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GC-MS profiling, antioxidant, antimicrobial activities, DNA cleavage effect of *Symphytum aintabicum* Hub.-Mor. & Wickens (Boraginaceae) and its anticancer activity on MCF-7 cell line

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Abstract: *Symphytum* species, generally known as comfrey, have been used for ancient times in folk medicine for wounds, bone breakages, rheumatism, liver problems, and innumerable potentialities. However, many of the species belonging to the genus *Symphytum* have not been analyzed with scientific methods and principles yet. Therefore, this work is a guide for determining and researching the main gaps in the literature. In this study, MeOH and petroleum ether (PE) extracts of *Symphytum aintabicum* Hub.-Mor. & Wickens were analyzed for their biological activities and GC-MS analysis, antioxidant, antimicrobial activities, DNA cleavage effect, and anticancer activity on the MCF-7 human breast cell line. The phytochemical constituents of the main composition of *S. aintabicum* extracts were determined. The MeOH extract was found to contain higher concentrations of phenolic 90.87 (±5.30) mg (GAE)/g extract and flavonoid 98.00 (± 9.72) mg (CE)/g extract substances in comparison to the PE extract. The extracts displayed significant ABTS activity and it was found that the IC_{50} values of the PE and MeOH extracts were calculated as 6.88 mg/mL (±0.11) and 0.032 mg/mL ±0.002), respectively. According to the data obtained, PE extract demonstrated more antimicrobial activity than the MeOH extracts. The PE and MeOH extracts was found to have strongest antimicrobial activity on *Micrococcus luteus*, *E. coli* (125 µg/mL; 250 µg/mL) and *Candida albicans* (1000 µg/mL), respectively. Additionally, the effects of plant extracts on DNA cleavage were investigated using pBR322 plasmid DNA, and it was found that the PE extract was more effective than the MeOH extract in both the presence and absence of H_2O_2. In the MTT assay, the lowest % cell viability values in the PE and MeOH extracts were 42.79% and 47.77% at 500 ppm, respectively. The IC_{50} values for the PE and MeOH extracts were 414.73 µg/mL and 443.31 µg/mL. Findings of this study show that *S. aintabicum* has shown interesting pharmacological and biological activities, and is a particularly suitable candidate for anticancer drug development.

Key words: Antimicrobial, anticancer, antioxidant, GC-MS, pBR322 plasmid DNA, *S. aintabicum*

1. Introduction

Plants were recorded in the first written sources, where they were used in the treatment of different ailments for thousands of years and Dioscorides' Materia Medica. Although the use of medicinal plants and their use in the field of treatment has decreased from time to time, it has increased in recent years and continues to increase with studies and observations. Sources of information about Anatolian medicinal plants date back to ancient times. Since the majority of Turkish people live in rural areas, they are closely related to wild plants. People use some wild plants as food, spice, dyestuff, and medicine (Gunther, 1968; Baytop, 1984; Kawarty et al., 2020). With the industrial revolution and the progress of organic chemistry, synthetic products have become the source of pharmacological treatment (Rates, 2001). However, due to the typically undesirable effect of synthetic drugs, the turn towards herbal drugs has increased significantly in recent years. Plants that increase self-protection power of the human body and raise the body's resistance against all kinds of problems are among the plants that are emphasized. The use of plants with this feature is increasing daily and provides a sizeable commercial income to countries that grow these plants (Stace, 1980).

Turkey has very remarkable and rich vegetation in terms of its available plant diversity. It may be seen that the use of the existing plant potential in Turkey in various industrial fields can be essential when studies carried out in the world are evaluated in general. The fact that Anatolia is a bridge between the flora of Southern Europe and Southwest Asia, as the origin and differentiation center of many genera and sections, and the high species endemism rate, probably related to ecological and phytogeographical differentiation, are other reasons for this richness (Dağcı et al., 2002). The number of natural plant species in Turkey is around 10.754, and 3.708 of these plants are endemic

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(endemism rate 34.8%) (Cosge et al., 2009). The genus *Symphytum* L. is in the tribus Boragineae Bencht & J. Presl (= *Anchusae* DC.) of the Boraginaceae Juss. (Langsrom and Chase, 2002). The family Boraginaceae consists of annual, biennial, and perennial herbs, shrubs, and trees, and there are 154 genera and 2500 species of this family in the world (Mabberley, 1997). This family is of great importance in terms of ornamental and medicinal plants. Species of the family contain valuable compounds, especially in their roots, such as pyrrolizidine alkaloids, red pigment, shikonin, a naphthoquinone derivative (Wollenweber et al., 2002; Orhan et al., 2011). The vernacular name of the genus *Symphytum* is "Karakafes otu", and *S.aintabicum* is known as "antep kafesotu" in Turkey (Baytop, 1997; Güner, 2012). Proteins, gum, phenolic acids, saponins, tannins, pyrrolizidine alkaloids, vitamins, especially vitamin B12, carotene, starch and high amounts of mineral substances have been detected in different part of species of genus *Symphytum* (Yakupoğlu, 2019). That is why these species have been used extensively in the diets of certain ethnic groups for many years (Rode, 2002). The roots and leaves of *Symphytum* species are used by communities, herbalists, and physicians in the therapy of bone fractures, sprains, skin problems, tendon rupture, hematomas, gastrointestinal ulcers, rheumatism, and lung congestion, liver problems, wound healing, gout, inflammation of the vessel wall with thrombosis, antifungal, antioxidant, and antiinflammatory effects (Rode, 2002; Talhouk et al., 2007; Salehi et al., 2019; Zengin et al., 2021).

However, many of the species belonging to the genus *Symphytum*, which have the potential for numerous medical uses, are still not scientifically researched. In this case, the lack of information is caused by the literature gap. Moreover, investigating the biological activities of unexplored species is extremely important to provide data on their potential to be used as a folk remedy. This case highlights the need for new studies. According to the literature review, there are a limited number of studies on the *S. aintabicum* plant (an endemic species). Meanwhile, there is no study as detailed as this study that included the parameters analyzed. Hence, in this study, aimed to determine biological features as (i) GC-MS analysis, (ii) antioxidant activity, (iii) antimicrobial activity, (iv) DNA cleavage effect (in particular, this parameter was made because the development of selective DNA cleavage natural nuclease sources led to the design of chemotherapeutic and antimicrobial drugs), as well as (iv) anticancer activity against MCF-7 cells (breast cancer is a serious health problem, accounting for about a quarter of all cancers in the world. In parallel with this, the discovery of biologically effective compounds that can be used in the treatment of various types of cancer has been among the most popular research topics in recent years. This parameter was made in order to contribute to the literature studies in this field) in different extracts (PE and MeOH) of *S. aintabicum* collected from Diyarbakır, in Turkey. This study is the first and original in many respects, as it is comprehensive enough to form a basis for forthcoming research.

2. Materials and methods

2.1. Plant material collection

S. aintabicum uniform raw plant materials were harvested from Eğil, Diyarbakır, 860 m (flowering period in 2020), then taxonomic identification of plant was carried out and vouchered by Dr. Alevcan Kaplan and kept at Batman University (Voucher no: 2020/010). The aerial parts of the plants were dehydrated in a shaded place for one week at room temperature. Dried plant samples were stored in ziplock bags until analysis. Afterwards, the dried samples were powdered and used for chemical analysis.

2.2. Extraction of plant materials for biological activity tests

The extraction of plant material (aerial parts) was carried out by the Soxhlet method. For this purpose, 30 g of the dried plant sample was ground into a cellulose thimble. To extract the nonpolar components, the cellulose thimble containing the plant material was placed in the Soxhlet extractor, and the extraction process was continued with PE (750 mL) until the color disappeared. At the end of this period, the plant material was removed from the cellulose thimble and dried. The same procedures were carried out with MeOH (750 mL) to extract of the polar components in the dried plant material. The PE and MeOH were removed from the environment with the help of a rotary evaporator (IKA RV10 D). The extraction yield was determined by the following equation:

$$\text{Extraction yield } (\%) = (g_{extract} / g_{dry\ plant\ material}) \times 100.$$

Here, $g_{extract}$: extraction weight of the dry extract in grams after the solvent has been completely evaporated, and $g_{dry\ plant\ material}$: the weight in grams of the dried plant material used in the extraction process. The extracts obtained at the end of the extraction process were decomposed in 100% dimethyl sulfoxide (DMSO) and stored at –20 °C until used in the subsequent analysis.

2.3. GC-MS profiling

2.3.1. Derivatization of plant extracts

Before derivatization, all glassware was soaked in 5% DMDCS (in toluene) solution at 60 °C overnight. The deactivated glass surfaces were washed and dried with toluene and MeOH, respectively. The derivatization of the PE and MeOH extracts of the plant was carried out using the MSTFA (containing 1% TMCS) reagent. For this purpose, 1–2 mg of the dehydrated extract was decomposed in pyridine (20 mg/mL MeOx-HCl) and incubated for 45 min at 70 °C on a thermal block. After this step, an equal

volume of MSTFA (containing 1% TMCS) was added and silanization was performed at 40 °C for 90 min.

2.3.2. GC-MS method of derivatized extracts

The derivatized samples were assayed using Agilent-7890B GC and MSD-5977A MS systems with an HP-5MS (19091S-433) capillary column. One microliter of the derivatized extracts was injected into the heater section at 300 °C with the help of an autosampler with an injection speed of 200 mL/min in the splitless mode. A gradient temperature program (0 min at 50 °C; ramp1 10 °C/min, 1 min at 120 °C; ramp2 10 °C/min, 1 min at 225 °C; ramp3 10 °C/min, 10 min at 300 °C) was followed. The samples separated in GC were ionized with a 70 eV ion source, and mass scanning was performed in the range of 25–700 Da. MS spectra were scanned in the NIST11 and MPW Drug libraries, and the results were evaluated according to their similarity percentages.

2.4. Antioxidant activity

2.4.1. Determination of the TPC

The total phenolic content (TPC) of the extracts was appraised according to the assay reported by Singleton and Rossi (1965) using the Folin-Ciocalteu reagent. For this purpose, the plant extract or the gallic acid standard (40 µL) was mixed with 1.8 mL of the Folin-Ciocalteu reagent (10 times prediluted with purified water). The mixture was left at room temperature for 5 min, and 1.2 mL of $NaHCO_3$ (7.5 %, w/v) was then added to the mix. Absorbance was read at 765 nm after leaving the mixture at room temperature (25 °C) for another 60 min. Aqueous solutions with known gallic acid concentrations in a series of 7.8–1000 mg/L were used for calibration, and R^2 (correlation coefficient) was found to be 0.9978 for the absorption plot. The results are expressed in mg gallic acid equivalent (GAE) / g extract. All assays were performed in triplicates.

2.4.2. Determination of the TFC

The total flavonoid content (TFC) of the extracts was evaluated according to the assay described by Zhishen et al. (1999). For this purpose, 1.25 mL of dH_2O and 75 µL of 5% sodium nitrite were added to 250 µL (1 mg/mL) of the extract solution and incubated for 5 min. Next, 150 µL of 10% $AlCl_3.6H_2O$ solution was added to the mixture and after mixing, 500 µL of 1 M NaOH and 275 µL of dH_2O were added consecutively to the mixture. The mixture was thoroughly mixed, and absorbance was read against water at 510 nm. The catechin calibration curve (0.0078–1 mg/mL) was drawn, and the results are expressed in mg catechin/g extract by calculating the total flavonoid content. All assays were performed in triplicates.

2.4.3. $ABTS^{\bullet+}$ assay

The $ABTS^{\bullet+}$ radical scavenging experiment of plant extracts was estimated on the procedure described by Re et al. (1999). According to this procedure, 7 mM of ABTS was mixed with an equal volume of 2.45 mM KPS (potassium persulfate) and incubated for 16–18 h at 25 °C in the dark. In the following step, the absorbance of the ABTS radical cation ($ABTS^{\bullet+}$) solution was adjusted to 0.700 (± 0.020) at 734 nm with a purified water. Next, 180 mL of the ABTS solution was added to 20 mL of various concentrations of the ligand and metal complexes (in DMSO). The mixture was then incubated for 30 min in the dark, and absorbance was read in a microplate reader. All assays were performed in triplicates. $ABTS^{\bullet+}$ scavenging abilities was calculated determined by the following equation.

$ABTS^{\bullet+}$ scavenging effect (%) = ((AB-AA)/AB) × 100.

AB: The absorbance of 20 µL of the DMSO and 180 µL $ABTS^{\bullet+}$ mixture,

AA: The absorbance of 20 µL of the extracts and 180 µL of the $ABTS^{\bullet+}$ mixture.

2.5. Antimicrobial activities

The antimicrobial activity of the extracts was determined using two gram-negative bacteria as *E. coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853) and two gram-positive bacteria which included *Staphylococcus aureus* (ATCC 25923) and *Micrococcus luteus* (NRRL B-4375) and a yeast called *Candida albicans* (ATCC 10231). The MIC (minimum inhibitory concentration) value of the extracts was assayed with the serial dilution method (CLSI, 2006). The microorganisms were incubated in NB medium at 37 °C for 18 h in an incubator, and the final concentration was adjusted to 10^6 cfu/mL. Stock solutions of the extracts were prepared at a concentration of 40 mg/mL. The extracts were dissolved in DMSO. All test microorganisms treated with dilutions of the extracts were incubated at 37 °C, the MIC value was determined as the first value at which growth did not. All assays were performed in triplicates.

2.6. DNA cleavage studies

The DNA cleavage effect of the different extracts (MeOH and PE) of *S. aintabicum* was tested on double-stranded pBR322 circular plasmid DNA by using the gel electrophoresis method (Eryılmaz et al., 2020). One microliter of pBR322 (0.25 µg/mL) was added to 3 µL of the appropriate concentrations of the plant extracts in DMSO, and the final volume was completed to 11 µL with distilled water. After 5 h of incubation at 37 °C, 2 µL of gel loading solution (6X) was added to each tube and loaded onto agarose gel prepared in 1% TBE buffer. Reactions were simplified in both the presence and absence of H_2O_2 (30 mM). Electrophoretic running was carried out in the electrophoresis system (Peqlab) at 80V for 90 min. After electrophoretic running, the gel was stained with EtBr and visualized with the imaging system (UVP PhotoDoc-lt Imaging System).

2.7. Cell culture and MTT cytotoxicity assay (Antiproliferative assay)

2.7.1. Cell lines, culture treatments

The MCF-7 (ATCC® HTB22™) cell line I used in this study was produced in 25 cm^2 or 75 cm^2 flasks by incubating it for 24 h in an incubator at 5% CO_2 and 37 °C using the RPMI-1640 (Roswell Park Memorial Institute) medium containing 0.2% sodium bicarbonate, 10% fetal bovine serum (FBS) and 1% penicillin / streptomycin. The MCF-7 cells were grown to reach a confluence rate of 80%, after which they were washed with PBS. The MCF-7 cells were passaged in 1 × Trypsin-EDTA.

2.7.2. MTT-based cytotoxicity assessment

The cytotoxicity experiment was performed to determine the cellular viability of the MeOH and PE extracts of the plant on the MCF-7 cell line. The MTT experiment was carried out based on the protocol described by Mosmann (1983) was optimized for the MCF-7 cell lines used in the assays.

First of all, to evaluate the cytotoxic concentration of the plant extracts with the MTT procedure, 96 plates were seeded with three replications and 1×10^4 MCF-7 cells in 100 µL for each well. After seeding, the cells were incubated for 24 h. After 24 h of incubation, the selected concentrations of the extracts dissolved in DMSO (1000-500-250-125-62.5-31.25-15.625-7.812 ppm) were seeded into the wells by 100 µL. Additionally, a negative control and a solvent control (DMSO) group were used. To see the effects of the concentrations, the MTT (100 µL per well) solution was added to the MCF-7 cells that were incubated in an oven for 24 h, and the resulting mixture was incubated for 2.5 h. After this, the MTT solution was removed from the wells, and the reaction was terminated with DMSO. Measurements were taken with a spectrophotometer at 570 nm.

2.8. Statistical analysis

For evaluation of MTT cytotoxicity assay, the 50% inhibitory concentration (IC_{50}) value was calculated by determining the % cell viability values using the Microsoft Excel program and added standard error bars to graphs. With the help of Microsoft Excel program, the applied concentration and % cell viability curve were determined and 50 % inhibitory concentration (IC_{50}) value was calculated with logarithmic slope graph. The IC_{50} value was calculated using the formula [Cytotoxicity = Test substance absorbance (mean) / control absorbance (mean)] × 100. The Kruskall–Wallis H-tests with Bonferoni correction were used to compare between groups and nonparametric measures because the data were not normally distributed. The significance level was set at 0.05. Data calculations were performed using SPSS 21 package program.

3. Results

The use of medicinal plants is increasing worldwide due to their few side effects and beneficial properties to treat and prevent many diseases. Interest in the chemical components of medicinal plant products has further increased their use in nutrition and biochemical research (Başgel et al., 2006). Roughly 60%–80% of the world's population still relies on conventional herbal medicines to treat common diseases (e.g., colds) and there is an increasing reliance on the use of traditional folk medicines in industrialized societies (Mothana et al., 2010). This paper reports the GC-MS profiling, antioxidant, antimicrobial activities, DNA cleavage effects and anticancer activity (MCF-7) of MeOH and PE extracts of *S. aintabicum*.

3.1. GC-MS profiling

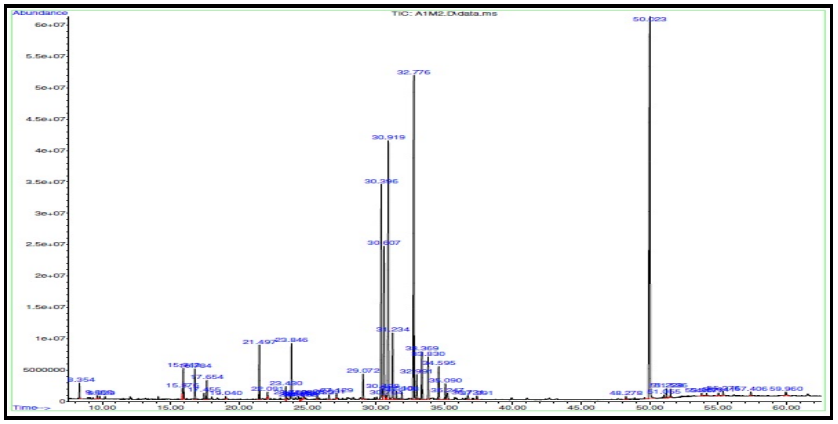
The GC-MS profiling of the MeOH and PE extracts of the aerial parts of *S. aintabicum* revealed the presence of eleven and twenty (thirty-one in total) constituents, respectively. The GC-MS chromatogram of the extracts is shown in Figure 1. The active principles with their retention time (RT) and peak area are presented in Table 1. The spectra of the compounds are matched with the NIST11 and MPW Drug libraries. It was determined that the identified compounds had various biological activities. Among the compounds identified in the plant extracts, the components with the highest peak areas for the MeOH extract were; sucrose, octakis(trimethylsilyl) ether (28.59%) and inositol, 1,2,3,4,5,6-hexakis-O-(trimethylsilyl)-, muco- (15.99%), while in the PE extract, these were heneicosane (13.06%) and hexadecanoic acid, trimethylsilyl ester (12.34 %).

3.2. TPC and TFC composition

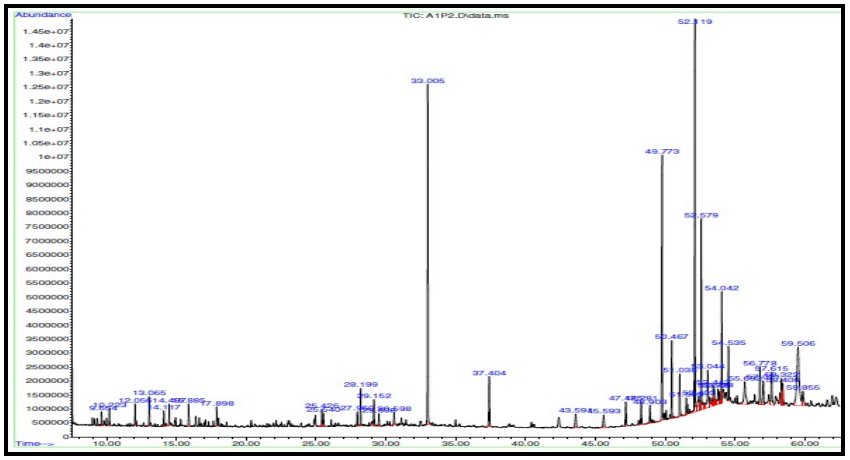
The total phenolics and flavonoid contents of the plant extracts were determined spectrophotometrically using the methods given in the experimental part, and the results are presented in Table 2. TPC was calculated in mg gallic acid equivalent (GAE)/g extract, and TFC was calculated in mg catechin equivalent (CE)/g extract. The results demonstrated that the phenolic and flavonoid contents of the MeOH extract were 90.87 (±5.30) mg (GAE)/g extract and 98.00 (±9.72) mg (CE)/g extract, respectively, whereas the TPC and TFC contents of the PE extract were consecutively 5.38 (±1.53) mg (GAE)/g extract and 59.64 (±7.91) mg (CE)/g extract.

3.3. $ABTS^{\bullet+}$ assay

The results on $ABTS^{\bullet+}$ inhibition by the two plant extracts (PE and MeOH) are summarized in Figure 2. According to these results, the ABTS radical scavenging effect of both extracts increased with their increasing concentrations. The IC_{50} values of the extracts were calculated as 6.88 mg/mL (± 0.11) and 0.032 mg / mL (± 0.002) for PE and MeOH, respectively. Antioxidant activity is expressed by



(A)



(B)

Figure 1. GC-MS profiling of the (A) MeOH, (B) PE extracts from *S. aintabicum*.

the IC_{50} (effective concentration) value, which expresses the amount of antioxidants consumed to reduce the initial $ABTS^{\bullet+}$ cation radical concentration by 50%. As the IC_{50} value decreases, an antioxidant the activity increases. The $ABTS^{\bullet+}$ reduction rate of the PE extract from *S. aintabicum* was higher than that of the MeOH extract. Remarkably, it may be stated that the antioxidant capacity and thus the biological features of the MeOH extract were higher than those of the PE extract.

3.4. Antimicrobial activities

The microbial inhibitory potentials of the two solvent extracts (MeOH and PE) of *S. aintabicum* are shown in Table 3. The results proved the presence of a different and selective effect antimicrobial activity of the solvent extracts against each microbial species that were analyzed. The solvent equivalent DMSO was tested for antimicrobial activity. In the comparison of the MIC values, it was seen that the PE extract was more effective on the selected test microorganisms than the MeOH extract. The MeOH extracts was found to have various rates of antimicrobial activity on selected microorganisms. The PE extract of *S. aintabicum* was influential on both gram-positive and gram-negative microorganisms, whereas it was found to have moderate antimicrobial activity on the yeasts. Conspicuously, it may be easily argued that the PE extract is a resource with a high potential for use in the improvement of new antimicrobial agents brought about by the problem of antibiotic resistance.

3.5. DNA cleavage studies

The DNA cleavage results are shown in Figures 3 and 4 for the MeOH and PE extracts, respectively. Figure 3 shows the DNA cleavage activity of the MeOH and PE extracts. Figure 4 shows the DNA cleavage activity of the MeOH and PE extracts in the presence of H_2O_2. As seen in Figure 3,

Table 1. Compounds defined by GC-MS analysis of the MeOH and PE extracts from *S. aintabicum*.

Number	Name of the compound	RT	Peak area (%)
	MeOH extract		
1	3,7-Dioxa-2,8-disilanonane, 2,2,8,8-tetramethyl-5-[(trimethylsilyl)oxy]-	15.947	1.30
2	Butanedioic acid, [(trimethylsilyl)oxy]-, bis(trimethylsilyl) ester	21.499	2.15
3	2-Methyl-1,3-bis(trimethylsilyloxy)butane	23.844	2.20
4	Pentanedioic acid, 3-methyl-3-[(trimethylsilyl)oxy]-, bis(trimethylsilyl) ester	24.126	1.43
5	D-Fructose, 1,3,4,5,6-pentakis-O-(trimethylsilyl)-, O-methyloxime	30.390	9.12
6	D-(+)-Xylose, tetrakis(trimethylsilyl) ether, trimethylsilyloxime	30.612	6.61
7	Cobalt, .eta.-5-cyclopentadienyl-.eta.-4-(2,4,5-triethyl-2-exo,3-dimethyl-1,2,5-	30.924	12.08
8	Inositol, 1,2,3,4,5,6-hexakis-O-(trimethylsilyl)-, muco-	32.779	15.99
9	4,6,4'-Trimethoxy-gris-3'-ene-3,2'-dione	33.373	2.19
10	Trimethylsilyl catechollactate tris(trimethylsilyl) ether	33.833	1.78
11	Sucrose, octakis(trimethylsilyl) ether	50.027	28.59
	PE extract		
12	Azelaic acid, bis(trimethylsilyl) ester	28.193	1.26
13	Tetradecanoic acid, trimethylsilyl ester	29.158	1.02
14	Hexadecanoic acid, trimethylsilyl ester	33.002	12.34
15	Octadecanoic acid, trimethylsilyl ester	37.410	2.61
16	Eicosanoic acid, trimethylsilyl ester	43.585	1.05
17	1-Docosanol, trimethylsilyl ether	47.177	1.32
18	1H-Pyrrole, 2,3,4,5-tetraphenyl-	48.275	1.02
19	Heptacosane	49.775	10.26
20	Tetracosan-1-ol trimethylsilyl ether	50.472	2.57
21	2,3-Diacetyl-6-methyl-4,5-diphenyl-o-diacylbenzole	51.036	1.70
22	Heneicosane	52.120	13.16
23	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	52.402	1.47
24	4-Hydroxy-3-methoxy-17-methyl-[2',5'-bis(mkethoxycarbonyl)pyrrolo]-[3',4' : 6,7]	52.580	6.89
25	Eicosane	54.049	4.09
26	3,3-[2,2-Dimethyl-1,3-propanediylbis(oxy)]-9,11-.alpha.-dihydro[1]benzopyrano[2'	54.539	2.02
27	Cholest-2-eno[3,2-a]naphthalene	55.697	2.63
29	4'-{[5-(2-Butyl-3-(4-methylphenyl))-2,1,3,4-1H-thiatriazol-2-on-1-yl]methyl}biph	56.781	2.04
28	Phenol, 4-[[5-(2-methyl-3-benzofuranyl)-1H-pyrrol-2-yl][5-(2-methyl-3-benzofuran	57.018	1.52
30	Benzo[b]naphtho[2,3-d]furan	57.612	2.18
31	(5.alpha.,6.alpha.)-4,5-Epoxy-6-methoxy-17b-hydroxy-17-propyl-3.beta.-phthalimid	59.512	9.27

the MeOH extract had little effect on the pBR322 DNA in both the presence and absence of H_2O_2. As seen in Figure 4, the PE extract opened the pBR322 DNA supercoiled structure and converted it into a linear form more than the MeOH extract did in both the presence and absence of H_2O_2.

3.6. MTT assay

The crude MeOH and PE extracts of the plant exhibited potential cytotoxic activity against the MCF-7 cell line at varying concentrations of 1000-500-250-125-62.5-31.25-15.625-7.812 ppm, respectively, and the percentage of cell viability was determined and is shown in Table 4. The cytotoxic activities of the extract are shown in Figure 5. Both extracts showed cytotoxic activity at various concentrations. In general, it may be stated that the PE extract had a higher cytotoxic effect than the MeOH extract. As seen in Table 4 and Figure 5, the lowest % viability values found for the PE and MeOH extracts

Table 2. TPC and TFC values of *S. aintabicum* different extracts.

Plant extracts	TPC	TFC
	mg (GAE)/ g extract	mg (CE)/g extract
PE	5.38 (±1.53)	59.64 (±7.91)
MeOH	90.87 (±5.30)	98.00 (±9.72)

*Values are reported as mean ± SD

were 42.79% and 47.77% at 500 ppm, respectively. The IC_{50} values for PE and MeOH extracts were 414.73 µg/mL and 443.31 µg/mL, respectively, where there was 50% cell death exhibiting cytotoxic activity against the MCF-7 cells at varying concentrations (Table 4). However, statistical evaluation of the cytotoxic effect of PE and MeOH extract of *S. aintabicum* is demonstrated in Tables 5 and 6, respectively.

4. Discussion

Nutrition and nutritional science studies in recent years have drawn attention to the potential antioxidant effect of plant products (Trouillas et al., 2003). Traditional herbs have been used to treat cancer for several millennia in various parts of the world, and herbal medicines are used worldwide, either alone or in combination with traditional therapeutics, to treat various diseases. It is known that especially plant-based bioactive compounds affect carcinogenic activity in various ways. They alter carcinogen metabolism, induce DNA damage, activate the immune system, inhibit cell cycle progression and promote apoptosis. They are also known to have chemotherapeutic and chemopreventive activity against cancer cells (Choromanska et al., 2017). As far as it is known, this study is the first work in which the biological features of the *S. aintabicum* species were studied and evaluated together, considering the studied parameters. This research aimed to reveal the phytochemical components and investigate the antioxidant, antimicrobial activities, DNA cleavage effect of *S. aintabicum* and its anticancer activity on the MCF-7 cell line.

The results of the GC-MS profiling (using GC-MS after derivatization) of the PE and MeOH extracts (Figure 1) of *S. aintabicum* with its major components and their composition percentages are summarized in Table 1. Thirty-one compounds in total were defined in the extracts tested in this study. For the MeOH extract, the GC-MS chromatogram of the active fraction demonstrated 11 compounds, of which three were dominant. Among the identified compounds, sucrose, octakis(trimethylsilyl) ether (28.59%) has been reported to act as a food preservative when used at sufficient concentrations (Naik et al., 2018) whereas inositol, 1,2,3,4,5,6-hexakis-O-(trimethylsilyl)-, muco- (15.99%) has been reported concerning the treatment of polycystic ovary syndrome (Wojciechowska et al., 2019), RNA export, DNA repair, and DNA recombination, in endocytosis and vesicular trafficking, and as an antioxidant (Raboy, 2003) having a significant anticancer effect in different in vivo and in vitro models, against the MCF-7 cell line (Tantivejkul et al., 2003). Likewise, various studies have reported the antimicrobial effect of sugars isolated from different plants (Petkova et al., 2017). Meanwhile, the GC-MS analysis of the PE extract clarified the presence of 20 compounds. Among the identified compound, heneicosane (13.06%) is known to be found in the pheromones of *Aedes aegypti* (Seenivasagan et al., 2009) and as well as having an antimicrobial activity (Vanitha et al., 2020) and anticancer activity (Swantara et al., 2019). Hexadecanoic acid, trimethylsilyl ester (12.34%), is known as an antioxidant,nematicide, hypocholesterolemic, and pesticide (Jain et al., 2012). Other phytochemicals found to be contained in both extracts are substances with many

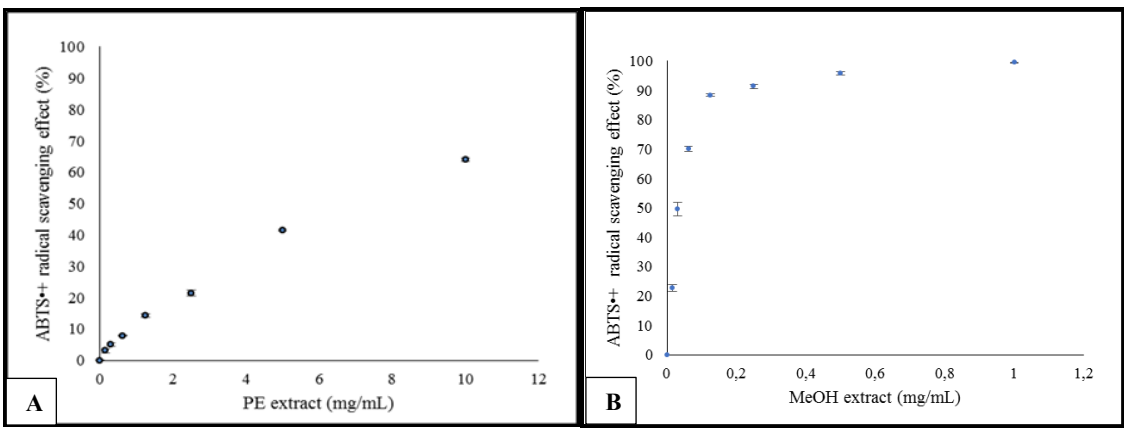


Figure 2. ABTS$^{•+}$ free radical scavenging effect of the PE (A) and MeOH (B) extract.

Table 3. MIC values on various microorganisms of *S. aintabicum* extracts (µg/mL).

Samples	Prokaryote				Eukaryote
	Gram (–)		Gram (+)		
	E. coli (ATCC 25922)	*P. aeruginosa* (ATCC 27853)	*S. aureus* (ATCC 25923)	*M. luteus* (NRRL B-4375)	*C. albicans* (ATCC 10231)
PE	250	500	500	125	1000
MeOH	5000	2500	5000	1250	1000
DMSO	>6000	>6000	>6000	>6000	>6000

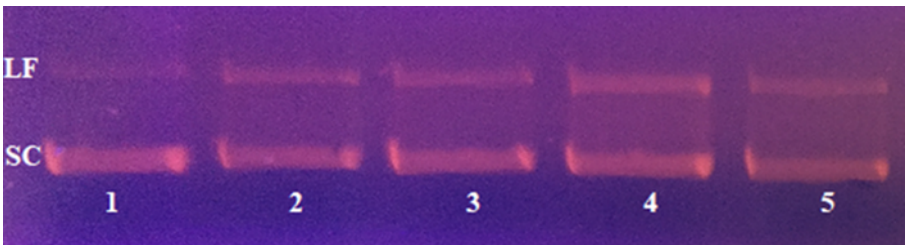


Figure 3. DNA cleavage activity of the MeOH and PE extracts of *S. aintabicum* (1. pBR322 control. 2. $MeOH_{extract}$ (3 mg/mL) + pBR322 DNA 3. $MeOH_{extract}$ (2 mg/mL) + pBR322 DNA 4. $PE_{extract}$ (3 mg/mL) + pBR322 DNA 5. $PE_{extract}$ (2 mg/mL) + pBR322 DNA) (LF: Lineer form, SC: Supercoiled form).

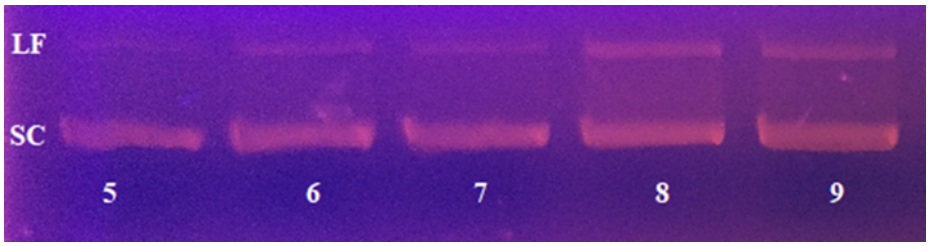


Figure 4. DNA cleavage activity of the MeOH and PE extracts of *S. aintabicum* in the presence of H_2O_2 (5. H_2O_2 + pBR322 control. 6. H_2O_2 + $MeOH_{extract}$ (3 mg/mL) + pBR322 DNA 7. H_2O_2 + $MeOH_{extract}$ (2 mg/mL) + pBR322 DNA 8. H_2O_2 + $PE_{extract}$ (3 mg/mL) + pBR322 DNA 9. H_2O_2 + $PE_{extract}$ (2 mg/mL) + pBR322 DNA) (LF: Lineer form, SC: Supercoiled form).

active biological properties used in pharmacology. The first study on the bioactive compounds of *S. aintabicum* was carried out by Zengin et al. (2021) using an HPLC-MS/MS system. The authors identified different classes of chemical compounds, including flavonoids, phenolic acids, secoiridoids, alkaloids, and xanthones. They reported that the solvent and extraction process affects the content of bioactive constituents. In this study, various bioactive compounds were obtained, since different solvents and extraction systems were used. Thus, this study reports the probability of the pharmaceutic nature of *S. aintabicum* with regard to its bioactive chemical constituents.

Although it has been known that increasing the consumption of herbal products is beneficial for health, it has not been known which specific components play a role in this beneficial effect. Moreover, many compounds found in plants attract more attention today, and the number of studies investigating their benefits and usage areas is increasing day by day (Raina et al., 2014). In this respect, here, it was also evaluated the biological features of various extracts of the *S. aintabicum* plant by using TPC, TFC test, and antioxidant activity tests based on ABTS assay. In the present study, the extraction yield values of the PE and MeOH were found to be 0.9% and 12.6%, respectively. TPC and TFC values in the extracts are shown in Table 2. The results demonstrated that the MeOH extract contained the highest amount of phenolic and flavonoid compounds values of 90.87 (±5.30) mg (GAE)/g extract and 98.00 (±9.72) mg (CE)/g extract, respectively. In the PE extract, the total amounts of phenolic and flavonoid compounds

Table 4. Percentage of cell viability of the MeOH and PE extract of *S. aintabicum* against the MCF-7 cells at varying concentrations.

Concentration (ppm)	$MeOH_{absorbans}$ (570nm)	$PE_{absorbans}$ (570nm)	$MeOH_{\% \text{ cell viability}}$	$PE_{\% \text{ cell viability}}$	MeOH IC_{50} (µg/mL)	PE IC_{50}(µg/mL)
(–) Control	1.74	1.24	100	100	443.31	414.73
1000	1.11	0.91	63.70	73.13		
500	0.83	0.53	47.77	42.79		
250	1.07	0.71	61.62	57.13		
125	1.50	0.77	86.01	62.27		
62.5	1.33	1.08	76.52	87.07		
31.25	1.73	1.16	99.31	93.22		
15.625	0.95	0.88	54.81	70.75		
7.812	1.87	1.06	107.45	85.18		
DMSO	1.61	1.56	92.38	125.90		

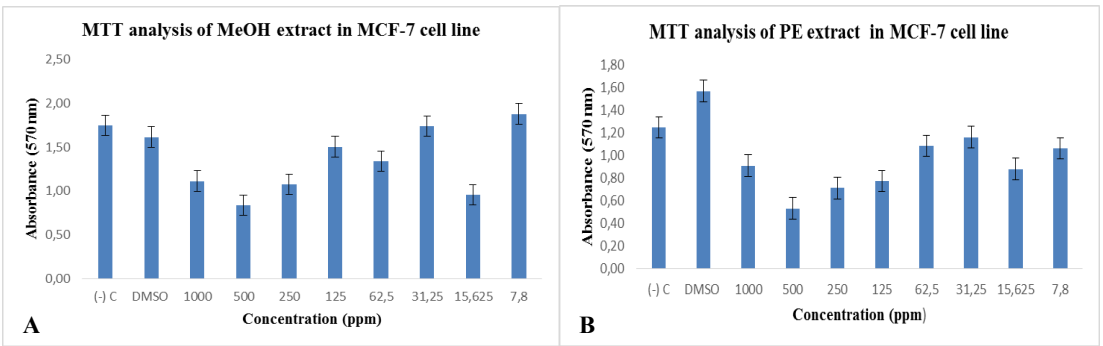


Figure 5. Effect of several concentration of *S. aintabicum* MeOH (A) and PE (B) extracts on cell proliferation in the MCF-7 cells. Cell viability was defined with the MTT assay. Error bars show standard error.

were calculated as 5.38 (±1.53) mg (GAE)/g extract and 59.64 (±7.91) mg (CE)/g extract, respectively. It was clearly seen that the total concentrations of phenolic and flavonoid compounds in the PE extract were lower than those of the MeOH extract. The antioxidant activities of extracts are due to their reduction of hydroperoxides, deactivating of free radicals, complexation of metal ions or a combination of these. It is thought that some of the antioxidant activity provided by these mechanisms is due to phenolics and flavonoids. Interestingly, in almost all biological activities analyzed in this study (except ABTS), the PE extract with relatively lower TPC and TFC was found to be more active and effective than the MeOH extract. This situation may be explained as follows, i) as known, plant secondary plant metabolites are grouped under three main groups as terpenoids, phenolics, and alkaloids. The PE extract of the plant, which constituted a material of this study, had low phenolic and flavonoid compound concentrations. However, its biological activity levels were high in this study. This suggested that the synergistic interaction of other secondary metabolites (such as terpenes, alkaloids) could have played a role in this result. Additionally, Kähkonen et al. (2001) considered that total phenolic content does not comprise all antioxidants such as ascorbic acid, carotenoid, and tocopherol. Moreover, Vinson et al. (2001) also had the idea that synergism between antioxidants in the mixture makes antioxidant activities dependent not only on the antioxidant concentration, but also on the structure and interaction between antioxidants ii) Another possible explanation for this situation was that the high biological activity of the PE extract may have been due to the quality of its compounds rather than quantity. Kaur and Kapoor (2002) thought that this event could be explained based on the high antioxidant activities of

Table 5. Statistical evaluation of the cytotoxic effect of concentrations of PE extract of *S. aintabicum*.

Concentration (ppm)	**Measurements**			**Kruskall–Wallis H test**		**Differences between groups ($p < 0.05$)**
	N	**Mean ± SD**	**Min–max**	**Chi-square (χ2) H**	***p* value**	
(-) C	3	1.24 ± 0.06	1.18–1.30			*(-)C-500ppm
DMSO	3	1.56 ± 0.35	1.17–1.87			NS
1000	3	0.91 ± 0.15	0.76–1.07			* DMSO-1000ppm
500	3	0.53 ± 0.05	0.46–0.56			* DMSO-500 ppm
250	3	0.71 ± 0.08	0.62–0.79			* DMSO-250 ppm
125	3	0.77 ± 0.12	0,69–0,91			* DMSO-125 ppm
62.5	3	1.08 ± 0.36	0.85–1.50	18.1	0.034	NS
31.25	3	1.16 ± 0.27	0.97–1.47			NS
15.625	3	0.88 ± 0.40	0.55–1.33			NS
7.8	3	1.06 ± 0.46	0.62–1.55			NS

* SD: Standard deviation; Min: Minimum value; Max: Maximum value; N: Number of observations
** $p < 0.05$ indicates that the differences between groups (concentrations) are statistically significant; $p > 0.05$ indicates that the differences between groups (concentrations) are not statistically significant.
*** NS: indicates that the differences between groups (concentrations) are statistically insignificant.

Table 6. Statistical evaluation of cytotoxic effect of concentrations of MeOH extract of *S. aintabicum*.

Concentration (ppm)	Measurements			Kruskall–Wallis H test		Differences between groups ($p < 0.05$)
	N	Mean ± SD	Min–Max	Chi-square (χ2) H	*p* value	
(-) C	3	1.74 ± 0.11	1.6–1.87			NS
DMSO	3	1.61 ± 0.07	1.55–1.69			NS
1000	3	1.11 ± 0.14	0.97–1.25			NS
500	3	0.83 ± 0.22	0.58–1.01			NS
250	3	1,07 ± 0,42	0.79–1.56			NS
125	3	1.50 ± 0.26	1.19–1.66			NS
62.5	3	1.33 ± 0.51	0.86–1.89	15.4	0.081	NS
31.25	3	1.73 ± 0.36	1.32–2.03			NS
15.625	3	0.95 ± 0.26	0.79–1.25			NS
7.8	3	1.87 ± 1.57	0.95–3.69			NS

* SD: Standard deviation; Min: Minimum value; Max: Maximum value; N: Number of observations
** $p < 0.05$ indicates that the differences between groups (concentrations) are statistically significant; $p > 0.05$ indicates that the differences between groups (concentrations) are not statistically significant.
*** NS: indicates that the differences between groups (concentrations) are statistically insignificant.

some special phenolic units, which may act as effective antioxidants rather than contributing to high total phenolic concentration. Zengin et al. (2021), calculated total phenolic and flavonoid compound concentrations of 35.50 (MAC/Water) to 112.25 mg GAE/g (MAC/MeOH) and 2.54 (MAC/Water) to 25.12 mg RE/g (HAE/MeOH) in *S. aintabicum* extracts prepared with various extraction techniques (HAE, infusion and MAC). Based on their findings, the yield of phenolics and flavonoids was affected by both the extraction techniques and the kind of solvent used. In a parallel study on the biological activities of the MeOH extracts of *S. anatolicum* Boiss. Varvouni et al. (2020) calculated the total phenolic and flavonoid amounts as 32.7 mg GAE/g extract and 13.3 mg RE/g, respectively. Sarikurkcu et al. (2019) confirmed that, among different extracts of *S. anatolicum*, a plant related to the one included

in this study, the highest amounts of TPC and TFC were, in aqueous and ethyl acetate extracts. Trifan et al. (2018) reported that various *Symphytum* species demonstrated strong antioxidant activity. Moreover, they stated that these species were plenty in phenolic compounds, and rosmarinic acid was the main phenolic compound of these species containing salvianolic acid. Accordingly, it may be stated that the differences in the studies on the total phenolic and flavonoid contents of plant extracts are due to factors such as species diversity, solvent system and extraction method.

Furthermore, in this paper, the ABTS radical scavenging measurement method was utilized to define the antioxidant activities levels of the PE and MeOH extracts of *S.aintabicum*. $ABTS^{\bullet+}$ was used in the study because both polar (MeOH) and nonpolar (PE) samples could be evaluated, and the maximum absorption used in this study was 760 nm, a wavelength not normally encountered in natural products, thus minimizing spectral interference. The results are depicted in Figure 2. As seen in Figure 2, the ABTS assay of both plant extracts increased with their increasing concentrations. The IC_{50} values of the extracts were calculated as 6.88 mg/mL (±0.11) and 0.032 mg/mL (± 0.002) for the PE and MeOH, respectively. As the IC_{50} value of the extracts decreased, their antioxidant activities increased. It is clearly seen that the IC_{50} value of the MeOH extract was lower than that of PE. Trouillas et al. (2003) reported a positive correlation between the antioxidant capacity of the plant they examined and its total phenolic compound concentration. It was emphasized that phenolic content is the basic factor that creates the antioxidant effect (Tello et al., 2008). In their study on *S. aintabicum*, Zengin et al. (2021) calculated the most important radical scavenging activity of infusion extracts in their ABTS experiments as 389.96 mg TE/g. On the other hand, they reported that the water extracts obtained with the HAE/Water method were weak ABTS radical scavengers with a value of 111.97 mg TE/g compared to the other extracts. In another study, Varvouni et al. (2020) calculated the ABTS radical scavenging activity of a MeOH extract of *S. anatolicum* as 197 ± 6 mg TE/g extract. Trifan et al. (2018) reported the ABTS activity of a 65% ethanolic extract of the root of *S. officinale* L. as 20.14 µg/mL. The ABTS activity of the root extraction of *S. officinale* with hot water (25 µg) was also determined to be 9.61 TE µg/mL (Thring et al., 2009). Neagu et al. (2010) reported the ABTS activity of EtOH and MeOH extracts of the root of *S. officinale* as 1152.01 TE µM/g and 874.81 TE µM/g, respectively. As seen the results of the present study and other studies in the literature, many *Symphytum* species show antioxidant activity, even though these studies have differed in terms of their methods, species, and solvents. It may be suggested that the MeOH extract of *S. aintabicum* can be used as a source of antioxidants.

The current study defined the antimicrobial activity and the minimum inhibitory concentration (MIC) of the MeOH and PE extracts of *S. aintabicum* using the serial dilution method. The MIC value for DMSO were >6000 µg/mL. Generally, the comparison of the MIC values demonstrated that the PE extract was more efficacious on the selected test microorganisms than the MeOH extract (Table 3). The PE extract of *S. aintabicum* was efficacious on both gram-positive (*M. luteus*; 125 µg/mL and *S. aureus*; 500 µg/mL) gram-negative (*E.coli*; 250 µg/mL and *P. aeruginosa*; 500 µg/mL) microorganisms whereas it was found to have moderate antimicrobial activity on yeast (*C. albicans*; 1000 µg/mL). It was clearly seen that the PE extract was found to have strongest antimicrobial activity on *M .luteus* and *E. coli*. However, the PE extract was observed to show intermediate antimicrobial activity on *P. aeruginosa* and *S. aureus*. The MeOH extract of *S. aintabicum* was efficacious on both gram-positive (*M. luteus*; 1250 µg/mL and *S. aureus*; 5000 µg/mL) gram-negative (*E. coli*; 5000 µg/mL and *P. aeruginosa*; 2500 µg/mL) microorganisms, whereas it was found to have strongest antimicrobial activity on yeast (*C. albicans*; 1000µg/mL). On the other hand, the MeOH extract was observed to show intermediate antimicrobial activity on *P. aeruginosa* and *M. luteus*. The MeOH extract showed low antimicrobial activity on *S. aureus* and *E. coli*. This study is the first to report antimicrobial activity in *S. aintabicum*. Gafar et al. (1989) reported the antimicrobial, antimycotic, and antiinflammatory effects of original products based on propolis and *S. officinale* extracts treating chronic marginal parodontopathy. Sumathi et al. (2011) researched the potential of various comfreys leaf extracts against different pathogenic strains. They found that the comfrey leaf extracts exhibited partial and strong obstruction against *Staphylococcus aureus*, *Bacillus subtilis*, *P. aeruginosa*, and *Salmonella typhi*. Many researchers have suggested that the antimicrobial contraption of action of comfrey leaves may be due to their phenolic content, which can behave by interfering with the permeability of the bacterial outer cell wall, binding to adhesives, or blocking DNA or RNA replication and transcription (Lee and Paik, 2016). In particular, many researches have been conducted on the antimicrobial effect of various extracts of *S. officinale* (Sumathi et al., 2016). Hence, one may suggest the PE extract of *S. aintabicum* can be used as a source of antimicrobial agents.

In recent years, DNA cleavage studies with various substances have attracted attention. In the biotechnology and pharmaceutical industries, the research and development of new nucleases that selectively break DNA will lead to the design of chemotherapeutic and antimicrobial drugs. The cleavage of plasmid pBR322

DNA was monitored by gel electrophoresis to investigate the DNA cleavage activities of the two different extracts of *S. aintabicum*. To the best of our knowledge, there is no study on the DNA cleavage activities of *S. aintabicum*, although various research studies have been carried out on this plant. The agarose gel electrophoresis is shown in Figures 3 and 4. H_2O_2 was used as a cutting activity enhancing agent. As shown in Figures 3 and 4, the PE extract opened the pBR322 DNA supercoiled structure and converted it into a linear form more than the MeOH extract in both the presence and absence of H_2O_2. The relatively fast immigration was the supercoil form (SC), and the slower moving immigration was the open circular form (LF), which was created from the supercoiled form when scission emerged on one strand. In their study on the DNA cleavage activities of aboveground and underground extracts of endemic *Heliotropium samolifolium* Bunge subsp. *erzurumicum* (Boraginaceae), Sağlam and Kandemir (2020), reported that the subsoil chloroform and aqueous extracts were more effective than the other extracts (hexane, ethyl acetate, ethanol) in the formation of the open ring form of pBR322 plasmid DNA. In a similar study with *Centranthus longiflorus* Steven subsp. *longiflorus*, above and below-ground ethanol extracts of *Centranthus longiflorus* subsp. *longiflorus* showed significant effects on pBR322 (Ayar et al., 2020). Özmen et al. (2011) researched the effects of *Ankyropetalum reuteri* Boiss. & Hausskn. extract on pBR322 plasmid DNA and showed that the superhelical form of pBR322 plasmid DNA was affected depending on the extract concentration. DNA cleavage agents that target the entirety of cellular DNA play a profound role in nonsurgical cancer therapy. It is expected that the breaking property of an anticancer agent with consistent and highly effective intracellular DNA will overcome the significant barrier in chemoresistance and radioresistance (Rebelein and Ward, 2018). It was concluded that this study could lead to future studies in terms of both being the first study of this kind and being a model study that can be used in anticancer drug design.

Remarkably, in this study, it has been demonstrated that the MeOH and PE extracts of *S. aintabicum* inhibited the proliferation of breast cancer cells. The results of the MTT test showed that both the MeOH and PE extracts had a cytotoxic influence on the MCF-7 cells at different rates in a concentration-dependent manner. As seen in Table 4 and Figure 5, the lowest % cell viability activity values in the PE and MeOH extracts were 42.79% and 47.77% at 500 ppm, respectively. There was also not much difference between the IC_{50} values of the PE (414.73 µg/mL) and MeOH (443.31 µg/mL) extracts. Statistical evaluation of the cytotoxic effect of PE and MeOH extract concentrations of *S. aintabicum* demonstrated in Tables 5 and 6, respectively. As seen in Table 5, there is a significant difference between the groups (concentration) in terms of measurement values ($p < 0.05$). While the values in the (-) C group were significantly higher than in the 500 ppm group, the values in the DMSO group were considerably higher than the 1000 ppm, 500 ppm, 250 ppm, and 125 ppm groups. There is no significant difference between the other groups measurement values ($p > 0.05$). As shown in Table 6, there is no significant difference between the measurement groups in terms of values. Nevertheless, it may be clearly seen that the PE extract displayed a higher level of cytotoxic activity than the MeOH. This may have resulted from the presence of other secondary metabolites (other than phenolic and flavonoids) with antioxidant activity, as mentioned above, and their synergistic interaction. This research is the first to report the anticancer activity of various extracts of *S. aintabicum* against breast cancer cells (MCF-7). Since there is no anticancer data for *S. aintabicum*, it was compared with other species belonging to the genus. Similarly, in studies conducted on different *Symphytum* species, the *S. asperum* Lepech., *S. officinale*, *S. caucasicum* M.Bieb., and *S. grandiflorum* DC. species were determined to contain caffeic acid-derived POCDPE has high levels of immunomodulatory, antioxidant, antiinflammatories, and anticancer activities (Merlani et al., 2018). Shrotriya et al. (2012) assessed the efficacy of a novel phytochemical phenolic polymer, namely p-DGA isolated from the roots of *S. caucasicum*, and its derivative form m-DGA against human prostate cancer cells. They found that p-DGA and m-DGA suppressed growth and induced death in prostate cancer cells with relatively less cytotoxicity against nonneoplastic hepG. In the literature review, it was seen that studies have focused on *S. officinale*. In vitro experiments performed by various researchers have shown that the leaves of *S. officinale* are effective against bone fractures, pneumonia, leukaemia, oral cancer, and skin cancer (Melo, 2011; Bhat, 2014). Albuquerque et al. (2007), in their study on medicinal and so-called miracle plants from a public market in Northeast Brazil, reported that the tea of the *S. officinale* plant sold by four different herbalists was used for asthma, ulcers, prostate problems, diabetes, leukemia, hepatitis, liver problems, gastritis, and inflammation in general. Additionally, this plant has medicinal effects, including proven wound healing effects, antiirritant activities, and moisturizing efficacy due to the presence of allantoin and mucilage. Nastić et al. (2018) reported that *S. officinale* showed the most cytotoxic effect in RD (12.76 ± 0.89), L2OB (19.39 ± 0.39), and Hep2c (29.88 ± 0.49) cancer line cells, respectively. Researchers have also stated that the cytotoxic activity of extracts may depend on the presence of different phytochemical compounds such as saponins, triterpenes, sterols, polyphenolics and may be closely related to the extraction technique that is used. The content of phytoconstituents in plant extracts is assigned

by various factors, including the properties of the applied solvent, extraction kinetics, and mass transfer. Briefly, in this research, the cytotoxic activity of the extracts may be partially imputed to their high phenolic contents, whereas secondary metabolites and synergistic efficacy presumably also contributed to the high cytotoxic activity of these extracts.

4. Conclusion

Consequently, traditional medicinal plants have a significant role in the treatment of some diseases in Turkey. In the present study, to the best of our knowledge, analysis of the DNA cleavage effects, antimicrobial activities and anticancer activites (MCF-7 cell line) of this plant was performed for the first time. The results clearly revealed that the PE extract exhibited strong antimicrobial, DNA cleavage, and anticancer (MCF-7 cells) activities. In contrast, the MeOH extract showed strong antioxidant activity due to its rich phenolic and flavonoid contents. These activities could be imputed to the synergistic interactions of secondary metabolites such as terpenes, alkaloids, phenolics, and flavonoids contained in the plant with each other and to quality rather than quantity. Moreover, the presence of various compounds which were identified by the GC-MS method in this research may have played a vital role in the biological activities of these extracts. In conclusion, these findings showed that *S. aintabicum* extracts can be used effectively in the medicine and pharmaceutical sectors. For future studies, it is recommended to isolate and purify the active ingredients of these extracts.

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Conflict of interest

No potential conflict of interest was reported by the author.

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