An-Najah National University
Faculty of Graduate Studies

DEVELOPMENT AND EVALUATION OF THE ANTICANCER, ANTIOXIDANT, AND ANTIMICROBIAL ACTIVITIES OF CYPRESS OIL NANOEMULGEL

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Dedication

To the most loving and kind father in the world who is supporting and encouraging me throughout my life.

To the greatest mother who is carrying my burdens, tiring on me, forgiving my mistakes and standing beside me until became what I am now.

To my supportive husband (Hamza) for being always there for me during my journey.

To the most affectionate sister (Wafaa') who is always there for me with her arms wide open for help, caring and kindness.

To my brothers (Abdul-Rahman, Ahmad and Firas) who are by my side no matter what.

To my children (Abdul-Rahman and Remah) who were the joy during my journey.

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My family, which doesn't have any words to express my love, thanks and gratitude to them for their psychological and moral support throughout the years of my studies and work in my research. I hope you will be proud of me.

Declaration

I, the undersigned, declare that I submitted the thesis entitled:

DEVELOPMENT AND EVALUATION OF THE ANTICANCER, ANTIOXIDANT, AND ANTIMICROBIAL ACTIVITIES OF CYPRESS OIL NANOEMULGEL

Unless otherwise referenced, I declare that the work provided in this thesis is the researcher's work and has not been submitted elsewhere for any other degree or qualification.

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Date: 1/7/2024

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Abstract

Cypress oil is a potent essential oil known for its wide range of therapeutic activities.

This study aimed to incorporate cypress oil into nanoemulgels to overcome their low solubility and high volatility.

Gas chromatography-mass spectrometry identified the chemical composition. The cypress oil nanoemulsion was optimized, and then it was incorporated with Carbopol hydrogel to produce cypress oil nanoemulgel. DPPH scavenger technique measured the cypress oil and its nanoemulgel antioxidant activity. Then, the droplet size, PDI, zeta potential, rheology, antimicrobial, anticancer, and anti-inflammatory activities were evaluated utilizing reference methods.

The chemical composition of cypress oil is predominantly composed of monoterpene hydrocarbons, with α-pinene as the major component (50.72%), followed by 3-b-carene (27.57%). The ternary phase diagram revealed that the nanoemulsion containing 40% Tween, 10% Span, and 50% cypress oil had an optimized droplet size of 105.28 ±2.12 nm and a PDI of 0.112 ±0.016 nm. The cypress nanoemulgel formulation showed no significant change in droplet size or PDI, while it has a zeta potential of -33 mV. Moreover, the antioxidant efficiency of cypress oil was IC_{50} = 14.7 ±0.3 μg/mL, while it was reduced to more than half for nanoemulgel with IC_{50}=6.6 ±0.13 μg/ml. Potent antibacterial activity was reported against several gram-positive and gram-negative bacteria, with inhibition zones in the 11–36 mm range. Also, effective antifungal activity was noticed against different species of *Candida albicans* in the range of 16–24 mm. The formulated nanoemulgel had better activity compared to the oil alone. Furthermore, it was more potent than the oil as an anticancer agent against Hep-G2 cells, with an IC_{50} of 39.81 μg/ml, followed by 58.88 and 61.65 μg/ml for MCF-7 and

HeLa cell line. It also demonstrated an anti-fibrotic effect with an IC_{50} of 63.09 µg/ml against LX-2 cell line. The cypress oil nanoemulgel was more selective for COX-2 than COX-1. In addition, the IC_{50} of the nanoemulgel toward COX-2 (13.96 µg/ml) was almost half the value for the oil (28.78 µg/ml).

The overall findings suggest that cypress oil nanoemulgel holds promise to counteract several skin infections and cancer cell lines. However, further *in vivo* studies are needed.

Keywords: Cypress essential oil; Nanoemulgel; Nanoemulsion; α-pinene.

Chapter One

Introduction and Theoretical Background

1.1 Background

1.1.1 Aromatherapy

The use of traditional medicine has been known since ancient times. According to the World Health Organization (WHO), "the growing interest in traditional medicine is not a return to the past but an innovative vision of overall patient care. Moreover, traditional medicine covers the primary healthcare needs of 80% of the inhabitants of the planet, particularly in certain Asian and African countries" (1). Consequently, the science of using traditional medicines, especially essential oils, is called "aromatherapy" (2, 3). This term was first introduced by French scientist called Gattefose that was working with fragrance. When his hand was burnt, he applied lavender oil on it which was able to reduce the pain and heal the burn with minimal or no scars at all. Subsequently, the use of essential oils in therapy vastly expanded in First World War to treat injured soliders (4).

According to the way that essential oils are used, aromatherapy can be classified into two types: if used orally, it is medical aromatherapy, while if applied topically, it is nursing aromatherapy (3).

1.1.2 Essential Oils

Essential oils were first used by Eygeption for many porposes, including cosmetics, religios ceremony, therapy and preparing mummies. Meanwhile, on the other side of the planet, chiense people used herbal plants and their extracts for medicinal reason which fastly extended to the indian culture. Moving forward in time and after the fall of the eygption empire, greeks learnt different way of using essential oils for medical therapy and they were amazed by their power of healing. Later on, essential oils reached the Arabian culture which were able to develop different ways for extracting them (4).

Essential oils are highly aromatic and can be obtained from the plant's flowers, buds, leaves, roots, bark, wood, rhizomes, twigs, fruit, peels, seeds, and resin (5, 6). Essential oils are extracted mainly by three different methods: distillation, mechanical expression,

and solvent extraction. These methods have a yield that usually ranges from 0.05% to 18% (5). Essential oils can also be found in animals or produced by microorganisms. Plants consider essential oils secondary metabolites because they are not made in intact cells. They contain hydrocarbons, alcohol, aldehydes, ketones, amines, nitrogen, and sulfur compounds (6).

Their use in the pharmaceutical industries has broadened in the twentieth century due to their therapeutic activity, which includes anti-inflammatory, analgesic, antimutagenic, antidiarrheal, antioxidant, anti-microbial, antidiabetic, antipyretic, cytotoxic, apoptotic, and insecticidal activity (6, 7). Moreover, essential oils have been found to be better alternatives to synthetic compounds due to their lack of or insignificant side effects (6, 8). In addition, plant-based products have shown activity against microbial strains that are resistant to the present synthetic drugs (8.).

One of the challenges with essential oils is the chemical diversity in their composition, which differs according to the conditions of the plant, location, harvesting conditions, extraction method, and storage (9). Moreover, essential oils have long carbon chains, which are susceptible to oxidation by light and heat. Also, they have high volatility and low solubility. These reasons limited the direct use of EO in the pharmaceutical industry and led to the incorporation of essential oils with different technologies, including nanotechnology (10).

1.1.3 Nanotechnology

The term “nanotechnology” was first proposed by Richard Feynman in 1959. This technology allowed the observation and manipulation of atoms and molecules on a very small scale. The particles are considered nanoscale when they are in the range of 0-100 nm. Although the cells are around 5000 times bigger than nanoscale particles, they have a complicated system that lets them interact biologically and physiologically with these tiny particles (11, 12).

Nanotechnology is applied in different fields like textiles, agriculture, aerospace, the environment, biotechnology, and pharmaceuticals. Applying nanotechnology to the field of pharmaceutics is called nanomedicine (12). The nanomedicine field is increasingly growing in order to understand drug delivery through different biological barriers and the interactions with biomolecules, cells, and tissues. It has been widely

used for delicate and poorly soluble drugs as a protection and as a drug carrier for poorly targeted drugs. It could also be used to control the release of drugs into the human body (13).

Nanomedicine showed great results as it improved the treatment of different diseases, eased the diagnosis, lowered the cost of effective treatment, and reduced the side effects. The Food and Drug Administration (FDA) has approved more than 50 drugs that are considered nanomedicine (14).

There are many types of nanocarriers, such as quantum dots, liposomes, micelles, dendrimers, nanoemulsions, polymer-drug conjugates, nanoparticles, and other therapeutic materials at the nanoscale. Nanoparticles like gold nanoparticles, silver nanoparticles, magnetic nanoparticles, and mesoporous silica nanoparticles could be bridgeable or inorganic. Nanoparticles are widely used due to their small size, enhancing cell penetration and targeting (12, 15).

Nanoemulsions are one type of applied nanotechnology in pharmaceuticals. They are colloidal systems made of two immiscible liquids, usually oil in water (o/w) or water in oil (w/o), forming nonmetric droplets stabilized by suitable surfactants. Nanoemulsion has more capacity than microemulsion (16, 17).

The primary distinction between microemulsions and nanoemulsions is that the latter are created naturally, whereas the former are made with the help of an energy input. Another significant difference is that the latter has a higher surfactant/co-surfactant content, which results in a smaller droplet size. Moreover, nanoemulsions can be created with great versatility to deliver various drug moieties with various properties. Triglycerides and essential oils are examples of lipids and oils that can be used to construct the oily phase to create nanoemulsions with various physicochemical and biological properties. Modifying the aqueous part by including various water-soluble substances (18).

Nanoemulsions are composed of transparent or translucent nanosized oils that are thermodynamically stable. They are stabilized by surfactant and co-surfactant, which covers the droplets with interfacial coating, forming droplets of a size range of 10-200 nm. Some of the great advantages that nanoemulsions could achieve are high drug

capacity, good stability, rapid digestion, resistance to degradation, controlled release of drugs, and a strong potential to increase bioavailability. The emulsion's nanoscale size increases the bioavailability of certain medications (16, 18).

However, it can be molded into different kinds of dosages, like foams, sprays, liquids, and creams. Different techniques are used to formulate nanoemulsions, like high-pressure homogenization, ultrasonication, microfluidization, and the titrimetric process (16, 18, 19).

In general, parenteral and non-parenteral delivery of substances to the skin or mucous membranes (transdermal) can be accomplished with nanoemulsions, nontoxic and nonirritating systems that have been applied lately in the cosmetic industry. Among the medications that use nanoemulsions for transdermal drug administration are insulin, nimesulide, plasmid DNA, gamma-tocopherol, caffeine, and aspirin (17). In addition, essential oils that demonstrate anticancer properties against breast cancer can be conveniently administered by nanoemulsions; one such oil is spearmint oil (20).

1.1.4 Cypress Oil

Cypress, or *Cupressus,* is one of many essential oils used for medicinal applications since ancient times. The cypress tree belongs to the *Cupressaceae* family, which can be found in warm, temperate climates like the Mediterranean, Asia, and North America. Naturally occurring on the southern Caspian Sea beaches in Iran, Syria, Turkey, Cyprus, Lebanon, Palestine, and a few Greek Islands (Crete, Rhodes, Samos, Kos, Symi, and Melos). According to its original location, there are many cypress trees:

- *Cupressus sempervirens*, native to the Middle East, North Africa, the Greek Islands, and Turkey.
- *Cypress lawsoniana,* which is found in Europe.
- *Cypress macrocarpa* and *Cypress arizonica* are found in the United States.
- *Cypress cashmeriana*, which is the cypress of Kashmir but originated in the Himalayas (21, 22).

All cypresses are employed as defensive, strip, and living fence trees in parks and gardens. They are also quite ornamental, especially in their early years. Also, they are drought-resilient. The Mediterranean cypress has a conical-shaped trunk and can grow

up to 20–30 meters in height. The primary characteristic that sets it apart from other species is the oil glands inside lengthy cavities on the backs of the Mediterranean cypress's dark green scale leaves (21-23).

By using these plants, wind damage to fields can be prevented. Numerous studies indicate that the species can exhibit horizontal and upright growth patterns, leading to its classification into a subspecific taxonomic status. It is widely believed that only the horizontal form existed before human intervention, as the upright form has been noted in horticulture since ancient or prehistoric times (22).

There are many species of cypress trees that vary in color, size, and shape, but generally they are large evergreen trees (Figure 1). Mediterranean, North American, and Asian cypress are the three primary groups. Among them are *Cupressus sempervirens L.*, *Cupressus atlantica Gaussen*, and *Cupressus dupreziana* A. Camus, together known as Mediterranean cypress (21-24).

Conventionally, the essential oil of *Cupressus* is such a great stimulant for the cardiovascular system that it has been used to heal blood problems such as varicose veins and hemorrhoids (21, 23). Moreover, it is used for respiratory problems like coughing (23, 25). It has also been used in cosmetics, hair care products, and deodorant products (21).

All these benefits of *Cupressus* essential oil are due to its phytochemical composition, which includes manoyl oxide, α-cadinol, sabinene, α-pinene, sandaracopimaradiene, two diterpenoids, p-pinene, one oxygenated sesquiterpene, bornyl acetate, mycene, carene, terpinolene, α-terpineol, p-cymene and terpene (21, 23). Consequently, these phytochemicals gave *Cupressus* essential oil its anti-microbial, antifungal, antiseptic, antioxidant, antidiabetic, anticancer, diuretic, and antispasmodic properties (21, 23, 25). Accordingly, these characteristics increase the chance of using this oil in medical products to treat several pathogenic infections.

Figure 1

Particulars of the morphological features of Cupressus species (24)

1.1.5 Antioxidant

Reactive oxygen species production rises and/or antioxidant defenses decline in cells and tissues under oxidative stress. The decline of antioxidant defense is mainly caused by poor diet, while the production of reactive oxygen species like oxygen radicals (O_2) and peroxide (H_2O_2) could be blamed for different reasons. First, increased oxygen concentration in the cell. Second, being exposed to certain toxins. Third, activating a significant phagocyte concentration at a certain location (26, 27).

High concentrations of free radicals can lead to serious problems, such as breaking and modifying DNA strands and highly important proteins and lipids. Consequently, this limits their capacity to function or makes them behave abnormally (26). It also increases the concentration of free intercellular calcium by damaging their transport channels, which leads to the activation of proteases that cleave the cytoskeleton. Moreover, when these radicals come into contact with iron ions in the cell, they create more damaging molecules like hydroxide. Oxidative damage is the term commonly used to describe these processes (27).

Nevertheless, the solution to these problems could be summarized by adding antioxidant particles to our cells, which strengthen their defense system. It can be accomplished by having a healthy diet or by administering antioxidant compounds through drugs. The mechanism of these antioxidants could be scavenging reactive oxygen species, preventing their production (by inhibiting phagocyte activation), attaching to transition metal ions, and blocking the production of ·OH, fixing damage, or any combination of the aforementioned methods (27).

1.1.6 Anticancer

The uncontrolled division of cells in one area of the body can lead to cancer, a condition that gradually spreads to neighboring tissues. There are around 200 distinct forms of cancer, with the most prevalent ones being breast, colon, lung, and prostate cancers (28). Cancer cells can multiply quickly by creating some cellular alterations such as the destruction of growth suppressors, a high nucleus-to-cytoplasm ratio, the prevention of apoptosis, the activation of angiogenesis, metastasis, and invasiveness (29). These changes happen quickly, making them the second cause of death globally. By the end of

2025, it is expected that there will be about 20 million more cases of cancer worldwide (28, 29).

Thus, one of the biggest challenges facing pharmaceutical companies and drug development researchers today is coming up with novel, effective ways to treat cancer. Nevertheless, chemotherapy, radiation therapy, surgery, hormone therapy, bone marrow and stem cell transplants, and a variety of anticancer medications are currently used as cancer treatment options. Many of the effective anticancer and chemotherapeutic medications of the past had their roots in natural substances. In actuality, a large number of well-known anticancer drugs, such as camptothecin, etoposide, paclitaxel, vinblastine, and vincristine, are derived from plants or their semi-synthetic analogs (28).

Numerous studies have shown that over half of medications are derived from natural substances and about 80% of chemotherapy agents are derived from natural sources. Researchers studying cancer have been interested in a number of natural products, including flavonoids, carotenoids, phenolic compounds, and terpenoids, which can be found in fruits, vegetables, spices, teas, herbs, and medicinal plants. These items are readily available, inexpensive, safe, and have minimal toxicity (30, 31).

Common chemotherapy agents have an impact on patients' survival, but they also have dangerous long-term effects and irreversible adverse effects. Additionally, research indicates that a variety of tumors may be resistant to the majority of conventional chemotherapy drugs. Chemotherapy remains, nevertheless, the most widely utilized therapeutic approach for the treatment of cancer. As a result, many researchers are looking for novel chemotherapy drugs with fewer adverse effects. Natural products are crucial for this aim. Using natural compounds to make cancer cells more sensitive to traditional chemotherapy compounds is one of the innovative therapeutic approaches for preventing chemotherapy resistance in cancer cells. Non-toxic natural substances fight cancer's chemoresistance by improving the effectiveness of chemotherapy drugs and lowering their associated side effects (29-31).

Moreover, natural compounds can occasionally be extracted from natural sources at a far lower price than they can be synthesized chemically. The low cost of the extraction procedure for artemisinin has been described as follows: One kilogram of pure artemisinine can be obtained from 250 kg of leaves by extracting 4 to 5 kg of raw

artemisinin. Conversely, approximately one ton of Catharanthus roseus leaves is required to produce 50 grams of crude vincristine sulfate (31).

However, the mechanism of action of several natural origin compounds is found to be working as "poisons" of this enzyme's relegation step. Several anticancer medications cause Top1-DNA lesions, DNA breakage, and, ultimately, cell death. Natural anticancer medications also work by two more typical mechanisms: first, they interfere with DNA structure by forming DNA adducts, as in the case of mitomycin C; second, they block microtubules, as in the case of epiphélones, taxanes (paclitaxel), and vinca alkaloids (vinblastine and vincristine). Disrupting microtubule activity, necessary for cell division, is the primary mode of action of the taxane class of anticancer medications (28, 31).

1.1.7 Antimicrobial

An antimicrobial is any natural, semi-synthetic, or synthetic substance that kills or inhibits the growth of bacteria with minimal or no harm to the host (32). Nevertheless, it's evident from the rising incidence of illnesses resistant to conventional drugs that new antimicrobial drugs are essential to modern medicine. We suggest that the best way to address the growing problem of antibiotic resistance is to generate novel drugs with antimicrobial properties derived from natural products, as combinatorial techniques have not produced viable medications (33).

Several herb and spice extracts and essential oils derived from thyme, oregano, parsley, cilantro, and cinnamon have shown antimicrobial activity *in vitro*. It has been demonstrated that different doses of these edible herb and spice extracts in the culture medium suppress the growth of certain bacterial strains. Alkaloids, phenolics and polyphenols, terpenoids and essential oils, lectins, and polypeptides are some of the phytochemicals groups with antimicrobial properties. Comparing phenolics and polyphenols to other phytochemical classes reveals that they have several antibacterial modes of action. They can combine with nucleophilic amino acids in proteins to produce an irreversible complex that inactivates the amino acids and causes bacteria to stop functioning. It has been observed that phenolic and polyphenols damage microbial membranes, deactivate microbial enzymes, and boost macrophage activity to initiate an immunological response (32, 34).

Several bacterial species are being studied continuously for the development of suitable antibacterial agents against them, such as *Staphylococcus aureus*, Methicillin-resistant *Staphylococcus aureus* (MRSA), *Klebsiella pneumoniae*, *Escherichia coli*, *Proteus mirabilis*, and *Pseudomonas aeruginosa*. However, *Staphylococcus aureus* is the most common cause of bacterial infections in humans worldwide. Also, about thirty percent of all humans carry various Staphylococcus aureus clones and suffer from various illnesses, including skin and soft tissue infections, bacteremia, pleuropulmonary disease, osteoarticular disease, and infective endocarditis. One of the challenges of MRSA, which is a family member of *Staphylococcus aureus*, is that the methicillin-resistant nature of *Staphylococcus aureus* may present a greater risk of nosocomial infections in humans and animals than *Staphylococcus aureus*. In addition, in both human and animal bodies, it can result in complex illnesses linked to the skin's structure. Also, it releases toxins that can lead to respiratory illnesses, urinary tract infections, food poisoning, and bloodstream diseases (35). Consequentially, more than 100,000 deaths per year are directly related to MRSA infections, which have a 64% higher mortality rate than other infections (36).

Klebsiella pneumonia (*K. pneumoniae*) is a gram-negative bacterium commonly found in the mouth, skin, and intestines and in natural habitats. *K. pneumoniae* is an opportunistic pathogen that can infect sick people severely and induce hospital-acquired diseases such as pneumonia, meningitis, septicemia, urinary tract infections, and soft tissue infections (37, 38). The capacity of this species to collect and transmit drug-resistance genes, such as extended-spectrum β-lactamase (ESBL), is widely recognized. Because ESBL-producing K. pneumoniae infections are particularly difficult to treat due to the organisms' developing resistance to numerous antibiotics, which very complicated mechanisms may produce, treatment options are limited (39, 40).

Another common gram-negative bacterium is *Escherichia coli* (*E. coli*), which does not generate spores and is typically motile due to its peritrichous flagella. E. coli most frequently causes urinary tract sepsis and acute urinary tract infections. It has also been linked to abscesses in several organ systems, sepsis, and newborn meningitis. In addition to causing acute enteritis in humans and animals, *E. coli* is also commonly linked to hemorrhagic colitis, also known as "bloody diarrhea" and "traveler's diarrhea," a disease that mimics diarrhea in humans (41, 42). Additionally, *E. Coli* is added to

water bacteriology since it is a helpful indicator of fecal contamination and has consequently gained significance in food and water hygiene (43).

Proteus mirabilis bacteria is present in the feces of numerous species, including humans. It is easily isolated from sewage and contaminated soils and streams. It seldom results in infection in healthy, normal people and is not a major invasive pathogen. Being an opportunist can spread illness when it finds a host that is weak. Urinary tract infections in healthy, normal individuals are rarely caused by it (44). However, another study by Sandra *et al.* reports that urinary tract infections are frequently caused by *Proteus mirabilis* in people with abnormal urinary tracts or indwelling catheter users (45).

Another undesirable human pathogen is *Pseudomonas aeruginosa* (*P. aeruginosa*), a common cause of nosocomial infections affecting individuals with weakened immune systems, tissue damage, and cystic fibrosis (46). It can easily develop resistance to numerous intravenous medications and is naturally resistant to the majority of oral medicines. Reduced permeability of cell membranes, elevated expression of efflux systems, the synthesis of enzymes that render antibiotics inactive, and alterations to the targets of antibiotics are among the resistance mechanisms. Antibiotic resistance contributes to the emergence of multi-drug resistant P. aeruginosa strains worldwide and causes a rise in treatment failures (47).

Candida albicans is categorized as an opportunistic fungus since it often only infects people who are immunodeficient or whose natural flora has been disrupted. Candida species are fungi that resemble yeast, with Candida albicans being the most common pathogen among them. Candida albicans is characterized by lesions that appear as white spots on the skin or mucous membranes. *Candida glabrata*, *Candida guilliermondii*, *Candida krusei*, *Candida parapsilosis*, and *Candida tropicalis* are some of the non-albicans species in this genus that are known to cause illness (48). These species have emerged as a result of the extensive use of fluconazole prophylaxis in HIV-positive patients, which are fluconazole-resistant strains, and they are becoming more common, particularly in individuals with late-stage AIDS (49). Diseases ranging from superficial mucosal infections to multiple catastrophic systemic infections, such as invasive candidiasis, a hospital-acquired fatal illness caused by many species of Candida, can be induced by specific abnormalities in their normal microenvironment. Between 40% and

60% of the patients who have this infection will die, and their morbidity rate will also be significant (50). Different Microbial species and linked diseases are clarified in Table 1 below.

Table 1

Different micrboial species and the linked dieases to them

Microbial species	Linked diseases
Staphylococcus aureus	• Skin and soft tissue infections • Bacteremia • Pleuropulmonary disease • Osteoarticular disease • Infective endocarditis
Klebsiella pneumonia	• Pneumonia • Meningitis • Septicemia • Urinary tract infections • Soft tissue infections
Escherichia coli (*E. coli*)	• Acute urinary tract infections • Hemorrhagic colitis, also known as "bloody diarrhea"
Proteus mirabilis	• Urinary tract infections
Pseudomonas aeruginosa	• Nosocomial infections
Candida albicans	• White spots on the skin or mucous membranes.

1.1.8 Anti-inflammatory

The primary enzyme in prostaglandin manufacture, prostaglandin-endoperoxide synthase (PTGS), sometimes referred to as cyclooxygenase (COX), functions as a peroxidase as well as a dioxygenase. There are two isozymes of COX: COX-2, which can be activated and mediates the inflammatory response, and COX-1, which is constitutively active. Since both COX isoforms are inhibited by aspirin and other nonsteroidal anti-inflammatory medications (NSAIDs), prostaglandin synthesis and inflammation are either blocked or both blocked. Gastric distress can result from COX-1 inhibition; however, selective COX-2 inhibitors, like celecoxib, have been developed

for analgesia without causing the same problem. However, COX-1 is assumed to be engaged in tissue homeostasis and cell-cell signaling (51, 52).

Plants and herbs are another source of anti-inflammatory agents other than drugs. In the case of arthritis and other inflammatory conditions, lipid peroxides are essential. Turmeric is the first recognized anti-inflammatory medication in India's traditional medical system. Numerous animal studies have demonstrated the anti-inflammatory properties of turmeric extract, curcuminoids, and volatile oils. The anti-inflammatory properties of the spice ingredients curcumin (found in turmeric), capsaicin (found in red pepper), and eugenol (found in cloves) have been shown in both *in vitro* and *in vivo* animal experiments. Curcumin's anti-inflammatory impact was shown to be on par with phenylbutazone in individuals who had undergone hernia surgery. When curcumin was administered to rheumatoid arthritis patients, there was a noticeable improvement. Capsaicin, as a pain reliever, has drawn a lot of interest. Topical cream administration in patients with rheumatoid arthritis and osteoarthritis (34).

1.2 Literature Review

The previously reported studies have shown the therapeutic effects of different essential oils, such as lavender oil, for alleviating sleeping disorders (53). Also, ginger oil improved the symptoms of nausea and vomiting (9). Moreover, tea tree oil using only 10% concentration has been successfully used against hemorrhoids (54). Another recent study showed a good effect on treating bacterial overgrowth in acne by adding essential oils to the clay-yoghurt mixture, like *Lavendula angutifolia* and *Salvia sclarea* (55).

Cypress oil has also been shown to be an effective healing tool. A recent study stated that cypress oil with a combination of other essential oils could alleviate different respiratory problems with significant antimicrobial effects, reduced toxicity, and improved anti-inflammatory results (25). Moreover, cypress oil has shown effective results as an eco-friendly insecticide against Culex pipiens (L.) larvae when prepared in nanoemulsion (56). Another study ensured that cypress essential oil is a good insecticide against flies, whose lifespan decreased when tested against it (57). On the other hand, cypress oil showed significant results as an antifungal when tested against ten different crop fungi (58).

According to the previous studies and the strong properties of essential oils, there is a growing interest in using them simply and cost-effectively to be suitable for developing new types of bactericidal plant-based nanosystems, such as cypress essential oil nanoemulsions for targeting various skin conditions (25, 59). Incorporating it into the nanoemulgel helps to get the most benefit from the superior properties of cypress oil, including antioxidant, antimicrobial, anticancer, and anti-inflammatory activities.

1.3 Significant of study

The medicinal application of EO is limited due to its high volatility, poor water solubility, and thermal instability (60). To solve this, it is proposed that EOs be incorporated into nanoemulgel. In the nanoemulsion, the EO can be entrapped in the nanoparticle matrix that works as a nanocarrier, enhancing time, targeting protection, and reducing toxicity (10). Besides, nanoemulgels have high physical stability, high bioavailability, low turbidity, and controlled release (61).

It is reported that many researchers have integrated EO into nanoemulgels to enhance their properties, like cinnamon EO nanoemulgel, which has shown great stability and other advantages over cinnamon EO alone (62). Zhang had also proved that *Monarda didyma* EO nanoemulgel did improve antimicrobial activity and decrease the rottenness of blueberries when used as a preservative over EO by itself (60). Additionally, when *zanthoxylum bungeanum* EO was incorporated in nanoemulgel, it was more stable with significant antioxidant and antibacterial activity, which made it possible to use it as a natural antioxidant, preservative, and treatment for hyperlipidemia (61). Therefore, nanoemulgels of EOs are an effective means to improve their activity and stability.

1.4 Aim of the Study

The main objective of this master's thesis was to develop a nanoemulgel composed of cypress essential oil that can effectively target a range of microbial skin conditions. Additionally, to evaluate the potential biological activities of this nanoemulgel will be examined in order to identify any such properties.

1.5 Objectives

1. To prepare and evaluate cypress oil nanoemulsion to optimize its particle size, PDI, and composition.
2. To prepare and evaluate cypress oil nanoemulgel.
3. To evaluate the antimicrobial activity of the cypress oil and its nanoemulgel.
4. To evaluate the anticancer activity of the cypress oil and its nanoemulgel.
5. To evaluate the ant-inflammatory activity of the cypress oil and its nanoemulgel.

Chapter Two

Materials and Methodology

2.1 Materials

Different manufacturers supplied the reagents and chemicals. The cypress essential oil was purchased from A2Z Beauty company, Ramallah, Palestine. Which was extracted from the leaves and the twigs of *Cupressus sempervirens* trees in Palestine. The Ultraviolet-visible (UV-Vis) spectra were recorded on a UV/Vis (JENWAY) using quartz cuvettes for antioxidant calculations. Carboxyvinyl polymer (Carbopol 940), Polysorbate 80 (Tween 80), Sorbitan oleate (Span 80) were obtained from CBC Co., Ltd., Japan. The culture media included dimethyl sulfoxide (DMSO), obtained from Riedel De Haen, Germany, and Mueller Hinton agar (produced by Becton, Dickinson, and Sparks Co. in France).

2.2 Methodology

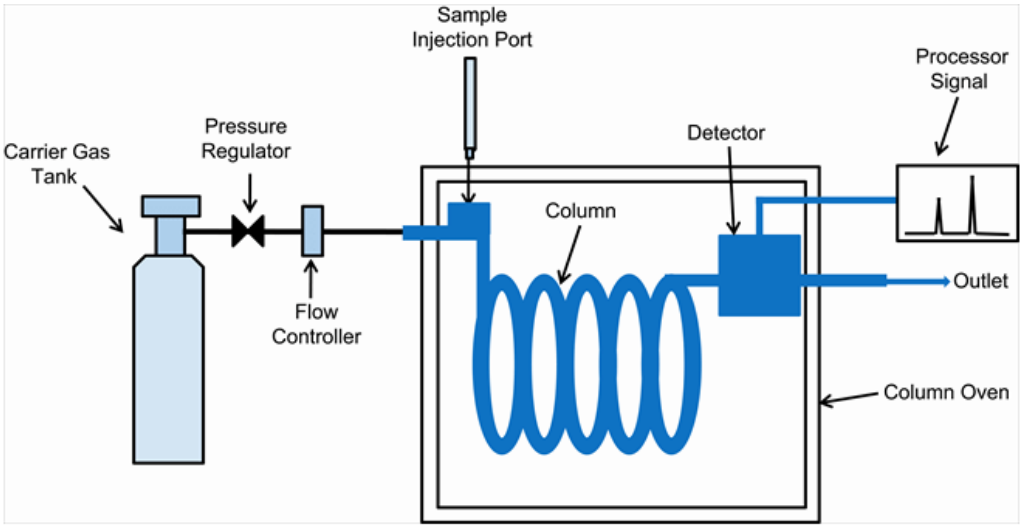
2.2.1 Gas Chromatography-mass spectrometry assessment of cypress essential oil (GC-MS)

The GC-MS approach was employed to determine the components of cypress essential oil. The analysis was conducted using a Perkin Elmer Clarus 500 gas chromatograph coupled with a Perkin Elmer Clarus 560 mass spectrometer illustrated in Scheme 1. A SLBTM-5ms fused-silica capillary column with dimensions of 30 m x 0.25 mm and a film thickness of 0.25 µm was employed for the separation process. The column temperature was programmed to increase by 4 °C each minute, starting at 50 °C and lasting for 5 minutes until reaching 280 °C.

Throughout the whole chromatographic run, helium was employed as a carrier gas at a consistent flow rate of 1 mL/min. At a temperature of 250 °C, a volume of 1 µl of the extraction being evaluated, dissolved in acetonitrile, was injected using a split mode with a split ratio of 1:50. The reference spectra of the chemical components of the essential oils were compared to the mass spectra of the National Institute of Standards and Technology's MS (NIST) Data Center. Additionally, the Kovats and retention indices of the essential oils were compared to the values provided in the literature. The Kovats Retention Index (KRI) was determined by utilizing the retention time measurement obtained from the hydrocarbon alkane standard (63).

Scheme 1

Schematic diagram of gas chromatography mass spectrometry



2.2.2 Preparation of cypress oil nanoemulsion

The cypress oil nanoemulsion was developed by combining various amounts of surfactant and co-surfactant (Tween 80 and Span 80) with cypress oil as shown in Table 2. Each formulation consisted of varying amounts of these three components, and several formulations were conducted to create a ternary phase diagram. Subsequently, a vortex apparatus was employed to thoroughly blend the components for a minimum duration of 3 minutes. Scheme 2 below illustrates the steps of formulating nanoemulsion. The optimal formulation was determined by reaching a consensus on the values of both droplet size and polydispersity indexes (PDI). Prior to assessing the droplet size, polydispersity index (PDI), and physical form, all formulations were emulsified in distilled water using moderate agitation (64).

Scheme 2

Steps of formulating nanoemulsion

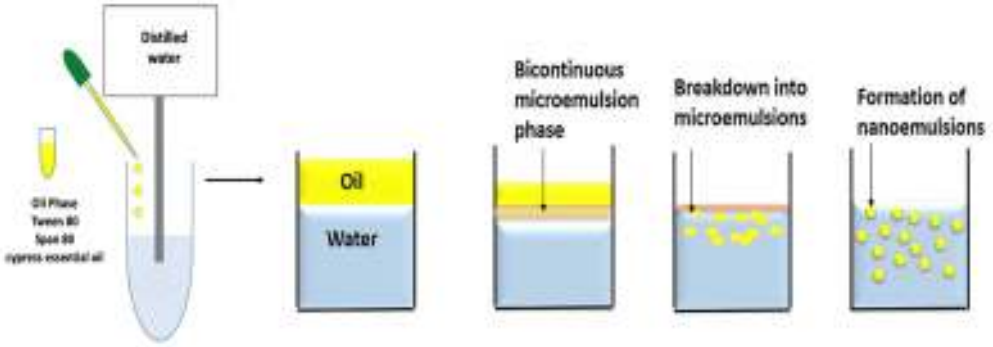


Table 2

Composition of differennt formulatiosn of nanoemulsion

	No.	tween 80 (mg)	span 80 (mg)	oil (mg)		No.	tween 80 (mg)	span 80 (mg)	oil (mg)
190	1	80	720	200	550	1	400	400	200
	2	64	576	360		2	320	320	360
	3	51.2	460.8	488		3	256	256	488
	4	40.96	368.6	590.4		4	204.8	204.8	590.4
	5	32.77	294.9	672.3		5	163.8	163.8	672.3
280	1	160	640	200	640	1	480	320	200
	2	128	512	360		2	384	256	360
	3	102.4	409.6	488		3	307.2	204	488
	4	819.2	327.7	590.4		4	245.8	163.8	590.4
	5	655.4	262.1	672.3		5	196.6	131.1	672.3
370	1	240	560	200	730	1	560	240	200
	2	192	448	360		2	448	192	360
	3	153.6	358.4	488		3	358.4	153.6	488
	4	122.9	286.7	590.4		4	286.7	122.9	590.4
	5	983	229.4	672.3		5	229.4	983	672.3
460	1	320	480	200	820	1	640	160	200
	2	256	384	360		2	512	128	360
	3	204	307.2	488		3	409.6	102.4	488
	4	163.8	245.8	590.4		4	327.7	819.2	590.4
	5	131.1	196.6	672.3		5	262.1	655.4	672.3

	No.	tween 80 (mg)	span 80 (mg)	oil (mg)
910	1	720	80	200
	2	576	64	360
	3	460.8	51.2	488
	5	294.9	32.77	672.3

2.2.3 Analysis of Droplet size and PDI for cypress oil prepared nanoemulsion

In this investigation, a master size analyzer (Brookhaven Instruments, Nano Brook Omni, New York) was utilized to measure the droplet size and PDI in the lab. Prior to conducting triplicate measurements, it is necessary to successfully perform the self-emulsifying process of essential oil nanoemulsion (64). The trials were conducted at a temperature of around 25°C, which is the standard room temperature. The results are shown as the average value plus or minus the standard deviation, based on a minimum of three measurements.

2.2.4 The selection of cypress oil nanoemulsion

The selection of the optimum nanoemulsion was based on the maximum amount of cypress essential oil used in the formulation and the minimum droplet size and PDI.

2.2.5 Formulation of cypress oil nanoemulgel

The cypress oil nanoemulgel formulations were prepared by incorporating Carbopol 940 at various concentrations (0.4%, 0.6%, 0.8%, and 1%) into the optimized nanoemulsion formulation. These exact compositions of the four formulations are shown in Table 3. The homogenization process was carried out meticulously for each formula until it precisely matched the target formula. Scheme 3 below shows the process of nanoemulgel formulation. Subsequently, the polydispersity, droplet size, and zeta potential were quantified (64).

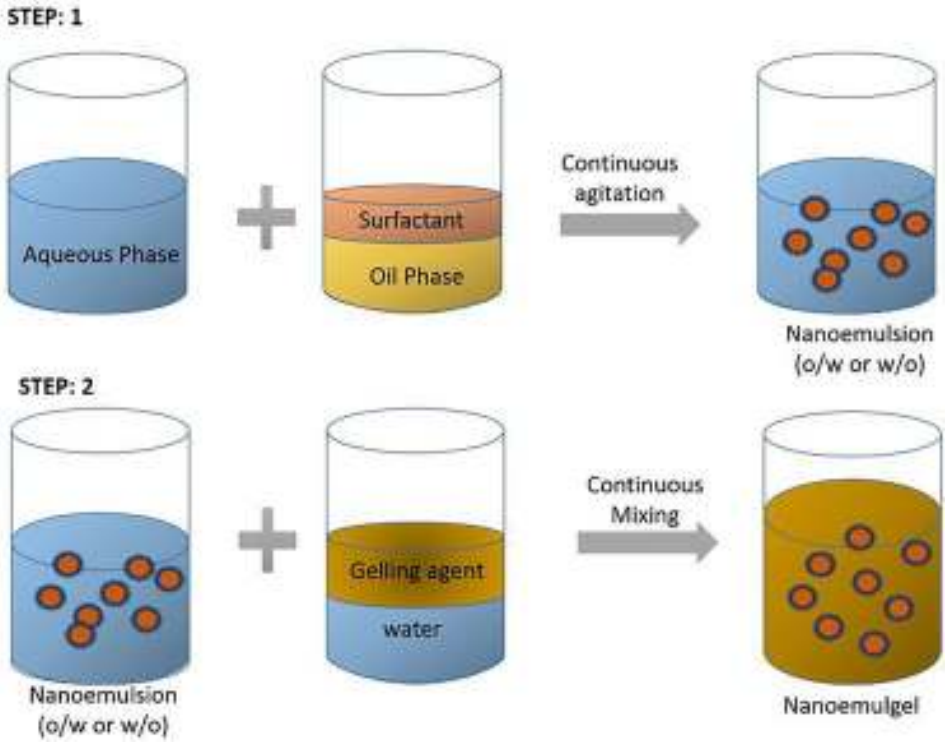
Table 3

Composition of four prepared formultions of cypress oil nanoemulgel

Carbopol Concentration	Cypress Oil	Tween 80	Span 80	Water	Carbopol
0.4%	2	1.6	0.4	14	2
0.6%	2	1.6	0.4	13	3
0.8%	2	1.6	0.4	12	4
1%	2	1.6	0.4	11	5

Scheme 3

The formulation of nanoemulsion and nanoemulgel



2.2.6 Physical characterization of cypress oil nanoemulgel formulation

Consistency, spreadability, homogeneity, visual appearance, and phase separation were tested visually during and after the preparation of the cypress oil nanoemulgel.

2.2.7 Rheological measurement of cypress oil nanoemulgel formulations

An investigation was conducted to assess the rheological properties of various cypress oil nanoemulgel formulations containing Carbopol 940 at concentrations of 0.4%, 0.6%, 0.8%, and 1%. A 7s-size spindle viscometer (Brookfield DVI, USA) was utilized to evaluate the test at a 25 °C temperature and a shear rate range of 0–100 rpm (65).

2.2.8 Assessment of cypress essential oil and its nanoemulgel antioxidant activity

The antioxidant activity of cypress essential oil and its nanoemulgel were assessed using the free 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging technique. A concentration of 100 µg/mL of essential oil stock solution was added to methanol. A solution of trolox, a standard reference, was prepared with a concentration of 100 µg/mL. The experiments were continued by placing a sequence of diluted solutions, derived from the stock solutions. The serial dilutions included concentrations of 2, 5, 7,

10, 20, 30, 50, and 80 µg/mL. A volume of approximately one milliliter of the current samples was combined with a solution of DPPH dissolved in methanol, with a concentration of 2 milligrams per milliliter. In addition, 1 mL of methanol was added to achieve a total working capacity of 3 mL. Following the aforementioned processes, the DPPH solution was produced again because to its high susceptibility to light. In order to prepare the blank control, DPPH was dissolved in methanol at a ratio of 1:2, without include the tested material. Simultaneously, fully functional solutions were cultivated at room temperature (22–25 ºC) in a dark laboratory for approximately 30 minutes. The absorbance was measured using a UV–Vis spectrophotometer (Shimadzu-UV-1800, Kyoto, Japan) at a specific wavelength of 517 nm. The equation shown below was utilized to compute the percentage of DPPH inhibition for cypress oil, using trolox as the reference chemical:

$$\text{DPPH inhibition } \% = \frac{(\text{AB} - \text{AS})}{\text{AB}} X\ 100\% \quad \text{.....(1)}$$

Where, AB is the absorbance measured for the prepared blank solution and AS is the absorbance measured of the prepared sample of cypress essential oil (66).

2.2.9 Antimicrobial assessment for cypress oil and its nanoemulgel

The antibacterial activity was assessed using the agar disk diffusion technique against six bacterial strains:

- MRSA
- *Klebsiella pneumoniae*
- *Escherichia coli*
- *Staphylococcus aureus*
- *Proteus mirabilis*
- *Pseudomonas aeruginosa*.

In addition, the antifungal activity of the substance was assessed against six strains of Candida, including:

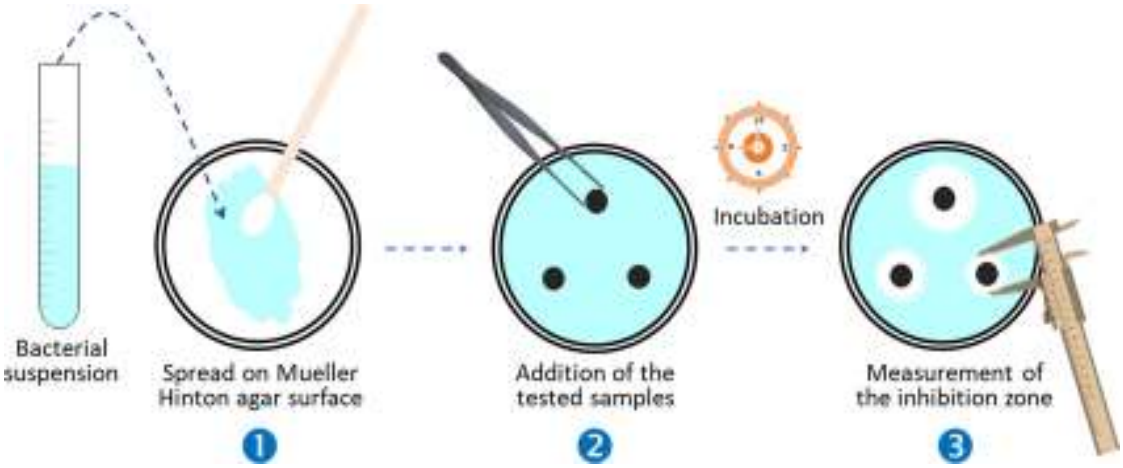
- *Candida albicans* (ATCC)
- *Candida parapsilosis* (151)
- *Candida tropicalis* (168)

- *Candida tropicalis* (260)
- *Candida albicans* (202)
- *Candida albicans* (209)

The methodology involved the process of plate punching, which entailed the placement of agar in two holes (A and B), each with a diameter of 6 mm. Cypress oil was inserted into hole A, whereas hole B contained a nanoemulgel containing cypress oil. The plates underwent a 24-hour incubation at 37 °C for antibacterial testing. The plates were incubated overnight at about 25 °C to evaluate the fungicidal efficacy. The width of the inhibitory zone played a crucial role in determining the antibacterial and antifungal activities. This technique is based on the dispersion of the essential oil onto the surface of a nutritious gelatin medium that has been infected. The size of the zone of inhibition (after incubation) is determined by the concentration, effectiveness, or ineffectiveness of the essential oil and nanoemulgel. Antibacterial assays were conducted on recently formed cultures (18-24 hours old) at a period of rapid development (67-69). The whole methodology is shown in Scheme 4 below.

Scheme 4

Illustration of disck diffusion method for antimibrobial activity assesment



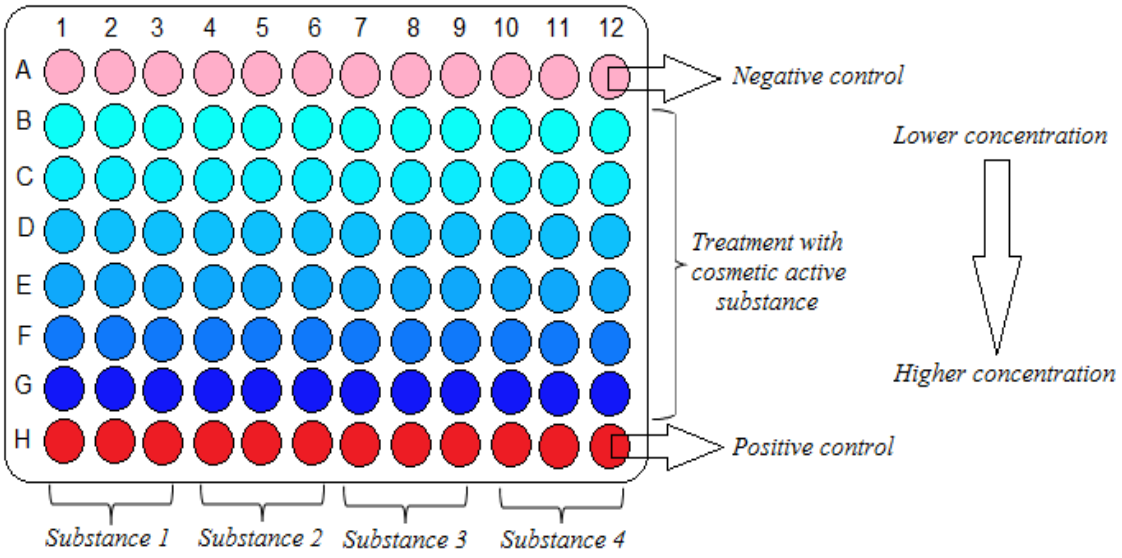
2.2.10 Anticancer assessment for cypress oil and its nanoemulgel

The anticancer assay was conducted on four different cell lines: HeLa (cervical cancer), Hep-G2 (hepatocellular carcinoma), LX-2 (hepatic stellate), and MCF-7 (breast cancer). The purpose was to determine the cytotoxicity of their IC_{50} values (the concentration required to inhibit 50% of cell growth) at different concentrations (62.5, 125, 250, 500, and 1000 µg/ml) compared to a positive control doxorubicin (DOX). The cells were

placed into 96-well plates with their respective culture medium. Approximately 1×10^3 cells were added to each well, with a volume of 100 µL per well. This was done in triplicate. The plates were then incubated for a period of 24 hours. Subsequently, the existing culture media were substituted with new, matching culture media that included different quantities of the extract. The mixture was then incubated for a duration of 72 hours. Next, the inhibitory impact of the essential oil and its nanoemulgel on cell proliferation was evaluated using the CellTilter 96® Aqueous One Solution Cell Proliferation (MTS) Assay, following the directions provided by the manufacturer (Promega Corporation, Madison, WI). Subsequently, 20 µL of MTS solution/100 µL was added to each well and incubated at 37 °C for about 2 hours. Absorbance was measured at a wavelength of 490 nm (63). The process of anticancer assessment is illustrated in Scheme 5 below.

Scheme 5

Illustration of the anticancer assesment of cypress oil nanoemulgel



2.2.11 Anti-inflammatory assessment for cypress oil and its nanoemulgel

The ability of cypress oil and its nanoemulgel to inhibit the formation of prostaglandin H2 (PGH2) from arachidonic acid (AA) by bovine COX-1 and human recombinant COX-2 was further analyzed using a screening assay kit (Item No. 460104) developed by Cayman Chemical Manufacturer (USA). This kit was utilized as a COX inhibitor. First, a standard curve was constructed using eight different doses of prostaglandin, along with a non-specific binding sample and a maximal binding sample as a reference

curve. Then, two concentrations (50 and 300 µg/mL) of cypress oil and its nanoemulgel were tested three times. Subsequently, calculations were made to determine the extent of inhibition exhibited by the sample plant using a multiple regression best-fit line. Thus, the 50% inhibitory concentration (IC_{50}) could be estimated at both the maximum and minimum concentrations. Then the results were compared with the inhibition concentration of positive control (celecoxib) (65).

2.2.12 Statistical analysis

The droplet size, PDI, antioxidant and anticancer and anti-inflammatory IC_{50} values were determined in triplicate for Cypress EO and its nanoemulgel. The obtained values were stated as means ± standard deviation (SD) and the attained results were compared using ANOVA. All the data were considered statistically significant when the *p* value was <0.05.

Chapter Three

Results

3.1 GC-MS analysis

Cypress oil analysis on GC-MS was carried out, resulting in the chromatogram in Figure 2 below. Further qualitative analysis of the chromatogram indicated that the oil consisted of at least 28 compounds listed in Table 4. Mainly, cypress essential oil is made of α-pinene (50.72%) and 3-b-carene (27.57%), which are monoterpene. Some other monoterpenes are present at more than 2%, such as limonene (2.99%) and α-terpinyl acetate (2.42%). Studies have shown that monoterpenes have excellent bioactive potential. Monoterpenoids like isoterpinolene (2.59%) are also present in this oil. Structure of the main components of cypress essential oil are listed in Table 5.

Figure 2

GC-MS chromatogram of 28 separated constituents from essential oil obtained from Cypress oil

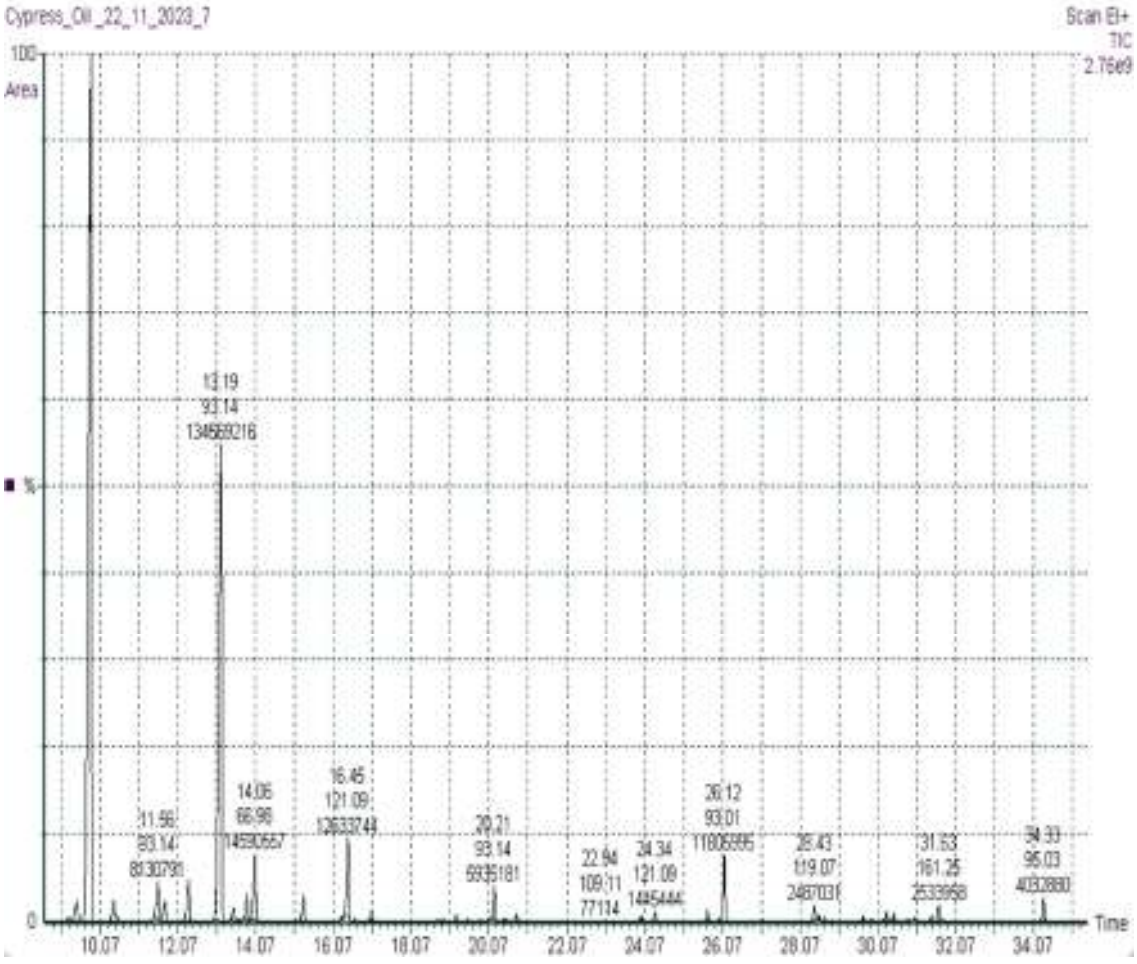


Table 4

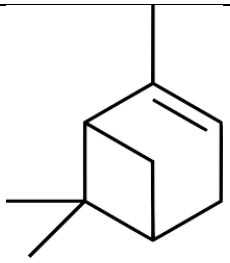
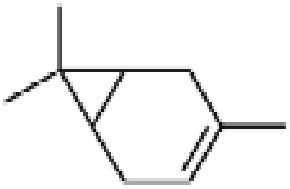
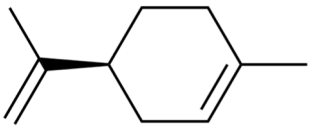
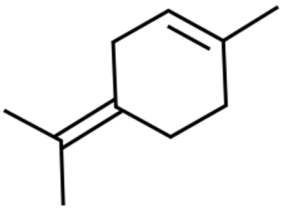
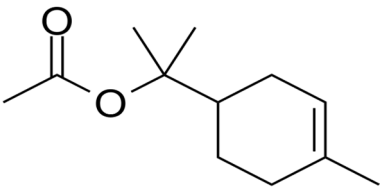
Chemical composition of cypress oil

Name	R.T	Area	% Area	KRI	L KRI
α-Pinene	9.84	247622160	50.72	931.9	932
3-b-Carene	13.19	134569216	27.57	1006.4	1006
Limonene	14.06	14590557	2.99	1026.8	1026
Terpinolene	16.45	12633744	2.59	1083.1	1083
α-Terpinyl acetate	26.12	11805995	2.42	1343.7	1343
Myrcene	12.35	8788412	1.80	987.4	987
Sabinene	11.56	8130791	1.67	969.9	970
Terpinen-4-ol	20.21	5935181	1.22	1171.5	1172
α-Fenchene	10.43	5214822	1.07	944.9	945
γ-Terpinene	15.3	5057238	1.04	1056.0	1056
p-cymene	13.84	4391783	0.90	1021.6	1022
β-Pinene	11.74	4247756	0.87	973.9	974
Epicedrol	34.33	4032880	0.83	1608.8	1608
δ-Cadinene	31.63	2533958	0.52	1517.3	1516
α-Cedrene	28.43	2487031	0.51	1413.8	1413
α-Terpinene	13.51	2235568	0.46	1013.9	1014
Linalool	17.06	1764148	0.36	1097.4	1097
Linalool propanoate	25.68	1659403	0.34	1330.5	1330
α-Thujene	9.45	1610123	0.33	923.2	923
3-Thujanol acetate	24.34	1445444	0.30	1291.1	1291
Germacrene D	30.49	1352776	0.28	1479.8	1479
γ-Muurolene	30.3	1204627	0.25	1473.7	1473
α-Terpinol	20.77	1181655	0.24	1184.7	1185
Camphor	19.23	870228	0.18	1148.5	1149
Bornyl acetate	23.99	800999	0.16	1281.3	1281
α-Humulene	29.69	771100	0.16	1454.2	1454
β-Caryophyllene	28.56	632216	0.13	1417.9	1418
β-Cedrene	28.69	602981	0.12	1422.1	1422
Sum		488172792	100		

Note. RT: Retention time, KRI: Kovats Retention Index, L KRI: Literature Kovats Retention Index.

Table 5

Structures of main components of Cypress essential oil

Compound	Structure
α-Pinene	
3-b-Carene	
Limonene	
Terpinolene	
α-Terpinyl acetate	

3.2 Droplet size and PDI for cypress oil prepared nanoemulsion

Several formulations were prepared using different concentrations of the three components: cypress oil, Tween 80 (the surfactant), and Span 80 (the co-surfactant). After determining the polydispersity index (PDI) and droplet size for all prepared cypress oil nanoemulsion formulations, we constructed a ternary phase diagram, as illustrated in Figure 3. The diagram facilitated the determination of the best formulation, which has the following characteristics, PDI less than 0.3 and droplet size less than 200 nm.

Remarkably, all formulated cypress oil nanoemulsion had a relatively small droplet size in the range of 191.13–105.28 nm. Furthermore, the polydispersity index (PDI) was below 0.299 for all formulations, indicating homogeneity of droplet size within the nanoemulsion due to the high-energy emulsification method. Accordingly, the optimum formulation that meets the specific previously mentioned properties is formulation number 6 from Table 6, it is also clarified in the pseudo ternary phase diagram by a red dot in Figure 3, which contained 40% Tween, 10% Span, and 50% cypress oil. Formulation 6 has a PDI of 0.112 ±0.016 nm and a droplet size of 105.28 ±2.12 nm.

Figure 3

Ternary diagram for several formulations containing Tween 80, Span 80 and cypress oil in different concentraions. Red circile pointing to the optimum formulation

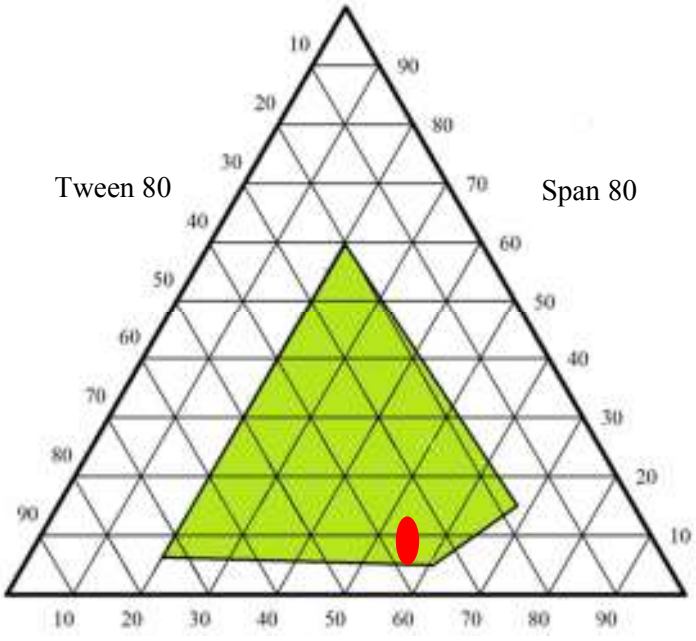


Table 6

Droplet size and polydipersity index (PDI) for different cypress oil nanoemulsion formulations labeling the optimum formulation in grey

No.	Tween 80	Span 80	Oil	Size (nm ± SD)	Polydispersity Index (PDI ± SD)
1	20	13	67	159.47 ±2.34	0.209 ±0.023
2	35	15	50	166.78 ±3.78	0.237 ±0.031
3	23	10	67	191.13 ±1.64	0.236 ±0.027
4	64	16	20	172.65 ±4.21	0.299 ±0.011
5	51	13	36	150.62 ±2.77	0.130 ±0.031
6	40	10	50	105.28 ±2.12	0.112 ±0.016
7	72	8	20	108.4 ±3.56	0.194 ±0.042

3.3 Physical characterization of cypress oil nanoemulgel

After choosing formulation six that meets the optimum properties of PDI and droplet size, Carbopol 940 was added to form the nanoemulgel. Four formulations were made containing Carbopol in different concentrations (0.4%, 0.6%, 0.8%, and 1%). Then they were compared and further analyzed according to their PDI, droplet size, and zeta potential to choose the best nanoemulgel formulation.

The nanoemulgel with a Carbopol concentration of 0.4% *w/w* was selected as an optimized formulation due to its small droplet size, low PDI, and high stability (zeta potential). The optimized gel formulation had a smooth, uniform appearance and was free of roughness. As shown in Figure 4 (A)**,** the droplet size of the nanoemulsion is compared with the four nanoemulgels. The chosen nanoemulsion's mean droplet size is 105.28 ±2.12 nm. Conversely, the droplet size is slightly higher, ranging from 107 to 113 nm for the nanoemulgel. Notably, the droplets with the lowest size is the one containing the lowest amount of Carbopol, only 0.4%. This indicates that the droplet size showed the slightest change (from 105 to 107 nm), 0.4%, compared to the other formulations**.** Figure 4 (B) compares the change in PDI between the four formulations, which ranged from 0.113 to 0.121, which was 0.112 for the nanoemulsion. It emphasizes the previous results because the lowest PDI is also for the formulation that contains 0.4% Carbopol.

The zeta potential of these formulations was tested, as illustrated in Figure 5**.** Overall, zeta potential values ranged from -33 to -39 mV. However, the zeta potential of the chosen nanoemulgel (-33 mV) was slightly affected by the addition of Carbopol in the formulation containing 0.4% of it (-37 mV), but the effect was much higher when the Carbopol amount was more than 1% (-39 mV), which underlines the previous results. Generally, there is no significant change in the droplet size, PDI, or zeta potential when Carbopol is incorporated.

Figure 4

(A) the droplet size of cypress oil nanoemulsion and its nanoemulgel containing 0.4, 0.6, 0.8, and 1% Carbopol. (B) PDI of the same formations in A

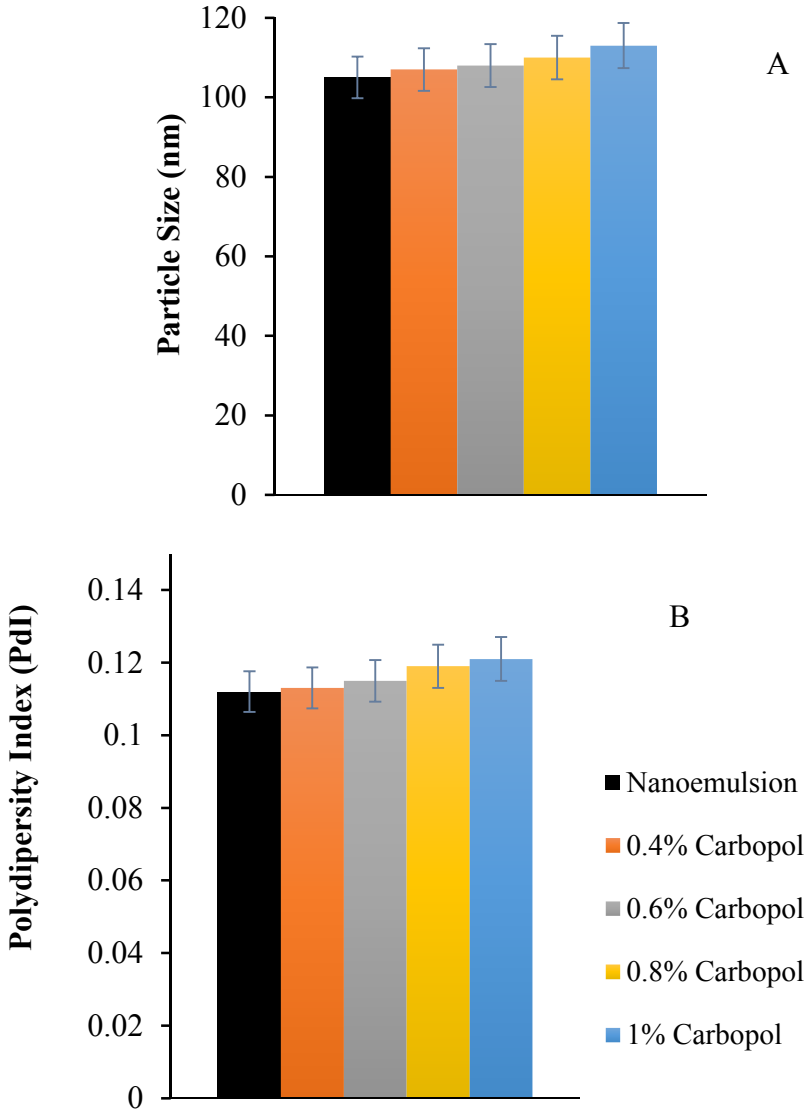
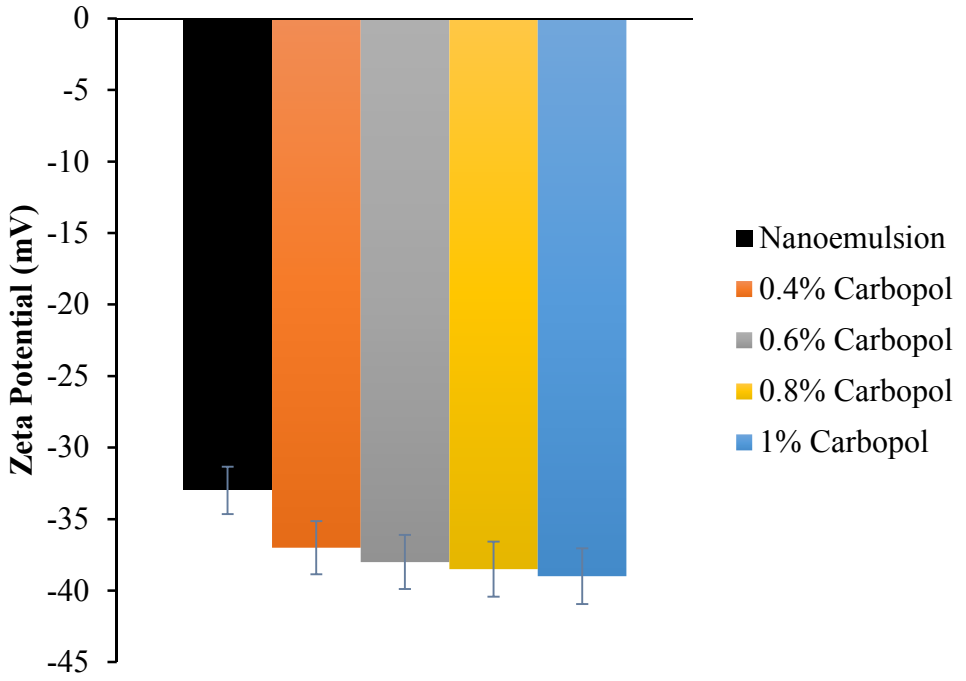


Figure 5

Zeta potential size of cypress oil nanoemulsion and its nanoemulgel containing 0.4, 0.6, 0.8 and 1% Carbopol

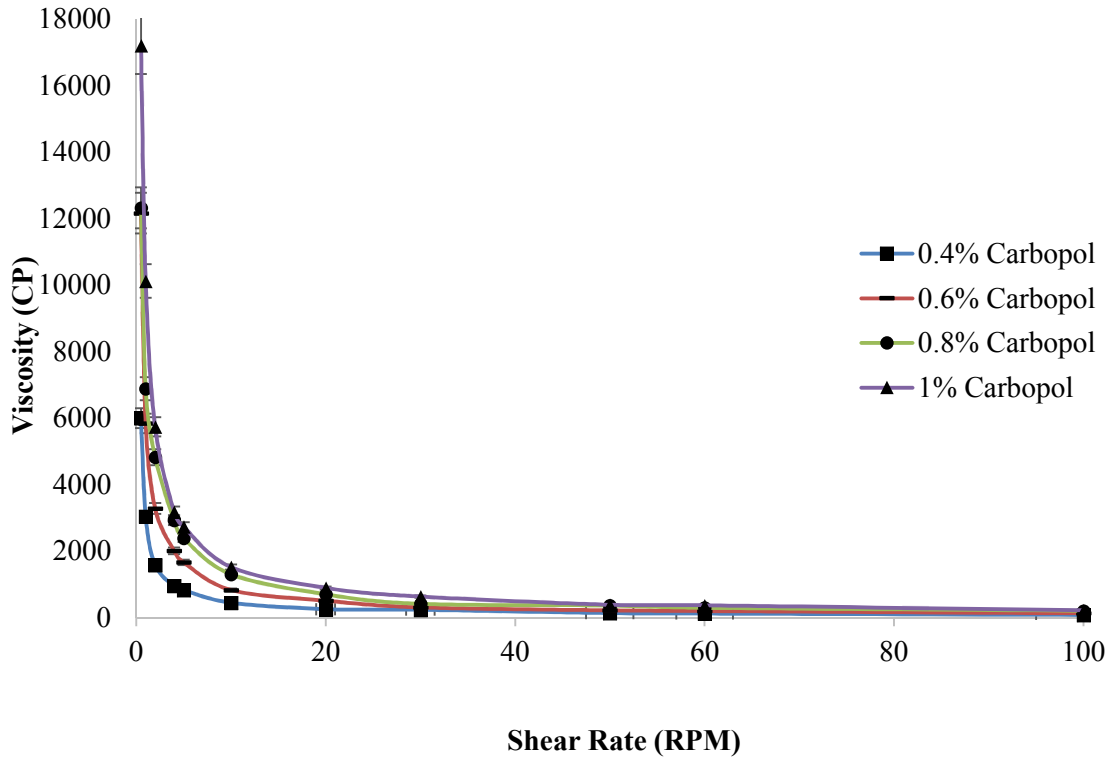


3.4 Rheological measurement of cypress oil nanoemulgel formulations

The rheology factor of the four-prepared cypress oil nanoemulgels containing 0.4%, 0.6%, 0.8%, and 1% Carbopol was analyzed to assess formulation flow characteristics that attribute to their quality and efficacy. The results are shown in Figure 6. It was reported that all formulations had the same behavior, lowering their viscosity as the shear rate increased, indicating pseudoplastic behavior.

Figure 6

Rheological behavior of cypress oil nanoemulgel containing 0.4, 0.6, 0.8 and 1% Carbopol

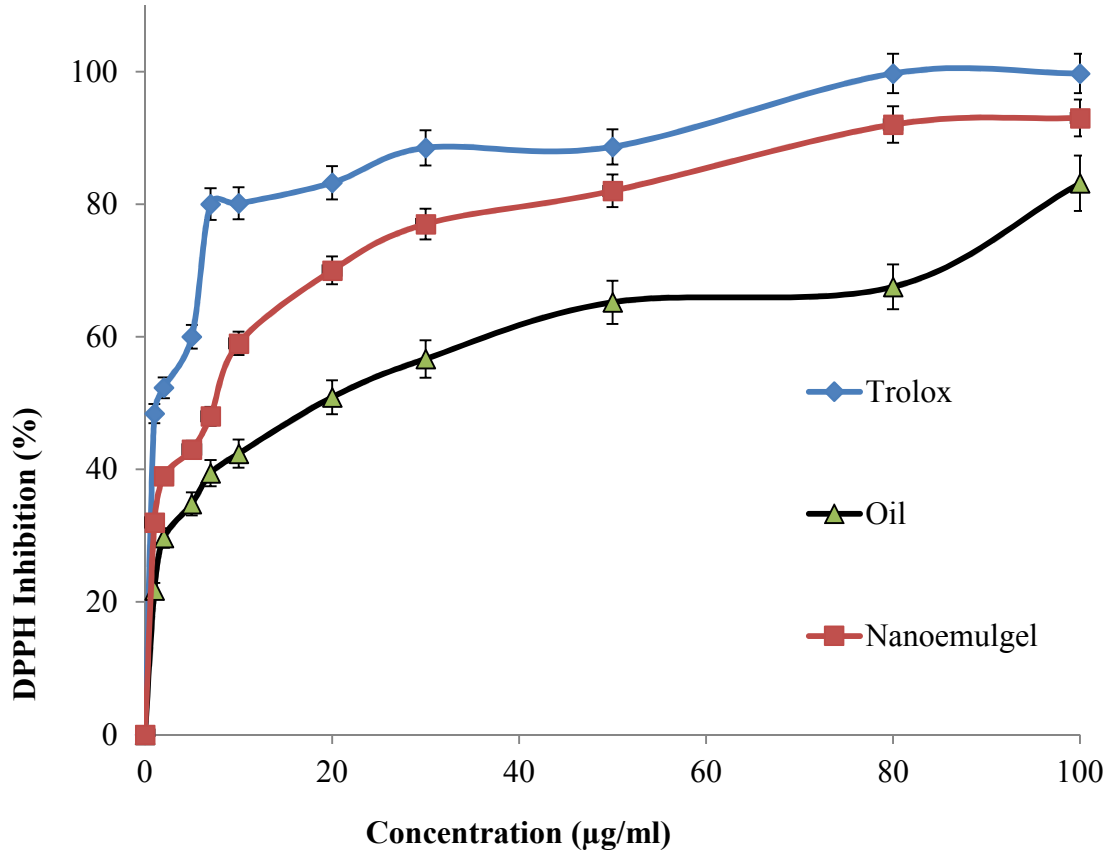


3.5 Antioxidant activity assessment for cypress oil and its nanoemulgel

The antioxidant activity of cypress oil was assessed using the DPPH technique by comparing it with a reference standard chemical (Trolox). The samples underwent testing at various concentrations, spanning from 0 to 100 mL, as seen in Figure 7. According to the findings, there is a direct relationship between the antioxidant activity and oil content. Cypress essential oil effectively inhibited DPPH free radical and converted its stable purple hue to yellow-colored an efficiency IC_{50} of 14.7 ±0.3 µg/mL, while nanoemulgel efficiency was IC_{50}=6.6 ±0.13 µg/ml. In comparison, Trolox had an IC_{50} of 2.7 ±0.2 µg/mL.

Figure7

The percentage of DPPH inhibition for cypress oil, its nanoemulgel and trolox



3.6 Antimicrobial assessment for cypress oil and its nanoemulgel

The cypress oil and its nanoemulgel were further analyzed for their antimicrobial activity using the disc diffusion method on six bacterial species, including gram-positive species (*Staphylococcus aureus* and MRSA) and gram-negative species (*Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris, and Pseudomonas aeruginosa*). Table 7 shows the test results and compares them to ampicillin, which is a well-known antibacterial agent. The inhibition zones ranged from 11 to 36 mm. Overall, the nanoemulgel inhibition zones were wider than the oil. For example, the inhibition zone increased by 8 mm for *S. aureus* bacteria when the oil was incorporated into the nanoemulgel. In other cases, like *P. vulgaris,* which is a gram-negative bacteria that was resistant to the oil by itself, when the cypress oil nanoemulgel was used, it had a diffusion zone of 11±0.4 mm.

Moreover, the cypress oil nanoemulgel showed antibacterial activity against three species of bacteria (*K. pneumonia, P. vulgaris,* and *P. aeruginosa),* which were resistant

to ampicillin. We found that K. pneumonia was the most sensitive gram-negative bacteria to cypress oil. Cypress oil was reported to have superior activity compared to ampicillin.

Table 7

Antibacterial activity of cypress oil, and its nanoemulgel compared with ampicillin. Inhibition zones measured in mm

Microorganism name	Oil	Nanoemulgel	Ampicillin
MRSA	22 ±0.4	28 ±0.3	36 ±0.2
S. aureus (ATCC 25923)	28 ±0.7	36 ±0.6	8 ±0.3
E. coli (ATCC 25922)	12 ±0.5	20 ±0.3	15 ±0.4
K. pneumonia (ATCC 13883)	12 ±0.8	19 ±0.6	Resistance
P. vulgaris (ATCC 8427)	Resistance	11 ±0.4	Resistance
P. aeruginosa (ATCC 9027)	Resistance	12 ±0.3	Resistance

The cypress oil nanoemulgel was also tested against fungi like *Candida albicans* and other species listed in Table 8. The results revealed that cypress oil has moderate activity compared with an antifungal agent named fluconazole, a commonly used medication. However, the results were promising, with inhibition zones ranging from 16 to 24 mm.

As noticed in the antibacterial activity, the nanoemulgel had wider inhibition zones than the oil itself, with differences reaching 7 mm as in *Candida albicans* (Clinical Strains 202).

Table 8

Antifungal activity of cypress oil, and its nanoemulgel compared with fluconazole. Inhibition zones measured in mm

Microbes name/number	Oil	Nanoemulgel	Fluconazole
Candida albicans (ATCC 90028)	20 ±0.6	26 ±0.5	12 ±0.1
Candida parapsilosis (Clinical strains 151)	24 ±0.4	29 ±0.7	26 ±0.4
Candida tropicali (Clinical strains 168)	16 ±0.3	23 ±0.5	23 ±0.3
Candida tropicalis (Clinical strains 260)	16 ±0.5	22 ±0.4	23 ±0.4
Candida albicans (Clinical strains 202)	20 ±0.4	27 ±0.6	26 ±0.5
Candida albicans (Clinical strains 209)	22 ±0.7	28 ±0.5	24 ±0.6

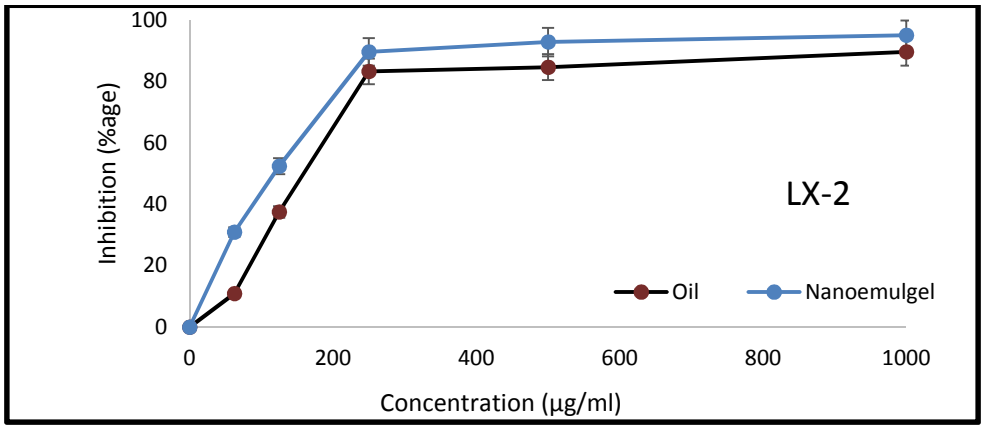
3.7 Anticancer assessment for cypress oil and its nanoemulgel

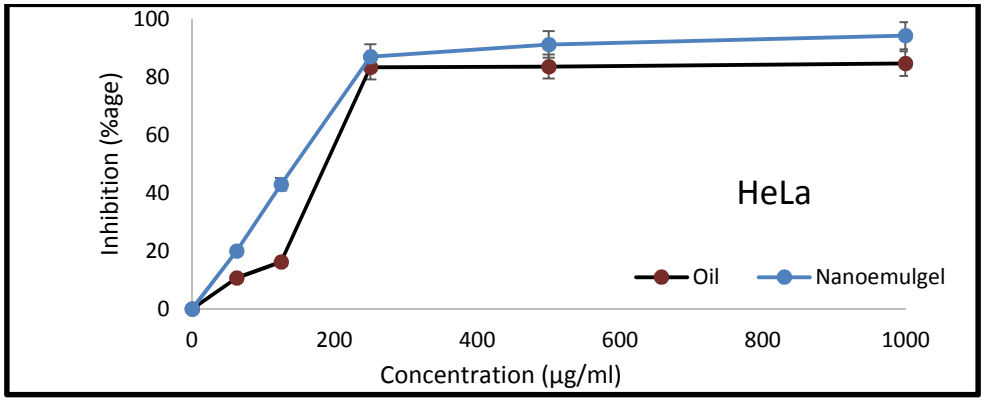
The anticancer activity of the cypress oil and its nanoemulgel was assessed by four different cell types and compared to a positive control DOX. The evaluating criteria depend on the percentage of cell inhibition caused by cypress oil and its nanoemulgel, proving whether they were able to damage or kill these cells. After incubation of four different cell lines, hepatic stellate (LX-2), breast cancer (MCF-7), hepatocellular carcinoma (Hep-G2), and cervical cancer (HeLa), staining with propidium iodide has been done for DNA staining. The treated cells were analyzed by flow cytometry for PI detection.

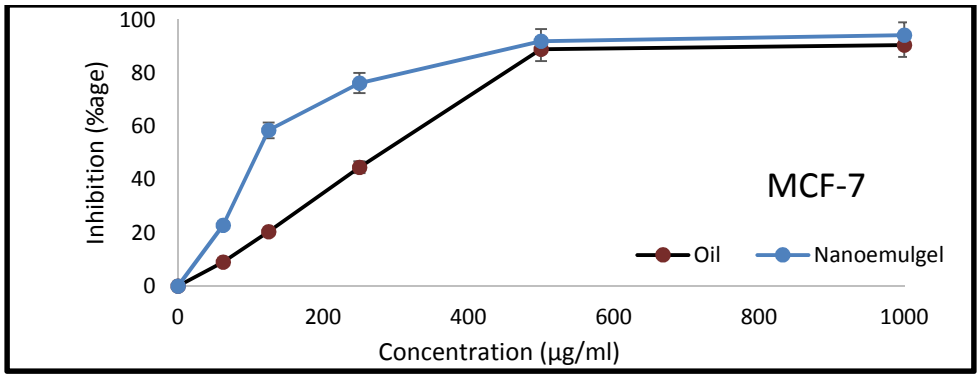
The cells were cultured in different concentrations (0–1000 µg/ml) of the formulation and the oil (Figure 8). The figures revealed that when these cells were incubated with cypress oil, their viability was reduced at different concentrations. This study revealed a dose-dependent cytotoxic effect on all cancer cells. The relationship between the concentration of oil and the number of dead cells was proportional. LX-2 and Hep G2 were the most sensitive, reaching 30% inhibited cells at relatively low concentrations of the nanoemulgel (<60 µg/ml). However, there was no change in the inhibited cells of these two lines or the HeLa cell line after 250 µg/ml. On the other hand, MCF-7 reached the steady state area later, at 500 µg/ml. Notably, nanoemulgel was more cytotoxic than the oil, even after reaching steady state.

Figure 8

Anticancer activity for the cypress oil and its nanoemulgel over four cell lines (LX-2, MCF-7, Hep G2, HeLa)







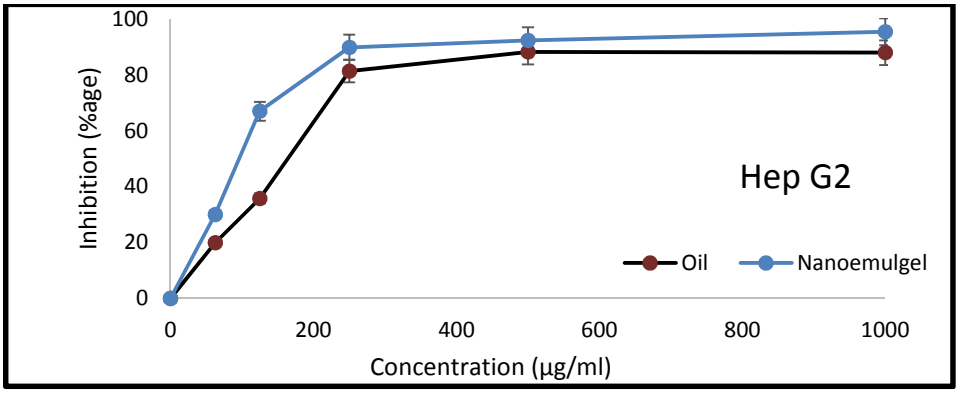
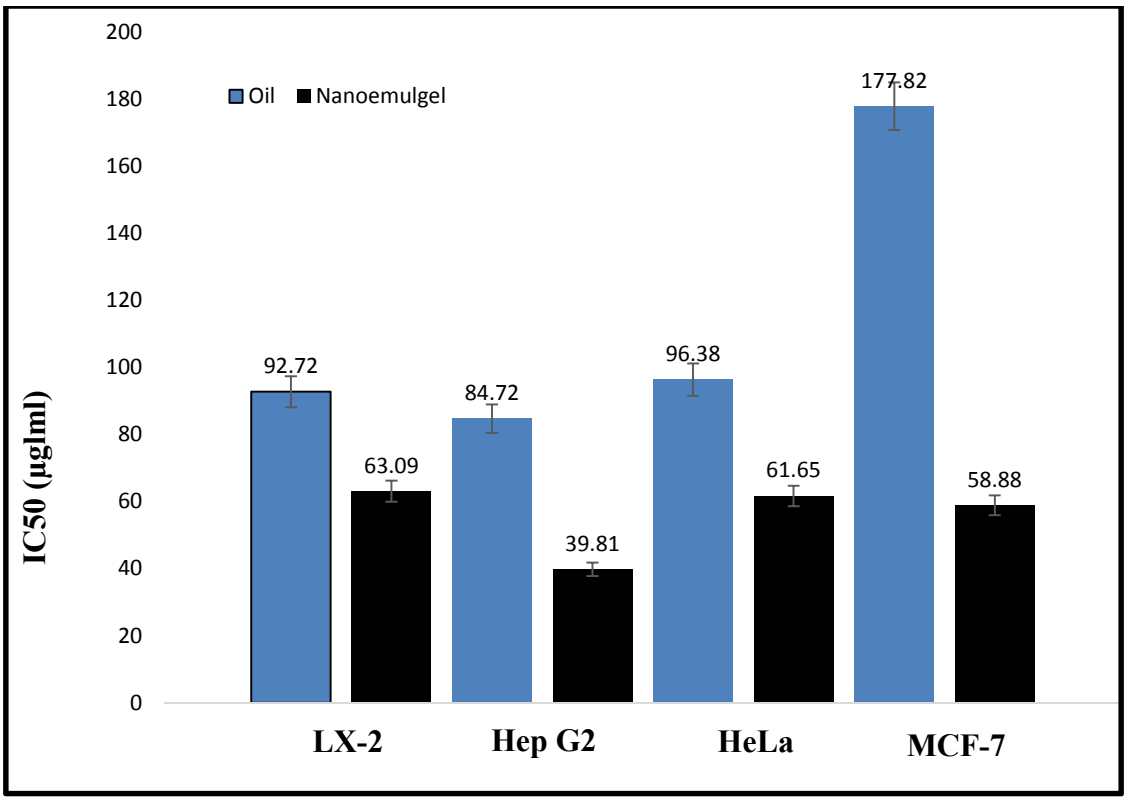


Figure 9 illustrates the calculation of the half-maximal inhibitory concentration (IC_{50}) for the cancer cells. Cypress oil nanoemulgel was most potent on Hep-G2 cells with an IC_{50} of 39.81 µg/ml, followed by 58.88, 61.65, and 63.09 µg/ml for MCF-7, HeLa, and LX-2, respectively. Compared to positive control DOX with an IC_{50} of 1.55±1.35, 0.434±0.271, 0.001±0.0013, 0.05±0.012 µg/ml for HeLa, Hep-G2, MCF, and LX-2, respectively. In general, cypress oil possesses great anticancer activity with an IC_{50} range of 39–63 µg/ml compared to the oil with an IC_{50} range of 84.7–277.82 µg/ml. Remarkably, there is a notable difference between the IC_{50} values of the oil and the nanoemulgel acting on MCF-7; it was 177.82 µg/ml for the oil but less than half (58.88 µg/ml) for the nanoemulgel. Conversely, the values of IC_{50} for the oil and its nanoemulgel were closer to each other for the rest of the cell lines, noting that the oil was always higher.

Figure 9

The IC_{50} of the oil and it nanoemulgel on four cell lines (LX-2, MCF-7, Hep G2, HeLa)



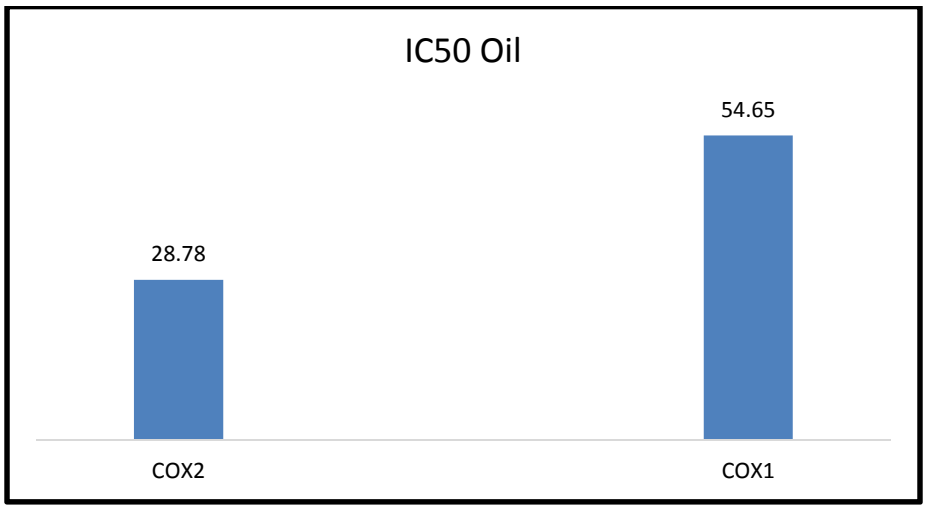
3.8 Anti-inflammatory assessment for cypress oil and its nanoemulgel

The anti-inflammatory activity is a great property to be found in topical creams or ointments. Therefore, it was assessed for the cypress oil and its nanoemulgel, as shown in Figure 10. The nanoemulgel had an IC_{50} of 34 and 13.96 µg/ml for COX-1 and COX-2, respectively. The results indicate strong anti-inflammatory properties for cypress oil nanoemulgel compared to positive control (Celecoxib), which had and IC_{50} of 5.72 ± 0.09 and 0.0152 ± 0.007 (µg/mL) for COX-1 and COX-2, respectively.

The Selectivity Index of both of them is shown in Table 9. Correspondingly, both of them were more selective for COX-2 than COX-1. In addition, the nanoemulgel's IC_{50} toward COX-2 (13.96 µg/ml) was almost half the value for the oil (28.78 µg/ml).

Figure 10

The IC_{50} (µg/ml) of the cypress oil and its nanoemulgel on both COX-1 and COX-2



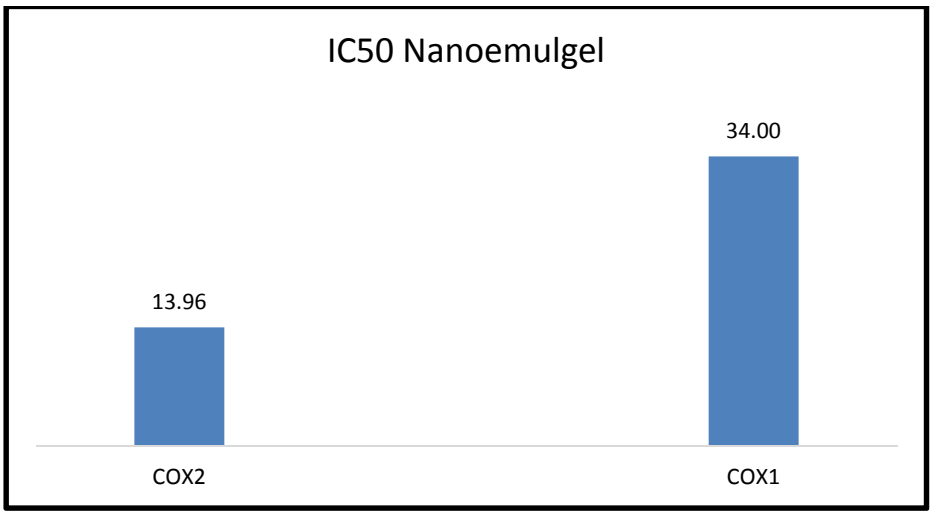


Table 9

Selectivity Index (SI) of cypress oil and its nanoemulgel. (SI= (COX-1 IC_{50})/ (COX-2 IC_{50}))

Formulation	Selectivity Index (SI)
Cypress oil	1.90
Nanoemulgel	2.44

Chapter Four:

Discussions and conclusions

4.1 Discussions

4.1.1 GC-MS analysis

The obtained results show that α-pinene (50.72%) and 3-b-carene (27.57%), two monoterpenes, make up the majority of cypress essential oil (21). Our results are very similar to the study made by Shaheen *et al.,* emphasizing that the main components of c*ypress atlantica* essential oil were found to be carene (23.5%) and α-pinene (46.3%). According to research by Ben Nouri et al., the primary component of cypress oil was α-pinene, accounting for 47.51% of the total composition. This was followed by δ-3-carene, α-terpinyl acetate, β-caryophyllene, and α-cedrol, which made up 7.40%, 4.11%, 4.53%, and 4.99% of the oil, respectively (70). Similarly, Selim *et al*. found that cypress oil contains 20 components, with α-pinene comprising 48.6% of the oil, δ-3-carene (22.1%), limonene (4.6%), and α-terpinolene (4.5%) (69). These studies (summarized in Table 10) underline that cypress oil is predominantly composed of monoterpene hydrocarbons, with α-pinene as a major constituent (21, 69, 70).

Table 10

Comparison between different studdies on the composition of cypress essential oil

Author	α-pinene	3-b-carene	Others
Current Study	50.72%	27.57%	limonene (2.99%) α-terpinyl acetate (2.42%)
Shaheen et al. (21)	46.3%	23.5%	
Ben Nouri et al. (70)	47.51%	7.40%	α-terpinyl acetate (4.11%) β-caryophyllene (4.53%) α-cedrol (4.99%)
Selim et al. (69)	48.6%	22.1%	limonene (4.6%) α-terpinolene (4.5%)

4.1.2 Droplet size and PDI for prepared nanoemulsion

According to the ternary phase diagram, formulation number 6 (Table 6) is the optimum formulation with a PDI of less than 0.3 and a droplet size of less than 200 nm contained 40% Tween, 10% Span, and 50% cypress oil. This formulation has a PDI of 0.112 ±0.016 nm and a mean droplet size of 105.28 ±2.12 nm. A small droplet size (less than 200 nm) aids in the diffusion of the droplet into the cell and, as a result, increases its bioavailability. In other words, it eases extravasations (71). Furthermore, the decreased size of the droplets and the increased surface area at the interface resulted in faster absorption and improved bioavailability (64).

The size distribution pattern of the droplets (PDI) indicates the homogeneity of the nanoemulsion. It plays an important role in determining the way droplets release their constituents, their suitability for intravenous injection, and the way they would act *in vivo* (71). Thus, the smaller PDI value means more homogeneity and, as a result, persistent stability and quality of the product across time (72-74). Weerapol *et al*. suggest that when the oil concentration increases without increasing the surfactant, it will increase the surface tension of the droplets; thus, they will tend to coagulate to minimize surface tension, which will finally have a high PDI value (75). However, it is recommended to have PDI lower than 0.3 for optimum stability and homogeneity of the colloidal (76). The selected nanoemulgel has a PDI value (0.112) very close to the optimum range.

A similar study incorporated *Rosmarinus officinalis* oil in nanoemulgel using 15% Span, 50% Tween, and 35% oil, resulting in a droplet size of 159.23 ±1.22 nm and 0.206 ±0.08 PDI (77). Both results concluded that smaller droplet size and PDI are attained when tween 80 surfactant is used in large quantities, while Span 80 surfactant is used in small quantities (less than half of Tween).

The non-ionic surfactants Tween 80 and Span 80, which have hydrophilic-lipophilic balances (HLB) of 15 and 4.3, work as emulsifiers to keep nanodroplets from sticking together and improve their physical stability. The surfactant has a strong hydrophilic or hydrophobic nature if its HLB value is high (78, 79). If the value is low, it means that the surfactant has a strong hydrophobic nature. Thus, using them both in significant quantities reduced droplet size by decreasing agglomeration. But, a study reported that

tween 80's concentration should always be optimized, as we did in our study, since using more amounts means a thicker adsorption layer, leading to an increase in the droplet size as in formulations 4 and 5 (80). However, the smaller hydrophilic head of span 80 explains its higher saturation surface concentration, allowing for structure that is more compressed at the interface, while the hydrophilic chains of Tween 80 occupy a bigger molecular area. This is why the optimum droplet size can be reached by using small amounts of span 80, while larger quantities are required from Tween (78-80).

4.1.3 Physical characterization of Cypress oil nanoemulgel

Carbopol was added to the formulation that contained 40% Tween, 10% Span, and 50% cypress oil in four percentages (w/w) of 0.4%, 0.6%, 0.8%, and 1% to formulate the nanoemulgel. The Nanoemulgel will enhance the stability of the formulation by reducing surface and interfacial tension. The nanoemulsion contains essential oils which are high volatile, but they are limited by their low visocosity, spreadability and retention time. Thus, nanoemulgel will improve stability, ease the oil application, and be more suitable for immediate or controlled release (81), (82).

Zeta potential typically determines the stability of the formulation. Researchers find that zeta potential values between -30 mV and 30 mV are more stable, as they are believed to cause clumps at higher or lower values (64). As a result, the nanoemulsion of formulation 6 has a -33 mV charge, which is considered stable since it is very close to the range motioned previously. However, the zeta potential of the chosen nanoemulsion (-33 mV) was slightly affected by the addition of carbopol in the formulation containing 0.4% of it (-37 mV).

Generally, there is no significant change in droplet size, PDI, or zeta potential when Carbopol is incorporated.

4.1.4 Rheological measurement of cypress oil nanoemulgel formulations

According to the results, all cypress oil nanoemulgel formulations had the same behavior: lower their viscosity as the shear rate increased. Therefore, all formulations are pseudoplastic. Since the rheology doesn't significantly change when more Carbopol is added, the 0.4% (w/w) carbopol was chosen. Consequently, lower viscosity is favorable because it will facilitate drug diffusion out of the vehicle and increase its

bioavailability. According to the rheology results, the formulation that contains 0.4% Carbopol with 107 nm, 0.113, and -37 mV for droplet size, PDI, and zeta potential was chosen and analyzed further for its antimicrobial, anticancer, and anti-inflammatory activity.

4.1.5 Antioxidant activity

The DPPH method was used to assess antioxidant activity, which revealed a remarkable reduction in it using cypress oil with an efficiency of IC_{50} = 14.7 ±0.3 µg/mL, while for trolox it was IC_{50} = 2.7 ±0.2 µg/mL. The antioxidant activity was enhanced by 2.23 folds for cypress oil when incorporated in nanoemulgel, resulting in IC_{50}=6.6 ±0.13 µg/ml.

The hydrogen-donating components of this substance were believed to be responsible for its exceptional ability to scavenge DPPH radicals and exhibit superior antioxidant activity. The DPPH radical is a persistent free radical that readily receives an electron or hydrogen radical to create a stable diamagnetic molecule (66, 70).

Al-Rajhi et al. reported that cypress oil had effective antioxidant activity with an IC_{50} of 8.97 µg/mL compared to ascorbic acid, which had an IC_{50} of 2.43 µg/mL(83). Shaheen et. al. similarly confirmed that cypress oil has excellent antioxidant activity along with its aqueous and alcoholic extracts (21). On the contrary, Ben Nouri et al. found higher values of IC_{50} using the same DPPH method, which was 151 µg/mL (70). The cypress oil's composition, which is roughly 83.7% made of monoterpenes like α-pinene, 3-b-careen, limonene, and α-terpinyl acetate, is thought to be the reason behind its antioxidant activity (84).

4.1.6 Antimicrobial assessment for cypress oil and its nanoemulgel

The disc diffusion method was used to study antimicrobial activity on six bacterial species, which include gram-positive species (*Staphylococcus aureus and MRSA*) and gram-negative species (*Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris, and Pseudomonas aeruginosa*). The inhibition zones ranged from 11 to 36 mm, concluding that the formulation had good antibacterial activity. Similarly, Al-Rajhi et al. reported that the inhibition zones of cypress oil when tested against *Escherichia coli* and *Staphylococcus aureus* were 33 and 23 mm, respectively (83). In addition to that, Selim

et al. found that cypress oil was effective against *Staphylococcus aureus* and *Klebsiella pneumoniae*, with inhibition zones of 7 mm (69). Mazari et al. observed inhibitory zones with diameters ranging from 6.8 to 15.6 mm for the bacteria *Staphylococcus aureus*, *Bacillus cereus*, *Enterococcus feacalis*, *Escherichia coli*, and *Pseudomonas aeruginosa* (85). The difference between our findings and the outcomes of other researchers may be attributed to the distinct sources of the microorganisms and the diverse origins of cypress oil and its extraction technique.

The antibacterial activity of cypress oil was found to be more active against gram-positive bacteria than gram-negative bacteria. Similar results were found by Nouri *et al.* who reported that the oil was more pronounced against gram-positive bacteria (70). In fact, essential oils are usually more pronounced against gram-positive bacteria, which is explained by the outer phospholipidic membrane in gram-negative bacteria, which is almost impermeable to lipophilic compounds (70). This membrane is not found in gram-positive bacteria, which allows contact of the lipophilic compounds in the essential oil with the cell constituents, causing either an increase in ion permeability and leakage of important intracellular constituents or damage to the bacteria enzyme (69). Cypress oil antibacterial activity is believed to be due to its chemical composition, of which its major components were found to be α-pinene (50.72%) and 3-b-carene (27.57%), as found in our study. Research has shown that these compounds possess superior antibacterial activity (69, 70).

The antifungal activity was tested against different species of fungi: *Candida albicans*, *Candida tropicalis*, and *Candida parapsilosis*. The outcome of the tests showed promising results, with inhibition zones ranging from 16 to 24 mm. Similarly, Al-Rajhi et al. reported that cypress oil had a diffusion zone of inhibition against *Candida albicans* of 32 mm (83). Recently, Galovičová et al. emphasized the antifungal activity of the oil against *Candida albicans* and *Candida tropicalis* with diffusion zones of 11.33 and 6.33 mm, respectively. However, Madar et al. explained the antifungal activity of cypress oil that is caused by specific terpenoids presented in its composition, which successfully inhibited the germination of four types of fungi: *Diplodia pinea f. sp. cupressi*, *Seiridium cardinale*, *Alternaria alternata*, and *Verticillium dahliae* (86). Emami et al. found the oil's strong antibacterial and antifungal activity when tested against *B. subtilis*, *Candida albicans*, *Escherichia coli*, and *Staphylococcus aureus* (87).

Similar to our findings, the researchers concluded that cypress essential oil has a noteworthy ability to act as an antifungal agent. In addition to that, Mazari et. al. reported wide inhibition zones ranging from 31 mm to 80 mm using cypress oil against three types of fungi: *Aspergillus flavus*, *Fusarium oxysporum*, and *Rhizopus stolonifera* (85). In our work, we detected significantly smaller inhibition zones, which nonetheless support the claim that cypress oil has significant potential for the control of microscopic filamentous fungi. Cypress essential oil may include phenolics, alkaloids, flavonoids, terpenoids, and polyacetylenes, which contribute to its antibacterial action. The plant extract's antibacterial effect is primarily ascribed to the presence of phenolic chemicals (69).

Overall, the nanoemulgel inhibition zones were wider than the oil, and some bacteria were resistant to the oil by itself, but when the cypress oil was incorporated into the nanoemulgel, it showed a wide diffusion area. The reason behind that is the smaller size of the nanoemulgel, which facilitates its penetration into the cell, thus increasing its bioavailability and the antibacterial activity of the essential oil (64, 88). However, small hydrophilic molecules like nanoemulgel prefer the transcellular route over the intercellular route to reach systematic circulation for transdermal application (89) (90). Moreover, the nanoemulsion droplets adsorb to the hydrophobic chain present in the cell membrane, causing intensive cell membrane permeability, leading to the leakage of intracellular molecules in the bacterial cell and their death (72, 91). As a result, the effectiveness of cypress essential oil in killing bacteria has generated interest in using cypress nanoemulgel to treat various diseases caused by the bacterial strains that were examined.

4.1.7 Anticancer assessment for cypress oil and its nanoemulgel

The formulation also showed anticancer activity against Hep G2, MCF-7, HeLa, and LX-2 with IC_{50} values of 39.81, 58.88, 61.65, and 63.09 µg/ml. A previous study by Fayed reviewed the antiproliferative effects of cypress essential oil and showed that it was effective in inhibiting human leukemia (HL-60) and acute promyelocytic leukemia NB4 cell lines at IC_{50} values of 365.41 and 333.79 µg/ml (92). Similarly, Galovičová *et al.* found that cypress oil was effective in reducing cells of the human breast cancer MDA-MB-231, colon cancer HCT-116 cell line, choriocarcinoma JEG-3, and chronic myelogenous leukemia K562 cell line. Still, it did not affect the viability of the human

lung fibroblast cell line MRC-5. Also, they suggested a time-dependent effect, as the number of cancer cells continued to decline after 72 hours (93). Orhan *et al.* found the leaf oil is cytotoxic against C32 cells with an IC_{50} value of 104.90 µg/mL. However, he discovered that cytotoxicity assays of the cone oil were completely inactive (94).

Nevertheless, *in vitro* studies by Shaheen et al. revealed increased apoptotic, antiproliferative, and cytotoxic effects that significantly lowered cell viability by inducing DNA breakage (21). From the obtained results, it was concluded that cypress oil has antiproliferative activity against cervical cancer (HeLa), hepatocellular carcinoma (Hep-G2), hepatic stellate (LX-2), and breast cancer cells (MCF-7). Also, it was most sensitive to hepatocellular carcinoma, suggesting that cypress oil could be used in the therapy of chronic hepatocellular carcinoma. Furthermore, LX-2 cell line is linked with liver fibrosis which can lead to liver failure and liver cirrhosis (95). Cypress oil nanoemulgel showed anti-fibrotic effect on LX-2 cell line with IC_{50}=63.09 µg/ml efficiency.

Fayed has found that the reason for the cytotoxic effect is α-pinene, which is the major component (50.72%) of cypress oil, because it showed cytotoxicity towards the rat brain cancer cell line (N2 neuroblastoma cells). The mechanism of α-pinene is believed to be the disturbance of the mitochondrial potential, production of reactive oxygen species, and growth of incaspase-3 activity in B16-F10 murine melanoma (21, 92).

Cypress oil nanoemulgels generally possess greater anticancer activity than the oil. The possible reason behind that is the fact that nanoemulgels facilitate the penetration of the oil into the cell due to their smaller size, larger surface area, and endocytosis process, which results in synergistic anticancer activity (75, 96). The cell uptake of the antiviral drug studied by Weerapol et. al. was enhanced 11-fold due to incorporating it in nanoemulsions. This synergetic effect could be clarified by the fact that surfactants used in the formulation could interrupt the cell membrane integrity, which results in leakage of intercellular molecules, leading to their death (75, 91). Moreover, enhanced permeability and retention effect (EPR) was one of the reasons for enhanced passive targeting of cancer cells, thus increasing the bioavailability of the nanodroplets (97, 98). These results suggest that cypress oil could be efficiently used as a therapy for different cancer types. Nonetheless, more studies are required.

4.1.8 Anti-inflammatory assessment for cypress oil and its nanoemulgel

According to the findings, both the cypress oil and its nanoemulgel were more selective for COX-2 than COX-1. In addition, the oil's IC50 was higher than the value of the nanoemulgel, indicating an improvement in the oil's anti-inflammatory activity when it was in the form of a nanoemulgel. Besides, the selectivity index increased by 1.28 folds when the cypress oil was incorporated into the nanoemulgel. Comparing the results to the positive control (Celecoxib), it was reported that the cypress oil and nanoemulgel have strong anti-inflammatory activity.

Also, Quan *et al.* improved the anti-inflammatory effects of 18-β-glycyrrhetinic acid by adding it to nanocrystals (99). The reason is that Nanovehicles increase bioavailability in the targeted cells by enhancing droplets' absorption because nanosized droplets have a larger surface area, leading to a better dissolution rate and enhanced solubility. In addition to that, cypress oil and its nanoemulgel have a lower IC_{50} for COX-2, which is better for the human body, as COX-2 is responsible for pain and inflammation. At the same time, COX-1 is involved in the regulation of maintaining stability in all physical systems inside the body, or what's called the homeostatic state (100).

4.2 Conclusion

The current work has effectively optimized and assessed the performance of cypress oil and its nanoemulgel. The primary constituents of cypress oil are α-pinene and 3-b-carene, which contribute to its potent properties. The optimum composition of the nanoemulgel consisted of nanoscale droplets, excellent stability, appropriate rheology, and exceptional uniformity. It comprised of 50% cypress oil, 40% tween, 10% span, and 0.4% (w/w) Carbopol 980. Which increased antioxidant activity of the oil by 2.23 folds. The formulation demonstrated strong antimicrobial activity against six bacterial species and six fungal species, including *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*. The inhibition zones reached 36 mm, much higher than the 28 mm seen for the oil alone. Furthermore, it effectively decreased the survival rate of hepatic stellate, breast cancer, hepatocellular carcinoma, and cervical cancer cells, with an efficacy beginning at an IC_{50} of 39.81–63.09 µg/ml. The nanoemulgel exhibited a higher degree of selectivity towards COX-2 compared to COX-1, as evidenced by its significantly low IC_{50} value (13.96 µg/ml) for COX-2. Significantly, the nanoemulgel showed a greater activity level than the oil alone. This indicates that the nanoemulgel

improved the characteristics of cypress oil by lowering the size of droplets and enhancing their capacity to enter the cell. The results demonstrate that adding cypress oil to the nanoemulgel system can potentially mitigate several skin conditions by improving its antibacterial, anticancer, and anti-inflammatory properties.

4.3 Limitations and Recommendations

Combining cypress oil with other potent essential oils is recommended to be formulated in nanoemulgels in further studies to enhance its antimicrobial, anticancer, and anti-inflammatory activity. Moreover, *in vivo* anti-inflammatory, antimicrobial, and anticancer analyses will be conducted to support the reported *in vitro* results.

List of Abbreviations

Abbreviation	Meaning
EO	Essential Oil
WHO	World Health Organization
DPPH	2,2-Diphenyl-1-picrylhydrazyl
COX	Cyclooxygenase
COX-1	Cyclooxygenase 1
COX-2	Cyclooxygenase 2
µl	Microliter
Mg	Milligram
UV-Vis	Ultraviolet-Visible
mV	MilliVolt
Carbopol 940	Carboxyvinyl polymer
PDI	Poly Dispersity Index
NIST	National Institute of Standards and Technology's
RT	Retention time
KRI	Kovats Retention Index
LKRI	Literature Kovats Retention Index
MRSA	Methicillin-resistant Staphylococcus aureus
IC_{50}	The half maximal Inhibitory concentration
LX-2	Hepatic stellate
HeLa	Cervical cancer
MCF-7	Breast cancer
Hep G2	Hepatocellular carcinoma
DOX	Doxorubicin

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جامعة النجاح الوطنية
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تطوير وتقييم الأنشطة المضادة للسرطان ومضادات الأكسدة ومضادات الميكروبات لمستحضر النانو ايملجل لزيت السرو

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قدمت هذه الرسالة استكمالا لمتطلبات الحصول على درجة الماجستير في العلوم الصيدلانية،
من كلية الدراسات العليا، في جامعة النجاح الوطنية، نابلس – فلسطين.

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الملخص

زيت السرو هو زيت أساسي قوي معروف بفعالياته العلاجية المتعددة. هذه الدراسة هدفت لتصنيع زيت السرو بتقنية النانواملجل للتغلب على سرعة تطايره وبطء ذوبانه. تم استخدام تقنية كروماتوغرافيا الغاز- قياس الطيف الكتلي لتحديد التركيب الكيميائي للزيت، كما تم استخدام محلول ثنائي فينيل -1-بيكريل هيدرازيل لقياس مدى قوة الزيت كمضاد للأكسدة. تراكيز المواد المستحلبة وزيت السرو تم دراستها من خلال حجم الحبيبات ومؤشر توزيع الحبيبات وثباتية الخلطة المحضرة. كاربوبول 940 أضيف إلى الخلطة المختارة لتكوين النانواملجل. ثم تم تقييم انسيابية الخلطة وفعاليتها كمضاد للأكسدة والبكتيريا والفطريات والالتهابات. التركيب الكيميائي للزيت مكون بمعظمه من ألفا-بينين (50.72%) و3-ب-كارين (27.57%). بالإضافة لذلك، فعالية الزيت كمضاد للأكسدة كانت بمقدار تثبيط نصفي يساوي 0.3± 14.7 مايكروغرام/مل مقارنة بمادة ترولكس 0.2± 2.7 مايكروغرام/مل. كما بين المخطط الثلاثي أن أفضل خلطة هي الي تحتوي على 40٪ من المستحلب توين و10٪ من المستحلب سبان و 50٪ من زيت السرو لكون حبيباتها تتسم بأصغر حجم 2.12± 105.28 نانومتر وكذلك أصغر مؤشر توزيع حجم 0.016± 0.112 نانومتر و أفضل جهد زيتا 33- ميلي فولت. تم تحويل هذه الخلطة لنانوايمل جل من خلال إضافة 0.4٪ (كتلة/كتلة) من مادة الكاربوبول 940. هذه الخلطة أظهرت فعالية ممتازة ضد أنواع مختلفة من البكتيريا بمناطق تثبيط تتروح بين11-36 ميلي متر. كما انها اثبتت فعاليتها ضد الفطريات بمناطق تثبيط تتروح بين 16-24 ميلي متر. كما أظهرت النتائج أن فعالية النانوايملجل أفضل بكثير من فعالية الزيت لوحده. إضافة إلى ذلك، كان النانواملجل بزيت السرو فعال أكثر من الزيت ضد الخلايا السرطانية الكبدية بمقدار تثبيط نصفي 39.81 مايكرو غرام/ مل، و خلايا سرطان الثدي بمقدار 58.88 و

سرطان عنق الرحم بمقدار61.65 و الخلايا النجمية الكبدية بمقدار 63.09. زيت السرو المحضر بتقنية النانواملجل أثبت انتقائيته لأنزيمات الأكسدة الحلقية-2 أكثر من أنزيمات الأكسدة الحلقية-1. كما تبين أن مقدار التثبيط النصفي للنانواملجل لأنزيمات الأكسدة الحلقية-2 13.96 مايكرو غرام/ مل نصف مقدارها للزيت لوحده 28.7 مايكرو غرام/مل. هذه النتائج السابقة تدل على أن زيت السرو المحضر بتقنية النانواملجل من الممكن استخدامه مستقبلا لمعالجة العديد من الأمراض التي تصيب الجلد.

الكلمات المفتاحية: زيت السرو العطري؛ نانومولجيل؛ مستحلب النانو؛ ألفا بينين.