Introduction

Today, with the increasing prevalence of bacterial infections and incessant utilization of multiple antibiotics and antimicrobial drugs, we are witnessing the widespread spread of antibiotic-resistant microbial species and their subsequent problems and complications.\(^1\)\(^-\)\(^3\) During the last few decades, infectious diseases have been a global health problem, attracting global attention as a threat to human health.\(^4\)\(^,\)\(^5\) Due to the high mortality rate of infectious diseases, many studies have been dedicated to prevent, control, and early diagnose them.\(^5\)\(^-\)\(^7\) In this regard, nanoencapsulation of essential oils (EOs), because of their transferability and high loading capacity, is one of our greatest hopes.\(^8\)\(^-\)\(^10\) The preparation of such formulations can increase the target compound’s solubility, stability, and biological activities, reduce its volatility, and improve its physical and chemical properties.\(^11\)

Some studies indicate that some plant extracts and their chemical compounds have antibacterial and antimicrobial effects, suggesting them as potential agents for treating infections.\(^12\)\(^,\)\(^13\) Accordingly, the potential antibacterial effects of the EOs deliver them suitable substitutes for antibiotics.\(^14\) Artemisia vulgaris is a species historically known for its medicinal applications, which had been called the “Mother of Plants” in the Middle Ages, and a herbaceous plant with high morphological and antioxidant effects.

Antibacterial and Antioxidant Effects of PLGA-Based Nanoparticles Modified With Chitosan-Folic Acid for Delivering Artemisia vulgaris Essential Oil

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Abstract

**Introduction:** Many diseases associated with oxidative stress threaten human health, exaggerated by antibiotic-resistant bacterial infections due to the increasing use of antibiotics. The aim of the present study was to evaluate the antioxidant and antimicrobial activities of a nanoemulsion prepared from *Artemisia vulgaris* essential oil (EO).

**Methods:** Poly (lactic-co-glycolic acid) (PLGA) nanoparticles (NPs) were synthesized in this study. The disk diffusion method, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) were used to investigate the antibacterial properties of the synthesized NPs. In addition, the microdilution method was utilized to examine the antimicrobial properties of the synthetic substance. Inhibitory concentrations (MIC and MBC) were determined against *Staphylococcus aureus*, *Micrococcus luteus*, *Escherichia coli*, and *Klebsiella pneumoniae*. Finally, the antioxidant activity of the nanoemulsion was evaluated using the 1,1-diphenyl-2-picryl-hydrazyl biochemical method.

**Results:** The findings of this study demonstrated that the synthesized NPs had significant growth inhibitory effects against Gram-positive bacteria with considerable inhibition of growth being observed for *S. aureus* and *M. luteus*.

**Conclusion:** In general, Artemisia EO seems to be beneficial for treating bacterial infections owing to its antimicrobial properties and antioxidant effects. Further therapeutic applications of this EO as a potential drug carrier are yet to be divulged in future studies.

**Keywords:** Artemisia essential oil, Nanoemulsion, Antibacterial, Antioxidant


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phytochemical diversity depending on the geographical location. Its plant, *A. vulgaris* herb, is used as a source of EOs, flavonoids, and sesquiterpenoid lactones due to their diverse biological activities. The European Pharmacopoeia has listed this species as a potential homeopathic raw material. The beneficial properties of the *A. vulgaris* plant extract, including hepatoprotective, antispasmodic, analgesic, estrogenic, cytotoxic, antibacterial, and antifungal effects, have been confirmed by many studies. In addition, some studies suggest the use of this species to produce cosmetics and its role as a valuable spice in the food industry.

*Artemisia vulgaris* has a long history as a medicinal plant in the treatment of human diseases. This medicinal plant has a wide range of therapeutic applications, and these activities are mainly attributed to the presence of different classes of secondary metabolites, including flavonoids, sesquiterpene lactones, coumarins, acetylenes, phenolic acids, organic acids, and mono- and sesquiterpenes. Studies on the morphology, anatomy, and phytochemistry of *A. vulgaris* have provided valuable information for a better understanding of how to concentrate on the most important therapeutic compounds of this species. Recently, phytochemical and pharmacological experiments have confirmed the therapeutic potential of the bioactive compounds of *A. vulgaris*, offering this plant various medical and biotechnological applications and highlighting the importance of developing effective methods for the controlled and efficient purification of its bioactive compounds to be used in modern medicine.

*Staphylococcus aureus*, *Micrococcus luteus*, *Escherichia coli*, and *Klebsiella pneumoniae* are among the most important human pathogenic bacteria and common causes of communicable diseases. These bacteria are highly resistant to antibiotics such as penicillin and ampicillin. Research represents that *E. coli* is the most common cause of urinary tract infections, along with *K. pneumoniae* and *Proteus*. Antibiotic resistance has significantly increased in the last few decades, as well as an increase in the side effects of using antibiotics such as colistin, which is currently the only treatment for infections caused by Gram-negative bacteria, including *K. pneumoniae*. Nowadays, the use of natural antimicrobial compounds along with antibiotics has acquired attention as a way to reduce the use and dosage of antibiotics. The primary purpose of this study was to determine the antioxidant and antibacterial effects of a nanoemulsion prepared from the *A. vulgaris* EO against pathogenic bacteria (with high clinical importance) by determining the minimum bactericidal concentration (MBC) and minimum inhibitory concentration (MIC).

**Materials and Methods**

**Bacterial Strains and Culture Conditions**

The strains used in this study were *S. aureus* ATCC 25923, *M. luteus* ATCC4968, *E. coli* ATCC1330, and *K. pneumoniae* ATCC700603, which were obtained from Iran’s Scientific and Industrial Research Organization.

**Nanoemulsion Preparation**

For nanoemulsion synthesis, the *A. vulgaris* EO was added to a solution containing PLGA/dichloromethane. Then, polyvinyl alcohol (2%) was added to the obtained solution and homogenized, and 10 mL of polyvinyl alcohol (0.1%) was added to the solution and incubated for 2 hours to evaporate the solvent.

**Evaluation of 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) Free Radical Inhibition**

The antioxidant activity was determined by the DPPH method. The basis of this method is the neutralization of DPPH free radicals produced in laboratory conditions by an antioxidant compound, causing a solution to change its color from purple to yellow, which can then be evaluated by spectrometry. Briefly, 750 µL of the DPPH solution (4 mg/100 mL MeOH) was incubated with 250 µL of nanoparticles (NPs). After 30 minutes of being placed in darkness at room temperature, the absorbance of the mixture was measured at 517 nm. Nanoemulsion (without the EO) diluted with ethanol and DPPH was used as a negative control, and the combination of glutathione and DPPH was employed as a positive control. The result of this test was expressed as half maximal inhibitory concentration (IC$_{50}$), indicating a concentration of the nanoemulsion that can scavenge 50% of DPPH free radicals.

**Disc Diffusion (Kirby-Bauer) Method**

This experiment was performed in three replicates using Gram-positive, namely, *S. aureus* (ATCC 25923) and *M. luteus* (ATCC 4968), as well as Gram-negative, namely, *E. coli* (ATCC 1330) and *K. pneumoniae* (ATCC 700603), bacteria. Blank discs were used to prepare antibiotic discs. Each blank disc was impregnated with the NPs synthesized using the EO of the herb at a concentration of 1 mg/mL. With the help of a sterile swab, the bacterial suspension (equal to the turbidity of 0.5 McFarland) was uniformly cultured on the Mueller-Hinton agar medium. Antibiotic discs were placed on the surface of the medium using sterile forceps at standard distances (1.5 cm from the edge of the plate and 2.4 cm from the center of two adjacent discs). The plates were incubated at 37 degrees Celsius in aerobic conditions for 24 hours. After this period, the diameter of the growth halo around each antibiotic was measured in millimeters using a ruler. Gentamicin antibiotic disc (as a control) was employed to confirm antibiotic sensitivity tests.

**Determining the Minimum Lethal and Minimum Inhibitory Concentrations**

To determine the MIC and MBC, the microbroth dilution
method was applied applying a 24-well microplate. The NPs synthesized with the herbal EO were prepared at the concentrations of 0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, and 2 mg/mL. Chloramphenicol antibiotic powder at a concentration of 100 μg/mL was employed as a control. After the inoculation of bacteria in the wells, the microplate was placed in an incubator for 24 hours at 37 °C. Next, the first well in which no growth was observed was indicative of the minimum growth inhibitory concentration (MIC), from which and higher dilutions, 10 μL was placed on a nutrient agar culture medium. The dilution with no sign of growth represented the minimum growth lethal concentration (MBC).  

Antimicrobial Activity Assessment by the Microdilution Method
In the microdilution method, 96-well microplates were used to investigate the antimicrobial effects of the nanoemulsion. Each well of the plate contained a combination of the culture medium, nanoemulsion, and microbial suspension. In this experiment, the inhibitory effects of different concentrations of NPs synthesized with the A. vulgaris EO were examined against S. aureus and M. luteus at 0 and 24 hours at 37 degrees Celsius. The wells contained 200 μL of the Mueller-Hinton Broth culture medium, 10 μL of the microbial suspension (at a concentration equivalent to 0.5 McFarland), and 10 μL of different concentrations of the synthetic material.

After microbial inoculation, the microplates were incubated at 37°C. Absorption at 630 nm in the microplates was read at zero time (i.e., before heating) and 24 hours (i.e., after heating) by an enzyme-linked immunosorbent assay reader device. After determining the absorbance of the wells, the growth inhibition percentage for the bacteria tested was calculated using equation (1).

\[
\text{Growth inhibition percentage} = \frac{\text{O} - \text{E}}{\text{O}} \times 100 \text{ Eq. (1)}
\]

where O is the absorbance of positive control after 24 hours (its absorbance at the zero time), and E indicates the absorbance of the sample containing the synthetic material and bacterial suspension at 24 hours (its absorbance at the zero time).

Statistical Analysis
The statistical analysis for the obtained data was calculated as the means ± standard deviation (SD) and performed by SPSS (16.0) software. The differences between the test groups were compared with the control group by the analysis of variance with the least significant difference test, and \( P < 0.05 \) was considered statistically significant.

Results
DPPH Scavenging Activity of NPs
Based on the results of Figure 1, the synthesized nanoemulsion neutralized DPPH free radicals in a concentration-dependent manner with an IC\(_{50}\) value of 111 μg/mL.

Results of the Disc Diffusion Test
In this study, to investigate the antibacterial properties of nanoemulsion synthesized by the A. vulgaris EO against bacteria (E. coli, K. pneumoniae, S. aureus, and M. luteus), the diameter of the halo of the non-growth zone around each antibiotic was measured in millimeters. The results revealed that E. coli and K. pneumoniae were resistant to synthesized nanoemulsion, while S. aureus and M. luteus were sensitive to this nanoemulsion as was evidenced by the formation of a non-growth zone (Figure 2).

MIC and MBC
No turbidity was observed after incubation at 37°C. The MIC values for S. aureus and M. luteus Gram-negative bacteria were 0.5 and 0.25 mg/mL, respectively. According to the results, the nanoemulsion had a lethal effect on these bacterial strains (M. luteus and S. aureus) at a concentration of 0.5 mg/mL (Table 1).

In the microdilution method, the inhibitory effects of different concentrations of the NPs synthesized by the A. vulgaris EO were evaluated against S. aureus and M. luteus after 0 and 24 hours of incubation at 37°C. At a concentration of 0.5 mg/mL, the nanoemulsion completely (100%) inhibited the growth of both bacteria (Figure 3).

Discussion
Considering the adverse effects of chemical drugs, it seems necessary to replace them with safe antimicrobial compounds, including EOs and plant extracts. This study aimed to determine the antibacterial and anti-oxidative effects of the nanoemulsion synthesized by the A. vulgaris EO. Our results confirmed the inhibitory effects of the nanoemulsion prepared via the herbal EO on the pathogenic bacteria, with more potent effects against Gram-positive than Gram-negative bacteria. Nanoemulsions have been proposed as novel promising antimicrobial agents that can disrupt the outer membrane integrity of coated bacteria and fungi. Nanoemulsions are thermodynamically suitable for being fused with lipid membranes, and this fusion is enhanced by electrostatic attractions between the cationic charge of the emulsion and the anionic charge of the pathogen, facilitating the cell lysis and death of the pathogen. Considering that nanoemulsions non-specifically disrupt the bacterial cell membrane, they do not lead to the formation of resistant strains; they are offered to be promising antimicrobial agents.

In this study, the nanoemulsion synthesized by the A. vulgaris EO inhibited the growth of S. aureus as evidenced by a non-growth halo zone with a diameter...
of 10 mm, which was comparable with the diameter of the non-growth zone calculated for gentamicin. This finding suggested that the prepared nanoemulsion exerted almost the same inhibitory effects as gentamicin. Consistently, previous studies reported similar inhibitory effects for the nanoemulsions prepared from the oregano EO, thyme, and cinnamaldehyde against *S. aureus*.

One study investigated the antibacterial and antioxidant effects of the ingredients of the EO of *Artemisia aucheri* Boiss. The main components of the EO were ρ-cymene, 8,1-cineol, linalool, trans-beta ocimene, gamma-terpinene, dodecane, octanol acetate, triene, borneol, lavandulol, and bornyl acetate as monoterpenic compounds, as well as chrysantheneyll.
ester, dehydroaromadendrin, and caryophyllene oxide as sesquiterpene compounds. The inhibitory effects of the EO against different bacterial strains were confirmed. The diameter of the non-growth halo of the EO was obtained at 19 mm for S. aureus (compared to 21 mm for gentamicin), which confirmed the inhibitory effects of this EO against Gram-positive bacteria.

In a study conducted in 2020, flaxseed oil was used for the synthesis of nanoemulsion, and the results revealed that the nanoemulsion of this oil had weaker antioxidant effects in comparison with the nanoemulsion of the EO. A comparison of the non-growth halos of A. vulgaris bacteria treated with the nanoemulsions of the linseed oil and the A. vulgaris EO approved the stronger antibacterial effects of the latter. Medicinal plants with proven pharmacological effects have long been employed in traditional medicine. According to the World Health Organization, the integration of traditional and complementary medicine can make a significant contribution to achieving universal health coverage and the provision of essential health services.

Artemisia vulgaris is one of the most well-known species of this genus, which is widely distributed in natural habitats around the world (Europe, Asia, North and South America, and Africa). For many centuries, this species has been mainly used to treat gynecological and digestive diseases. Recently, research has proven that this species has antioxidant, blood lipid lowering, hepatoprotective, antispasmodic, analgesic, estrogenic, cytotoxic, antibacterial, antifungal, blood pressure lowering, and broncholytic effects. Different uses have been suggested for this plant species due to its rich chemical compounds, including EOs, flavonoids, sesquiterpene lactones, phenolic acids, coumarins, and other groups of metabolites. The genus belongs to the Artemisinae subtribe, the Anthemideae sub-family of the Asteraceae family, and has more than 500 species. These species are distributed all over the world, especially in Europe, East Asia, America, North Africa, and Australia. The plants of this herbaceous family are annual, biennial, and perennial and generally grow as small bushes and semi-shrubs.

Some studies have documented the antioxidant activity of the whole plant, as well as its aerial parts, leaf extract, and EO applying modern techniques such as lipid peroxidation, protein glycation, xanthine oxidase, and DPPH radical scavenging tests. Moreover, a study performed in 2019 confirmed the growth inhibitory effects of the EOs of A. vulgaris against different bacteria, which was attributed to its high levels of 8-cineole and β-thujone. On the other hand, the oil extracted from the underground parts of the plant showed only little activity against the mentioned pathogens due to the low levels of 1,8-cineole and no content of β-thujone in the root.

**Conclusion**

In this study, the antibacterial growth inhibitory effects of the nanoemulsions prepared by the A. vulgaris EO were demonstrated against Gram-positive bacteria. Given that the growth of S. aureus and M. luteus was considerably suppressed in the presence of the synthetic material, the EO of A. vulgaris is suggested to be employed to treat infections caused by these bacteria. It is also recommended that future studies investigate the antimicrobial properties of the nanoemulsions synthesized by the EO of this plant against a wide range of other microorganisms such as parasites and fungi, as well as in inflammatory and neoplastic diseases due to their potential pro-apoptotic, anti-proliferative, and antioxidant activity. The obtained results revealed the potential of the herb as a viable source of antimicrobial and antioxidant compounds to be used in combination with broad-spectrum antibiotics.

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**Authors’ Contribution**

Study conception and design: M.A.; M.G.; E.Y.; A.E.; Data collection: M.A.; M.G.; E.Y.; Analysis and interpretation of results: M.A.; M.G.; E.Y.; A.E.; All authors reviewed the results and approved the final version of the manuscript.

**Competing Interests**

None.

**Ethical Approval**

Not applicable.

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