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Essential Oils (Eos) as the Advantages of its Microencapsulation in Cosmetic Industry

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ABSTRACT

Essential oils are aromatic and volatile liquids extracted from plants. Essential oils and their labile constituents have been widely used to prevent and treat human diseases. They have been widely used for medicinal, bacterial, fungicidal, antioxidant, allelochemical, cosmeceutical, and pharmaceutical, agricultural, and food industries. Extensive documentation on the antimicrobial properties of essential oils and their constituents has been carried out by several workers. Although the mechanism of action of a few essential oil components has been elucidated in many pioneering works in the past, detailed knowledge of most of the compounds and their mechanism of action is still lacking. This knowledge is particularly important for the determination of the effect of essential oils on different microorganisms, how they work in combination with other antimicrobial compounds, in cosmetic products; essential oils (EOs) play a major role as fragrance ingredients. They can optimize its proprieties and preservation, as well as the marketing image of the final product. Microencapsulation of EOs can protect and prevent the loss of volatile aromatic ingredients and improve the controlled release and stability of these core materials. The importance of EOs for cosmetic industry and its microencapsulation was reviewed in this study. Also, a brief introduction about the preparation of microparticles was presented. Some of the most important and usual microencapsulation techniques of EOs, as well as the conventional encapsulating agents, were discussed.

***Key words*:** Essential oils; microencapsulation; Antimicrobial Activity; Cosmetic Industry

1) INTRODUCTION

By the evolution of science, the scientific fields received the medicinal properties of plants and have a great interest because of their low toxicity, pharmacological activities, economic viability, antimicrobial activities, cosmeceutical industries, and food preservation in essential oils. Among compounds of natural origin, biological activities have been shown by essential oils from aromatic and medicinal plants and have received particular attention because of their radical-scavenging properties. Several pathologies such as cancer, deterioration of the organoleptic and hygienic quality of food, and neurodegenerative diseases have been attributed to free radicals. Massive use of antibiotics has resulted in the emergence of resistance against them, which is another problem affecting public health. The use of natural ingredients in consumer products has a very long history. Apart from being a source of food, many plant species biosynthesize and accumulate extractable substances with economic and health importance. Industrial oils, flavour and fragrances, resins, gums, natural rubber, waxes, surfactants, dyes, pharmaceuticals, pesticides and many specialty products are raw materials that have been used in several consumer products, such as cosmetics, herbal medicines, and pharmaceuticals. Furthermore, the consumer demand for nutritional, medicinal and cosmetic products derived from natural sources has been increasing in last decade. Essential oils are a complex liquid mixture of volatile, lipophilic and odoriferous compounds biosynthesized by living organisms, predominantly aromatic plants. The major plants families from which EOs are extracted include Asteraceae, Myrtaceae, Lauraceae, Lamiaceae, Myrtaceae, Rutaceae and Zingiberaceae, the dicotyledonous angiosperm plant families. They are secondary metabolites produced in cytoplasm and plastids of plant cells and stored in secretory cells, cavities, canals, epidemic cells or glandular trichomes. Present in different parts of the plants (buds, flowers, leaves, stems, twigs, seeds, fruits, roots, wood, bark or rhizome), EOs are usually extracted by processes of steam distillation, solid-phase extraction, cold pressing, solvent extraction, supercritical fluid extraction, hydrodistillation or simultaneous distillation–extraction. The food industry primarily uses essential oils as flavorings; they represent an interesting source of natural antimicrobials for food preservation. However, application of essential oils as food

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preservatives requires detailed knowledge about their properties, i.e., the minimum inhibitory concentration (MIC), the range of target organisms, the mode of action, and the effect of food matrix components on their antimicrobial properties. The purpose of this review is to provide an overview of current knowledge about the antimicrobial mode of action of essential oil constituents, and to identify research avenues that can facilitate implementation of essential oil constituents as natural food preservatives in foods.

Table 1: Minimum inhibitory concentration (MIC) values of some essential oils against different bacteria.

Plant from Which Essential Oil is Derived	Micro-organisms	MIC Values
Cymbopogan citraus	*Escherichia coli*	0.6 µL/mL
	Staphylococcus aureus	0.6 µL/mL
Satureja montana	*Lactobacilus acidophilus*	8.66 µL/mL
Origanum vulgarae	*Escherichia coli*	1600-1800ppm
	Staphylococcus aureus	800-900ppm
Lavandula officinalis	*Escherichia coli*	2000ppm
	Staphylococcus aureus	1000-1200ppm
Cinnamomum zeylanicum	*Acinetobacter*	8 mg/mL
	Protreus vulgaris	8mg/mL
Psidia arguta	*Enterococcus fecalis*	8mg/mL
	Staphylococcus epidermidis	0.25mg/mL
Piper betle	*Acinetobacter*	8mg/mL
	Proteus vulgaris	4mg/mL
Pimento diocia	*Klebsiella pneumoniae*	4mg/mL
	Staphylococcus epidermidis	1mg/mL
Psidia terebinthina	*Proteus vulgaris*	8mg/mL
	Enterecococcus fecalis	8mg/mL
	Acinetobacter	16mg/mL
Thymus vulgaris	*Clostridium perfringens*	1.25mg/mL
Salvia sclarea	*Staphylococcus epidermidis*	1.5-2mg/mL

2) MAIN CHEMICAL COMPONENT OF ESSENTIAL OILS RESPONSILBLE FOR ANTIMICROBIAL ACTIVITY

Antimicrobial Activity of Essential Oils

In recent years there has been a growing interest in researching and developing new antimicrobial agents from various sources to combat microbial resistance. Therefore, greater attention has been paid to the screening of antimicrobial activity and its evaluation methods. Several bioassays such as well diffusion, disk-diffusion, and broth or agar dilution are well known and commonly used methods [1]. The lowest concentration of antimicrobial agent that completely inhibits growth of the organism in micro-dilution wells or tubes as detected by the unaided eye is called minimum inhibitory concentration (MIC). The most appropriate bioassays for the determination of MIC value are these dilution methods, as these bioassays offer the possibility of estimating the concentration of the tested antimicrobial agent in the agar (agar dilution) or broth medium (macro dilution or micro-dilution) (Table 1). The most common estimation of bactericidal activity is the determination of minimum bactericidal concentration (MBC) which is defined as the concentration killing 99.9% or more of the initial inoculums.

Biological activity of essential oils from five Lamiaceae species, namely, *Mentha piperita*, *Lavandula angustifolia*, *Mentha pulegium*, *Salvia lavandulifolia* and *Satureja montana* was determined by Nikolíc et al. for their antimicrobial, cytotoxic properties, and chemical composition. *Pseudomonas aeruginosa*, *Streptococcus pyogenes*, *Streptococcus mutans*, *Streptococcus sanguis*, *Streptococcus salivarius*, *Enterecoccus feacalis* and *Lactobacillus acidophilus* were the seven bacterial species, representing clinical specimens, along with fifty-eight clinical oral Candida spp. isolates with three reference strains used in the study. *Satureja montana* essential oil proved to be the most potent, and also significant antimicrobial activity was exhibited by all essential oils against all tested microorganisms [2]. In another study, the antibacterial activity of the essential oil from dried leaves of oregano (*Origanum vulgare*) that were fully formed, and leaves and flowers of lavender (*Lavandula officinalis*) was reported. The lowest values of minimum inhibitory concentration were yielded by oregano essential oil against *E. coli* with an MIC value of 1600–1800 ppm, whereas the MIC value of lavender essential oil was 2000 ppm. On the other hand, MIC value of oregano essential oil was 800–900 ppm and the MIC value of lavender essential oil was 1000–1200 ppm against *S. aureus*. The higher content of phenolic compounds was reported to be the cause for this inhibition [3]. Aumeeruddy-Elalfi et al. evaluated the antimicrobial properties of essential oils against eighteen microorganisms (bacterial and fungal isolates) that were isolated from seven exotic and two endemic medicinal plants of Mauritius. Using the micro broth dilution assay, significant antibacterial activities were recorded with low minimal inhibitory concentration for eight essential oils except for *Salvia officinalis*, where the recorded activity was comparable with the activity of antibiotics [4]. It has been reported that the antibacterial activity of fruit of *Eucalyptus globulus* showed an inhibition effect against the pathogenic strains *Pseudomonas aerugenosa*, *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Listeria innocua*, with an MIC value of 3–4 mg mL^{-1}. The MBC value of bactericidal effect varied between 3.6 and 9.0 mg mL^{-1}, which demonstrated that all the tested bacteria were sensitive to the essential oil of *Eucalyptus globulus* fruits [5].

Likewise, it was reported that the secondary essential oil (SEO) of *Mentha citrata* showed antibacterial activity against all eight tested bacterial strains of Gram-positive bacteria, namely, *Staphylococcus aureus* (MTCC 96), *Staphylococcus epidermidis* (MTCC 435) and *Streptococcus mutans* (MTCC 890), as well as Gram-negative bacteria, namely, *Pseudomonas aerugenosa* (MTCC 741), *Klebsiella pneumoniae* (MTCC 109), *Escherichia coli* (DH5α), *Escherichia coli* (MTCC 723) and *Salmonella typhimurium*

(MTCC 98), with an MIC value of 50–1000 µg/mL, while primary essential oils (PEOs) were active against seven strains with an MIC value of 250–1000 µg/mL [6]. Furthermore, a recent study assessed antimicrobial activity of six commonly used Brazilian condiments, viz., *Ocimum basilicum* L. (basil), *Rosmarinus officinalis* L. (rosemary), *Origanum majorana* L. (marjoram), *Mentha piperita* L. var. Piperita (peppermint), *Thymus vulgaris* L. (thyme) and *Pimpinella anisum* L. (anise) against a *Clostridium perfringens* strain. The MIC value for thyme essential oil was 1.25 mg mL−1 and 5.0 mg mL−1 for both marjoram and basil essential oil. Similarly, the three condiments, namely peppermint, rosemary and anise, showed MIC values of 10 mg mL^{-1}. With the exception of anise oil, which was only bacteriostatic, bactericidal activity was shown by all the oils at their respective MICs [7]. Tomáš et al. evaluated the antibacterial activity of the essential oil of *Epilobium parviflorum* Schreb against five microorganisms (*Pseudomonas aeruginosa*, *Staphylococcus aureus, Enterococcus faecalis, Escherichia coli, and Candida albicans*) using a microdilution method. Inhibition in the growth of all tested bacteria was observed.

An increase in bacterial resistance to antibiotics and the lack of new antibiotics introduced into the market resulted in a need to find alternative strategies so as to cope with infections resulting from drug-resistant bacteria [8]. Development of alternatives for antibiotics and the discovery or developments of adjuvants are amongst the potential strategies proposed [9]. In order to increase or restore antimicrobial efficacy against multi-drug-resistant bacteria, some efforts have been made. Addition of essential oils to antibiotics can induce a reduction in the antimicrobial MIC and the maximum effect has been observed with aminoglycosides, such as amikacin. It has been shown in assays that geraniol demonstrates good activity in modulating drug resistance of various Gram-negative bacterial species (*Enterobacter aerogenes*, *E. coli*, *P. aeruginosa*) by targeting efflux pumps and could restore susceptibility to drugs in strains that over-express efflux pumps. This modification of drug resistance by EOs is more evident for drugs such as chloramphenicol, β-lactams and fluoroquinolones. *S. aureus* is a common Gram-positive bacterium that can cause pathogenic conditions including food-borne diseases and infections ranging from minor localized skin disturbances to life-threatening deep tissue and systemic illness. Different components of EOs obtained from *Alpinia pahangensis*, *Origanum vulgare, Origanum dictamnus, Mentha piperita, Lavandula hybrida, Zataria multiflora* and *Hofmeisteria schaffneri* have been tested against *S. aureus*, and all were found to possess potential inhibitory activity [10, 11]. Furthermore, combination of essential oil with an antimicrobial agent produces a synergistic effect against multidrug-resistant S. aureus, and in many cases, a substantial decrease in the MIC has been observed [12].

Mechanism of Action

The most appropriate method for determining the bactericidal effect as well as a strong tool for obtaining information about the dynamic interaction between the anti-microbial agent and the microbial strain is the time-kill test. Also, a time-dependent or a concentration-dependent antimicrobial effect is revealed by the time-kill test.

Table 2: Mechanism of action of certain oils against different micro-organisms.

Plant from Which Essential Oil is derived	Micro-Organism Targeted	Mechanism of Action
Allium sativum	*Escherichia coli*	Induced leakage
Litsea cubeba	*Escherichia coli*	Destruction of outer and inner membrane
Foeniculum vulgare	*Shigella dysenteriae*	Loss of membrane integrity and increased permeability Foodborne and other pathogenic bactria
Piper nigrum	*Escherichia coli*	Cell becomes pitted, shriveled and leakage of intercellular material
Cumimm cyminum	*Bacillus cereus*	Changes in cytoplasm
Dipterocarpus gracilis	*Bacillus cereus*	Disruption of cell membrane
Ocimum gratissimum	*Pseudomonas aeruginosa*	Permeablized membrane
Coriaria nepalensis	*Candida*	Inhibition of ergosterol biosynthesis and isolates disruption of membrane integrity
Curcuma longa	*Aspergillis flavus*	Inhibition of ergosterol biosynthesis
Origanum vulgare	*Staphylococcus aureus*	Permeabilized membrane
Mentha longifolia	*Micrococcus luteus*	Cell wall damage

Li *et al.* reported that the kinetic curves (antibacterial) of Litsea cubeba oil at 0.0625% (v/v) was able to prolong the lag phase growth of *E. coli* cells to approximate 12 h while the cells were completely killed at 0.125% (v/v) within 2 h, as shown by transmission electron microscopy [13]. Destruction of the *E. coli* outer and inner membrane might be due to the penetration of the *Litsea cubeba* oil with the observation of many holes and gaps on the damaged cells, which led to killing them eventually. Therefore, a broad application of the *Litsea cubeba* oil in the antimicrobial industry would be possible due to its antimicrobial properties. The time-kill assay of *Foeniculum vulgare* (Fennel) oil against *Shigella dysenteriae* revealed destruction of the membrane integrity [14]. Similarly, it was reported that the leaf essential oil of *Forsythia koreana* acted on the cytoplasmic membrane against food-borne and other pathogenic bacteria, resulting in loss of membrane integrity and increased permeability [15].

Factors determining the activity of essential oils are composition, functional groups present in active components, and their synergistic interactions. The antimicrobial mechanism of action varies with the type of EO or the strain of the microorganism used. It is well known that in comparison to Gram-negative bacteria, Gram-positive bacteria are more susceptible to EOs [16,17]. This can be attributed to the fact that Gram-negative bacteria have an outer membrane which is rigid, rich in lipopolysaccharide (LPS) and more complex, thereby limiting the diffusion of hydrophobic compounds through it, while this extra complex membrane is absent in Gram-positive bacteria which instead are surrounded by a thick peptidoglycan wall not dense enough to resist small antimicrobial molecules, facilitating the access to the cell membrane. Moreover, Gram-positive bacteria may ease the infiltration of hydrophobic compounds of EOs due to the lipophilic ends of lipoteichoic acid present in cell membrane.

It has been shown in several reports that the bioactive components present in EOs might attach to the surface of the cell, and thereafter penetrate to the phospholipid bilayer of the cell membrane. The structural integrity of cell membrane is disturbed by their accumulation, which can detrimentally influence the cell metabolism causing cell death [18]. *E. coli* treated with black pepper essential oil (BPEO) became deformed, pitted, shriveled, because BPEO led to the leakage, disorder and death by breaking cell membrane [19]. Zhang et al. determined the mechanism behind the antibacterial activity of cinnamon EO against *E. coli* and *S. aureus* and reported that the bacterial cell membrane was destroyed after addition of cinnamon EO at the MIC level, whereas addition of cinnamon EO at the MBC levels resulted in the killing of the bacterial cell [19]. In addition to this, cinnamon EO led to increase in the electric conductivity of samples at the first few hours due to leakage of small electrolytes rapidly, concentration of proteins and nucleic acids in cell suspension and 3–5 fold decreased bacterial metabolic activity as reflected by the results of membrane potential. EO from *Dipterocarpus gracilis* inhibited the growth of *Bacillus cereus* and *Proteus mirabilis* by acting on the cytoplasmic membrane as one of its targets. These activities could be exploited for food preservation in the food industry [20]. Further, it has been reported that action of EOs on the integrity of cell membrane changes the membrane permeability which leads to loss of vital intracellular contents like proteins, reducing sugars, ATP and DNA, while inhibiting the energy (ATP) generation and related enzymes leading to the destruction of cell and leakage of electrolytes [21,22]. Antimicrobial activity of EOs is therefore attributed to a cascade of reactions involving the entire bacterial cell [23]. As reported in a study, essential oil from mustard presented 10 times more bactericidal/bacteriostatic effect than cinnamon essential oil [24]. Ahmad et al. [25] showed that antifungal activity of *Coriaria nepalensis* essential oil (CNEO) against *Candida* isolates is due to the inhibition in the biosynthesis of ergosterol and disruption in the integrity of membrane. Similarly, another study described the utility in designing new formulations for candidosis treatment because of the antifungal activity of coriander essential oil on *Candida* spp., in which it was reported that the fungicidal effect of coriander essential oil is a result of damage in the membrane of cytoplasm and subsequent leakage of intracellular components such as DNA. Likewise, disruption of the fungal cell endomembrane system including the plasma membrane and mitochondria, i.e., the inhibition of ergosterol synthesis, malate dehydrogenase, mitochondrial ATPase, and succinate dehydrogenase activities was related to the antifungal activity of natural essential oil (EO) derived from turmeric (*Curcuma longa* L.) against *Aspergillus flavus* [26].

Components of Essential Oils with Antimicrobial Activity

The major constituents of EOs can constitute up to 85%, whereas other components are present in trace amounts. α-phellandrene (36%) and limonene (31%) in *Anethum graveolens* leaf oil, d-limonene (over 80%) in citrus peel oils, α-phellandrene (36%) and limonene (31%) in *Anethum graveolens* leaf oil, carvacrol (30%) and thymol (27%) in *Origanum compactum* oil, α/β-thujone (57%) and camphor (24%) in *Artemisia herba-alba* oil, carvone (58%) and d-limonene (37%) in *Anethum graveolens* seed oil, and menthol (59%) and menthone (19%) in *Mentha piperita* oil are among the constituents present at relatively higher concentrations in essential oils. Generally, the biological properties of the essential oils are determined by their major components including two groups of distinct bio-synthetical origin. Terpenes and terpenoids comprise the main groups whereas aromatic and aliphatic constituents comprise the other group, all characterized by low molecular weight.

Terpenes and Terpenoids

Several isoprene units (C_5H_8) upon combination result in the production of hydrocarbons called terpenes. Occurring in the cytoplasm of plant cells, biosynthesis of terpenes proceeds via the mevalonic acid pathway starting from acetyl-CoA. Having a backbone of hydrocarbons, cyclases can rearrange terpenes into cyclic structures, thus forming monocyclic or bicyclic structures. Terpene biosynthesis consists of synthesis of the isopentenyl diphosphate (IPP) precursor, IPPs being added repetitively to form the prenyldiphosphate precursor of the various classes of terpenes, terpene-specific synthetase modification of the allylic prenyldiphosphate to form the terpene skeleton, and finally, secondary enzymatic modification (redox reaction) of the skeleton to attribute functional properties to the different terpenes. Monoterpenes ($C_{10}H_{16}$) and sesquiterpene ($C_{15}H_{24}$) are the main terpenes, but longer chains such as diterpenes ($C_{20}H_{32}$), triterpenes ($C_{30}H_{40}$), etc., also exist. *p*-cymene, limonene, menthol, eugenol, anethole, estragole, geraniol, thymol, γ-terpinene, and cinnamyl alcohol are among the examples of some constituents of essential oils with antimicrobial activity (Figure 1). Angelica, bergamot, lemongrass, mandarin, mint, caraway, celery, citronella, coriander, eucalyptus, geranium, petitgrain, pine, juniper, lavandin, lavander, lemon, orange, peppermint, rosemary, sage, and thyme are among the representatives of plants with some of these compounds. Oxygenated monoterpene (β-fenchol) and oxygenated sesquiterpene (α-eudesmol) were identified as the two main bioactive constituents in the essential oil

obtained from fresh leaves of *Eucalyptus teretecornis* with a minimum inhibitory amount (MIA) of 28 µg and 10 µg against *Alternaria alternata* [27]. Similarly, another study reported β-fenchol and linalool as the two antimicrobial components in essential oil obtained from the fresh leaves of *Zanthoxylum alatum* [28].

Biochemical modifications of terpenes via enzymes that add oxygen molecules and move or remove methyl groups result in the formation of terpenoids. Terpenoids can be sub-divided into alcohols, phenols, esters, aldehydes, ethers, ketones, and epoxides. Thymol, carvacrol, linalool, linalyl acetate, citronellal, piperitone, menthol, and geraniol are the examples of terpenoids. In one study, *α*-cedrol was reported as the bioactive constituent of the essential oil from fresh leaves of *Thuja orientalis* with a minimum inhibitory amount (MIA) of 30.5 µg against *A. alternate*.

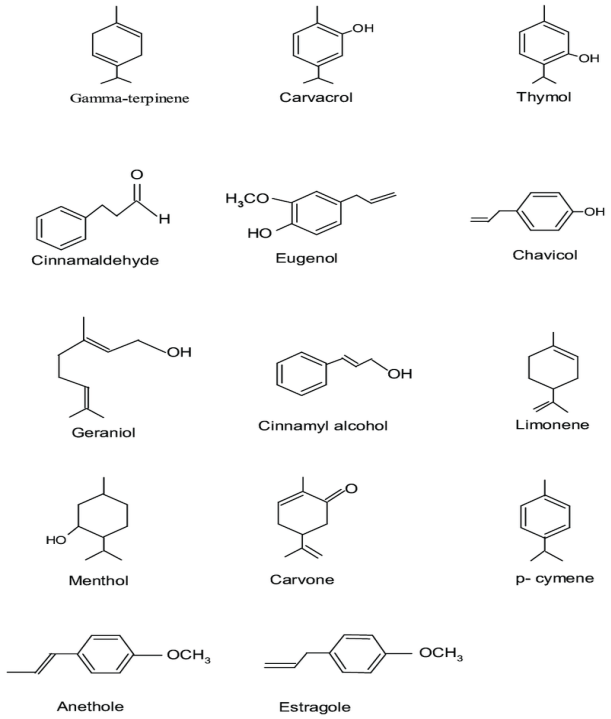


Figure 1: Some representative bioactive compounds present in essential oils.

Monoterpenoid phenols present in the essential oil of *Origanum vulgare*, thyme, pepperwort and wild bergamot are carvacrol or cymophenol. Diarrheal toxin production by *Bacillus cereus* and growth of vegetative bacteria were inhibited by carvacrol. The precursor of carvacrol is *p*-cymene which is a monoterpene with a benzene ring without any functional groups on its side chains. When used alone, *p*-cymene is not an efficient antimicrobial compound, but the activity of compounds like carvacrol is potentiated by *p*-cymene and polymyxin B nona peptide. It has been shown that *p*-cymene is hydrophobic in nature and causes swelling of the cytoplasmic membrane to a greater extent. Also, *p*-Cymene had an effect on the synthesis of protein in *E. coli* cells.

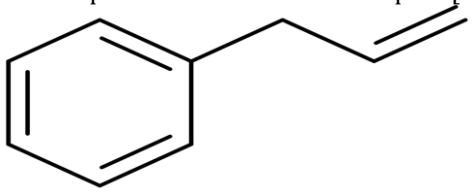
It is expected that the antimicrobial action of phenolic compounds such as thymol and carvacrol is attributed to structural and functional damages in the cytoplasmic membrane. The primary mode of antibacterial action of thymol is not completely understood, but is believed to involve disruption of outer and inner membrane and interaction with membrane proteins and intracellular targets. Thymol (or 2-isopropyl-5-methylphenol), a natural monoterpene phenol derivative of cymene, is isomeric with carvacrol present in thyme essential oil and is extracted from *Thymus vulgaris* (common thyme) and various other plants. In a study by Di Pasqua et al. interaction of thymol with membrane proteins was further supported by exposing *Salmonella enterica* to sub-lethal concentrations of thymol, and accumulation of outer membrane proteins in misfolded pattern and upregulation of genes involved in synthesis of outer membrane proteins was also observed. The citrate metabolic pathway was also impaired by thymol and many enzymes involved directly or indirectly in ATP synthesis. Intracellular action of thymol indicates that it affects important energy-generating processes, which lower the ability of a cell to recover after exposure to thymol. Studies pertaining to investigation of the mode of action of thymol against yeast and fungi point towards the interaction of thymol with the cell envelope and intracellular targets. It has been shown that thymol disrupted vesicles and cell membranes, and impaired biosynthesis of ergosterol in *Candida* strains, which consequently affected the integrity of cell membrane because membrane fluidity and asymmetry is regulated by ergosterol similarly to cholesterol in animal cells. Rao et al. proposed that specific signaling pathways are activated by thymol in yeast, rather than causing non-specific lesion of membranes. This was based on the observation that cytosolic Ca^{2+} bursts caused by thymol and transcription responses similar to those in Ca^{2+} stress and nutrient starvation are activated. Moreover, an increase in the permeability of *P. aeruginosa* and *S. aureus* cells was observed in ethidium bromide (fluorescence nuclear stain), dissipated pH gradients irrespective of glucose availability, and leakage of inorganic ions. These results were in accordance with a study that utilized a mixture of thymol and carvacrol. A major constituent of oregano is carvacrol (a phenolic monoterpenoid). Carvacrol is one of the most extensively studied essential oil constituents together with its closely related isomer thymol. EOs rich in carvacrol have been reported to possess remarkable antimicrobial activity [29,30]. Although the outer membrane is affected by carvacrol, the cytoplasmic membrane is thought to be its site of action, causing passive transport of ions across the membrane. As an adaptation mechanism to maintain optimal membrane function and structure, it has been proposed that cells exposed to carvacrol change the fatty acid composition of the membrane because of the effect of carvacrol on fluidity. It has been demonstrated that carvacrol affects the outer membrane of Gram-negative bacteria.

According to Friedman et al. based on the time they take to produce significant action, essential oils can be divided into the following two types: compounds that act slowly and compounds with fast action. Examples of some antimicrobials considered as fast acting compounds are carvacrol, cinnamaldehyde, and geraniol, since they

inactivate organisms like *E. coli* and *Salmonella* in a short time of five minutes. It was reported that time duration of 30–60 min was required to show efficient antimicrobial activity for the compounds acting slowly. Carvacrols' mechanism of antifungal activity is similar to thymol, showing H^+ homeostasis and disruption of Ca^{2+}, up- and down-regulation of gene transcription similar to that found in Ca^{2+} stress and nutrient starvation, disruption of membrane integrity, and impairment of biosynthesis of ergosterol in *Candida* strains. Silva-Angulo et al. showed that citral exhibited antilisterial activity against *L. innocua* and *L. monocytogenes* and can be applied in active packaging to control possible recontamination of foods or in combination with other preservation technologies [31]. Similarly, Klein et al., determined the antimicrobial activity of six essential oil components against the potential food spoilage bacteria *Aeromonas hydrophila*, *Escherichia coli*, *Brochothrix thermosphacta*, and *Pseudomonas fragi* for single use and in combination with each other [32]. They further showed that, for single use, the most effective oil components were thymol (bacteriostatic effect starting from 40 ppm, bactericidal effect with 100 ppm) and carvacrol (50 ppm/100 ppm), followed by linalool (180 ppm/720 ppm), α-pinene (400 ppm/no bactericidal effect), 1,8-cineol (1400 ppm/2800 ppm), and α-terpineol (600 ppm/no bactericidal effect).

Phenylpropenes

In plants, synthesis of phenylpropenes occurs from the amino acid precursor phenylalanine, constituting a subfamily among the various groups of organic compounds called phenylpropanoids. A relatively small proportion of essential oils is composed of phenylpropenes, and the phenylpropenes that have been most thoroughly studied are safrole, eugenol, isoeugenol, vanillin, and cinnamaldehyde. Eugenol, which is a clear to pale yellow oily liquid is extracted from clove oil, nutmeg, cinnamon, basil, and bay leaves. A study reported eugenol as the antifungal bioactive molecule from *Cinnamomum tamala*, with a minimum inhibitory amount of 9.5 and 8.2 µg against *Alternaria alternata and Curvularia lunata*, respectively [33]. Eugenol has also been shown to cause deterioration of the cell wall, lysis of cells, and prevention of enzyme action in *Enterobacter aerogenes* [34]. The antimicrobial activity of phenylpropenes is dependent on the selected microbial strains, the kind and number of substituents on the aromatic ring, and experimental parameters such as temperature and medium chosen for growth, etc. Cinnamaldehyde is a flavor- and odor-giving organic compound. Being a pale yellow viscous liquid, it occurs naturally in the bark of cinnamon trees and other species of the genus *Cinnamomum.* It is found as growth inhibitor of *Escherichia coli* and *Salmonella typhimurium* but does not disintegrate the outer membrane or deplete the intracellular ATP pool [34].



Phenylpropenes

Nano-Encapsulation of Essential Oils for Enhancing Their Antibacterial Effect

A process resulting in the formation of small capsules with many useful properties by surrounding droplets of the bioactive in nature with a coating, or embedding them in a homogeneous or heterogeneous matrix is called encapsulation. Oil encapsulation may retard or even prevent thermo-oxidation reactions, leading to a widening of the intended range of enrichment purposes for food commodities [35]. Bioactive oils are commonly used for their pharmaceutical, cosmetic and nutritional properties. Generally, EOs are volatile substances sensitive to oxygen, light, moisture, and heat. These reported special characteristics could diminish their applicability in cosmetics, food and pharmaceutical industries. Thus, encapsulation is one of the most efficient methods for the formulation of bioactive oils and various studies have been developed in this aspect. The encapsulation system is selected in line with the intended usage of the final formulation, which can vary depending on the size, shape, or nature of selling components. The growing interest in the use of essential oils as natural antimicrobials and preservatives in the food industry has been driven in the last years by the growing consumer demand for natural products with improved microbial safety, and fresh-like organoleptic properties. Nano-emulsions efficiently contribute to support the use of EOs in foods by increasing their dispersibility in the food areas where microorganisms grow and proliferate, by reducing the impact on the quality attributes of the product, as well as by enhancing their antimicrobial activity [36].

Beyki et al. [37] showed that MIC values of free as well as chitosan–cinnamic (CS–Ci) acid nanogel-encapsulated *Mentha piperita* essential oils against *A. flavus* under sealed condition were 2100 and 500 ppm, respectively. Contrary to this, when tested under non-sealed conditions, the encapsulated oils performed better (800 ppm), while within the concentration range tested (up to 3000 ppm) the free oils failed to cause complete inhibition. As a carrier for essential oils in order to enhance their antimicrobial properties, these findings revealed the promising role of CS–Ci nanogel. A higher in vitro bactericidal action of nano-emulsions loaded with essential oils of lemongrass, clove, thyme or palmarosa against *Escherichia coli* has been reported as these nano-emulsions achieved log-reductions of 4.1, 3.6, 2.8 or 3.9, respectively, after a contact time of 30 min. In the case of nano-emulsions containing lemongrass or clove essential oils, faster and enhanced inactivation kinetics were also observed compared to their respective coarse emulsions [38]. Herculano et al. nano-encapsulated *Eucalyptus staigeriana* essential oil (ESO) using cashew gum (CG) as wall material with sizes of nano-emulsions ranging from 27.70 nm to 432.67 nm with negatively charged surfaces. The antimicrobial activity of nanoparticles against *Listeria monocytogenes* (Gram-positive) and *Salmonella Enteritidis* (Gram-negative) was evaluated by determining their minimum bactericidal concentration, The data from MBC showed greater antibacterial activity against Gram-positive bacteria, due to a likely synergistic effect between the ESO and CG. Thus, the data mentioned above suggest

that the nanoparticles of ESO have potential to be used as natural food preservatives [39]. Another study revealed superior performance of encapsulated *Zataria multifloria* essential oil (ZEO) by chitosan nanoparticles (CSNPs) under both in vivo and in vitro conditions as compared to unmodified ZEO against *Botrytis cinerea*. The in vivo experiment also showed that at a 1500 ppm concentration, the encapsulated oils significantly decreased both disease severity and incidence of *Botrytis*-inoculated strawberries during 7 days of storage at 4 °C followed by 2–3 days at 20 °C. In another study, the role of CSNPs as a controlled release system for EOs has been suggested [40]. Fatih and Tornuk produced novel water-soluble and thermally stable chitosan nanoparticles loaded with different levels (1%, 1.2%, 1.4% and 1.5%) of summer savory (*Satureja hortensis* L.) essential oil using an ionic gelation method. NPs loaded with essential oils exhibited strong antibacterial activity against *Staphylococcus aureus*, *Listeria monocytogenes* and *Escherichia coli* depending on the concentration of EO encapsulated. It was concluded that the summer savory EO-loaded chitosan NPs were highly adapted to excessive environmental factors such as high temperature and acidic pH, possessing high bioactive properties convenient for future food processing and packaging applications [41]. Also, Shengjiang et al. prepared blended cloves/cinnamon essential oil nano-emulsions using Tween 80 and ethanol as surfactant and co-surfactant, respectively. Even at far lower concentrations, the nano-emulsion showed higher antimicrobial activity against the four tested microorganisms *Escherichia coli, Bacillus subtilis, Salmonella typhimurium*, and *Staphylococcus aureus*. This shows that blended cloves/cinnamon essential oil nano-emulsions have the potential to be a natural antimicrobial agent in the food industry [42].

Mohammadi et al. [43] studied the performance of *Cinnamomum zeylanicum* essential oil (CEO) when encapsulated by CSNPs under both in vitro and in vivo conditions in comparison with unmodified CEO against *Phytophthora drechsleri*. The in vivo study showed that at a concentration of 1.5 g/L the encapsulated oils of *Cinnamomum zeylanicum* significantly decreased both disease severity as well as incidence of *Phytophthora* in inoculated cucumbers over 7 days of storage at 4 °C followed by 2–3 more days at 20 °C. Furthermore, the shelf life of cucumbers with CEO-CSN coating was extended up to 21 days at 10 ± 1 °C while uncoated fruit were unmarketable in less than 15 days. In addition to this, CEO-CSN coated fruits were firmer, maintained color, water content, had improved microbiological and physicochemical quality and showed lower microbial counts throughout storage. Thus, CEO-CSN coatings can be an effective method to extend cucumber shelf life. Similarly, Guerra-Rosas et al. assessed the antimicrobial activity of nano-emulsions containing oregano, thyme, lemongrass or mandarin essential oils and high methoxyl pectin during a long-term storage period (56 days) against *E. coli* and *Listeria innocua*. Regardless of the EO type, a higher antimicrobial activity was detected against *E. coli* as compared to *L. innocua*. Significant damage in the *E. coli* cells for both the cytoplasm and cytoplasmic membrane led to cell death which was revealed by the images of transmission electron microscopy (TEM). The antimicrobial activity of the nano-emulsions was found to be strongly related to the EO type rather than to their droplet size. The smallest droplet size (11 ± 1 nm) of the lemongrass-pectin nano-emulsion had a higher antimicrobial activity, reaching 5.9 log reductions of the *E. coli* population. Nevertheless, nano-emulsion of the freshly-made oregano, thyme and mandarin EO–pectin led to 2.2, 2.1 or 1.9 *E. coli* log-reductions, respectively. However, a significant decrease in the antimicrobial activity was observed during storage regardless of the EO type, which was related to the loss of volatile compounds over time [44]. Besides EOs, there are numerous reports available on the antimicrobial efficacy of nanoparticles. Prabhu and Poulose reviewed various mechanisms of antimicrobial action of NPs, especially silver NPs [45], which are potential antimicrobial agents [46]. Silver NPs create pits and thus anchor and penetrate the cell wall, causing release of free radicals followed by structural change in the cell membrane leading to a greater influx of the antibacterial agent through cytoplasmic membrane because of increased cell permeability, resulting in cell death. Another mechanism of the silver nanoparticles is the formation of free radicals, by which the cells die. It has been suggested by electron spin resonance spectroscopy studies that silver nanoparticles form free radicals, when in contact with bacteria, and these free radicals have the ability to make the cell membrane porous by damaging it which can ultimately lead to cell death. Moreover, silver nanoparticles attacking DNA bases can also inhibit signal transduction and cell wall formation, and interact with respiratory enzymes, liberating reactive oxygen species which is followed by cell death.

Over the last decade, several research studies have focused on the synergy between EOs and various types of NPs for their superior antimicrobial efficacy. Cinnamaldehyde, a representative of EO, showed a strong synergistic activity with silver NPs against spore-forming *Bacillus cereus* and *Clostridium perfringens*. Bacterial kill curve analysis revealed rapid bactericidal action exerted by this combination of antimicrobial agents, while extensive damage to the cell envelope was evidenced by electron and atomic force microscopy [47].

Release of silver ions by nanoparticles can inactivate many enzymes by interacting with the thiol groups. An enhancement in the antimicrobial potential of EOs can be attained by encapsulating with various nanomaterials e.g., solid lipid NPs, liposomes, polymeric NPs and nano-emulsions, where the inside core consists of EO while nanomaterial forms the outer nano-capsule. Essential oils upon nano-encapsulation exhibit physical stability, decreased volatility and protection from environmental interactions (e.g., light, oxygen, moisture, pH), enhanced bioactivity and reduced toxicity. Small nano-emulsion droplets are able to bring the EOs close to the cell membrane surface, improving the accessibility to microbial cells and enabling the membranes of the cells to be disrupted, possibly by altering the integrity of phospholipid bilayer or by interfering with the embedded phospholipid bilayer active transport proteins [48]. In

order to modulate drug release i.e., burst release and/or controlled release, this represents a promising approach [49]. The in vitro release study of encapsulated oregano EO with chitosan NPs revealed an initial burst effect followed by slow release of drug (EO) [50,51]. Similarly, efficient biocidal activity against *Stegomyia aegypti* larvae was observed due to a slow and sustained release of EO by chitosan/ cashew gum nano-encapsulation. The effect of EO nano-emulsions on yeast cells has also been addressed in several studies among which *Zygosaccharomyces bailii* and *Saccharomyces cerevisiae* are the most investigated. Yeast cells required longer incubation times with respect to bacterial inactivation, when exposed to carvacrol, cinnamaldehyde, and d-limonene nano-emulsions [52] and exhibited lower minimum inhibitory concentration for encapsulated d-limonene [53]. In another study, an enhancement in the antimicrobial activity of carvacrol loaded in polylactic glycolic acid nano-capsules was reported due to significant transformation in the rheological characteristics of bacterial biofilm that potentially facilitated the activity of carvacrol [54]. EOs are protected from enzymatic degradation by the nano-carriers which transform them into powder and help to achieve the desired therapeutic levels for the required time duration to the target tissues with reduction in number of doses and may also ensure an optimal pharmacokinetic profile [49]. Thus, the combination of various EOs with their inherent antimicrobial activity with other potent antimicrobial agents like NPs may greatly enhance their antimicrobial activity by complementing each other with the involvement of various mechanisms against different types of pathogens. Therefore, combinations of different antimicrobials appear to be the best strategy for controlling multidrug resistant microbes.

Study of Synergistic Antimicrobial Activity of Essential Oils

There is a need to find alternative strategies to deal with infections resulting from drug-resistant bacteria, due to an increase in antibiotic-resistant bacteria and the lack of new antibiotics being brought onto the market. Development of alternatives to antibiotics and the discovery or development of adjuvants is amongst the potential strategies proposed. Combination of antibiotics with other drugs that are non-antibiotic is one such possibility. Another such possibility is the combination of antibiotics with adjuvants or antimicrobials selected from nature's reservoir of bioactive compounds. An overview pertaining to synergism between plant metabolites and antibiotics has been provided by Hemaiswarya et al. and according to them promising adjuvants of antibiotics may be represented by phytochemicals. Essential oils (EOs) and their components form part of the group of phytochemicals that is said to have such effects, according to in vitro studies [55].

Synergism between Constituents of Essential Oils

Antimicrobial activity of a given essential oil may depend on one or two of the major constituents only that make up the entire oil. In accordance with the increasing level of evidence, the ratio in which the main active constituents are present may not be the only factor responsible for the inherent activity of essential oils, but the interactions between these and minor constituents in the oils are also important.

Synergism between Different Essential Oils

Bag and Chattopadhya showed that the coriander/cumin seed oil combination showed synergistic antibacterial interactions with an FICI ranging from 0.25 to 0.5 [56]. In a study by Hossain et al. eight essential oils (EOs) of plants, namely, eucalyptus, tea tree, basil, oregano, cinnamon, mandarin, peppermint, and thyme were evaluated for their ability to inhibit growth of *Aspergillus niger, Penicillium chrysogenum, Aspergillus flavus*, and *Aspergillus parasiticus* and it was reported that a combined formulation of oregano with thyme essential oil resulted in a synergistic effect, thus showing an enhancement in the efficiency against *A. flavus, A. parasiticus* and *P. chrysogenum*. A synergistic effect was also exhibited by mixtures of peppermint and tea tree essential oil against *A. niger*, and thyme and cinnamon against *A. flavus*. Also, a synergistic effect was observed against *P. chrysogenum* by combining oregano essential oil with essential oils of cinnamon, tea tree, thyme and mint, and an individual mixture of mint with thyme [57]. Similarly, a synergistic effect against *A. flavus* was produced by combination of oregano and thyme, cinnamon and thyme and oregano and mint essential oils. Also, combination of mint with tea tree oil showed a synergistic effect against *A. niger*. It was revealed that the combination of some particular oils produced synergism as a result of the combined activities of two or more constituents of essential oils. Because pathogens cannot easily acquire resistance to multiple components of two or more essential oils, such an increase in the fungistatic activity would be advantageous in pre- and post-harvest protection [58].

Synergism between Essential Oils and Antibiotics

In a study conducted by Rosato et al. it was reported that oregano oil in combination with gentamicin exhibited synergism against *B. cereus*, *B. subtilis* and one strain of *S. aureus*. *Zataria multiflora* (Shiraz oregano) essential oil exhibited synergistic activity with vancomycin against methicillin-sensitive *Staphylococcus aureus* (MSSA) and 12 clinical isolates of MRSA, although the FIC data for individual strains were not stated. In another study, Australian tea tree (*Melaleuca alternifolia*) volatile oil combinations with aminoglycoside antibiotics were investigated. *E. coli*, *Yersinia enterocolitica*, *Serratia marcescens* and one strain of *S. aureus* were among the bacterial species for which synergism was found with gentamicin. The FIC index was found to be at borderline between additivity and synergism against *Acinetobacter baumannii*, *B. subtilis* and another strain of *S. aureus*. Also, tea tree oil along with tobramycin had synergism against *E. coli* and *S. aureus*. It was found that aminoglycosides inhibit protein synthesis and tea tree oil damages the bacterial cytoplasmic membrane; this was possibly an example of multi-target synergy. Ampicillin and gentamicin along with clove oil have been tested for synergism against a number of periodontic pathogens. FIC indices of less than 0.5 were found for ampicillin against *Streptococcus mutans*, *S. sobrinus* and *Streptococcus gordonii* and for gentamicin against *Streptococcus sanguinis*, *S. criteci* and *Porphyromonas gingivalis*. In an in

vitro study conducted by Duarte et al. the combination of coriander essential oil with gentamicin, chloramphenicol, ciprofloxacin, and tetracycline against *Acinetobacter baumannii* showed effectiveness, which was therefore an indicator of a possible synergistic interaction against two reference strains of *Acinetobacter baumannii* (LMG 1025 and LMG 1041) with an FIC index of 0.047 and 0.37, respectively. This study indicated that this in vitro interaction could improve the antimicrobial effectiveness of tetracycline, ciprofloxacin and gentamicin and may contribute to re-sensitize *Acinetobacter baumannii* for the action of chloramphenicol.

In most of the cases, examination of combination of *Eucalyptus camaldulensis* essential oils with conventional antibiotics (gentamycin, ciprofloxacin, and polymyxin B) showed synergistic antibacterial effect even in some re-sensitized multidrug-resistant (MDR) *A. baumannii strains*. Time-kill curves confirmed the synergistic interaction of *E. camaldulensis* essential oil and polymyxin B combination resulting in a reduced bacterial count under very fast detection limit, i.e., after 6 h of incubation [59].

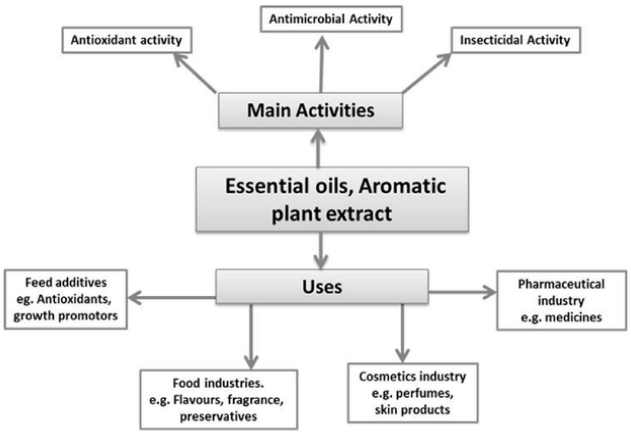


Figure 2: Essential oils as application with use

3) IN COSMECEUTICAL INDUSTRY

Essential oils are complex mixtures containing dozens of substances of varying chemical composition at different concentrations. They are characterized by the compounds present at highest concentration which determine the flavour, fragrance and biological properties of the EOs [60].

The importance of EOs for health, beauty and wellness dates back to ancient times. In recent decades, the concern about the possible risks of synthetic ingredients for the human health has been increasing. Therefore, the use of natural compounds to enhance health and beauty of human has been showing an increasing trend line. EOs are remarkable used in nowadays cosmetic industry.

Cosmetics companies produce diverse kinds of products, such as personal and beauty care, hair care, cleanser, perfume and make-up. The cosmetics industry is continuously innovating and improving products for consumers. As a result, the cosmeceutical products (products that contain one or more bioactive compound and are intended to improve health and beauty) have increasingly become popular [61]. As shown in Table (3), EOs may be present in the formulations of the various categories cosmetics. An EO can be named by the common name and/or specie of the plant from which it was extracted. When only the common name is mentioned, it is understood that the EO can be obtained by the various species related to that plant.

Table 3. Example of essential oils present in the different categories of cosmetic products

Type of Cosmetic Products	Essential oils
Skin care and maintenance Softener/smoothing(emollients)	Fenugreek *(Trigonella foenumgraeceum)*
Moisturizers	Chamomile
Anti-ageing	Vanillin, Sandalwood, Olive, Borage, Evening primrose, Chamomile
Repairing (anti-chapping and anti-wrinkling agent)	Camellia *(Camellia japonica), Centella asiatica,* Hippophae,
Repairing (anti-acne agents)	Rosemary *(Rosmarinus officinalis)*
Sunscreens	Lavender *(Lavandula stoechas),* Oregano (*Origanum majorana),* Aloe Vera
After-sun	Tea tree, Methol, patchouli,
Cosmetic textiles	Sage *(salvia officinalis)* Rosemary, Aloe Vera
Cleansing Shampoo	Rosemary *(R. officinalis)*
Soaps	Sweet orange (*Citrus sinensis* L.) Lavender (*Lavendula angustifolia, Lavendula latifolia,*
Dentifrices and toothpastes	Sage (*S. officinalis*) Clove Eucalyptus Peppermint (*Mentha piperita*) Menthol mint (*Mentha arvensis*) Myrrh (*Commiphora myrrha)*
Dour improvement Perfumes, deodorants, and antiperspirants	Lemon (*Citrus limonum* L.) Sweet orange (*C. sinensis* L.) Geranium (*Pelargonium graveolens* L.) Clove (*Syzygium aromaticum*)

In the past, cosmetic industry used EOs mainly due to their well-known fragrance properties. However, other interesting properties of certain EOs have been studied, demonstrated and used in cosmetic products [62].

Essential oils are the ingredients added to natural origin products with fragrance. Once they contain several components with fragrance properties well blended, EOs improve the odour of a product. Flower EOs, such as rose, tuberose, narcissus, gardenia, jasmine and *Lavandula officinalis*, remain the most popular aroma ingredients in

the cosmetic industry. Other EOs commonly used in cosmetics for the same purpose are patchouli (*Pogostemon cablin*), citronella (*Cymbopogon winterianus*), sandalwood (*Santalum álbum*), bergamot (*Citrus* aurantium), rosemary (*Rosmarinus officinalus*), mint (*Mentha piperita*) and vetiver (*Chrysopogon zizanioides*) [62]. Many studies showed that EOs (e.g., *Artemisia afra*, *Pteronia incana* and *Rosmarinus officinalis*) can be used as preservative ingredients in cosmetics, because of their anti-microbial proprieties against a wide range of bacterial strains. Generally, the anti-microbial activity in decreasing order of some common EOs are reportedly oregano, clove, coriander, cinnamon, thyme, mint, rosemary, mustard and sage**.** Yorgancioglu et al. studied the effect of some natural EOs (*Thymus vulgaris, Origanum onites*, *Eucalyptus globulus* and *Mentha piperita*) with reported anti-microbial efficacy in cosmetic formulations containing collagen hydrolysate. The anti-microbial effectiveness of cosmetic formulations was performed against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Enterococcus faecalis, Aspergillus fumigatus* and *Candida albicans*. They concluded that formulations containing 2% of *T. vulgaris* or *O. onites* EOs have highest anti-microbial activity, and both are effective against *S. aureus, E. coli, A. fumigatus* and *C. albicans.*

Furthermore, currently, EOs are widely used in cosmetic products with pharmacological properties, the named cosmeceutical products. Geranium oil, for example, is used in cosmetics as cleansing for over-oily skin, acne and eczema. The anti-inflammatory activity of *Agathosma betulina, Eriocephalus africanu* and *Eriocephalus punctulatus* EOs are reported [62]. Chamomile EOs also has anti-inflammatory effects and is used for treating inflammation of skin and prevention of other skin disorders. Ursolic acid, a compound of rosemary EOs, promotes collagen build-up and elastin synthesis, prevents wrinkles and increases blood circulation in the skin and scalp. In fact, there are several properties and bioactivities, which make EOs so attractive for cosmetic industry. However, due to its physicochemical nature, the cosmetic benefits of EOs are not entirely availed. In this context, microencapsulation has been reported as an effective process to overcome these limitations.

Microencapsulation of EOs

Microencapsulation is the process that one material or a mixture of materials is coated with or entrapped within another material or system. The coated material is called active or core material and may be solid, liquid or gas, and the coating material is called shell, wall material, carrier, or encapsulating agent. Microparticles consist in a multicomponent structure with a diameter of 1–1000 µm. Commonly, microspheres are described as a matrix system, in which the active ingredient is dispersed/ dissolved in the carrier matrix. Microcapsules have at least one discrete domain of active agent and sometimes more (reservoir system). As a result, the microcapsule consists of a layer of an encapsulating agent that isolates and protects the active substance, avoiding its inadequate exposure. Microcapsules can have regular shape (e.g. spherical, tubular and oval) or irregular shape.

Microencapsulation technology was first introduced in the 1930s with a publication that described gelatin microcapsules obtained by the coacervation technique. However, the first large-scale application of microencapsulation dates 1950s, when the American company National Cash Register (NCR) used complex coacervation for development of carbonless copy paper. Nowadays, microencapsulation has numerous applications in different industrial fields, such as food, textile, pharmaceutical [64, 65], cosmetic [66] and agrochemical [67] industries. This technique allows the improvement and/or modification of the characteristics and properties of the active material, as well as its protection, stabilization and slow release.

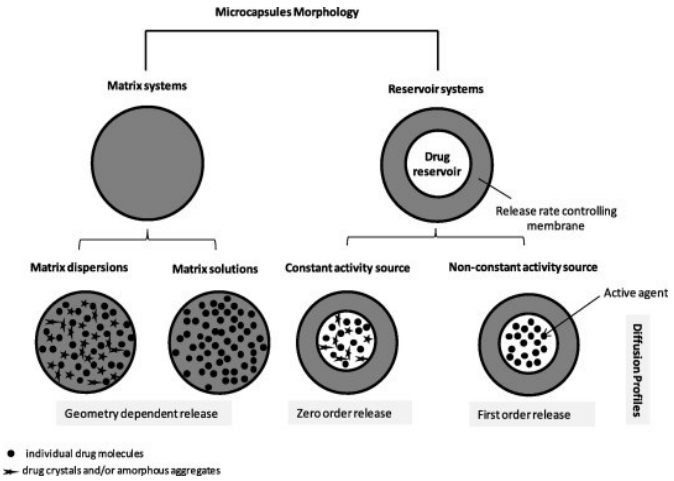


Figure 3: Microencapsulation of essential oils with biodegradable polymeric carriers for cosmetic applications (Source doi: 10.1111/ics.12232)

Encapsulation techniques

Currently, there are numerous methods of microencapsulation, and this number continues to increase as companies' market products from their patented and innovative microencapsulation technologies. Numerous methods allow encapsulating an active material depending on the type of the material to be encapsulated, the release characteristics of the encapsulated compound, the application and regulatory considerations. It may influence the final characteristics and properties of microparticles. Although a range of techniques have been reported for microencapsulation, they can widely be divided into three main categories: (i) chemical processes (e.g. interfacial and *in situ* polymerization methods); (ii) physiochemical processes (e.g. coacervation (phase separation), and emulsification solvent evaporation/ extraction); and (iii) physical–mechanical processes (e.g. air suspension method, pan coating, spray drying, spray chilling, spray cooling and fluid bed coating). Some of the most important and usual microencapsulation techniques are discussed below. Table (4) summarizes the microencapsulation methods discussed, presenting the particle size, advantages and disadvantages of these methods.

Table 4: Particle size produced by different microencapsulation technologies and their advantages and disadvantages

Microencapsul-ation technique	Particle size(μm)	Advantages	Limitations
Simple coacervation	20-200	High encapsulation efficiency	Expensive method
	5-200	Efficient control of particle size	Aggregation of particles Hard scalling-up Evaporation of volaties Possibility of dissolution of active compound into the processing solvent Oxidation of product
Spray drying	1-50	Simple Versatile Low process cost Easy scaling-up technique	Particles no uniform Low oil loading Require further processing Possibility of lost low-boiling point aromatics
Spray chilling	20-200	Suitable for water-soluble materials	High process costs Require special handling and storage conditions
Fluid bed spray coating	>100	Low operational costs High thermal efficiency process	Long time process
Emulsification	0.1-100	Small droplets Narrow particle size distribution	Low encapsulation efficiency Production of high quantity residual solvent
Interficial polymerization	0.5-1000	Easy scaling-up technique Fast High encapsulation efficiency	Difficult to control Production of high quantity residual solvent Possibility of non-biodegradable and/or non-biocompatible monomers

Coacervation (phase separation)

Phase separation microencapsulation consists in obtaining two immiscible liquid phases from a solution that contains a dispersed macromolecule. The liquid or solid to be encapsulated is dispersed in a solution of a macromolecule (wall material). Through different methods, the encapsulating polymer is induced to separate as a viscous liquid phase (coacervate). This separation process is known as coacervation. The macromolecule is present at high and low concentrations in the coacervate phase and in the supernatant phase, respectively. Under certain conditions, coacervate phase forms a continuous layer which coats the material to be encapsulated. The formed microparticles can be collected by centrifugation or filtration, and thereafter washed with the appropriate solvent, dried and hardened by thermal, cross-linking or desolvation techniques. Therefore, coacervation is a three-step process: (i) formation of an oil-in-water (o/w) emulsion (active compound is dispersed in the aqueous phase and polymer is dissolved in the organic phase); (ii) deposition of the liquid polymer coating upon the core material; and (iii) stabilization and hardening the coating material to form a self- sustaining microcapsule (Figure 2).

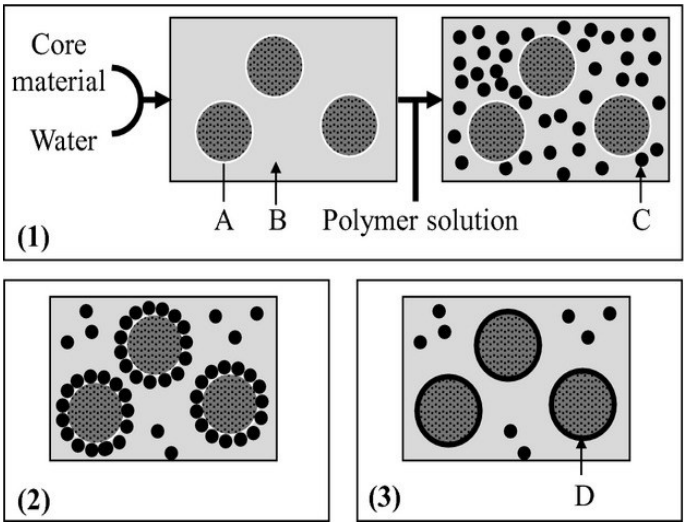


Figure.2: Generic scheme of microencapsulation process by coacervation: (1) formation of an oil-in-water (o/w) emulsion [core material (A) is dispersed in water (B), and polymer (C) is dissolved in the organic phase]; (2) deposition of the liquid polymer coating upon the core material; and (3) hardening the coating material to form a self-sustaining microcapsule (D). Adapted from Lamprecht and Bodmeier (Source doi: 10.1111/ics.12232)

This technique allows high encapsulation efficiency and efficient control of particle size. Additionally, it can provide protection against degradative reactions, prevent the loss of volatile aromatic ingredients, control release and improve the stability of the flavour and EOs core materials. However, the most common problem is agglomeration of microcapsules. It is also operationally complex, requiring careful control of experimental conditions, and expensive. In some cases, the stabilization of the microcapsules using high temperature, extreme pH values or cross-linking agents is required, which limits the encapsulation chemically and thermo-labile materials such as proteins and polypeptides. Other limitations are evaporation of volatiles (e.g. flavours and EOs) during processing, dissolution of active compound into the processing solvent and oxidation of product.

Interfacial and in situ polymerization

There are different polymerization techniques for microencapsulation of active compounds. Reactions

between oil-soluble and water-soluble monomers can result in interfacial polymerization to form polymeric microparticles, whose size is determined by the droplet size of the emulsion. Alternatively, interfacial polymerization can result in reaction of two reactive monomers dispersed in one phase, induced to polymerize at the interface or in the dispersed phase and precipitate at the interface. Under these conditions, the surface of the core material is coated by a polymer. In this technique are used polymers formed by monomers that have preferential solubility for one of the phases, such that polymerization only happens at the interface. Typically, the interfacial polymerization involves the reaction between a diacyl chloride and an amine or alcohol, resulting polyester, polyurea, polyurethane or polycarbonate polymers. Scarfato *et al*. produced polyurea microcapsules by interfacial polymerization in o/w emulsion, coating lemon balm (*Melissa officinalis* L.), lavender (*Lavandula angustifolia* Miller), sage (*Salvia officinalis* L.) and thyme (*T. vulgaris* L.) EOs. The EO loadings were in the range of 25–50wt%.

In situ polymerization is a chemical microencapsulation technique similar to interfacial polymerization. However, *in situ* polymerization, no reactants are included in the core material. The polymerization of a single monomer directly occurs on the particle surface. Chung et al. prepared microcapsules containing thyme oil by *in situ* polymerization, using melamine–formaldehyde pre-polymer as a wall material, and achieved 78% of loading efficiency of thyme oil in microcapsules using 2% sodium lauryl sulfate as emulsifier.

Polymerization technique is generally a fast and easy scale-up method and provides high encapsulation efficiency. However, polymerization reaction is difficult to control and requires large quantities of organic solvents, and monomers may be non-biodegradable and/or non-biocompatible.

4) CONCLUSION

In this work, a briefly review about the use of EOs and the advantages of its microencapsulation in cosmetic industry was presented. The multimillionaire cosmetic industry continues to invest in research and development of innovative products to satisfy the needs of increasingly demanding consumers for products formulated with natural and nutraceutical ingredients differentiate from competition and add value to the product. The use of EOs as cosmetic ingredients has several advantages, such as enhancing the cosmetic properties and preservation, and marketing image of the final product. In recent years, a significant effort has been made to microencapsulate cosmetic ingredients, because of its ability to conserve and protect the active compounds from degradation and evaporation, as well as its controlled release. Consequently, the microencapsulation of EOs is extremely attractive for formulation cosmetics and personal care products. Although there are many microencapsulation techniques, multidisciplinary cooperation is needed to improve them for its efficient large-scale implementation and take full advantage of this technology. Additionally, it is important to develop a better understanding about the biological activities of microencapsulated EOs for its safety use in cosmetics and the modulation the release of active ingredient.

An attractive application of essential oils and their constituents is in food products to prolong the shelf-life of foods by limiting growth or survival of microorganisms. The organoleptic impact of essential oils and their components in food products currently limits their usage to spicy foods normally associated with herbs, spices, or seasonings. Synergistic interactions should therefore be exploited to lower the organoleptic impact and thereby facilitate the use in a broader range of products. Synergistic blends that have commercial interest must be evaluated under the relevant environmental conditions which reflect the food matrixes.

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