



Original Article

Asian Pacific Journal of Tropical Biomedicine



apjtb.org

doi: 10.4103/apjtb.apjtb_515_24

Antioxidant, antimicrobial, and anticancer effects of *Achillea filipendulina* L. against colon cancerTutku Tunç^{1✉}, Şeyda Akın², Okan Aykaç³, Ceylan Hepokur^{4✉}, Serap Duran⁴, Hülya Özpınar⁵¹Sivas Cumhuriyet University, Faculty of Pharmacy, Department of Pharmaceutical Microbiology, Sivas, Türkiye²Sivas Cumhuriyet University, Faculty of Medicine, Department of Medical Biology, Sivas, Türkiye³Sivas Cumhuriyet University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Sivas, Türkiye⁴Sivas Cumhuriyet University, Faculty of Pharmacy, Department of Biochemistry, Sivas, Türkiye⁵Sivas Cumhuriyet University, Faculty of Pharmacy, Department of Pharmaceutical Botany, Sivas, Türkiye

ABSTRACT

Objective: To investigate the antioxidant, antimicrobial, and anticancer effects of *Achillea filipendulina*.**Methods:** Phytochemical content was analyzed by GC-MS. Antioxidant activity was assayed by DPPH, total antioxidant status, total oxidant status, and iron reduction tests. Antimicrobial activity was assessed using the MIC assay. Anticancer activity was detected via MTT assay, followed by analysis of the apoptotic process using annexin V and caspase 3/7. *miR-519d* and *Dvl-1* expression were assessed by RT-PCR, and molecular docking analysis was also performed.**Results:** The ethanol extract of *Achillea filipendulina* showed the strongest anticancer effect. A significant increase in *miR-519d* expression was observed in HT29 cells, while a decrease in *Dvl-1* expression was noted. DPPH, FRAP, total antioxidant status, and total oxidant assays showed high antioxidant activity. The hexane and ethanol extracts were effective against *Streptococcus mutans* (MIC<50 µg/mL), while the ethanol extract was effective against *Enterococcus faecalis* and *Candida albicans* (MIC<50 µg/mL). Molecular docking studies also confirmed that ethanol extracts could act on the Wnt pathway. GC-MS analysis showed high level of eucalyptol and borneol content in the extract.**Conclusions:** Ethanol extract of *Achillea filipendulina* has anticancer effect and can be used in colon cancer treatments.**KEYWORDS:** *Achillea filipendulina*; Wnt signaling pathway; miRNA-519d; Dvl-1; Colon cancer; Antioxidant; Antimicrobial

1. Introduction

Colon cancer is the third most common in the world after lung and breast cancer. Chemotherapy and radiotherapy have proven to be highly effective in cancer treatment. However, the use of these treatments is limited due to acute toxicities and the drug resistance of tumors. In the fight against cancer, the success rates of treatment are also very low in case of late diagnosis. For this reason, new treatment methods are being developed every year and scientists are looking for new treatment methods through natural ingredients[1].

Significance

There are few studies in the literature investigating the effects of *Achillea filipendulina* Lam. extract on cancer. Our study shows high level of borneol and eucalyptol secondary metabolites in the extract. *Achillea filipendulina* extract has high antioxidant activity and could induce apoptosis in colon cancer by increasing *miR-519d* and decreasing *Dvl-1* expression. It can be used in colon cancer treatments.

✉To whom correspondence may be addressed. E-mail: tutkutunc58@hotmail.com (T. Tunç); cozoaya@gmail.com (C. Hepokur)

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-Non Commercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

©2024 Asian Pacific Journal of Tropical Biomedicine Produced by Wolters Kluwer-Medknow.

How to cite this article: Tunç T, Akın Ş, Aykaç O, Hepokur C, Duran S, Özpınar H. Antioxidant, antimicrobial, and anticancer effects of *Achillea filipendulina* L. against colon cancer. Asian Pac J Trop Biomed 2024; 14(12): 540-550.

Article history: Received 31 August 2024; Revision 14 September 2024; Accepted 28 November 2024; Available online 26 December 2024

Targeting hundreds of mRNAs, micro RNAs (miRNAs), which are roughly 20-25 bp in length, actively participate in the regulation of gene expression. MicroRNAs, which are associated with many diseases, are suggested to act as oncogenes or tumor suppressors, especially in cancer development[2]. Recent research on the function of C19MC microRNAs in normal cell physiology and during tumorigenesis has identified microRNA-519d (miR-519d), a C19MC member, as a regulator of cell migration, invasion, and interaction with the extracellular matrix[3]. Both direct and indirect evidence have demonstrated that p53 is a transcriptional modulator of miR-519d and that miR-519d is up-regulated or down-regulated in response to p53 overexpression or silencing, respectively. The targets of miR-519d and the biological processes it regulates have not been fully elucidated. Studies have focused on the tumor suppression function of miR-519d in different cancer types such as gastric cancer, hepatocellular carcinoma, ovarian cancer, and breast cancer[4,5]. Considering the relationship of miR-519d with many pathways (Wnt and AMPK, *etc.*), the antiproliferative, apoptotic, and anti-metastatic effects of plant extracts in cancer cells may be related to controlling the changes in miR-519d expression.

The Wnt signaling pathway is responsible for tumor development, especially in hepatocellular, pancreatic, gastric, and colorectal cancers[6,7]. The canonical Wnt signaling pathway (also known as the Wnt/ β -catenin signaling pathway) is one of the most representative signaling pathways involved in colon cancer development[8]. Dvl proteins play a central role as scaffold proteins in transducing Wnt signaling to control various cellular functions such as proliferation, stem cell renewal, and migration[9]. In colorectal cells, the Dvl-1 protein has shown elevated expression and nuclear localization. The nuclear localization of Dvl-1 has the potential to enhance the expression of additional proteins that facilitate the Wnt signaling pathway[10]. This makes Dvl-1 an important therapeutic target and suggests that changes in Dvl-1 expression should be evaluated in drug therapies.

Achillea filipendulina (*A. filipendulina*) L. belongs to the genus *Achillea*, a member of the Asteraceae (Compositae) family. The genus comprises 85 species distributed across Europe and Asia, with several species also found in North America. In Turkey, there are 48 species (54 taxa)[11] of *Achillea*. *Achillea* species are utilized in the treatment of various ailments such as gout, arthritis, malaria, sciatica, cardiovascular diseases, and gastrointestinal disorders. Additionally, they are believed to possess wound healing, antibacterial, antidiabetic, gastroprotective, and antioxidant properties[12,13].

In some studies on *A. filipendulina* species, the phytochemical components and biological properties of essential oils and ethanol extracts obtained from the flowers and leaves have been examined. Ethanol extracts are rich in phenolic acids, flavonoids, and biologically active metabolites such as tannins and saponins, while essential oils contain high amounts of oxygenated monoterpenoids[14]. The anticarcinogenic effect of *A. filipendulina*

has been studied in MCF-7 and MDA-MB-468 breast cancer cell lines[15], but its efficacy in colon cancer cell lines has rarely been studied. This study aimed to investigate the antioxidant, antimicrobial, and anticancer efficacy of *A. filipendulina* L. flower extracts. Furthermore, it studied the changes in miR-519d and Dvl-1 expression levels to predict the effects of plant extracts on Wnt signaling and offer preliminary insights for more comprehensive research in future studies.

2. Materials and methods

2.1. Materials and reagents

Water, ethanol, *n*-hexane, and ethyl acetate used as solvents in this study were purchased from Merck. 2,2-Diphenyl-1-picrylhydrazyl, trolox, AlCl₃, KCH₃COO, quercetin, and ascorbic acid were purchased from Sigma-Aldrich. DMEM (high glucose), fetal bovine serum (FBS), phosphate-buffered saline (PBS), Capricorn, 1% penicillin-streptomycin BI used in cell culture, and MTT have been provided from Sigma-Aldrich.

2.2. *A. filipendulina* L. material and preparation of extracts

A. filipendulina L. specimens were obtained from the Zeytinburnu Medicinal Plants Garden. In this way, a sample group grown under standardized conditions was studied. The species were identified by Hülya Ozpınar, a faculty member of Sivas Cumhuriyet University, Faculty of Pharmacy, Department of Pharmaceutical Botany. The collected *A. filipendulina* L. was washed with tap water, then distilled water, and dried in the laboratory for 2 weeks. The dried samples were ground and 100 g of the sample was extracted twice in 1 L of different solvents (water, ethanol, *n*-hexane, and ethyl acetate) at 45 °C for 45 min in an ultrasonic bath. The extracts were then evaporated at 30 °C using an evaporator. The extracts obtained were stored at -20 °C for use in experimental procedures[15].

2.3. GC-MS analyses and total flavonoid content determination

For GC-MS, a 7890A (Agilent) model GC-MS instrument with HP-5MSIU capillary column (30 m×250 μ m×0.25 μ m) and 5975C (Agilent) inert MSD mass detector was used. The GC-MS used an electronisation system with an ionization energy of 70 eV and 99.999% purity helium (He) carbon dioxide. Carbon dioxide was introduced into the column as the He carrier phase with a flow rate of 1.5 mL/min and an initial pressure of 17.897 psi. After maintaining the initial temperature of 50 °C for 2 min, the temperature increase in all stages was 5 °C/min and increased to 80 °C (2 min at temperature), 100 °C (1 min at temperature), 150 °C

(1 min at temperature), 240 °C (1 min at temperature) and 270 °C (7 min at temperature), respectively[16].

The total flavonoid content of the extracts was determined according to the method developed by Chang *et al.*[17]. The principle of the method is based on the formation of acid-stable complexes of the $AlCl_3$ with C-4 keto group and C-3 or C-5 hydroxyl groups of flavones and flavonols. In this method, 80% ethanol, 10% $Al(NO_3)_3$ solution, and 1 mol/L KCH_3COO solution were added and treated with the sample. It was incubated at room temperature in the dark for 40 min and measured at 415 nm. According to this method, quercetin was used as a standard[18].

2.4. Determination of antioxidant properties

2.4.1. Determination of DPPH radical scavenging activity

DPPH determination was performed according to the method developed by Cuendet *et al.* DPPH radical (2,2-diphenyl-1-picrylhydrazyl) is a commercially available radical and was dissolved in a 100 μ M ethanolic solution of this radical. According to this method, the sample was treated with DPPH radical and kept in the dark for 50 min, and then absorbance was measured at 517 nm in a spectrophotometer (Ascorbic acid was used as standard)[19].

2.4.2. Determination of iron (Fe^{3+}) reducing power in extracts

Antioxidants, like some reducing agents, cause the reduction of the Fe^{3+} -ferricyanide complex to Fe^{2+} . In the method, the color of the test solution changes from yellow to green depending on the reducing power of the sample under test. This green color gives maximum absorbance at 700 nm and increasing absorbance indicates increasing reducing power. According to this method, trolox was used as a standard antioxidant compound[20]. The FRAP antioxidant power of *A. filipendulina* extracts was calculated as trolox equivalent.

2.4.3. Total antioxidant status (TAS) and total oxidant status (TOS)

Total antioxidant status assay kit (Rel Assay Diagnostics, Turkey) was used to evaluate TAS values and total oxidant status assay kit (Rel Assay Diagnostics, Turkey) was used to evaluate TOS values. Different extracts of *A. filipendulina* were analyzed three times each. Results were reported as mmol Trolox equivalent/L for TAS and μ mol H_2O_2 equivalent/L for TOS[20].

2.5. Antimicrobial activity

The microorganisms used for activity determination were *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 13883), *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 29213), *Enterococcus faecalis* (ATCC 29212), *Streptococcus mutans* (ATCC 25175), *Bacillus cereus* (ATCC 11778),

Candida albicans (ATCC 10231) and were obtained from American Type Culture Collection (Rockville, MD, USA). Mueller Hinton Broth (Oxoid, UK) was used for bacterial cultures and Sabouraud Dextrose Broth (Himedia, USA) for fungi. Cultures were cultured at 37 °C for 24–48 h.

Minimum inhibition concentration (MIC) method was used to determine the antimicrobial activity of *A. filipendulina* extracts. A 10 μ L sample (100 μ g/mL) was added to the first row of U-bottom 96 plates, followed by two-fold serial dilutions at a total of 10 concentrations. Bacteria and yeasts were adjusted to a McFarland turbidity of 0.5, the final inoculum was diluted to 5×10^5 CFU/mL for bacteria and 0.5×10^3 – 2.5×10^3 CFU/mL for yeasts, and 10 μ L was added to the wells[21,22].

The plates were incubated at 37 °C for 24 h for bacteria and 48 h for yeasts. At the end of incubation, 2,3,5-triphenyl tetrazolium chloride (Merck, Germany) solution (2 mg/mL) was added to each well (50 μ L) for better observation of growth. The plates were incubated at 37 °C for 2 h. The concentration of the first well in which a decrease in formazan color was observed due to the presence of viable microorganisms in the wells was considered MIC. MIC results were evaluated as effective ($MIC < 100 \mu$ g/mL), moderate ($100 < MIC \leq 625 \mu$ g/mL), and weak ($MIC > 625 \mu$ g/mL) according to reference sources[23,24].

2.6. Anticancer study

2.6.1. Cells

In this study, human colon carcinoma HCT116 (No. CCL-247), human colon adenocarcinoma HT29 (No. HTB-38) and healthy mouse fibroblast L929 (No: CRL-2148) cell lines were grown and cultured in DMEM (high glucose) medium containing 10% (*v/v*) FBS, 1% penicillin-streptomycin, 5% CO_2 , 95% humidity and 37 °C in an incubator.

2.6.2. MTT assay

The cytotoxic effects of *A. filipendulina* ethanol extract, which was found to be the most effective antioxidant, on colon and healthy cells were performed using the MTT method. Firstly, cells were seeded in plates at 5×10^4 cells/well, respectively. All cells were treated with extracts at different concentrations (100, 50, 25, 12.5, 6.25, 3.125, 1.56 μ g/mL) and incubated for 24 h. After incubation, MTT solution was added and incubated for 4 h. After incubation, 200 μ L DMSO was added to the cells and absorbance was read at 575 nm[25].

2.6.3. Microscopic imaging

To examine the morphological appearance of the cells, ethanol extracts of *A. filipendulina* at different doses (5, 15, 20, 40 μ g/mL) were applied to the cell lines and fixed with 10%, 25%, 50%, and 100% methanol for 3 min consecutively. The differences after

fixation were visualized by light microscopy.

2.6.4. Annexin V assay

HT29, HCT116, and L929 cells were seeded in 6 plates of approximately 5×10^5 cells per well and left for 24 hours of incubation. Ethanol extract at IC_{50} dose was added to the cells and incubated for another 24 h. After the incubation period, cells were detached using trypsin, collected, and resuspended in PBS containing 1% FBS. The cell suspension was then mixed with an equivalent volume of Annexin V & PI reagent following the manufacturer's instructions. The Muse Cell Analyser from Merck Millipore (USA) was used to quantify various cell populations including live cells, dead cells, early apoptotic cells, and late apoptotic cells[26].

2.6.5. Caspase 3/7 analysis

Caspase 3/7 analysis was performed using Muse Caspase-3/7 Kit (MARKA). HT29, HCT116, and L929 cells were added to the wells as 5×10^5 seeded in a 6-well plate, and left for 24 hours of incubation. Cells were treated with ethanol extract at IC_{50} dose and incubated again for 24 h. Cells were collected in a 50 μ L cell suspension volume in each Eppendorf tube. Then, cells were treated with 5 μ L of caspase 3/7 working solution and incubated at 37 °C for 30 min. Then, cells were treated with 150 μ L 7-amino actinomycin D (7-ADD) (2 μ L 7-AAD + 148 μ L PBS) dye for 5 min at room temperature in the dark. The Muse Cell Analyser (Merck, Millipore, USA) was then used to analyze the cells[27].

2.6.6. RT-PCR

Total RNA was isolated from HT29, HCT116, and L929 cells treated with *A. filipendulina* ethanol extracts (1 mg/10 mL). The RNeasy Mini Kit (QIAGEN, catalog no: 74104) was used for total RNA isolation. Cells treated with the vehicle (0.1% DMSO) for 48 h were collected as the control, and total RNA was isolated. In this study, the expressions of *miR-519d* and *Dvl-1* genes in HT29, HCT116, and L929 cells treated with *A. filipendulina* ethanol extracts were analyzed using the RT-PCR method. To quantify total RNA samples previously isolated from cell lines, the RNAs were analyzed using ultraviolet-visible spectroscopy (UV-VIS) (Maestro NANO). Total RNA concentration (ng/ μ L) and purity (A260/A280) of all samples were evaluated. cDNA synthesis was performed from total RNA following the kit protocol (RNase Inh. High Capacity, catalog no: C03-01-20). To standardize the RNA concentration for the reverse transcription reaction, each sample stored at -80 °C was adjusted to 100 ng/ μ L using nuclease-free water. The cDNA synthesis reaction consisted of 2 μ L of 10 $\times$ reaction buffer, 1 μ L of dNTP mixture, 2 μ L of random hexamers, 1 μ L of reverse transcriptase, 0.5 μ L of RNase inhibitor, 3.5 μ L of RNase-free water, and 10 μ L of total RNA, resulting in a final volume of 20 μ L. This mixture was then subjected to incubation in the Bio-Rad Thermo Cycler at 25 °C for 10 min, 37 °C for 120 min, and 85 °C for 5 min. For the RT-PCR reaction, the RT-PCR SYBR-Green MasterMix kit

was used along with the following optimized primers: *miR-519d* (gene ID: 574480) forward 5'-AACAAAGCAAAGTGCCTCCC-3' and reverse 5'-GTCGTATCCAGTGCAGGGT-3', *Dvl-1* (gene ID: 1855) forward 5'-GCATAACCGACTCCACCATTGTC-3' and reverse 5'-GATGGAGCCAATGTAGATGCCG-3', *RNU6* gene forward 5'-CCAUGCUCUUCUACUCCU-3' and reverse 5'-CACUGAUGUCGGUUAGUU-3' and *GAPDH* gene forward 5'-ATGTTCCAATATGATTCCACCC-3' and reverse 5'-ATGAGTCCTTCCACGATACC-3'. *GAPDH* gene and the *RNU6* gene were used as internal controls for gene expression analysis. For the RT-PCR step, each reaction was replicated three times for both groups. Reaction components were prepared as follows: 6 μ L of nuclease-free water and 10 μ L of SYBR Green Mastermix (A.B.T.TM, cat. no: Q03-02-01), 2 μ L of cDNA, 1 μ L of forward primer, and 1 μ L of reverse primer. The reaction mixtures were plated and subjected to incubation using the Roche LightCycler[®] 96 with the following protocol: Initial incubation for 5 min at 95 °C for one cycle; 40 cycles of 15 s at 95 °C and 1 min at 60 °C for 2-step amplification; melting curve analysis was conducted in one cycle with 10 s at 95 °C, 1 min at 65 °C, and 97 °C for 1 s to determine specificity. In the RT-PCR step, reactions were repeated three times for both cell lines. In the expression analysis, the formulas $\Delta Ct = Ct(\text{target}) - Ct(\text{reference})$ (normalization), $\Delta\Delta Ct = \Delta Ct(\text{experimental}) - \Delta Ct(\text{control})$, and $2^{-\Delta\Delta Ct}$ (fold change, FC) are used, respectively. When calculating fold regulation, if the FC > 1, the fold regulation (FR) value is equal to FC. If the FC < 1, the FR is expressed as FR = -1/FC[28]. The analysis of RT-PCR expression data was based on the $\Delta\Delta Ct$ ($\Delta\Delta Cq$) method[28] using the Data Analysis Center provided by Qiagen[29] (<https://www.qiagen.com/de/shop/genes-and-pathways/data-analysis-center-overview-page/>).

2.7. Molecular docking studies

PDB:7AFW was screened according to certain purification properties and selected as the protein to be studied. PDB:7AFW protein was previously studied by Ridwan *et al.* based on the target region[30]. The codes of the amino acids in the residue region were 205, 206, 207, 208, 243, 246, 247, 248, and 251. According to the GC-MS results of *A. filipendulina*, different and potential compounds in the most active extract, *i.e.* ethanol extract, were drawn in 2D structure and ligands were created. Then Glide-2021 (Schrödinger[®]) interface was used for the docking study. In the first step, protein preparation was performed. OPLS_2005 was performed by excluding all other chains and waters except chain A. The surface containing our specific amino acids was ensured while creating the grid area where the target will bind. In the second step, the compounds drawn in 2D were selected and the LigPrep step was prepared according to the OPLS_2005 strength. As the third step, LigDock was performed by selecting the appropriate conditions for the docking step, and images and results were obtained[30].

2.8. Statistical analysis

The analyses were conducted using the SPSS 22.0 software (SPSS, Chicago, IL, USA). Measurement data were presented as mean±SD. Comparisons between the two independent groups were performed using either an independent *t*-test or a Mann-Whitney *U* test. Statistical analyses involving multiple groups were conducted using either one-way ANOVA or the Kruskal-Wallis test. Normal distribution was assessed using the Shapiro-Wilk test, one of the normality tests. The mean IC₅₀ values were calculated, and the averages were graphed using the SigmaPlot 12.0 software. *P*<0.05 was considered significant.

3. Results

3.1. GC-MS analysis result and total flavonoid content

It was observed that each solvent yielded different component profiles, with the highest number of components extracted using hexane (Figure 1).

According to GC-MS results, eucalyptol (1,8-cineole) (S)-2,5-dimethyl-3-vinylhex-4-en-2-ol (santolina alcohol) and borneol were the main components of all extracts (Supplementary Tables 1-4).

The analysis of *A. filipendulina* extracts obtained with different solvents showed that the plant is a rich source of biologically active compounds. Ethanol extract showed the highest value with (90.32±2.43) µg/mL flavonoid content. The flavonoid contents in other solvents were as follows: water (25.63±1.32) µg/mL, ethyl acetate (43.43±1.30) µg/mL, *n*-hexane (56.32±2.39) µg/mL.

3.2. DPPH radical scavenging activity, ferric reducing antioxidant power (FRAP), TAS and TOS levels

Ethanol extract showed the highest antioxidant activity with the

lowest DPPH value of (18.13±1.53) µg/mL and the highest FRAP value of (432.32±3.43) mg/g. Ethyl acetate and *n*-hexane extracts showed high TAS values (1.25 and 1.39 mmol/L) and the ethanol extract showed the highest TOS value around 7.89 µmol H₂O₂ equivalent/L (Table 1).

3.3. Antimicrobial activity

The hexane and ethanol extracts were found to be effective on *Streptococcus mutans* (MIC<50 µg/mL). Besides, the ethanol extract was found to be effective on *Enterococcus faecalis* and *Candida albicans* (MIC<50 µg/mL).

3.4. MTT assay result and microscopic imaging

IC₅₀ values of all extracts in L929 were high, indicating the safety in healthy cells. The IC₅₀ value of the ethanol extract was (15.68±1.32) µg/mL against HT29 cells, which was stronger than other extracts (*P*<0.001) (Table 2), and it was also shown in the light microscope images (Figure 2). As a result, subsequent experiments were conducted using the ethanol extract.

3.5. Apoptosis analysis result

Ethanol extract of *A. filipendulina* induced 42.9% apoptosis in HT29 cells and 14.7% apoptosis in HCT116 cells. Necrosis was found to be 4.25% in HT29 and 8.70% in HCT116 (Figure 3A). Caspase 3/7 activities were 72% in HT29 cell line and 94.75% in HCT116 cell line (Figure 3B).

3.6. Expression levels of miR-519d and Dvl-1

Supplementary Table 5 reports FR and FC of *miR-519d* and *Dvl-1* expression levels after ethanol extract treatment in HT29, HCT116, and L929 cell lines. Compared to the control group, the expression of

Table 1. DPPH, FRAP, TAS, and TOS of *Achillea filipendulina* Lam. extracts (mean±SD, *n*=3).

Extracts	DPPH (µg/mL)	FRAP (mg/g)	TAS (mmol/L)	TOS (µmol/L)
Water	25.32±1.50	213.43±2.42	1.11±0.09	4.52±0.56
Ethanol	18.13±1.53	432.32±3.43	0.78±0.05	7.89±0.43
Ethyl acetate	24.53±0.53	242.23±3.43	1.25±0.03	6.72±0.75
<i>n</i> -Hexane	56.42±2.33	250.93±1.93	1.39±0.03	6.12±0.19
Ascorbic acid	5.93±0.09	-	-	-
Trolox	-	-	1.50±0.02	4.12±0.38

FRAP: Ferric reducing antioxidant power; TAS: Total antioxidant status; TOS: Total oxidant status.

Table 2. IC₅₀ values of different solvents of *Achillea filipendulina* in colon cancer (HCT116, HT29) and mouse fibroblasts (mean±SD, µg/mL).

Extracts	HCT116	HT29	L929
Water	17.10±0.93	37.43±1.42	127.13±2.12
Ethanol	20.80±0.94	15.68±1.32	80.38±0.78
Ethyl acetate	22.97±1.31	48.43±1.54	120.38±2.39
<i>n</i> -Hexane	13.56±1.03	39.92±2.91	98.83±1.78

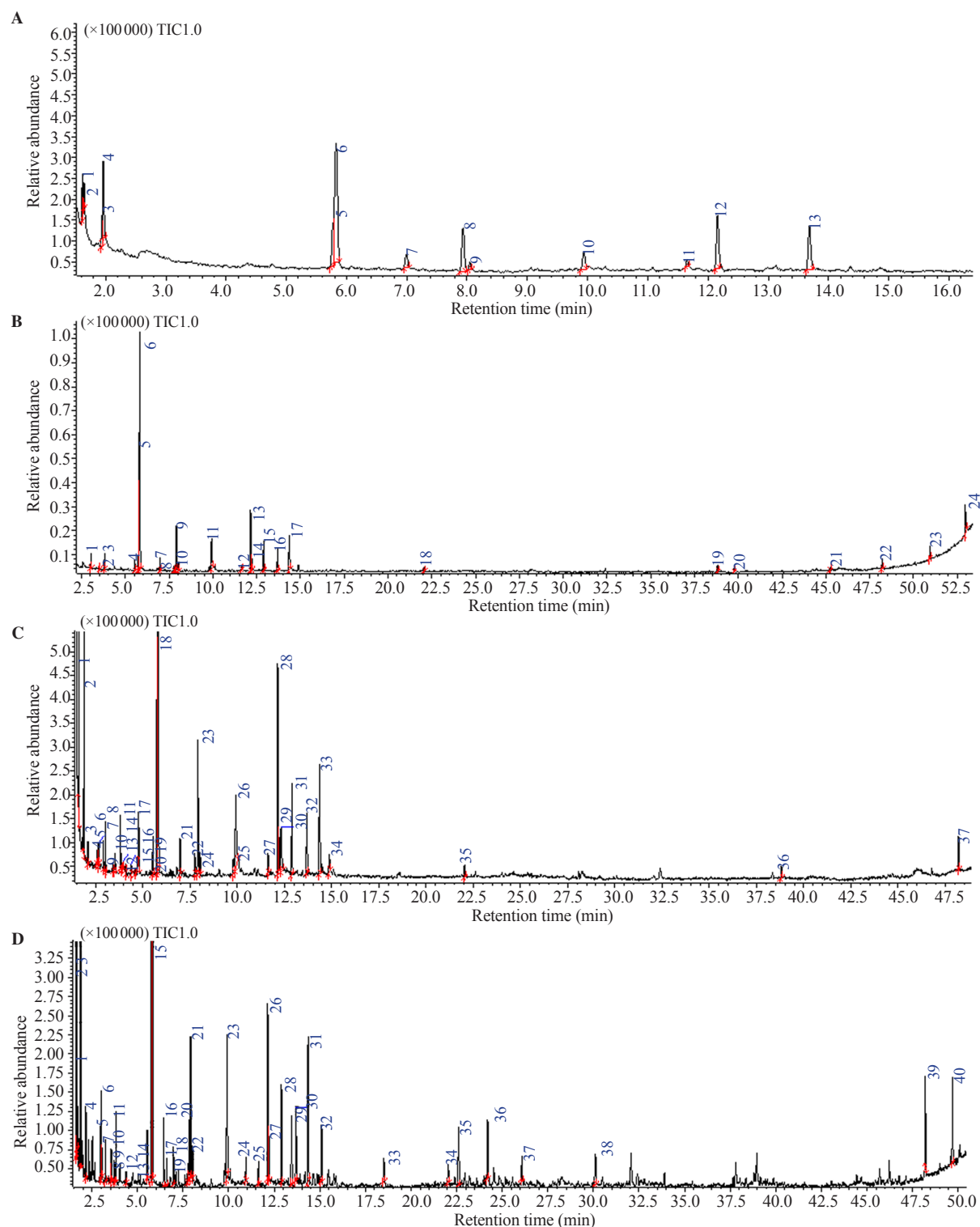


Figure 1. GC-MS chromatograms of *Achillea filipendulina* extracts in water (A), ethanol (B), ethyl acetate (C), and *n*-hexane (D) solvents.

the *miR-519d* gene was upregulated in HT29 cells ($FC=2.53\pm0.73$, $P=0.03$), HCT116 cells ($FC=2.62\pm1.10$, $P=0.08$), and L929 cells ($FC=1.16\pm0.24$, $P=0.33$) compared to the control group. Additionally, the expression of the *Dvl-1* gene was downregulated in HT29 cells ($FC=0.36\pm0.24$, $P=0.08$), HCT116 cells ($FC=0.46\pm0.26$, $P=0.19$), and L929 cells ($FC=0.79\pm0.19$, $P=0.85$) compared to the control group (Figure 4).

3.7. Molecular docking

According to Table 3, the closest results to the docking data in our reference molecule capecitabine molecule on average, especially based on docking and MMGBSA dGBind values, were found to belong to the borneol molecule with PUBCHEM ID 64685. Figure 5 shows the hydrogen bonds responsible for docking in the bond

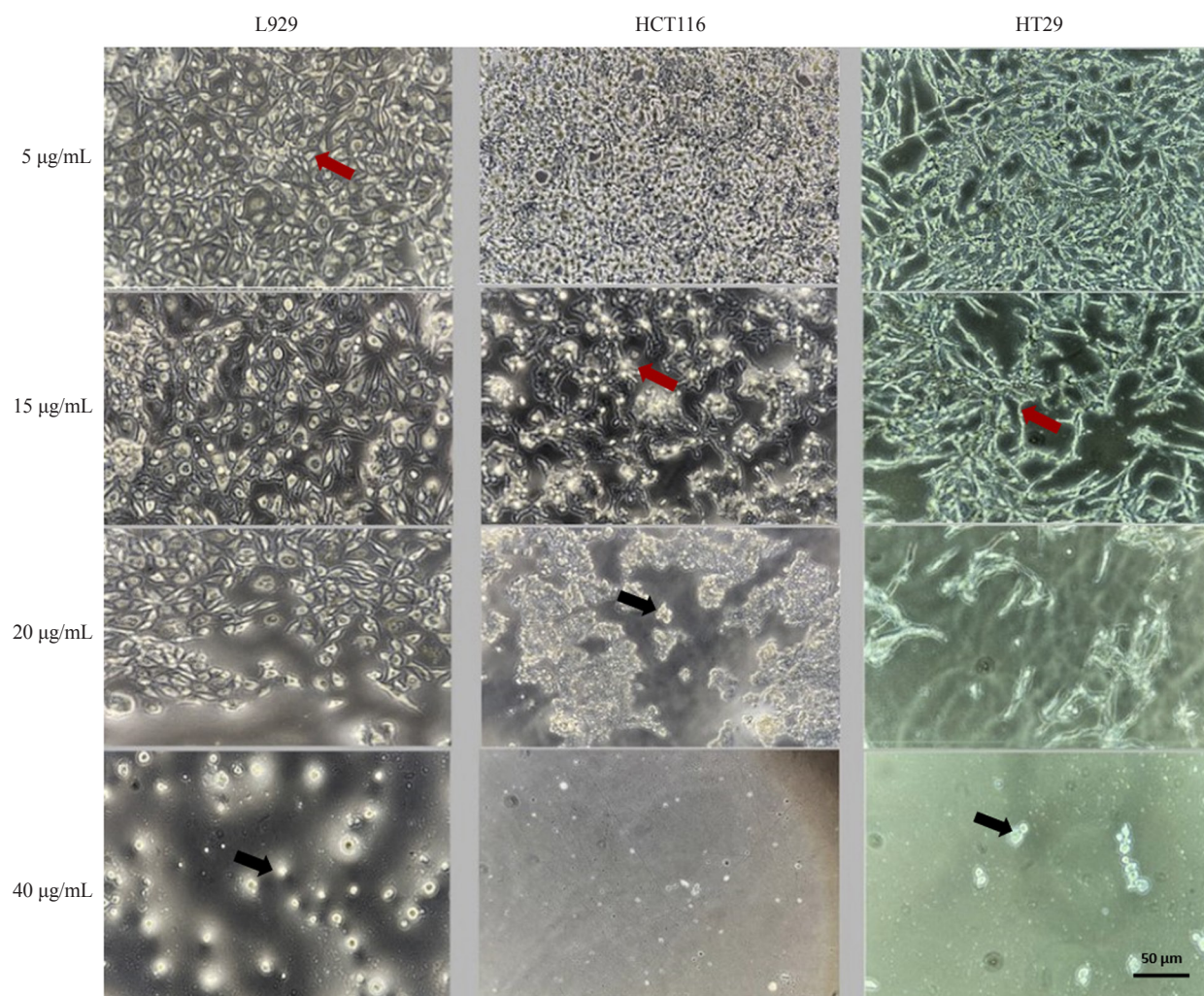


Figure 2. Inverted microscopy images of HCT116, HT-29, and L929 cells treated with *Achillea filipendulina* ethanol extract at different concentrations (5, 15, 20, 40 µg/mL). Red arrows indicate live cells and black arrows indicate early and late apoptotic cells. In all cells, as the extract concentration increases, there is a decrease in the number of living cells. Magnification: 400×.

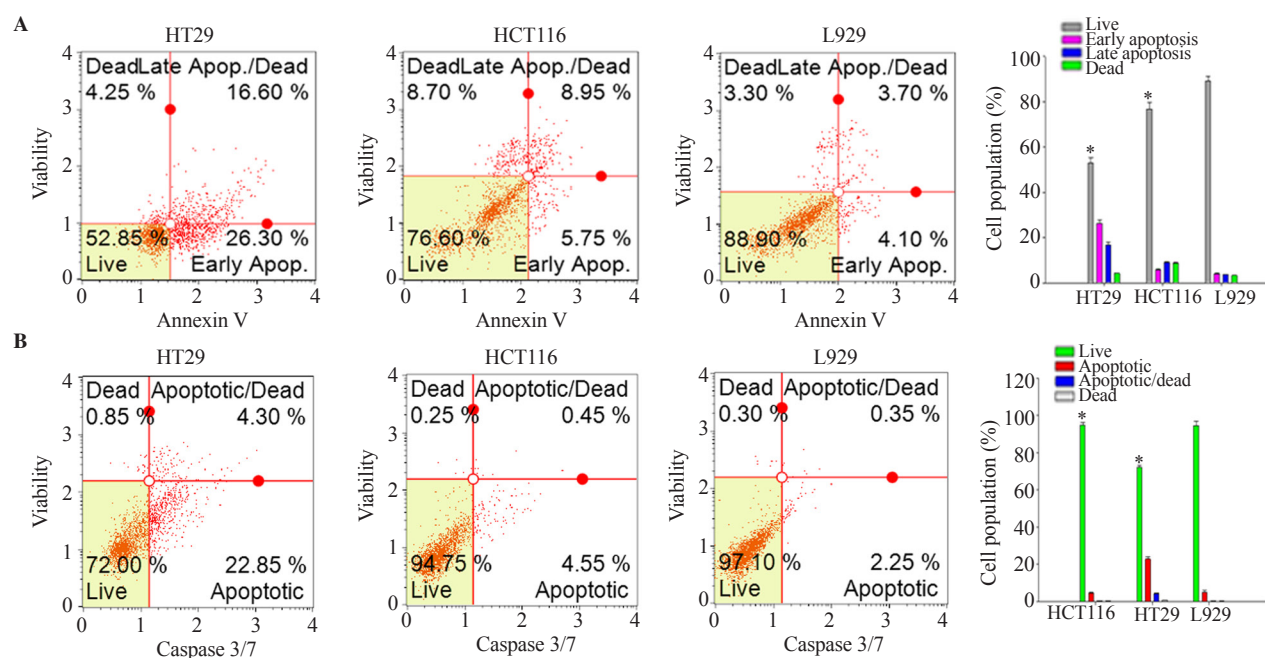


Figure 3. Annexin V assay (A) and caspase 3/7 activity (B) of cells after 24 hours of *Achillea filipendulina* ethanol extract treatment. * $P < 0.05$ compared with the control group (L929 cell).

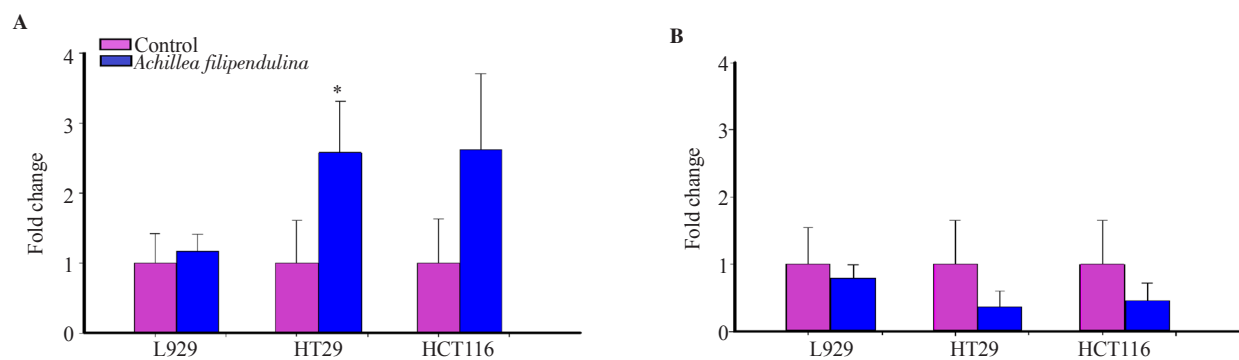


Figure 4. Effect of *Achillea filipendulina* ethanol extract on *miR-519d* (A) and *Dvl-1* (B) expression levels in L929, HT29, and HCT116 cells. * $P < 0.05$ compared with the control group.

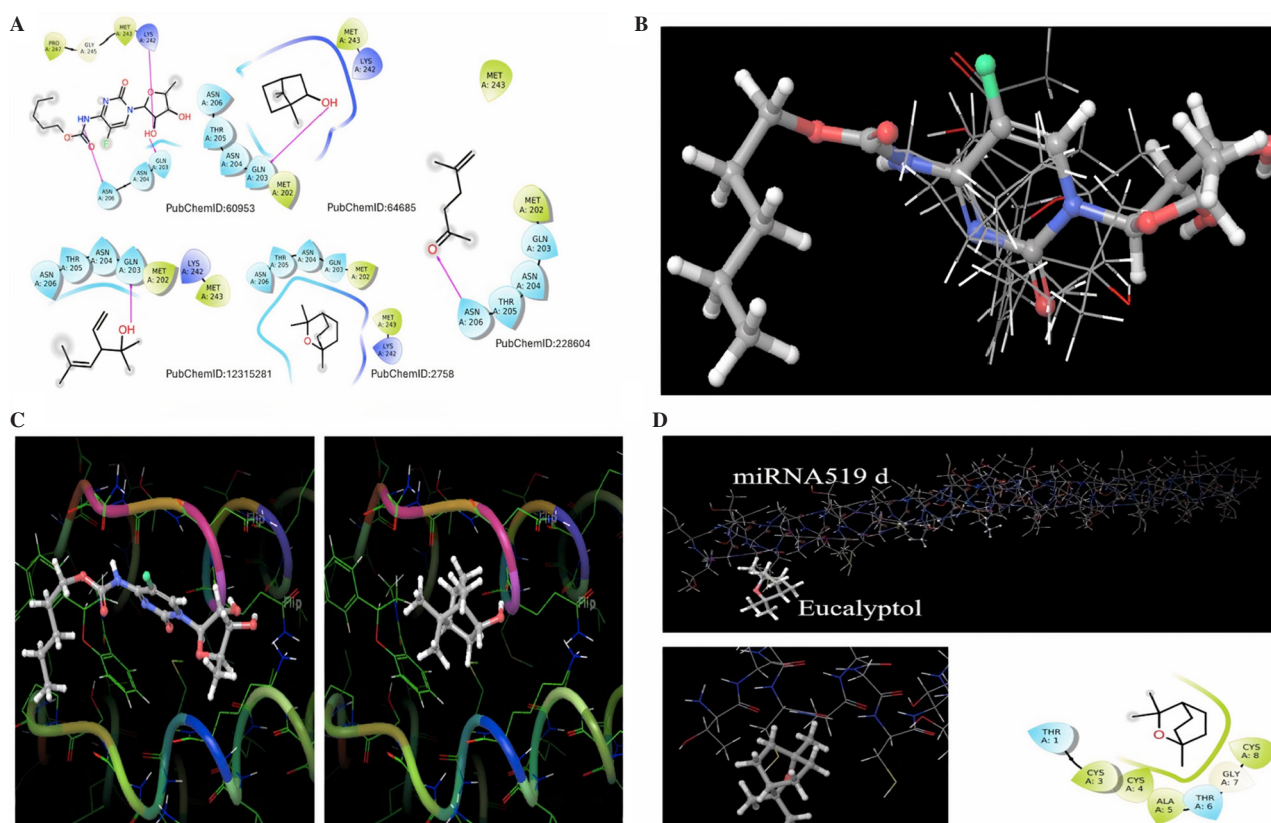


Figure 5. (A) PDB ID: 2D representation of ligand interaction results of compounds in ethanol extract in the 7AFW target residue domain (Capecitabine as a reference compound). (B) Superimposition image of the compounds in the ethanol extract concerning the active substance capecitabine. (C) 3D ligand interaction images of compounds with PubChem ID 60953 and PubChem ID 64685. (D) PubChem ID 2758 and miR-519d 2D and 3D ligand interaction images.

interactions of drug targets and molecules.

4. Discussion

This study provides evidence of the antioxidant, antimicrobial, and anticancer properties of *A. filipendulina* Lam. ethanol extracts *via* Wnt signaling by upregulating *miR-519d*, a tumor suppressor, and downregulating *Dvl-1* expression.

GC-MS analysis shows that 13 different compounds were found

in water solvent, 24 different compounds in ethanol solvent, 37 different compounds in ethyl acetate solvent, and 40 different compounds in *n*-hexane solvent. In all three solvents, the compound santolina alcohol [(S)-2,5-dimethyl-3-vinylhex-4-en-2-ol] was found in the largest amounts, which is similar to the studies by Alkhatib *et al.*[31] and Vojoudi *et al.*[32]. The second active ingredient was eucalyptol (1,8-cineole) in all four solvents. Bruchhage *et al.*[33] showed that the eucalyptol inhibited the Wnt/ β -catenin pathway. Another study showed that eucalyptol suppressed human colorectal cancer proliferation by inducing apoptosis *via* Wnt/ β -catenin

Table 3. *In silico* results.

PubChem ID	Docking score	XP GScore	Glide GScore	Glide EModel	Prime energy	MMGBSA dG bind
60953*	–3.940	–3.940	–3.940	–38.091	–7052.2	–36.76
64685	–3.089	–3.089	–3.089	–20.521	–6970.2	–35.40
12315281	–2.523	–2.523	–2.523	–21.223	–7036.4	–41.52
2758	–2.175	–2.175	–2.175	–13.691	–6995.4	–27.94
228604	–2.097	–2.097	–2.097	–15.386	–7018.8	–27.77

*Used as a reference since it is a drug with proven efficacy.

pathway and p38[34].

A. filipendulina L. induced cell death, especially in HT29 cell line. HCT116 is known to be a highly aggressive cell line with little or no differentiation capacity, while HT29 has an intermediate differentiation capacity to enterocytes and mucin-expressing lineages. Our study showed that the IC₅₀ value in HCT116 cell line was (20.80±0.94) µg/mL, (15.68±1.32) µg/mL in HT29 cell line, and (80.38±0.78) µg/mL in L929 cell line. According to the American National Cancer Institute criteria, the IC₅₀ value of plant extract candidates should be below 30 µg/mL[35]. Therefore, it can be said that the extracts are suitable for colon cancer.

The study shows that the apoptosis percentage was 42.90% in HT29 and 14.7% in HCT116. Necrosis was found to be 4.25% in HT29 and 8.70% in HCT116. When we looked at the caspase 3/7 pathway, it was found to be 72% in HT29 cell line and 94.75% in HCT116 cell line. This suggests that *A. filipendulina* ethanol extract may induce apoptosis by caspase 3/7 mechanism in HT29 cell line. According to the study by Moghadam *et al.*, *A. filipendulina* showed IC₅₀ values of 386 and 248 µg/mL against MCF-7 and MDA-MB-468, respectively, and Annexin V assay revealed that *A. filipendulina* can induce cell apoptosis[14].

The effects of *A. filipendulina* L. ethanol extract on the expression of tumor suppressor miR-519d were also investigated. Our study findings show that ethanol extract increased the expression of *miR-519*. miR-519d was found to suppress Wnt/β-catenin signaling and subsequently inhibit cell epithelial-mesenchymal transition in gastric cancer[4]. Studies have reported that increased expression of miR-519d in cancer cells regulates G₁/S transition, proliferation, apoptosis, cell invasion, and epithelial-mesenchymal transition[5,36,37]. Similarly, miR-519d has been shown to reduce drug resistance to 5-Fu and increase sensitivity to chemotherapy by directly targeting CCND1 in colorectal cancer[36]. Down-regulation of miR-519d-3p has been reported to be associated with lower survival rate, tumor size, TNM stage, and lymph node metastasis[5]. Furthermore, overexpressed miR-519d was found to suppress Rab10 expression and promote increased expression of cell apoptosis-related genes *Bax*, *Bcl-2*, and *p53*, and autophagy-related genes *Beclin1* and *Atg5*[38]. In a study conducted by Pereira and colleagues in 2018, it was emphasized that p53 and p21 levels increased in HCT-15 and NCI-H460 cells treated with the hydroethanolic extract of *Achillea millefolium* (*A. millefolium*) and phenolic components in *A. millefolium* inhibited cell proliferation[39]. In a study examining

the secondary metabolites of *A. millefolium* and *A. filipendulina* plants by GC-MS, the presence of similar metabolomic profiles between these two species was noted[40]. This suggests that similar to *A. millefolium*, *A. filipendulina* may also be of clinical importance. In this study, miR-519d expression increased in all cells treated with *A. filipendulina* Lam. ethanol extract, and the increase in the HT29 cell line was significant ($P<0.05$).

In our study, a decrease in *Dvl-1* gene expression level was observed in cells treated with ethanol extracts compared to control cells. Recently, it has been suggested that the expression of Dvl proteins is increased in cancer and its nuclear localization may enhance the activation of the Wnt/β-catenin signaling pathway[10]. In support of this hypothesis, three Wnt pathway genes, *Sarp1*, *frizzled (Fz)* and *disheveled (Dvl)*, were found to show increased expression upon examination of intestinal mucosa in patients at risk of colon cancer[41]. In a study conducted in breast cancer cell lines, three conserved major lysines that undergo acetylation and deacetylation cycles in Dvl-1 proteins were found to function as a potent modulator of the Wnt pathway, triggering cell migration, proliferation, cell cycle progression[8] and residues K69 and K285 were found to regulate nuclear import of Dvl-1 and trigger the expression of proteins involved in Wnt signaling[10]. This information suggests that Dvl-1 is involved in cancer. In this study, we observed that *Dvl-1* levels in HTC116 and HT29 cancer cells treated with ethanol extracts of *A. filipendulina* decreased. This suggests that *A. filipendulina* has a suppressive effect on the Wnt signaling pathway.

When the molecular modeling of the main chemical molecules in the extracts was compared, it was observed that their activities were almost identical, but it was thought that the borneol molecule (PubChem ID: 64685) was found differently in the ethanol extract according to the GC-MS results due to the synergistic/additive effect.

The interaction of the active components of *A. filipendulina* ethanol extract with proteins in the Wnt/β-catenin pathway and miR-519d in colon cancer was investigated using molecular docking methods. Compared to reference compounds and capecitabine, docking and MMGBSA scores showed that borneol, one of the components of the ethanol extract, may be effective in modulating the Wnt pathway. The study by Li *et al*[42] also shows that borneol can induce apoptosis in cancer cells. Future research may benefit from isolating pure borneol from *A. filipendulina* using other colon cell lines and examining the correlation with the activity of this compound. In

addition, a limitation of our study is that it was not supported by molecular dynamics simulations.

Several species in the genus *Achillea* have pharmacological properties such as antimicrobial, anti-inflammatory, anti-allergic, and antioxidant activities. In our study, according to DPPH, FRAP and flavonoid content, the ethanol extract of *A. filipendulina* has high antioxidant properties. The TOS level determines the formation of oxidants and the TAS level determines the antioxidants that defend against free oxygen radicals. Low TOS and high TAS values indicate that the antioxidant properties of the plant are high. Water and *n*-hexane extracts showed better antioxidant activity than ethanol extract. In MIC results using 8 different standard pathogenic microorganisms frequently used in scientific studies, ethanol extract was found to be effective on *Streptococcus mutans*, *Enterococcus faecalis*, and *Candida albicans*, thus having antimicrobial activity.

In conclusion, *A. filipendulina* L. plant exhibits antioxidant, antimicrobial, and anticancer properties and the effects of its active components should be investigated by purification.

Conflict of interest statement

The authors declare that there is no conflict of interest.

Acknowledgments

We gratefully acknowledge the support of TUBITAK-2209-A University Students Research Projects Program for the antioxidant activity assay of this research.

Funding

The antioxidant assays part of this study was financially supported by the TUBITAK 2209-A University Student Project (Project Number: 1919B012111858).

Data availability statement

The data supporting the findings of this study are available from the corresponding authors upon request.

Authors' contributions

TT and CH were responsible for writing the original draft, review, editing, and investigation, as well as project administration; ŞA participated in writing the original draft, review, editing and

investigation; OA, HO and SD were involved in investigation and writing.

References

- [1] Onur E, Nalbantsoy A, Kışla D. Immunotherapy and the potential of propolis in cancer immunotherapy. *Food Health* 2018; **4**(4): 231-246.
- [2] Budakoti M, Panwar AS, Molpa D, Singh RK, Büsselberg D, Mishra AP, et al. Micro-RNA: The darkhorse of cancer. *Cell Signal* 2021; **83**: 109995.
- [3] Xie L, Sadovsky Y. The function of miR-519d in cell migration, invasion and proliferation suggests a role in early placentation. *Placenta* 2016; **48**: 34-37.
- [4] Yue H, Tang B, Zhao Y, Niu Y, Yin P, Yang W, et al. MIR-519d suppresses the gastric cancer epithelial-mesenchymal transition *via* Twist1 and inhibits Wnt/ β -catenin signaling pathway. *Am J Transl Res* 2017; **9**(8): 3654.
- [5] Ye X, Lv H. MicroRNA-519d-3p inhibits cell proliferation and migration by targeting TROAP in colorectal cancer. *Biomed Pharmacother* 2018; **105**: 879-886.
- [6] Rennoll S, Yochum G. Regulation of *MYC* gene expression by aberrant Wnt/ β -catenin signaling in colorectal cancer. *World J Biol Chem* 2023; **6**(4): 290-300.
- [7] Liu Y, Huang D, Wang Z, Wu C, Zhang Z, Wang D, et al. LMO2 attenuates tumor growth by targeting the Wnt signaling pathway in breast and colorectal cancer. *Sci Rep* 2016; **6**: 36050. doi: 10.1038/srep36050.
- [8] Sharma M, Castro-Piedras I, Simmons GE Jr, Pruitt K. Dishevelled: A masterful conductor of complex Wnt signals. *Cell Signal* 2018; **47**: 52-64.
- [9] Zhao H, Ming T, Tang S, Ren S, Yang H, Liu M, et al. Wnt signaling in colorectal cancer: Pathogenic role and therapeutic target. *Mol Cancer* 2022; **21**(1): 144.
- [10] Castro-Piedras I, Sharma M, Brelsfoard J, Vartak D, Martinez EG, Rivera C, et al. Nuclear dishevelled targets gene regulatory regions and promotes tumor growth. *EMBO Rep* 2021; **22**: e50600. doi: 10.15252/embr.202050600.
- [11] Akyalcin H, Arabaci T, Yildiz B. Pollen morphology of six *Achillea* L. sect. *Achillea* (Asteraceae) species in Turkey. *Turk J Bot* 2011; **35**(2): 183-201.
- [12] Afshari M, Rahimmalek M. Variation in essential oil composition, anatomical, and antioxidant characteristics of *Achillea filipendulina* Lam. as affected by different phenological stages. *J Essent Oil Res* 2021; **33**(3): 283-298.
- [13] Asghari B, Mafakheri S, Zengin G, Dinparast L, Bahadori MB. In-depth study of phytochemical composition, antioxidant activity, enzyme inhibitory and antiproliferative properties of *Achillea filipendulina*: A good candidate for designing biologically active food products. *J Food Meas Charact* 2020; **14**: 2196-2208.
- [14] Moghadam MH, Mosaddegh M, Irani M. Programmed cell death in breast adenocarcinoma induced by *Achillea filipendulina*. *Med Plants – Int J Phytomed Relat Ind* 2019; **11**(4): 435-439.
- [15] Karakol P, Saraydin SU, Bozkurt M, Hepokur C, Inan ZD, Turan M.

- Anticancer effects of *Urtica dioica* in breast cancer. *Asian Pac J Cancer Prev* 2022; **23**(2): 673.
- [16] Ozpinar H. Anti-helminthic activity of *Myrtus communis* L. fruit ethanol extract on nematodes of *Caenorhabditis elegans* and the determination of possible active ingredients. *Hittite J Sci Eng* 2021; **8**(3): 267-272.
- [17] Chang C, Yang M, Wen H, Chern J. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *J Food Drug Anal* 2002; **10**: 178-182.
- [18] Shraim AM, Ahmed TA, Rahman MM, Hijji YM. Determination of total flavonoid content by aluminum chloride assay: A critical evaluation. *LWT* 2021; **150**: 111932. doi: 10.1016/j.lwt.2021.111932.
- [19] Cuendet M, Hostettmann K, Poterat O, Dyatmiko W. Iridoid glucosides with free radical scavenging properties from *Fagraea blumei*. *Helv Chim Acta* 1997; **80**(4): 1144-1152.
- [20] Toprak G, Sabancilar İ, Özcan N. Total oxidant status (TOS), total antioxidant status (TAS), ferritin and vitamin D levels in patients with chronic hepatitis C. *Microbiol Infect Dis* 2020; **4**(2): 1-4.
- [21] CLSI. *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically*. 9th Edition, CLSI document M07-A9. Pennsylvania, USA: Clinical and Laboratory Standards Institute; 2012.
- [22] CLSI. *Reference method for broth dilution antifungal susceptibility testing of yeasts*. 2nd edition. CLSI document M27-A2. Pennsylvania, USA: Clinical and Laboratory Standards Institute; 2012.
- [23] Kuete V. Potential of Cameroonian plants and derived products against microbial infections: A review. *Planta Med* 2010; **76**(14): 1479-1491.
- [24] Awouafack MD, McGaw LJ, Gottfried S, Mbouangouere R, Tane P, Spiteller M, et al. Antimicrobial activity and cytotoxicity of the ethanol extract, fractions and eight compounds isolated from *Eriosema robustum* (Fabaceae). *BMC Complement Altern Med* 2013; **13**: 1-9.
- [25] Tunç T, Hepokur C, Kariper A. Synthesis and characterization of paclitaxel-loaded silver nanoparticles: Evaluation of cytotoxic effects and antimicrobial activity. *Bioinorg Chem Appl* 2024; **2024**. doi: 10.1155/2024/9916187.
- [26] Lin W, Ye H. Anticancer activity of ursolic acid on human ovarian cancer cells via ROS and MMP mediated apoptosis, cell cycle arrest and downregulation of PI3K/AKT pathway. *J BUON* 2020; **25**(2): 750-756.
- [27] Panicker NG, Balhamar SO, Akhlaq S, Qureshi MM, Rehman NU, Al-Harrasi A, et al. Organic extracts from *Cleome droserifolia* exhibit effective caspase-dependent anticancer activity. *BMC Complement Med Ther* 2020; **20**: 1-3.
- [28] Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2^{−(Delta Delta C(T))} method. *Methods* 2001; **25**(4): 402-408. doi: 10.1006/meth.2001.12.
- [29] GeneGlobe Qiagen. Maryland: qPCR and Data analysis. [Online] Available from: <https://www.qiagen.com/de/shop/genes-and-pathways/data-analysis-center-overview-page/> [Accessed on 9 May 2024].
- [30] Ridwan S, Erlina LI, Bahtiar AN, Sukmawati DE. Identification and *in silico* analysis of inhibitor on the Wnt/ β -catenin signaling pathway as potential drug for colon cancer. *Int J Appl Pharm* 2023; **15**: 111-120.
- [31] Alkhatib R. Chemical composition of essential oils total phenols and antioxidant activity of *Achillea fragrantissima* and *A. santolina* grown in Syria. *Jordan J Pharm Sci* 2024; **17**(3): 594-602.
- [32] Vojoudi S, Sefidkon F, Salehi Shanjani P. Essential oil variation of *Achillea filipendulina* populations in farm condition. *J Essent Oil Res* 2024; **36**(2): 164-172.
- [33] Bruchhage KL, Koennecke M, Drenckhan M, Plötze-Martin K, Pries R, Wollenberg B. 1, 8-cineol inhibits the Wnt/ β -catenin signaling pathway through GSK-3 dephosphorylation in nasal polyps of chronic rhinosinusitis patients. *Eur J Pharmacol* 2018; **835**: 140-146.
- [34] Luca T, Napoli E, Privitera G, Musso N, Ruberto G, Castorina S. Antiproliferative effect and cell cycle alterations induced by *Salvia officinalis* essential oil and its three main components in human colon cancer cell lines. *Chem Biodivers* 2020; **17**(8): e2000309. doi: 10.1002/cbdv.202000309.
- [35] Widiandani T, Tandian T, Zufar BD, Suryadi A, Purwanto BT, Hardjono S, et al. *In vitro* study of pinostrobin propionate and pinostrobin butyrate: Cytotoxic activity against breast cancer cell T47D and its selectivity index. *J Public Health Afr* 2023; **14**(1): 6.
- [36] Yu G, Zhang T, Jing Y, Bao Q, Tang Q, Zhang Y. miR-519 suppresses nasopharyngeal carcinoma cell proliferation by targeting oncogene URG4/URGCP. *Life Sci* 2017; **175**: 47-51.
- [37] Huang R, Lin JY, Chi YJ. miR-519d reduces the 5-fluorouracil resistance in colorectal cancer cells by down-regulating the expression of CCND1. *Eur Rev Med Pharmacol Sci* 2018; **22**(9): 2869-2875.
- [38] Zhang YJ, Pan Q, Yu Y, Zhong XP. microRNA-519d induces autophagy and apoptosis of human hepatocellular carcinoma cells through activation of the AMPK signaling pathway via Rab10. *Cancer Manag Res* 2020; **12**: 2589-2602.
- [39] Pereira JM, Peixoto V, Teixeira A, Sousa D, Barros L, Ferreira ICFR, et al. *Achillea millefolium* L. hydroethanolic extract inhibits growth of human tumor cell lines by interfering with cell cycle and inducing apoptosis. *Food Chem Toxicol* 2018; **118**: 635-644.
- [40] Gonulalan EM, Nemutlu E, Demirezer LO. A new perspective on evaluation of medicinal plant biological activities: The correlation between phytomics and matrix metalloproteinases activities of some medicinal plants. *Saudi Pharm J* 2019; **27**(3): 446-452.
- [41] You XJ, Bryant PJ, Jurnak F, Holcombe RF. Expression of Wnt pathway components frizzled and dishevelled in colon cancer arising in patients with inflammatory bowel disease. *Oncol Rep* 2007; **18**(3): 691-694.
- [42] Li FF, Fang J, Ma Q, Fu H, Mao JP. Natural borneol liquid induced cancer cell apoptosis. *China Biotech* 2013; **33**(5): 22-27.

Publisher's note

The Publisher of the *Journal* remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Edited by Tan BJ, Liang Q

Antioxidant, antimicrobial, and anticancer effects of *Achillea filipendulina* L. against colon cancer

Running Title: Antioxidant, antimicrobial, anticancer effects of *Achillea filipendulina*

Tutku Tunç^{1*}, Şeyda Akın², Okan Aykaç³, Ceylan Hepokur^{4*}, Serap Duran⁴, Hülya Özpınar⁵

^{1*}*Sivas Cumhuriyet University, Faculty of Pharmacy, Department of Pharmaceutical Microbiology, Sivas, Türkiye*

²*Sivas Cumhuriyet University, Faculty of Medicine, Department of Medical Biology, Sivas, Türkiye*

³*Sivas Cumhuriyet University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Sivas, Türkiye*

⁴*Sivas Cumhuriyet University, Faculty of Pharmacy, Department of Biochemistry, Sivas, Türkiye*

⁵*Sivas Cumhuriyet University, Faculty of Pharmacy, Department of Pharmaceutical Botany, Sivas, Türkiye*

Corresponding Author 1:

Tutku TUNÇ

E-mail address: tutkutunc58@hotmail.com

Corresponding Author 2:

Ceylan HEPOKUR

E-mail address: cozsoya@gmail.com

Supplementary Table 1. GC-MS chromatogram of *Achillea filipendulina* extracts in water solvent.

Peak No	Retention time	Start time	End time	Area, %	Compound name
1	1.614	1.6	1.625	3.93	4-Hydroxy-2-butanone
2	1.642	1.63	1.655	1.93	2-Butanone, 3-hydroxy-
3	1.936	1.92	1.945	2.78	2,3-Butanediol
4	1.962	1.945	2	8.68	2,3-Butanediol
5	5.785	5.725	5.785	7.72	Eucalyptol (1,8-cineole)
6	5.827	5.785	5.88	31.7	(S)-2,5-Dimethyl-3-vinylhex-4-en-2-ol
7	6.992	6.95	7.04	3.11	3,5-Hexadien-2-ol, 2-methyl-
8	7.931	7.875	7.985	10.52	5-Hexen-2-one, 5-methyl- (CAS)
					7-Oxabicyclo[4.1.0]heptane, 1-methyl-
9	8.047	8.01	8.08	1.38	2,2,5,5-Tetramethyl-4-vinyl-3-hydroxytetrahydrofuran
10	9.932	9.88	9.985	4.49	Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, endo-
11	11.645	11.625	11.68	1.03	4.beta.-Hydroxyachipendol
12	12.157	12.11	12.2	11.94	4.alpha.-Hydroxyachipendol
					<i>trans</i> -Ascaridol glycol
13	13.68	13.625	13.745	10.78	3-Hexyne-2,5-diol, 2,5-dimethyl- (CAS)

Antioxidant, antimicrobial, and anticancer effects of *Achillea filipendulina* L. against colon cancer

Running Title: Antioxidant, antimicrobial, anticancer effects of *Achillea filipendulina*

Tutku Tunç^{1*}, Şeyda Akın², Okan Aykaç³, Ceylan Hepokur^{4*}, Serap Duran⁴, Hülya Özpınar⁵

^{1*}*Sivas Cumhuriyet University, Faculty of Pharmacy, Department of Pharmaceutical Microbiology, Sivas, Türkiye*

²*Sivas Cumhuriyet University, Faculty of Medicine, Department of Medical Biology, Sivas, Türkiye*

³*Sivas Cumhuriyet University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Sivas, Türkiye*

⁴*Sivas Cumhuriyet University, Faculty of Pharmacy, Department of Biochemistry, Sivas, Türkiye*

⁵*Sivas Cumhuriyet University, Faculty of Pharmacy, Department of Pharmaceutical Botany, Sivas, Türkiye*

Corresponding Author 1:

Tutku TUNÇ

E-mail address: tutkutunc58@hotmail.com

Corresponding Author 2:

Ceylan HEPOKUR

E-mail address: cozsoya@gmail.com

Supplementary Table 2. GC-MS chromatogram of *Achillea filipendulina* extracts in ethanol solvent.

Peak No	Retention time	Start time	End time	Area, %	Compound name
1	3.027	3	3.07	1.41	1,4-Hexadiene, 2,5-dimethyl-3-vinyl-
2	3.53	3.495	3.555	0.54	2-Pinene
3	3.814	3.78	3.855	1.74	2,2-dimethyl-3-methylene-bicyclo [2.2.1]heptane
4	5.541	5.485	5.58	1.6	Benzene, 1-methyl-2-(1-methylethyl)-
5	5.767	5.715	5.785	13.52	Eucalyptol (1,8-cineole)
6	5.816	5.785	5.885	32.53	(S)-2,5-Dimethyl-3-vinylhex-4-en-2-ol
7	6.98	6.94	7.035	1.68	3,5-Hexadien-2-ol, 2-methyl-
8	7.771	7.73	7.805	0.67	5-Isopropyl-2-methylbicyclo[3.1.0]hexan-2-ol
9	7.922	7.875	7.975	6.13	5-Hexen-2-one, 5-methyl- (CAS)
10	8.036	7.99	8.085	1.12	2,2,5,5-Tetramethyl-4-vinyl-3-hydroxytetrahydrofuran
11	9.925	9.88	10.035	5.46	Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, endo-
12	11.64	11.61	11.68	0.6	4.beta.-Hydroxyachipendol
13	12.15	12.095	12.19	8.73	4.alpha.-Hydroxyachipendol
14	12.199	12.19	12.25	1.52	1-Methyl-1-(4-methyl-3-cyclohexen-1-yl)ethyl acetate (1R,5S,6R)-2,7,7-Trimethylbicyclo[3.1.1]hept-2-en-6-yl acetate
15	12.9	12.855	12.95	3.98	
16	13.694	13.64	13.75	3.28	2-Norbornanol, 1,3,3-trimethyl-, acetate, endo- 2,3-Dioxabicyclo[2.2.2]oct-5-ene, 1-methyl-4-(1- methylethyl)-
17	14.363	14.305	14.425	5.62	
18	22.046	21.985	22.105	0.87	8-Isopropyl-1-methyl-5-methylene-1,6-cyclodecadiene
19	38.81	38.77	38.875	1.01	Pentadecanoic acid
20	39.745	39.705	39.785	0.47	Hexadecanoic acid, ethyl ester
21	45.239	45.18	45.28	0.56	Ethyl (9z,12z)-9,12-octadecadienoate
22	48.207	48.16	48.26	1.55	Octacosane <n->
23	50.941	50.91	50.975	1.27	Tricosane <n->
24	52.962	52.915	53.045	4.15	Nonyl tetracosyl ether

Antioxidant, antimicrobial, and anticancer effects of *Achillea filipendulina* L. against colon cancer

Running Title: Antioxidant, antimicrobial, anticancer effects of *Achillea filipendulina*

Tutku Tunç^{1*}, Şeyda Akın², Okan Aykaç³, Ceylan Hepokur^{4*}, Serap Duran⁴, Hülya Özpınar⁵

^{1*}*Sivas Cumhuriyet University, Faculty of Pharmacy, Department of Pharmaceutical Microbiology, Sivas, Türkiye*

²*Sivas Cumhuriyet University, Faculty of Medicine, Department of Medical Biology, Sivas, Türkiye*

³*Sivas Cumhuriyet University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Sivas, Türkiye*

⁴*Sivas Cumhuriyet University, Faculty of Pharmacy, Department of Biochemistry, Sivas, Türkiye*

⁵*Sivas Cumhuriyet University, Faculty of Pharmacy, Department of Pharmaceutical Botany, Sivas, Türkiye*

Corresponding Author 1:

Tutku TUNÇ

E-mail address: tutkutunc58@hotmail.com

Corresponding Author 2:

Ceylan HEPOKUR

E-mail address: cozsoya@gmail.com

Supplementary Table 3. GC-MS chromatogram of *Achillea filipendulina* extracts in ethyl acetate solvent.

Peak No	Retention time	Start time	End time	Area. %	Compound name
1	1.612	1.555	1.64	19.07	Acetic acid, propyl ester
2	1.883	1.845	1.92	4.11	Propanoic acid, ethyl ester
3	2.096	2.075	2.125	0.35	Benzene, methyl-
4	2.558	2.535	2.58	0.24	Acetic acid, butyl ester (CAS)
5	2.64	2.615	2.65	0.42	Methanone, dicyclopropyl-
6	2.664	2.65	2.695	0.35	1-Butanol, 3-methyl-, acetate (CAS)
7	2.92	2.89	2.96	0.64	1,2-Propanediol, 1-acetate
8	3.029	3	3.065	1.04	Hexane, 2,2,3,3-tetramethyl-
9	3.416	3.39	3.45	0.18	1,4-Hexadiene, 2,5-dimethyl-3-vinyl-
10	3.53	3.495	3.585	0.56	Octane, 3,6-dimethyl- (CAS)
11	3.813	3.78	3.85	1.31	2-Pinene
12	3.907	3.88	3.93	0.11	2,2-dimethyl-3-methylene-bicyclo [2.2.1]heptane
13	3.958	3.94	3.98	0.12	Pentane, 2,2,3,3-tetramethyl- (CAS)
14	4.104	4.07	4.135	0.21	Nonane, 2-methyl-
15	4.386	4.34	4.435	0.38	Octane, 3,6-dimethyl- (CAS)
16	4.613	4.585	4.66	0.18	1,6-Octadien-3-ol, 3,7-dimethyl-, acetate
17	4.776	4.74	4.82	1.41	1,2-Ethanediol, diacetate
18	5.539	5.48	5.585	1.35	Undecane
19	5.76	5.71	5.78	9.74	Benzene, 1-methyl-2-(1-methylethyl)-
20	5.81	5.78	5.875	22.66	Eucalyptol (1,8-cineole)
21	6.975	6.935	7.025	1.17	(S)-2,5-Dimethyl-3-vinylhex-4-en-2-ol
22	7.757	7.715	7.805	0.69	3,5-Hexadien-2-ol, 2-methyl-
23	7.917	7.87	7.975	4.55	Octadecane, 1-chloro-
24	8.031	7.995	8.075	0.65	5-Hexen-2-one, 5-methyl- (CAS)
25	9.795	9.735	9.865	0.77	2,2,5,5-Tetramethyl-4-vinyl-3-hydroxytetrahydrofuran
26	9.92	9.87	10.045	3.87	<i>cis</i> -Chrysanthenol
27	11.635	11.59	11.68	0.61	Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, endo-
28	12.146	12.095	12.185	7.3	4.beta.-Hydroxyachipendol
29	12.19	12.185	12.27	1	4.alpha.-Hydroxyachipendol
30	12.322	12.285	12.45	2.04	.Alpha.-irone B
31	12.894	12.835	12.95	3.15	2-hydroxypropane-1,3-diyl diacetate (1R,5S,6R)-2,7,7-Trimethylbicyclo[3.1.1]hept-2-en-6-yl acetate
32	13.689	13.64	13.74	2.22	2-Norbornanol, 1,3,3-trimethyl-, acetate, endo-
33	14.357	14.3	14.44	4.61	2,3-Dioxabicyclo[2.2.2]oct-5-ene, 1-methyl-4-(1-methylethyl)-
34	14.879	14.825	14.935	0.78	Linalool oxide (2)
35	22.039	21.985	22.105	0.53	8-Isopropyl-1-methyl-5-methylene-1,6-cyclodecadiene
36	38.815	38.755	38.885	0.56	Tridecanoic acid

37	48.202	48.155	48.255	1.08	Octacosane <n->
----	--------	--------	--------	------	-----------------

Antioxidant, antimicrobial, and anticancer effects of *Achillea filipendulina* L. against colon cancer

Running Title: Antioxidant, antimicrobial, anticancer effects of *Achillea filipendulina*

Tutku Tunç^{1*}, Şeyda Akın², Okan Aykaç³, Ceylan Hepokur^{4*}, Serap Duran⁴, Hülya Özpınar⁵

^{1*}*Sivas Cumhuriyet University, Faculty of Pharmacy, Department of Pharmaceutical Microbiology, Sivas, Türkiye*

²*Sivas Cumhuriyet University, Faculty of Medicine, Department of Medical Biology, Sivas, Türkiye*

³*Sivas Cumhuriyet University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Sivas, Türkiye*

⁴*Sivas Cumhuriyet University, Faculty of Pharmacy, Department of Biochemistry, Sivas, Türkiye*

⁵*Sivas Cumhuriyet University, Faculty of Pharmacy, Department of Pharmaceutical Botany, Sivas, Türkiye*

Corresponding Author 1:

Tutku TUNÇ

E-mail address: tutkutunc58@hotmail.com

Corresponding Author 2:

Ceylan HEPOKUR

E-mail address: cozsoya@gmail.com

Supplementary Table 4. GC-MS chromatogram of *Achillea filipendulina* extracts in *n*-hexane solvent.

Peak No	Retention time	Start time	End time	Area. %	Compound name
1	1.623	1.605	1.66	1.22	Furan, tetrahydro-2,5-dimethyl- (CAS)
2	1.689	1.66	1.715	3.83	Cyclohexane, methyl-
3	1.878	1.85	1.915	35.9	Benzene, methyl-
4	2.166	2.145	2.19	0.61	Hexane, 2,3,3-trimethyl- (CAS)
5	2.976	2.95	2.99	0.6	2,2-Dimethyltetrahydrofuran
6	3.02	2.99	3.055	1.32	1,4-Hexadiene, 2,5-dimethyl-3-vinyl-
7	3.236	3.205	3.275	0.51	Propane, 2-methyl-1-propoxy-
8	3.524	3.5	3.54	0.3	2-Pinene
9	3.556	3.54	3.59	0.39	Hydroperoxide, 1-ethylbutyl (CAS)
10	3.728	3.705	3.765	0.29	Hydroperoxide, 1-methylpentyl (CAS)
11	3.808	3.78	3.845	0.87	2,2-dimethyl-3-methylene-bicyclo [2.2.1]heptane
12	4.369	4.34	4.405	0.16	2(10)-Pinene
13	5.532	5.5	5.585	0.85	Benzene, 1-methyl-4-(1-methylethyl)-
14	5.754	5.705	5.77	6.31	Eucalyptol (1,8-cineole)
15	5.801	5.77	5.865	15.46	(S)-2,5-Dimethyl-3-vinylhex-4-en-2-ol
16	6.434	6.39	6.475	1.18	5-Isobutylnonane
17	6.605	6.56	6.64	0.44	5-Isobutylnonane
18	6.967	6.925	7.005	0.71	3,5-Hexadien-2-ol, 2-methyl-
19	7.761	7.725	7.785	0.37	4-Thujanol
20	7.823	7.785	7.86	0.97	5-Isobutylnonane
21	7.907	7.865	7.96	2.52	5-Hexen-2-one, 5-methyl- (CAS)
22	8.007	7.97	8.055	0.67	Decane, 4-methyl-
23	9.906	9.865	9.965	2.74	Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, endo-
24	10.942	10.91	10.975	0.38	Dodecane
25	11.627	11.6	11.675	0.39	4.beta.-Hydroxyachipendol
26	12.139	12.095	12.17	3.12	4.alpha.-Hydroxyachipendol
27	12.193	12.185	12.245	0.6	(E)-4-((1R,5S)-2,5,6,6-Tetramethylcyclohex-2-en-1-yl)but-3-en-2-one-rel-
28	12.89	12.845	12.94	1.8	(1R,5S,6R)-2,7,7-Trimethylbicyclo[3.1.1]hept-2-en-6-yl acetate
29	13.449	13.41	13.5	1.34	2,6,10-Trimethyltridecane
30	13.687	13.645	13.755	1.61	2-Norbornanol, 1,3,3-trimethyl-, acetate, endo-
31	14.352	14.295	14.415	3.33	2,3-Dioxabicyclo[2.2.2]oct-5-ene, 1-methyl-4-(1-methylethyl)-
32	15.092	15.04	15.145	1.37	Octadecane
33	18.509	18.465	18.565	0.57	Pentadecane <n->
34	22.044	21.98	22.1	0.46	1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1-methylethyl)-, [s-(E,E)]-
35	22.611	22.55	22.68	1.41	Heptadecane, 8-methyl-
36	24.204	24.145	24.25	1.31	Hexadecane, 2,6,10,14-tetramethyl-
37	26.079	26.045	26.125	0.44	Eicosane <n->
38	30.114	30.045	30.17	0.87	2,6,10-Trimethyltridecane
39	48.202	48.17	48.25	1.45	Octacosane <n->

40	49.702	49.665	49.74	1.33	Hexacosane
----	--------	--------	-------	------	------------

Antioxidant, antimicrobial, and anticancer effects of *Achillea filipendulina* L. against colon cancer

Running Title: Antioxidant, antimicrobial, anticancer effects of *Achillea filipendulina*

Tutku Tunç^{1*}, Şeyda Akın², Okan Aykaç³, Ceylan Hepokur^{4*}, Serap Duran⁴, Hülya Özpınar⁵

^{1*}*Sivas Cumhuriyet University, Faculty of Pharmacy, Department of Pharmaceutical Microbiology, Sivas, Türkiye*

²*Sivas Cumhuriyet University, Faculty of Medicine, Department of Medical Biology, Sivas, Türkiye*

³*Sivas Cumhuriyet University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Sivas, Türkiye*

⁴*Sivas Cumhuriyet University, Faculty of Pharmacy, Department of Biochemistry, Sivas, Türkiye*

⁵*Sivas Cumhuriyet University, Faculty of Pharmacy, Department of Pharmaceutical Botany, Sivas, Türkiye*

Corresponding Author 1:

Tutku TUNÇ

E-mail address: tutkutunc58@hotmail.com

Corresponding Author 2:

Ceylan HEPOKUR

E-mail address: cozsoya@gmail.com

Supplementary Table 5. *miR-519d* and *Dvl-1* gene expression levels in colorectal cell and healthy cell lines treated with ethanol extracts.

Gene	<u>HT29</u>			<u>HCT116</u>			<u>L929</u>		
	Fold regulation	Fold change	<i>P</i>	Fold regulation	Fold change	<i>P</i>	Fold regulation	Fold change	<i>P</i>
<i>miR-519d</i>	2.53	2.53±0.73	0.03*	2.62	2.62±1.10	0.08	1.16	1.16±0.24	0.33
<i>Dvl-1</i>	-2.75	0.36±0.24	0.08	-2.17	0.46±0.26	0.19	-1.26	0.79±0.19	0.85

**P*<0.05 was considered significant.