High anticancer efficacy of solid lipid nanoparticles containing *Zataria multiflora* essential oil against breast cancer and melanoma cell lines

Alireza Valizadeh¹, Ali Asghar Khaleghi², Ghazaal Roozitalab² and Mahmoud Osanloo³*

Abstract

**Background:** The cancer burden is rising rapidly worldwide, and it annually causes about 8.8 million deaths worldwide. Due to chemical drugs' side effects and the emergence of resistance, the development of new green drugs has received much attention. We aimed to investigate whether solid-lipid nanoparticles containing essential oil of *Zataria multiflora* (ZMSLN) enhanced the anticancer efficacy of the essential oil against breast cancer (MDA-MB-468) and melanoma (A-375) cells.

**Results:** ZMSLN was prepared by the high-pressure homogenizer method; particle size 176 ± 8 nm, polydispersity index 0.22 ± 0.1, entrapment efficiency 67 ± 5%. The essential oil showed a dose-dependent antiproliferative effect on MDA-MB-468 and A-375 cells at all examined concentrations (75, 150, 300, 600, and 1200 μg/mL). Interestingly, after treating both cells with 75 μg/mL of ZMSLN, their viabilities were reduced to under 13%.

**Conclusion:** The finding showed that ZMSLN had a distinct antiproliferative efficacy; it could thus be considered a green anticancer candidate for further in vivo and in vivo studies.

**Keywords:** Malignancy, Cell Survival, Phytochemicals, Herbal Medicine

Introduction

Cancers have many financial, emotional, and physical burdens in all societies and comprise approximately 17% of global deaths [1]. Although cancer incidence trends are declining by 3.1%/year in men, it was stable in women (from 2009 to 2012) [2]. Cardiovascular disease and cancer are the first and second leading causes of death worldwide [3]. Breast cancer is defined as uncontrolled cell proliferation or growth, becoming the most prevalent cancer in many Asian countries [4, 5]. Melanoma, or cancer of melanocytes in skin tissue, is another prevalent form of cancer with an increasing rate in the last decades worldwide [6]. Due to its heavy burdens on societies in different countries, more attention is required to prevent melanoma morbidity, incidence, and mortality [7].

Bone marrow depression, extreme fatigue, alopecia, loss of self-esteem or immunity, and a remarkable decline in white blood cell count are common side effects of synthetic or semisynthetic anticancer drugs [7, 8]. Thus, the identification and development of novel anticancer using natural products with fewer serious adverse effects have received more attention [9, 10]. *Zataria multiflora* Bioss, the *Lamiaceae* family, is one of the most important medicinal plants in Pakistan, Afghanistan, and southern Iran; it is applied as an...
antiseptic and antitussive in respiratory tract disorders [11]. The Z. multiflora essential oil (ZMEO) also possesses many biological activities, including anticancer and antioxidant effects [11, 12]. EOs (such as ZMEO) are generally hydrophobic; loading them into nanocarriers is one of the most effective ways to improve their performance in laboratory and animal researches [13, 14]. Solid-lipid nanoparticles (SLN) possess advantages over other colloidal systems, such as improved physical stability and sustaining the drug release [15, 16].

We describe here SLNs containing ZMEO (ZMSLN) model for natural indexing products with anticancer effects against breast cancer (MDA-MB-468) and melanoma (A-375) cells.

Materials and Methods
MDA-MB-468 (ATCC HTB-132 and A-375 (ATCC CRL-1619) as breast cancer and melanoma cell lines were purchased from Pasteur Institute of Iran. Stearic acid, tween 80, span 60, phosphate-buffered saline (PBS) tablets, penicillin-streptomycin, trypsin, dimethyl sulfoxide (DMSO), absolute ethanol (99.8%), and MT (3-(4, 5-dimethyl thiazolyl)-2, 5diphenyltetrazolium bromide) were bought from Merck Co. (Germany). Shellmax (China) and Gibco (USA) supplied cell culture medium (RPMI 1640) and fetal bovine serum (FBS). ZMEO was bought from Zardband Pharmaceutical Co. (Iran).

Preparation of ZMSLN
ZMSLN was prepared according to our described high-pressure Homogenizer method [17]. ZMEO (1% v/v) was first dissolved in stearic acid 4% w/v (temperature = 85 °C) and a hot span 60 2% w/v (lipophilic surfactant). After that, the prepared mixture was dispersed in a hot surfactant solution (tween 80—4%) and homogenized (1 min, 8000 RPM) using a high-shear Homogenizer (D-91126 Schwabach, Heidolph, Germany). The obtained pre-emulsion was then homogenized, three cycles, at high pressure, 500 bar, using an APV Micron Lab 40 (APV Systems, Unna, Germany) thermostated at 90 °C. Moreover, free SLNs were also prepared in the same procedure; only ZMEO was not used.

Investigation particle size and morphology of the prepared SLNs
The size of ZMSLN and free SLNs were analyzed by Dynamic Light Scattering (DLS, the model of 9900, K-one Nano Ltd., Korea). The formulation was poured into a quartz cell and situated in the hole of the device for analysis. To determine the shape of ZMSLN and free SLNs, it was first diluted two times with distilled water; one drop was then impregnated on a copper grid (200-mesh carbon-coated). Next, it was stained with phosphotungstic acid (2%) solution, dried at room temperature, and subjected to a TEM device, Philips CM30 TEM microscope (Netherlands).

Entrapment efficiency
ZMEO was first diluted in ethanol in a 150–350 μg/mL concentration, and absorbance was then screened (200–400 nm) to determine the maximum absorption wavelength (λ max). The standard calibration curve (with regression equation) for ZMEO was then plotted (see Fig. 3). For determining entrapment efficiency, ZMSLN was first centrifuged for 20 min at 25,000 RPM and then filtered using a syringe filter (pore size 0.22 μm; n = 3). ZMEO content in the supernatant was then determined by the UV-Visible method; absorbance at λ max was placed in the regression equation.

Entrapment efficiency = (initial drug amount–drug supernatant/injection volume) × 100

MTT assay
The in vitro cytotoxicity of ZMEO and ZMSLN on the breast cancer (MDA-MB-468) and melanoma (A-375) cancer cells was estimated by MTT assay according to our previous study with slight modification [18]. ZMEO was dissolved (4800 μg/mL) in PBS solution containing 0.5% DMSO; required serial dilutions were also prepared using the same solvent. The cells were first seeded (1 × 10^4 cells/well) in 96-well culture plates overnight. Wells content was then replaced with 75 μg.mL^-1 RPMI complete medium (containing 10% FBS and penicillin/streptomycin 1%). The cells were treated with various concentrations of ZMEO and ZMSLN (75, 150, 300, 600, and 1200 μg.mL^-1), as well as free SLNs; treated plates were incubated for 24 h (37 °C and 5% of CO2 concentration). Wells’ content was then discarded and washed with PBS to the removed milky color of ZMSLN. After that, 100 μL of MTT reagent (0.5 mg/mL dissolved in RPMI) was treated for each well and incubated for 4 h at 37 °C and 5% of CO2 concentration. Finally, 100 μL/well DMSO was added to solubilize the precipitate formazan crystals to the obtained purple color. The absorbance (A) of wells was measured at 570 nm by the ELISA Plate Reader. Cell viabilities at different concentrations were calculated by eq. 2. Six well/plates were considered the control group, contained with PBS solution containing 0.5% DMSO (25 μL) and RPMI (75 μL).

Cell viability = (A sample/A control) × 100

Statistical Analysis
All the tests were carried out in triplicate; results were expressed as mean ± SD. CalcuSyn software
version, BIOSOFT, UK) was applied for calculating the 
IC50s of the samples. One-way ANOVA (SPSS software 
v. 22, USA) with the least significant difference test 
(L.S.D) at the 5% level was used to compare anticancer 
activities.

**Results**

**Characterization of size, morphology, and entrapment 
efficiency**

DLS characterized the ZMSLN with a particle size of 
176 ± 8 nm and a polydispersity index of 0.22 ± 0.1 (see 
Fig. 1). The morphology of ZMSLN was demonstrated 
by TEM; it showed a spherical shape with a toothed 
shell-like (see Fig. 2). Moreover, a polluted calibration 
curve for determining the amount of ZMEO is depicted 
in Fig. 3. Noted, λ max of ZMEO was obtained at 236 
nm, and the entrapment efficiency was achieved at 67 ± 
5%.

**In vitro anticancer activity**

As shown in Fig. 4A, MDA-MB-468 cancer cells have 
susceptibility to ZMSLN at all concentrations. Besides, 
the results showed that the survival of MDA-MB-468 
cancer cells was under 50% at a concentration of 
≥600 μg.mL-1 in the treating group with ZMEO. The 
difference between the anticancer activity of ZMEO and 
ZMSLN was significant (P < 0.001) at all concentrations. 
Moreover, a significant difference (P < 0.001) is observed 
amongst the control group and treated cells with free 
SLNs; viability was reduced to 79%.

From Fig. 4B, the viability of the A-375 cell line in 
the presence of ZMEO and ZMSLN showed a signifi-
cant reduction under 50% at all concentrations. The 
results demonstrated that ZMSLN had significantly 
higher anticancer activity than ZMEO at all concen-
trations (P < 0.001). Besides, the viability of the cell 
after treatment with free SLNs was significantly (P < 
0.001) reduced (to ~82%) in comparison with the 
control group.

IC50 values of ZMEO against MDA-MB-468 and A-
375 cells were obtained at 380 and 59 μg.mL-1. Because 
even the lowest concentration of the ZMSLN reduced 
cell viability of both cell lines below ~13%, IC50 of 
ZMSLN was thus not calculating precisely. However, it 
could be concluded that ZMSLN had a distinct antipro-
liferative effect.
Breast and skin cancer are the two most frequent types of cancers worldwide [19, 20]. The commonest life-threatening malignancy in women is breast cancer; it is the cause of 14% of all cancer-related fatalities [21, 22]. MDA-MB-468 without estrogen and progesterone receptors and HER2 non-expression (triple-negative) is an invasive and poorly differentiated cell line. It is one of the commonly used cell lines in the literature [23, 24]. Skin cancer is the most common and preventable carcinoma around the world, and rising annual incidence has made it a pre-eminent public health threat [25]. Malignant melanoma is a type of skin cancer and is responsible for most skin cancer deaths; it begins with the abnormal proliferation of cells known as melanocytes [26]. Amongst melanoma cell lines, A-375 cells are aggressive with low susceptibility to chemotherapy [27, 28].

Natural products, especially EOs, have been widely used for their therapeutic properties as chemopreventive/chemotherapeutic agents in vitro and in vivo studies [29–31]. For instance, in our previous study, chemical compositions of ZMEO (the used batch in the current study) was first investigated; carvacrol (30.2%), thymol (25.2%), o-cymene (10.7%), c-terpinene (6.1%), and α-pinene (3.616%) were identified as five major compounds. Its anticancer effects were then evaluated against breast cancer cell lines, including MCF-7, MDA-MB-175, and MDA-MB-231; IC₅₀ values were obtained.
as 76, 70, and 104 μg.mL$^{-1}$ [32]. Furthermore, the anticancer effect of major ingredients of some EOs has been repeatedly investigated on different cancer cells [33]. For instance, anticancer effects of ZMEO main constituents, i.e., thymol and carvacrol (monoterpenoids and monoterpenes), were reported [33–36]. For instance, thymol and carvacrol at lower concentrations had antioxidant activity and at higher doses had antiproliferative and apoptotic effects [37]. The cytotoxicity of carvacrol with IC$_{50}$ 380 μM was more potent than thymol with IC$_{50}$ 497 μM against human non-small cell lung carcinoma after a 24 h incubation period [37]. Recently anticancer effects of thymol and carvacrol were investigated against the SKOV-3 ovarian cancer cell line by Elbe et al.; thymol was more potent than carvacrol [38].

SLNs contain a hydrophobic solid matrix core with one layer of a phospholipid coating [39]. The high entrapment efficiency for hydrophobic drugs in the core, well-tolerated composition due to physiologically similar lipids, prolonged drug release feature, low toxicity, biodegradable composition, and high stability makes SLNs promising candidates for cancer therapy and pharmaceuticals usage [40, 41]. It was thus chosen in the current study to entrap ZMEO inside the core. However, some reports on the loading of EOs into SLNs have been found. In one research, SLNs containing Croton argyrophyllus EO with the particle size of 201.4 ± 2.3 nm and entrapment efficacy 89.63 ± 1.02% was reported; an improvement in antioxidant properties of EO in the treatment of neurodegenerative diseases was observed [42]. In another research, SLNs containing ZMEO were prepared to increase its antifungal activity efficiency. The entrapment efficacy of ZMSLN was 64.6 ± 3.8%, with a particle size of 134 ± 7 nm. ZMSLN also facilitated the applicability of ZMEO as antimicrobials and antifungal medication [43, 44]. SLNs loaded with Eugenia caryophyllata EO were presented a unique nanoparticulate system with a vast antimicrobial activity. The particle size was between 397 ± 10 nm and 786 ± 11, with entrapment efficacy at approximately 70% [43]. Another study aimed to prepare SLNs (with a mean size of 113.3 ± 3.6 nm and entrapment efficacy of 80% ± 1%) for the oral delivery of two EOs. The SLNs increased the antitumoral activity of EOs in H22-bearing Kunming mice [44]. In 2013, Moghimpour et al. designed and prepared a SLN from ZMEO using hot homogenization and precipitation methods; the prepared SLNs had a mean size of 650 nm [45]. Ehsanfar et al. applied the ultrasonic Homogenizer method to produce several SLNs with different sizes [46].

Furthermore, several studies showed that formulating EO in nanocarriers leads to higher activity. For example, Nasseri et al. prepared ZMSLN with a particle size of 255 ± 3 nm to improve and control antifungal efficiency in some fungal pathogens [47]. In our previous study, antibacterial activity increased by reducing the particle size of two EOs of Zataria multiflora and Mentha piperita [48]. In addition, the leishmanicidal activity of Cinna- momum zeylanicum EO against Leishmania tropica and Leishmania major was increased when size was reduced to the nanoscale range, 52 ± 4 nm [18]. Also, curcumin-loaded SLN had greater antiproliferative activity on breast cancer cells than the bulk of curcumin [49]. Besides, the anticancer activity of variabilin was improved by reducing the size to nano range (IC$_{50}$ 8.94 μM) compared with free variabilin (IC$_{50}$ 87.74 μM) against the PC-3 cell line [50].

In the current study, the anticancer activity of ZMEO and ZMSLN was evaluated; results represented that ZMEO at the nanoscale size (176 nm) had a substantially more anticarcinogenic effect compared with bulk form against MDA-MB-468 and A-375 cancer cell lines. It may relate to the higher efficiency of cellular uptake and intracellular absorption of ZMSLN than the bulk form of ZMEO [51]. Moreover, in the current study, no dose-response effects were observed in cells treated with ZMSLN; viability of MDA-MB-468 cells was reduced to ~10%, and A-375 cells viability was reduced to ~5% at a concentration range of 150–1200 μg.mL$^{-1}$. This is due to the maximum capacity of this nanoformulation at examined concentrations; with decreasing concentrations, lower efficacies become visible. In our previous report, the anticancer properties of chitosan nanoparticles containing Citrus sinensis and Citrus limon essential oils at examined concentrations were also not dose-dependent [52]. However, due to the promising efficacies of the prepared nanoformulation (ZMSLN) in a wide range of concentrations (150–1200 μg.mL$^{-1}$), further research to investigate toxic effects on normal cells and other cancer cell lines in vitro and animal models are suggested.

**Conclusions**

Solid-lipid nanoparticle-containing Z. multiflora EO (ZMSLN) with a particle size of 176 ± 8 nm, polydispersity index 0.22 ± 0.1, and entrapment efficiency 67 ± 5% was first prepared. The comparative study showed that ZMSLN had a higher anticancer activity on tested cancer lines (MDA-MB-468 and A-375) than the EO treatment group. Furthermore, after treating both cell lines with the lowest examined concentration of ZMSLN, 75 μg/mL, their viabilities were decreased under %13. ZMSLN could thus be introduced as a green potent antiproliferative candidate. However, more studies should be evaluated to reveal the exact mechanism of anticancer activity of ZMSLN.

**Acknowledgments**

Not applicable.
Authors' contributions

AV analyzed the data and was the main draftsman of the manuscript. AA. Kh revised the manuscript. GhR reviewed the literature. MO designed the study and carried out all laboratory examinations. All authors contributed to drafting the manuscript and approved the final version.

Funding

Fasa University of Medical Sciences financially supported this research, grant number 99126.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Declarations

Ethics approval and consent to participate

The ethical committee has ethically approved this research; IRIFUMSREC.1399.139. This research did not involve in vivo or human study, so consent to participate did not use.

Consent for publication

Not Applicable.

Competing interests

None.

Author details

1Department of Medical Nanotechnology, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran. 2Noncommunicable Diseases Research Center, Fasa University of Medical Sciences, Fasa, Iran. 3Department of Medical Nanotechnology, School of Advanced Technologies in Medicine, Fasa University of Medical Sciences, Fasa, Iran.

Received: 29 April 2021 Accepted: 20 September 2021

Published online: 30 September 2021

References

1. Cancer. [https://www.who.int/health-topics/cancer#tab=tab_1].
2. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. CA Cancer J Clin. 2016;66(1):7–30.
3. Nagai H, Kim YH. Cancer prevention from the perspective of global cancer burden patterns. J Thorac Dis. 2017;9(3):448–51.
4. What Is Breast Cancer? [https://www.cdc.gov/cancer/breast/basic_info/what-is-breast-cancer.html#:~:text=Breast%20cancer%20is%20a%20disease,more%20than%20tumor%20deform%20breast].
5. Youn HJ, Han W. A Review of the Epidemiology of Breast Cancer in Asia: Focus on Risk Factors. Asian Pac J Cancer Prev. 2020;21(4):867–80.
6. Matthews NH, Li WQ, Qureshi AA, Weinstock MA, Cho E. Epidemiology of Melanoma. In: Ward WH, Farma JM, editors. Cutaneous Melanoma: Etiology and Therapy. Brisbane (AU): Codon Publications; 2017.
7. Mohamed JM, Alqahtani A, Ahmad F, Krishnaraju V, Kalpana K. Pectin co-functionalized dual layered solid lipid nanoparticle made by solubilize curcumin for the targeted potential treatment of colorectal cancer. Carbohydr Polym. 2021;252:117180.
8. Nurgali K, Jagoe RT, Abalo R. Adverse effects of cancer chemotherapy: Anything new to improve tolerance and reduce sequelae? Front Pharmacol. 2018;9:245.
9. Rayan A, Rayn J, Falah M. Nature is the best source of anticancer drugs: Indexing natural products for their anticancer bioactivity. PLoS One. 2017;12(11):e0178925.
10. Abbedinpour N, Ghanbariasad A, Taghinezhad A, Osanloo M. Preparation of Nanoemulsions of Mentha piperita Essential Oil and Investigation of Their Cytotoxic Effect on Human Breast Cancer Lines. BioNanoScience. 2021;11:428–36.
11. Azadi M, Jamali T, Kiannnehz M, Kavoosi G, Ardestani SK. In-vitro (2D and 3D cultures) and in-vivo cytotoxic properties of Zataria multiflora essential oil (ZEO) emulsion in breast and cervical cancer cells along with the investigation of immunomodulatory potential. J Ethnopharmacol. 2020;257:112865.
12. Kavoosi G, Rabiee F. Zataria multiflora: chemical and biological diversity in the essential oil. J Essent Oil Res. 2015;27(5):428–36.
13. Gafur A, Sukamdani GY, Kristi N, Maruf A. From bulk to nano-delivery of essential phytochemicals: recent progress and strategies for antibacterial resistance. 2020;8(43):9825–9835.
14. Rathore C, Rathbone MJ, Chellappan DK, Tambaruwa MM. Nanocarriers: more than tour de force for thymoquinone. Expert Opin Drug Deliv. 2020;17:479–94.
15. Rosiaux Y, Jannin V, Hughes S, Marchaud D. Solid lipid excipients as matrix agents for sustained drug delivery. Expertise Applications in Formulation Design and Drug Delivery. J Control Release. 2015;237–71.
16. Rajpoot K. Solid lipid nanoparticles: a promising nanomaterial in drug delivery. Curr Pharm Des. 2010;15(37):3943–59.
17. Keldari HR, Moemenbehollah-Fard MD, Morteza-Semnani K, Amooghezfar F, Shahrkari-Namadi M, Saeedi M, et al. Solid lipid nanoparticles (SLN)s containing Zataria multiflora essential oil with no-cytotoxicity and potent repellent activity against Anopheles stephensi. J Parasit Dis. 2021;45(1):101–8.
18. Ghanbariasad A, Valizadeh A, Ghadimi SN, Fereidouni Z, Osanloo M. Nanoformulating Cinnamonum zeylanicum essential oil with an extreme effect on Leishmania tropica and Leishmania major. J Drug Deliv Sci Technol. 2021;63:102436.
19. Waks AG, Winer EP. Breast cancer treatment: a review. JAMA. 2019;321(3):288–300.
20. Lomas A, Leonardi-Bee J, Bath-Hextall F. A systematic review of worldwide incidence of nonmelanoma skin cancer. Br J Dermatol. 2012;166(5):1069–80.
21. Sharma GN, Dave R, Sanadja Y, Sharma P, Sharma K. Various types and management of breast cancer: an overview. J Adv Pharm Technol Res. 2010;1(2):109.
22. McGuire A, Brown JA, Kerin MJ. Metastatic breast cancer: the potential of mRNA for diagnosis and treatment monitoring. Cancer Metastasis Rev. 2015;34(1):145–55.
23. Wang WL, Porter W, Burghardt R, Safe SH. Mechanism of inhibition of MDA-MB-468 breast cancer cell growth by 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin. Carcinogenesis. 1997;18(5):925–33.
24. Androustopoulos V, Arroo RR, Hall JF, Suchitan S, Potter GA. Antiproliferative and cytostatic effects of the natural product eupatorium on MDA-MB-468 human breast cancer cells due to CYPI-mediated metabolism. Breast Cancer Res. 2008;10(3):R99.
25. Gordon R. Skin cancer: an overview of epidemiology and risk factors. Semin Oncol Nurs. 2013;29(3):160–9.
26. Uong A, Zon Li. Melanocytes in development and cancer. J Cell Physiol. 2010;222(2):38–41.
27. Maksimović-Ivančić D, Bulatović M, Dederer D, Bensing C, Golic I, Korać A, et al. The interaction between SBA-15 derivative loaded with Ph (3) Sn (CH 2) 6 OH and human melanoma A375 cell line: uptake and stem phenotype loss. J Biol Inorg Chem. 2019;24(2):223–34.
28. Massi D, Brusa D, Merelli B, Ciano M, Audrito V, Serra S, et al. PD-L1 marks a subset of melanomas with a shorter overall survival and distinct genetic and morphological characteristics. Ann Oncol. 2014;25(12):2433–42.
29. Leal PF, Braga ME, Sato DN, Carvalho JE, Marques MO, Meireles MAA. Functional properties of spice extracts obtained via supercritical fluid extraction. J Agric Food Chem. 2003;51(8):2520–5.
30. Soylu EM, Kurt Ş, Soylu S. In vitro and in vivo antifungal activities of the essential oils of various plants against tomato grey mould disease agent Botrytis cinerea. Int J Food Microbiol. 2010;143(3):183–9.
31. Panth N, Manandhar B, Pauldel KR. Anticancer activity of Punicia granatum (pomegranate): a review. Phytother Res. 2017;31(4):568–78.
32. Ghanbariasad A, Osanloo M. Development of two stable green nanofluidicant formulations containing Zataria multiflora essential oil with no-cytotoxicity and potent repellent activity against Anopheles stephensi. J Parasit Dis. 2021;45(1):101–8.
33. Günes-Bayir A, Kocyigit A, Guler EM, Dadak A. In Vitro Hormetic Effect of thymol on in vitro and in vivo antifungal activities of the essential oils of various plants against tomato grey mould disease agent Botrytis cinerea. Int J Food Microbiol. 2010;143(3):183–9.
34. Panth N, Manandhar B, Pauldel KR. Anticancer activity of Punicia granatum (pomegranate): a review. Phytother Res. 2017;31(4):568–78.
35. Ghanbariasad A, Osanloo M. Development of two stable green nanofluidicant formulations containing Zataria multiflora essential oil with no-cytotoxicity and potent repellent activity against Anopheles stephensi. J Parasit Dis. 2021;45(1):101–8.
36. Bouhtit F, Najar M, Moussa Agha D, Melki R, Najimi M, Sadki K, et al. New Anti-Leukemic Effect of Carvacrol and Thymol Combination through Synergistic Induction of Different Cell Death Pathways. Molecules. 2021; 26(2):410.

37. Ozkan A, Erdogan A. A Comparative Study of the Antioxidant/Prooxidant Effects of Carvacrol and Thymol at Various Concentrations on Membrane and DNA of Parental and Drug Resistant H1299 Cells. Nat Prod Commun. 2012;7(12):1557–60.

38. Elbe H, Yigitturk G, Cavusoglu T, Baygar T, Ozgul Oral M, Ozturk F. Comparison of ultrastructural changes and the anticarcinogenic effects of thymol and carvacrol on ovarian cancer cells: which is more effective? Ultrastruct Pathol. 2020;44(193–202.

39. Thukral DK, Dumoga S, Mishra AK. Solid lipid nanoparticles—apromising drug delivery system. Curr Drug Deliv. 2014;11(6):771–91.

40. Gordillo-Galeano A, Mora-Huertas CE. Solid lipid nanoparticles and nanostructured lipid carriers: a review emphasizing on particle structure and drug release. Eur J Pharm Biopharm. 2018;133:265–308.

41. Zhao Y, Chang YX, Hu X, Liu CY, Quan LH, Liao YH. Solid lipid nanoparticles for sustained pulmonary delivery of Yuxingcao essential oil. Preparation, characterization and in vivo evaluation. Int J Pharm. 2017;516(1–2):364–71.

42. Souto EB, Severino P, Marques C, Andrade LN, Durazzo A, Lucarini M, et al. Croton argyrophyllus Kunth essential oil-loaded solid lipid nanoparticles: evaluation of release profile, antioxidant activity and cytotoxicity in a neuroblastoma cell line. Sustainability. 2020;12(18):7697.

43. Fazly Bazzaz B, Khameneh B, Namazi N, Iranshahi M, Davoodi D, Golmohammadzadeh S. Solid lipid nanoparticles carrying Eugenia caryophyllata essential oil: the novel nanoparticulate systems with broad-spectrum antimicrobial activity. Lett Appl Microbiol. 2018;66(6):506–13.

44. Shi F, Zhao JH, Liu Y, Wang Z, Zhang YT, Feng NP. Preparation and characterization of solid lipid nanoparticles loaded with frankincense and myrrh oil. Int J Nanomedicine. 2012;7:2033–43.

45. Hossainelahi A, Alizadeh A, Abdolghorani M. Investigating Characterizations and Antifungal Effects of Solid Lipid Nanoparticles (SLNs) Loaded with Essential Oil of Citrus Aurantifolia on Isolated Malassezia Strains. Arch Adv Biosci. 2020;11(3):43–55.

Publisher’s Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.