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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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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.[6,7] 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

phytochemical diversity depending on the geographical location.[15] Its plant, *A. vulgaris* herb, is used as a source of EOs, flavonoids, and sesquiterpenoid lactones due to their diverse biological activities.[16] The European Pharmacopoeia has listed this species as a potential homeopathic raw material. The beneficial properties of the *A. vulgaris* plant extract, including hepatoprotective,[17] antispasmodic,[18] analgesic,[19] estrogenic,[20] cytotoxic,[21] antibacterial,[22] and antifungal effects,[23] 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.[24, 25]

Artemisia vulgaris has a long history as a medicinal plant in the treatment of human diseases.[26] 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.[27,28] 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.[24] 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.[26]

Staphylococcus aureus, *Micrococcus luteus*, *Escherichia coli*, and *Klebsiella pneumoniae* are among the most important human pathogenic bacteria and common causes of communicable diseases.[29,30] These bacteria are highly resistant to antibiotics such as penicillin and ampicillin.[31] Research represents that *E. coli* is the most common cause of urinary tract infections, along with *K. pneumoniae* and Proteus.[32] 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*.[33,34] Nowadays, the use of natural antimicrobial compounds along with antibiotics has acquired attention as a way to reduce the use and dosage of antibiotics.[35-37] 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* ATCC4698, *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.[38]

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.[39]

Disc Diffusion (Kirby-Bauer) Method

This experiment was performed in three replicates using Gram-positive, namely, *S. aureus* (ATCC 25923) and *M. luteus* (ATCC 4698), 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).[40]

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 absorption of the wells, the growth inhibition percentage for the bacteria tested was calculated using equation (1).

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

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).[41]

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.[42,43] 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.[44] 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.[45,46] 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.[47]

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

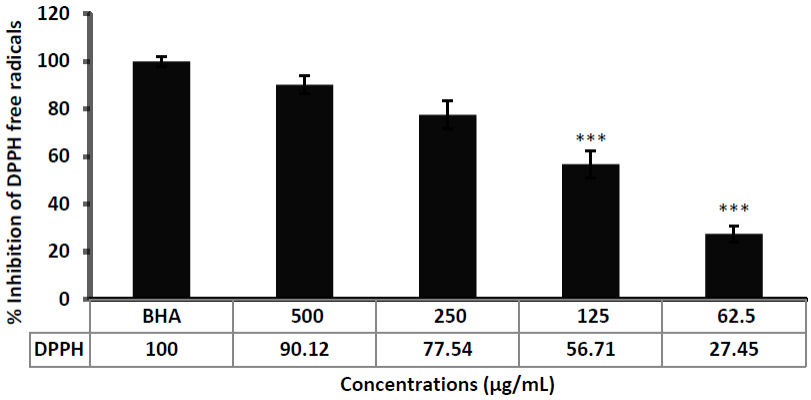

Figure 1. The Antioxidant Activity of Nanoparticles by DPPH Assay. *Note*. DPPH: 1,1-diphenyl-2-picryl-hydrazyl; BHA: Butylated hydroxyanisole. The experiment was conducted in triplicate. $^{***}P<0.001$ indicated a significant difference as compared to the BHA scavenging activity

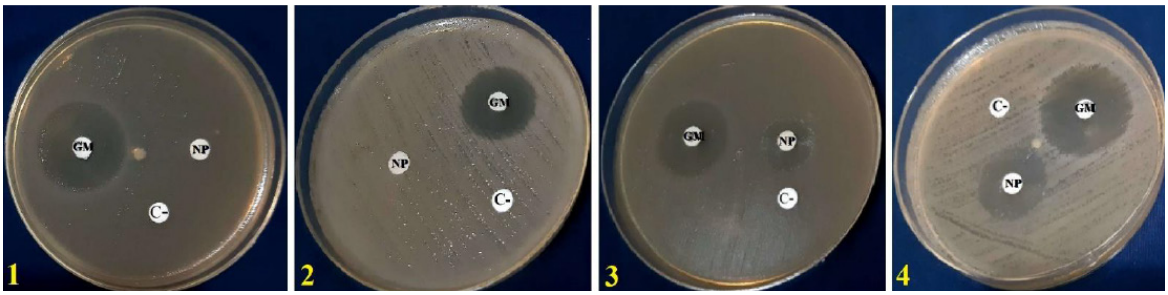

Figure 2. NP: Nanoparticle, C-: Negative control, and GM: Gentamicin. *Note*. (1) *Escherichia coli*, (2) *Klebsiella pneumoniae*, (3) *Staphylococcus aureus*, and (4) *Micrococcus luteus*

Table 1. Results of Disk diffusion, MIC, and MBC

Test	Materials	*Micrococcus luteus* ATCC 4698	*Staphylococcus aureus* ATCC 25923	*Klebsiella pneumoniae* ATCC 700603	*Escherichia coli* ATCC 25922
Disk diffusion	Nano (1 mg/mL)	21±0.1 mm	16±0.1 mm	Effectless	Effectless
	Gentamicin (10 µg) [Control]	27.33±0.1 mm	21.66±0.1 mm	24.66±0.1 mm	23.66±0.1 mm
MIC	Nano	250±00 ug/mL	500±00 ug/mL	Effectless	Effectless
	Chloramphenicol [Control]	100±5 ug/mL	100±2 ug/mL	100±00 ug/mL	100±1 ug/mL
MBC	Nano	500±3 ug/mL	500±00 ug/mL	Effectless	Effectless
	Chloramphenicol [Control]	100±2 ug/mL	100±2 ug/mL	100±1 ug/mL	100±1 ug/mL

Note. MIC: Minimum inhibitory concentration; MBC: Minimum bactericidal concentration.

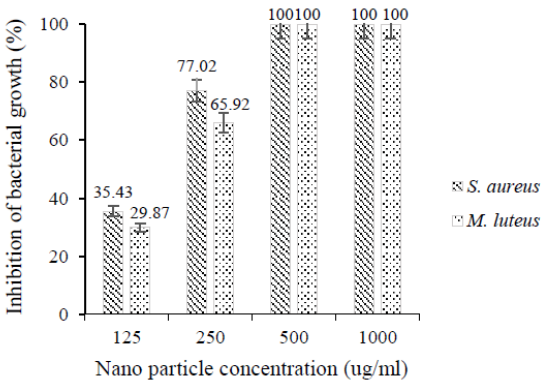

Figure 3. Growth Inhibition Percentage of *Staphylococcus aureus* and *Micrococcus luteus* by Nanoparticles by the Microdilution Method

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*.[48] One study investigated the antibacterial and antioxidant effects of the ingredients of the EO of *Artemisia aucheri Boiss*.[49] The main components of the EO were identified as ρ-cymene, 8,1-cineol, linalool, trans-beta ocimene, gamma-terpinene, dodecane, octanol acetate, triene, borneol, lavandulol, and bornyl acetate as monoterpene compounds, as well as chrysanthenyl

ester, dehydroaromadendrin, and caryophyllene oxide as sesquiterpene compounds.[50] 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.[51] A comparison of the non-growth halos of *Aureus* bacteria treated with the nanoemulsions of the linseed oil and the *A. vulgaris* EO approved the stronger antibacterial effects of the latter.[52] Medicinal plants with proven pharmacological effects have long been employed in traditional medicine.[53] 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.[54]

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. [55] 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.[26] 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.[26]

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.[56,57] 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.[58]

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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References

1. Bottalico L, Charitos IA, Potenza MA, Montagnani M, Santacroce L. The war against bacteria, from the past to present and beyond. Expert Rev Anti Infect Ther. 2022;20(5):681-706. doi:10.1080/14787210.2022.2013809
2. Taghavizadeh Yazdi ME, Darroudi M, Amiri MS, et al. Antimycobacterial, anticancer, antioxidant and photocatalytic activity of biosynthesized silver nanoparticles using *Berberis integerrima*. Iran J Sci Technol Trans A Sci. 2022;46(1):1-11. doi:10.1007/s40995-021-01226-w
3. Asgari HT, Es-Haghi A, Karimi E. Anti-angiogenic, antibacterial, and antioxidant activities of nanoemulsions synthesized by *Cuminum cyminum* L. tinctures. J Food Meas Charact. 2021;15(4):3649-3659. doi:10.1007/s11694-021-00947-1
4. Coker RJ, Hunter BM, Rudge JW, Liverani M, Hanvoravongchai P. Emerging infectious diseases in southeast Asia: regional challenges to control. Lancet. 2011;377(9765):599-609. doi:10.1016/s0140-6736(10)62004-1
5. Ashna M, Es-Haghi A, Karimi Noghondar M, Al Amara D, Taghavizadeh Yazdi ME. Greener synthesis of cerium oxide nanoemulsion using pollen grains of *Brassica napus* and evaluation of its antitumour and cytotoxicity properties. Mater Technol. 2022;37(8):525-532. doi:10.1080/10667857.2020.1863558

6. Wang L, Wang Y, Jin S, et al. Emergence and control of infectious diseases in China. Lancet. 2008;372(9649):1598-1605. doi:10.1016/s0140-6736(08)61365-3
7. Es-Haghi A, Javadi F, Taghavizadeh Yazdi ME, Amiri MS. The expression of antioxidant genes and cytotoxicity of biosynthesized cerium oxide nanoparticles against hepatic carcinoma cell line. Avicenna J Med Biochem. 2019;7(1):16-20. doi:10.34172/ajmb.2019.04
8. Noore S, Rastogi NK, O'Donnell C, Tiwari B. Novel bioactive extraction and nano-encapsulation. Encyclopedia. 2021;1(3):632-664. doi:10.3390/encyclopedia1030052
9. Farhangi MJ, Es-Haghi A, Taghavizadeh Yazdi ME, Rahdar A, Baino F. MOF-mediated synthesis of CuO/CeO2 composite nanoparticles: characterization and estimation of the cellular toxicity against breast cancer cell line (MCF-7). J Funct Biomater. 2021;12(4):53. doi:10.3390/jfb12040053
10. Es-Haghi A, Soltani M, Karimi E, Namvar F, Homayouni-Tabrizi M. Evaluation of antioxidant and anticancer properties of zinc oxide nanoparticles synthesized using *Aspergillus niger* extract. Mater Res Express. 2019;6(12):125415. doi:10.1088/2053-1591/ab5f72
11. Barradas TN, de Holanda e Silva KG. Nanoemulsions of essential oils to improve solubility, stability and permeability: a review. Environ Chem Lett. 2021;19(2):1153-1171. doi:10.1007/s10311-020-01142-2
12. Manandhar S, Luitel S, Dahal RK. In vitro antimicrobial activity of some medicinal plants against human pathogenic bacteria. J Trop Med. 2019;2019:1895340. doi:10.1155/2019/1895340
13. Mobaraki F, Momeni M, Taghavizadeh Yazdi ME, Meshkat Z, Silanian Toosi M, Hosseini SM. Plant-derived synthesis and characterization of gold nanoparticles: investigation of its antioxidant and anticancer activity against human testicular embryonic carcinoma stem cells. Process Biochem. 2021;111(Pt 1):167-177. doi:10.1016/j.procbio.2021.09.010
14. Omonijo FA, Ni L, Gong J, Wang Q, Lahaye L, Yang C. Essential oils as alternatives to antibiotics in swine production. Anim Nutr. 2018;4(2):126-136. doi:10.1016/j.aninu.2017.09.001
15. Szopa A, Pajor J, Klin P, et al. *Artemisia absinthium* L.-importance in the history of medicine, the latest advances in phytochemistry and therapeutical, cosmetological and culinary uses. Plants (Basel). 2020;9(9):1063. doi:10.3390/plants9091063
16. Koul B, Taak P, Kumar A, Khatri T, Sanyal I. The *Artemisia* genus: a review on traditional uses, phytochemical constituents, pharmacological properties and germplasm conservation. J Glycomics Lipidomics. 2017;7(1):142. doi:10.4172/2153-0637.1000142
17. Gilani AH, Yaeesh S, Jamal Q, Ghayur MN. Hepatoprotective activity of aqueous-methanol extract of *Artemisia vulgaris*. Phytother Res. 2005;19(2):170-172. doi:10.1002/ptr.1632
18. Khan AU, Gilani AH. Antispasmodic and bronchodilator activities of *Artemisia vulgaris* are mediated through dual blockade of muscarinic receptors and calcium influx. J Ethnopharmacol. 2009;126(3):480-486. doi:10.1016/j.jep.2009.09.010
19. Ashok PK, Upadhyaya K. Evaluation of analgesic and anti-inflammatory activities of aerial parts of *Artemisia vulgaris* L. in experimental animal models. J Biol Act Prod Nat. 2013;3(1):101-105. doi:10.1080/22311866.2013.782761
20. Lee SJ, Chung HY, Maier CG, Wood AR, Dixon RA, Mabry TJ. Estrogenic flavonoids from *Artemisia vulgaris* L. J Agric Food Chem. 1998;46(8):3325-3329. doi:10.1021/jf9801264
21. Jakovljević MR, Grujičić D, Vukajlović JT, et al. In vitro study of genotoxic and cytotoxic activities of methanol extracts of *Artemisia vulgaris* L. and *Artemisia alba* Turra. S Afr J Bot. 2020;132:117-126. doi:10.1016/j.sajb.2020.04.016
22. Ahmadizadeh C, Monadi A, Rezaie A, Ghiami Rad M, Jafari B. Antibacterial activity of methanolic extract and essence of Sagebrush (*Artemisia vulgaris*) against pathogenic bacteria. Life Sci J. 2018;15(5):69-73. doi:10.7537/marslsj150518.12
23. Soares LB, Tucci EC, Gonçalez E, Felicio RC, Felicio JD. Acaricide and fungicide effects of the *Artemisia vulgaris* essential oil. Annu Res Rev Biol. 2015;5(4):285-292. doi:10.9734/arrb/2015/13190
24. Bisht D, Kumar D, Kumar D, Dua K, Chellappan DK. Phytochemistry and pharmacological activity of the genus *Artemisia*. Arch Pharm Res. 2021;44(5):439-474. doi:10.1007/s12272-021-01328-4
25. Amiri MS, Taghavizadeh Yazdi ME, Rahnama M. Medicinal plants and phytotherapy in Iran: glorious history, current status and future prospects. Plant Sci Today. 2021;8(1):95-111. doi:10.14719/pst.2021.8.1.926
26. Ekiert H, Pajor J, Klin P, Rzepiela A, Ślesak H, Szopa A. Significance of *Artemisia vulgaris* L. (common mugwort) in the history of medicine and its possible contemporary applications substantiated by phytochemical and pharmacological studies. Molecules. 2020;25(19):4415. doi:10.3390/molecules25194415
27. Hussein RA, El-Anssary AA. Plants secondary metabolites: the key drivers of the pharmacological actions of medicinal plants. In: Builders PF, ed. Herbal Medicine. Rijeka: IntechOpen; 2018. doi:10.5772/intechopen.76139
28. Taghavizadeh Yazdi ME, Darroudi M, Amiri MS, et al. Anticancer, antimicrobial, and dye degradation activity of biosynthesised silver nanoparticle using *Artemisia kopetdaghensis*. Micro Nano Lett. 2020;15(14):1046-1050. doi:10.1049/mnl.2020.0387
29. Jafari-Sales A, Bagherizadeh Y, Malekzadeh P, Ahmadi B, Bonab FR. Evaluation of the antimicrobial effects of essential oil of *Reseda lutea* L. on pathogenic bacteria: *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Escherichia coli*. Arch Clin Microbiol. 2017;8(3):41. doi:10.4172/1989-8436.100041
30. Mousavi-Kouhi SM, Beyk-Khormizi A, Amiri MS, Mashreghi M, Taghavizadeh Yazdi ME. Silver-zinc oxide nanocomposite: from synthesis to antimicrobial and anticancer properties. Ceram Int. 2021;47(15):21490-21497. doi:10.1016/j.ceramint.2021.04.160
31. Bao L, Peng R, Ren X, Ma R, Li J, Wang Y. Analysis of some common pathogens and their drug resistance to antibiotics. Pak J Med Sci. 2013;29(1):135-139. doi:10.12669/pjms.291.2744
32. Daza R, Gutiérrez J, Piédrola G. Antibiotic susceptibility of bacterial strains isolated from patients with community-acquired urinary tract infections. Int J Antimicrob Agents. 2001;18(3):211-215. doi:10.1016/s0924-8579(01)00389-2
33. Li J, Nation RL, Turnidge JD, et al. Colistin: the re-emerging antibiotic for multidrug-resistant gram-negative bacterial infections. Lancet Infect Dis. 2006;6(9):589-601. doi:10.1016/s1473-3099(06)70580-1
34. Taghavizadeh Yazdi ME, Amiri MS, Hosseini HA, et al. Plant-based synthesis of silver nanoparticles in *Handelia trichophylla* and their biological activities. Bull Mater Sci. 2019;42(4):155. doi:10.1007/s12034-019-1855-8
35. Aiyegoro OA, Okoh AI. Use of bioactive plant products in combination with standard antibiotics: implications in antimicrobial chemotherapy. J Med Plants Res. 2009;3(13):1147-1152.
36. Taghavizadeh Yazdi ME, Housaindokht MR, Sadeghnia HR, Esmaeilzadeh Bahabadi S, Amiri MS, Darroudi M. Assessment of phytochemical components and antioxidant activity of *Rheum turkestanicum* Janisch. Stud Med Sci. 2020;31(2):75-

81.
37. Zarei M, Karimi E, Oskoueian E, Es-Haghi A, Taghavizadeh Yazdi ME. Comparative study on the biological effects of sodium citrate-based and apigenin-based synthesized silver nanoparticles. Nutr Cancer. 2021;73(8):1511-1519. doi:10.1080/01635581.2020.1801780
38. Gutiérrez-Valenzuela CA, Esquivel R, Guerrero-Germán P, et al. Evaluation of a combined emulsion process to encapsulate methylene blue into PLGA nanoparticles. RSC Adv. 2018;8(1):414-422. doi:10.1039/c7ra12296a
39. Prieto JM. Procedure: Preparation of DPPH Radical, and Antioxidant Scavenging Assay. DPPH Microplate Protocol. 2012:7-9.
40. Najafi M, Nakhaei Moghaddam M, Yousefi E. The effect of silver nanoparticles on pyocyanin production of *Pseudomonas aeruginosa* isolated from clinical specimens. Avicenna J Med Biotechnol. 2021;13(2):98-103. doi:10.18502/ajmb.v13i2.5529
41. Casey JT, O'Cleirigh C, Walsh PK, O'Shea DG. Development of a robust microtiter plate-based assay method for assessment of bioactivity. J Microbiol Methods. 2004;58(3):327-334. doi:10.1016/j.mimet.2004.04.017
42. Shamasi Z, Es-Haghi A, Taghavizadeh Yazdi ME, Amiri MS, Homayouni-Tabrizi M. Role of *Rubia tinctorum* in the synthesis of zinc oxide nanoparticles and apoptosis induction in breast cancer cell line. Nanomed J. 2021;8(1):65-72. doi:10.22038/nmj.2021.08.07
43. Taghavizadeh Yazdi ME, Amiri MS, Nourbakhsh F, Rahnama M, Forouzanfar F, Mousavi SH. Bio-indicators in cadmium toxicity: role of HSP27 and HSP70. Environ Sci Pollut Res Int. 2021;28(21):26359-26379. doi:10.1007/s11356-021-13687-y
44. Chouhan S, Sharma K, Guleria S. Antimicrobial activity of some essential oils-present status and future perspectives. Medicines (Basel). 2017;4(3):58. doi:10.3390/medicines4030058
45. Shah P, Bhalodia D, Shelat P. Nanoemulsion: a pharmaceutical review. Syst Rev Pharm. 2010;1(1):24-32.
46. Arazmjoo S, Es-Haghi A, Mahmoodzadeh H. Evaluation of anti-cancer and antioxidant properties of nanoemulsions synthesized by *Nigella sativa* L. tincture. Nanomed J. 2021;8(1):57-64. doi:10.22038/nmj.2021.08.06
47. Gupta S, Bansal R, Maheshwari D, Ali J, Gabrani R, Dang S. Development of a nanoemulsion system for polyphenon 60 and cranberry. Adv Sci Lett. 2014;20(7-9):1683-1686. doi:10.1166/asl.2014.5579
48. Soufi S, Aminzare M, Azar HH, Kamali K. Comparative study on the antibacterial properties of nanoemulsion of *Zataria multiflora* essential oil fortified with cinnamaldehyde against common foodborne pathogens. Nat Prod J. 2020;10(5):631-641. doi:10.2174/2210315509666191111105918
49. Mojtahed Zadeh Asl R, Niakousari M, Hashemi Gahruie H, Saharkhiz MJ, Mousavi Khaneghah A. Study of two-stage ohmic hydro-extraction of essential oil from *Artemisia aucheri* Boiss.: antioxidant and antimicrobial characteristics. Food Res Int. 2018;107:462-469. doi:10.1016/j.foodres.2018.02.059
50. Tajadod G, Mazooji A, Salimpour F, Samadi N, Taheri P. The essential oil composition of *Artemisia vulgaris* L. in Iran. Ann Biol Res. 2012;3(1):385-389.
51. Mazonde P, Khamanga SMM, Walker RB. Design, optimization, manufacture and characterization of efavirenz-loaded flaxseed oil nanoemulsions. Pharmaceutics. 2020;12(9):797. doi:10.3390/pharmaceutics12090797
52. Keykhasalar R, Homayouni-Tabrizi M, Ardalan P. Antioxidant property and bactericidal activity of *Linum usitatissimum* seed essential oil nanoemulsion (LSEO-NE) on *Staphylococcus aureus*. Int J Infect. 2020;7(2):e101639. doi:10.5812/iji.101639
53. Singh S, Sedha S. Medicinal plants and their pharmacological aspects. FPI. 2018;1(4):156-170.
54. World Health Organization (WHO). WHO Global Report on Traditional and Complementary Medicine 2019. WHO; 2019.
55. Abad MJ, Bedoya LM, Apaza L, Bermejo P. The *Artemisia* L. genus: a review of bioactive essential oils. Molecules. 2012;17(3):2542-2566. doi:10.3390/molecules17032542
56. Temraz A, El-Tantawy WH. Characterization of antioxidant activity of extract from *Artemisia vulgaris*. Pak J Pharm Sci. 2008;21(4):321-326.
57. Matvieieva N, Drobot K, Duplij V, et al. Flavonoid content and antioxidant activity of *Artemisia vulgaris* L. "hairy" roots. Prep Biochem Biotechnol. 2019;49(1):82-87. doi:10.1080/10826068.2018.1536994
58. Munda S, Pandey SK, Dutta S, Baruah J, Lal M. Antioxidant activity, antibacterial activity and chemical composition of essential oil of *Artemisia vulgaris* L. leaves from Northeast India. J Essen Oil Bearing Plants. 2019;22(2):368-379. doi:10.1080/0972060x.2019.1602083