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RESEARCH ARTICLE

Anticancer and Antibacterial Efficacy of Nanoemulsion of *Matricaria chamomilla* Essential Oil

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ABSTRACT

Conclusions: Cancers and bacterial infections are significant global public health challenges and economic issues. Nanostructures containing essential oils have been considered a suitable alternative as therapeutic agents. In this study, the chemical composition of *Matricaria chamomilla* essential oil (EO) as herbal medicine was investigated using GC-MS analysis. β-hymacalene (23%), limonene (10%), azonol (9%), and α-farnesene (9%) were identified as major compounds. Different tween 20 and tween 80 concentrations were screened to prepare an optimum nanoemulsion containing the EO. It showed a circular shape with droplet size and Zeta potential of 135 ± 8 nm and -21 ± 2 mV, and ATR-FTIR confirmed the successful loading of the EO in droplets. In vitro biological assays demonstrated that the nanoemulsion exhibited significant potency with IC50 values of 30, 311, 544, 88, and 213 µg/mL against A-375, A-431, *Escherichia coli, Pseudomonas aeruginosa*, and *Staphylococcus aureus*, respectively, compared to the unformulated EO. These findings prove the merit of the prepared nanoemulsion as a suitable candidate for further research against other types of cancer, various pathogens, and in vivo studies.

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BACKGROUND

In 2023, the United States recorded approximately 2 million new cancer cases, leading to 609,820 deaths. Among these, skin cancer was notably prevalent, with 105,000 cases and 13,000 related fatalities [1]. Research on developing new treatments often starts with cell line studies. For melanoma skin cancer studies, the A-375 cell line, derived from human melanoma, serves as a crucial model for investigating the disease's pathophysiology and potential therapies [2]. Researchers mostly utilize the A-431 cell line, derived from human epidermoid carcinoma, in studies concentrating on non-melanoma skin cancers. This cell line is exclusively noteworthy for examining the most common forms of skin cancer, squamous cell carcinoma (SCC) and particularly basal cell carcinoma (BCC) [3].

Beyond skin cancers, bacterial invasions pose a substantial risk to dermal wellness. The U.S. Food and Drug Administration (FDA) categorizes a range of conditions under Acute Bacterial Skin and Skin Structure Infections (ABSSSIs). This classification includes diverse skin irritations such as cellulitis, erysipelas, infected wounds, and significant cutaneous abscesses, all of which present serious challenges to skin health [4, 5]. These infections can

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lead to severe complications if untreated, and rising antibiotic resistance has heightened the urgency to find alternative treatments. Furthermore, microorganisms affect human skin and damage textiles by causing discoloration, staining, and fiber decomposition, ultimately weakening materials and accelerating decay [6, 7]. Different bacterial pathogens contribute to various skin issues, from mild irritations to severe infections. For instance, *Escherichia coli*, commonly found in the gut, can cause skin infections, wound infections, and cellulitis [8]. *Staphylococcus aureus,* a well-known skin pathogen, is linked to conditions such as folliculitis, impetigo, cellulitis, abscesses, and Staphylococcal scalded skin syndrome [9, 10]. *Pseudomonas aeruginosa*, which often affects immunocompromised individuals, can lead to hot tub folliculitis, wound infections, and ecthyma gangrenosum [11].

The increasing prevalence of drug resistance has sparked a resurgence of interest in herbal remedies, particularly essential oils (EOs) and plant extracts, as potential alternative therapies [12, 13]. Among these, *Matricaria chamomilla*, customarily referred to as German chamomile, is distinguished for its diverse biological activities. Research has affirmed that this plant exhibits a wide spectrum of lucrative properties, including but not limited to antioxidant, anti-inflammatory, pain-relieving, and antimicrobial effects. Additionally, it has shown promise in managing diabetes, combating parasites, alleviating allergies, reducing anxiety, inducing sedation, and alleviating depression. Further studies have disclosed its potential as an anticonvulsant, blood pressure depressor, lipid-lowering agent, and cognitive enhancer [14, 15]. Besides, its antibacterial efficacy has been demonstrated against *Enterococcus faecalis*, *Staphylococcus aureus*, *Aspergillus flavus*, *Candida albicans*, and *Bacillus cereus* [16-18]. Additionally, *M. chamomilla* EO has shown the potential to inhibit psoriatic skin injury and inflammation. These effects were investigated in vitro and in vivo models, including clinical samples from psoriasis patients and an imiquimod-induced psoriatic-like skin inflammation model in mice [19]. Furthermore, *M. chamomilla* has been the focus of scientific studies for treating various skin conditions [20, 21].

To fully harness the medicinal potential of EOs, it is crucial to enhance their stability, solubility, bioavailability, and efficacy. One promising solution is the development of nanoformulations incorporating EOs [22, 23]. Among these, nanoemulsions stand out due to their simple, cost-effective preparation methods and the use of safe materials, making them highly effective formulations on the nanoscale [24, 25]. In this study, a nanoemulsion of *M. chamomilla* EO was prepared, and a comprehensive comparison of the cytotoxic and antibacterial effects of the EO and its nanoemulsion was conducted against A-375 and A-431 cancer cells, as well as the bacteria *S. aureus, E. coli,* and *P. aeruginosa*.

MATERIALS AND METHODS

Materials

The researchers utilized Tween 20 and Tween 80, which were obtained from Merck Chemicals (USA). The essential oil (EO) of *M. chamomilla*, extracted from its bark, was purchased from the Tabib Daru Company (Iran). The human cancer cell lines, including both malignant melanoma (A-375) and epidermoid carcinoma (A-431), were procured from Iran's Pasteur Institute. Likewise, the study used several bacterial strains, containing *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), and *Staphylococcus aureus* (ATCC 25923), which the same institution also provided.

GC-MS process

Analysis of *M. chamomilla* EO's chemical components was conducted using a GC-MS instrument featuring an HP-5MS column, following previously established protocols [26]. The analysis was performed using an HP-5MS silica fused column under specific operational parameters. The temperature protocol began at 40 °C and moderately increased to 250 °C at 3 °C per minute, conserving this final temperature for a duration of 60 minutes. The system's injection port was maintained at 250 °C, while the detector operated at 230 °C. Helium served as the carrier gas in the system, with operational specifications including a 25 mL/min split flow, 6 mL/min septum purge, and a column flow rate maintained at 1 mL/min. Mass spectral data were collected using full scan mode, employing 70 eV ionization energy, scanning across a mass range of 50–550 m/z. Component identification was accomplished by comparing retention indices and mass spectra against the Wiley7n.l MS computer library database. Retention indices were calculated through interpolation of the linear temperature-programmed data from

the gas chromatogram. The relative quantities of various compounds in the EO were determined through the normalization of peak areas in the *M. chamomilla* EO analysis.

Preparation and characterizations of nanoemulsion containing M. chamomilla EO

The fabrication of *M. chamomilla* EO nanoemulsion was accomplished through spontaneous emulsification methodology (Figure 1). The process involved combining 10 µL of EO with varying concentrations of tween 20 and tween 80, mixed independently for 3 minutes under room temperature conditions at 2000 rpm. The mixture was then gradually supplemented with distilled water to achieve a final volume of 5000 µL, followed by continuous agitation for 40 minutes (2000 rpm, RT). The resulting emulsions underwent size analysis using a DLS instrument (K-One Nano, Ltd, Korea). Optimal characteristics were defined as droplet dimensions below 200 nm and a size distribution index (SPAN) less than 1, as per established standards [27]. The optimized nanoemulsion formulation was further characterized through zeta potential measurements and visualized using transmission electron microscopy (TEM Philips EM 208S, Netherlands). A control nanoemulsion (NE(-EO)) was similarly prepared, excluding the *M. chamomilla* EO component. The successful incorporation of EO within the nanoemulsion structure was verified through ATR-FTIR spectroscopy, analyzing spectra across the 400-3500 cm-1 range using a Bruker Tensor II Spectrometer (Germany).

Nanoemulsion stability was assessed through comprehensive short-term and long-term evaluations. The short-term durability assessment amalgamated three distinct testing protocols: centrifugation, heating-cooling cycles, and freeze-thaw cycles.

Centrifugation Test: The nanoemulsion was centrifuged at 22,000 g for 30 minutes at three different temperatures: -4°C, +4°C, and 25°C. This test aimed to identify any phase separation, such

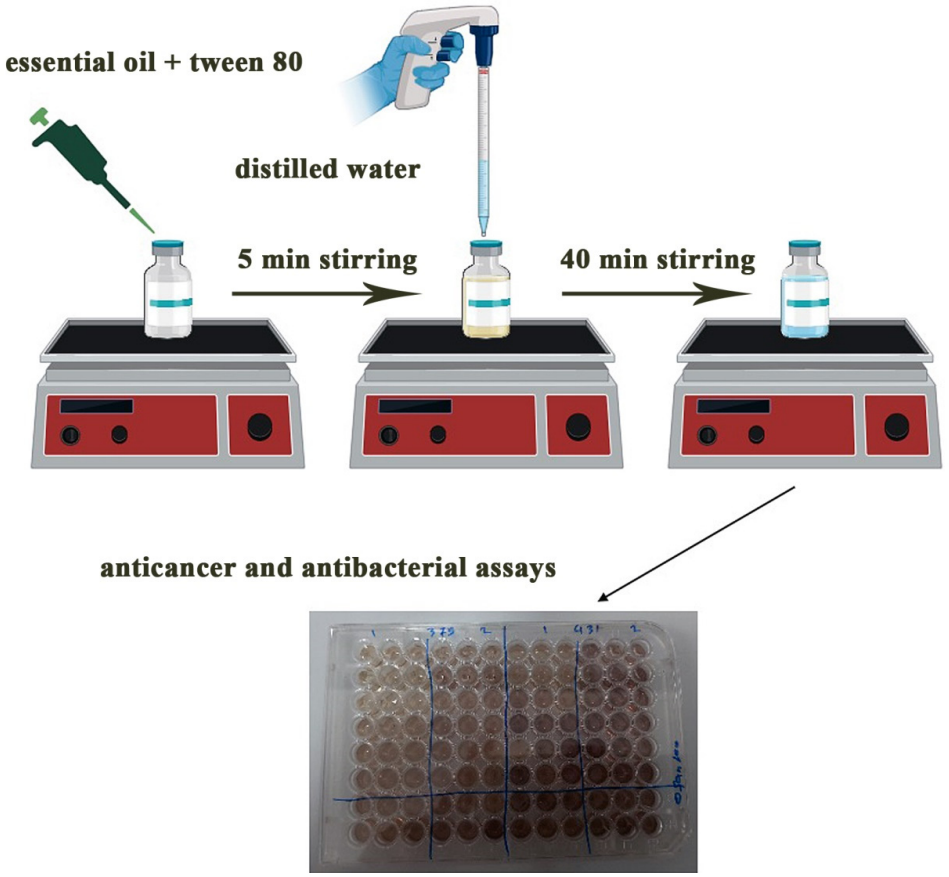

Fig. 1. Preparation process of the nanoemulsion containing *M. chamomilla* EO and next steps

as creaming, sedimentation, or biphasic behavior under these varying thermal conditions.

Heating-Cooling Cycle: The nanoemulsion was subjected to six consecutive storage cycles, each lasting 48 hours, in alternating conditions of 4°C (refrigeration) and 45°C (Bain-Marie). This test simulates temperature fluctuations to determine the emulsion's ability to withstand variations between cold and warm environments.

Freeze-Thaw Cycle: The nanoemulsion's constancy was measured through six sequential freeze-thaw cycles, intermittent between -25°C and ambient temperature (25°C), with each cycle lasting 48 hours. This rigorous testing protocol was designed to evaluate the formulation's stability under extreme temperature fluctuations. Following each cycle, the nanoemulsion underwent a comprehensive assessment, including a visual examination for potential instability indicators such as phase separation, sedimentation, or cream formation. Additionally, DLS analysis was performed to track any modifications in the droplet size distribution pattern.

Upon completing these short-term stability assessments, the nanoemulsion proceeded to long-term stability evaluation. This extended analysis involved storing samples at two distinguished temperatures (4°C and 25°C) over a six-month duration. Throughout this period, the formulation was regularly monitored for any signs of physical instability, including the formation of biphasic systems, creaming phenomena, or sediment development.

Investigation of cytotoxic effects

Cell viability of A-375 and A-431 cell lines exposed to *M. chamomilla* EO and its nanoemulsion was assessed using the MTT assay protocol. The empirical procedure involved culturing cells in DMEM perfect medium (enriched with 10% FBS and 1% antibiotics) at a compactness of 104 cells per well in 96-well plates. The cells were incubated for 24 hours (37 °C, 5% CO2) till reaching 80% confluence. Subsequently, the culture medium was exchanged with a combination of fresh DMEM perfect medium (50 µL per well) and varying concentrations of test samples (31-1000 µg/mL) prepared in PBS solution. The control group received PBS solution (50 µL per well), while the negative control group was treated with NE(-EO). After a 24-hour incubation period, the medium was replaced with MTT solution (0.5 mg/mL in DMEM, 50 µL per well). After 4 hours of incubation, the organized formazan crystals were solubilized using DMSO (100 µL per well). Cell viability was calculated by comparing the optical density measurements of treated samples to the control group at 570 nm, using a Synergy HTX Multi-Mode Reader (USA).

Investigation of antibacterial effects

The antibacterial properties against bacterial strains, including *E. coli*, *P. aeruginosa*, and *S. aureus,* were determined through a microdilution technique utilizing 96-well plates. The experimental procedure involved preparing bacterial suspensions in Muller-Hinton broth adjusted to 0.5 McFarland standard (equivalent to 1.5 × 108 CFU/mL). These suspensions were then blended with various concentrations of test samples (31-1000 µg/mL in PBS solution) in identical volumes (50:50 µL) per well. PBS solution and NE(-EO) were administered to the control and negative control groups, respectively, at 50 µL per well. After 24 hours of incubation, bacterial growth prevention was measured by comparing the optical density readings of treated samples with the control group at 630 nm, exploiting a plate reader.

Statistical analyses

Experimental procedures were performed in triplicate to ensure authenticity, with outcomes expressed as mean values accompanied by standard deviations. To calculate the comparative effectiveness of *M. chamomilla* essential oil and its nanoemulsion formulation, an Independent Sample T-test was employed using GraphPad Prism software. Statistical significance was defined at $P < 0.05$. Furthermore, the IC_{50} values for the test samples were calculated utilizing the free version of CalcuSyn software (BIOSOFT, UK).

RESULTS

Identified compounds in *M. chamomilla* EO using GC-MS analysis are listed in Table 1. The four main compounds included β-himachalene (23%) and limonene (10%), as well as azunol (9%), a-farnesene (9%).

Table 2 shows that various tween 20 and 80 concentrations were examined to formulate a nanoemulsion containing *M. chamomilla* EO. The optimal conditions, characterized by a droplet size greater than 200 nm and a SPAN value below 1, were achieved in sample 4. The DLS profile of

Table 1. Chemical composition of *M. chamomilla* EO by GC-MS analysis

Retention Time (min)	Compound	Area	%	Kovats Index
11	**limonene**	**13125168176**	**10**	**683**
17	4-Terpineol	8767401867	6	841
28	*trans*-caryophyllene	6901544740	5	1035
29	α.-himachalene	6192319688	4	1060
30	**α-farnesene**	**11843027504**	**9**	**1074**
30	ɣ-himachalene	8888301188	6	1086
31	**β-himachalene**	**27434197102**	**23**	**1109**
35	2,6-di-butyl-2,5-cyclohexadiene-1,4-dione	1798629738	1	1182
37	α-bisabolol oxide B	1786175251	1	1214
37	1,3-Dimethyl-5-n-hexyladamantane	1553785683	1	1226
38	bisabolone oxide	1414781794	1	1234
38	mesitylene	2589443005	2	1244
39	ar-turmerone	2641816671	2	1253
39	α-Atlantone	1683682106	1	1261
40	bisabolol oxide A	4675170605	3	1286
41	**azunol**	**13039458851**	**9**	**1310**

Table 2. Prepared nanoemulsions containing *M. chamomilla* EO

No	*M. chamomilla* EO (µL)	tween 20 (µL)	tween 80 (µL)	Droplet size (nm)	SPAN	No. peak
1	10	10	--	252	1.04	1
2	10	12	--	308	1.06	2
3	10	14	--	1040	0.97	1
4	10	--	10	135	0.99	1
5	10	--	12	203	0.97	1
6	10	--	14	285	1.7	2

*All samples reached 5000 µL by distilled water

this sample (135± 8 and SPAN 0.99) is presented in Figure 2A. Additionally, the Zeta potential measured was -21 ± 2 mV (Figure 2B), indicating good stability, and the droplets were observed to be circular in shape, as shown in Figure 2C.

The nanoemulsion containing *M. chamomilla* EO exhibited remarkable stability during short-term and long-term evaluations. As illustrated in Figures 3A and 3B, the nanoemulsion retained its structural integrity in the short-term tests, including centrifugation, heating-cooling, and freeze-thaw cycles. No signs of sedimentation, creaming, or biphasic separation were observed throughout the centrifugation at 22,000 g or during the temperature stress tests. The droplet size, measured at 125 ± 5 nm, remained consistent, with a SPAN value of 0.99, indicating a narrow size distribution and uniformity in droplet size. Additionally, the zeta potential was measured at -19 ± 2 mV, suggesting good electrostatic stability and resistance to particle aggregation.

In the long-term stability analysis, after six months of storage at 4°C and 25°C, the nanoemulsion showed no visible signs of instability, such as sedimentation, creaming, or phase separation. Both short- and long-term assessments verified the nanoemulsion's robust stability.

The ATR-FTIR spectral analysis of *M. chamomilla* EO (Figure 4) revealed multiple characteristic peaks: a band at 3481 cm^{-1} indicating OH groups involved in hydrogen bonding; signals at 2960 and 2914 cm^{-1} representing –CH groups;

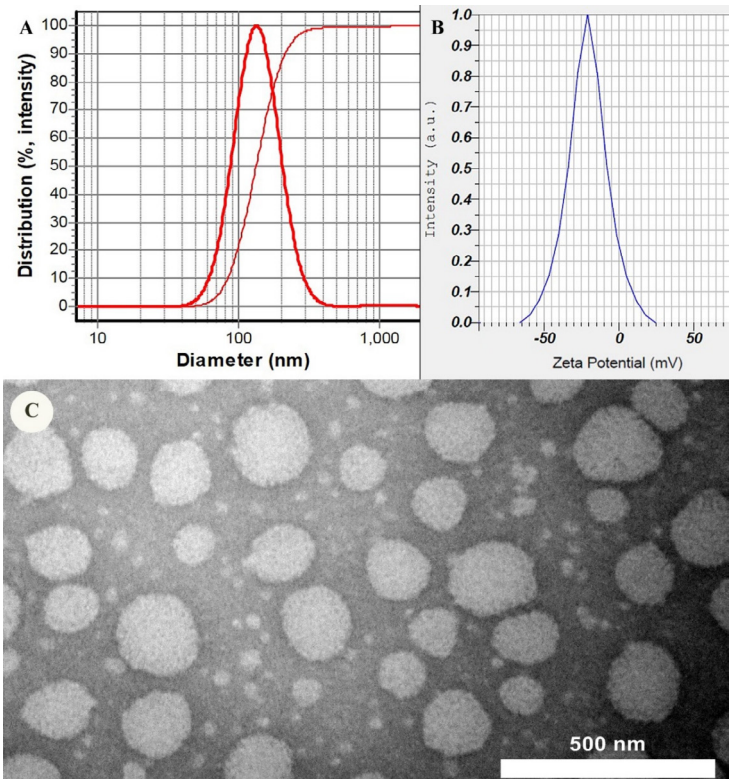

Fig. 2. Characterization of the optimum nanoemulsion containing *M. chamomilla* EO; A: DLS profile, B: Zeta potential profile, and C: TEM image

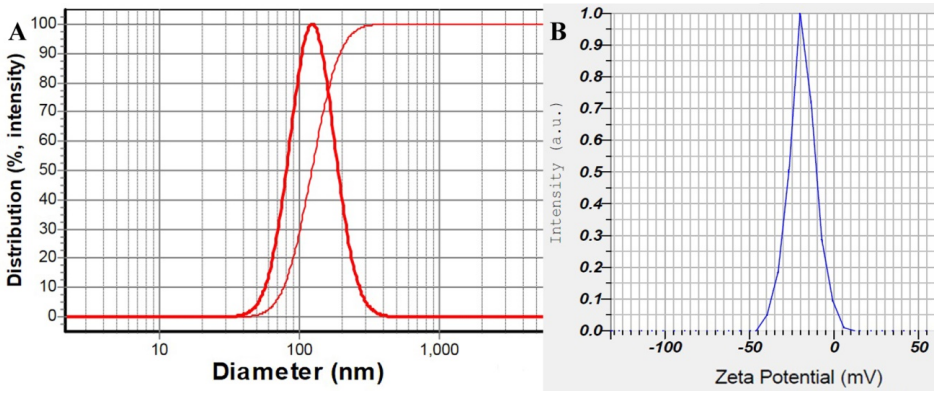

Fig. 3. Characterization of the optimum nanoemulsion containing *M. chamomilla* EO after short-term stability analyses; A: DLS profile and B: Zeta potential profile

a peak at 2358 cm^{-1} denoting CO2; bands at 1715 and 1673 cm^{-1} identifying carbonyl groups. The aromatic ring C=C skeletal vibrations were evidenced by peaks at 1626 and 1444 cm^{-1}. C-O stretching vibrations manifested at 1121 and 1155 cm^{-1}. Benzene ring C-H vibrations appeared at 887 cm^{-1}, while alkene vibrations were detected at 798 cm^{-1}.

The spectrum of blank nanoemulsion (NE(-EO)) exhibited a broad band between 3200-3600 cm^{-1}, characteristic of OH stretching vibrations resulting from water-tween 80 hydrogen bonding interactions. Additional features included C-H stretching at 2923 cm^{-1}, carbonyl (C=O) stretching of tween 80 at 1733 cm^{-1}, CH2 bending at 1460 cm^{-1}, and a distinctive C-O stretching band at 1078 cm^{-1}.

The nanoemulsion's ATR-FTIR spectrum displayed several key features: OH stretching at

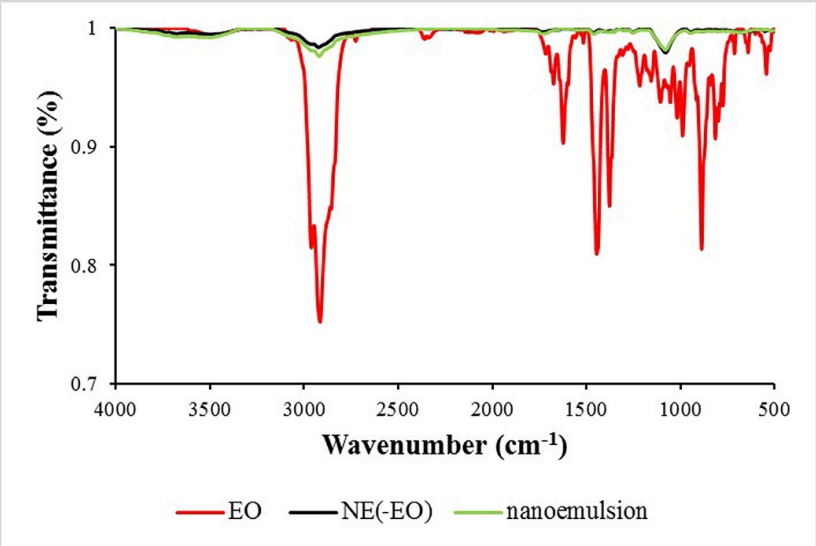

Fig. 4. ATR-FTIR spectra of *M. chamomilla* EO, NE(-EO), and nanoemulsion containing *M. chamomilla EO*

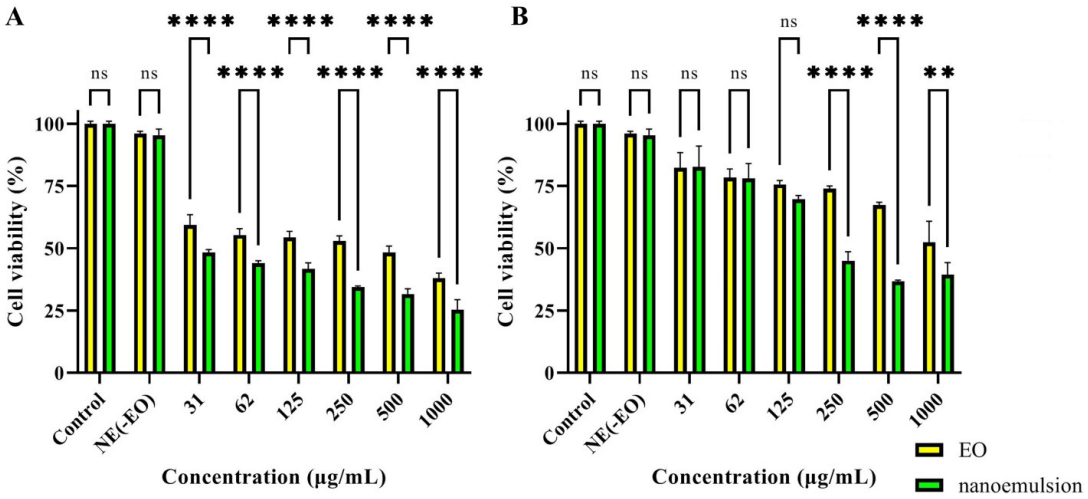

Fig. 5. Cytotoxic effects of *M. chamomilla* EO and its nanoemulsion; A: A-375 and B: A-431 cells
ns: not significant, **: $P < 0.01$, and **: $P < 0.0001$

Table 3. Obtained IC_{50} values (µg/mL) of *M. chamomilla* EO and its nanoemulsion

Samples	Factors	A-375	A-431	*E. coli*	*P. aeruginosa*	*S. aureus*
***M. chamomilla* EO**	**IC_{50}**	219	2765	1411	152	544
	LCL-UCL*	133-361	982-7734	894-2225	121-190	300-987
nanoemulsion	**IC_{50}**	30	331	544	88	213
	LCL-UCL	21-43	235-465	347-852	66-118	162-282

*Lower Confidence Limit-Upper Confidence Limit

3472 cm^{-1}, indicating hydrogen bonding among EO, tween 80, and water components; C-H stretching at 2918 cm^{-1} from both EO and tween 80; a prominent carbonyl stretching band at 1730 cm^{-1}; CH2 bending at 1463 cm^{-1}; and C-O stretching at 1078 cm^{-1}. The presence of characteristic bands from both EO and blank nanoemulsion components confirmed successful EO incorporation into the nanoemulsion system.

The cytotoxic effects of *M. chamomilla* EO and its nanoemulsion on A-375 and A-431 cells are illustrated in Figure 5. The negative control sample, i.e., NE(-EO), did not impact cell viability. As summarized in Table 3, significant differences ($P < 0.05$) were observed between the IC_{50} values of *M. chamomilla* EO and its nanoemulsion in both cell lines. Additionally, the nanoemulsion exhibited greater efficacy against A-375 cells than A-431 cells, with the difference being statistically significant ($P < 0.05$).

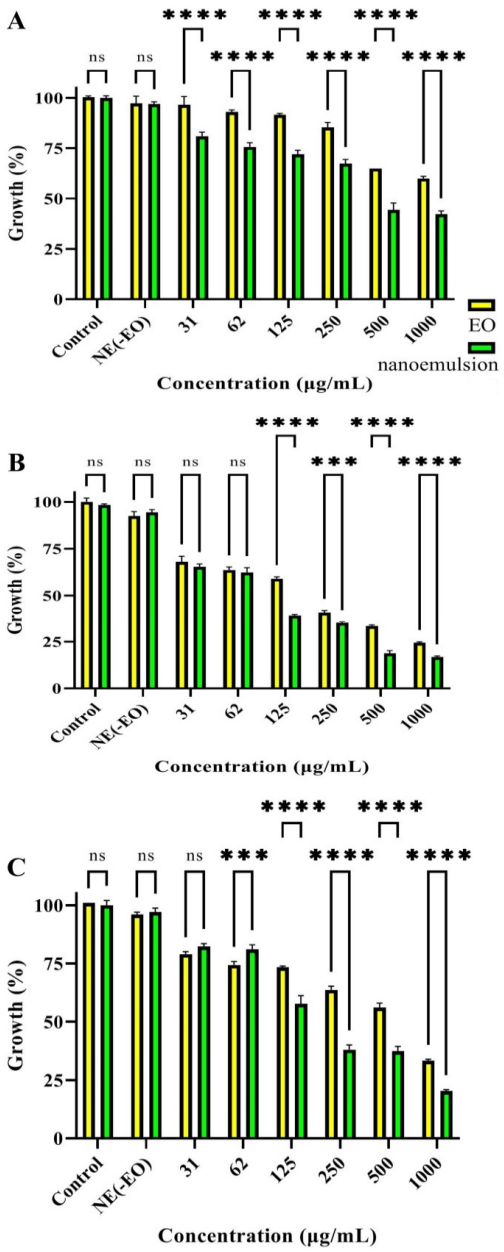

Fig. 6. Antibacterial effects of *M. chamomilla* EO and its nanoemulsion; A: *E. coli*, B: *P. aeruginosa*, and C: *S. aureus*
ns: not significant, ***: $P < 0.001$, and **: $P < 0.0001$

The antibacterial tests demonstrated that the nanoemulsion displayed superior efficacy to the unformulated *M. chamomilla* EO across all three bacterial strains, as shown in Figure 6. The negative control sample, i.e., NE(-EO), did not affect bacterial growth. As detailed in Table 3, the IC_{50} values of the nanoemulsion were significantly lower than those of *M. chamomilla* EO ($P < 0.05$) for all three bacteria. Notably, the highest antibacterial efficacy was observed against *Pseudomonas aeruginosa*.

DISCUSSIONS

This research utilized *M. chamomilla* EO to investigate both its anticancer and antibacterial potential. While the specific mechanisms behind these properties in *M. chamomilla* EO are not fully elucidated, insights can be drawn from the diverse biochemical pathways through which other EOs operate. The antibacterial effects of EOs generally encompass several strategies: They can compromise bacterial cell membrane integrity, resulting in the escape of vital cellular components and subsequent cell death. Some EOs inhibit the development of bacterial biofilms, protective structures that enhance antibiotic resistance. EOs can also disrupt bacterial quorum sensing, a communication network used by bacteria to synchronize group behaviors, including virulence factors. Furthermore, compounds in EOs may stimulate oxidative stress within bacterial cells, causing damage to proteins, lipids, and DNA. Lastly, EOs have been shown to deter the function of crucial bacterial enzymes essential for survival and pathogenicity [28-30].

EOs exert anticancer effects through several proposed mechanisms. They can induce apoptosis, or programmed cell death, in cancer cells by activating various pathways. Some EOs hinder the cell cycle, preventing cancer cells from dividing and proliferating. Additionally, certain EOs can disrupt angiogenesis, the process of forming new blood vessels, thereby depriving tumors of essential nutrients. EOs also possess anti-inflammatory properties that help reduce inflammation often linked to cancer progression. Furthermore, they can modulate critical signaling pathways involved in cancer cell proliferation, survival, and metastasis. Lastly, EOs may induce autophagy, a process of cellular self-digestion, as well as senescence, a state in which cancer cells permanently cease dividing. Together, these mechanisms contribute to the potential of EOs as therapeutic agents in cancer treatment [31-33].

The research focused on preparing nanoemulsions containing *M. chamomilla* EO using varying concentrations of tween 20 and tween 80. It was found that the final nanoemulsion formulated with tween 80, which has a hydrophilic-lipophilic balance (HLB) value of 15.0, was more lipophilic than tween 20, which has an HLB value of 16.7. In spontaneous emulsification processes, correctly matching the HLB values of the surfactant and the EO is essential for ensuring effective nanoemulsion formulation [34, 35]. Subsequently, the anticancer effects of *M. chamomilla* EO and its nanoemulsion were evaluated on A-375 and A-431 cancer cell lines. The calculated IC_{50} values were 30 µg/mL for A-375 and 331 µg/mL for A-431, indicating promising results, particularly for A-375. According to the National Cancer Institute and Geran protocol criteria, herbal medicines with IC_{50} values below 20 µg/mL and between 21–200 µg/mL are considered to have highly and moderately cytotoxic effects, respectively [36]. The enhanced efficacy of the *M. chamomilla* EO nanoemulsion is likely due to improved bioavailability and stability, which may increase the interaction of the active compounds with cellular membranes and bacterial walls [37].

Research findings suggest that *M. chamomilla* represents anticancer traits in vitro, exclusively against breast cancer cell lines MCF-7 and MDA-MB-468. One study demonstrated that the hydroalcoholic extract of *M. chamomilla* suppressed cancer cell invasion and migration in a manner dependent on both time and dosage [38]. Another research documented the cytotoxic effects of *M. chamomilla* root extract on MCF-7 cells, with an IC_{50} value of 1954 ± 4.2 µg/mL [39]. Moreover, the efficacy of *M. chamomilla* EO has been examined across various cancer cell lines, producing unlike IC_{50} values: 208.54 ± 1.39 µg/mL for A549 (non-small cell lung cancer), 315.44 ± 1.17 µg/mL for MCF-7 (breast cancer), 197.52 ± 0.98 µg/mL for PC3 (prostate cancer), and 638.79 ± 1.15 µg/mL for HEK293 (human embryonic kidney) cells. These findings emphasize the adoptive effectiveness of *M. chamomilla* EO against different cell types [40]. Furthermore, studies have shown that nanoformulations of other EOs, such as *Zingiber ottensii* and *Trachyspermum ammi*, enhance anticancer activity against MCF-7 breast and HT-29 colon cancer cells compared to their unformulated counterparts [41, 42]. Similarly,

nanoformulations containing lavandin EO have shown promising effects against neuroblastoma, breast cancer, lymphoblastic leukemia, and colorectal adenocarcinoma [43]. These findings suggest that nanoformulations can improve drug delivery efficiency, increase cellular uptake, and induce apoptosis in cancer cells more effectively than unformulated EOs [44, 45]. This underscores the potential of utilizing nanoformulations to enhance the therapeutic efficacy of natural compounds like *M. chamomilla*.

The current study assessed the antibacterial efficacy of *M. chamomilla* EO and its nanoemulsion against bacterial species *E. coli*, *P. aeruginosa*, and *S. aureus*. Results indicated that both forms effectively inhibited bacterial growth, with a comparative analysis of IC_{50} values revealing that the nanoemulsion had significantly lower IC_{50} values than the EO. Specifically, for *E. coli* and *S. aureus*, the IC_{50} values for the nanoemulsion were 544 µg/mL and 213 µg/mL, respectively, approximately 60% lower than the corresponding values for the EO, which were 1411 µg/mL and 544 µg/mL. Notably, *P. aeruginosa* showed a particularly low IC_{50} of 88 µg/mL when treated with the nanoemulsion. According to existing classifications, herbal antibacterials with IC_{50} values below 100 µg/mL are considered highly effective, while those ranging from 100 to 500 µg/mL demonstrate moderate activity [46]. The promising results of the nanoemulsion, especially against *P. aeruginosa*, suggest the need for further investigation into its mechanisms of action and optimization for enhanced therapeutic outcomes.

CONCLUSION

This study successfully formulated an optimum nanoemulsion of *M. chamomilla* EO, enhancing its stability and bioavailability. The comparative evaluation demonstrated that the nanoemulsion exhibited significantly improved anticancer and antibacterial activities compared to the non-formulated essential oil. Specifically, the nanoemulsion showed higher cytotoxicity against A-375 and A-431 cancer cell lines and more potent antibacterial effects against *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. These findings suggest that nanoemulsions represent a promising approach for improving the therapeutic efficacy of essential oils in both cancer treatment and antibacterial applications. Further studies are recommended to explore the underlying mechanisms and assess the potential for clinical translation.

DECLARATIONS

Ethics approval and consent to participate

This study has been ethically approved by the Fasa University of Medical Sciences (IR.FUMS.REC.1402.090). Since this research did not involve human study, the informed consent form was thus not used.

Consent for publication

Not applicable.

Availability of data and materials

All data are available upon reasonable request from the corresponding author.

Competing interests

None.

Funding

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Acknowledgments

Not applicable.

Authors' contributions

ZQ prepared nanoemulsion and performed anticancer tests. HA drafted MS in cooperation with MF. SK performed antibacterial tests. EZ interpreted ATR-FTIR spectra. MO designed the study, analyzed data, and drafted the MS. All authors contributed to the drafted MS and approved the final MS.

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