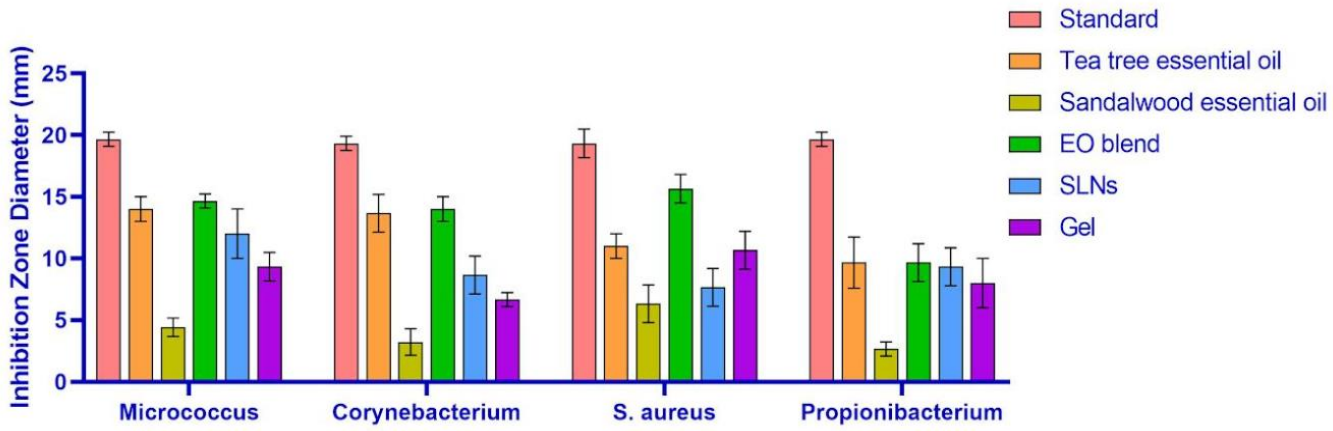


Figure 4.9: Antibacterial activity of EO blend loaded SLNs and components against isolated microbes.

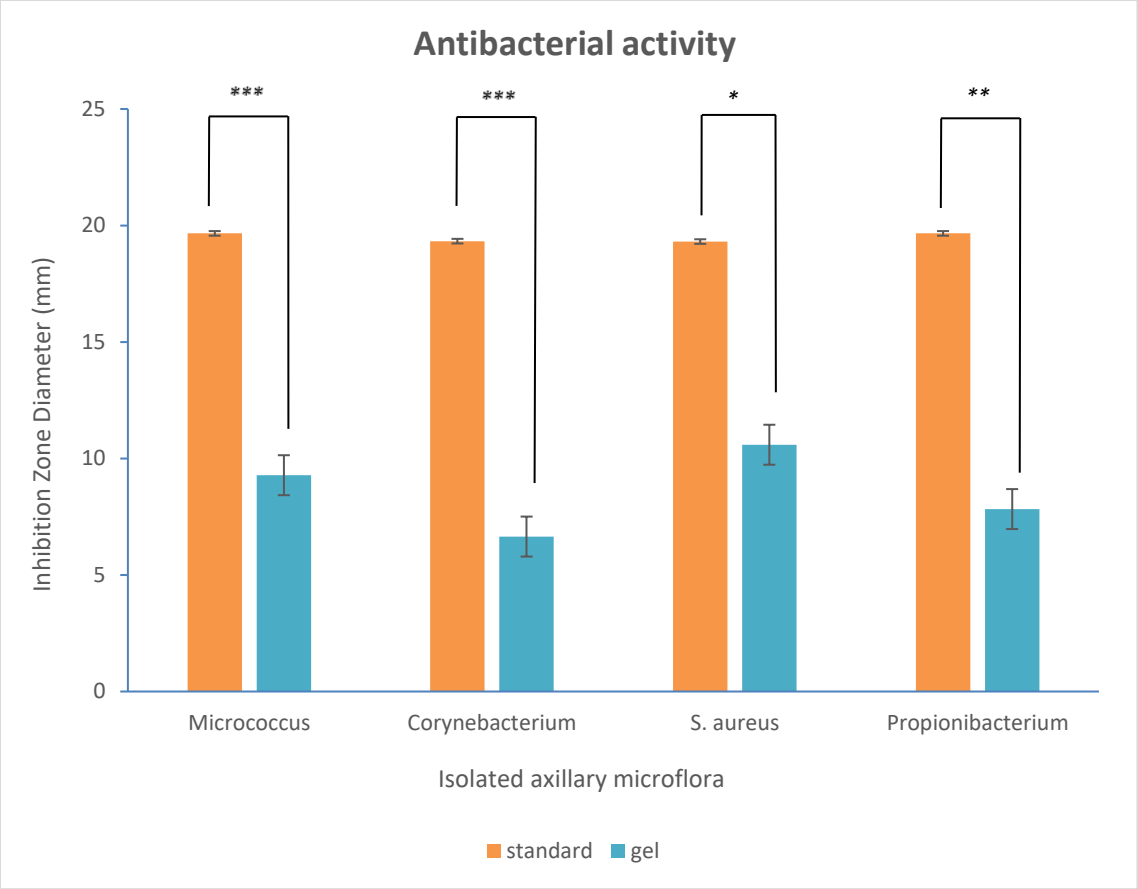


Figure 4.10: The bar graph shows the t-test results for the antibacterial activity.

CHAPTER 5: DISCUSSION

In this study we analyzed the antibacterial effects of EO blend by incorporating them to specific type of delivery systems which have the properties to deliver the lipid contents to the target site and maintain its originality. Human skin contains a variety of microorganisms that are divided into groups based on how they affect health and disease. Therefore, the creation of a healthy symbiotic relationship between microorganisms and humans may emerge from the selective inhibition of the growth of microorganisms responsible for diseases and offensive odors. By taking this piece of applied knowledge, the current formulation is designed. The cosmetic industry is becoming more and more interested in the family of compounds known as essential oils. They have a variety of biological qualities, such as those that are antibacterial, antifungal, anti-inflammatory, or antioxidant, which can be used to support wellness, attractiveness, and health. The essential oils which are used i.e., Tea tree and Sandalwood essential oil are increasingly now used in applications besides the scent industry. These natural preservatives are now being used in numerous formulations for hair and skin care as well as a seemingly unlimited array of applications that are constantly expanding (Suksuwan et al., 2021). Consequently, these have developed into vital elements that contribute to the ideal physical wellness. However, the safety concerns related with the use of EOs in the finished goods cannot be hidden by the significant contribution that they have made to the current growth of the cosmetic industry towards greener and eco-sustainable products, necessitating caution in their dosage. Thus, it is evident that EOs constitute a significant supply of bioactive molecules for the beauty sector, even though a careful examination of their application conditions is required.

On the other hand, the use of Tea tree and Sandalwood essential oil in cosmetics and toiletries is advantageous not only in terms of the cosmetic advantages of the products and their function as preservatives, but also because it enhances the brand image of commercial goods. This makes it vital to advance research toward a deeper comprehension of the biological functions of these compounds and any potential toxicological implications to open new doors for the creation of cosmetics based on them. Therefore, it is required to conduct more systematic experiments evaluating the actual efficacy of essential oils in final formulations, considering the cosmetic industry's interest in substituting conventional actives for greener bioactive substances. The olfactory system (sense of smell) and armpits serve as entry points for deodorant and antiperspirant chemicals into the body. That implies that whichever components are present in the deodorant sticks and sprays we use every day are entering your bloodstream ((Arora et al., 2022) . More toxins and synthetic components are ingested into our body with each application. This is a step toward a clean-living, natural skincare regimen, switching to a fantastic natural deodorant. Both our skin and the environment benefit from using this natural deodorant. It is equally as effective as the conventional roll-ons and spray cans we might be accustomed to. Due to its ability to kill microorganisms, tea tree oil is a fantastic all-natural deodorant and antiperspirant substitute. Tea tree oil is helpful for our skin since it can shield it from bacteria in addition to its ability to moisturize. 4-Terpineol, a colourless to pale yellow chemical with demonstrable antibacterial activity, is present in the essential oils derived from tea tree leaves. In fact, it makes up the majority of tea tree oil, and the tea tree may be the only plant in the world with as much 4-Terpineol in its leaves and branches. Several studies have revealed that this substance appears to boost white blood cell activity, enhancing the body's resistance to germs. This indicates that tea tree oil has a built-in propensity to combat bacteria and germs (Bilia et al., 2014) (Binic et al., 2013) (Edris, 2007)

(Isman, et al., 1990). Sandalwood oil is perfect for products like lotions and deodorants because it is also a disinfectant and has hydrating qualities. It will treat any irritations or breakouts while also removing the skin's surface of dangerous microorganisms. The wax, Spermaceti was used because it is devoid of any kind of smell, which made it very suitable for using in this formulation. Also, it will provide the best representation of the fragrances used and will not hinder or mask the fragrance which is used at the last step in formulation preparation. The lecithin and Tween-80 were used and they played their role as the particle stabilizer and acting as surfactant and co-surfactant. The amount of lecithin greatly affects the particle size. The average particle size we observed from SEM analysis is 50nm. The semisolid consistency was achieved from the method used. It is carried out as this is suitable for the topical applications. The EO blend loaded in SLNs was carried out to receive maximum effects of both oils used. The benefits which mainly include, are antibacterial, anti-inflammatory, moisturizing and soothing properties (Subasinghe et al., 2013).

When they come into touch with the skin surface, the liposomes, SLNs, have exceptional adhesive properties. Along with amazing skincare benefits, SLNs offer a huge potential for use in delivering active substances and treating dermatological conditions. These properties of lipid carrier systems make them ideal to be used for the topical formulation preparation. The formulation incorporated with EO blend loaded in SLNs were tested against the main bacteria responsible for sweat odor. *Corynebacterium* and *Staphylococcus* are two of the main bacteria found in the microbiota of human skin. These two bacterial genera may work together and provide one another with protection, which affects the relative amounts of each species. *Propionibacterium acnes*, a member of the *Propionibacterium* genus, generates propionic acid and is responsible for the elderly who are bedridden and have a terrible stench. This resident microbiota is primarily made up of Gram-positive bacteria from the genera *Staphylococcus*, *Micrococcus*, *Corynebacterium*, and

Propionibacterium, according to traditional culture-based microbiological research and were successfully isolated.

The antibacterial testing of the designed formulation was carried out using agar well diffusion assay. The formulation and its components were tested against each bacterial strain isolated. The results showed that the gel contains the SLNs with loaded EO blend possess the antibacterial properties against the odor causing bacteria. The designed formulation has proved that it can be used for eco-friendly skin formulation with no side effects. This formulation can be used on a commercial scale to provide public alcohol, aluminium, and other toxins free (Reddy and Shariff, 2013). From the t-test applied on the formulation, we found out that P-values of *Micrococcus*, *Corynebacterium, S. aureus* and *Propionibacterium* are 0.005, 0.003, 0.01 and 0.002 respectively. The results show that relationships are significant. The smallest value from the t-test result is observed in the *Corynebacterium*, which shows that the designed formulation is having the best antibacterial activity against this bacterial strain which is proved from the literature that it is the main bacteria which is mainly responsible for the malodor in human sweat. The possible reasons for the low values of antibacterial zones and the *p* values are that the SLNs provide slow and sustained therapeutic agent release. They must have to reach their melting point for the melting of the lipid and then releasing the agents incorporated in them. As the lipid used has a melting point slightly above the human body temperature it will be needing a shorter period to transfer its loaded agents to the target site (Kauul et al., 2018).

CHAPTER 6: CONCLUSION

The solid lipid was first used as a liquid phase, and then it was combined with a thickening agent to develop a gel formulation that consists of incorporated the solid lipid nanoparticles. Different techniques of characterization, including UV-Vis spectroscopy, FTIR, and scanning electron microscopy, have shown that this process resulted in the manufacture of an efficient formulation. The final deodorant gel had a scent, consistency, and viscosity that was skin and environment friendly. The deodorant formulation is free of alcohol, aluminium, and other toxins. The antioxidant scavenging activity of the nanoparticles-loaded gel was measured at 52%. *Micrococcus, Corynebacterium, Staphylococcus aureus,* and *Propionibacterium* were all inhibited by the gel. This shows that the gel consists of SLNs and these SLNs have been loaded with the EO blend with antibacterial properties. On the other hand, the inhibitory zones produced by the gel were slightly smaller than those produced by either the essential oil mix or the solid lipid nanoparticles on their own. The formulation may be altered even more to accommodate other alterations that will result in an even higher level of antibacterial activity.

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