An-Investigation onto the potential Antimicrobial, Anticancer, and Anti-inflammatory Activates of a Origanum Vulgare Leaf Oil Self-Nanoemulsifying System

Ahmad M Eid

ahmadeid@najah.edu

An-Najah National University

Mohammed Hawash

An-Najah National University

Abdullah Al-Saleh

An-Najah National University

Bashar Hawash

An-Najah National University

Adham Faqeeh

An-Najah National University

Amr Hussein

An-Najah National University

Salah Hussein

An-Najah National University

Research Article

Keywords: Anticancer, Antimicrobial, Self-nanoemulsion, Origanum vulgare, Anti-inflammatory, Self-emulsifying system

Posted Date: June 24th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-9567169/v1

Additional Declarations: No competing interests reported.

An-Investigation onto the potential Antimicrobial, Anticancer, and Anti-inflammatory Activates of a *Origanum Vulgare* Leaf Oil Self-Nanoemulsifying System

Ahmad M Eid[1,2*], Mohammed Hawash[1], Abdullah Al-Saleh[1], Bashar Hawash[1], Adham Faqeeh[1], Amr Hussein[1], Salah Hussein[1]

[1]Department of Pharmaceutical Chemistry and Technology, Faculty of Pharmacy, An-Najah National University, Nablus, Palestine,
[2]Department of Cosmetic and Skin Care, Faculty of Pharmacy, An-Najah National University, Nablus, Palestine

Corresponding Author
Ahmad M Eid
Department of Pharmaceutical Chemistry and Technology, Faculty of Pharmacy, An-Najah National University, P.O. Box 7, Nablus, Palestine.
Department of Cosmetic and Skin Care, Faculty of Pharmacy, An-Najah National University, P.O. Box 7, Nablus, Palestine.
ahmadeid@najah.edu

Abstract

Purpose: This study aimed to formulate a self-nanoemulsion system using *Origanum vulgare* (*O. vulgare*) leaf oil and investigate the antibacterial, anticancer, and anti-inflammatory properties.

Method: The formulation a nanoemulsion from *O. vulgare* leaf oil involved the self-nanoemulsifying technique, with Span 80 and Tween 80 acting as the emulsifying agents. The particle size, polydispersity index (PDI), antibacterial, cytotoxic, and anti-inflammatory properties were examined.

Results: The nanoemulsion formulation with a PDI of 0.216 and a particle size of 89.61 nm was identified as the optimal formulation. The formulation showed a zeta potential of -33.15 ± 2.3 mV. The AFM values were in the range of 30-150 nm. The *O. vulgare* leaf oil self-nanoemulsion showed significant antimicrobial activity against MRSA, *S. aureus,* and *P. vulgaris,* with zone of inhibition diameters of 53 ±0.5, 52 ± 0.5, and 48 ± 0.5 mm, respectively, and minimum inhibitory concentration (MIC) against MRSA, *S. aureus*, *P. vulgaris*, *E. coli*, and *K. pneumonia* of 0.19 µg/mL. A significant anticancer activity against HeLa, Caco-2, MCF-7, Hep-G2, B16-F7, and LX-2 cancer cell lines with IC_{50} values of 7.58 ± 0.5, 7.24 ± 0.5, 8.23 ± 0.5, 10.02 ± 0.5, 9.6 ± 0.5, 12.02 ± 0.5 µg/mL, respectively, which were superior to those of the original oil. Regarding its anti-inflammatory effects, *O. vulgare* leaf oil demonstrated activity against both COX-1 and COX-2, with greater selectivity for COX-2.

Conclusions: Accordingly, a novel *O. vulgare* leaf oil self-nanoemulsion was developed, illustrating a promising step forward in the development of pharmacological dosage forms.

***Keywords*:** Anticancer; Antimicrobial; Self-nanoemulsion; *Origanum vulgare*; Anti-inflammatory; Self-emulsifying system.

Introduction

Herbology, which some call folk medicine, has been one of the most widely used healing methods. Some are still using these traditional practices to this day. It involves the use of botanicals and plant extracts to treat various diseases, including inflammation, mental disorders, cardiovascular disease, and immune system support. Moreover, many medical professionals recommend the use of botanical, herbal treatments and complementary and alternative medicine (CAM) methodologies in managing specific diseases [1]. Aromatherapy has gained value increasingly for its therapeutic properties in encouraging overall wellness, as it is a holistic healing method that uses the fragrance of plant based essential oils. Using phytoconstituent-derived essential oils has played a crucial part of the evolution of the therapeutic potential of aromatherapy as a complementary medical system [2].

Oregano, scientifically called *Origanum vulgare* (*O. vulgare*) of the *Lamiaceae* family, has been used traditionally for its Pharmacological benefits, as in dyspepsia, dental caries, rheumatoid arthritis and disorders of the urinary and respiratory systems in houses and is extensively spread across Asia [3]. The terpenoid and phenolic compounds in *O. vulgare* leaf oil, such as thymol, carvacrol, γ-terpinene, p-cymene, sabinene, caryophyllene, germacrene, and spathulenol, have shown great antioxidant capacity and antimicrobial effect. Previous studies have also reported the anti-carcinogenic, anti-viral, anti-fungal, potent anti-inflammatory, and anti-hyperglycaemic effects of this plant. Furthermore, its anti-inflammatory properties prevent chronic inflammation, which is essential for cell growth and cancer formation, and inhibit activities involving angiogenesis, metastasis, and cell division [4].

Oregano vulgare leaf oil shows a potent anti-inflammatory property through several mechanisms. It inhibits cyclooxygenase (COX) and lipoxygenase (LOX) enzymes, which have a crucial part in the synthesis of pro-inflammatory mediators like prostaglandins and leukotrienes, leading to inflammation and cell division. Furthermore, compounds in oregano oil suppress the activation of NF-κB, a key regulator of inflammation and immune responses, reducing the expression of pro-inflammatory genes and cytokines. Its capacity to modulate cytokine production and activity further helps regulate the inflammatory cascade and maintain immune homeostasis [5].

Oregano is a commonly used plant in the Mediterranean diet, which has been linked to a lower chance of developing colon cancer, but only a few data on its anti-cancer effect are available. *O. vulgare* leaf oil encourages cell death and growth inhibition in a dose-dependent and time-dependent way. It may be that the alterations in the levels of glutathione, as well as the rise in its oxidized form, are involved in the cell death induced by the extract of Oregano, it seems to be the

reason for the activation of both intrinsic and extrinsic apoptotic pathways of cancer cells, the findings and results suggest that the quantity in the Mediterranean diet can have a proapoptotic effect on cancer cells [6]. The researches published between 1950–2022 were reviewed to explore the preventative and therapeutic effect of oregano on cancer. Origanum species contain essential oil consisting of monoterpenes like carvacrol, p-cymene, γ-terpinene and thymol, which are known for their anticarcinogenic effect, making oregano a good choice in cancer treatment [7].

Notable antibacterial and antimicrobial properties of *O. vulgare* leaf oil against microorganisms that are resistant to multiple drugs, such as *E. coli*, *S. aureus*, *C. albicans* and *P. aeruginosa,* have been published, the primary mechanisms of action are inhibition of the enzymes, efflux pump inhibition, adenosine triphosphate (ATP) depletion, inhibition of biofilm formation and membrane damaging [8]. Thymol and carvacrol are the two main phenolic compounds that make up around 78–85% of *O. vulgare* essential oils and are mainly responsible for their antimicrobial effect [9]. Decontamination of surfaces in contact with food by gaseous *O. vulgare* oil, although its effectiveness should be evaluated since it depends on several factors such as the target microorganisms, food-contact surface material, humidity, temperature, and contact time [10].

Nanoemulsions are widely used for encapsulation, protection, and delivering lipophilic ingredients to liquid foods, so this aids in the effective transportation and penetration of active ingredients through a semipermeable membrane [11]. Literature has revealed that nanoemulsions are emulsions characterised by very tiny droplet sizes, below 100 nm. This fact aids in the creation and development of systems, devices, and structures by controlling and manipulating their forms and dimensions at the level of nanometers, including nanocomposites, nanostructures, and nanoparticle aggregates [12, 13]. One of these nanotechniques that is being increasingly used is self-nanoemulsifying systems, which have shown a huge improvement in the world of pharmaceutics and the pharmaceutical industry [14].

In the past few years in drug and food research, the self-nanoemulsions have gained growing attention due to their great capacity to enhance the solubility of poor solubility medications. Despite the fact that the nanoemulsions were considered important vehicles for drugs that have hydrophobic properties, nonetheless, there are still several potentially limiting and restrictive factors to overcome. [15]. Self-nanoemulsifying technologies are employed to enhance the bioavailability of oral drugs that show poor water solubility in water. The size of droplets is a significant factor affecting medications release, distribution and absorption. Creation of self-emulsifying formulations includes the mixing of oils and surfactants, which affect the concentration of surfactant and the lipophilic/hydrophilic [16].

This study's main aim is to develop a self-nanoemulsifying system of *Origanum vulgare* (oregano) leaf oil and assess its multifaceted biological activities that include anti-inflammatory, antimicrobial, and anticancer effects. The purpose is to optimise the formulation for enhanced bioavailability and efficacy, providing a potential therapeutic platform for treating bacterial infections, inflammatory conditions, and cytotoxic growths.

Material and Methods

Chemical and Reagents

The used chemicals in this study include polysorbate 80 (Tween 80) and sorbitan monooleate (Span 80), which were purchased from Al-Shams company. We also used Muller-Hinton agar, designed by Becton Dickinson France. Dimethyl sulfoxide (DMSO) was acquired from Riedel-de Haën, Germany. The *O. vulgare* essential oil was purchased from O&3 Limited, England. Extraction of *O. vulgare* oil was done by the steam distillation technique, using the leaves of this plant. All materials in this experiment were of analytical value. The Mueller–Hinton agar (MHA) contained beef extract as part of the standard MHA formulation (17.5 g of casein acid hydrolysate, 1.5 g of starch, 2 g of beef extract, and 17 g of agar) that is supplied by France by Becton, Dickinson, and Sparks Co., Sparks, MD, USA.

Gas chromatography-mass spectrometry (GC-MS) for phytochemical profiling of oregano leaf oil.

To analyse the fatty acid methyl esters (FAMEs) in *O. vulgare* leaf oil, we used a Gas Chromatography-Mass Spectrometry (GC-MS) protocol. First, we weighed out 100 mg of the oregano oil and mixed it with 10 mL of a sodium hydroxide solution (0.5 M in methanol). Under reflux, this mixture was heated for 10 minutes using a heating mantle to fully break down the sample through hydrolysis. After this step, we added 10 mL of a 14% boron trifluoride (BF_3) solution prepared in menthol to the reaction flask and repeated the reflux process for another 10 minutes. The derivatised FAMEs were then ready for GC-MS characterisation. Following esterification, 50 mL of heptane was introduced into the reaction flask, and the mixture was refluxed for another 10 minutes. The heptane phase with the fatty acid methyl esters (FAMEs) derived from *O. vulgare* leaf oil was isolated for analysis after the cooling process. Characterization was performed using a PerkinElmer Clarus 500 gas chromatograph interfaced with a Clarus 560 mass spectrometer. The system employed an SLB TM-5ms fused silica helium

gas (≥99.999%) as the mobile phase, flowing at 1 mL/min. Samples (1 µL) were injected into a 290°C heated inlet with a 50:1 split ratio.

The GC oven temperature protocol began at 100°C (held for 4 minutes), followed by a gradual ramp of 3°C per minute to 240°C, which was maintained for 15 minutes. Mass spectral data were acquired after a 9 minutes solvent delay, scanning ions between 50-500 amu. Ionisation occurred at 70 eV, and the detector voltage was auto-optimised during system calibration. Compound identification relied on the National Institute of Standards and Technology's MS Data Center. Furthermore, their retention indices and Kovats were compared to values found in the literature.[17-19].

Preparation of *O. vulgare* leaf oil self-nanoemulsifying formulation

For the purpose of determining the most appropriate self-nanoemulsifying formulation for *O. vulgare* leaf oil, a pseudoternary phase diagram was developed. To develop the system, various self-nanoemulsifying formulations were prepared. *O. vulgare* leaf oil, surfactant (Tween 80) and cosurfactant (Span 80) are included in the formulations in different quantities. To prepare each formulation, the three components were weighed and subsequently mixed for two min utilising a vortex mixer produced by VELP Scientifica in Europe. This study utilised the master size analszer (Brookhaven Instruments, Nano Brook Omni, New York) to assess the size of droplet and the polydispersity index (PDI). The oil nanoemulsion experienced self-emulsification. The ideal formulation was selected by evaluating the droplet size, polydispersity index (PDI), and the concentration of the oil. All measurements were conducted at room temperature in triplicate [20].

PDI and droplet size analysis of *O. vulgare* leaf oil self-nanoemulsifying system.

To assess the size of droplet and PDI of the *O. vulgare* leaf oil self-nanoemulsifying formulation, the self-emulsification process was performed on each formulation in 50 ml of distilled water, and subsequently a master-size analyser was used for analysis (Brookhaven Instruments, Nano Brook Omni, New York) to determine the optimal self-nanoemulsifying formulation. Before measurement, the formulation was exposed to gentle stirring for two min with a magnetic stirrer [21].

Zeta Potential Measurement of the Optimal *O. vulgare* Oil Self-Nanoemulsifying Formulation

This research assesses the surface charge and droplet dispersion stability of a self-nanoemulsifying formulation of *O. vulgare* leaf oil utilising the NanoBrook Omni. After the self-emulsification of the formulation in water, the zeta potential was assessed three times, in the same way carried out in the previous test**.**

Atomic force microscopy (AFM) analysis of the self-nanoemulsifying formulation of *O. vulgare* Leaf oil

A core AFM (Nanosurf, Switzerland) was implemented to evaluate the shape and size of the self-nanoemulsifying formulation of *O. vulgare* oil. The nanoemulsions were diluted with water prior to their use in the AFM investigation. After an attenuated nanoemulsion solution was drop-cast onto mica substrates, the samples were subsequently vacuum desiccated at 120°C. The probe force constant was 5 Nm−1, and the tapping mode operated at a resonance frequency of 150 kHz. The Gwyddion software was employed to analyse the photographs.

Antimicrobial Evaluation of *O. vulgare* Leaf Oil and its Nanoemulsion

Six bacterial strains, namely *Methicillin-resistant Staphylococcus aureus* (MRSA), *Proteus vulgaris, Klebsiella pneumoniae, Escherichia coli*, *Pseudomonas aeruginosa and Staphylococcus aureus*, were employed in an antibacterial assay using the microdilution method for the evaluation of the efficacy of *O. vulgare* oil and its nanoemulsion compared to ampicillin antibiotics. All of these bacteria can be accessed through the American Type Culture Collection (ATCC). Additionally, *Candida albicans* was employed for the antifungal evaluation and compared with fluconazole.

Minimum Inhibitory Concentration (MIC) Measurement of *O. vulgare* leaf oil and its Nanoemulsion Using Agar Dilution Method

Muller Hinton agar, utilised for culture medium preparation, was developed in France by Becton, Dickinson, and Sparks Co., located in Sparks, MD, USA. In this technique, the MIC endpoint was determined using the lowest concentrations of *O. vulgare* leaf oil and its nanoemulsion, at which, in these concentrations, there will be a complete inhibition of microbial growth
[22].

Zone of Inhibition Determination of *O. vulgare* leaf oil and its Nanoemulsion Using Agar Dilution Method

Muller Hinton agar is also used. Each litre of the sterile water comprised the following components: 17.5 g casein acid hydrolysate, 1.5 g starch, 2 g beef extract, and 17 g agar. After the components were thoroughly combined, they were simmered with gentle stirring to promote dissolution. After autoclaving for 20 minutes at 121°C, the agar was permitted to cool prior to being transferred to new Petri plates. A flat surface was utilised to ensure consistency in height and width. Agar was ultimately stored at a of temperature 4 to 8°C. Agar diffusion was used to assess antibacterial and antifungal characteristics. Four apertures, each measuring 6 mm in diameter, were created in the agar plates and labelled A, B, C, and D. In hole B, *O. vulgare* leaf oil was applied, while hole A contained only DMSO. Hole C contained a self-nanoemulsifying formulation of *O. vulgare* leaf oil, while hole D was filled with an emulsion lacking oil. The antibacterial assessment involved incubating the petri plates at 37°C for 24 hours, whereas the antifungal evaluation required incubation at 25°C. Measurement of the inhibitory zone diameter was performed to ascertain its antibacterial and antifungal properties [23]. DMSO serves as a negative control and prevents cell death, and the emulsion without oil helps assess the effect of the oil in the formulation.

Anticancer Evaluation of *O. vulgare* Leaf Oil and its Nanoemulsion

This study investigates the toxic effects of *O. vulgare* leaf oil and its self-nanoemulsifying formulation on various cancer cell lines, including Hep-G2 (hepatocellular carcinoma cells), HeLa (human cervical epithelioid carcinoma cells), MCF-7 (breast cancer cells), Caco-2 (human colorectal adenocarcinoma), LX-2 (human hepatic stellate cell line) and B16F7 (murine melanoma cell line), The cancer cell lines were purchased from the American Type Culture Collection (ATCC, USA) and maintained at the laboratories of An-Najah National University. And the cultured cells were in RPMI 1640 medium (Biological Industries, Cromwell, CT, USA), and the cells were fed with 1% penicillin/streptomycin, 10% fetal bovine serum, and 1% L-glutamine. Cell cultures were kept at 37°C in a humidified environment with 5% CO_2 [24].

Cells were inoculated in triplicate into 96-well plates using 100 µL of each growth medium for the experimental configuration. Each well contained approximately 5000 cells. After 24 hours of incubation, fresh medium with *O. vulgare* leaf oil was added to the cultures at concentrations of 250, 125, 62.5, 31.25 and 15.625 µg/mL. The cells were cultured for an additional 72 hours. The Cell Titer 96® Aqueous One Solution Cell Proliferation (MTS) Assay from Promega Corporation, (Madison, WI, USA) was used to evaluate potential anti-proliferative effects, in accordance with the manufacturer's guidelines. Each well received the MTS solution after treatment and was

incubated at 37°C for more than two hours. Absorbance was quantified at 490 nm to assess the effect of plant oil on the proliferation of cells [25].

Anti-inflammatory Evaluation of *O. vulgare* Leaf Oil and its Nanoemulsion.

To investigate the ability of oil from *O. vulgare* leaves in inhibiting the transformation of arachidonic acid (AA) into prostaglandin E_2 (PGE_2) and its inhibition properties on some of the inflammatory mediators, for instance, TNF-α, we utilised a Cayman Chemical Company (USA) COX inhibitor screening assay reagent (Item No. 460104). The 50% inhibitory concentration (IC50) was determined by examining two different concentrations of 50 and 300 µg/mL of every compound (leaf oil from *O. vulgare* and its nanoemulsion) in duplicate experiments. The inhibition degree demonstrated by the sample was assessed by employing the best-fitting line derived from a multiple regression analysis. This was achieved in accordance with the guidelines in the assay by utilising a standard curve comprising eight distinct concentrations of prostaglandins, in addition to a maximum binding sample and non-specific binding sample. Inhibition percentages at each concentration were utilised to determine the IC_{50} [26].

3. Results

***O. vulgare* leaf oil Gas chromatography-mass spectrometry (GC–MS) characterization**

Based on the GC-MS analysis (Table 1, Figure 1), carvacrol (71.28%) was the most predominant constituent in the *O. vulgare* oil, followed by crithmene (8.22%), dolcymene (8.18%), and caryophyllene (4.83%) with other active compounds such as linalool, eucalyptol, camphor, and other compounds detected in trace amounts.

Table 1: The natural active constituents of *O. vulgare* leaf oil characterized by GC-MS.

Name	R.T	Area	% Area	KRI	L KRI
Carvacrol	24.76	56669400	71.28	1303.0	1303
Crithmene	15.46	6534195	8.22	1056.2	1056
Dolcymene	14	6503920	8.18	1023.9	1024
Caryophyllene	28.72	3842161	4.83	1423.1	1423
Linalool	17.21	1228902	1.55	1101.0	1101
Eucalyptol	14.31	1187568	1.49	1030.8	1031
Camphor	19.02	1031573	1.30	1147.4	1147

Terpilene	13.67	557286	0.70	1016.6	1017
Humulene	29.85	506884	0.64	1459.3	1459
Nopinene	11.92	443043	0.56	977.9	978
Limonene	14.23	406468	0.51	1029.0	1029
Comphene	10.67	173790	0.22	950.2	950
Beta-Myrcene	12.5	152573	0.19	990.7	991
Alfa-Pinene	9.95	143536	0.18	934.3	934
Ethyl amyl ketone	12.31	119844	0.15	986.5	987
		79501143	100.00		

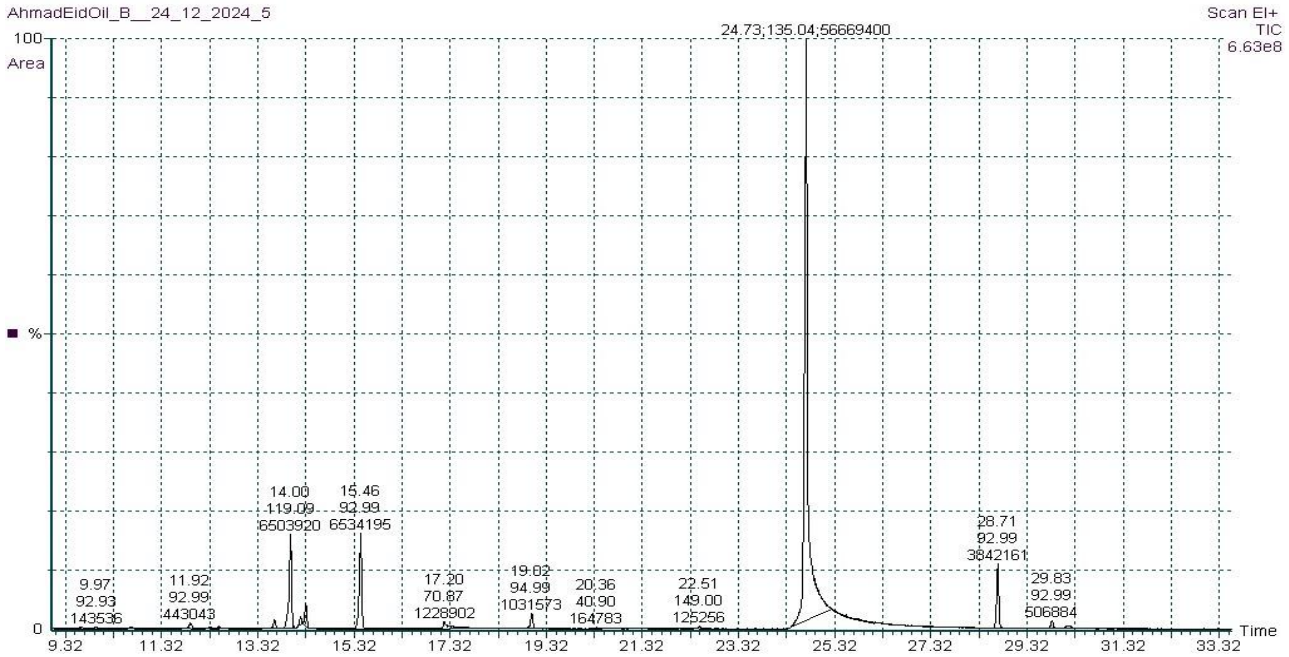


Figure 1. Gas chromatography-mass spectrometry chromatogram of *O. vulgare* leaf oil.

Analysis of *O. vulgare* leaf Oil self-nanoemulsifying formulation Droplet Size, PDI and Zeta Potential

Ternary phase diagrams were created using various concentrations of the surfactant (Tween 80), co-surfactant (Span 80), and *O. vulgare* oil to determine the suitable and optimal self-nanoemulsifying system formulation, with a PDI below 0.3 and a droplet size below 200 nm (Fig. 1). After comparing multiple formulations, the optimal formulation was 29% Tween, 4% Span 80, and 67% *O. vulgare* leaf oil, as shown in Table 2. This formulation has a PDI of 0.216 ± 0.12 and a droplet size of 89.61 ± 1.8 nm.

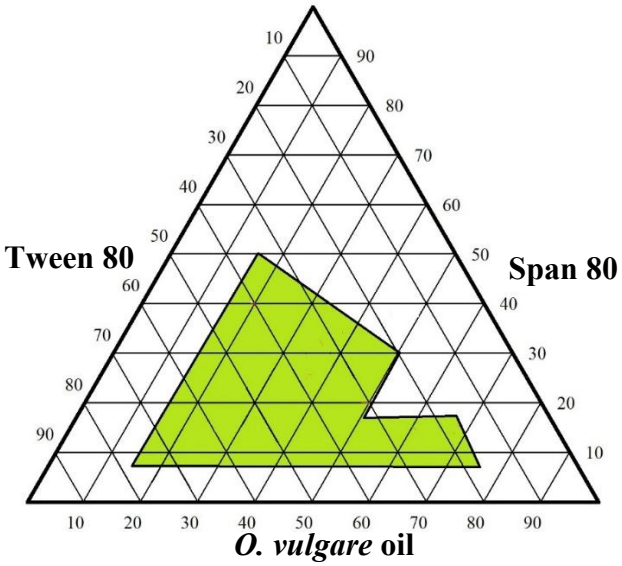


Figure 2*:* *O. vulgare* oil self-nanoemulsifying formulations pseudoternary phase diagrams.

Table 2: The selected *O. vulgare* oil self-nanoemulsifying optimum formulation**.**

Tween 80 (%)	Span 80 (%)	Oil (%)	Droplet size (nm)	Polydispersity Index (PDI)
29	4	67	89.61 ± 1.8	0.216 ± 0.12

Zeta potential of the optimum *O. vulgare* oil self-nanoemulsifying formulation was -33.15 ± 2.3 mV.

Atomic Force Microscope of the self-nanoemulsifying formulation of *O. vulgare* leaf oil

AFM tests were used to assess the diameter and shape of the self-nanoemulsifying formulation of *O. vulgare* oil. The shape of the nanoemulsion was almost spherical (Figure 3). The diameter ranged from 50 to 130 nm.

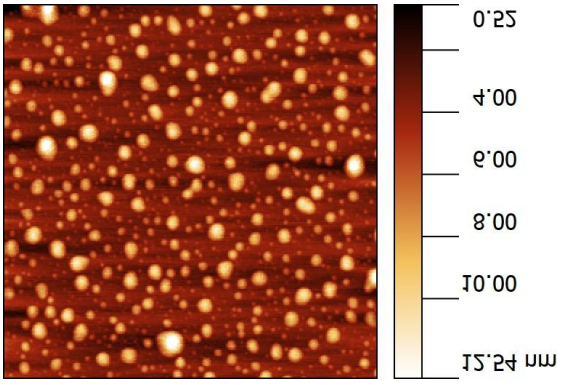


Figure 3: AFM image for *O. vulgare* oil self-nanoemulsifying formulation

Antimicrobial Activity of *O. vulgare* Oil and Its self-nanoemulsifying formulation

The antibacterial studies conducted on various gram-positive and gram-negative bacterial strains using *O. vulgare* oil and its nanoemulsion showed different results compared to those observed when using control-positive antibiotics and antifungal agents such as ampicillin and fluconazole. Based on the measurement of the zone of inhibition diameter (in mm), it was observed that the oil demonstrated a more pronounced effect on *P. vulgaris* and *MRSA* in comparison to the standard drug ampicillin, which had a diameter of 44 ± 0.5 mm for both, and on *K. pneumonia* in comparison to ampicillin, which had a diameter of 36 ±0.5 mm. On the contrary, the nanoemulsion exhibited a more pronounced impact on *P. vulgaris*, MRSA and *S. aureus* than both the oil and the standard drug (ampicillin), as evidenced by the zone of inhibition diameters of 53 ± 0.5 mm, 52 ± 0.5 mm and 48 ± 0.5 mm, respectively. Furthermore, the oil's activity on *Candida albicans* was comparable to that of the standard drug (fluconazole), whereas the nanoemulsion exhibited a stronger and more pronounced effect in comparison to the fluconazole, as evidenced by the zone of inhibition of 49 ± 0.5 mm diameter, as in Table 3 below.

Table 3: Antimicrobial Activity (zone inhibition diameter mm ±0.5) of *O. vulgare* oil and its self-nanoemulsifying formulation against ampicillin and fluconazole.

Microorganism	***O. vulgare* oil**	***O. vulgare* oil self-nanoemulsion**	**Ampicillin**	**Fluconazole**
MRSA	44	52	26	--
***S. aureus* (ATCC 25923)**	38	48	42	--
***K. pneumonia* (ATCC 13883)**	36	45	28	--
***E. coli* (ATCC 25922)**	26	36	33	--
***P. aeruginosa* (ATCC 9027)**	18	26	38	--
***P. vulgaris* (ATCC 90270)**	44	53	18	--
***C. albican* (ATCC 90028)**	40	49	--	12

The minimum inhibitory concentration (MIC) values for the nanoemulsion were consistently lower (0.19–0.95 µg/ml) than those of the oil alone (0.39–1.56 µg/ml). So, the self-nanoemulsion was more effective than ampicillin against MRSA, *S. aureus*, *K. pneumoniae*, *E. coli*, and *P. vulgaris* and comparable to fluconazole against *C. albicans*. These findings suggest the significant

improvement of antimicrobial potential of *O. vulgare* oil by nanoformulation, as shown in Table 4.

Table 4: Antimicrobial Activity (MIC value µg/ml ±0.05) of the *O. vulgare* oil and its self-nanoemulsifying formulation against ampicillin and fluconazole.

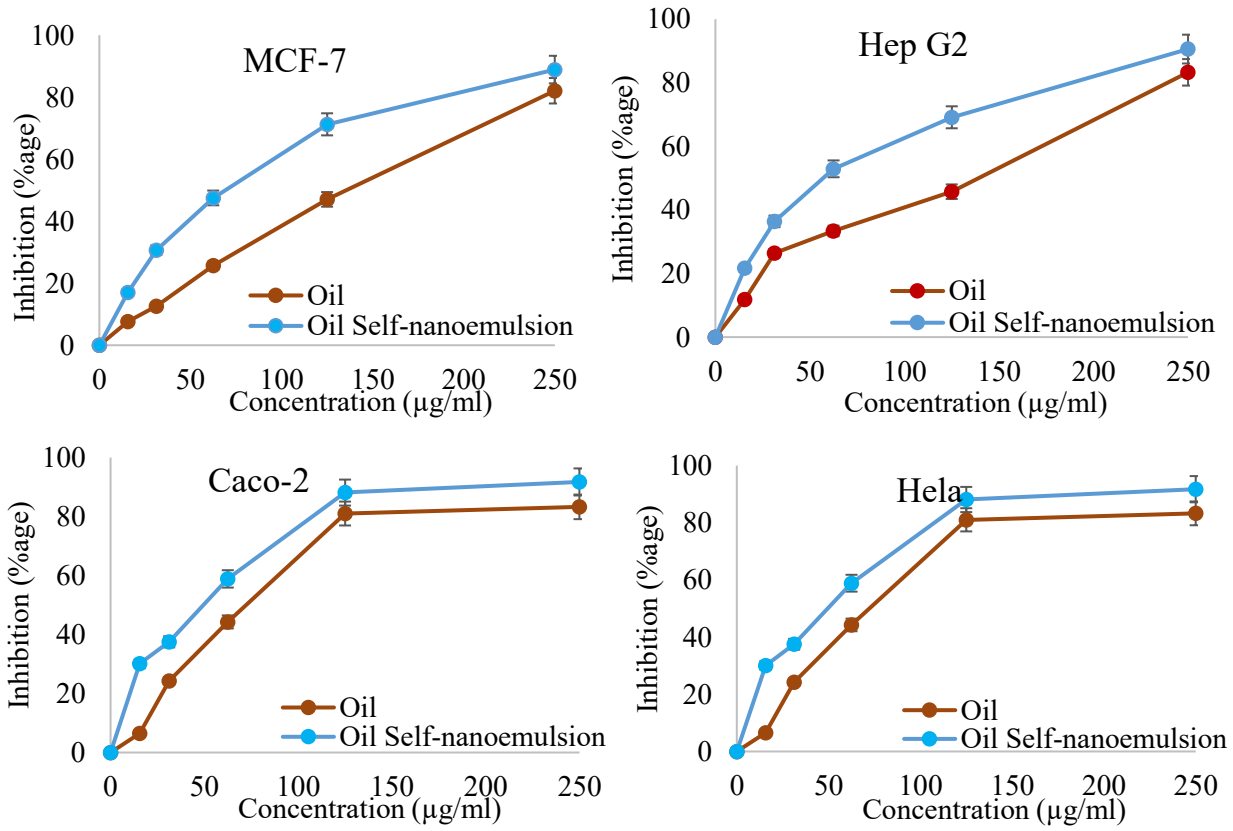
Microorganisms	***O. vulgare* oil**	***O. vulgare* oil self-nanoemulsion**	**Ampicillin**	**Fluconazole**
MRSA	0.73	0.19	32	-
***S. aureus* (**ATCC 25923)	0.73	0.19	6.25	-
K. pneumonia (ATCC 13883)	0.73	0.19	12.5	-
E. coli (ATCC 25922)	0.73	0.19	3.12	-
***P. vulgaris* (**ATCC 8427)	0.39	0.19	3.25	-
P. aeruginosa (ATCC 9027)	1.56	0.73	100	-
C. albicans (ATCC 90028)	1.25	0.95	-	3.12

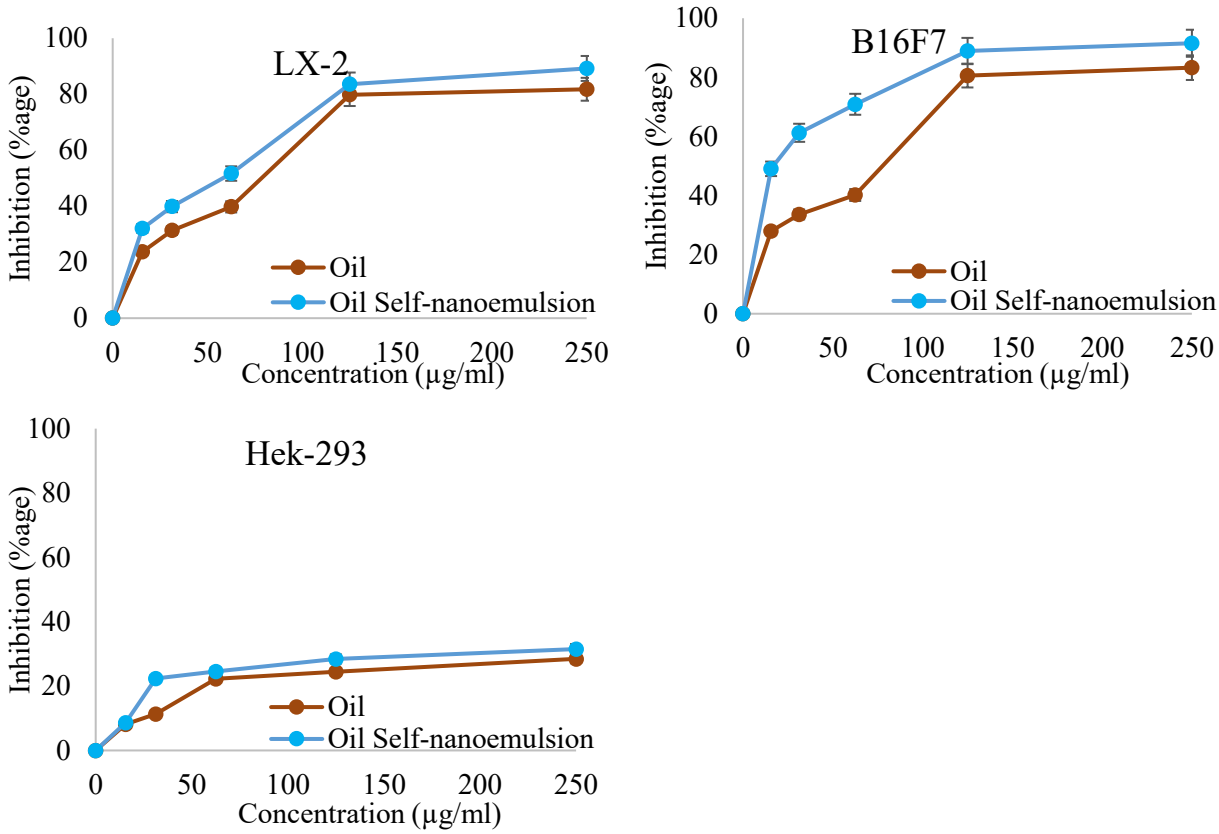
Anticancer Activity of *O. vulgare* leaf Oil and Its self-nanoemulsifying formulation

In this study, we examined the potential anticancer effects of *O. vulgare leaf* oil and its nanoemulsion formulation using seven different cancer cell lines: MCF-7, Hep G2, Caco-2, HeLa, LX-2, B16F7 and Hek-293. MCF-7 cells, a breast cancer cell line derived from the pleural effusion of a 69-year-old woman in 1970. The risk of developing breast cancer depends on many things, including being a woman and lifestyle factors such as being overweight or obese, alcohol, the contraceptive pill, hormone replacement therapy (HRT), and smoking. Hep G2 cells is a cell line exhibiting epithelial-like morphology that was isolated from a hepatocellular carcinoma of a 15-year-old, White, male youth with liver cancer. Excessive alcohol consumption, smoking and medication are prominent factors contributing to the development of this cancer type. Caco-2 cells were established from a human colorectal adenocarcinoma in 1970. Age, race, diet, low physical activity and the presence of inflammatory bowel disease are the risk factors for colorectal adenocarcinoma. HeLa cells, originating from cervical cancer specimens, are recognised for their significant contribution to fatal cancer cases in women. Key factors associated with this type of cancer include smoking, use of oral contraceptives, and infection by human papillomavirus (HPV). LX-2 cells, which represent a human hepatic stellate cell line, are frequently utilised in liver disease research, including studies on cancer and cirrhosis, and have become a standard model for studying the fibrosis of the liver. These cells were derived from non-cancerous human hepatic

tissue. B16F7 cells, a murine melanoma cell line, are commonly employed in melanoma-related studies, given their origin from a melanoma tumor in a C57BL/6 mouse strain. Key factors contributing to melanoma include fair skin, light-colored hair and eyes, the presence of dysplastic nevi (atypical moles), exposure to certain chemicals, and genetic predisposition. Hek-293 cells, generated in 1973, are not a cancer cell line derived from human embryonic kidney cells, they are a type of immortalised cell line widely utilised due to their ability to grow and divide continually, and we use them to test the safety of *O. vulgare* leaf oil on human cells.

Following the cytotoxic tests, we obtained compelling findings, as delineated in Figure 4. These findings elucidate the relationship between the concentration of *O. vulgare* leaf oil and its nanoemulsion, plotted against the percentage of inhibited growth of the cancer cell. There was a discernible influence of both the oil and its nanoemulsion, as evidenced by the escalated suppression of cancer cell growth with increasing concentrations of the oil and its nanoemulsion. This emphasise the significant impact of the oil and its nanoemulsion on these cells ability to proliferate.





Figures 4: Cytotoxic effects of *O. vulgare* oil and *O. vulgare* oil self-nanoemulsifying formulation.

Figure 5 elucidates the IC_{50} values pertaining to *O. vulgare* oil and its oil self-nanoemulsion concerning different cancer cell lines. Their efficacy against cancer cells diminishes proportionally with increasing IC_{50} values. Hela and Caco-2 cells exhibited the highest susceptibility, with IC_{50} values of 12.08 ± 0.5 µg/mL and 7.58 ± 0.5 µg/mL for Hela, and 12.58 ± 0.5 µg/mL and 7.24 ± 0.5 µg/mL for Caco-2, for the oil and its nanoemulsion, respectively. MCF-7 cells followed, with IC_{50} values of 14.12 ± 0.5 µg/mL and 8.23 ± 0.5 µg/mL, respectively. Sensitivity was also observed in B16F7 cells, displaying IC_{50} values of 14.85 ± 0.5 µg/mL and 9.6 ± 0.5 µg/mL for the oil and its nanoemulsion. LX2 cells exhibited moderate susceptibility, with IC_{50} values of 14.79 ± 0.5 µg/mL and 12.02 ± 0.5 µg/mL, respectively. Hep G2 cells showed relatively lower sensitivity, with IC_{50} values of 18.1 ±0.5 µg/mL and 10.02 ± 0.5 µg/mL for the oil and its nanoemulsion. Conversely, Hek 293 cells (the non-cancerous line) exhibited the least susceptibility, with IC_{50} values of 6556.01 ± 0.5 µg/mL and 2846,33 ± 0.5 µg/mL for the oil and its nanoemulsion, respectively. These findings, determined through drug concentration measurements in the

medium, indicate that the oil self-nanoemulsion formulation enhanced the anticancer efficacy of *O. vulgare* oil while maintaining minimal toxicity toward normal cells.

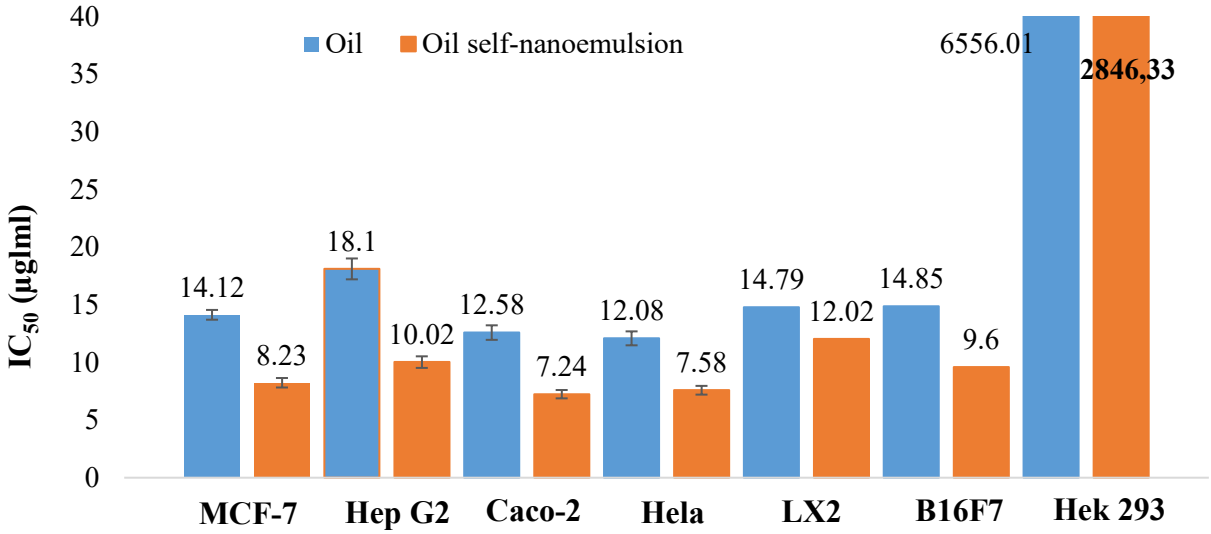


Figure 5: The IC_{50} values ± 0.5 (µg/mL) of *O. vulgare* oil and *O. vulgare* oil self-nanoemulsifying formulation against different cancer cells line.

Anti-inflammatory Activity of *O. vulgare* Leaf Oil and Its Self-nanoemulsion Formulation

The anti-inflammatory activity of *O. vulgare* leaf oil as well as its self-nanoemulsion formulation was performed by examining the cyclooxygenase enzymes (COX) activity. The crude oil and its nanoemulsified form were tested at two different concentrations (50 and 300 µg/mL, specifically assessing their effects on inhibiting the enzymatic metabolism of arachidonic acid into prostaglandin E2 using catalytic pathways by cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX−2). The findings from the *in vitro* experiments clearly established that crude oil hydroxylated significantly inhibiting both cyclooxygenase isoforms 1 and 2. Nevertheless, following nanoemulsification, the difference between the concentrations strikingly changed due to a significant improvement in the inhibitory capacity of the self-nanoemulsion formulation (most pronounced against COX-1). The high increase of inhibition activity compared with the crude oil is illustrated in Fig. 6.

The inhibitory effects on COX-1 and COX-2 enzymes are presented in the accompanying figure, showing both IC_{50} values and percentage inhibition for *O. vulgare* leaf oil and its nanoemulsion formulation. As expected, higher IC_{50} values correlated with reduced anti-inflammatory activity. At 50 µg/mL, the crude oregano oil effectively inhibited both COX isoforms, with calculated IC_{50}

values of 60.30 ± 1.1 µg/mL for COX-1 and 31.55 ± 0.7 µg/mL for COX-2. The nanoemulsion formulation showed markedly stronger COX-1 and COX-2 inhibition (IC_{50} = 33.12 ± 0.7 and 16.50 ± 0.7 µg/mL, respectively), representing a significant improvement over the crude oil. A selectivity index (SI) value above 1 shows that a drug is more focused on COX-2, which helps lower the chances of stomach problems that usually happen with all COXs but are mostly reduced for COX-1 when the SI value is lower. The SI of *O. vulgare* leaf oil self-nanoemulsion (2.00) is much higher than that of crude oil (1.91). The SI of *O. vulgare* leaf oil self-nanoemulsion (2.00) is significantly higher than that of crude oil (1.91).

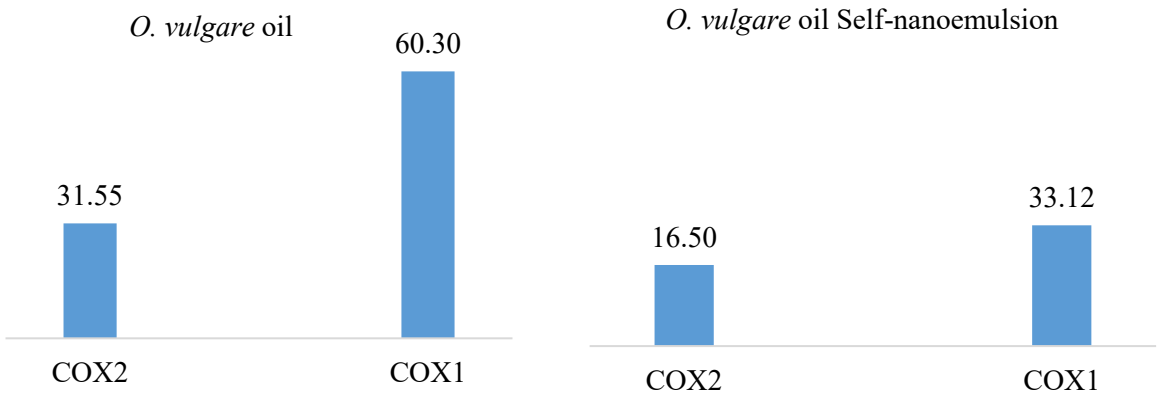


Fig. 6 The IC_{50} values (µg/mL) of the anti-inflammatory activity of *O. vulgare* leaf oil and its Self-nanoemulsion.

4. Discussion

Previous research highlights the remarkable bioactive potential of *O. vulgare* leaf oil, positioning it as a promising candidate for pharmaceutical applications. Based on these results, our study looked into whether nano-emulsification could make it more bioactive. We used GC-MS to find out what phytochemicals were in the oil. The chemical profile established in this study deviates noticeably from previously documented literature. In addition, a previous study shows that carvacrol (25.6%), p-cymene (12.3%) and linalool (8.71%) were found as the primary and major chemical constituents present along with considerable amounts of thymol and γ-terpinene [27]. In another study, 2-methen-1-ol was the most abundant component with a percentage of important Fr. (36.33%), terpinene-4-ol (9.01%) and carvacrol methyl ether (5.14%) in the composition of the mixture, which corresponds to other profiles [28]. There is partial contract with the earlier findings, as the current results demonstrate considerable variation in composition. In this study analysis, carvacrol was the main constituent of the oil, as its concentration was 71.28% of the total content. Then followed by p-cymene (8.22%), γ-terpinene (8.18%), and caryophyllene (4.83%). There are

some minor constituents, including linalool (1.55%), eucalyptol (1.49%), and camphor (1.30%), whereas compounds such as terpinene, humulene, nopinene, and limonene were detected only in trace amounts.

Such discrepancies in chemical profiles are likely attributable to variations in factors such as geographical origin, environmental conditions, and extraction methodology. These considerations are critical when aiming to standardise oregano oil for pharmaceutical applications.

In order to find the optimal *O. vulgare* oil self-nanoemulsifying system, several formulations with varying amounts of *O. vulgare* oil and surfactants (Tween 80 and Span 80) were prepared. A ternary phase diagram directed the selection of formulations and helped identify the composition of the best stability and emulsification performance formulation. The polydispersity index (PDI) was used to assess droplet uniformity; high-quality, stable systems typically show a low PDI (preferably <0.3) and small particle sizes (<200 nm). Our formulation met these criteria. The best-performing mixture contained 67% *O. vulgare* oil, 29% Tween 80 and 4% Span 80. These nonionic surfactants were chosen for their complementary HLB values (Tween 80: 15; Span 80: 4.3), which promote the formation of fine, homogeneous droplets while reducing potential skin irritation. The formulation demonstrated stability with a PDI of 0.261 and an average droplet size of 89.61 nm, confirming effective self-nanoemulsification. These findings align with previous studies emphasising the importance of surfactant selection in drug delivery systems [29].

Conversely, in the case of *O. vulgare* oil self-nanoemulsifying formulation, droplet AFM image is shows being spherical. The diameter range (50–130 nm) may be consistent with the effective diameter readouts from DLS [30]. A critical parameter that shows the stability of a nanoemulsion over time is measuring its zeta potential. This metric shows whether particles are likely to flocculate (remain dispersed) or cluster together (destabilising the system) by measuring the electrostatic charge on droplet surfaces [31]. Typically, dispersions are classified as stable or unstable if their zeta is above +30 or below -30 mV, respectively. If the zeta potential of the droplets is greater than +30 mV or less than -30 mV, it is considered stable. In our case, this *O. vulgare* oil self-nanoemulsifying formulation had a zeta potential of −33.15 mV, which seems to indicate that droplets repel each other electrostatically (strong repulsive force), a strong sign of long-term stability because droplets do not tend to aggregate. In addition to stability, we worked on making a self-nanoemulsifying system with *O. vulgare* oil and investigating its medicinal potential as an antibacterial, anti-inflammatory, and anticancer agent. The goal of this testing was to connect the oil's known phytochemical richness (such as its high carvacrol concentration) with

its real-world medicinal uses. This was a step toward proving that it may be used in future drug delivery systems.

Essential oils from plants, such as *O. vulgare*, are well-known for their ability to fight germs, reduce inflammation, and protect against free radicals. Many studies back up these claims, but one that stands out is the one that showed oregano oil's strong effect on *Propionibacterium* acne and *Staphylococcus epidermidis*, which cause acne [32]. Based on this foundation, our research investigated the efficacy of *O. vulgare* leaf oil and its self-nanoemulsifying formulation against a wider array of pathogens, including *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, MRSA**,** *Staphylococcus aureus*, *Candida albicans*, and the notoriously resilient *Pseudomonas aeruginosa*.

Interestingly, the raw oil didn't work very well against P. aeruginosa, but the nanoemulsion version worked much better. However, both types had considerable effects on the other microorganisms that were tested. We think that the nanoemulsion works better because the size of the droplets is much smaller (<200 nm), which increases the surface area and makes it easier for microbial cells to interact with it. The nanosized oil droplets probably pass through bacterial and fungal membranes more easily, bringing higher concentrations of bioactive chemicals like carvacrol to the target areas. This mechanistic benefit may elucidate why nanoemulsions frequently serve as a link between conventional plant extracts and contemporary medicinal formulations.

Past research showed how nanoemulsification makes plant-based oils more effective at killing germs to put our findings in context. For example, one study made a clove oil nanoemulsion that worked better than both raw clove oil and the antibiotic amikacin at stopping microbes from growing. This is a great illustration of how nanotechnology may improve natural substances [33]. Researchers developing a nanoemulgel from Nigella sativa oil noted markedly enhanced antibacterial properties relative to the non-formulated oil [34], a trend that corresponds with our findings.

These similarities point to a bigger trend: nanoemulsions might be able to help plant oils get around problems like low solubility or quick breakdown. Our research corroborates this, demonstrating how the self-nanoemulsion of *O. vulgare* leaf oil connects traditional herbal extracts with contemporary medicinal standards.

Growing evidence suggests that *O. vulgare* essential oil may be able to combat cancer, but scientists are still trying to figure out how it works. A study examining Sicilian oregano leaf oil on aggressive breast cancer cells (MDA-MB-231 and hormone-responsive MCF-7 lines) demonstrated a dose-dependent decrease in cancer cell viability, presumably associated with its

elevated phenolic content [35]. These results correspond with a growing interest in plant-derived chemicals; yet, problems remain. A distinct study of the principal antimicrobial constituents of oregano oil—carvacrol, trans-cinnamaldehyde (TCA), and eugenol—uncovered a significant limitation: although these chemicals inhibited cervical cancer (HeLa) cells, they concurrently inflicted damage on non-cancerous fibroblasts (CCD-1123Sk) in cytotoxicity assays.

Researchers employed techniques such as MTT/LDH assays, flow cytometry, and RT-qPCR to validate this non-selective toxicity [36], emphasising the necessity for a tailored delivery strategy to reduce off-target effects.

The anticancer activities of *O. vulgare* leaf oil and its self-nanoemulsion were evaluated against seven cell lines: LX-2, HeLa, MCF-7, Hep G2, B16F7, and Hek 293 to see how well they worked against cancer. The self-nanoemulsion was better than the raw oil in most cells. The maximum cytotoxic effect was seen in Hela, Caco-2, MCF-7, Hep-G2, B16-F7, and LX-2 cells. Hek 293 had the least harmful effect on cells because it is not a malignant cell and was examined for safety.

The increase in the activity of the *O. vulgare* leaf oil self-nanoemulsion is due to very small droplet size (89.61 nm) and better solubility, which probably improved its penetration deeper into cell membranes and transported its bioactive substances, like carvacrol, more effectively. This mechanistic benefit, where smaller particles operate as "targeted carriers", could explain why nanoemulsions make the oil's therapeutic effects stronger while reducing non-specific interactions, which is a problem that commonly happens with crude plant extracts.

Our results show that self-nanoemulsification greatly increases the COX-1 and COX-2 inhibitory action of *O. vulgare* leaf oil. This is probably because important bioactive chemicals like carvacrol become more available. The formulation's nanoscale droplets (~90 nm) may enable deeper cellular penetration, enhancing contact with the COX-1 hydrophobic active site, a strategy corroborated by molecular docking studies indicating carvacrol's preferential affinity for COX-2 over COX-1 [37]. This selectivity is in line with new plans to make anti-inflammatory drugs that are safer. Non-selective COX inhibitors, like aspirin, can cause problems in the stomach because they lower COX-1 levels too much [38]. The dual COX inhibition of crude oil is similar to traditional herbal therapies, but the self-nanoemulsion's more precise targeting could help with chronic inflammation with fewer side effects [39]. Future research should investigate synergies with COX-2 targeted medications and confirm efficacy in *in vivo* models of inflammation.

Conclusion

In conclusion, this study was able to develop a novel stable self-nanoemulsifying system for *O. vulgare* leaf oil utilising Tween 80 (29%) and Span 80 (4%) as surfactants. The optimised self-nanoemulsion showed big improvements in the bioactivity over the crude oil. It had a uniform droplet size of 89.61 nm, a low polydispersity index of 0.216, and a zeta potential of -33.15 mV, and AFM confirmed that the nanoemulsion droplets were spherical with diameters between 50 and 130 nm. Metrics that show better colloidal stability and bioavailability. The self-nanoemulsion showed better bioactivity, especially against infections like MRSA, *E. coli*, and *Candida albicans*. It also showed more cytotoxicity in cancer cell lines, including HeLa, MCF-7, and Caco-2, and it showed tremendous promise against COX-2 enzymes. These improvements are because the system's nanosized droplets let cells get through and concentrate bioactive substances including carvacrol (71.28%), p-cymene, and γ-terpinene at their target areas. The formulation's effectiveness looks promising, but earlier studies have shown that some of its parts may not be selective and could affect both healthy and cancerous cells. This evidence shows that future versions need to have targeted delivery systems. *O. vulgare* self-nanoemulsion, which is high in phenolics and terpenes, is a strong candidate for use in medicine, especially in therapies that fight inflammation, bacteria, and cancer. To transition from laboratory success to clinical use, subsequent research must focus on *in vivo* safety evaluations, dose optimisation, and investigations into selective drug delivery methods. This discovery is in line with initiatives around the world to use nanotechnology to improve plant-based medicines. The self-nanoformulation of *O. vulgare* oil could be a model for the next generation of natural drug development.

Declarations

Abbreviations

O. vulgare: *Origanum vulgare*

PDI: Polydispersity index

MIC: Minimum inhibitory concentration

CAM: Complementary and alternative medicine

FAMEs: Fatty acid methyl esters

DMSO: Dimethyl sulfoxide

COX: Cyclooxygenase

ATCC: American Type Culture Collection

AFM: Atomic Force Microscope

Hep-G2: Hepatocellular carcinoma cells

HeLa: Human cervical epithelioid carcinoma cells

MCF-7: Breast cancer cells

Caco-2: Human colorectal adenocarcinoma

LX-2: Human hepatic stellate cell line

B16F7: Murine melanoma cell line

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

None.

Authors' contributions

Ahmad M Eid: Conceptualization, Methodology, Writing–original draft, Writing–review & editing, Supervision, Validation, Formal analysis. The rest of the authors: Writing, Formal analysis.

Acknowledgments

The authors would like to acknowledge the Faculty of Pharmacy at An-Najah National University.

References:

1. Ernst E: **Complementary medicine: common misconceptions**. *J R Soc Med* 1995, **88**(5):244-247.
2. Vora LK, Gholap AD, Hatvate NT, Naren P, Khan S, Chavda VP, Balar PC, Gandhi J, Khatri DK: **Essential oils for clinical aromatherapy: A comprehensive review**. *J Ethnopharmacol* 2024, **330**:118180.
3. Habibi E, Shokrzadeh M, Ahmadi A, Chabra A, Naghshvar F, Keshavarz-Maleki R: **Genoprotective effects of Origanum vulgare ethanolic extract against**

cyclophosphamide-induced genotoxicity in mouse bone marrow cells. *Pharm Biol* 2015, **53**(1):92-97.

4. Enayatifard R, Akbari J, Babaei A, Rostamkalaei SS, Hashemi SMH, Habibi E: **Anti-Microbial Potential of Nano-Emulsion form of Essential Oil Obtained from Aerial Parts of Origanum Vulgare L. as Food Additive**. *Adv Pharm Bull* 2021, **11**(2):327-334.
5. Fatima K, Luqman S, Meena A: **Carvacrol Arrests the Proliferation of Hypopharyngeal Carcinoma Cells by Suppressing Ornithine Decarboxylase and Hyaluronidase Activities**. *Front Nutr* 2022, **9**:857256.
6. Savini I, Arnone R, Catani MV, Avigliano L: **Origanum vulgare induces apoptosis in human colon cancer caco2 cells**. *Nutr Cancer* 2009, **61**(3):381-389.
7. Emire Z, Yabalak E: **Can Origanum be a hope for cancer treatment? A review on the potential of Origanum species in preventing and treating cancers**. *Int J Environ Health Res* 2023, **33**(9):894-910.
8. Soltani S, Shakeri A, Iranshahi M, Boozari M: **A Review of the Phytochemistry and Antimicrobial Properties of Origanum vulgare L. and Subspecies**. *Iran J Pharm Res* 2021, **20**(2):268-285.
9. Kokkini S, Karousou R, Dardioti A, Krigas N, Lanaras T: **Autumn essential oils of Greek oregano**. *Phytochemistry* 1997, **44**(5):883-886.
10. Pinto L, Cervellieri S, Netti T, Lippolis V, Baruzzi F: **Antibacterial Activity of Oregano (Origanum vulgare L.) Essential Oil Vapors against Microbial Contaminants of Food-Contact Surfaces**. *Antibiotics* 2024, **13**(4):371.
11. Preeti, Sambhakar S, Malik R, Bhatia S, Al Harrasi A, Rani C, Saharan R, Kumar S, Geeta, Sehrawat R: **Nanoemulsion: An Emerging Novel Technology for Improving the Bioavailability of Drugs**. *Scientifica (Cairo)* 2023, **2023**:6640103.
12. Acevedo-Fani A, Salvia-Trujillo L, Rojas-Graü MA, O. M-B: **Edible films from essential-oil-loaded nanoemulsions: Physicochemical characterization and antimicrobial properties**. *Food hydrocoll* 2015, **47**:168-177.
13. Ojha B JV, Gupta S, Talegaonkar S, Jain K. : **Nanoemulgel: a promising novel formulation for treatment of skin ailments**. *Polym Bull* 2022, **79**(7):4441-4465.
14. Eid AM, El-Enshasy HA, Aziz R, Elmarzugi NA: **The preparation and evaluation of self-nanoemulsifying systems containing Swietenia oil and an examination of its anti-inflammatory effects**. *Int J Nanomedicine* 2014, **9**:4685-4695.

15. Wang Y, Li D, Lin H, Jiang S, Han L, Hou S, Lin S, Cheng Z, Bian W, Zhang X *et al*: **Enhanced oral bioavailability and bioefficacy of phloretin using mixed polymeric modified self-nanoemulsions**. *Food Sci Nutr* 2020, **8**(7):3545-3558.
16. Yang S, Gursoy RN, Lambert G, Benita S: **Enhanced oral absorption of paclitaxel in a novel self-microemulsifying drug delivery system with or without concomitant use of P-glycoprotein inhibitors**. *Pharm Res* 2004, **21**(2):261-270.
17. Wei X KI, Kim S, Zhang X. : **Compound identification in GC-MS by simultaneously evaluating the mass spectrum and retention index.** *Analyst* 2014, **139**(10):2507-2514.
18. Vinaixa M SE, Neumann S, Navarro M, Salek RM, Yanes O. : **Mass spectral databases for LC/MS- and GC/MS-based metabolomics: State of the field and future prospects**. *Trends Anal Chem* 2016, **78**:23-35.
19. Jaradat N, Al-Lahham S, Abualhasan MN, Bakri A, Zaide H, Hammad J, Hussein F, Issa L, Mousa A, Speih R: **Chemical Constituents, Antioxidant, Cyclooxygenase Inhibitor, and Cytotoxic Activities of Teucrium pruinosum Boiss. Essential Oil**. *Biomed Res Int* 2018, **2018**:4034689.
20. Vartak R, Menon S, Patki M, Billack B, Patel K: **Ebselen nanoemulgel for the treatment of topical fungal infection**. *Eur J Pharm Sci* 2020, **148**:105323.
21. Đorđević SM, Cekić ND, Savić MM, Isailović TM, Ranđelović DV, Marković BD, Savić SR, Timić Stamenić T, Daniels R, Savić SD: **Parenteral nanoemulsions as promising carriers for brain delivery of risperidone: Design, characterization and in vivo pharmacokinetic evaluation**. *Int J Pharm* 2015, **493**(1-2):40-54.
22. Balouiri M, Sadiki M, Ibnsouda SK: **Methods for in vitro evaluating antimicrobial activity: A review**. *J Pharm Anal* 2016, **6**(2):71-79.
23. Eid AM, Jaradat NA, Al-Masri M, Issa L, Zubidat F, Asrawi H, Ahmad S: **Development and Antimicrobial Evaluation of Eruca Sativa Oil Nanoemulgel with Determination of the Oil Antioxidant, Sun Protection Factor and Elastase Inhibition**. *Curr Pharm Biotechnol* 2020, **21**(3):244-255.
24. Jaradat N, Abualhasan MN, Qadi M, Issa L, Mousa A, Allan F, Hindi M, Alhrezat Z: **Antiamylase, Antilipase, Antimicrobial, and Cytotoxic Activity of Nonea obtusifolia (Willd.) DC. from Palestine**. *Biomed Res Int* 2020, **2020**:8821319.
25. Eid AM, Hawash M: **Biological evaluation of Safrole oil and Safrole oil Nanoemulgel as antioxidant, antidiabetic, antibacterial, antifungal and anticancer**. *BMC Complement Med Ther* 2021, **21**(1):159.

26. Hawash M JN, Hameedi S, Mousa A. : **Design, synthesis and biological evaluation of novel benzodioxole derivatives as COX inhibitors and cytotoxic agents.** *BMC Chem* 2020, **14**:54.
27. Plata-Rueda A, Zanuncio JC, Serrão JE, Martínez LC: **Origanum vulgare Essential Oil against Tenebrio molitor (Coleoptera: Tenebrionidae): Composition, Insecticidal Activity, and Behavioral Response**. *Plants (Basel)* 2021, **10**(11).
28. Tejada-Muñoz S, Cortez D, Rascón J, Chavez SG, Caetano AC, Díaz-Manchay RJ, Sandoval-Bances J, Huyhua-Gutierrez S, Gonzales L, Chenet SM *et al*: **Antimicrobial Activity of Origanum vulgare Essential Oil against Staphylococcus aureus and Escherichia coli**. *Pharmaceuticals (Basel)* 2024, **17**(11):1430.
29. Chrismaurin F, Dwiastuti R, Chabib L, Yuliani SH: **The effect of olive oil, tween 60 and span 20 on physical characteristics of quercetin nanoemulgel**. *Int J Appl Pharm* 2023, **15**:212-217.
30. Khaksarian M, Bahmani M, Taherikalani M, Ashrafi B, Rafieian-Kopaei M, Abbasi N: **Biosynthesis of titanium dioxide nanoparticles using Hypericum perforatum and Origanum vulgare extracts and their main components, hypericin and carvacrol as promising antibacterial agents**. *J Tradit Chin Med* 2022, **42**(2):167-175.
31. Salopek B, Krasic D, Filipovic S: **Measurement and application of zeta-potential**. *Rud-geol-naft zb* 1992, **4**(1):147.
32. Taleb MH, Abdeltawab NF, Shamma RN, Abdelgayed SS, Mohamed SS, Farag MA, Ramadan MA: **Origanum vulgare L. Essential Oil as a Potential Anti-Acne Topical Nanoemulsion-In Vitro and In Vivo Study**. *Molecules* 2018, **23**(9).
33. Anwer MK, Jamil S, Ibnouf EO, Shakeel F: **Enhanced Antibacterial Effects of Clove Essential Oil by Nanoemulsion**. *J Oleo Science* 2014, **63**(4):347-354.
34. Sultan MH, Javed S, Madkhali OA, Alam MI, Almoshari Y, Bakkari MA, Sivadasan D, Salawi A, Jabeen A, Ahsan W: **Development and Optimization of Methylcellulose-Based Nanoemulgel Loaded with Nigella sativa Oil for Oral Health Management: Quadratic Model Approach**. *Molecules* 2022, **27**(6):1796.
35. Di Liberto D, Iacuzzi N, Pratelli G, Porrello A, Maggio A, La Bella S, De Blasio A, Notaro A, D'Anneo A, Emanuele S *et al*: **Cytotoxic Effect Induced by Sicilian Oregano Essential Oil in Human Breast Cancer Cells**. *Cells* 2023, **12**(23).

36. Ranjitkar S, Zhang D, Sun F, Salman S, He W, Venkitanarayanan K, Tulman ER, Tian X: **Cytotoxic effects on cancerous and non-cancerous cells of trans-cinnamaldehyde, carvacrol, and eugenol**. *Sci Rep* 2021, **11**(1):16281.
37. Landa P, Kokoska L, Pribylova M, Vanek T, Marsik P: **In vitro anti-inflammatory activity of Carvacrol: Inhibitory effect on COX-2 catalyzed prostaglandin E(2) biosynthesis**. *Arch Pharm Res* 2009, **32**:75-78.
38. Vane JR, Botting RM: **The mechanism of action of aspirin**. *Thromb Res* 2003, **110**(5-6):255-258.
39. Aman RM, Ibrahim AHI, and Meshali MM: **Novel Clove Essential Oil Nanoemulgel Tailored by Taguchi's Model and Scaffold-Based Nanofibers: Phytopharmaceuticals with Promising Potential as Cyclooxygenase-2 Inhibitors in External Inflammation**. *Inter J Nanomed* 2020, **15**:2171-2195.