

Antitumoral effects of *Santolina chameacyparissus* on non-small cell lung cancer cells

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Abstract

Santolina chameacyparissus (Santo) is an evergreen plant which is traditionally used for its anti-inflammatory effects in various diseases. In this study, we aimed to explore the effects of Santo in non-small cell lung cancer cells. We extracted volatile oil from the plant and evaluated cytotoxicity, apoptosis, and motility effects of the extract on two non-small cell lung cancer (NSCLC) cell lines; one is a patient derived and the other one is a commercially available A549 cells. We also identified its components via GC/MS and investigated possible targets of the major components of the plant using qPCR and docking studies. Cytotoxicity tests showed dose dependent cell killing activity and flow cytometry assays exposed apoptotic effects of Santo. The essential oil also remarkably decreased migration rate of A549 cells, therefore we evaluated the expression levels of epithelial to mesenchymal transition related genes E-cadherin and Vimentin ratio, *ZEB1* and *SNAIL* and another motility related gene *Ezrin*. Santo did not change the expression of EMT related genes but decreased *Ezrin* levels. According to the results of the GS/MS analysis, Artemisia ketone and Camphor were identified as major molecules of the extract. Docking analysis also revealed that artemisia ketone, the major component of the Santo extract, potentially showed strong binding to the active site of ezrin protein and both artemisia ketone and camphor had ability to bind DNA. The results of the present study indicate that Santo and its components artemisia ketone and camphor are promising anti-cancer agents, and their potential in targeting DNA and oncogenic proteins in the lung cancer cells seems worth to focus on this plant in cancer related drug discovery science.

Keywords: essential oil, lung cancer, antitumor agents, ezrin, santolina chameacyparissus, molecular docking, artemisia ketone, camphor

1. Introduction

Aromatic plants have been used since ancient times for their medicinal properties. These properties can be partially or wholly related to their volatile oil fractions which can be also referred as essential oil (Merle et al., 2004). Various essential oils have been used to treat illnesses at different periods in the history can now be reached at the market and subject to regulation in most countries (Baser and Buchbauer, 2010). The diverse therapeutic potentials of essential oils have attracted the attention of many researchers to investigate their anticancer activity because of the limitations and adverse effects of common antitumor agents.

Santolina chameacyparissus also known as Cotton Lavender or Gray Santolina, is an evergreen plant that belongs to the Asteraceae family which has grayish leaves and yellow flowers. It is an aromatic plant, mostly found in Mediterranean

region and traditionally used for pain release of premenstrual syndrome, treatment for infections and infestations and digestive disorders due to its anti-inflammatory effects (Mete, 2019). Studies in the literature from Tunisia, Algeria and India also support antimicrobial and antiparasitic activities of the plant (Suresh et al., 1997; Boudoukha et al., 2016, Bel Hadj Salah-Fatnassi et al., 2017) and recently, possible cytotoxic effects of Santo's on cancer cells have also been shown (Elsharkawy, 2014). Even though the components of the essential oils are different between these studies, oxygenated monoterpenes and sesquiterpenes consist most of the essential oil derived from Santo. Among monoterpenes, limonene, peryllil alcohol have been shown to prevent breast, liver, lung, and other cancers (Crowell, 1999), and p-cymene and thymoquinone contribute to autophagic cell death in ovarian, breast and colon adenocarcinoma cells (Ashrafzadeh et al.,

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2019). There are other individual studies focusing on the effects of other components of essential oils derived from various herbals such as β -pinnen, myrcene, artemisia but there still little is known about the effects of Santo's anti-tumoral activity and which of its compounds might have which of the effect remains uncertain.

In this study, to explore anticancer effects of *Santolina chamaecyparissus*, we derived essential oil from the plant, from southern part (Osmaniye city) of Turkey and identified its components via GS/MS. We used two non-small cell lung cancer (NSCLC) cell line; one was a patient derived and the other one was a commercially available, and evaluated the antitumoral activity of *Santolina chamaecyparissus* on these cells. Via docking studies, we speculate Ezrin is a possible target of Artemisia ketone which came forward as the major component of the plant.

2. Materials and methods

2.1. Isolation of the essential oil

On April 2018, leaves were collected from the *S. chamaecyparissus* plant and tree of *C. aurantium* grown in the Karacaoglan campus of Osmaniye Korkut Ata University, Osmaniye, which is 20 km away from the East Mediterranean Sea (37°02'303"N; 36°13'0.91"E) and 150 m above sea level. The plant samples were diagnosed by Dr. Fuat BOZOK with the Flora of Turkey and the East Aegean Islands (Davis, 1982). The shade-dried samples were powdered in a Waring blender and hydrodistilled for three hours using a Clevenger according to the method recommended in the current European Pharmacopoeia to obtain essential oils (European Pharmacopoeia, 2008). The essential oils were dried over anhydrous sodium sulphate and stored in an amber vial in the dark at 4°C for further use.

2.2. Gas chromatography-mass spectrophotometry analysis

The components and the quality of the extracted essential oil was investigated using gas chromatography (GC, Agilent Technologies 6890N Network system) with flame ionization detection (FID) detector GC/FID and gas chromatography combined with a mass selective detector GC/MS (Ulukanli, et al., 2015). Retention indices (RI) calculations were also confirmed with the results obtained from alkanes (C8-C32). Further, RIs of the constituents were compared with the authentic substances. Mass spectra data of the tested complex were crosschecked with the earlier data in the GC/MS databases of the Wiley 2001 library data (NIST 02 version 2.62) and the published references (Adams, 2009).

2.3. Establishment of primary cell line (SA7) and cell culture conditions

Pathologist approved lung squamous cell carcinoma tumor specimens was derived from a 61-year-old male patient who was operated at the Çukurova University Medical Center and given consent to join the research. Ethical approval was obtained from the Local Ethical Board (Number: 2018-73). Small fragments of the tumor tissue (1 mm³) washed twice in

PBS and re-suspended in cell culture medium (RPMI-1640 supplemented with 10% fetal bovine serum (FBS) and 1%, 200 IU/ml penicillin-streptomycin) then plated in flasks. Cell-culture medium was replaced every three to five days and the cells were cultivated using trypsin to eliminate fibroblasts before starting experiments. A549 cells were provided from Gaziantep University and maintained with the same conditions with SA7 primary cells. Both A549 cells and SA7 cells were cultured under standard conditions at 37°C with 5% CO₂.

2.4. siRNA transfection, western blot, and antibodies

Ezrin siRNA (Ambion Life #s1496) and ON-TARGET plus non-targeting control siRNA (Dharmacon, #D001818-02) were transfected as previously described (Saygideger-Kont et al., 2016) using Lipofectamine 2000. For western blot, cells were lysed with RIPA buffer (50mM Tris HCl pH 7.4, 1%Nonidet p-40, 0.5% Na-deoxycholate, 0.1% SDS, 150mM NaCl, 2mM EDTA, 50mM NaF) supplemented with protease and phosphatase inhibitors and DNase. For protein quantification, bicinchoninic acid assay was performed and samples were boiled in SDS-PAGE sample buffer as previously described (Saygideger-Kont et al., 2016). Antibodies were as follows: Ezrin (E8897) from Sigma-Aldrich, Actin (C-11) from Cell Signaling, Alexa-Fluor mouse (A21235) secondary and DAPI (D1306) from Thermo Fisher.

2.5. Immunofluorescence (IF) and microscopy

IF staining of A549 cells to confirm Ezrin expression was performed as previously described (Saygideger-Kont et al., 2016). Basically, cells were seeded onto collagen coated coverslips in 12-well plates and fixed with 3.7% paraformaldehyde in PBS, permeabilized and blocked with 1% BSA, 10% goat serum and 0.5% Triton X-100 in PBS before incubation with primary and secondary antibodies and DAPI in the dark. Nikon Eclipse Ti-S microscope with Nikon Intensilight C-HGFI illuminator used to detect images.

2.6. Cell viability

Santo was tested for its cytotoxic effect against two NSCLC cell line, one is a new primary squamous cell lung cancer cell line SA7 and other one is a well-studied lung adenocarcinoma cell line A549, using (MTT) Thiazolyl Blue Tetrazolium Bromide method as previously described (Saygideger-Kont et al., 2016). Briefly, SA7 cells (1.0×10⁴/200 mL/well) were cultured in a 96-well plate for overnight at 37°C, 5% CO₂ and 80% humidity in their respective medium containing 10% FBS and 1% AB. After 24h old medium was removed and the cells were incubated with 0-500 µg/mL concentrations of the oils for 24 and 48h at 37°C, 5% CO₂. Cells with 0.1% DMSO (vehicle control) and cisplatin (positive control) were also incubated at the same conditions. After incubations, 20µL of MTT solution (5 mg/mL in PBS buffer) was added and the cells were further incubated at 37°C, 5% CO₂ for 4h to metabolize MTT by viable cells. After MTT treatment, the supernatants were carefully removed, 50µL DMSO was added to each well and then absorbance was measured at 630 nm subtracted from optical density at 570 nm in a multi-well plate reader.

2.7. Flow cytometry

To determine the apoptotic effects of the essential oils on the cells in vitro, Annexin V staining was performed according to the protocol of BD Pharmingen™ FITC Annexin V Apoptosis Detection Kit. For quantitative analysis, 200 µM and 400 µM concentrations of essential oil were tested on A549 cells. Cell suspension (1.0×10^7 cells/mL) in serum-free medium was incubated with the respective compound in 6-well plates in the CO₂ incubator. At the 24th and 48th hour, the cells were harvested and incubated with FITC-Annexin V and PI. The fluorescence emission of FITC-Annexin-V-stained cells was measured at 633 nm (Red laser) in a flow cytometer (Beckman Coulter/CytoFLEX, United States). Dots represent cells as follows: lower left quadrant, normal cells (FITC⁻/PI⁻); lower right quadrant, early apoptotic cells (FITC⁺/PI⁻); upper left quadrant, necrotic cells (FITC⁻/PI⁺); upper right quadrant, late apoptotic cells (FITC⁺/PI⁺).

2.8. Scratch assay

A549 cells were plated in 6cm plates in regular growth medium containing 10% FBS. After 24h, a straight-line scratch was made on cell layers using a sterile 1mL disposable pipette tip and washed with PBS. Then, the cells treated either with the essential oil (200 µg /mL) or with PBS in the growth media. Images of cell migration were taken using inverted microscope at 0h and 24h after the scratch. Gap lengths from various points of the scratch were measured using Image J.

2.9. Quantitative Polymerase chain reaction (qPCR) and primers

Total RNA was extracted from A549 cells using RNA isolation kit (Macherey-Nagel, Germany) and reverse transcribed using High-Capacity cDNA Reverse Transcription kit (ThermoFisher Sci.) according to their protocols. qPCR was performed on Light Cycler 480 Instrument (Roche) in 96 well-plate in triple form for each condition using SYBR green mix (Ampliqoon). 18S rRNA was used as internal control gene and fold differences were calculated using $2^{-\Delta\Delta CT}$ method which $\Delta\Delta CT$ symbolize (Normalized control sample CT)-(Normalized treated sample CT) (Table 1).

Table 1. Primers used in the experiments

Gene name		Primer sequence
18S rRNA	F	5'-cttagagggacaagtggcg-3'
	R	5'-acgctgagccagtcagtgta-3'
E-Cadherin	F	5'-gtcattgagcctggcaatttag-3
	R	5'-gttgagactcctccattcctc-3'
Vimentin	F	5'-cagcttcaagtgcctttctg-3
	R	5'-ctgtaggagtgtcggtgtt-3
Ezrin	F	5'-catcactgaggcagagaagaac-3'
	R	5'-tgtcattgtggctccttattc-3'
SNAIL	F	5'-gcatgcccagctagaaa-3
	R	5'-ggtaatgtgtgggtccaata-3
ZEB1	F	5'-cttctcacactctgggtcttattc-3'
	R	5'-cgttctccgcttctctttac-3'

2.10. Molecular docking studies

In this process, Discovery Studio (DS) 2019 (Dassault

Systèmes, 2019) was used to exert molecular docking for understanding interactions between 3,3,6-Trimethylhepta-1,5-dien-4-one (Artemisia ketone, AK) and Ezrin. Molecular docking includes three steps. First step, the small compound as ligand and human ezrin model structure were prepared using Gaussian 09 (Frisch et al., 2009) and DS 2019 (Phang et al., 2016) software for molecular docking calculations. The ligand, artemisia ketone was drawn and minimized at DFT/B3LY/6-31G* level by using G09. The protein crystal structure of ezrin (PDB code: 4RM9) (Phang, et al., 2016) was taken from the Protein Data Bank. The target protein subsequently optimized using CHARMM forcefield and the adopted-basis Newton-Raphson (ABNR) method (Chattaraj et al., 2011) available in the DS 2019 protocol until the root mean square deviation (RMSD) gradient was < 0.05 kcal/mol Å². Second step, the active sites were determined by using define and edit binding site subprotocol of DS software. Lastly, *Dock Ligands* (CDOCKER) was applied using the default settings. The best pose of the related compound was defined based on the docking score, binding energy and root mean square deviation (RMSD) values.

2.11. Statistical analysis

Three replicates for each experiment were performed and values were expressed as the mean and standard deviation. Data were subjected to Prism (v. 8.2) for column analysis, dose-response analysis and for graphics. $P < 0.05$ was counted as significant when indicated.

3. Results

3.1. Characterization of the essential oil derived from Santo

The oil extracted from *S. chamaecyparissus* by hydro distillation had a pale-yellow color and fragrant pleasant odor. The components, percentages, and retention index of the constituent of the essential oil of Santo is shown in Table 2. Artemisia ketone (33.36%), and camphor (20.86%) were found to be the major compounds of the essential oil. The other increased components were determined as alpha-bisabolol (6.65%), β -Phellandrene (5.61%), camphene (4.63%), sabinene (2.12%), β -pinene (1.77%), myrcene (2.13%), santolina triene (1.4%), and endo-borneol (2.02%).

Table 2. Chemical composition of the essential oil from *Santolina chamaecyparissus* assessed by GC-MS

Name	% Area
Santolina triene	1.488
Camphene	4.639
Sabinene	2.123
beta-Pinene	1.772
Myrcene	2.129
β -Thujene/beta phellandrene	5.611
Artemisia ketone	33.360
Artemisia alcohol	1.930
Camphor	20.864
alpha-bisabolol	6.658
endo-Borneol	2.023

The components of the local essential oil were different from previously published Santo oils (Bel Hadj Salah-Fatnassi et al., 2017; Suresh et al., 1997; Ortiz de Elguea-Culebras et al., 2018; Djeddi et al., 2012) that major component was Artemisia ketone (AK) in the present study. AK is an enone and was found effective in malarial and other parasitic diseases (Radulovic et al., 2013). The plant part and harvesting period usually effect the composition of the corresponding essential oil. However, the quality of an essential oil also depends on several factors which must be taken into consideration such as plant age, vegetation cycle, geographic situation, mode of extraction (Lota et al., 1999; Ferhat et al., 2006) and methods of analysis (Mondello et al., 1995; Boussaada and Chemli, 2007). The differences between the results of this study and previous reports may be attributed to these variables.

3.2. Santo had cytotoxic effect on lung cancer cells inducing apoptosis

In cell viability assay we tested different concentrations (0 to 1000 µg/mL) of *Santo* on lung cancer cells A549 and SA7 for 24h and 48h. Compound had cytotoxic effect on the cells and IC50 values at 24th hour was calculated between 92 to 100µg/mL for SA7 and 200 to 240µg/mL for A549 in replicated experiments (Fig. 1). At the 48th hour, IC50 values did not differ with 24h.

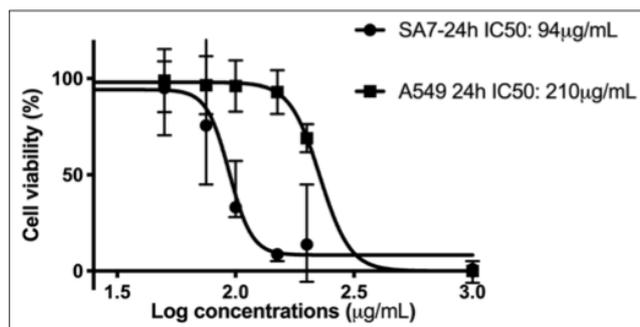


Fig. 1. Cell viability assay. Evaluation of the cytotoxic effect of Santo on SA7 and A549 cells was performed via MTT test with increasing concentrations of Santo (0 to 1000 µg/mL) for 24 hours. Complex had cytotoxic effects on both cells and IC50 values were calculated using Prism V8

We further evaluated the induction of apoptosis in A549 cells for 24h and 48h. We treated cells with 200 µg/mL and 400 µg/mL concentrations of essential oils and used FITC-Annexin V/PI flow cytometry assay to analyze apoptotic cell ratio. The percentage of apoptosis was found to be higher in the increased dosages and prolonged treatments (Figs. 2A and 2B) and we have concluded that compatible with the cytotoxicity experiments, the essential oil had dose-dependent cytotoxic effects. In addition, the results revealed that Santo had its cytotoxic effect in the apoptotic way. Different studies evaluated Santo extract on cancer cell lines and found different results ranging between high cytotoxicity (has triterpene activity) (Elsharkawy and Aljohar, 2016), to low cytotoxicity against A549 cells (Elsharkawy, 2014). The letter, the extract consisted mainly of oxygenated monoterpene compounds rich in Curcumin, Thujol, limonene oxide, Bornyl, Eucalyptol,

alpha-bisabolene epoxide, Carveol, caryophyllene oxide and Camphor while in our study main component of Santo was artemisia ketone and camphor which might explain the differences between the results.

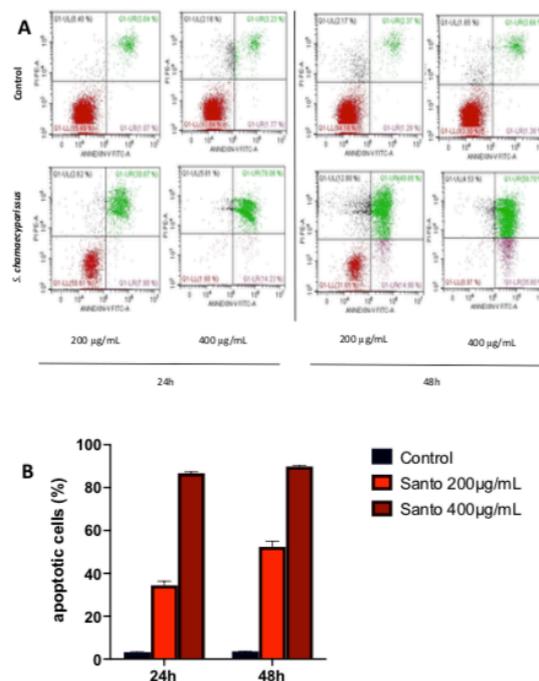


Fig. 2. Flow cytometry analysis of Santo treated A549 cells. Cells were treated with 200 and 400 µg/mL Santo and equal amounts of DMSO as control. Flow cytometry was performed at the 24th and 48th hours of the treatment. In each square image of flow cytometry, upper (late apoptotic cells/green) and lower (early apoptotic cells/purple) right panels show the apoptotic cells of the population (A). Total apoptotic cell rates are given with the bar graph (B)

3.3. Santo suppressed cell motility in A549 cells

We investigated the role of Santo, on the motility of A549 cells via scratch assay. The measurements showed a decreased gap closure on with *Santo* treatment at the end of the 24th hour, comparing to the control cells (Fig. 3A). The percentage of the migrations are shown in Fig. 3B and were significantly different between the two groups ($p < 0.0001$). The results suggested that, suppressing cell motility in A549 cells, Santo also had another anti-tumoral effect in lung cancer cells.

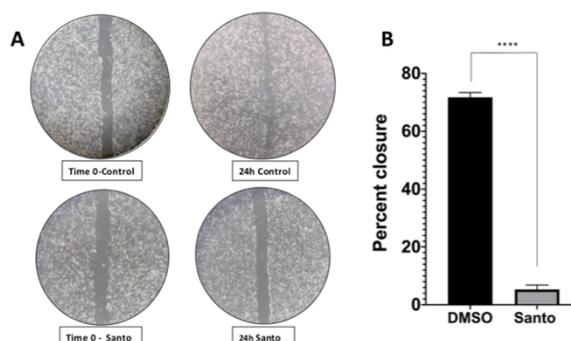


Fig. 3. Effects of Santo on A549 cell migration. Representative images from the scratch assay at time 0, and 24h in the presence of 200 µg/mL Santo (A). The migratory level (%) of A549 cells were quantified by the gap closure and analyzed via Prism (**** $p < 0.001$) (B)

3.4. Santo did not change *E-Cadherin* / *Vimentin* mRNA ratio but suppressed *Ezrin* expression

To evaluate the underlying mechanisms of Santo's inhibiting cell motility, we evaluated mRNA expression of Epithelial to Mesenchymal Transition (EMT) related genes *E-Cadherin*, *Vimentin*, *Snail* and *Zeb1*, and additionally *Ezrin* which codes a cell membrane-cell skeleton connected protein and takes place in cell motility in cancer cells including A549 and claimed to control TGF- β 1 induced EMT in these cells (Chen MJ et al., 2014). The qPCR results regarding with EMT related genes were non-specific in repeated experiments and *E-Cadherin* / *Vimentin* ratio did not change after Santo treatment in A549 cells which did not explain the motility suppression after the essential oil treatment (Figs. 4A and 4B). On the other hand, *Ezrin* expression was significantly down regulated ($p=0.04$) with Santo treatment which might be the responsible for the inhibition of cell motility in A549 cells (Fig. 4A).

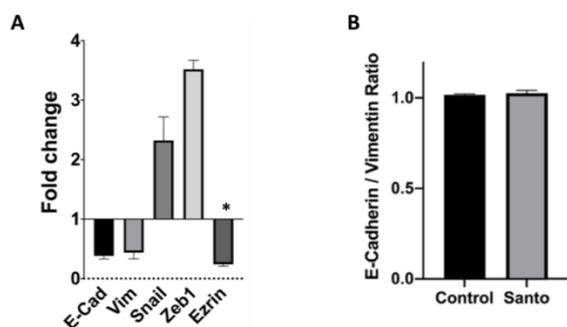


Fig. 4. qPCR analysis of *Ezrin* and EMT related gene expression. *E-Cadherin*, *Vimentin*, *Snail* and *Zeb1* and additionally *Ezrin* primers used for qPCR analysis. 18S used as internal control. The mRNA expression of EMT related genes were non relevant that both epithelial marker *E-Cadherin* and mesenchymal marker *Vimentin* down regulated after Santo treatment while *Snail* and *Zeb1* expressions were increased. Interestingly, Santo significantly suppressed *Ezrin* mRNA expression ($p=0.04$) (A). *E-cadherin/Vimentin* ratio did not differ between Santo treated or DMSO treated A549 cells (B)

3.5. A549 cells expressed ezrin protein and knockdown of *Ezrin* suppressed cell motility

After analyzing qPCR results, we decided to knock-down *Ezrin* in A549 cells to see if the motility effect would be like Santo treated cells. Before knock-down experiments, we evaluated *ezrin* expression in regularly growing A549 cells with immunofluorescent staining and showed that the cells expressed considerable amount of *ezrin* especially located in and behind the cell membrane (Fig. 5A). We then knock-downed *Ezrin* via siRNA technique and performed scratch assay in both *Ezrin* siRNA and scrambled RNA treated cells. The results revealed that *Ezrin* down regulation suppressed cell motility in A549 cells alike with Santo treatment (Figs. 5B and 5C). We used western blot to confirm *Ezrin* knock down (Fig. 5D).

3.6. Docking Studies showed potential strong artemisia ketone- *Ezrin* binding

In the section 3.1, we have demonstrated that the two major

component of Santo used in this research were Artemisia Ketone (AK) and Camphor. Since Santo consisted of multiple compounds, we focused on AK and camphor to speculate their potential anti-tumor activities and designed docking studies to evaluate the interaction between these two complexes and *Ezrin*, and DNA.

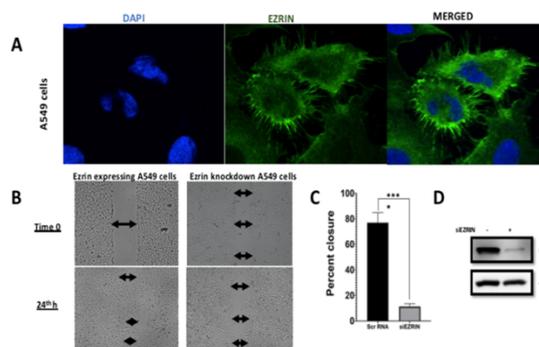


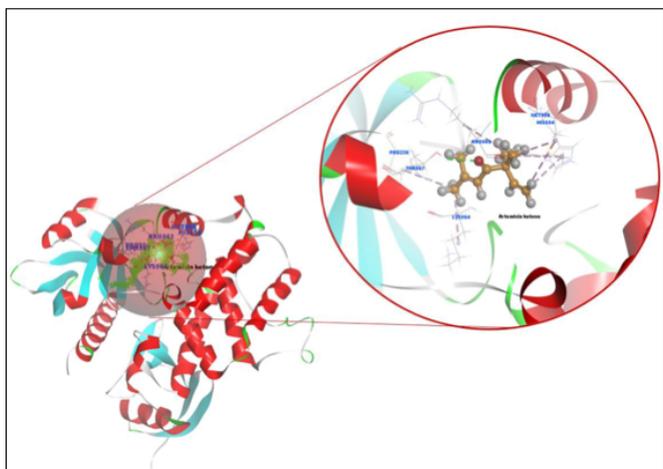
Fig. 5. *Ezrin* knockdown in A549 cells. We first evaluated *Ezrin* expression and localization in A549 cells via immunofluorescent staining. A549 cells expressed significant amount of *Ezrin* and localized in and around the cell membrane as well as cytoplasm (A). After *Ezrin* knockdown with siRNA, we performed scratch assay (B), and as a result *Ezrin* knockdown significantly suppressed A549 cell motility ($p<0.001$) multiple gap measurements are made using image J and percent closure is shown in the bar graph (C). Confirmation of *Ezrin* knockdown via western blot is given (D)

4. Discussion

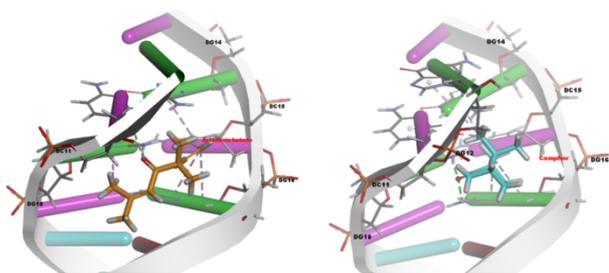
Among these docking evaluation, AK-*Ezrin* complex had the strongest interaction on one hydrogen bond at Thr567 (2.798 Å) and had eight hydrophobic interactions with Met558 (4.954 Å), Pro236 (5.00 Å), Lys564 (4.462 Å), Met558 (3.903 Å), Arg562 (4.897 Å) and His554 (4.510, 4.828, 4.526 Å), respectively (Fig. 6). The hydrogen bond at Thr567 might inhibit the activity of *Ezrin* protein because *ezrin* is known to change its structure from closed to open form to complete its job in the motility and invasiveness of the cancer cells (Antelmi et al., 2013). The detailed information about the mentioned complex can be seen in Supp. Table 3. The results of the docking calculations between AK and *ezrin*, comprising of binding energy, docking score and RMSD values are given in Table 3. Specifically, binding energy of any compound shows how the ligand interaction with the target. In this case, the compound, having a low binding energy value to *Ezrin* (-11.413 kcal / mol) supports a strong binding potential. AK, as mentioned before, is a compound that is effective to inhibit malaria (Radulovic et al., 2013) and a recent research suggest that anti-malarial compounds had ability to bind *Ezrin* in surface plasmon resonance (SPR) and immunoprecipitation and immunoblotting experiments (Celik et al., 2015). Besides, some of these compounds inhibited cell migration and pulmonary metastasis of osteosarcoma cells in-vitro as well as embryonic development in zebrafish assays in-vivo (Celik et al., 2015). This literature, combining with the findings of this research, support the idea of AK's being a potential *ezrin* inhibitor in Santo extract.

Table 3. Binding Energy, Docking score and RMSD values of Artemisia ketone and Ezrin

Name	Binding Energy (kcal/mol)	Docking score	RMSD (Å)
Artemisia ketone	-11.4131	-20.1775	0.0890

**Fig. 6.** Top scoring Ezrin along with the crystal structure for compound, Artemisia ketone (orange carbons) shows one H-bonding of the O atom of ketone group to Thr567 (2.798 Å) and eight hydrophobic interactions of the related compound at Met558 (4.954 Å), Pro236 (5.00 Å), Lys564 (4.462 Å), Met558 (3.903 Å), Arg562 (4.897 Å) and His554 (4.510, 4.828, 4.526 Å), respectively

We also studied DNA docking of AK and Camphor which the results indicate weaker binding comparing to AK-Ezrin binding (Fig. 7). Since DNA is a complex biomolecule to be affected from every biochemical change occurring in the cells, the interaction of small compounds with DNA might generate significant effects. The DNA bindings of these two molecules provide that AK-DNA interaction is more stable than Camphor-DNA interaction, based on their calculation of binding energies. In the meantime, these interactions are revealed that the related compounds are intercalating agents on DNA. The results regarding DNA are in Table 4.

**Fig. 7.** We also studied DNA docking of AK and Camphor which the results indicate weaker binding comparing to AK-Ezrin binding**Table 4.** Binding Energy, Docking score and RMSD values of Artemisia ketone and Camphor against DNA

Name	Binding Energy (kcal/mol)	Docking score	RMS D (Å)
Artemisia ketone-DNA	-5.480	-9.1629	0.0067
Camphor-DNA	-5.410	-34.6270	0.0064

In this research, the essential oil extracted from *Santolina chamaecyparissus*, widely growing around southern part of Turkey, was found to show in vitro anti-cancer activity on A549 cells. The extract induced apoptosis, inhibited cell motility and downregulated *Ezrin* expression which is a protein that is involved in cell motility and other oncogenic pathways. The major component of the oil Artemisia ketone showed potential strong binding to ezrin protein in the docking studies as potential inhibitor candidate of the protein. Moreover, both major components of Santo are shown to interact with DNA, theoretically. These findings obviously need further investigation to understand the interaction between the Santolina extract and oncogenic proteins such as ezrin in cancer cells as well as *in-vivo* experiments.

Conflict of interest

All authors declare no support, financial or otherwise, from any organization for the submitted work.

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