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RESEARCH ARTICLE

Membrane Damaging and Protective Effects of *Gypsophila bicolor*'s Grossh. (Caryophyllaceae) Root Extract on H1299, A549, and A431 Cells

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ABSTRACT

Objective: This study aims to determine the effects of the *Gypsophila bicolor* Grossh. root extract on cell viability and to investigate its protective (antioxidant) effects against hydrogen peroxide (H_2O_2)-induced cytotoxicity and membrane damage in A431, A549, and H1299 cells.

Materials and Methods: The study uses the Cell Titer-BlueR Cell Viability assay to determine the cytotoxic effects of catechol on cells and the fluorometric method to determine cellular malondialdehyde (MDA) levels. The study uses DPPH free radical scavenging, superoxide anion scavenging, hydroxyl radical scavenging, and reducing power assays to determine the antioxidant capacity of the *G. bicolor* root extract.

Results: The IC_{50} values were respectively calculated as 60, 200, and 70 µg/mL in the H1299, A549, and A431 cells incubated with the root extract for 24 h. The IC_{50} values of H_2O_2, a strong oxidizing agent, were found to be 50, 400, and 295 µM, respectively. The most effective cytoprotective concentrations against H_2O_2 cytotoxicity in the cells pre-incubated with low concentrations of root extract were found to be 5 µg/mL for the A549 and A431 cells and 10 µg/mL for the H1299 cells. MDA levels increased in cells exposed to H_2O_2 and the root extract (IC_{50} and IC_{70}) but decreased in the cells pre-incubated with low doses of root extract prior to H_2O_2 exposure. The root extract's antioxidant capacity has also been supported by other tests.

Conclusion: While the root extract caused membrane damage in cells due to high concentrations, it showed a protective effect against H_2O_2 at low concentrations.

Keywords: *Gypsophila bicolor*, Anticancer, Antioxidant, Oxidant, Malondialdehyde

INTRODUCTION

Lung cancer is one of the most common cancers in both men and women worldwide. Because of the cancer's systemic nature with a high rate of recurrence and clinically nonspecific symptoms, most patients are diagnosed at an advanced stage, with the standard treatment still being anticancer drugs.[1] However, the majority of current anticancer drugs have significant side effects with poor prognoses. Therefore, a great need exists to develop a novel anti-cancer drug with low toxicity and high potency. In recent years, the fact that most anticancer drugs are obtained from natural extracts has made plants the focus of drug research.[2] Thus, a popular approach among scientists has been to focus on phytotherapy in cancer treatment. However, extracts obtained from plants for phytotherapy contain many bioactive components, and living things cannot safely use these components without knowing their therapeutic concentrations.

The current study uses this approach to investigate the dose-dependent anticancer and antioxidant effects of *Gypsophila bicolor* root extract on different cancer cell lines. Members of this genus are known as *çöven* in Turkish and soapwort in English. One study demonstrated *Gypsophila oldhamiana gypsogenin* (GOG) to be able to stimulate apoptosis and suppress the proliferation of the NCI-N87 (human gastric cancer cell line), observing GOG be able to decrease the vascular endothelial growth factor (VEGF) and matrix metalloprotein (MMP-9) expression in a dose-dependent manner while increasing the expression of Bax proteins and caspase-3.[3] Another study revealed the effects of *Gypsophila oldhamian*a root extract, which is rich in triterpenoid saponins,

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on SMMC-7721 (human hepatoma) and L02 (normal human liver) cells to vary in a concentration-dependent manner. As a result of the cytotoxic assay, that study calculated the IC_{50} value of TGOE in SMMC-7721 cells as 19.50 ± 3.63 µg/mL and 40.48 ± 3.74 µg/mL in L02 cells, thus concluding the root extract to show selective toxicity against cancer cells. Those results suggested that *G. oldhamiana* root extract may have potentially beneficial effects against hepatocellular carcinoma.[4] Studies in the literature have shown many plant extracts with high saponin and phenolic content similar to *G. bicolor* to have concentration-dependent antioxidant and anticancer properties.[4-7] The concentration of an antioxidant substance plays an important role in its effect on oxidation reactions. Factors such as the structure of the antioxidant in question, the conditions in which oxidation takes place, and the change in the structure of the oxidized substance are effective in this case. High concentrations of antioxidants are known to gain pro-oxidant properties. As a result, antioxidants can act as prooxidants by causing damage at increasing concentrations while protecting the membrane against oxidants at low concentrations. Recent studies have shown the toxic and antiprooxidant properties of molecules with this feature to change in a dose-dependent manner and to be unsafe for humans when used without proper awareness.

Therefore, the current study investigates the concentration-dependent cytoprotective and membrane protective effects of *G. bicolor* root extract against hydrogen peroxide (H_2O_2), a strong oxidant, on H1299, A549, and A431 cells. The study also evaluates the cytotoxic and membrane-damaging effects of the root extract at higher concentrations on cell lines. The study uses four different complementary *in vitro* tests to evaluate the antioxidant properties of the *G. bicolor* root extract.

MATERIALS AND METHODS

Obtaining the *G. bicolor* Root Extract

G. bicolor root extract was obtained from Akdeniz University, Faculty of Engineering, Department of Food Engineering, and prepared using 80% methanol and modifying Güçlü-Üstündağ and Mazza's method.[8,9]

DPPH (2,2'-diphenyl-1-picrylhydrazyl) Assay

DPPH is a stable radical molecule used to measure the free radical scavenging effect of natural compounds. A hydrogen donor antioxidant compound is used to reduce it to yellow diphenyl picryl hydrazine. The absorbance of the generated yellow compound is measured colorimetrically. This spectrophotometric experiment utilizes the stable DPPH radical as a reagent.[10] Using this method, 50 µl was taken from samples of the various concentrations that had been prepared by dissolving the *G. bicolor* root extract in methanol and put into tubes. 5 mL of the DPPH solution prepared in 0.004% (w/v) methanol was added to each tube. The absorbance of the samples was measured at 517 nm after 30 min of incubation at room temperature. Five replicate measurements were made. The absorbance value of the root extract at different concentrations was assessed against the blank control (50 µl methanol). The DPPH radical's percentage of inhibition (%I) value was computed as follows:

$$\%I = ((A_{control} - A_{sample}/A_{control}) \times 100) \qquad (1)$$

The obtained % I value was plotted against concentrations, with the concentration value that ensures 50% color lightening being computed as the 50% inhibition (EC_{50}) value. Butylhydroxytoluene (BHT), ascorbic acid, and α-tocopherol were utilized as positive controls for comparison. The selected concentration range was determined as a result of preliminary trials.

Hydroxyl Radical Scavenging Activity

The study uses the 2-deoxyribose oxidation method to test *G. bicolor*'s ability to scavenge hydroxyl radicals.[11] The method is based on measuring the absorbance of the generated reactive products through the degradation of deoxyribose by the hydroxyl radical that is released by the Fe/Ascorbate/EDTA/H_2O_2 system at a wavelength of 532 nm. 100 µl of deoxyribose (60 mM), 100 µl of $FeCl_3$ (1 mM), 100 µl of EDTA (1 mM), and 100 µl of ascorbic acid, as well as 100 µl of H_2O_2 (12 mM) were added to the root extract samples at different concentrations. The mixture was left for one hour at a temperature of 3°C. Afterward, the reaction was stopped by adding 1% thiobarbituric acid (TBA) and 2.8% trichloroacetic acid (TCA) to the reaction mixture. The reaction mixture was boiled for 15 min, then cooled on ice and extracted with *n*-butanol. The color intensity of the resulting-colored complex was measured at 532 nm against *n*-butanol (blank control). BHT is a synthetic antioxidant and additive and was employed as a positive control. The selected concentration range was determined as a result of preliminary trials. The percentages of inhibition of the plant extract and the standard were calculated using Equation 1.

Reduction Potential Method

The reduction potential was determined by Oyaizu.[12] As another method based on free radical seizure activity, the reduction potential method shows high absorbance to imply a high reduction potential. 1 mL of different concentrations of root extract samples was combined with 2.5 mL of phosphate buffer (0.2 M) and 2.5 mL of potassium ferricyanide [$K_3Fe(CN)_6$] solution (%1 w/v). The mixture was incubated at 50 °C for 20 min. The mixture was then centrifuged at 1000 x g for 10 min with 2.5 mL (10% w/v) of TCA added. 2.5 mL of this mixture was then taken from the top and mixed with 2.5 mL of deionized water and 0.5 mL (0.1 percent) $FeCl_3$, after which the absorbance at 700 nm was measured against the blank control. Ascorbic acid was employed as a positive control. The selected concentration range was determined based on the preliminary trials.

Superoxide Anion Scavenging Activity

The superoxide anion scavenging activity of the *G. bicolor* root extract was measured using the method described by Liu.[13] This method is based on reducing the yellow NBT to its blue-violet-colored formazan derivative through the superoxide radical produced by the NADH/PMS/O_2 system. Low absorbance levels are obtained when the reaction environment includes compounds with superoxide anion scavenging activity. The production of superoxide anions occurred in a 3 mL Tris-HCl buffer (16 mM, pH 8.0) that contained 1 mL of NADH (78 µM) solution, 1 mL of NBT (50 µM) solution, and sample solutions. A 1 mL phenazine methosulfate (PMS) solution (10 µM) was added to the mixture to start the reaction. The mixture was then incubated for 5 min at 25 °C, and the absorbance was measured against a blank sample at 560 nm. Ascorbic acid was utilized as the positive control. The selected concentration range was determined based on the preliminary trials. Superoxide radical scavenging activity (%) was calculated by Equation 1. Values in all *in vitro* antioxidant assays are presented as the mean of five replicate measurements.

Cancer Cell Culture

Human lung cancer (H1299 and A549) and epidermoid carcinoma (A431) cell lines were acquired from the American Type Culture Collection in Rockville, Maryland. The cells were cultured in an RPMI 1640 medium containing 10% fetal bovine serum and 1% antibiotic - antimycotic solution at 37 °C within a humid atmosphere containing 5% CO_2.

Cytotoxicity Analysis

The H1299, A549, and A431 cells (10,000 cells/well, monolayer) were trypsinized to remove them from the surface of the flask to which they were adhered. The trypan blue (1:1 ratio) assay was used to count cells while transferring them to well plates (96 well plates). The method is based on the principle that live cells do not take up the dye, but dead cells do, with the number of cells per mL being calculated using the live and dead cells on a hemocytometer slide. 200 µl of cell suspension was dispensed into 96 well plates at $1x10^4$ cells per well. The medium used in the wells was removed and replaced after a 24 h incubation at 37 °C. The *G. bicolor* root extract was diluted with a medium and administered to the wells containing cells at doses ranging from 20 to 400 µg/mL. The control group consists of cells that had received no treatment. The cells were incubated for 24 h in an incubator at 37 °C. The viability of the cells was determined using the Cell Titer-Blue® Cell viability assay kit after incubation. This method is based on the ability of live cells to convert resazurin into the fluorescent substance resorufin, whereas dead cells cannot make fluorescent-signaling products because their metabolic capacity is rapidly depleted. After 5 sets of 24-hour incubations, 20 µl of the Cell TiterBlue® Cell viability assay kit was added to each well and incubated at 37 °C for 1 h. The reduction of cells from resazurin to resorufin was calculated by measuring the excitation at 560 nm and emission values at 590 nm in a fluorescent spectrophotometer (PerkinElmer LS55).[14] The percentage of live cells was calculated by comparing the mean absorbance value of the cell-only control group with the mean absorbance values of cells incubated with different concentrations of the *G. bicolor* root extract. The results are expressed as a percentage of live cells. H_2O_2 cytotoxicity on the H1299, A549, and A431 cells was measured in the same way. Cells were pre-incubated with different concentrations of the root extract (2.5-20 g/mL) for 1 h before 24 h of the H_2O_2 treatment to measure the root extract's antioxidant effect against H_2O_2 cytotoxicity (IC_{50} and IC_{70}). Each concentration and control were replicated five times. The IC_{50} values were calculated based on Equation 1. The root extract was dissolved in 0.5% dimethyl sulfoxide (DMSO).

Determining the Malondialdehyde Levels

The malondialdehyde (MDA) levels were measured using Wasowicz, Neve, and Peretz's method,[15] whose basic principle is that MDA is a product of lipid peroxidation resulting from membrane damage and reacts with TBA. The resulting compound is extracted in the butanol phase, and fluorescence spectrophotometry (PerkinElmer LS 55) is used to read the excitation at 525 nm and emission values at 547 nm. The MDA levels in the H1299, A549, and A431 cells were measured after 24 h of exposure to various root extract concentrations (IC_{50}, IC_{70}). To measure the root extract's membrane protective effect against H_2O_2, the cells were pre-incubated for 1 h at various concentrations (2.5-20 µg/mL) of the root extract before being exposed to the IC_{50} and IC_{70} concentrations of H_2O_2 and then treated with H_2O_2 (IC_{50} and IC_{70}) for 24 h. The root extract is dissolved in a 0.5% DMSO solution. The cells were centrifuged for 10 min at 600 x g after being taken from the culture environment. The cells were washed with a phosphate buffer, sonicated for 3 sets of 15 sec with 50 mM potassium phosphate (pH 7.2) containing 1 µg/ mL leupeptin and 1 mM PMSF, and centrifuged for 45 min at 15,000 x g. The MDA levels were determined in the supernatant. Protein was determined according to the Bradford method using bovine serum albumin as a standard.[16]

Statistical Analysis

The program Minitab Release 13.0 was used to analyze the data statistically.[17] The ANOVA GLM (General Linear Model) procedure was used to compare between groups.

RESULTS

The study has conducted four *in vitro* assays (i.e., the DPPH radical scavenging, superoxide anion scavenging, hydroxyl radical scavenging, and reduction potential assays) to investigate

the potential *in vitro* antioxidant properties of the methanol extract from *G. bicolor* roots. The results have been compared to positive controls (i.e., BHT and ascorbic acid).

The study utilized the DPPH assay to measure the antiradical capacity of the *G. bicolor* root extract relative to the activities of synthetic antioxidants such as ascorbic acid, α-tocopherol, and BHT. While the root extract showed a 35.2% radical scavenging activity at 3 µg/mL, the radical scavenging activities of α-tocopherol, ascorbic acid, and BHT were found to be 34.8%, 34.8%, and 25.8%, respectively. This study's extract also showed radical scavenging activity close to the positive controls (Table 1).

The hydroxyl radical scavenging efficacy of the *G. bicolor* root extract increases depending on dosage, as shown in Figure 1. The scavenging activities of 250 µg/mL root extract, α-tocopherol, and BHT were 64.27%, 83.64%, and 87.64%, respectively, with the respective EC_{50} values of 108.51, 53.33, and 24.17 µg/mL being found. The root extract's hydroxyl radical scavenging activity was about 25% lower than that of the positive controls.

The reducing power assay was performed to measure *G. bicolor*'s ability to reduce Fe^{3+} to Fe^{2+}. The reduction potential was found to be 3.06 in the 1,000 µg/mL of the root extract and 3.62 in the 1,000 µg/mL of ascorbic acid (Figure 1). Here the root extract acted as an antioxidant through electron donation.

G. bicolor showed lower superoxide anion scavenging activity than the positive control of ascorbic acid, as shown in Figure 1. At a concentration of 100 µg/mL, the root extract and ascorbic acid exhibited superoxide anion scavenging activity of 57.24% and 65.04%, respectively. While the root extract's EC_{50} value was found 87.35 µg/mL, the EC_{50} of ascorbic acid was found 76.88 µg/mL, at the same concentration.

Many studies have stated antioxidants to act as pro-oxidants as well as antioxidants on cells depending on the concentration and to exert a biphasic effect. The prooxidant or antioxidant effect of an antioxidant is based on the structure of the antioxidant, the conditions in which the oxidation takes place, and the structure of the oxidized substance that makes it unsafe for living things. Therefore, knowing at what concentrations an antioxidant acts as a prooxidant or an antioxidant is important. This study has shown the *G. bicolor* root extract to act as a prooxidant and to have an anti-cancer effect when applied to cancer cells at increasing concentrations.

The viability of H1299, A549, and A431 cancer cells exposed for 24 h to increasing concentrations of the root extract (20, 40, 80, 100, 150, 200, 250, 300, 350, 400 µg/mL) was significantly and increasingly reduced up to 150 µg/mL, with no significant change in viability being observed at concentrations higher than 150 µg/mL ($p \leq 0.05$; Figure 2A).

After 24 h of incubation, the root extract IC_{50} values were

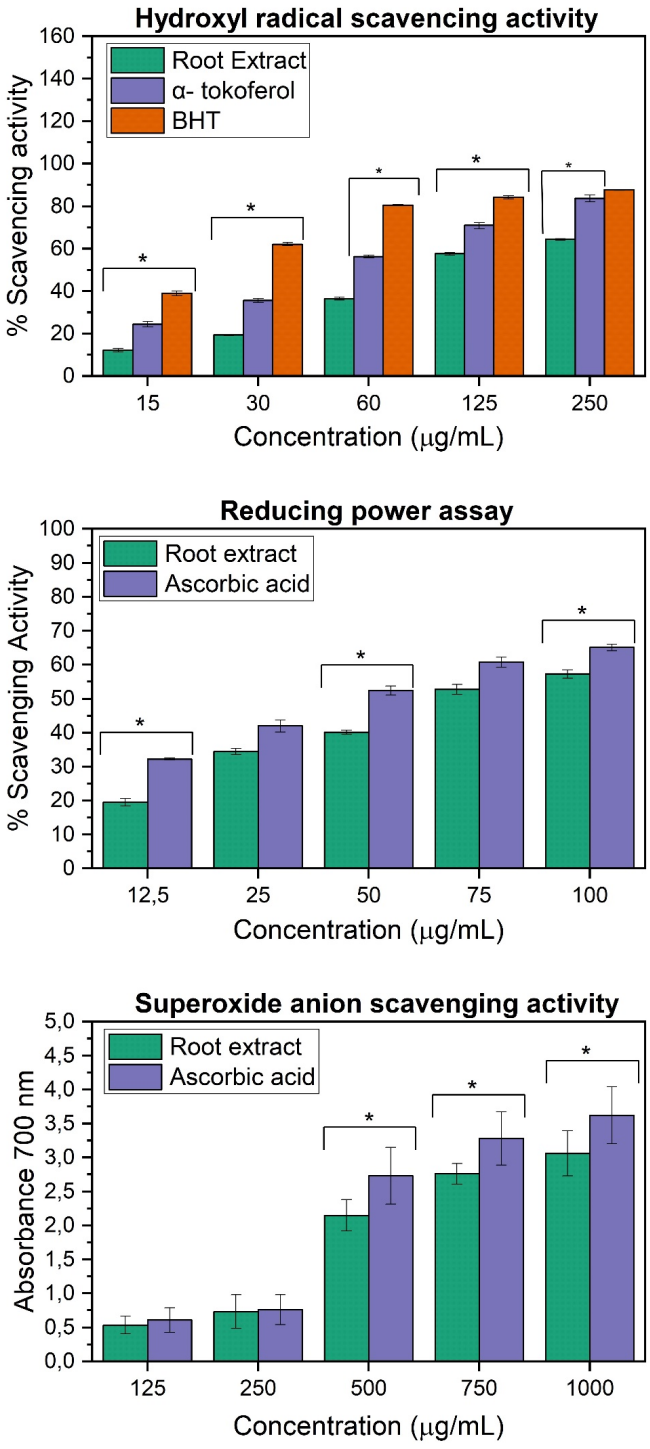

Figure 1. Hydroxyl radical scavenging activity, reducing power, and superoxide anion scavenging activity of root extract and positive controls, respectively. Data were expressed as the mean ± SD for 5 separate experiments. *: $p<0.05$

calculated as 60, 200, and 70 µg/mL for the H1299, A549, and A431 cell lines, respectively (Figure 2A). The toxic effect of the root extract was found to be three times more sensitive in the H1299 and A431 cells than in the A549 cells. The IC_{50} and IC_{70} values of H_2O_2, a powerful oxidizing agent, were computed for

Table 1. Scavenging capacity of *G. bicolor* root extract to DPPH radical (%).

Sample	0.1 µg/mL	0.2 µg/mL	0.4 µg/mL	0.6 µg/mL	0.8 µg/mL	1 µg/mL	3 µg/mL
G.bicolor	16.9 ± 0.1*	18.4 ± 0.4*	19.7 ± 0.4*	24.5 ± 0.7*	28.6 ± 0.4*	32.7 ± 0.4*	35,2 ± 0.8*
α-tocopherol	17.4 ± 0.5*	19.5 ± 0.1*	23.7 ± 0.3*	24.2 ± 0.1*	27.8 ± 0.8*	29.9 ± 0.3*	34,8 ± 0.3*
Ascorbic acid	17.8 ± 0.2*	20.3 ± 0.5*	22.7 ± 0.6*	26.1 ± 0.4*	28.0 ± 0.1*	33.2 ± 0.1*	34,8 ± 0.1*
BHT (Butyl hydroxytoluene)	12.8 ± 0.5*	15.1 ± 0.4*	15.3 ± 0.1*	17.7 ± 0.4*	22.9 ± 0.2*	23.3 ± 0.9*	25,8 ± 0.4*

*Denotes a statistically significant difference when compared to control values ($p < 0.05$) ± standard deviation (SD). Each datum in the table is an average of five repetitions.

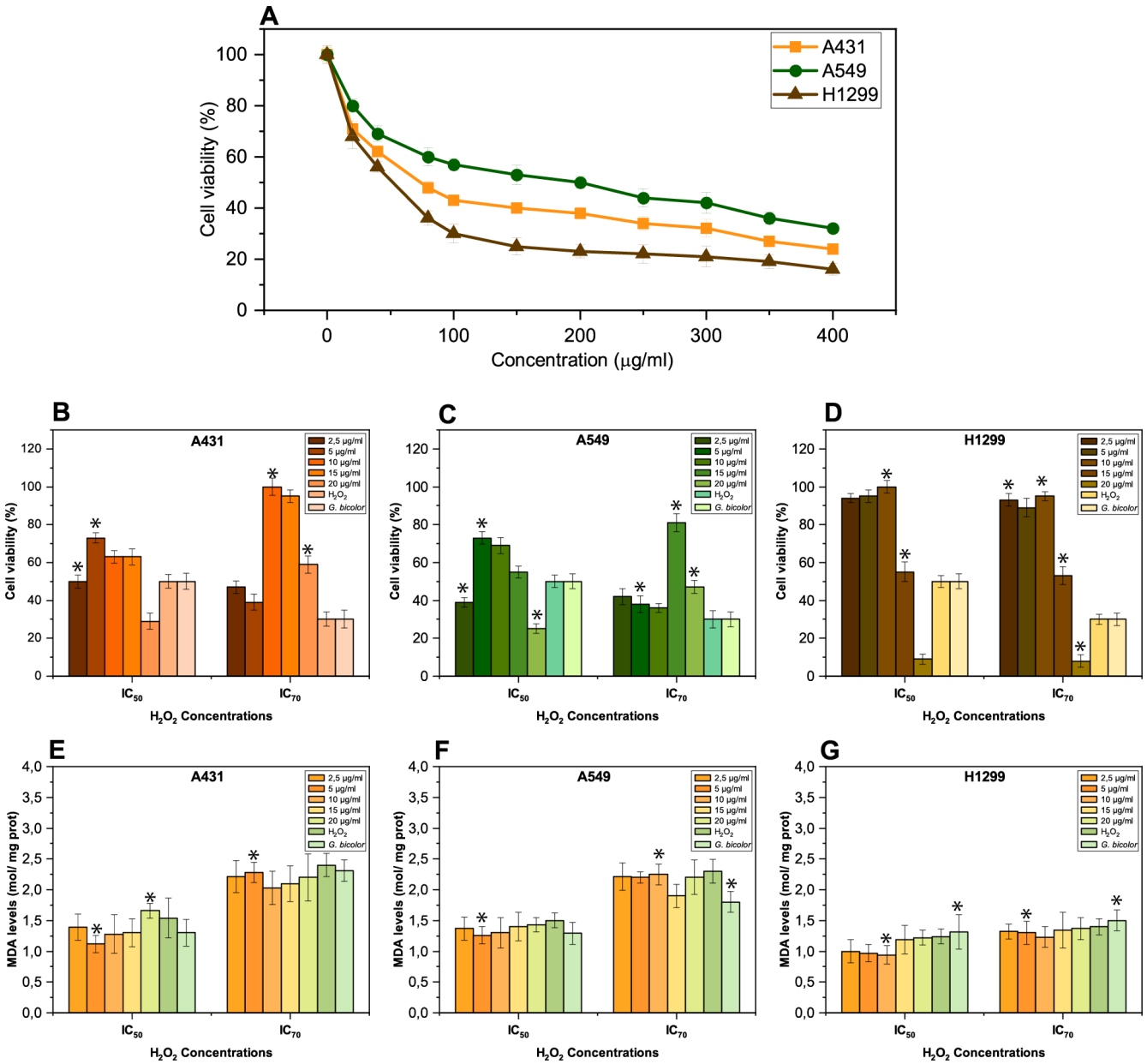

Figure 2. The dose-dependent cytotoxic effect of *G. bicolor* root extract in A549, H1299, and A431 cells (A). The protective (antioxidant) effect of *G. bicolor* root extract concentrations (<IC_{50}) on A431 (B), A549 (C), and H1299 (D) against H_2O_2 cytotoxicity. Dose-dependent membrane damaging effects of the root extract from *G. bicolor* on A431 (E), A459 (F), and H1299 (G). Results are presented as viability ratio compared with the control group (treated with only the medium-untreated cells). Values are expressed as the mean of three separate trials with three replications (ANOVA with Tukey test, $p<0.05$)

Table 2. IC values of root extract and H_2O_2 on the cells.

Cells	Root extract /IC_{50}	Root extract /IC_{70}	H_2O_2/IC_{50}	H_2O_2/IC_{70}
H1299	70 ± 0.09*	300 ± 0.23*	295 ± 0.16*	450 ± 0.51*
A549	200 ± 0.49*	400 ± 0.32*	400 ± 0.96*	547 ± 0.81*
A431	60 ± 0.78*	100 ± 0.1*	50 ± 0.32*	180 ± 0.46*

*Denotes a statistically significant difference when compared to control values ($p < 0.05$) ± standard deviation (SD). Each datum in the table is an average of five repetitions.

the H1299 cells (50 µM and 400 µM), A549 cells (400 µM and 547 µM), and A431 cells (295 µM and 354 µM; see Table 2).

The cytotoxicity results for the *G. bicolor* root extract applied to the cells in high concentrations showed the extract to reduce cell viability by acting as a strong oxidant similar to H_2O_2. Table 2 shows the IC values for the root extract and H_2O_2 on cells. The *G. bicolor* root extract has a dose-dependent antiproliferative effect, indicating it to perhaps be a potential agent for cancer treatments.

However, pre-incubating the cells with low concentrations of the root extract attenuated the cytotoxic effect induced by H_2O_2, a powerful oxidizing agent. The maximum cytoprotective activity of the root extract against the IC_{50} concentration of H_2O_2 cytotoxicity was found to be 5 µg/mL for the A549 and A431 cells and 10 µg/mL for the H1299 cells. In addition, the concentrations at which the root extract showed maximum cytoprotective activity against IC_{70} H_2O_2 cytotoxicity were calculated as 10 µg/mL for the H1299 and A431 cells and 15 µg/mL for the A549 cells (Figures 2B-2D).

As a result, the *G. bicolor* root extract shows cytotoxic effects on the H1299, A549, and A431 cells at high concentrations while significantly reducing the cytotoxic effects caused by H_2O_2 applied after incubating the cells with the root extract at low concentrations. In this case, the root extract shows an antioxidant or prooxidant effect depending on the concentration. The cytotoxic effect from the H_2O_2 application after the preincubation with the root extract was found to be lower than the cytotoxic effect of H_2O_2 alone, which reveals the root extract's cytoprotective effect.

At high concentrations, antioxidants can cause membrane damage by acting as prooxidants, while at lower concentrations they can protect the membrane against oxidants by expressing their antioxidant effect. Oxidative stress created by pro-oxidants in a cell leads to membrane damage by lipid peroxidation and increases the MDA level. In the H1299 cells treated with the IC_{50} and IC_{70} root extract, the quantity of MDA respectively increased by around 5 and 5.7 times compared to the control cells. Similarly, MDA levels in the A431 cells treated with the IC_{50} and IC_{70} concentrations of the root extract were respectively found to be 3.9 and 7 times higher than in the control cells. MDA levels respectively increased 4.6- and 6.4-fold in the A549 cells. H_2O_2 is a powerful oxidizing agent well known for causing oxidative stress and cell membrane damage. Compared to the control cells, MDA levels were found to be 4.8 and 5.4 times higher in the H1299 cells treated with IC_{50} and IC_{70} H_2O_2, respectively. Likewise, MDA levels in the A549 cells treated with IC_{50} and IC_{70} H_2O_2 were determined to be 5.4 and 8.2 times higher than in the control cells. This increase was respectively 4.6 and 7.2 times higher in the A431 cells (Figures 2E, 2F, and 2G).

The study's results from the cytoprotective effect test obtained dosage values where the root extract protects against membrane damage caused by H_2O_2 in cells. To demonstrate the membrane protective effect of the study's root extract, the cells were exposed to IC_{50} and IC_{70} concentrations of H_2O_2 for 24 h after being pre-incubated with low concentrations (2.5-20 µg/mL) of root extract.

The IC_{10} (5 µg/mL) root extract concentration showed the highest membrane protective effect against membrane damage caused by IC_{50} H_2O_2 in the A549 and A431 cells (Figures 2E, 2F, and 2G). Meanwhile, the IC_{15} (10 µg/mL) root extract concentration had the greatest membrane protective effect in the H1299 cells against IC_{50} H_2O_2-induced membrane damage (Figure 2).

When examining the root extract's membrane protective effect against IC_{70} concentrations of H_2O_2, the IC_{15} (10 µg/mL) root extract concentration had the highest protective activity in the H1299 and A431 cells, and the IC_{20} (15 µg/mL) concentration in the A549 cells (Figure 2). The membrane-damaging effect from the H_2O_2 application after being pre-incubated with the root extract was found to be lower than the membrane-damaging effect of H_2O_2 alone, thus revealing the root extract's membrane protective effect. The concentration with the highest cytoprotective effect against H_2O_2 in each cell was also observed to be the concentration with the highest membrane protective effect.

DISCUSSION

One study's DPPH test results showed low concentrations (1 and 5 µg/ mL) of the methanol root extract of *Gypsophila pilulifera* Boiss.&Heldr. to have higher free radical scavenging activity than that of the extracts from *Gypsophila arrostii Guss*. and *Gypsophila simonii* Hub.-Mor.[18] Another study found the antiradical activity of the saponin-rich leaf extract (26.3 mmol Trolox/kg.dw) and the fruit extract (26.9 mmol Trolox/kg.dw) of *Asparagus albus* L. to be higher than that of its rhizome extract (20.5 mmol Trolox/kg.dw).[19] Yet an-

other study found the hydroxyl radical scavenging activity of the *Pouteria campechiana* seed polysaccharide (EC_{50}: 98.72 µg/mL) to be lower than ascorbic acid (EC_{50} = 8.92 µg/mL).[20] One other study showed the hydroxyl radical scavenging activity of the polysaccharide extract (92.62%) of *Polygonum cillinerve* (Nakai) Ohwi to be close to the positive control of ascorbic acid (91.83%).[21] The reducing activity of the *Indigofera tinctoria* L. extract, which has high saponin and flavonoid content, has also been found to be lower than the control, similar to *G. bicolor*.[22] One study found the EC_{50} values for *Pouteria campechiana* (Kunth) Baehmi's polysaccharide and ascorbic acid superoxide anion scavenging activity to be 358.22 and 7.74 g/mL, respectively.[20] The superoxide anion scavenging activity of *P. campechiana* polysaccharide was seen to be lower than that of the *G. bicolor* root extract. One study investigated the cytotoxic effect of the fresh extract from the tuber *Romulea tempskyana* Freyn. on H1299 and Hep-G2 cells determined IC_{50} values of 76.15 µg/mL and 94.79 µg/mL, respectively, and also found low concentrations of the fresh extract from *R. tempskyana* tuber (5-25 µg/mL) to significantly protect cancer cells from H_2O_2 cytotoxicity.[23] Other studies have shown various extracted saponins from the genus *Clematis*, including *Clematis lasiandra* Maxim and *Clematis argentilucida* (H.Lév.&Vaniot) W.T.Wang, to have considerable anti-cancer activity.[23,24] For instance, researchers have isolated seven novel cytotoxic triterpenoid saponins from *C. lasiandra.* Of these, five extracts demonstrated IC_{50} values ranging from 1.40-19.50 µmol/L in human tumor cell lines (i.e., HL-60, HepG-2, and SGC-7901).[23] One study comparatively investigated the antihypertensive, anticholinesterase, anturease, anti-tyrosinase, and anti-elastase enzyme inhibition and anticancer activities of *in vivo* (collected male and female root, stem, and leaf parts) and *in vitro* samples (germinated root, stem, and leaf parts) of *Pistacia khinjuk* stocks and found all the samples analyzed for anticancer activity to have cytotoxic effects against MCF-7 (human breast cancer) and HT-29 (colon cancer) cell lines. However, the study stated the in vivo samples had higher biological activity than the *in vitro* samples.[25] Another study investigated the effect of saponins obtained from garlic on the stress created by hypoxic conditions in PC12 cells and showed garlic saponins at concentrations of 0-5 ng/mL to significantly reduce hypoxic stress and to increase cell viability by 73% in 72 h.[26] One study found the methanol extract of the above-ground parts of *G. bicolor* and *Gypsophila ruscifolia* Boiss. to have cytotoxic effects on MCF-7 cells (IC_{50} <100 µg/mL) but failed to observe any cytotoxic effect in the A-549 (non-small cell lung carcinoma) and AGO-1522 cells (human fibroblast cell) at the tested doses of the extracts (0.01-1 mg/mL).[27] Another study analyzed the biological activities; gas chromatography and mass spectrometry (GC-MS) analysis; and antioxidant, antimicrobial, antioxidant, antimicrobial, DNA fragmentation, and anticancer activity of methanol (MeOH) and petroleum ether (PE) extracts of *Symphytum aintabicum* Hub.-Mor.&Wickens regarding MCF-7 cells. The IC_{50} values of the PE and MeOH extracts were 414.73 µg/mL and 443.31 µg/mL, respectively. That study's findings indicated *S. aintabicum* shows interesting pharmacological and biological activities and is a particularly suitable candidate for anticancer drug development.[28] One study examining the effect of saponins extracted from *Panax quinquefolius* L. leaves on acetaminophen (APAP)-)-induced liver toxicity in rats observed saponins to decrease the increased hepatic MDA levels.[29] Another study investigated the protective effect of saponins obtained from *Platycodon grandiflorum* A. DC. on hypoxia/reoxygenation-induced oxidative stress in cardiomyocytes (H9c2), observing *P. grandiflorum* saponins at a concentration of 20 µM to significantly decrease the MDA level and to cause an increase in superoxide dismutase and catalase activity.[30]

CONCLUSION

The results from the current study's four *in vitro* tests have revealed the root extract's antioxidant properties as well as hydroxyl, superoxide, and DPPH radical scavenging activity, and reducing power activity. The root extract shows antioxidant properties by protecting cancer cells against the oxidizing effect of H_2O_2, which supports the results presented above. The study's results also observed the root extract to act as a prooxidant at high concentrations, thus causing cytotoxic and membrane-damaging effects. However, the cytotoxic and membrane damage effects were reduced in cells that had been preincubated with low concentrations of the root extract compared to cells that had been exposed to H_2O_2 alone, thus revealing the extract's antioxidant effect. Due to the root extract showing biphasic effects such as being an antioxidant or prooxidant in cells depending on its dosage, foods containing *G. bicolor* root extract should be consumed carefully. This report showcases the potential the root extract has for decreasing the toxicity of hydrogen peroxide in cancer cells. However, further investigation on pharmacokinetics, pharmacodynamics, and toxicology is required before any clinical recommendations on root extraction supplementation can be made.

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