


Original Article

## Cytotoxicity of Artemisia Vulgaris Essential oil Encapsulated in SLN on Breast Cancer Cell Line (MCF7)

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Received: 2021-04-12

Revised: 2021-06-06

Accepted: 2021-06-26

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**Cite this article as:**

Pooladi M, Teimouri M, Odoumizadeh M. Cytotoxicity of Artemisia Vulgaris Essential oil Encapsulated in SLN on Breast Cancer Cell Line (MCF7). Archives of Advances in Biosciences 2021:12(3)

**Abstract**

**Introduction:** Research has revealed that breast cancer is the second most common cancer among women and that the compounds in Artemisia plant have good anti-tumor potential. Nanomaterials significantly increase solubility, stability, and effective drug delivery. The aim of this study was to evaluate the efficacy of Nano-form of Artemisia essential oil in comparison with non-Nano-form in inhibiting MCF7 cells.

**Materials and Methods:** After extracting essential oil from Artemisia Vulgaris plant, nanoparticles containing Artemisia Vulgaris essential oil were synthesized through homogenizer and sonication method. Different properties of nanoparticles including particle size, zeta potential, and morphology were measured. Finally, the toxicity effect of solid lipid nanoparticles containing essential oil on MCF7 cancer cell line was examined adopting MTT technique. **Results:** The results indicated that tDCS significantly reduced the scores on DDQ and OCDUS in the active tDCS group compared to the sham tDCS group ( $P < .05$ ).

**Results:** The results of cellular effect of lipid nanoparticles containing Artemisia Vulgaris annua on MCF7 cells showed that increasing the concentration of lipid nanoparticles containing Artemisia Vulgaris essential oil, compared to purified essential oil, reduced the survival rate of MCF7 cells.

**Conclusions:** The findings show that lipid nanocarriers raise the release rate of essential oil compared to pure essential oil. Increase in concentration of lipid nanoparticles containing Artemisia essential oil reduces the survival rate of breast cancer cells. The IC<sub>50</sub> obtained for unbaked essential oils and nanoparticles containing essential oils were 1105 and 262  $\mu\text{g/ml}$ , respectively. Thus, it can be concluded that nanoparticle essential oil of this plant is effective in reducing the IC<sub>50</sub> of the drug and increasing cytotoxicity.

**Keywords:** Artemisia Vulgaris, MCF7, SLN, Breast Cancer

## 1. Introduction

Encapsulation of plant essential oils is one of the most effective methods to increase the solubility and stability of essential oils in adverse environmental conditions and to control their active compounds. Compared to other encapsulation systems, the development of Nano-sized formulations of bioactive compounds have more advantages due to their small size which enables these systems to have higher intracellular absorption and to be targeted. Because of the fatty nature of plant essential oils, Solid Lipid Nanoparticles (SLN) can be used as a useful option to transport plant essential oils given their biocompatibility, low toxicity and good degradability along with the ability to encapsulate hydrophobic biological compounds. *Artemisia vulgaris* L, known as (mugwort), belongs to the Asteraceae family of plants, a genus of more than 500 species scattered worldwide. This plant is traditionally used to treat a wide range of ailments including gastrointestinal disorders, headaches, nosebleeds, muscle spasms, epilepsy, circulatory problems, menopausal problems, fever, rheumatism, asthma, gout, infertility, contact dermatitis, and bacterial infections [1-3]. As these substances have been shown to have anti-cancer potential by inducing apoptosis in various cancer cell lines, interest in the use of essential oils as pharmacological agents has recently increased. There is considerable evidence that the active compounds in the essential oils of various *Artemisia* species are responsible for their anti proliferative effect on cancer cells. Previous evidence suggests that the hydroalcoholic extract of the dried leaves of this plant has cytotoxic effects on human hepatocytes. HepG2 is induced by apoptotic induction [4, 5]. Aqueous extracts of *Artemisia vulgaris* have also been

reported to induce apoptosis in prostate, breast, and colon cancer cell lines. In addition, *A. vulgaris* extracts have been shown to sensitize breast cancer cells MDA-MB-231 and MDA-MB-468 to TRAIL [6]. In one study, the essential oil of *Artemisia vulgaris* (leaves and buds) was extracted and its chemical compounds were identified by gas chromatography (GC/MS) mass spectrometry [8]. The results of 22 compounds in the essential oil of *A. vulgaris* L. indicated that the major constituents of the essential oil were Germacrene D (25.25%), Caryophyllene (20%),  $\alpha$ -Gingerbread (15%), and Borneol (11%). The main components of essential oils such as caryophylline,  $\alpha$ -Zingburn, Borneol, and R-Curcumin have all been reported to induce apoptosis in various cancer cell lines in humans [9]. Additionally, studies on the effect of *Artemisia* essential oil on cancer cells in 2004 showed that *Artemisia Annul* can induce apoptosis in SMMC-7721 liver cancer cells [10]. Furthermore, a study on the evaluation of the antioxidant properties of *Artemisia* on cancer cells revealed that the essential oils of three different species of *Artemisia* called *Artemisia absinthium*, *Artemisia santonicum*, *spicigera* have antifungal effects and that they are antioxidants [11]. To date, few studies have been conducted on *Artemisia vulgaris* than on other *Artemisia* species. For example, Asghari et al. (2012) first identified the chemical compounds in *Artemisia absinthium* oilseeds, showing that these compounds have a lethal effect on the bacteria *Escherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes*. These chemical compounds were determined by GC-MS method: Decane,  $p$ -Cymene, 1,8-Cineole, Linalool,  $p$ -Mentha-8-ol, Triene, Borneol, Lavandulol, Bornyl Acetate,

Chrysanthenyl Acetate, Dehydro Aromadenderene, and Caryophyllene Oxide were among them [12].

This study set out to investigate the effect of *Artemisia vulgaris* in SLN on Breast Cancer Cell Line (MCF7). Thus, the present study aimed to synthesize and develop a new method for preparing lipid nanoparticles using the essential oil of *Artemisia vulgaris*. Further, the cytotoxic effects of lipid nanoparticles containing *Artemisia vulgaris* essential oil on MCF7 breast cancer cell line were assessed by MTT assay.

## 2. Materials and Methods

### 2.1. Plant Collection and Essential Oil Extraction

*Artemisia Vulgaris* plant was collected in Jiroft city of Kerman province in late spring/early summer of 2021 and was identified by herbarium number (PMP-1648) at Faculty of Pharmacy of Tehran University of Medical Sciences. Then, 500 g of dry plant powder was ground and essential oil was extracted by Clevenger for 4 hours. The amount of essential oil obtained was about 2.5 mg (equivalent to 5% w/w). Qualitative assessment to identify compounds in *Artemisia Vulgaris* essential oil by gas chromatography (HP-5973) equipped with HP-INNOWAX pole column with a length of 60 meters and an inner diameter of .320 mm and a thickness of .50 mm of stationary phase. Temperature programming was chosen so that the initial temperature of the oven was 80°C; then, it was raised to 230°C at a rate of 30°C per minute and the temperature was maintained for 5 minutes. It was then raised to 250°C and kept constant for 10 minutes. The temperature of the detector injection chamber was 250°C and the volume of the injection sample was .1 µl. Helium carrier gas with a purity of 99.999 and a flow rate

of 1.5 ml/min and ionization energy of 70 electron volts was selected. Normal C8-C32 series alkanes were injected under the same conditions to determine the Kovats index.

### 2.2. Preparation Method of SLN Containing *Artemisia Vulgaris* Essential Oil

Melt High-Pressure Homogenizer was used to fabricate nanoparticles. For this study, 2 types of lipophilic surfactants (Span 60 solid and Span 80 liquid), one hydrophilic surfactant (Twin 20) and 5 types of solid fats (Stearic Acid, Palmitic Acid, Cholesterol, Bisux and Glyceryl Mono Stearate) were selected. For this purpose, the lipid phase containing lipophilic surfactant, essential oil, and fat reaching 70°C is added to the aqueous phase containing surfactant co-heated with the fat phase under milk homogenizers. The initial emulsion is then homogenized at a pressure of 250 bar in 3 consecutive cycles by a High-Pressure Homogenizer. Once finished, the sample is cooled to ambient temperature to form nanoparticles. Placebo was prepared according to the mentioned method. The studied formulas are given in Table 1.

### 2.3. Investigating Physicochemical Properties of Nanoparticles

When determining particle size, scattering index, and zeta potential, the parameters were measured at room temperature at a 90° angle using a Zeta Sizer (Malvern UK ZS series). To investigate the parameters, the device was calibrated based on the aqueous phase (RI = 1.3) and then passed through a needle filter with a size of .22 µm and the particle size, zeta potential, and particle size distribution were analyzed in 3 replications it placed.

### 2.4. Investigating Morphology of Nanoparticles Containing Essential Oil

The best nanoparticles were diluted with deionized water according to the amount of essential oil loading and particle size in a ratio of 1 to 10. The sample was then placed on a copper grid large enough to form a thin layer. Next, one to two drops of 2% phosphotungstic acid were added. After one minute, the excess material was removed from the copper grid with a paper filter to create a thin layer for imaging. As the next step, the sample was allowed to dry at room temperature. Then, the morphology of nanoparticles was observed by transmission electron microscope at different magnifications.

### 2.5. Determining the Amount of Essential Oil Release

Membrane diffusion technique (Dialysis) nanoparticles were used to calculate the essential oil release. To this end, a 12,000 dalton dialysis bag was used, which was hydrated in deionized water for 24 h before the test. For the release test, the release of selected formulations in the acetate buffer medium was taken.

### 2.6. Stability Study of SLN Containing *Artemisia Vulgaris* Essential Oil

Stability study was carried out based on particle size changes, particle size distribution, and zeta potential. The two formulations were placed in dark glass containers (to protect against light), with their lids completely closed. They were placed at room temperature (25°C) and refrigerator temperature (4°C) for one week. After one-month and three-month intervals, they were examined.

### 2.7. Cytotoxicity Test

Cell culture: For this test, human breast carcinoma cells (MCF7), prepared from Pasteur Institute of Iran, were used. Growth medium DMEM was employed.

### 2.8. Study Grouping

In order to investigate the anti-cancer effects of essential oil loaded on lipid nanoparticles, cells were cultured for 24 h in 96-well plates in the following groups with specific concentrations. MCF7 control group: MCF7 cells were cultured without factor treatment.

- Group 1: *Artemisia vulgaris* essential oil (24 h treatment with concentrations of 10, 50, 100, 500 and 1000 µ/ml),
- Group 2: SLN containing *Artemisia vulgaris* essential oil (24 h treatment with concentrations of 10, 50, 100, 500 and 1000 µg/ml).
- Group 3: SLN without *Artemisia vulgaris* essential oil 24h treatment with concentrations of 10, 50, 100, 500, and 1000 µg/ml).
- Negative control: Dimethyl Sulfoxide (DMSO) 5% with 24 h treatment (DMSO was used in negative control as a solvent in essential oil).

### 2.9. Toxicity and Cell Viability Test

Cell viability was determined by MTT assay. To measure toxicity, MCF7 cells were cultured separately at a concentration of 104 per well in 96 plates for 24 h. Cells were then treated with the same volume of fresh medium and different concentrations of essential oils and SLN containing essential oils (10, 50, 100, 500 and 1000 µg/mL) were injected in wells in five iterations. Twice, after 24 h, 20 µl of MTT solution with a concentration of 5 mg/mL was added to each well and incubated for 4 h. After that, the surface was removed and 150 µl of DMSO was added to each well. There was a centrifuge at each step to remove the fluid. Adsorption at a wavelength of 570 nm was recorded using a microplate reader (ELISA reader). Finally,

the surviving and killing percentage was calculated.

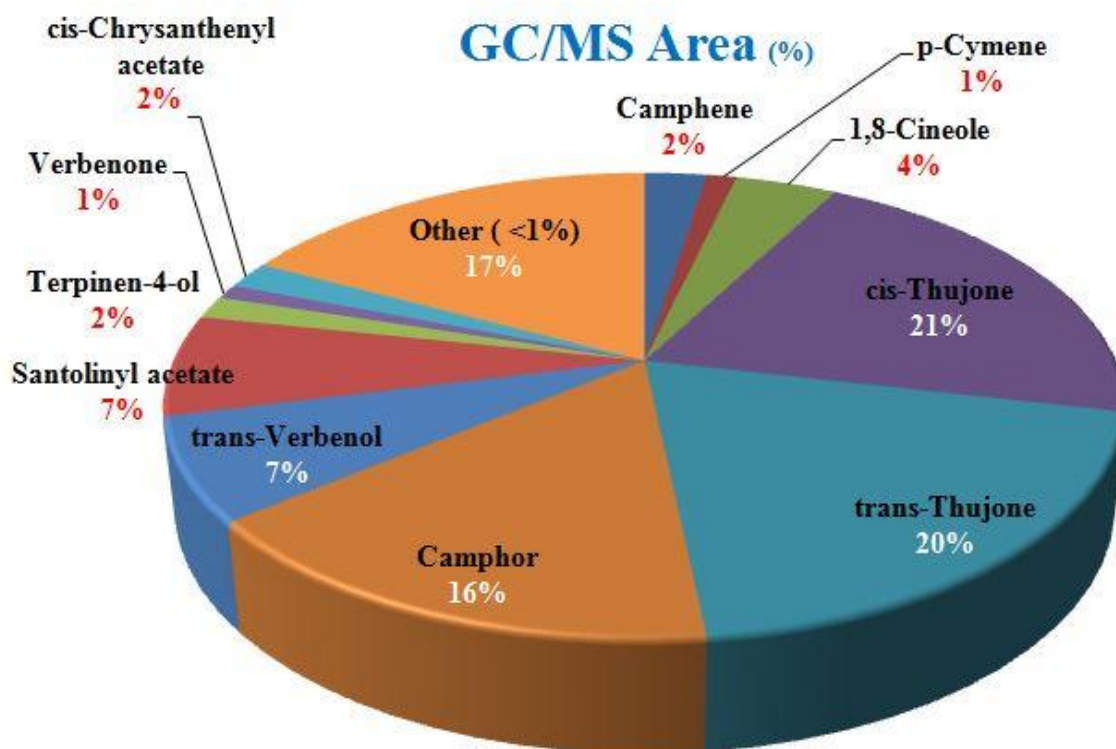
### 2.10. Statistical Analysis

In this study, SPSS software version 22 was used for data analysis. Two-way ANOVA was performed to analyze the statistical results. Significance of the results was measured based on  $P \leq 0.05$ .

## 3. Results

### 3.1. Results of Compounds in *Artemisia Vulgaris* Essential Oil

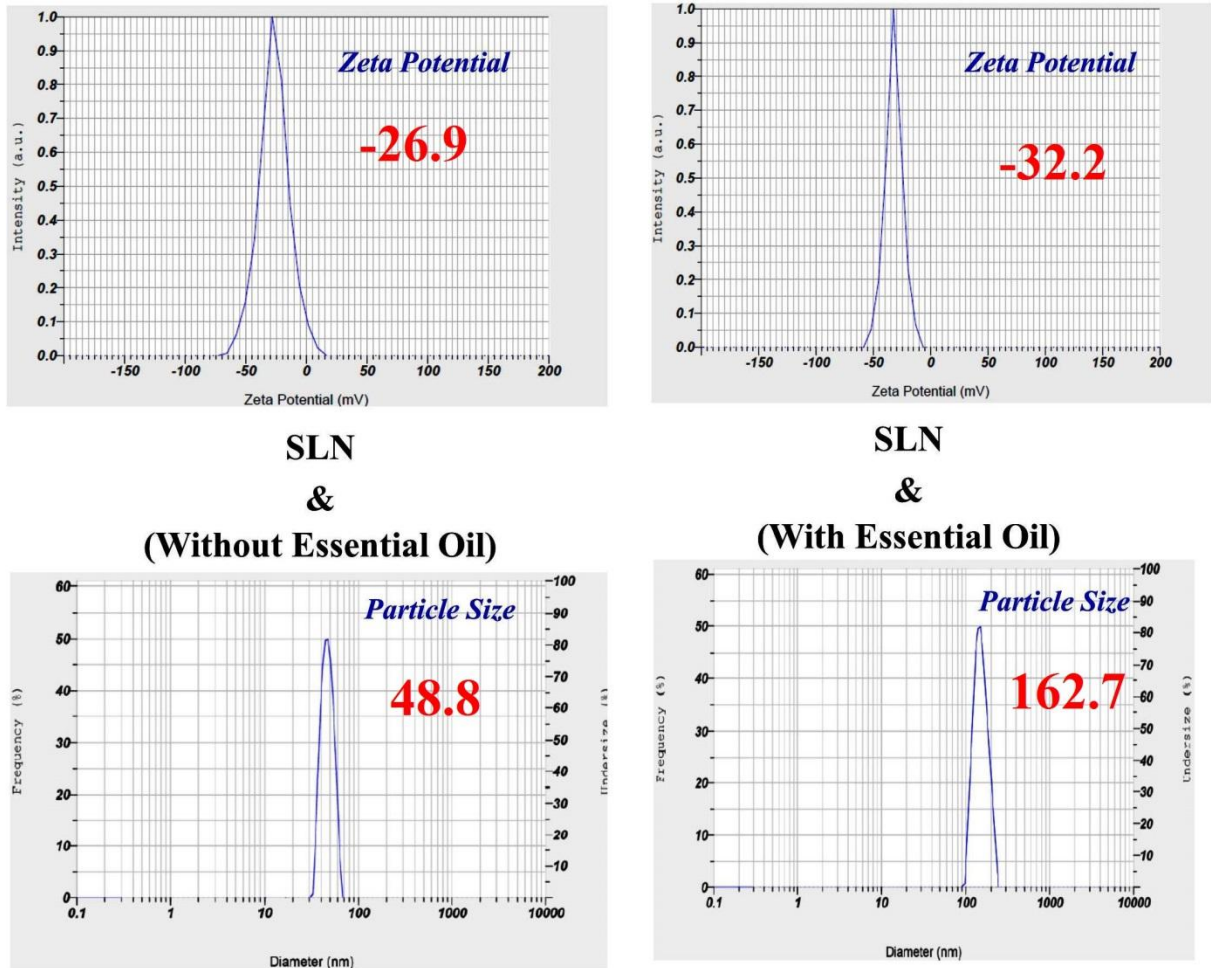
By examining the spectra obtained from gas chromatography (GC/MS), calculating inhibition indices, and comparing the mass spectra of compounds with standard compounds, 56 different compounds in *Artemisia vulgaris* essential oil were identified. The major constituents were cis-Thujone, trans-Thujone, Camphor, and trans-Verbenol with 20.67, 19.78, 15.78, and 7.1%, respectively (listed in Figure 1).



**Figure 1.** Percentages of components in the essential oil of *Artemisia Vulgaris* by GC/MS.

The mean values of particle size and zeta potential of essential oil were formulated by DLS, and then shown in figure 2. The zeta potential of SLN containing essential oil in this study was -32.2, indicating a suitable negative charge and acceptable stability of particles. SLN made without essential oils

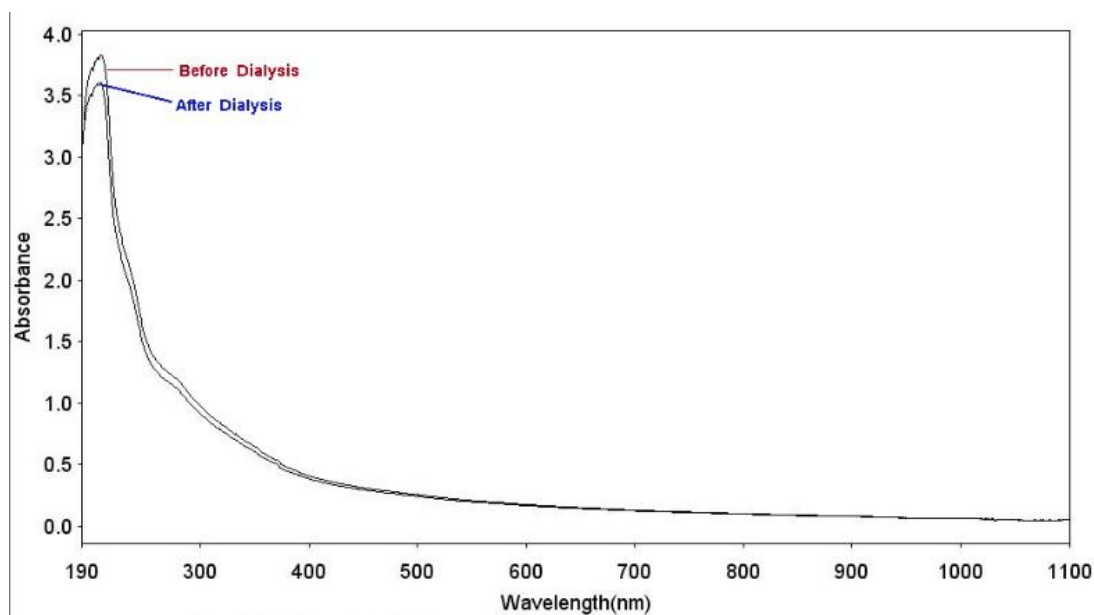
also had a zeta potential of -26.9. The sizes of SLN containing essential oil with and without essential oil were 162.7 and 48.8 nm, respectively. Figure 2 depicts the particle size and zeta potential of essential and non-essential oil nanoparticles.



**Figure 2.** Zeta potential and particle size obtained by DLS for both SLN groups with and without essential oil.

Regarding the percentage of loaded essential oil, the results of spectrophotometer absorption in the range of 190 to 1100 nm were measured for the solution before and after passing through the dialysis bag. Since different substances are present in the essential oil of the plant, the amount of adsorption was averaged throughout this spectrum. For the main solution before

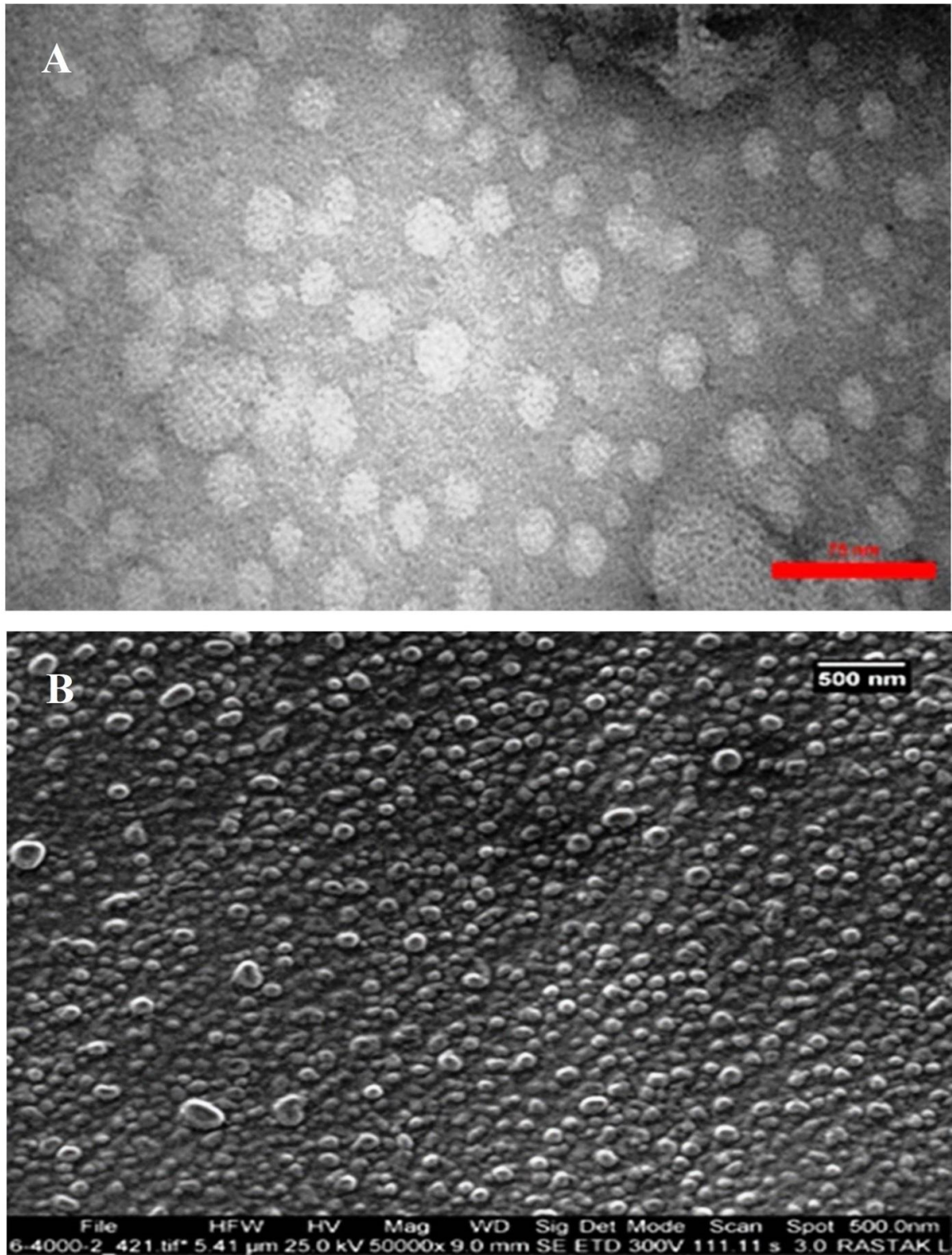
passing through the dialysis bag, this was .409 on average; and in the case of the solution after passing through the dialysis bag was .377. Figure 3 presents the spectrophotometric absorption diagram of a solution of nanoparticles containing essential oil before and after passing through the dialysis bag.



**Figure 3.** Spectrophotometric diagram of a solution of nanoparticles containing essential oil before and after passing through the dialysis bag.

Results of morphology study of lipid nanoparticles containing essential oil and images from TEM and SEM microscopes

are shown in Figure 4. The images showed that the shape of the nanoparticles was spherical.



**Figure 4.** SEM (A) and TEM (B) electron microscope images of lipid nanoparticles

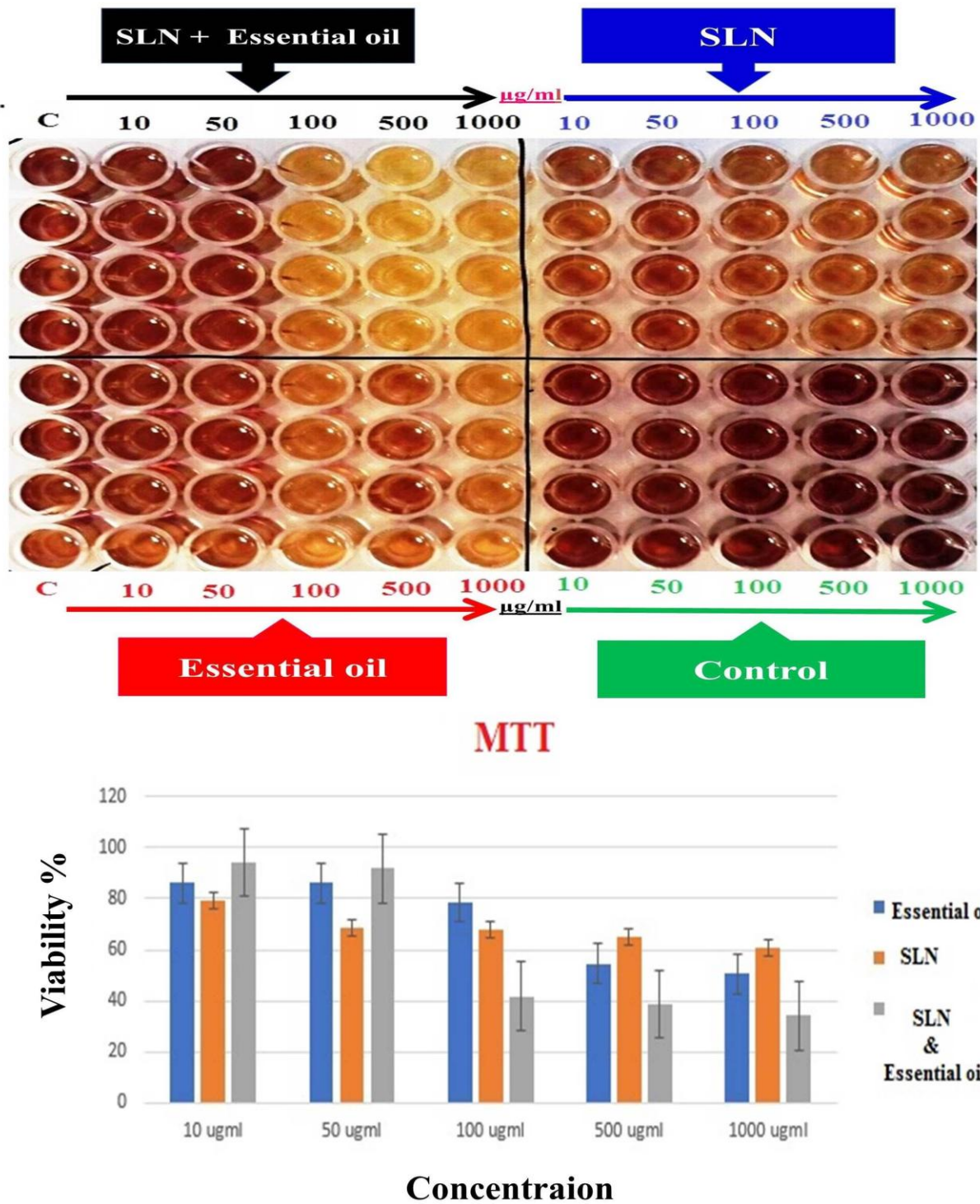
### 3.2. Results of Determining the Maximum Wavelength

According to the spectra obtained from the spectrophotometer, the maximum absorption of essential oil at a wavelength of 274 nm was obtained. Thus, the standard essential oil calibration curve was drawn at a wavelength of 274 nm.

### 3.3. MTT Test Results

Figure 5 depicts the results of the MTT test on different test groups. After comparing the MTT results of different groups using ANOVA test, the following conclusions were obtained. At a concentration of 50  $\mu\text{g/ml}$ , all groups decreased in viability percentage and there was no significant difference between the essential oil group and the essential oil-treated nanoparticles group in terms of survival percentage. At a concentration of 100  $\mu\text{g/ml}$ , all groups

significantly decreased in viability percentage and there was a significant difference in terms of viability between the essential oil group and the essential oil-treated nanoparticles group ( $P < .05$ ), meaning that essentially treated nanoparticles reduced the survival rate of cancer cells more than pure essential oil. Essential oil group and essential oil-treated nanoparticles at concentrations of 500 and 1000  $\mu\text{g/ml}$  showed a significant difference regarding survival percentage in the group treated with non-essential oil compared to cells treated with non-essential oil nanoparticles ( $P < .05$ ). This finding indicates the effectiveness of the essential oil and the formulation considered in comparison with lipid nanoparticles without essential oil. The  $\text{IC}_{50}$  values of different treatments with essential oil, non-essential oil and non-essential nanoparticles in 24-hour treatment are shown in Table 2.



**Figure 5.** MTT test (24 h) results for different concentrations (10, 50, 100, 500, and 1000) and groups (SLN, Essential oil, and SLN & Essential oil)

## 4. Discussion

In the present study, the anticancer effects of solid lipid nanoparticles of *Artemisia vulgaris* essential oil on MCF7 were investigated. In previous studies, the effects of essential oil of this plant as an anti-cancer have been studied [2, 13, 14], but this study has paid special attention to the role of nano-essential oil in anti-cancer effects. Herbal products have been considered one of the treatment methods so that now 60% of anti-cancer agents are obtained from medicinal plants and other natural sources. In fact, the use of alternative supplements is an alternative to the harmful effects of synthetic drugs. One of these valuable medicinal plants is *Artemisia*, which belongs to the Asteraceae family [15-17]. Given the gravity of breast cancer and the antioxidant potential of *Artemisia vulgaris* L, this study was conducted to examine the toxicity effects of lipid nanoparticles containing plant essential oil in MCF7 breast cancer cells. One of the most important factors regarding the use of drug compounds in living organisms is the study of the toxicity of the drug used. In other words, a product is considered a suitable drug when it has no toxic side effects [18]. Toxicity of drugs loaded in SLN is an area researchers have conducted experiments on [19]. In the present study, gas chromatography (GC/MS) technique, calculation of inhibition indices, and comparison of mass spectra of compounds with standard compounds led to identification of 56 different compounds in the essential oil of *Artemisia vulgaris*. The major constituents are *cis*-Thujone (20.67%), *trans*-Thujone (19.78%), Camphor (15.78%), and *trans*-Verbenol (7.1%).

According to the spectra obtained from the spectrophotometer, the maximum adsorption of essential oil was obtained at a wavelength of 274 nm. Therefore, our experimental findings show that lipid nanocarriers have increased the release rate of essential oil compared to pure essential oil. Increase in concentration of lipid nanoparticles containing *Artemisia* essential oil reduces the survival rate of breast cancer cells (MCF7). Our results were in agreement with the findings of Gharehmatrossian et al. (2012) indicating that this essential oil has antioxidant and cell killing properties and has the potential to be used in cancer treatment [20]. They also showed that increasing the concentration of *Artemisia* essential oil reduces the viability of cancer cells; in other words, they have a dose-dependent effect. Similarly, Sharif et al. (2015) showed that the methanolic essential oil of *A. aucheri* has the ability to inhibit the growth of cancer cells in a concentration-dependent manner [21]. Kordezageneh et al. showed that lipid nanoparticles containing *Ariemisia* essential oil increase the expression of Bax gene and Bcl-2 gene. Since Bax and Bcl-2 are important genes in the apoptotic pathway, lipid nanoparticles containing the essential oil of this plant activate the apoptotic pathway in breast cancer cells [22]. Emami et al. (2009) in their study on the toxicity of *Artemisia Annua* species on gastric cancer cells demonstrated that methanolic essential oil of *Artemisia* plant significantly inhibits the process of apoptosis [23]. In addition, Ahmadi et al. (2015) argued that the essential oil of this plant has many anti-proliferative effects on neuroblastoma cells and this is due to its effect on Bax and Bcl2

genes [24]. Based on the results of the present study and other studies, the mechanisms of anticancer activity are such that after treatment with *Artemisia* essential oil, cytochrome c is released from mitochondria and caspase-3 activity is increased. As a result, the activity of this apoptotic protease leads to division of Poly ADP Ribose Polymerase induce apoptosis and DNA breakdown, ultimately inhibiting the activity of the anti-apoptotic factor NF-KB. In this way, the activity of anti-apoptotic factor is suppressed and cell death is stimulated and induced. As a result of the interactions of specific proteins of the Bcl-2 family, cytochrome C enters the cytosol from the space between the two mitochondrial membranes and forms a complex called apoptosome, activating the cascading pathway of caspases and programming the cell to die [25, 26]. Pre-apoptotic members of this family are Bak and Bax, whose increased expression releases cytochrome C into the cytosol and initiates apoptosis. By dephosphorylation of Bak and its release from the cytosolic protein attached to serine 3-3-14, this Bak protein binds to Bcl-2 and prevents its interaction with Bax protein. With Bax oligomerization, the permeability of mitochondrial membrane increases and Cytochrome C is released from the space between the two membranes and enters the cytosol. It then binds to the adaptive protein Apaf1 and the initiator caspase (caspase 9) to form the apoptosome complex, which in turn activates the activator caspase (caspase 3), leading to cell death. Therefore, it seems that the treatment of lipid nanoparticles containing *Artemisia* essentially prevents cell division by inducing apoptosis [27-29]. Baek et al. incorporated the drug Wogonin,

which acts on many cancers, into solid lipid nanoparticles. They observed that the rate of cell death of solid lipid nanoparticles combined with vugunin increased and this drug compound was able to inhibit poly (ADP-ribose) polymerase in MCF-7 breast cancer cells [30]. Also, Shafi et al. 2012 showed that the anti-proliferative effects of *Artemisia Absinthium* extract on human breast cancer cells can cause apoptosis in two cancer cell lines by modulating Bcl-2 family proteins and the MEK/ERK pathway. This may lead to its possible development as a therapeutic agent for breast cancer further research [31]. Mughees and Waged in 2019 came to the conclusion that nanoparticles from the plant extract *Artemisia* effectively prevent the proliferation of breast cancer cells (MCF-7 & MDA MB-231) and induce apoptosis [32]. In addition, Li et al, using the MTT method, showed that *Artemisia Absinthium* L. leaf extract reduced MCF-7 cancer cell viability so that IC50 of crude extract was found to be 25 µg/ml. The results indicated that the plant extract causes the production of ROS and significantly reduces the mitochondrial membrane potential ( $\Delta\Psi_m$ ). It also leads to blockade of MCF-7 cells in the sub-stage G1 of the cell cycle and eventually apoptosis due to concentration-induced 4'-6-diamidino-2-phenylindole staining. Moreover, the plant extract also reduces the colony formation potential of MCF-7 cells in a dose-dependent manner. Their study showed that the ethanolic extract of *Artemisia Absinthium* L. has strong anti-proliferative properties against breast cancer cells. Given that, the extract can be used directly to treat breast cancer or chemical ingredients may be used after separation [33].

## 5. Conclusion

The findings of this study suggest that the lipid nanoparticles synthesized from the essential oil of *Artemisia Vulgaris* act as an inhibitor of breast cancer cell growth and that they have the potential to be used in the pharmaceutical industry and various biological applications, thus being introduced as a drug supplement to the pharmaceutical industry.

## References

1. Lai F, Sinico C, De Logu A, Zaru M, Müller RH, Fadda AM. SLN as a topical delivery system for *Artemisia arborescens* essential oil: in vitro antiviral activity and skin permeation study. *International journal of nanomedicine*. 2007;2(3):419.
2. Ehsanfar P, Teimouri M, Pooladi M. Investigating Characterizations and Antifungal Effects of Solid Lipid Nanoparticles (SLNs) Loaded with Essential Oil of *Citrus Aurantifolia* on Isolated *Malassezia* Strains. *Archives of Advances in Biosciences*. 2020;11(3):43-55.
3. Militello M, Settanni L, Aleo A, Mammina C, Moschetti G, Giammanco GM, Blázquez MA, Carrubba A. Chemical composition and antibacterial potential of *Artemisia arborescens* L. essential oil. *Current Microbiology*. 2011;62(4):1274-81.
4. Teimouri M, Pooladi M. Anti-Angiogenic and Anti-Proliferative Effects of *Physalis Alkekengi* Hydroalcoholic Extract on Breast Cancer in Mice. *Journal of Fasa University of Medical Sciences*. 2021;11(1): 3684-91.
5. Sharmila K, Padma PR. Anticancer activity of *Artemisia vulgaris* on hepatocellular carcinoma (HepG2) cells. *Int J Pharm Pharmaceut Sci*. 2013;5(3):479-83.
6. Demir S, Turan I, ALIYAZICIOĞLU Y. Antioxidant properties of *Primula vulgaris* flower extract and its cytotoxic effect on human

## Acknowledgement

The present study was sponsored by Department of Biology, Roudehen branch, Islamic Azad University.

## Conflict of interest

The authors declare no conflict of interest.

cancer cell lines. *Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi*. 2019;22(1):78-84.

7. Amiel E, Ofir R, Dudai N, Soloway E, Rabinsky T, Rachmilevitch S.  $\beta$ -Caryophyllene, a compound isolated from the biblical balm of gilead (*Commiphora gileadensis*), is a selective apoptosis inducer for tumor cell lines. *Evidence-based complementary and alternative medicine*. 2012;2012.

8. Fan S, Chang J, Zong Y, Hu G, Jia J. GC-MS analysis of the composition of the essential oil from *Dendranthema indicum* Var. *Aromaticum* using three extraction methods and two columns. *Molecules*. 2018;23(3):576.

9. Ahmad A, Van Vuuren S, Viljoen A. Unravelling the complex antimicrobial interactions of essential oils—the case of *Thymus vulgaris* (Thyme). *Molecules*. 2014;19(3):2896-910.

10. Li Y, Li MY, Wang L, Jiang ZH, Li WY, Li H. Induction of apoptosis of cultured hepatocarcinoma cell by essential oil of *Artemisia annul* L. *Sichuan da xue xue bao. Yi xue ban*= *Journal of Sichuan University. Medical science edition*. 2004;35(3):337-9.

11. Kordali S, Cakir A, Mavi A, Kilic H, Yildirim A. Screening of chemical composition and antifungal and antioxidant activities of the essential oils from three Turkish *Artemisia*

- species. *Journal of agricultural and food chemistry*. 2005;53(5):1408-16.
12. Asghari G, Jalali M, Sadoughi E. Antimicrobial activity and chemical composition of essential oil from the seeds of *Artemisia aucheri* Boiss. *Jundishapur journal of natural pharmaceutical products*. 2012;7(1):11.
  13. Jamali T, Kavooosi G, Ardestani SK. In-vitro and in-vivo anti-breast cancer activity of OEO (*Oliveria decumbens* vent essential oil) through promoting the apoptosis and immunomodulatory effects. *Journal of ethnopharmacology*. 2020;248:112313.
  14. Periasamy VS, Athinarayanan J, Alshatwi AA. Anticancer activity of an ultrasonic nanoemulsion formulation of *Nigella sativa* L. essential oil on human breast cancer cells. *Ultrasonics sonochemistry*. 2016;31:449-55.
  15. Shahzadi I, Mehmood F, Ali Z, Ahmed I, Mirza B. Chloroplast genome sequences of *Artemisia maritima* and *Artemisia absinthium*: Comparative analyses, mutational hotspots in genus *Artemisia* and phylogeny in family Asteraceae. *Genomics*. 2020;112(2):1454-63.
  16. Valles J, Garcia S, Hidalgo O, Martin J, Pellicer J, Sanz M, Garnatje T. Biology, genome evolution, biotechnological issues and research including applied perspectives in *Artemisia* (Asteraceae). *Advances in botanical research*. 2011;60:349-419.
  17. Venugopal K, Ahmad H, Manikandan E, Arul KT, Kavitha K, Moodley MK, Rajagopal K, Balabhaskar R, Bhaskar M. The impact of anticancer activity upon *Beta vulgaris* extract mediated biosynthesized silver nanoparticles (ag-NPs) against human breast (MCF-7), lung (A549) and pharynx (Hep-2) cancer cell lines. *Journal of Photochemistry and Photobiology B: Biology*. 2017;173:99-107.
  18. Liu Y, Huo N, Dong L, Wang Y, Zhang S, Young HA, Feng X, Gu YQ. Complete chloroplast genome sequences of Mongolia medicine *Artemisia frigida* and phylogenetic relationships with other plants. *PloS one*. 2013;8(2):e57533.
  19. Das S, Ng WK, Tan RB. Are nanostructured lipid carriers (NLCs) better than solid lipid nanoparticles (SLNs): development, characterizations and comparative evaluations of clotrimazole-loaded SLNs and NLCs?. *European journal of pharmaceutical sciences*. 2012;47(1):139-51.
  20. Gharematrossian S, Popov YU, Ghorbanli M, Safaeian S. Antioxidant activities and cytotoxic effects of whole plant and isolated culture of *Artemisia aucheri* Boiss. *Asian Journal of Pharmaceutical and Clinical Research*. 2012;5(4):95-8.
  21. Sharif M, Ziaei H, Azadbakht M, Daryani A, Ebadattalab A, Rostami M. Effect of methanolic extracts of *Artemisia aucheri* and *Camellia sinensis* on *Leishmania major* (in vitro). *Turkish Journal of Medical Sciences*. 2007;36(6):365-9.
  22. Kordezangeneh M, Irani S, Mirfakhraie R, Esfandyari-Manesh M, Atyabi F, Dinarvand R. Regulation of BAX/BCL2 gene expression in breast cancer cells by docetaxel-loaded human serum albumin nanoparticles. *Medical Oncology*. 2015;32(7):1-9.
  23. Emami SA, Rabe SZ, Ahi A, Mahmoudi M, Tabasi N. Study the cytotoxic and pro-apoptotic activity of *Artemisia annua* extracts. *Pharmacologyonline*. 2009;3:1062-9.
  24. Ahmadi F, Mojarrab M, Ghazi-Khansari M, Hosseinzadeh L. A semipolar fraction of petroleum ether extract of *Artemisia aucheri* induces apoptosis and enhances the apoptotic response to doxorubicin in human neuroblastoma SKNMC cell line. *Research in pharmaceutical sciences*. 2015;10(4):335.
  25. Rabe ST, Emami SA, Iranshahi M, Rastin M, Tabasi N, Mahmoudi M. Anti-cancer properties of a sesquiterpene lactone-bearing fraction from *Artemisia khorassanica*. *Asian*

Pacific Journal of Cancer Prevention. 2015;16(3):863-8.

26. Nazeri M, Mirzaie-Asl A, Saidijam M, Moradi M. Methanolic extract of *Artemisia absinthium* prompts apoptosis, enhancing expression of Bax/Bcl-2 ratio, cell cycle arrest, caspase-3 activation and mitochondrial membrane potential destruction in human colorectal cancer HCT-116 cells. *Molecular Biology Reports*. 2020;47(11):8831-40.

27. Zhai DD, Supaibulwatana K, Zhong JJ. Inhibition of tumor cell proliferation and induction of apoptosis in human lung carcinoma 95-D cells by a new sesquiterpene from hairy root cultures of *Artemisia annua*. *Phytomedicine*. 2010;17(11):856-61.

28. Shamas-Din A, Kale J, Leber B, Andrews DW. Mechanisms of action of Bcl-2 family proteins. *Cold Spring Harbor perspectives in biology*. 2013;5(4):a008714.

29. Zou H, Yang R, Hao J, Wang J, Sun C, Fesik SW, Wu JC, Tomaselli KJ, Armstrong RC. Regulation of the Apaf-1/caspase-9 apoptosome by caspase-3 and XIAP. *Journal of Biological Chemistry*. 2003;278(10):8091-8.

30. Baek JS, Na YG, Cho CW. Sustained cytotoxicity of wogonin on breast cancer cells by encapsulation in solid lipid nanoparticles. *Nanomaterials*. 2018;8(3):159.

31. Shafi G, Hasan TN, Syed NA, Al-Hazzani AA, Alshatwi AA, Jyothi A, Munshi A. *Artemisia absinthium* (AA): a novel potential complementary and alternative medicine for breast cancer. *Molecular biology reports*. 2012;39(7):7373-9.

32. Mughees M, Wajid S. *Artemisia absinthium* nanoparticles induce apoptosis in breast cancer cells via inhibiting vesicular trafficking related proteins. *Annals of Oncology*. 2019;30:vii9.

33. Li L, Wang J, Miao S, Ma S, Shi X, Wen A. Apoptosis induction and reactive oxygen species generation by *Artemisia absinthium* l. leaf extract in MCF-7 breast carcinoma cells. *Pharmacognosy Magazine*. 2020;16(69):422.

**Tables:**

<b>Formula</b>	<b>AR</b>	<b>Col</b>	<b>BW</b>	<b>GMS</b>	<b>SA</b>	<b>PA</b>	<b>T 20</b>	<b>SP 60</b>	<b>SP 80</b>	<b>Water</b>
AR-SLN <sub>1</sub>	0.2	0.05	0.75	-	-	-	0.2	0.4	-	0.4
AR-SLN <sub>2</sub>	0.2	0.05	0.75	-	-	-	0.2	-	0.4	-
AR-SLN <sub>3</sub>	0.2	0.05	-	0.75	-	-	0.2	0.4	-	98.4
AR-SLN <sub>4</sub>	0.2	0.05	-	0.75	-	-	0.2	-	-	98.4
AR-SLN <sub>5</sub>	0.2	0.05	-	-	0.75	-	0.2	0.4	-	98.4
AR-SLN <sub>6</sub>	0.2	0.05	-	-	0.75	-	0.2	-	0.4	98.4
AR-SLN <sub>7</sub>	0.2	0.05	-	-	-	0.75	0.2	0.4	-	98.4
AR-SLN <sub>8</sub>	0.2	0.05	-	-	-	0.75	0.2	-	0.4	98.4

**Table 1.** Formulation of Artemisia Vulgaris essential oil encapsulated in SLN. AR: Artemisia Vulgaris; Col: Cholesterol; BW: Bees Wax; GMS; Glycerol Monostearate; SA: Stearic Acid; PA: Palmitic Acid; T 20: Tween 20; SP 60: Span 60; SP 80: Span 80

<b>Study Groups</b>	<b>Treatment (24 h)</b>		
	<b>Essential Oil</b>	<b>SLN &amp; (Without Essential Oil)</b>	<b>SLN &amp; (With Essential Oil)</b>
<b>IC50 µg/ml</b>	1105	15034	262

**Table 2.** IC50 levels of different study groups in treatment 24 h.