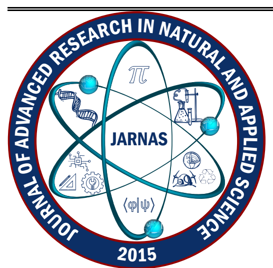

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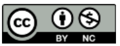

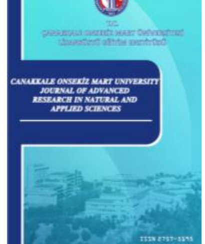

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Anticancer, Antioxidant, Antimicrobial Activities, and HPLC Analysis of Alcoholic Extracts of *Parthenocissus quinquefolia* L. Plant Collected from Çanakkale

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Abstract − This study aimed to contribute to revealing the health effects of the *Parthenocissus quinquefolia* L. (PQ) plant by examining the biological activities of various extracts of the plant. An ethnobotanical important medicinal plant, PQ belongs to the Vine family (Vitaceae) and is known for its medicinal uses. Herein, fruit and leaf alcoholic extracts of the plant exhibited anticancer activity on triple negative breast cancer (TNBC) cell lines, estrogen receptor positive (ER+) (MCF-7), and human colon adenocarcinoma (HT-29) cancer cell lines. Furthermore, fruit methanol extracts displayed strong radical scavenging activity with low IC50 values such as 0.51 mg/mL, 0.80 mg/mL, 0.84 mg/mL, and 1.45 mg/mL. The highest TEAC values (18.72 mM Trolox/mg, 9.90 mM Trolox/mg, and 8.41 mM Trolox/mg) were calculated for the extracts of red leaves. The antifungal activity was determined against *Candida albicans* as fungal pathogen. The cell apoptosis with methanol extract was obtained between 20 μg/mL and 70 μg/mL for 48 h and 72 h treatments by Hoechst 33258 staining of nuclei under a fluorescence microscopy. HPLC analysis was performed for methanol extracts to determine major bioactive components. Consequently, this plant extracts may guide to new strategies and may be uses in medicinal applications for further investigations.

Keywords − *Anticancer, Antimicrobial, Antioxidant, HPLC, Parthenocissus quinquefolia* L.

1. Introduction

Plants are widely used worldwide in folk medicine and medicine for cancer therapy and other diseases [1]. Due to the significance of plants for human health, research on their bioactive properties has increased [2]. Compared to using traditional drugs to reduce side effects caused by chemotherapy, there are many advantages to the usage of plant-based medicines [3]. Natural antioxidants and many phytochemicals have been recently proposed against cancer therapy due to their importance in signaling pathways [4]. Plant bioactive components have a significant role in inhibiting cancer cells because of their structural diversity [5]. The harmful effects of these free radicals on cellular systems cause many diseases, such as cancer [6].

Bioactive components from the plant extracts act as antioxidants, and hereby, free radicals can be neutralized.

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Phenolic compounds contain biologically active secondary metabolites with strong antioxidant and antiradical properties [7,8]. Plant-derived antioxidants have become an important research topic for the last two decades [9,10]. Oxidative stress (OS) has a key role in the stimulation of many diseases, such as immunosuppressive, diabetes mellitus, cancer, and neurodegenerative diseases. Thus, antioxidants have an important role in destroying free radicals that cause OS [11]. Phenolics play an important role against microbial activities and infections [12]. Phenolic compounds such as quercetin and ellagic acid are recognized to be beneficial to human health, reducing the risk of degenerative diseases by reducing OS [13].

Parthenocissus quinquefolia L. (PQ) belongs to the Vitaceae family and is known for its many pharmacological activities, including cancer chemoprotective effect, antimicrobial, antiviral, anti-rheumatism, arthritis, gastrointestinal, antidiabetic, diuretic, anti-inflammatory, and anti-cholesterol activities [14-16]. It was indicated that PQ was an ethnobotanical important medicinal plant with broad-spectrum biological activities, including antifungal, antibacterial, and antioxidant [14,17]. The plant's bark has been employed in traditional medicine as an expectorant and tonic. PQ roots have been used in infusions [15]. Pharmacologically active plants are a rich source of compounds with special therapeutic potentials and are still of great importance for identifying new drug leads [18].

Herein, this study focused on the biological activity studies, including anticancer, antioxidant, and antimicrobial, with various extracts of the PQ plant collected from Çanakkale/Türkiye. The morphological differences in cancer cells were determined under fluorescent microscopy. Determination of the bioactive content of methanol extracts of fruit and leaves of the plant was carried out by HPLC analysis. The flowchart is given in Figure 1.

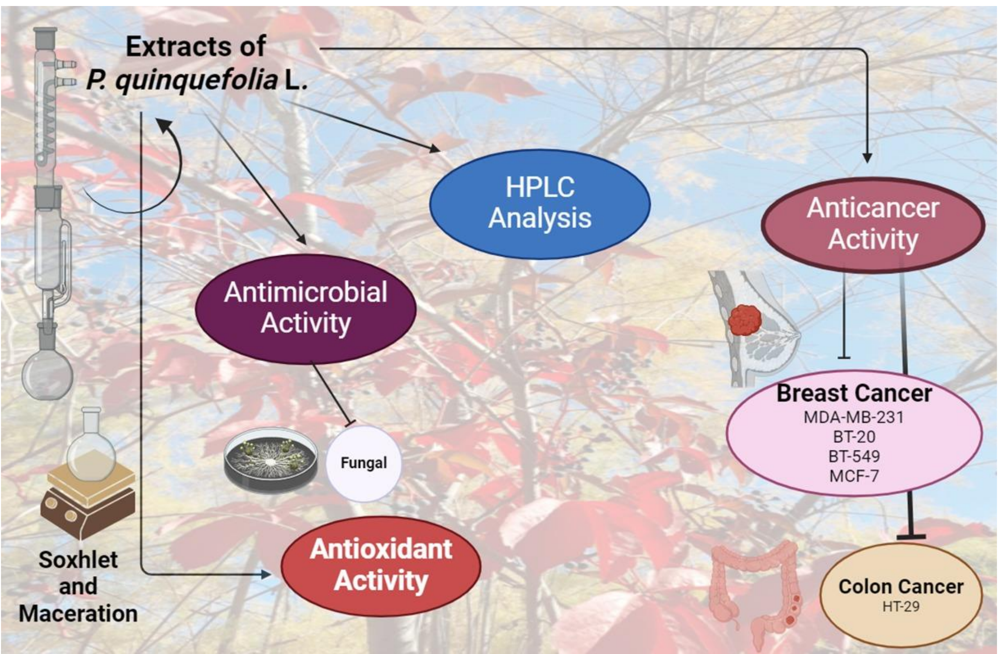


Figure 1. A representative flowchart

2. Materials and Methods

Ethanol (EtOH), *n*-hexane, ethyl acetate (EtOAc), methanol (MeOH), and dichloromethane (DCM), DPPH (2,2-diphenyl-1-picrylhydrazil), trolox, F-C reagent, and ABTS were obtained from Sigma-Merck.

2.1. Plant Preparation

This plant was collected from Çanakkale Onsekiz Mart University Terzioglu Campus-Türkiye. The plant was identified and kept in Çanakkale Botanik Bahçesi Herbaryumu (CBB 003110-Herb. Hort. Bot. Canakkalensis). The fruits and leaves were dried without direct sunlight in the air, and then ground. The photos of *PQ* were shown in Figure 2. The extracts were prepared by using Soxhlet method with *n*-hexane, DCM, EtOAc, MeOH, and EtOH:H_2O (75:25), respectively, according to our previously reported study [19]. The other extract was prepared by directly using MeOH and then ethanolic solution. Maceration was performed at room temperature by using MeOH [20]. The solvent was removed by using rotary evaporator (IKA RV10). The plant extracts were kept in the refrigerator at +4°C. The obtained extracts were given in Table 1.

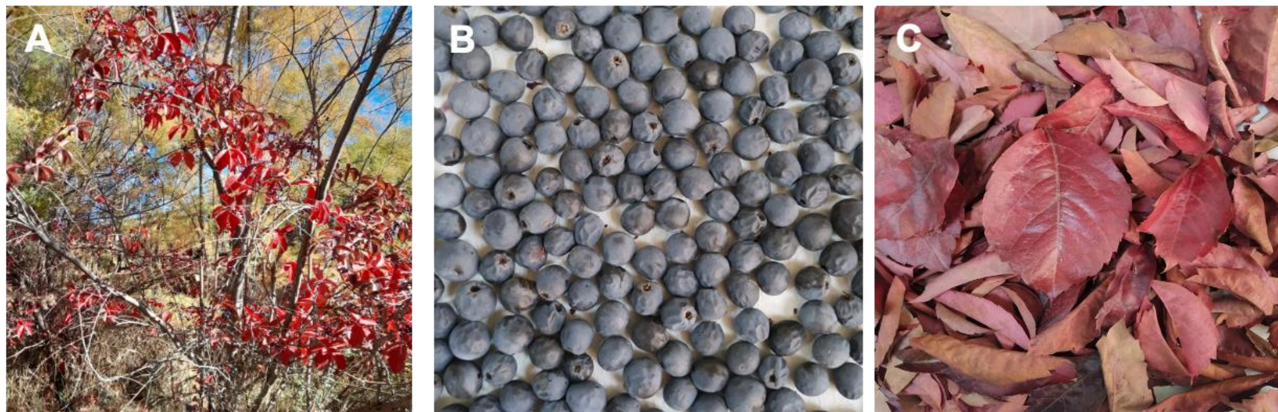


Figure 2. Photos of *PQ* were taken by the authors. A) collected from Çanakkale, B) fruits, C) red leaves

Table 1. The prepared extracts (**1-7**)

Extracts	Codes
MeOH extract of red leaves (gradually)	**1**
MeOH extract of fruits (gradually)	**2**
Aqueous ethanol extract after directly methanol of fruits	**3**
MeOH extract of fruits (maceration)	**4**
MeOH extract of fruits (directly)	**5**
MeOH extract of red leaves (directly)	**6**
Aqueous ethanol extract after directly methanol of red leaves	**7**

2.2. Anticancer Activity

2.2.1. Cell Culture

Human triple negative breast cancer cell lines (TNBC) (MDA-MB-231, BT-20, BT-549), and ER+ (MCF-7), and human colon cancer (HT-29) cell lines were gift. Dulbecco's containing 2 mM L-Glutamine Modified Eagle Medium (DMEM) was used for the cultivation. 1% Penicillin/streptomycin (PS) and 10% fetal bovine serum (FBS) were added to culture media and the cells were kept in a 37°C incubator containing 5% CO_2 incubator [21,22]. For anticancer activity, stock solutions of the extracts were prepared at 10 mg/mL.

2.2.2. Clonogenic Assay and Cell Cytotoxicity

Colony inhibition on breast and colon cancer cells were carried out. The effect of the extracts at various concentrations was examined for 8-12 days. Crystal violet was used for staining. The cytotoxicity was

determined by MTT (Thiazole Blue) assay for 48 h with time- and dose-dependent manner studies. The absorbances were measured by a plate reader (Thermo) at 570 nm. The cell viability (%) was calculated and compared to dimethyl sulfoxide (DMSO). Statistically significant results were indicated as asterisk [21-23].

2.2.3. Wound Healing Assay

The cell migration analysis was performed in 6-well plates. The cells were scratched in the middle with the help of a pipette tip and washed with phosphate buffer saline (PBS). The extracts (**1** and **2**) were applied to cancer cells. Then, microscope images were taken for different time points and the Image J program was used [21].

2.2.4. Fluorescent microscopy

The most active extract in cell-based assays was applied to cancer cells for 48 h and 72 h treatments. Paraformaldehyde (4%) was used to fix the cells and Hoechst 33258 dye was used for staining of nuclei. The morphological changes were examined under a fluorescence microscope (Zeiss AXIO Scope A1) [21,24].

2.3. Antioxidant Activity

The antioxidant capacities were investigated with DPPH and trolox equivalent antioxidant capacities (TEAC) assays according to a reported study. 10 mg/mL stock solutions were prepared for each extract and then, intermediate solutions were prepared freshly and used. All experiments were repeated three times [20].

2.3.1. DPPH Assay

To determine antioxidant capacity, stock solutions prepared in MeOH were used at 10 mg/mL concentration. IC_{50} values were calculated by the help of measured absorbances at 517 nm [20,25].

2.3.2. TEAC Assay

The TEAC method was performed by the form of ABTS radical cation as a result of the oxidation of ABTS reagent with persulfate. TEAC values obtained at various concentrations were performed by using the Trolox standard curve [20].

2.3.3. Analysis of The Total Phenolic Amount

F-C method was used according to the previously reported study. The absorbances measured at 725 nm spectrophotometrically were used to calculate the total phenolic amount. The results were given as mg gallic acid equivalent [20].

2.4. Antimicrobial Activity

Disc diffusion method was used to examine the effect of plant extracts against various strains including *Staphylococcus aureus*-ATCC29213, *Bacillus subtilis*-ATCC6633), *Bacillus cereus*-ATCC10876, *Escherichia coli*-ATCC25952, *Pseudomonas aeruginosa*-ATCC27853. To grow bacterial strain cultures in the agar disc diffusion method, 30 g of tryptic soy broth (TSB) medium solution was prepared in 1 L water and autoclaved. To grow, 40 g of tryptic soy agar (TSA) medium solution was prepared in 1 L water and autoclaved. TSA medium is then transferred to petri dishes as 20 mL. One bead of the bacterial strain removed

from -80°C was transferred to 15 mL of TSB medium solution and incubated overnight in a shaking water bath (37 °C). Solid agar petri dish 50 µL of the bacterial suspension was added to the surface and seeded with a sterile cell spreader. In these aseptic conditions, 6 mm diameter sterile discs were placed in the middle of the agar plates and mass was placed on these discs known plant extracts were placed. Petri dishes were incubated at 37°C overnight. Then, the inhibition zone diameters around the samples with a caliper were measured to determine the antimicrobial activity [26].

2.5. HPLC Analysis

To identify the major phenolic components, MeOH extracts of fruit (**2**) and red leaf (**1**) of *PQ* were used. HPLC analysis was carried out by using ACE Generix5 C18 (250x4.6 mm) 5 um column 30°C at Igdir University Research Laboratory Practice and Research Center. The instrument conditions were given as follows: injected volume (10 µL), flow (0.8 mL/min). The DAD detector was set at wavelengths 300/200 nm and 300/4 nm. The solvent system was 83% (0.1 phosphoric acid in water) 17% (100% ACN). Standards such as catechin, isoquercitrin, baicalin, oleuropein, hesperidin, chlorogenic acid, naringin, resveratrol, quercetin, chrysin, 6-hydroxy flavone, curcumin, gallic acid, tannic acid, caffeic acid, rutin, *t*-ferulic acid, resveratrol, apigenin were used in the methods.

2.6. Statistical Analysis

Statistical analysis was performed by using GraphPad Prism.

3. Results and Discussion

3.1. Effects of Plant Extracts on Cancer Cell Cytotoxicity

MTT assay is based on the reduction of MTT tetrazolium compound and has been carried out in this study. The cytotoxic effects of these extracts on short-term cell proliferation at increasing concentrations from 40 to 200 µg/mL for 48 h treatments were determined on TNBC, ER+ MCF-7, and HT-29 cells. The cytotoxicity of mature red leaves and fruit extracts of *PQ* contributed to reduce the inhibition of cell viability in the studied cells. We detected the antiproliferative effects of MeOH and ethanolic extracts for fruits and leaves obtained from PQ. According to our findings, the cytotoxic effect of extracts (**2** and **6)** in MDA-MB-231 cells at µg/mL concentrations were obtained at higher for 48 h time-dependent. MDA-MB-231-cells were treated with Doxorubicin (Dox) (0.05 µM) for 48 h in combination therapy with the extracts, the cell viability decreased at 40 µg/mL with the combination of the extracts (Figure 3a).

As seen in Figure 3d, cell viability in BT-549 cells was dramatically decreased by all extracts at 160 µg/mL concentration, respectively, for 48 h as a time-dependent. According to the cancer cell cytotoxicity, we obtained the cell inhibition in BT-20 cells for 48 h, the most effective extracts were determined, and all extracts displayed high cytotoxicity at 80 µg/mL (Figure 3f). In the light of these findings, the most significant antiproliferative effect of *PQ* on ER+ MCF-7 cells was observed at approximately 80 µg/mL of extract **4** (Figure 3g). According to the obtained cell cytotoxicity data in HT-29 cells for 48 h, the extracts (**3** and **7)** had higher cytotoxic effects (Figure 4a,4b). Based on our above-mentioned results, plant extracts showed higher cytotoxicity in five cancer cell lines for 48 h. As far as is known, it has been reported for the first time that *PQ* plant extracts have strong cytotoxic effects against studied cancer cell lines.

According to the literature findings, Summiya et al. [10] emphasized the necessary of using *PQ* in cancer research study. The extracts of four different plant species including *PQ* were used for the cytotoxicity of different cancer cells, cell cycle arrest, and apoptosis studies [27]. In addition, anticancer activity of aqueous ethanol extract of *PQ* leaves was reported for KB cell lines in A431 and subG1 [27]. In another study involving the *P. tricuspidata* plant, cytotoxicity, antioxidant and antimicrobial activity were examined. Cytotoxicity analyses were performed on MDA-MB-361 and MDA-MB-453 cell lines [28]. Based on our findings, antiproliferative effects of *PQ* fruit and leaf extracts were observed in MeOH extracts for all breast cancer cell lines for 48 h treatments. Although the results exhibited MeOH extracts were found to be significant due to their cytotoxicity, the effect on the inhibition of colonies with ethanolic extract was determined more significant in long-term inhibition.

In a recent study, combination studies of natural bioactive compounds and doxorubicin (Dox) in various types of cancer were highlighted [29]. The combination therapy studies of Dox with curcumin [30], apigenin [31], naringenin [32] and quercetin in breast cancer cells have been reported. Furthermore, the prepared hydrogel using the *PQ* plant extract as a crosslinker reported as a potential drug-carrier in combination with Dox for cancer therapy studies [21].

3.2. Inhibition Effect of Plant Extracts on Colony Formation

To determine the effects of fruit and leaf extracts on the colony formation, a dose-dependent manner study was performed in cancer cells. Our results indicated that all extracts (**1-7**) decreased the number of colonies in TNBC MDA-MB-231 cell line at 45 μg/mL compared to control cells (Figure 3b). To understand the role of reducing the colonies, the treatments at various volumes were performed with the extracts (**2-7**) in BT-549 cells. According to this, the results showed that all extracts inhibited the colony formation at 40 μg/mL (Figure 3c). The extracts significantly inhibited the colony formation in BT-20 cells at 45 μg/mL. Moreover, it has been determined that extract **4** obtained by maceration has a highly strong inhibitor effect on the colony formation (Figure 3e).

The inhibitor effect of extracts (**1-6**) on ER+ MCF-7 cells was determined to be similar as BT-20 cells (Figure 3g). According to colony formation inhibition study in HT-29 colon cells, although other extracts exhibited inhibitor potential at 50 μg/mL, the extracts (**1-4**) showed the colony inhibition at 45 μg/mL (Figure 3a). Overall, all extracts were determined with their inhibition effect on breast and colon cancer cell lines. As far as is known, we report for the first time these results may exhibit that *PQ* includes high cytotoxic compounds.

3.3. Effect of the Extracts on Cell Migration

It was used a scratch test to measure the spread of cancer cells [21]. For this purpose, highly effective MeOH extracts (**1** and **2**) were applied to HT-29 cells. Blocked the migration of HT-29 cells with extract **1** at 160 μg/mL (24 h) and 200 μg/mL (48 h) and extract **2** at 80 μg/mL (24 h) and 160 μg/mL (48 h). These results may indicate that the extracts of *PQ* can block aggressive cells for metastasis (Figure 4c).

3.4. Effect of the Extracts on Morphological Changes

Herein, the fluorescence microscopy was used for fruit MeOH extract **2** of *PQ* for the first time. Nuclei (blue) was stained with Hoechst to observe the effects of extract **2** on the morphologic changes in breast cancer cells. MDA-MB-231 and MCF-7 cells were treated with extract **2** in the increasing volumes of 20-80 μg/mL for 48 h and 72 h and observed under a fluorescence microscopy. It was also determined that extract **2** contributed to

morphological changes on cancer cells as dose- and time-dependent. Extract **2** induced apoptosis in both cancer cells, and the apoptotic cells increased by the increasing doses of extract **2**. It was the most active extract that caused the morphological changes in cancer cells (Figure 5). As is indicated in the literature, it was observed that the condensed chromatin, marginalized nuclei, and apoptotic features such as fragmentation in doxorubicin- and extract-treated cells by a fluorescence microscopy [33].

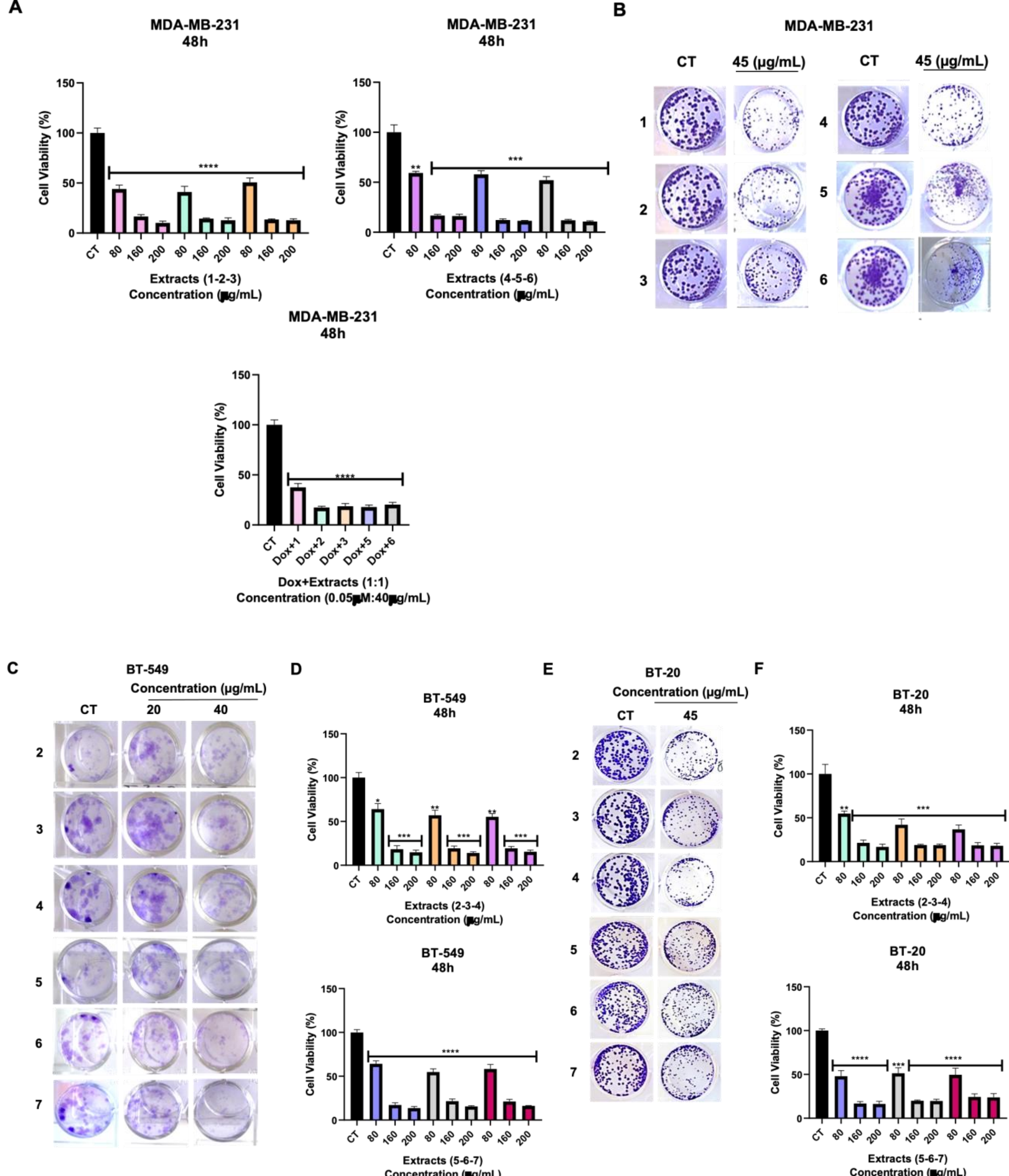


Figure 3. Cell cytotoxicity and colony inhibition assay in breast cancer cells. A,D,F,G) The extracts decreased the cell cytotoxicity with MTT assay in time- and dose-dependent manner. B,C,E,G) The colonies were dramatically inhibited by the extracts (**1-7**) (8-12 days)

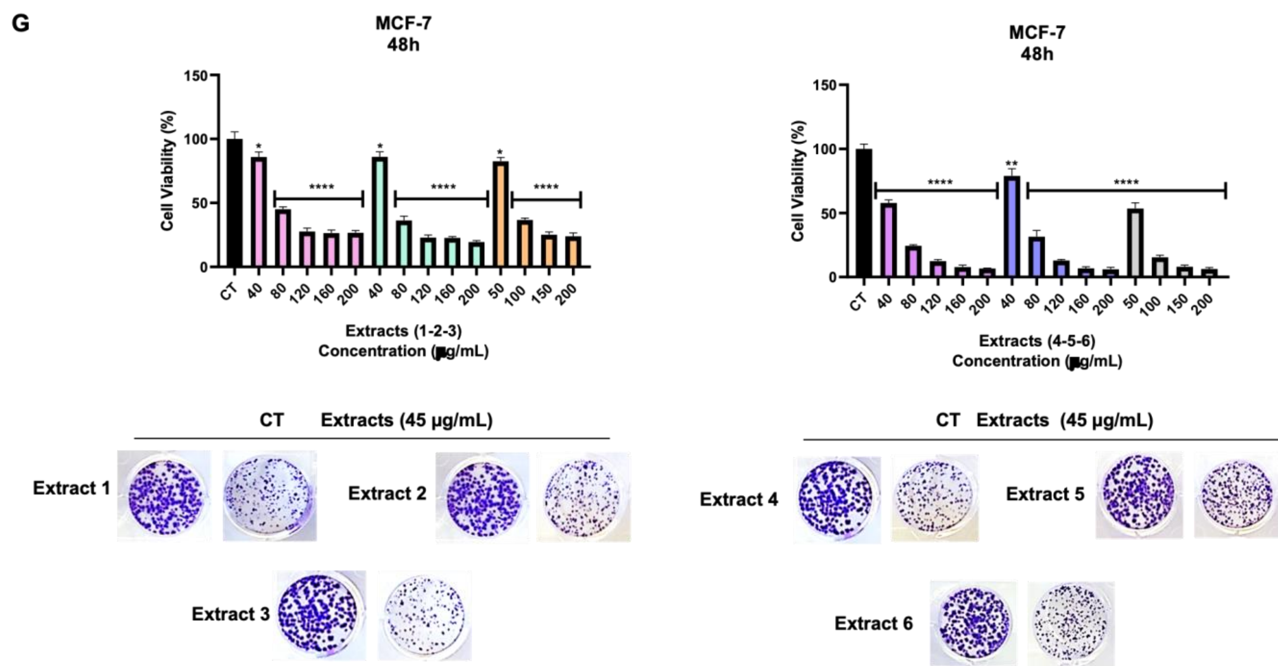


Figure 3. (Continued)

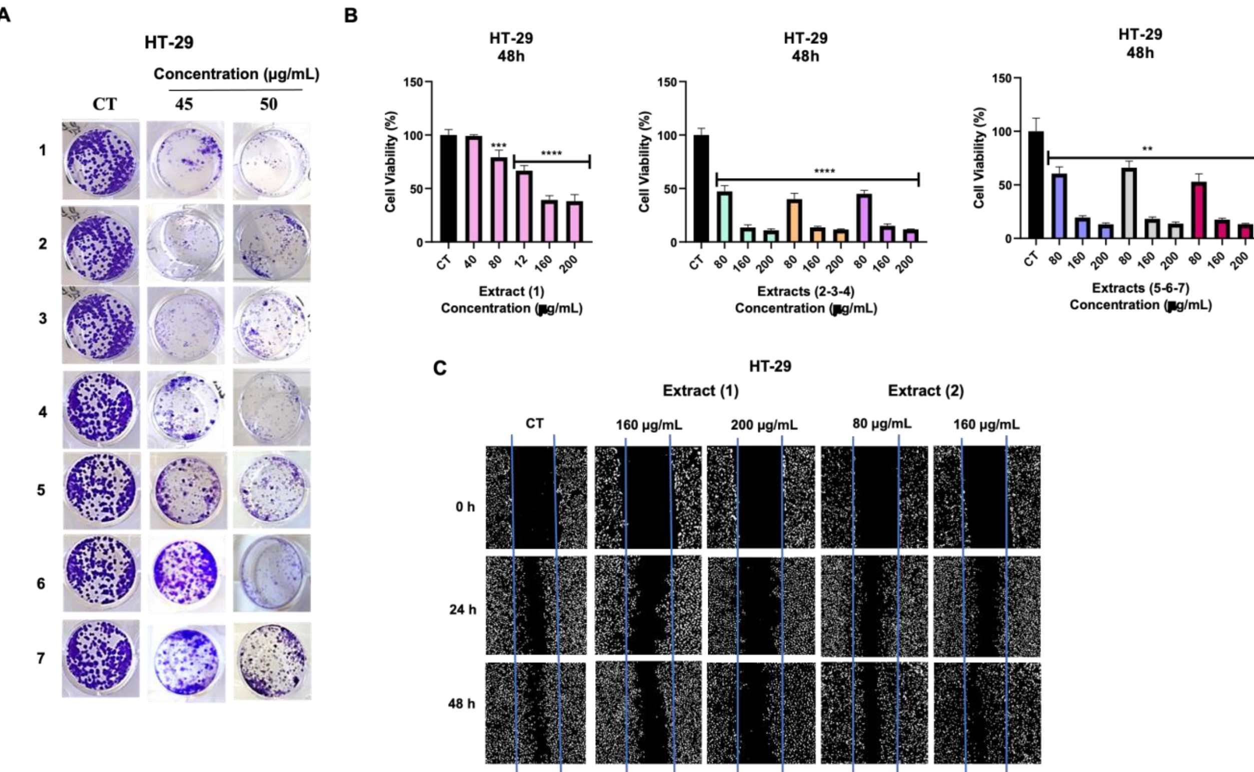


Figure 4. Cell cytotoxicity and colony formation inhibition assay in HT-29 colon cancer cells. A) The colonies were dramatically inhibited by the increasing doses of the extracts (9-12 days). B) The extracts decreased the cell cytotoxicity with MTT assay following the 48 h treatments. C) Wound healing assay in HT-29 cells. HT-29 cells were blocked by the extracts (**1** and **2**)

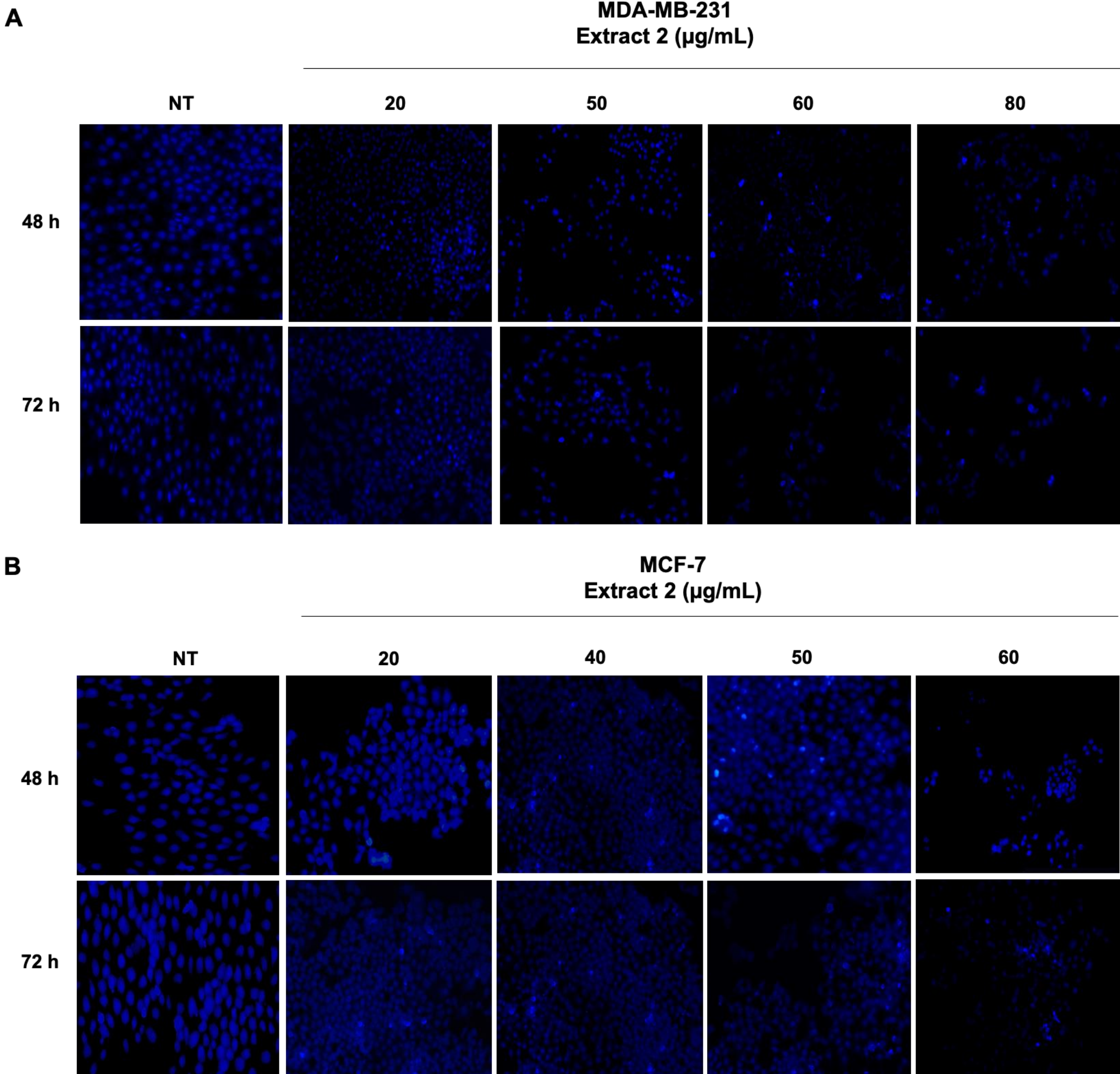


Figure 5. Morphological changes following the treatment with the extract 2 were detected by Hoechst staining under a fluorescence microscopy

3.5. Total Phenolic Content and Antioxidant Activity

In terms of phenolic component amounts, the extracts (**1** and **2**) were evaluated with their high phenolic amounts. However, extract **5** was determined with its low phenolic content. In this study, red leaf extracts displayed high total phenolic content. The gallic acid values in 100 µL samples of the extracts were calculated and the total phenolic component amounts were determined in 10 mg/mL extract. As a result, the extracts were determined as equivalent to gallic acid per mg extract. It is known that aqueous extracts contain glycoside linked structures. It was reported that the extracts from fruits and leaves of *PQ* plant included alkaloids, terpenoids, flavonoids, saponins, tannins, and glycoside metabolites [14,16]. The findings are given in Table 2. In DPPH analysis, the 50% inhibition values were calculated as the IC_{50} value. Accordingly, the results show that the fruit MeOH (extract **3**) and fruit MeOH maceration (extract **4**) showed higher scavenging activity with the DPPH radical, respectively. However, the extracts (**1, 6**, **7**) obtained from red leaves did not exhibit strong radical scavenging activity with DPPH analysis (Table 2).

Table 2. IC_{50} inhibition and TEAC values and total phenolic amount of extracts

Extracts	DPPH IC_{50} values (mg/mL)	TEAC (mM Trolox/mg)	Total phenolic content (mg GA/10 mg extract)
1	11.82±0.32	9.90±0.21	9.40±0.49
2	0.80±0.01	0.86±0.03	9.54±0.45
3	0.51±0.11	1.33±0.03	3.17±0.11
4	0.84±0.03	0.88±0.03	1.43±0.11
5	1.45±0.06	0.46±0.02	0.57±0.08
6	12.60±0.45	8.41±0.33	2.83±0.18
7	10.22±0.36	18.72±0.31	1.52±0.33

According to the TEAC method, the highest activity was obtained from extracts (**7** and **1**) with 18.72±0.31 mM Trolox/mg and 9.90 ±0.21 mM Trolox/mg values, respectively. TEAC values were found to be quite low in fruit extracts. These results indicated that extracts from red leaves have more activity in TEAC analysis (Table 2). In the reported studies, the stems and leaves of *PQ* of DPPH scavenging activity were detected for aqueous (322.34±0.01) and ethanol extracts (1398.85±0.01) [34]. The DPPH assay was reported with IC_{50} value of 13.6±0.34 µg/mL, 18.24±1.43 µg/mL, 28.84±2.20 µg/mL, and 38.28±2.79 µg/mL for aqueous, chloroform, ethanol, and *n*-hexane stem extracts, respectively, and the IC_{50} value of 27.08±3.41 µg/mL, 19.67±0.70 µg/mL, 24.32±1.02 µg/mL,14.25±0.65 µg/mL aqueous, chloroform, ethanol, and n-hexane bark extract, respectively [10]. Finally, chloroform extracts of fruits and leaves of *PQ* from Pakistan have been reported with its best antiradical activity [16].

The vine family is known for its many pharmacological activities such as cancer chemoprotective effect, antimicrobial, antiviral, anti-rheumatism, arthritis, gastrointestinal system problems, antidiabetic, diuretic, anti-inflammatory, anti-cholesterol activity. Phytochemical screening was carried out by extracting the plant collected from Iraq and Pakistan with different solvents [35,36]. A study was conducted to evaluate the antioxidant capacities of *PQ* leaves and fruits collected from Pakistan. The extracts were obtained from the fruits and leaves of the plant with the help of various solvents. Its antioxidant activity was examined by phytochemical analysis and DPPH, FRAP, TFC methods. Phytochemical screening of various extracts from *PQ* revealed the presence of its secondary metabolites (alkaloids, flavonoids, terpenoids etc.). As a result, the antioxidant potential of chloroform and ethanolic extracts was found to be higher. Thus, it has been stated that the traditional use of *PQ* leaves and fruits may be possible for the treatment of human diseases [13,14]. DPPH and FRAP antioxidant activity results of various extracts of *PQ* bark and roots collected from Pakistan were compared with standard antioxidants and plant extracts were found to have better antioxidant potential. For this reason, it has been stated that it can be used in the treatment of cancer and aging in the future [35]. DPPH, FRAP and total phenolics were determined in aqueous and ethanol extracts of the roots and leaves of the plant collected from Thailand [34].

Based on our results, the radical scavenging activities and TEAC values for the active extracts indicated that the extracts from fruits and red leaves could have promising bioactive components. The phenolic components are generally extracted with solvents in the changing polarities such as ethyl acetate, alcohol, and alcohol-water mixtures [37]. Extraction with polar solvents is resulted in the extracts containing largely phenolic components [38,39]. In order to obtain major antioxidants from various parts of plants such as leaves the effect of different solvent types such as hexane, MeOH, and EtOH was analyzed. It was found that MeOH extract

was more effective than EtOH extract in the large amount of phenolic content obtained from walnut fruits [40,41].

Analysis on the antioxidant potential is used as an indicator of phenolic content [42]. Among them, DPPH is widely used and the oldest known method. Antioxidant capacity of plant extracts can be determined with TEAC. In addition, the antioxidant activity values may also depend on various factors such as solvent and method for extraction [43]. Besides, free radicals and reactive oxygen species (ROS) have a significant impact on biological systems. OS has a role in the varying types of diseases such as cancer, Alzheimer's disease, and diabetes. Natural compounds as antioxidants from plants protect biological systems and provide human health [44]. Thus, we have thought that these active plant extracts may contribute to reducing the ROS in the biological system.

3.6. Antimicrobial Activity

To examine the inhibitory effect of plant extracts on some microorganisms that cause various infectious diseases, antimicrobial activity was determined by using the minimum inhibitory concentration measurement and disk diffusion method [45]. *S. aureus*, *B. cereus*, *E. coli*, *P. aeruginosa*, *E. faecalis*, *B. subtilis*, and *C. albicans* are microorganism strains used in the study. Bacterial growth was controlled with an empty disc (C) placed on petri dishes. It is known that many medicinal plants are traditionally used to treat diseases caused by microorganisms [46,47]. Antibacterial effects of various extracts of *PQ* plant on gram positive (*S. aureus, B. cereus, E. faecalis, B. subtilis*) and gram negative (*E. coli, P. aeruginosa*) bacterial and fungal pathogen (*C. albicans*) were evaluated and the results of the zone diameters are given in Table 3. Also, the results of a few of them (*B. cereus, E. coli and C. albicans*) are given in Figure 7. All plant extracts showed very promising results against all tested microorganisms. The antibacterial properties found in the extracts of this *PQ* may possibly be attributed to some phenolic compounds. It was determined that the effects of the plant, especially the extract **4**, on various test bacteria were more effective than the other plant solutions. The main reason for the different results obtained may be due to phytochemicals (Table 3) such as flavonoids, vitamins, and sterols in the plant structure [48]. Overall, these studies show that *PQ* extracts have potential antimicrobial activity against and fungi and may serve as potential sources for the future use of these extracts as new bactericidal agents in the treatment and prevention of infections. In the literature, MeOH extract of *PQ* fruits showed antifungal effects on *A. solani* pathogen [14].

Table 3. Antimicrobial activity of the extracts of *PQ* by disc diffusion method (Zone of inhibition in mm at 300 µg/disc)

Extracts	*E. faecalis*	*S. aureus*	*B. cereus*	*B. subtilis*	*E.coli*	*P. aeruginosa*	*C. albicans*
1	8	7	8	8	8	8	8
2	7	7	9	8	7	8	7
3	8	9	9	7	NI	9	7
4	11	10	10	9	8	10	9
5	NI	8	9	NI	NI	9	7
6	8	9	9	7	NI	10	7
7	7	9	8	7	NI	9	8

NI: No inhibition zone observed.

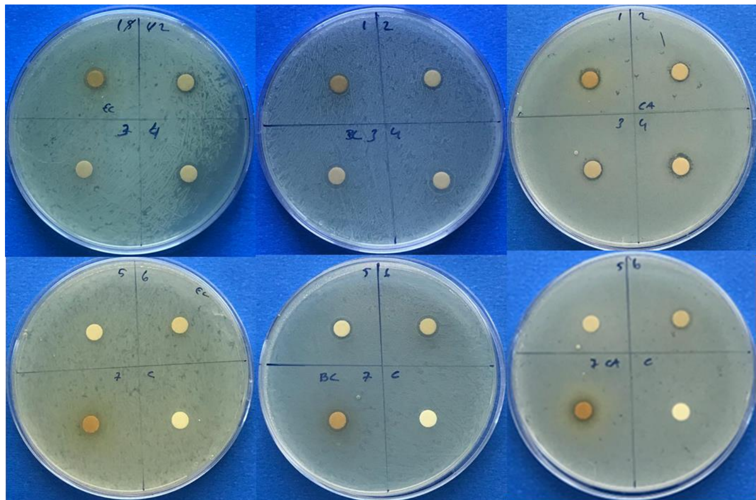


Figure 6. E. coli, A (1,2,3,4) and B (5,6,7,C); B. cereus, C (1,2,3,4) and D (5,6,7,C); C. albicans, E (1,2,3,4) and F (5,6,7,C) (extracts (1); (2); (3); (4); (5); (6); (7); control disk **(C)**)

In addition, it is reported that *PQ* ethanolic extract of leaves has inhibitory effects against *A. hydrophila* (59.68%) and *A. caviae* (55.90%) which cause gastroenteritis disease [34]. In the reported antimicrobial activity study, gram-positive and -negative bacteria and fungi *S. aureus*, *S. epidermidis*, *M. luteus*, *E. faecalis*, *B. subtilis*, *B. cereus*, *E. coli*, *P. aeruginosa*, *K. pneumoniae* and *C. albicans* strains were studied [28].

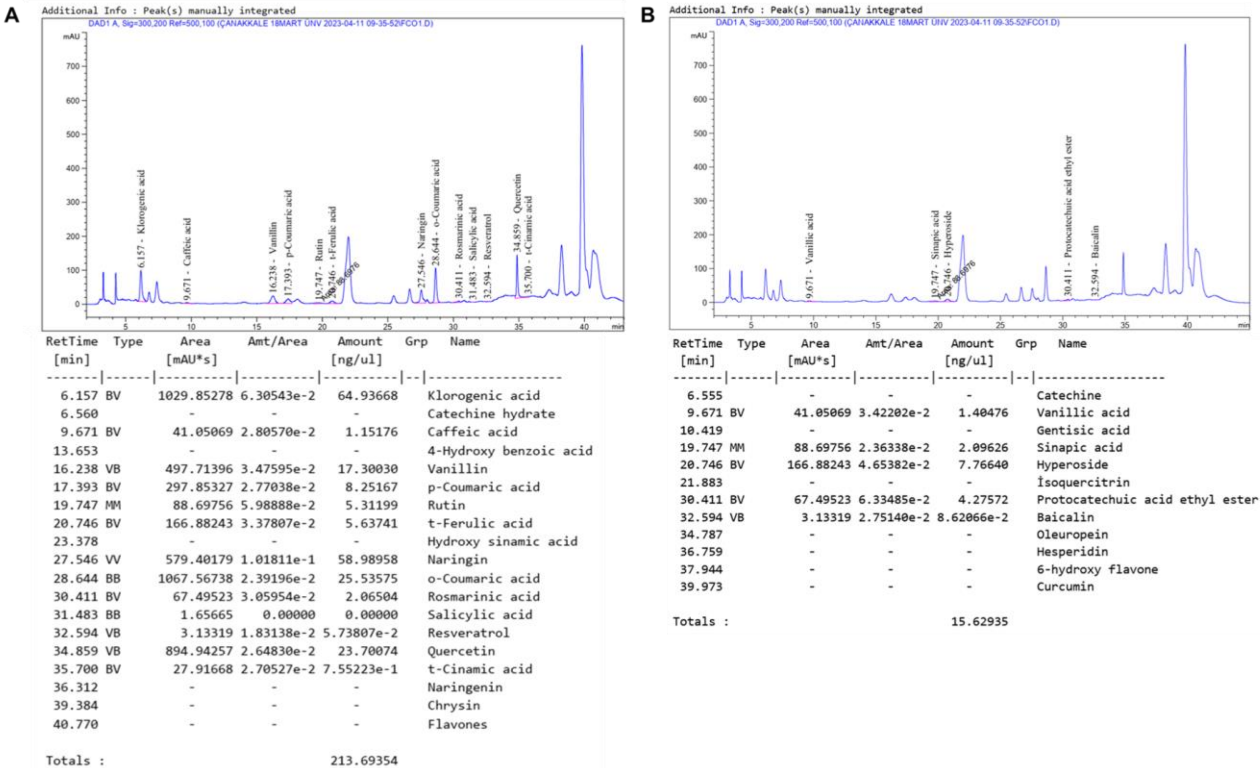
3.7. The Secondary Metabolites were Determined by HPLC Analysis

Herein, the secondary metabolites in extracts (**1** and **2**) were determined by HPLC analysis with different methods. The phenolic contents including rutin (19.747), caffeic acid (9.671), naringin (27.546), resveratrol (32.594), quercetin (34.859), baicalin (32.594) were determined in extract **1** (Figure 7a,7b). The phenolic compounds of extract **2** were determined as rutin (19.488), naringin (27.578), resveratrol (32.404), and baicalin (32.404) (Figure 8a,8b). The retention times of phenolic contents of extract **1** were calculated as caffeic acid (9.653), isoquercitrin (27.013), naringin (29.299), resveratrol (30.545), quercetin (35.008) (Figure 9a). The retention times of phenolic contents of extract **2** were calculated as caffeic acid (9.620), ellagic acid (22.233), isoquercitrin (26.815), naringin (29.272), resveratrol (30.283) (Figure 9b).

According to HPLC studies with the *PQ* [14,36]. Mohamed et al. [14] used HPLC-UV for the determination of flavonoid compounds such as rutin and myricetin in MeOH extracts of *PQ* fruits. Spectroscopic analysis of its secondary metabolites was carried out [36]. In the other study it was reported that tropane glycosidic alkaloid, sennoside C and rutin were identified from ethanolic extracts of *PQ* obtained from petroleum ether fraction by HPLC [36]. The fruits of the plant collected from Tunisia were dried at room temperature and anthocyanins were identified by HPLC [17].

4. Conclusion

Herein, *PQ* extracts from fruits and red leaves were biologically investigated in detail. First, the extracts were performed gradually in the increasing polarity from apolar to polar solvents. Some extracts were obtained by directly polar solvents such as MeOH and aqueous EtOH or aqueous EtOH after MeOH. According to our results, all plant extracts contributed to anticancer activity in the changing doses on five different cancer cell lines for cancer therapy. As is expected, MeOH extracts of fruits displayed strong radical scavenging activity at low IC_{50} values such as 0.51 mg/mL, 0.80 mg/mL, 0.84 mg/mL, and 1.45 mg/mL.



A

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [ng/ul]	Grp	Name
6.157	BV	1029.85278	6.30543e-2	64.93668		Klorogenic acid
6.560		-	-	-		Catechine hydrate
9.671	BV	41.05069	2.80570e-2	1.15176		Caffeic acid
13.653		-	-	-		4-Hydroxy benzoic acid
16.238	VB	497.71396	3.47595e-2	17.30030		Vanillin
17.393	BV	297.85327	2.77038e-2	8.25167		p-Coumaric acid
19.747	MM	88.69756	5.98888e-2	5.31199		Rutin
20.746	BV	166.88243	3.37807e-2	5.63741		t-Ferulic acid
23.378		-	-	-		Hydroxy sinamic acid
27.546	VV	579.40179	1.01811e-1	58.98958		Naringin
28.644	BB	1067.56738	2.39196e-2	25.53575		o-Coumaric acid
30.411	BV	67.49523	3.05954e-2	2.06504		Rosmarinic acid
31.483	BB	1.65665	0.00000	0.00000		Salicylic acid
32.594	VB	3.13319	1.83138e-2	5.73807e-2		Resveratrol
34.859	VB	894.94257	2.64830e-2	23.70074		Quercetin
35.700	BV	27.91668	2.70527e-2	7.55223e-1		t-Cinamic acid
36.312		-	-	-		Naringenin
39.384		-	-	-		Chrysin
40.770		-	-	-		Flavones

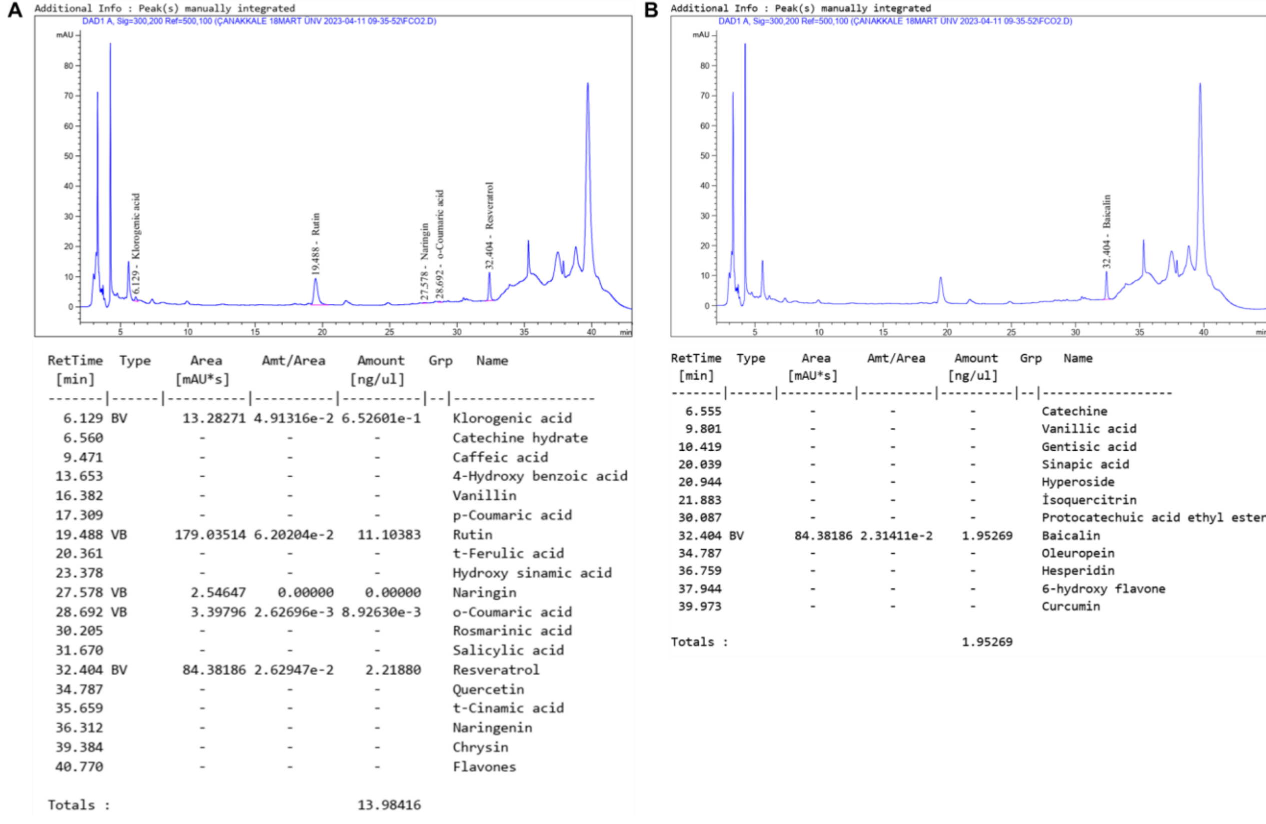
Totals : 213.69354

B

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [ng/ul]	Grp	Name
6.555		-	-	-		Catechine
9.671	BV	41.05069	3.42202e-2	1.40476		Vanillic acid
10.419		-	-	-		Gentisic acid
19.747	MM	88.69756	2.36338e-2	2.09626		Sinapic acid
20.746	BV	166.88243	4.65382e-2	7.76640		Hyperoside
21.883		-	-	-		İsoquercitrin
30.411	BV	67.49523	6.33485e-2	4.27572		Protocatechuic acid ethyl ester
32.594	VB	3.13319	2.75140e-2	8.62066e-2		Baicalin
34.787		-	-	-		Oleuropein
36.759		-	-	-		Hesperidin
37.944		-	-	-		6-hydroxy flavone
39.973		-	-	-		Curcumin

Totals : 15.62935

Figure 7. HPLC spectrum of extract 1



A

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [ng/ul]	Grp	Name
6.129	BV	13.28271	4.91316e-2	6.52601e-1		Klorogenic acid
6.560		-	-	-		Catechine hydrate
9.471		-	-	-		Caffeic acid
13.653		-	-	-		4-Hydroxy benzoic acid
16.382		-	-	-		Vanillin
17.309		-	-	-		p-Coumaric acid
19.488	VB	179.03514	6.20204e-2	11.10383		Rutin
20.361		-	-	-		t-Ferulic acid
23.378		-	-	-		Hydroxy sinamic acid
27.578	VB	2.54647	0.00000	0.00000		Naringin
28.692	VB	3.39796	2.62696e-3	8.92630e-3		o-Coumaric acid
30.205		-	-	-		Rosmarinic acid
31.670		-	-	-		Salicylic acid
32.404	BV	84.38186	2.62947e-2	2.21880		Resveratrol
34.787		-	-	-		Quercetin
35.659		-	-	-		t-Cinamic acid
36.312		-	-	-		Naringenin
39.384		-	-	-		Chrysin
40.770		-	-	-		Flavones

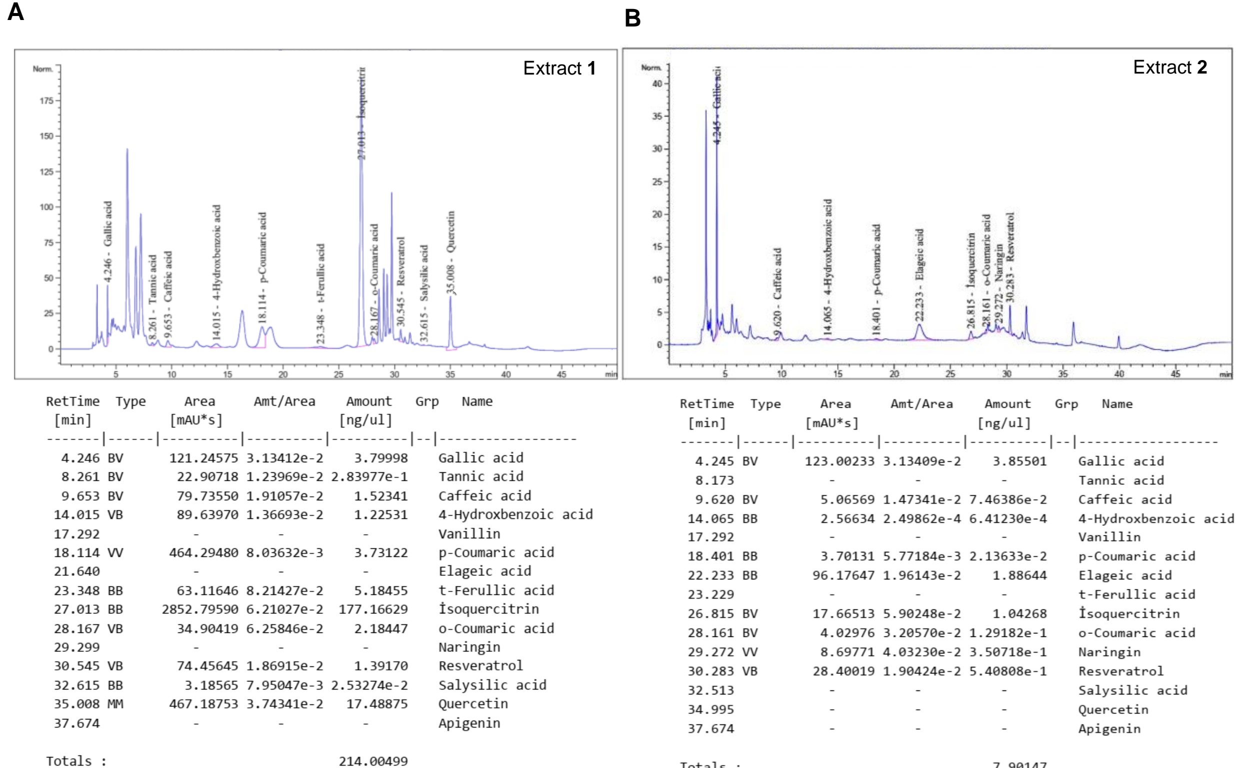
Totals : 13.98416

B

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [ng/ul]	Grp	Name
6.555		-	-	-		Catechine
9.801		-	-	-		Vanillic acid
10.419		-	-	-		Gentisic acid
20.039		-	-	-		Sinapic acid
20.944		-	-	-		Hyperoside
21.883		-	-	-		İsoquercitrin
30.087		-	-	-		Protocatechuic acid ethyl ester
32.404	BV	84.38186	2.31411e-2	1.95269		Baicalin
34.787		-	-	-		Oleuropein
36.759		-	-	-		Hesperidin
37.944		-	-	-		6-hydroxy flavone
39.973		-	-	-		Curcumin

Totals : 1.95269

Figure 8. HPLC spectrum of extract 2 with two different methods



Extract 1

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [ng/ul]	Grp	Name
4.246	BV	121.24575	3.13412e-2	3.79998		Gallic acid
8.261	BV	22.90718	1.23969e-2	2.83977e-1		Tannic acid
9.653	BV	79.73550	1.91057e-2	1.52341		Caffeic acid
14.015	VB	89.63970	1.36693e-2	1.22531		4-Hydroxbenzoic acid
17.292		-	-	-		Vanillin
18.114	VV	464.29480	8.03632e-3	3.73122		p-Coumaric acid
21.640		-	-	-		Elageic acid
23.348	BB	63.11646	8.21427e-2	5.18455		t-Ferullic acid
27.013	BB	2852.79590	6.21027e-2	177.16629		İsoquercitrin
28.167	VB	34.90419	6.25846e-2	2.18447		o-Coumaric acid
29.299		-	-	-		Naringin
30.545	VB	74.45645	1.86915e-2	1.39170		Resveratrol
32.615	BB	3.18565	7.95047e-3	2.53274e-2		Salysilic acid
35.008	MM	467.18753	3.74341e-2	17.48875		Quercetin
37.674		-	-	-		Apigenin
Totals :				214.00499		

Extract 2

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [ng/ul]	Grp	Name
4.245	BV	123.00233	3.13409e-2	3.85501		Gallic acid
8.173		-	-	-		Tannic acid
9.620	BV	5.06569	1.47341e-2	7.46386e-2		Caffeic acid
14.065	BB	2.56634	2.49862e-4	6.41230e-4		4-Hydroxbenzoic acid
17.292		-	-	-		Vanillin
18.401	BB	3.70131	5.77184e-3	2.13633e-2		p-Coumaric acid
22.233	BB	96.17647	1.96143e-2	1.88644		Elageic acid
23.229		-	-	-		t-Ferullic acid
26.815	BV	17.66513	5.90248e-2	1.04268		İsoquercitrin
28.161	BV	4.02976	3.20570e-2	1.29182e-1		o-Coumaric acid
29.272	VV	8.69771	4.03230e-2	3.50718e-1		Naringin
30.283	VB	28.40019	1.90424e-2	5.40808e-1		Resveratrol
32.513		-	-	-		Salysilic acid
34.995		-	-	-		Quercetin
37.674		-	-	-		Apigenin
Totals :				7.90147		

Figure 9. HPLC spectrum of extracts 1 and **2**

The highest TEAC values including 18.72 mM Trolox/mg, 9.90 mM Trolox/mg, and 8.41 mM Trolox/mg were calculated for red leaf extracts. The antifungal activity was determined against *C. albicans* as a fungal pathogen. All plant extracts showed very promising results against all tested microorganisms.

In conclusion, we may emphasize that *PQ* may be a promising medicinal plant for breast and colon cancers due to the cell cytotoxicity, cell migration, and inhibition effect on colony formation. It was obtained that MeOH extracts of fruits and red leaves led to apoptosis determined by Hoechst staining in MDA-MB-231 and ER+ MCF-7 cells. Additionally, major bioactive components in plants such as rutin, quercetin, resveratrol, naringenin, catechin, curcumin were identified by HPLC analysis. As a result, bioactive components of *PQ* may contribute to further research to be used in plant-based cancer studies.

Author Contributions

The first author directed the project and supervised this study's findings. All authors analyzed the experiments. All authors contributed to the writing of the manuscript. The first, fourth, fifth, and sixth authors reviewed and edited the manuscript. All authors read and approved the final version of the manuscript.

Conflicts of Interest

All the authors declare no conflict of interest.

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