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Review Article

Antibacterial Properties of Nanoemulsions Based on Almond or Lavender Essential Oils

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Abstract

Biphasic dispersions of two liquids, oil in water (O/W) or water droplets in oil (W/O), stabilized by a surfactant are called nanoemulsions. The most effective surfactant is a non-ionic surfactant like Tween 80, which can be used as a solubilizer in different medicinal items, an emulsifier in food, or a surface-active ingredient in soap manufacture and cosmetics. Nanoemulsions consist of droplets that have a diameter ranging from 20 to 200 nm and are thermodynamically stable systems. The composition of the system and the homogenization technique determine the droplet size. Regarding solubilization, drug formulations made with nanoemulsions are more capable than those made with micellar solutions. Essential oils are highly valuable for use in the culinary, cosmetic, and pharmaceutical industries because of their rich chemical composition, which includes fatty acids, proteins, carbohydrates, minerals, and vitamins. Essential oil-based nanoemulsions help to preserve their bioactive components, which are sensitive to light, heat, and oxygen. On the other hand, they improve the dispersibility and stability of these components, guaranteeing their efficacy and purity. Some essential oils have antimicrobial qualities. The chemical makeup of almond and lavender oils as well as their advantageous health benefits is covered in this paper. A thorough analysis of the last five years' worth of research on the creation and description of nanoemulsions is provided.

Keywords: Nanoemulsions; Essential Oils; Almond Oil; Lavender Oil

Introduction

Nanoemulsions

Emulsions are colloidal systems made up of a surfactant, which is required to lessen the interfacial tension between the two phases, and two immiscible liquids [1]. Nanoemulsions (NE) are a particular kind of emulsion that have droplets that are tiny in diameter, often between 20 and 200 nm. Because of their small size, these droplets seem transparent or bluish-transparent. They are therefore ideal for covering fruits and vegetables so that their original texture and colour are preserved [2]. NE can be formed in oil in water (O/W) wherein oil droplets are dispersed in the continuous aqueous phase and water in oil (W/O) structures wherein water droplets are dispersed in the continuous oil phase (Figure 1). Nanoemulsions, in terms of physico-chemical properties, are positioned between microemulsions and traditional emulsions.

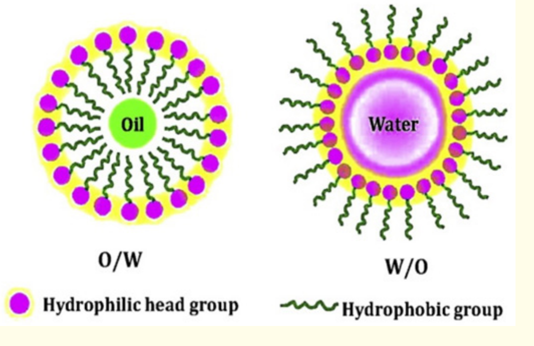

Figure 1: Nanoemulsion structure [3].

Systems made up of surfactant, oil, and water operate as a kinetic barrier, keeping the phases from coming to kinetic equilibrium and causing the droplets to swell in size. The formulation's stability is also determined by the polydispersity index at 24 °C and 4 ºC. The development of instability in nanoemulsions, such as flocculation and coalescence, is evaluated by electrophoresis. Eliminating the charge can weaken the emulsion's stability by lowering the forces that repel nanodroplets from one another, which prevents flocculation and coalescence [4].

The droplet size and polydispersity index of the nanoemulsion depend on the Hydrophilic-Lipophilic Balance (HLB) value, surfactant concentration, and sonication time. The HLB value of the surfactant used can influence the structural composition and stability of nanoemulsions, making it a crucial step in creating a stable nanoemulsion to determine the required HLB value of the essential oil. Kinetic stability can be achieved when the interfacial tension between the water and oil phases is minimized [5].

Using high-frequency ultrasonography, a stable nanoemulsion is created. High-energy emulsification techniques, such as ultrasonic, high-pressure, and high-speed homogenization, are frequently used to prepare nanoemulsions [6]. Nanoemulsions have a long shelf life and are simple to create using little energy, such as heat and mixing [7]. The number of published research and review papers is evidently growing from year to year (Figure 2).

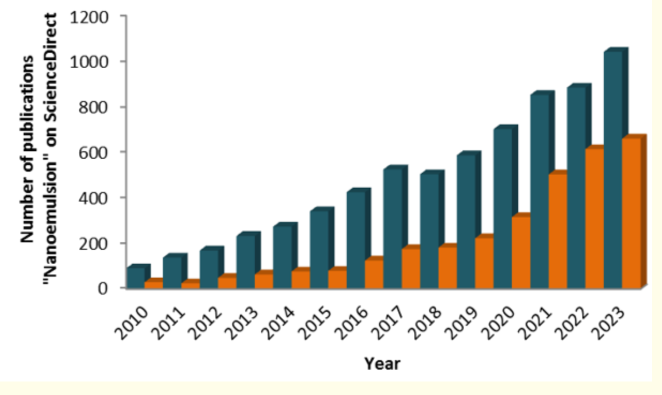

Figure 2: Number of publications "Nanoemulsion" on ScienceDirect for period of 13 years.

Application of nanoemulsions

Lipophilic medications can be made more soluble in water by using nanoemulsions, which increases the drug's bioavailability in the bloodstream. Oral administration of nanoemulsions has demonstrated encouraging outcomes in terms of augmenting medication bioavailability, permeability, and therapeutic activity in addition to boosting drug effectiveness at the target site [8]. Controlling the release rate of antioxidant-loaded nanoemulsions in the human body can typically maximize their therapeutic efficacy. Antioxidant compound release patterns can be predicted using kinetic release models, which also make it easier to pinpoint the critical factors affecting the release rate [9].

Characteristics of essential oils

Terpenoids are the primary constituents of nutmeg essential oil (Myristica fragrans) and lavender essential oil (*Lavandula dentata*), which were first identified in traditional medicine. These are intricate combinations of substances that are light- and oxygen-sensitive as well as having important pharmacological characteristics. They are substances with antibacterial, anti-inflammatory, anticancer, hypoglycemic, anticonvulsant, and insecticidal qualities, yet they are mostly utilized in food and fragrances. Nonetheless, there are a few things to take into account that prevent their use and biological action. Since they have high oil/water distribution coefficients and are hydrophobic, low water solubility frequently restricts production and sustainability. This restriction limits their applicability to hydrophilic goods like makeup. There is currently a lot of research being done on the use of essential oils as a natural antibacterial agent and how well they preserve food. There is also significant customer demand for these products. However, a major disadvantage for their practical application is the loss of bioactivity of essential oils as a result of changing climatic circumstances. When oils are exposed to light, oxidation, and high temperatures, they lose stability [4]. Nanotechnology is used to enhance stability, ensuring quality and effectiveness [10-12]. More specifically, the efficacy and durability of essential oils may be increased by encasing them in a suitable polymer matrix that is both biodegradable and biocompatible. Because they are more easily dispersed than other nanocapsulated systems, nanoemulsions help make essential oils more useful in real-world applications [13].

Mechanism of antibacterial action

The most important mechanism through which essential oils exert their antibacterial effects is by disrupting the structural membrane of microbial cells. According to research, nanoemulsion may be able to bind to protein locations on the cell membrane by penetrating the phospholipid bilayer structure of the membrane. This causes the cell membrane's structure and organization to alter, making it more permeable and allowing vital components to escape. This finally results in cell lysis and death [14].

Mechanism of antifungal action

According to research findings, the volatile chemicals found in essential oils have been found to significantly restrict the growth of fungi. These compounds act both directly on the fungi and indirectly, by absorbing and spreading through the fungi into the growth medium. By interfering with sterol production, the antifungal action mechanism causes damage to the cytoplasmic membrane. Three primary routes govern how essential oil systems based on nanoemulsions improve their interaction with microbial cell membranes. First, improved cooperation with cytoplasmic membranes leads to higher reactivity; this is achieved through passive transport through the outer cell membrane and an increased surface-to-volume ratio. Because more active molecules are able to pass across biological membranes, essential oils that include lipophilic components are more active when droplet size falls. Second, the fusion of emulsifier droplets with the phospholipid bilayer of the cell membrane promotes the targeted release of essential oils at particular locations. Third, the concentration of essential oils at the site of action is increased through electrostatic interaction between charged nanoemulsion droplets and microbial cell walls [15].

Almond oil

Prunus dulcis, or almonds, are among the nuts that are produced most commonly in the globe. They are prized for their flavor, a variety of nutritious components (proteins, fats, polyphenols, and vitamin E), and adaptability to many preparations [16-17]. Fatty acids, proteins, and carbohydrates are abundant in almond oil. Owing to its profusion of vitamins and minerals, it is frequently utilized in medicine for therapeutic purposes. For example, the zinc and B vitamin complex in almond oil helps to maintain healthy skin [18-19].

Lavander oil

The *Lamiaceae* family includes the *genus Lavandula*, which includes a variety of shrubs and plants used in medicine and cooking [20]. Plants and spices that contain essential oils (EO) are valued and have a significant role in the food, cosmetics, and pharmaceutical industries [21]. The use of lavender essential oils in the pharmaceutical industry is significantly limited due to their suboptimal physicochemical characteristics, such as limited water solubility [22]. Monoterpenoids, which are primarily concentrated in the flowers and other higher sections of the plant, are primarily responsible for the pleasant aroma of lavender. Higher amounts of linalyl acetate and linalool and lower concentrations of camphor are found in lavender oils, which are highly prized in cosmetics [21]. Lavender has antibacterial, antifungal, antidepressant, sedative, antioxidant, anti-inflammatory, and spasmolytic effects. These qualities are utilized in traditional medicine to treat a wide range of illnesses. It is well-known for its therapeutic properties, which include easing the symptoms of psychological stress, sleeplessness, and digestive issues - particularly in aromatherapy, as well as acting as an antibacterial. It is known as a therapeutic agent used to alleviate symptoms of psychological stress, insomnia, and digestive disorders, especially in aromatherapy, neuralgia, and as an antiseptic [23-25].

Methods for preparing nanoemulsions

The dilution method of nanoemulsions is one of the techniques for preparing them. It has been discovered that the amount of oil phase present in the base solution is related to the nanoemulsion droplet size [26].

In their study, Badr., *et al*. examined the antibacterial, antioxidant, and stability properties of lavender essential oil, as well as that of its nanoemulsions and constituents, camphene (95%), and α-terpinyl acetate (95%) [15]. In comparison to lavender oil (IC50 = 1828.25 mg/L), which demonstrated stronger and more significant antioxidant activity, α-terpinyl acetate (an oxygenated monoterpene) and camphene (a hydrocarbon monoterpene) demonstrated stronger and more significant antioxidant activity to the lowest IC50 values (647.08 and 868.40 mg/L, respectively). The nanoemulsion of lavender oil shown a noteworthy capability for scavenging (IC50 = 261.66 mg/L), trailed by that of α-terpinyl acetate (IC50 = 374.46 mg/L) and camphene (IC50 = 510.23 mg/L). The antibacterial properties of lavender oil, camphene, α-terpinyl acetate, and their nanoemulsions against Gram-positive *S. aureus* and Gram-negative *S. typhimurium* revealed that, in comparison to lavender oil, α-terpinyl acetate and camphene exhibit exceptional activity against both bacterial strains. Lavender oil demonstrated decreased activity compared to α-terpinyl acetate, which was a more superior active product followed by camphene. All of the compounds are still less active than ceftriaxone. The findings showed that, in comparison to lavender oil, pure camphene and α-terpinyl acetate exhibit greater toxicity. Lavender oil had the lowest effectiveness against *A. flavus* and *A. niger*, whereas α-terpinyl acetate and camphene were the most effective treatments. When compared to pure lavender oil and monoterpenes, all compounds were more effective against both fungus, according to the nanoemulsion data that were obtained.

In their research, Luciano., *et al*. [27] used citral, liquid paraffin, Tween 80, pepsin from pig gastric mucosa, pancreatin from pig pancreas, and pig bile extract to show the impact of digestible versus indigestible citral nanoemulsions on human gut microbes. Different lipid phase compositions were used to generate nanoemulsions: either 100% w/w of citral or 20% w/w of citral combined with 80% w/w of paraffin or maize oil, two types of lipid carriers that can be either digestible or indigestible. A coarse emulsion was then formed by combining 5% w/w of each lipid phase with 1% w/w Tween 80 and water in a mixer running at 6500 rpm for two minutes.

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In the study by Santamaria., *et al*. [2], medium-chain triglycerides were employed along with food-grade carvacrol with a purity > 98%, non-ionic surfactant Tween 80, potassium hydroxide 85%, and oleic acid with a purity of 88% to create nanoemulsions using the low-energy approach. They weigh the desired composition of the surfactant combination (Tween 80 and oleic acid) before adding a blend of oil with varying ratios of medium-chain triglycerides to carvacrol. To achieve the liquid crystal region or microemulsion phase, varying amounts of water with the matching potassium hydroxide concentration are added for each composition and continuously mixed using a vibrating mixer, as per the phase diagram. For the second step of formation, the heated mixture is placed in a test tube heater set at 70º C. The purpose of raising the temperature is to enable the crystalline phase to have a reduced viscosity, which makes it easier for components to combine and mix. The remaining water is added to the heated liquid at a rate of 25º C (dropwise) as the test tube vibrates in the second step of creation. One of the important factors in the creation of nanoemulsions is the adding rate. A nanoemulsion is created by gradually adding the remaining water over a two-minute period. The mixture is agitated for a further minute after the nanoemulsion forms. The emulsions have a final water content of 85% w/w, with a 10% w/w surfactant mixture (oleic-oleate)/Tween 80 and a 5% w/w blend of oil (carvacrol/medium-chain triglycerides). In the carvacrol medium-chain triglycerides/Tween 80-oleic acid-potassium oleate/water system, oil-in-water nanoemulsions are produced using the low-energy phase inversion composition method. 5% w/w is the ultimate concentration of the oil blend. Some formulations, such as nanoemulsions made using the high-energy emulsification and low-energy phase inversion composition procedures, had diameters of 19-30 nm, which were lower than those of formulations previously used to make edible films. To achieve small, stable nanoemulsions, it is necessary for the emulsification pathway to pass through the region where liquid crystals or direct microemulsions without excess oil appear. When water is added, the induced change in the spontaneous curvature of the surfactant causes the trapping of the total oil present in the liquid crystal or microemulsion in droplets, without the need for high shear or other high-energy methods. Although, in the studied range, the average diameter of the nanoemulsion increases with the carvacrol/medium-chain triglyceride ratio, there must be an optimal ratio for low values outside the range because nanoemulsions cannot be formed without carvacrol, indicating that carvacrol aids in packing the surfactant mixture in the droplet interlayer. The emulsification channel must cross the area where liquid crystals or direct microemulsions without extra oil occur in order to produce small, stable nanoemulsions. Without the use of high shear or other high-energy techniques, the addition of water induces a change in the surfactant's spontaneous curvature, which traps all of the oil in the liquid crystal or microemulsion in droplets. Since carvacrol is necessary for the formation of nanoemulsions and because carvacrol helps pack the surfactant mixture in the droplet interlayer, even though the average diameter of the nanoemulsion increases with the carvacrol/medium-chain triglyceride ratio in the studied range, there must be an ideal ratio for low values outside the range. Nanoemulsion formation is dependent on medium-chain triglycerides because they significantly slow down the Ostwald ripening destabilization mechanism. According to the experimental design, the carvacrol/medium-chain triglyceride ratio should be 45/55 for maximum stability [2].

Sadeghian., *et al*. [28] created flaxseed oil nanoemulsions by dissolving Tween 80 (5% w/w) in distilled water and mixing the mixture for 15 minutes. The water phase (95% w/w) and oil phase (5% w/w) were combined and homogenized using a mixer set at 13,000 rpm for five minutes at room temperature to create a coarse emulsion. Clove essential oil (CEO) and pomegranate peel extract (PPE) were introduced separately to the water and oil phases prior to emulsification, with a 10% w/w concentration for each phase. After homogenizing the coarse emulsions under high pressure and ultrasonically sonicating them for ten minutes, nanoemulsions were created. The ultrasound's amplitude and on/off cycle were 80% and 0.7, respectively. An ice bath was used to regulate the temperature rise that occurred during sonication. To guarantee consistent delivery of actual output power, acoustic intensity, and acoustic density to each sample, sonication conditions (such as type of probe or emitter surface, probe location in the middle of the sample at a fixed depth of 1 cm, volume of coarse emulsion, and type of container) were maintained. More oxidative instability was caused by the use of ultrasound emulsification, a higher storage temperature, and the lack of antioxidants. Pomegranate peel extract was not as successful as clove essential oil in reducing the rate at which the flaxseed oil-in-water nanoemulsion (FNE) oxidized. The outcomes demonstrated the potential use of flaxseed oil in water nanoemulsion as a component in the preparation of beneficial foods and drinks.

In two stages, Sharma., *et al*. [29] made the clove oil nanoemulsion. Eugenol, the primary ingredient in essential clove oil, is thought to have some antibacterial properties. Using a high-speed magnetic stirrer, pre-emulsification was used in the first step to create coarse emulsions. The final stable emulsion with nano-sized particles was obtained using ultrasonic treatment in the second step. Clove oil (1–10% w/v) was used as the core material for the inner oil phase (O), and deionized water was used to form the outer aqueous phase (W) by coating it with whey protein concentrate (WPC-70) (0.1-1% w/v). A magnetic stirrer was used to combine the two phases and create coarse emulsions. Clove oil stable O/W nanoemulsions were created by sonicating (on ice) for ten minutes using an ultrasonic apparatus. Using the minimal bactericidal concentration (MBC) and minimal inhibitory concentration (MIC) from the agar well diffusion procedures, the antibacterial activity of the produced nanoemulsion was assessed. Clove oil (1-4%) emulsions showed little antibacterial effect, which may be related to the lower concentration. In order to conduct a more potent antibacterial investigation against E. coli and B. subtilis, the O/W nanoemulsion containing 10% clove oil with 0.5% whey protein concentrate was chosen for this study. The minimal inhibitory concentration of the most stable clove oil nanoemulsion, as depicted in Figure 3, was 50 µl, which was half of the minimal inhibitory concentration of free clove oil (100 µl) against B. subtilis and *E. coli*.

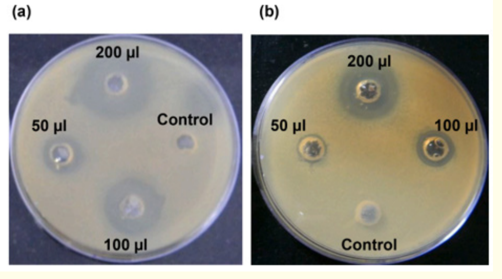

Figure 3: Minimal inhibitory concentration as the zone of inhibition: (a) clove oil (10%) encapsulated in a nanoemulsion prepared using 0.5% whey protein concentrate; and, (b) non-encapsulated free clove oil (10%) in dimethyl sulfoxide (DMSO), against *E. coli* [29].

The nanoemulsion and free clove oil had minimal inhibitory concentration values that were marginally lower than the minimal bactericidal concentrations. When compared to free clove oil, the nanoemulsion demonstrated superior bactericidal properties or a decrease in the number of bacteria against *B. subtilis* and *E. coli*. Clove oil's enhanced hydrophilicity in the aqueous environment and its smaller size as a nano-produced particle within the nanoemulsion may be the cause of this, supporting the oil's potential as an antibacterial agent. It was shown that the clove oil nanoemulsion exhibited greater resistance to *E. coli* as compared to *B. subtilis*. Given that they are Gram-positive and Gram-negative, there may be a difference in the makeup of their cell walls, which could account for this. Because E. coli has a distinct cell wall with a periplasmic gap, antimicrobial drugs cannot easily destroy it. The lipopolysaccharide-enriched membrane of Gram-negative bacteria serves as a direct barrier to a variety of antimicrobial substances, which causes resistance to antibiotic molecules.

Ibrar., *et al*. [30] developed a nanoemulsion of neomycin based on garlic and ginger essential oils to demonstrate their effective action for the rapid treatment of wound healing. In order to create the nanoemulsion, 100 milliliters of neomycin sulphate solution was sonicated for 30 minutes at room temperature with two to three drops of surfactant. Next, drop by drop, 10 milliliters of the essential oil solution was added. In order to prevent the drug and essential oil from degrading, the temperature was kept between 20 and 25 ºC throughout addition. A solution of ascorbic acid at the same concentration was utilized as a control, and nanoemulsion solutions (20 µg/ml) were created by dispersing in ethanol. In comparison to garlic-neomycin sulfate and ginger-neomycin sulphate nanoemulsions, garlic-ginger nanoemulsion combinations and neomycin sulfate shown decreased antibacterial activity. This is explained by the antagonistic effects of the combined composition of ginger and garlic. When compared to neomycin sulphate - 1 (solution), the majority of nanoemulsions showed increased activity, suggesting that they are more effective at inhibiting microbial growth and inflammation. The ointment containing neomycin sulfate-2 did not exhibit a zone of inhibition. A few of the recognized causes of skin infections are E. coli, S. aureus, and Bacillus species. All of these microorganisms have the potential to, in one way or another, result in life-threatening wound infections and even hospitalization of patients with immunodeficiency disorders and hematologic cancers. Due to the comparable dosage of essential oils utilized in the study, nearly all nanoemulsions have a similar antioxidant potential, i.e., < 99%. Antioxidant activity is essential for wound healing, particularly for reducing inflammation.

Black pepper essential oil (BPEO) was utilized by Nie., *et al*. [31] to create a nanoemulsion with Tween 80. Certain antioxidant benefits of black pepper essential oil (BPEO) have been discovered. In addition to acting as a natural antioxidant, BPEO nanoemulsion has antifungal properties against *B. theobromae* and *C. gloeosporioides* as well as bacteriostatic properties against *S. aureus* and *E. coli*. The antibacterial action of BPEO nanoemulsion was enhanced for several bacteria, according to analysis of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). However, for *E. coli* and *S. aureus*, the obtained MIC was the same for both EO and the emulsion/nanoemulsion. The antibacterial efficiency of nanoemulsions produced through microfluidization was notably superior when it came to *E. Coli*. Longer ultrasound exposure raises the system's output temperature, which can be ascribed to high temperatures and the breakdown of volatile chemicals in the presence of essential oils.

Das., *et al*. [32] used ripe bananas, Tween 80, glycerol, acetic acid, chitosan powder, and pure cumin essential oil to illustrate the antibacterial and antibiofilm properties of the nanoemulsion coating made from these ingredients and how it affects the shelf life and quality of bananas. On a hot plate, chitosan and water were combined for ten minutes at 40°C using a magnetic stirrer until the mixture turned transparent. The chitosan solution was then made plastic by adding 0.75 ml/g of glycerol. To get a transparent solution, a 1.5% concentration of cumin essential oil nanoemulsion was added to the chitosan solution and stirred for 30 minutes. Subsequently, 1% (w/v) Tween 80 was added as an emulsifier, and a magnetic stirrer was used to continually agitate the mixture. After that, the emulsion was treated with an ultrasonic probe sonicator running at 300 W and a frequency of 20 kHz for 1800 s in order to reduce the mixture's particle size to the nanoscale. One centimetre above the beaker's bottom was where the probe was placed. The temperature was maintained at 25 °C, and the pulse duration of 2 seconds on and 2 seconds off was appropriately set. The characteristics of nanoemulsions were then ascertained. Only a portion of the produced nanoemulsion's potential to eradicate *S. typhi* and *E. coli* from food surfaces may be inferred from its antibacterial activity against planktonic organisms. These two bacteria have been shown to build biofilms on food surfaces, which may play a significant role in their ability to survive, spoil, and fight antibiotics.

Oil/oil nanoemulsion	Antibacterial activity against *E. coli*		Antibacterial activity against *Staphylococcus Aureus*		Ref.
	MIC (mg mL^{-1})	MBC (mg mL^{-1})	MIC (mg mL^{-1})	MBC (mg mL^{-1})	
Carvone	0.78	1.56	0.78	1.56	[33]
Carum Copticum	75.00	150			[34]
Clove	0.5	1.0	0.25	2	[35]
Origanium vulgare	0.29		0.59		[36]
D-limonene nanoemu	0.336	1.682	0.336	1.682	[37]
Cineole-containing	14.0		14.0		[38]
N. mahanesis EO	1.25	2.27			[39]
Cinnamon bark oil	0.019	0.019	0.039	0.039	[5]
Cinnamon bark oil nano-emulsion	0.009	0.019	0.019	0.019	[5]
Black pepper oil	12.5	25	12.5	25	[31]
Black pepper nanoemulsion	12.5	25	12.5	25	[31]

Table 1: The minimum inhibitory concentration and minimum bactericidal concentration for reference strains of *E. coli* and *S. aureus.*

*MIC: Minimum inhibitory concentration

**MBC: Minimum bactericidal concentration

Conclusion

This paper describes how to prepare essential oil-based nanoemulsions and discusses their antibacterial and antioxidant properties against pathogenic microorganisms that cause disruptions to the structural membrane of microbial cells. Nanoemulsion droplet sizes typically fall between 20 and 200 nm, and because of their small diameter, they appear transparent or bluish-transparent at these sizes. The created nanoemulsions consist mostly of water, oil, co-surfactant, and surfactant. Systems with water, oil, and surfactant constitute a kinetic barrier that keeps phases from coming to kinetic equilibrium, which causes the droplet size to increase. The nonionic surfactant Tween 80, also known as Polysorbate 80, is important to their preparation since it is employed as a mouthwash solubilizer, an emulsifier in food, and a surface-active ingredient in soaps and cosmetics, including eye drops. It is also used in a number of pharmaceutical items. Polysorbate 20, another nonionic surfactant produced by ethoxylation of sorbitan monolaurate, is also utilized in addition to Polysorbate 80. Its stability and relative non-toxicity make it suitable for use in many pharmaceutical, scientific, and household applications as an emulsifier and detergent. The microemulsion dilution method is the most widely used technique for creating nanoemulsions.

Conflict of Interest

The author declares there is no any financial interest or any conflict of interest exists.

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