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Biological evaluation and *in silico* molecular docking studies of *Abies cilicica* (Antoine & Kotschy) Carrière) resin

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ABSTRACT

Abies cilicica (Antoine & Kotschy) Carrière) is an endemic species of industrial and medicinal importance. The resin obtained from this plant is effective for different diseases. The chemical content of the ethanol extract of *Abies cilicica* resin by GC-MS method, its antimicrobial activity on 12 different microorganisms by MIC value, antibiofilm activity on six microorganisms by Christensen method and biological activities on three different cancer cell lines by MTT method were investigated in this study. The findings showed that antimicrobial and antibiofilm activity was high in *L. monocytogenes*. The resin extract showed strong anticancer activity on all three DU-145, HeLa and SaOS-2 cells. The major component of the resin was abietic acid. A molecular docking simulation of the major compounds from resin extracts was conducted. In this study, in which the strong biological properties of *A.cilicica* resin were revealed, which suggests that the resin can increase the inhibition of cancer cell growth while strengthening the immune system against pathogenic microorganisms. Molecular docking analysis performed supported the experimental observations. Further extensive studies will support the possibility of using this resin as a therapeutic agent.

1. Introduction

Abies cilicica ((Antoine et Kotschy) Carrière), also known as Cilician Fir, is an endemic tree species belonging to the Pinaceae family. The plant's natural range is Turkey's Southeast Anatolia Region [1]. This species, frequently used as a raw material in the landscape and furniture industry, has different biological activities (such as cytotoxic, antimicrobial, antioxidant, and anti-inflammatory) [2–5].

Resin, one of the defense mechanism products of plants, is secreted to protect the plant from microbiological threats and parasites. Although resins have been used as industrial raw materials for centuries, they are used in the treatment of different diseases with the phenolic compounds and essential oils they contain [2,6-8].

Many antimicrobial activity studies have been conducted with the resin known to have antiseptic properties. Since resins secreted from different plant species, in general, have different properties regarding content, the microbial agents it acts on may vary [8–10].

Research and applicability of herbal treatment methods on pathogenic microorganisms have gained considerable importance in recent years. Thus, studies on plants with high antimicrobial activity have accelerated.

The efficacy of some infective microorganisms may not end with the treatment of an extract with high antimicrobial activity alone. Other properties of the pathogen that procure it to survive must be known and inhibited.

The ability of pathogenic microorganisms to produce biofilm on the host makes it very challenging to treat the infection [11,12]. The resin and its derivatives produced by plants can show antibiofilm activity. For example, *Boswellia spp. oleogum* resin used in traditional medicine in Iran is known to prevent biofilm formation [13]. Similarly, it is claimed that the resin obtained from the *Ferula assa-foetida* plant has antifungal and antibiofilm properties [14].

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There are some studies showing the antimicrobial activity of rosin and resin compounds obtained from Cilician Fir. In these studies, it was stated that rosin and resin components showed high antimicrobial activity [3,5]. However, to our knowledge, there is no study investigating the antimicrobial activity and antibiofilm activity of *A.cilicica* together in the literature.

Predicting interactions between proteins and molecules is critical for decoding a wide range of biological processes and for understanding protein activities, as well as enabling drug development. Blind docking of proteins/ligands is a potent tool for investigating receptor binding locations and ligand binding poses [15,16] It has found widespread use in pharmacological and biological research. For the ligand docking (selected compounds from the composition part of ethanol extract of *A. cilicica* resin Fig. 1), CB-Dock2 is used. The discovery of chemicals that promote apoptosis by targeting both intrinsic and extrinsic apoptotic pathways assisted in the understanding of the mechanism behind the growth of tumor cells, which in turn can lead to the creation of effective cancer treatments. Because they can influence the cell cycle, apoptosis evasion, angiogenesis, and metastasis, phytocompounds have been increasingly popular in recent years [17].

This study aimed to investigate the antimicrobial and antibiofilm activity of ethanol extract of resin obtained from *Abies cilicica* and anticancer activity on prostate, cervix and osteosarcoma cell lines (DU-145, HeLa and SaOS-2). A molecular docking simulation of the major compounds from resin extracts was conducted.

2. Material and methods

The resin used in the study was collected from *Abies cilicica* (Kotschy & Antoine) trees in 2019 from the flora of Kahramanmaraş (Göksun, Tekir Plateau (1400 m)), Turkey.

3. Chemical composition with derivatization method

3.1. Obtaining resin extract

The solid resin was softened with 80% ethanol. The resulting mixture was shaken for 24 hours and then filtered with filter paper to remove unpurificate particles, and the extract was made ready.

3.2. Derivatization procedure

The solid resin sample (10 mg) was weighed and dissolved by 10 mL ethanol placed in a clean-glass test tube. The solvent was evaporated to incomplete dryness under a constant nitrogen flow (0.6 mL.min-1) at 40°C. Then, the residue was redissolved with 1 mL of N,O-Bis (trimethylsilyl) trifluoroacetamide (BSTFA), and trimethylchlorosilane (TMCS) (99:1, v/v) mixture used as a silylation reagent (Supelco-PA, USA). Following, the dissolved mixture, placed in an amber gas chromatography-mass spectrometry (GC-MS) sample vial, was incubated at a constant temperature of 70°C for 16 hours in the drying-oven for the derivatization procedure. Eventually, a derivatized sample was stored at 4°C until it was applied to the GC-MS under the determined conditions, which are described in detail below [18]. The resulting derivative was chemically stable for at least a week.

3.3. Gas chromatography-mass spectrometry (GC/MS) and GC analysis of resin extract

The components in the extract and their relative percentages were identified by the gas chromatography/mass spectrometry method. GC-MS analyses were studied with a mass spectrometer detector. Helium gas was used as carrier gas at a constant flow rate of 1.5 mL per minute, and 1 μL injection volume was programmed at a rate of five minutes in splitless mode. Subsequent run was set at 300°C for two minutes. Total run time was 60 minutes [19].

3.4. Preparation of extract for cytotoxicity

3 g of Cilician fir resin was vortexed until dissolved in 3 ml of absolute ethanol and left for 24 hours. Prepared of extract was filtered with Whatman filter paper No.1 and was diluted to different concentrations.

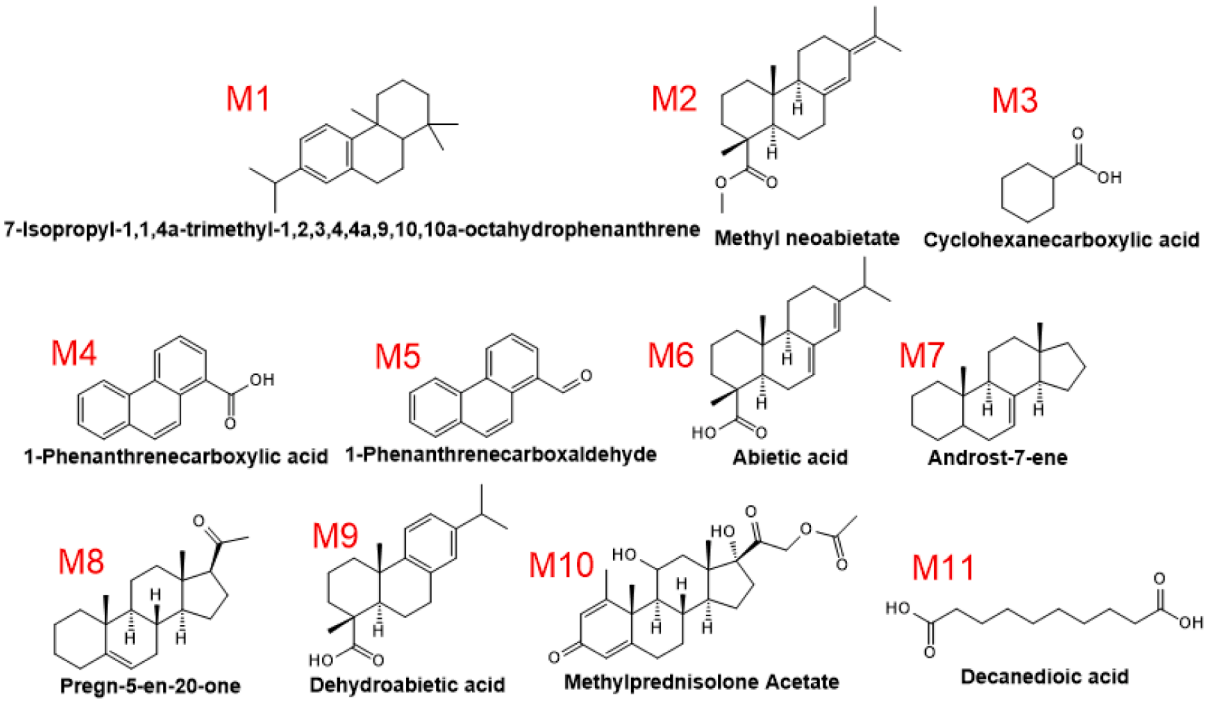

Fig. 1. Selected compounds for molecular docking as a composition part of ethanol extract of *A.cilicica* resin.

3.5. Anticancer assay

The effect of ethanol extract of Cilician fir's resin on Prostate (DU-145), Cervix (HeLa) and Osteosarcoma (SaOS-2) cancer cells was investigated. Skehan's method [20] of MTT 3- (4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide) was used in the present study. According to this method, cells were treated with trypsin and plated in 0.1 mL of complete culture medium at a density of 1×10^5 cells per well in 96-well plates (Corning, USA) and allowed to bind for 24 hours. 2 µL of test substance at concentrations ranging from 0.001-1 mg/mL (0.001, 0.005, 0.01.05, 0.1, 0.3, 0.5, 1 mg/mL) was added to each well containing cells. The prepared extract was diluted with ethanol at the desired concentrations. Plates were incubated at 37°C in an environment with 5% CO_2. After 24, 48 and 72 hours of incubation with components of different concentrations, MTT (5 mg/mL dissolved in 0.01 mL/well Phosphate buffered saline (PBS) was added directly to all wells and incubated at 37°C for two hours. and 100 mL of Dimethyl Sulfoxide (DMSO) was added to each well. After mixing with a mechanical plate shaker for 15 minutes, the absorbance of the plates was recorded at 570 nm on a microplate reader (Bio-Tek, USA).

3.6. Antimicrobial activity assay

The antimicrobial activity of the resin, which was obtained from *Abies cilicica* trees, was evaluated quantitatively using the broth microdilution method according to the Clinical Laboratory Standard Institute (CLSI) guidelines [21]. In the study, 12 indicator microorganisms were used, including 5 gram-positive (*Staphylococcus aureus ATCC 25923, Listeria monocytogenes* ATCC 7644, *Bacillus cereus* ATCC 10987, *Enterococcus faecalis* ATCC 29212, *Bacillus subtilis* ATCC 6633), 5 gram-negative (*Escherichia coli* ATCC 11229, *Pseudomonas aeruginosa* ATCC 27853, *Proteus vulgaris* ATCC 7829, *Klebsiella pneumoniae* ATCC 10031, *Shigella boydii* ATCC 9905) and 2 yeast (*Candida tropicalis* ATCC 750, *Candida albicans* ATCC 10231) species. The stock solution of resin was prepared by dissolving 3 gr of resin in 3 ml absolute ethanol and filter sterilized using a 0.22 µm pore size syringe filter. Afterwards, by taking a sample from here, dilutions were prepared in various concentrations in the range of 25 mg/ml to 0.05 mg/ml in Mueller Hinton Broth (MHB). The study was briefly conducted as follows. After 18 ±2 h incubation at 37°C in MHB medium, the densities of indicator microorganisms were adjusted McFarland standard No: 0.5 using the same medium. Firstly, dilution series of samples (100 µl) were transferred to microtiter plates, and then an adjusted indicator microorganism (100 µl) was added to the wells. After all the microtiter plates were incubated at 37°C for 24 h, the absorbance was recorded at 600 nm on a microplate reader (Bio-Tek, USA) and the lowest concentration that inhibits the indicator microorganism growth was determined as the minimum inhibitory concentration (MIC) value. Just before the determining MIC values, 10 µl samples were taken from each well and inoculated Muller Hilton Agar (MHA) using the spot inoculation method. The minimum bactericidal concentration (MBC) value was determined as the lowest concentration that inhibits the growth of indicator microorganisms by ≥ 99.9% after incubation of agar plates at 37°C for 24 hours. In addition to using piperacillin/tazobactam (8:1) and fluconazole as reference antimicrobial agents, indicator microorganism suspension and sterile MHB was used as the negative and positive control, respectively. All assays were performed in triplicate.

3.7. Antibiofilm activity assay

Antibiofilm activity was determined by modifying the semi-quantitative method, also known as the Christensen method, first proposed by Christensen et al. 1985 [22]. In this study, only six indicator microorganisms were used, including *S. aureus ATCC 25923, L. monocytogenes* ATCC 7644, *E. faecalis* ATCC 29212, *P. aeruginosa* ATCC 27853, *K. pneumoniae* ATCC 10031 and *C. albicans* ATCC 10231. Indicator microorganisms and solution of resin were prepared as in MIC determination with the difference that 2% glucose-added Tryptic Soy Broth (TSB) medium was used instead of MHB. Also, dilutions of resin were prepared in sub-MIC concentrations in the range of 5 mg/ml to 0.05 mg/ml in 2% glucose-added TSB medium. For this study, 100 µl resin samples plus 100 µl indicator microorganism (100 µl) were transferred to microtiter plate wells and incubated at 37°C for 48 h for biofilm formation. At the end of the incubation period, supernatant was removed, wells washing with sterile distilled water three times and left to dry at 60°C for 45 min. For dyeing, 200 µl of 1% crystal violet solution was added to each well and kept for 15 min. After the crystal violet solution was removed from the wells and washed again (with sterile distilled water three times), for the destaining, 150 µl of ethanol was added to the wells. Lastly, 100 µl samples were taken from each well and transferred to a new microtiter plate and the absorbance was recorded at 600 nm on microplate reader (Bio-Tek, USA). Indicator microorganism suspension and sterile 2% glucose-added Tryptic TSB was used as the negative and positive control, respectively. All assays were performed in triplicate and the average of three repetitions was used for calculations. The percentage of preventing biofilm formation was calculated with the "$[(OD_{NC} - OD_S) / OD_{NC}] *100$" formula ($OD_{NC}$: OD negative control and OD_S: OD sample) [23,24].

3.8. Statistical analysis

All experiments were run in triplicate and the results were expressed as mean±SD. Data were analyzed with GraphPad Prism7 and IC_{50} growth inhibition values were determined.

3.9. Molecular docking

In this docking approach, four processes for highly automated protein-ligand blind docking are automatically performed: a. data input (protein. pdb), (ii) data processing, (iii) cavity identification and docking, and (iv) visualization and analysis. The provided ligand will be processed in the first stage regarding the generation of the lowest energy 3D conformation by adding hydrogens and partial charges. Afterwards, the CB-Dock2 server [15] will examine the provided protein, add missing side-chain and hydrogen atoms, deliver notifications about missing residues in a protein and remove co-crystallized waters and other het groups. The cavity discovery and docking process begins with template matching, which searches the generated complex database for existing complexes with comparable proteins and ligands. The docking was performed on the 4MAN (apoptotic protein) [25] protein retrieved from Protein databank [26]. The affinity of protein-ligand interaction is evaluated from the docking score [27,28]. The docking poses (both 2D interaction plots and 3D poses are presented in the Supporting section of the article, M1 to M11). The affinity of those ligands toward the protein was evaluated by their docking score presented in Fig. 4.

4. Results and discussion

4.1. Chemical composition

The chemical content of the ethanol extract of the *A.cilicica* species was first derivatized by silylation and then revealed using the GC-MS method. While "Abietic acid, trimethylsilyl ester" (10.63%) was determined as the major component in the silylated resin extract, it was followed by "Methyl neoabietate" with 6.62%. In the resin extract dissolved with ethanol, the major component was determined as "Abietic acid" (29.28%). It was followed by "Podocarp-7-en-3.beta.-ol, 13.beta.-methyl-13-vinyl" with 8,49%. The silylated sample and ethanol extract sample were compared regarding chemical composition, while the total values of the compounds (85,79% for silylated sample and 86,74 for ethanol extract) were close to each other, it was determined that the number of components detected in the silylated sample was higher than

the number of samples determined in the ethanol extract (Table 1).

In a study in which the acidic components of *Abies cilicica subsp. isaurica* resin were examined using the colophony technique, it was stated that abietic acid was the main component [29]. In another study, the chemical components of the resin belonging to the *Abies nordmanniana* species were investigated and the findings showed that the major component was a-pinene and abietic acid [30].

Although the techniques for obtaining chemical contents vary, it is seen that the main components of many species belonging to the genus Abies are a-pinene and abietic acid. It has been reported that abietic acid, which inhibits lipoxygenase activity, has an anti-inflammatory effect [31]. In this study, it was seen that Cilician Fir resin was rich in abietic acid. When evaluated from this point of view, it was understood that *Abies cilicica* (Antoine & Kotschy) Carrière) resin had a pharmaceutical value.

4.2. Anticancer activities of ethanol extracts of resin from Abies cilicica ((Antoine & Kotschy) Carrière) on DU-145, HeLa and SaOS-2 Cells

Fig. 2 shows changes in cell inhibition for 24, 48 and 72 hours versus increasing concentrations of DU-145, HeLa and SaOS-2 cell lines. As seen in Fig. 2, parallel to the increase in resin extracts, DU-145, HeLa and SaOS-2 mortality rates also increased. The low IC_{50} value ''the high concentration of complex required for killing 50% of DU-145, HeLa and SaOS-2 cancer cells'' indicated high anticancer activities. An increase in anticancer activities of ethanol extracts of *A.cilicica* resin was observed in DU-145, HeLa and SaOS-2 cells depending on time and dose. In this study, IC_{50} values were observed between 24, 48 and 72 hours. When resin extract was examined in DU-145, HeLa and SaOS-2 cells, the most active was the DU-145 cell. It was for 24, 48 hours, and the IC50 value was 35.41 ± 2.43 μg/ml and 27.91 ±1.17 μg/ml, respectively. The extract was in the most active SaOS-2 cells within 72 hours and the IC50 value was 23.45 ±2.67 μg/ml (Table 2). As a result, it was determined that ethanol extracts of resin from *A. cilicica* had high anticancer activity in all three-cell lines.

Similarly, the effect of ethanol extract of *A. cilicica* resin on six different cancer cells (HepG2, PC-3, U-87, MCF-7, MDA-MB-231, HT-29) was previously investigated and the highest cytotoxicity was detected in endometrial adenocarcinoma cancer cells according to the 48-hour MTT test results. However, it was also among the results of the present study that it did not have any cytotoxic effect on Hepatocellular carcinoma (Hep G2) [4]. Studies showing the effect of Cilician Fir resin on cancer are quite limited in the literature. The fact that the resin extract, which appears to have anticancer activity, creates different degrees of inhibition on different cancer cells indicates that more comprehensive in vitro studies should be carried out with this extract.

4.3. Antimicrobial and antibiofilm activity

As a result of the antimicrobial activity studies performed with the broth micro-dilution method, it was found that the *A. cilicica* resin exhibited antimicrobial activity against all the indicator microorganisms used. In the present study, it was determined that the strongest activity was against *L. monocytogenes,* while the weakest activity was found against *P. vulgaris and S. boydii.* Also, when the obtained MIC values were compared with the literature data, it was seen that the antimicrobial activity was weak. MIC and MBC values are given in Table 3.

Antibiofilm activity was evaluated by preventing biofilm formation and it was observed that the resin showed antibiofilm activity even at sub-MIC concentration. As a result of the study performed at various concentrations in the range of 5 mg/ml to 0.05 mg/ml, the lowest concentration that the resin exhibited antibiofilm activity was determined at 0.312 mg/ml. The percentages of preventing biofilm formation at a concentration of 0.312 mg/ml are given in Table 3. As seen in Table 3, *A. cilicica* resin showed strong antibiofilm activity against *L. monocytogenes, E. faecalis* and *C. albicans.* Howbeit, it showed

Table 1
The chemical composition of ethanol extract of silylated *A.cilicica* resin.

Retention time	Compound name	Silylated Resin	Ethanol+Resin
33,892	Androst-7-ene, (5.alpha.)-		1,62
35,684	estr-4-en-17.beta.-o1		1,44
38,827	Decanedioic acid, dibutyl ester		1,01
40,369	Cyclohexanecarboxylic acid, 1,3-dimethyl-2-[2-[3-(1-methylethyl) phenyl]ethyl]-, methyl ester, (1. alpha.,2.alpha.,3.alpha.)-	1,32	2,95
40,439	1-Phenanthrenecarboxaldehyde, 7-ethenyl-1,2,3,4,4a,4b,5,6,7,9,10,10a-dodecahydro-1,4a,7-trimethyl-, [1R-(1.alpha.,4a.beta.,4b.a		1,5
40,983	Tributyl acetylcitrate		1,45
41,947	Dehydroabietylamine	1,18	
42,075	Retinol	0,66	5,03
42,234	Pimaric acid TMS	1,55	2,76
42,377	7-Isopropyl-1,1,4a-trimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene	1,94	3,07
43,014	Abietic acid, trimethylsilyl ester	10,63	4,48
43,33	Pimaric acid TMS		7,04
43,532	Dehydroabietic acid, trimethylsilyl ester	1,51	
43,744	17.alpha.-Hydroxypregnenolone, bis (trimethylsilyl) ether	1,06	
43,855	Podocarp-7-en-3.beta.-ol, 13.beta.-methyl-13-vinyl-		8,49
44,304	1-Phenanthrenecarboxylic acid, 1,2,3,4,4a,4b,5,6,7,9,10,10a-dodeca-hydro-1,4a-dimethyl-7-(1-methyle-thylidene)-, [1R-(1.alpha.,4a	2,97	8,30
44,501	Methyl abietate		3,3
44,705	Androstan-17-one, 3,11-bis[(trime-thylsilyl)oxy]-, O-(phenylmethyl) oxime, (3.alpha.,5.alpha.,11.beta.)	2,97	
45,01	Methylprednisolone Acetate		5,02
45,026	3,16,20-Tris[(trimethylsilyl)oxy]-5. alpha-pregnane, (3.beta.,16. alpha.,20.alpha.)	1,53	
45,463	2,2,4-Trimethyl-4-(4′-trimethylsilyloxyphenyl)chromane	5,02	
45,97	Methyl neoabietate	6,62	
46,115	Abietic acid		29,28
46,256	2,5-bis(dimethylchlorosilyl)furan	2,65	
46,451	1,2-bis(trimethylsiloxy)-1,1,2,2-tetraphenylethane	1,35	
46,561	Chol-9(11)-en-24-oic acid, 3-(acetyloxy)-12-oxo-, methyl ester, (3. alpha.,5.beta.)-	2,46	
46,894	Pregn-5-en-20-one, 3,21-bis[(trime-thylsilyl)oxy]-, (3.beta.)- (CAS)	2,68	
47,085	2,2′-Diethoxyisodiopyrin	2,75	
47,384	7-Oxodehydroabietic acid, trimethylsilyl ester	2,65	
47,506	3.Beta.-Hydroxy-Androst-5,16-Ene	1,88	
47,813	(1R,2R,3S,4R,5S,8S,11R,12R)-12-Formyloxy-2,11:3,8-dicyclocembr-15-en-5-ylacetate	2,23	
48,012	Dehydroabietic acid, trimethylsilyl ester	4,18	
48,433	Abietic acid, trimethylsilyl ester	3,33	
48,83	N,N,N',N'-Tetra(trimethylsilyl)-1,2-bis(aminothio)benzene	4,75	
49,38	Propenoic acid, 3-phenyl-, 2-[(benzoisothiazol-3-yl-1,1-dioxide) methylamino]ethyl ester	0,54	
49,581	Propanoic acid, 2-methyl-2-[(trime-thylsilyl)oxy]-, trimethylsilyl ester	1,69	
49,73	Pyrimidine, tetrakis[(trimethylsilyl) oxy]-	1,65	
50,024	.beta.-Eudesmol, trimethylsilyl ether	2,45	
50,359	2-Hydroxy-4-(methylsulfonyl) isophthalic acid tritms	1,99	

(*continued on next page*)

Table 1 (*continued*)

Retention time	Compound name	Silylated Resin	Ethanol+Resin
50,708	1-(1-Methyl-2,2-D2-2-Trimethylsilyloxyethyl)-4-(2-Methyl-2-Trimethylsilyloxypropyl)Benzene	3,31	
51,205	5.beta.-Cholestane-3.alpha.,7.alpha.,12.alpha.,24.alpha.,25-pentol TMS	0,89	
51,48	Acetic acid, [bis[(trimethylsilyl)oxy] phosphinyl]-, trimethylsilyl ester	1,12	
		85,79	86.74

exisluous antibiofilm activity against *S. aureus, K. pneumonia*, especially *P. aeruginosa*. Even at concentrations above 0.312 mg/ml, including the MIC value, so strong activity was not detected against this 3 indicator microorganism (data not shown here).

Kizil et al. (2002) previously investigated the antimicrobial activity of Cilician fir resin on various microorganisms using the paper disc diffusion method and stated that the resin showed a high rate of antimicrobial activity [5]. It should be kept in mind that the minimum inhibitory concentrations (MIC) values obtained from this study provide more specific and accurate results. In addition, by evaluating the results of these two studies together, studies should be conducted to develop antibiotics with the fraction of terpenes that give this resin antimicrobial activity. When the antimicrobial activity and antibiofilm activity of the resin extract are evaluated together, it can be said that it is highly effective especially against *L. monocytogenes, E. faecalis* and *C. albicans* microorganisms.

In a previous study, it was seen that results similar to the results of this study were obtained on a different plant resin. In addition to the antifungal properties of *Ferula assa-foetida* resin, has been reported to inhibit biofilm formation on *Candida* species as well as exhibit antifungal activity [14].

4.4. Molecular docking studies

Molecular docking analysis provides important clues about the power of the interaction with biological systems of studied molecules. It should be noted that more negative docking score values represent the more powerful interactions. The M4 ligand has a strong affinity toward the studied protein and (as seen from Fig. 3, 2D interaction fingerprints) interacts with protein pocket binding sites via: van der Waals interactions (PRO 120, PHE 121, LEU 166, TRP 173 protein's amino acid sidechains), π-donor hydrogen bonding (ASNN 169), π-σ bonding interaction (TRP 173) and π-alkyl interactions. However, M10 possesses apart the van der Waals interactions (ASN 169, PRO 120, TRP 173, GLY 125), also π-π T-shaped (ARG 124 and PRO 165) and π-alkyl interactions (PRO 165). As a result, the findings suggest that the components of the selected extract can be effective against some cancer types. In the future, these components can be considered in drug design studies.

From the docking score represented in the Fig. 4, two most potent ligands (M4 and M1) were analyzed in detail.

5. Conclusion

If the immune system is weakened as a result of the treatments applied to cancer patients, the mortality rate of the patients may increase significantly with infective agents. In such cases, a microorganism

Table 2
Comparison of IC_{50} values between ethanol extract of *A.cilicica* resin on different cells after 24 h, 48 h and 72 h of incubation.

Cells	IC_{50} (µg/ml) 24h	48h	72h
DU-145	35,41±2,43	27,91±1,17	24,2±0,86
HeLa	42,5±3,04	38,87±2,68	27,88±4,01
SaOS-2	49,47±4,27	28,35±3,43	23,45±2,67

IC_{50}: Half-maximal inhibitory concentration, SD*: Standard deviation.

Table 3
Antimicrobial and antibiofilm activity of *A. cilicica* resin.

Indicator microorganism	MIC values mg/ml	Reference antimicrobial agents	MBC values mg/ml	% preventing biofilm formation (for 0.312 mg/ml)
S. aureus	5	0.0625[a]	>25	59.35±1.67
L. monocytogenes	0.2	0.0625[a]	1	98.22±1.03
E. faecalis	0.5	0.0625[a]	6.25	93.64±5.82
B. cereus	0.5	0.125[a]	>25	ND
B. subtilis	0.5	0.0156[a]	>25	ND
E. coli	5	0.0156[a]	6.25	ND
P. vulgaris	6.25	0.0313[a]	6.25	ND
P. aeruginosa	5	0.0156[a]	>25	13.76±1.06
K. pneumonia	5	0.0313[a]	>25	44.72±3.74
S. boydii	6.25	0.0625[a]	6.25	ND
C. tropicalis	5	0.0156[b]	6.25	ND
C. albicans	0.5	0.0156[b]	25	95.31±2.90

*ND: Not detected;
[a] : piperacillin/tazobactam (8:1);
[b] : fluconazole

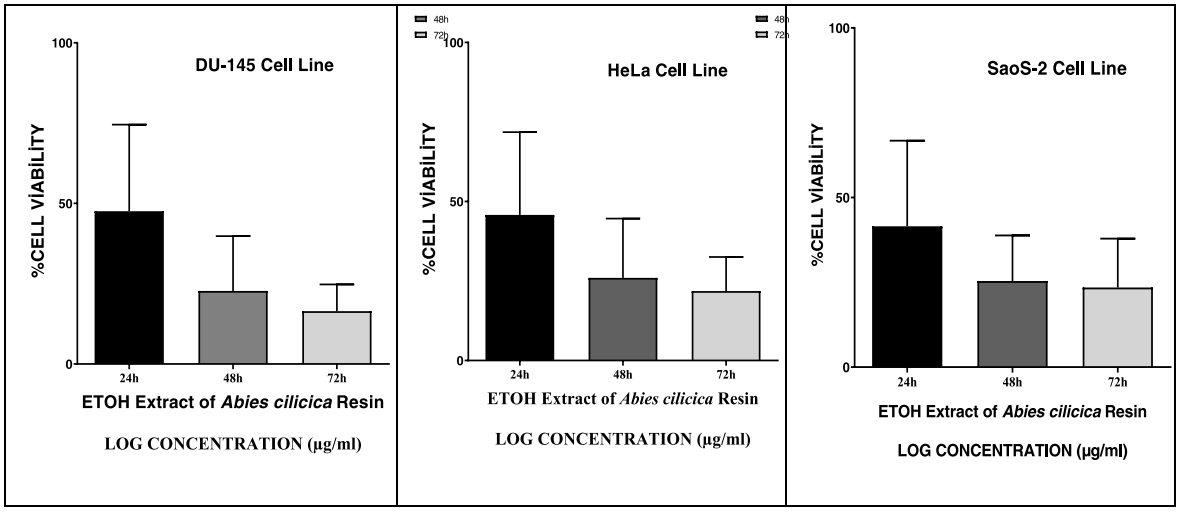

Fig. 2. Anticancer activities of ethanol extract of *A.cilicica* resin on DU-145, HeLa and SaOS-2 cell lines.

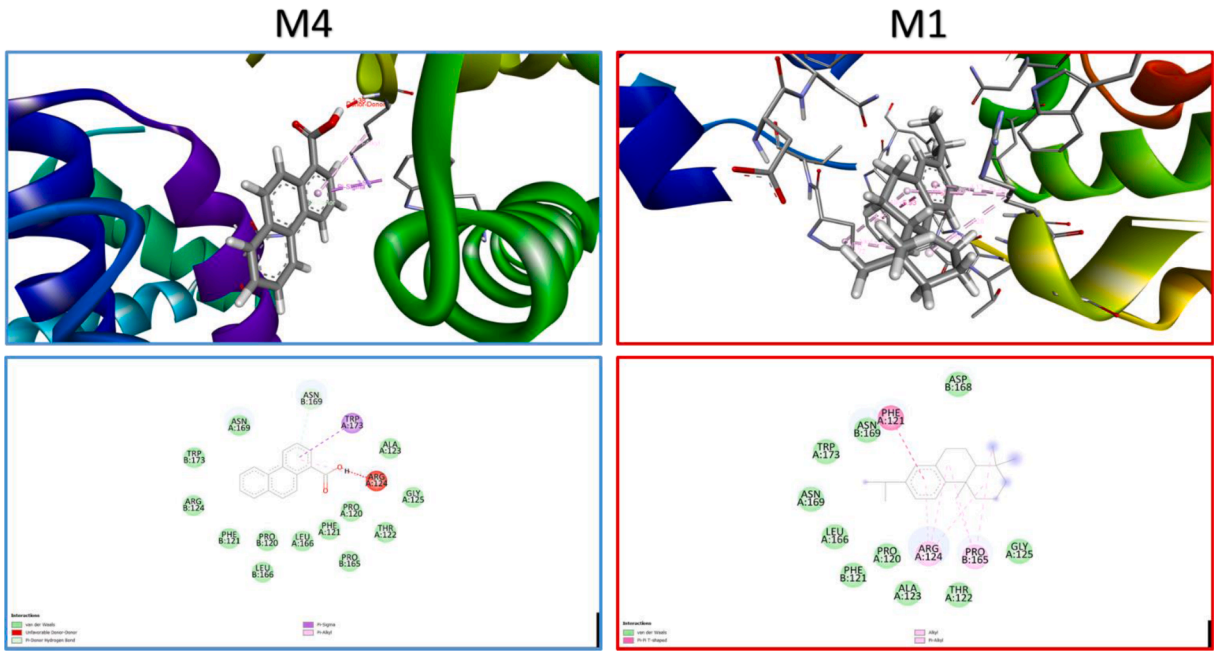

Fig. 3. Selected compound for molecular docking as a composition part of ethanol extract of *A.cilicica* resin.

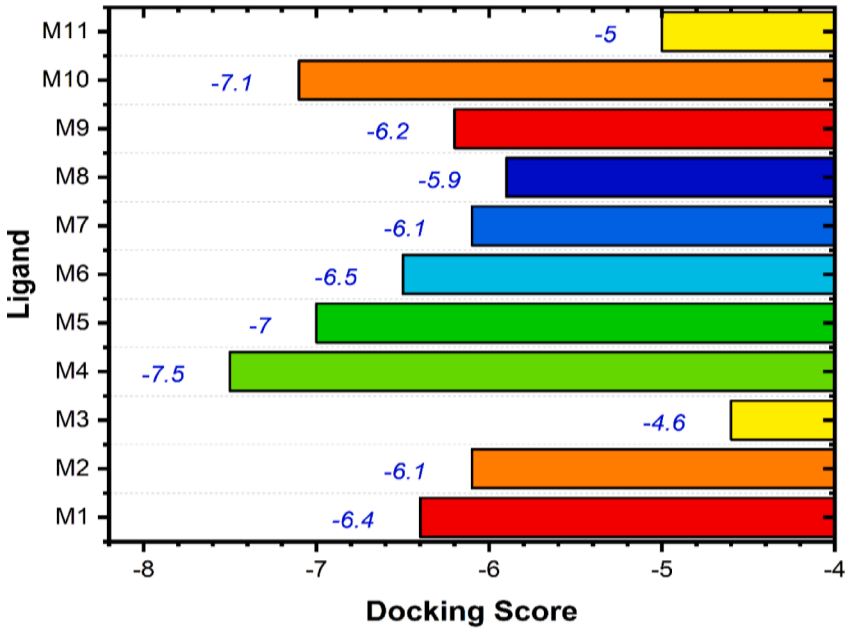

Fig. 4. Docking score of the selected ligands (as represented in Fig. 1) toward the protein pdb id: 4MAN (Apoptosis Regulator/Inhibitor).

with a biofilm mechanism will pose a great risk for the patient. The fact that the treatment agents to be applied show both anticancer activity and antibiofilm activity will greatly contribute to the healing mechanism of the disease. In this study, different biological properties of the ethanol extract of *Abies cilicica* resin revealed. To our knowledge, this is the first study investigating the antibiofilm activity of the resin of this plant in the literature. This extract, which shows cytotoxic effects on different cancer cells, it also shows antimicrobial and antibiofilm activity on different microorganisms, suggesting that it has an important potential for combined treatment applications on diseases that cause immune system damage, such as cancer. In addition, the inflammation-suppressing effect of the abietic acid contained in the resin is also critical to support the immune system. Molecular docking pointed out that the extract of the *A.cilicica* resin contains some potential potent ligands for the regulation of the apoptotic protein.

Given all these, it is predicted that *Abies cilicica* ((Antoine & Kotschy) Carrière) resin can be used as an important therapeutic agent with more comprehensive studies in the future.

CRediT authorship contribution statement

Gulsen Guclu: Investigation, Methodology, Formal analysis, Visualization, Validation, Writing – original draft, Writing – review & editing. **Ayca Tas:** Investigation, Methodology, Validation, Formal analysis, Writing – review & editing. **Emine Dincer:** Methodology, Formal analysis. **Esra Ucar:** Investigation, Methodology, Validation, Formal

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Declaration of Competing Interest

The authors declare no competing interests.

Data availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.molstruc.2023.135740.

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