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Exploring the Antimutagenic and Antigenotoxic Potential of *Arbutus unedo* L. Fruits

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Abstract

Objective: This study aimed to comprehensively evaluate the phytochemical composition, antioxidant capacity, genoprotective potential, and genotoxic safety of *Arbutus unedo* L. (strawberry tree) fruit ethanolic (EtOH) and aqueous extracts.

Materials and Methods: Ethanolic and aqueous extracts of *A. unedo* fruits were analysed for total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity using cupric reducing antioxidant capacity (CUPRAC), ferric reducing antioxidant power (FRAP), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays. High-performance thin-layer chromatography (HPTLC) was employed to check the presence of some specific phenolic compounds. Genotoxicity and genoprotection were assessed using the Ames test, alkaline comet assay, and cytokinesis-block micronucleus (CBMN) assay in Chinese hamster ovary (CHO-K1) cells.

Results: The EtOH extract showed significantly higher TPC (46.46 ± 0.56 mg gallic acid equivalent [GAE]/g) and TFC (14.23 ± 1.18 mg catechin equivalent [CE]/g) than the aqueous extract (TPC: 33.66 ± 1.80 mg GAE/g; TFC: 8.90 ± 0.24 mg CE/g), which was consistent with stronger antioxidant activity in the CUPRAC (114.86 ± 5.33 mg Trolox equivalent [TE]/g), FRAP (47.48 ± 1.70 mg TE/g), and DPPH (89.86 ± 0.83 mg TE/g) assays. No mutagenic or genotoxic effects were detected in either extract in all genotoxicity tests. Moderate antimutagenic activity was observed only at the highest concentration (5000 µg/plate) of the EtOH extract in the TA100 strain with S9 metabolic activation in the Ames test. However, no significant DNA protection was observed against doxorubicin-induced damage in either the comet or micronucleus assays.

Conclusion: *Arbutus unedo* fruit extracts, particularly the EtOH extract, are rich in phenolic antioxidants and do not exhibit genotoxic effects under the tested conditions. These findings support the safe use of *A. unedo* fruits in the food industry and their potential as natural sources of antioxidants.

Keywords: *Arbutus unedo*, antioxidant activity, micronucleus assay, Comet Assay, Ames test

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INTRODUCTION

Arbutus unedo L., commonly referred to as the strawberry tree, belongs to the genus *Arbutus* in the *Ericaceae* family and is predominantly distributed throughout the Mediterranean region. Its distribution extends across Northeastern Africa, the Canary Islands and Western Asia, Western, Central, and Southern Europe as well as the Mediterranean region of Türkiye (1). The species typically thrives on arid, rocky slopes and hillsides or within pine forests, particularly in the Taurus mountains, from sea level up to elevations of 600 meters (2). *Arbutus unedo* is an evergreen perennial that can appear as either a shrub or a small tree. While its usual height ranges from 1.5 to 3 meters, it can reach up to 9 meters under favorable environmental conditions (3). Uniquely, the plant bears both fruit and flowers simultaneously during the winter months. Its inflorescences are composed of clustered, small, cream-colored, lantern-shaped flowers. The alternate leaves are simple and oblanceolate with a dark green hue, leathery texture, short petioles, and serrated margins. The fruits are globular, orange-red, rough in texture, and can reach up to 2 cm in diameter (1,4).

Wild edible plants like *A. unedo* play a significant role in traditional diets and community health practices. Its fruits have historically been incorporated into regional foods and beverages, including fermented drinks and fruit preserves. Extracts obtained from *A. unedo* fruits have demonstrated various pharmacological effects, such as antioxidant, anti-inflammatory, antimicrobial and antiproliferative activities (1,3). Triterpenoids such as lupeol and betulinic acid isolated from its fruit extracts have shown inhibitory effects on cyclooxygenase-2 (COX-2) activity (5). Additionally, the leaves are known for their astringent properties, antiplatelet effects, and potential urinary antiseptic, anti-inflammatory, antidiarrheal, antihypertensive, and antidiabetic activities. In traditional folk medicine, *A. unedo* has been used for its antiseptic, diuretic, and laxative properties, as well as for managing arterial hypertension (6). Despite its established health benefits and wide traditional use, the genotoxic safety profile of *A. unedo* fruit extracts remains insufficiently studied. Given that certain phytochemicals may exert genotoxic effects under specific conditions, a comprehensive safety evaluation is warranted. Therefore, the present study aims to assess the mutagenic/antimutagenic and genotoxic/antigenotoxic effects of aqueous and ethanolic extracts prepared from the ripe fruits of *A. unedo*, using pharmacognostic and toxicological approaches to provide a comprehensive safety profile.

MATERIALS AND METHODS

Plant Material

The ripe fruits of *A. unedo* L. were collected in October 2022 from the vicinity of the Yeditepe University campus, located in the Ataşehir district of Istanbul, Türkiye. The plant species was identified by one of us (HK) and a voucher specimen was deposited at the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Yeditepe University, Istanbul, Türkiye (Herbarium No: YEF 22043).

Preparation of Extracts

A 400 g of fresh fruits were cleaned and lyophilized in order to remove water. For the ethanol extract (EtOH), 20 g of ground sample was macerated in 200 mL of EtOH at room temperature for 3 days, followed by extraction at 40°C for 3 hours. The mixture was then filtered, and the solvent was evaporated under reduced pressure using a rotary evaporator. The concentrated extract was dispersed in H2O, frozen in refrigerator and subsequently lyophilized (yield: 30.15%). For the aqueous extract (infusion), 20 g of ground material was mixed with 200 mL of boiling distilled water. After 15 minutes, the mixture was filtered, and lyophilized (yield: 44.14%).

Phytochemical Analysis

High-Performance Thin-Layer Chromatography (HPTLC)

Sample test solutions were prepared at a concentration of 20 mg/mL in methanol (MeOH) and applied as 5 µL bands onto 20 × 10 cm glass-backed HPTLC plates (Merck, Darmstadt, Germany) precoated with silica gel 60 F_{254}. Standard compound solutions of isoquercitrin, myricitrin, quercitrin and arbutin (50 µg/mL) was applied as 2 µL bands (8 mm in length) using a 100 µL Hamilton syringe and a semi-automated Linomat 5 sample applicator (CAMAG, Muttenz, Switzerland). Chromatographic separation was performed in a twin-trough chamber pre-saturated for 20 minutes with a mobile phase consisting of ethyl acetate, dichloromethane, acetic acid, formic acid, and water in a ratio of 100:25:10:10:11 (v/v/v/v/v). The plate was developed to a distance of 7 cm, then dried using a stream of cold air for 2 minutes. For derivatization, the developed plate was heated at 105°C for 3 minutes using a TLC plate heater III (CAMAG, Muttenz, Switzerland). Subsequently, it was sequentially derivatized by immersion in NP reagent (prepared by dissolving 1 g of 2-aminoethyl diphenylborinate in 200 mL of ethyl acetate) and PEG reagent (10 g of polyethylene glycol 400 in 200 mL of dichloromethane) using the chromatogram immersion device (CAMAG, Muttenz, Switzerland). The derivatized plate was then photographed under 366 nm UV light using an HPTLC imaging system. All procedures were performed using WinCATS software (CAMAG, version 128 1.4.8.2031). Identification of the compounds in the samples was achieved based on their retardation factor (R_f) values and fluorescence band characteristics (7).

Total Phenolic Content (TPC)

In a 96-well plate, 25 µL of each sample (2.5 mg/mL), gallic acid (standard) or water (blank) was mixed with 125 µL of 10% Folin-Ciocalteu reagent and 100 µL of 7.5% $NaHCO_3$. After 30 minutes incubation in the dark at room temperature, absorbance was measured at 760 nm. Results were expressed as mg gallic acid equivalents per gram dry extract (mg GAE/g) (7,8).

Total Flavonoid Content (TFC)

Total flavonoid content was determined using sodium nitrite, aluminium chloride and sodium hydroxide. In a 24-well plate, 200 µL of aqueous extract or catechin (standard), and 50 µL of ethanolic extract (20 mg/mL) were tested. After sequential addition of 30 µL 5% $NaNO_2$, 30 µL 10% $AlCl_3$, 200 µL 1 M NaOH, and 340 µL water, mixtures were incubated at room temperature. Absorbance was measured at 510 nm. Results were expressed as mg catechin equivalents per gram dry extract (mg CE/g) (9).

Antioxidant Activity Assays

DPPH Radical Scavenging Capacity Assay

In a 96-well plate, 20 µL of extract, ethanol (blank), or Trolox (standard) was mixed with 280 µL of 0.1 mM 2,2-diphenyl-1-picrylhydrazyl (DPPH) in ethanol. After incubation in the dark for 30 minutes, absorbance was measured at 520 nm. Results were expressed as mg Trolox equivalents per gram extract (mg TE/g) (10).

Cupric Ion Reducing Antioxidant Capacity (CUPRAC) Assay

In a 96-well plate, 30 µL of sample, standard, or blank was mixed with 80 µL each of 1×10^{-2} M $CuCl_2$, 7.5×10^{-3} M neocuproine, and 1 M ammonium acetate buffer, followed by 30 µL methanol. After 30 min at room temperature, absorbance was measured at 450 nm. Results were expressed as mg TE/g extract (11,12).

Ferric Reducing Antioxidant Power (FRAP) Assay

In a 96-well plate, 20 µL of sample, Trolox standard, or blank were mixed with 280 µL of FRAP reagent ($FeCl_3$, TPTZ, sodium acetate buffer, 1:1:10). After incubation at room temperature for 6 minutes, absorbance was measured at 593 nm. Results were expressed as mg TE/g extract (13).

Genotoxicity Assessment

Mutagenicity and Antimutagenicity Assays

The bacterial reverse mutation assay (Ames test) was performed using *Salmonella typhimurium* strains TA98 (frameshift mutations) and TA100 (base-pair substitutions), with and without metabolic activation (±S9), following Maron and Ames (14). Aqueous and ethanolic *A. unedo* fruit extracts (10–5000 µg/plate) were tested. A maximum concentration of 5000 µg/plate was selected, in line with OECD Test Guideline 471 recommendations for non-cytotoxic and non-toxic substances. Serial dilutions were then applied to determine concentration-dependent effects. Dimethyl sulfoxide (DMSO) served as the negative control; 4-nitro-o-phenylenediamine (NPD) and sodium azide (SA) as positive controls for −S9 (TA98/TA100), and 2-aminofluorene (2-AF) for +S9.

Each test plate contained the test compound or control, bacterial culture, top agar, and either phosphate buffer (−S9) or S9 mix (+S9). After incubation at 37°C for 48 hours, revertant colonies were counted. All experiments were performed in triplicate; results were expressed as mean ± SD.

The mutagenic index (MI) was calculated as follows:
MI = A/B

where A = average number of revertant colonies in the presence of sample, B = average number of revertant colonies in the negative control. An MI value of ≥2 was considered indicative of a mutagenic effect.

For the antimutagenicity assay, the number of revertant colonies on plates containing only the mutagen was considered as 100% (0% inhibition). The percentage inhibition of mutagenicity was calculated using the following formula (Formula 1):

Formula 1= (A−B) / (A−C) × 100

where A = average number of revertants with mutagen only, B = average number of revertants with mutagen and test sample, C = average number of spontaneous revertants. Inhibition rate of 40% or more was defined as strong antimutagenicity, 25–40% inhibition as moderate antimutagenicity. Inhibitory effects of less than 25% were considered as weak and were not recognized as a positive result (15). Both mutagenicity and antimutagenicity experiments were performed in triplicate for all concentrations, as well as for the negative and positive controls.

Genotoxicity and Antigenotoxicity Assessment

Cell Line and Culture Conditions

Chinese hamster ovary (CHO-K1) cells (ATCC® CCL-61™) were cultured in Ham's F-12 medium (Gibco, NY, USA) supplemented with 10 % fetal bovine serum (FBS; Gibco, NY, USA) and 1% penicillin-streptomycin (Gibco, NY, USA). Cells were maintained in a humidified incubator at 37°C with 5% CO_2 and sub-cultured every three days to ensure exponential growth.

The genotoxic and antigenotoxic potential of *A. unedo* extracts were evaluated in CHO-K1 cells using the alkaline comet assay and the cytokinesis-block micronucleus (CBMN) assay. The test concentrations were determined by first performing a range-finding cytotoxicity assay in CHO-K1 cells. The highest concentration tested (500 µg/mL) represented the maximum non-cytotoxic dose, and lower concentrations were selected in descending order to evaluate dose-response relationships. The comet assay was carried out to assess DNA strand breaks following the protocol previously described with slight modifications (16).

Alkaline Comet Assay

CHO-K1 cells (3 × 10^5 cells/well) were seeded in 6-well plates and treated with *A. unedo* extracts (10–500 µg/mL) for 4 hours. Dimethyl sulfoxide (DMSO; 0.5%) and 1 µM doxorubicin were used as negative and positive controls. After treatment, cells were centrifuged (1500 rpm, 5 minutes), resuspended in PBS and mixed with 1% low-melting agarose. This mixture was layered onto slides pre-coated with 1.5% high-melting agarose. After solidification, slides were lysed (1 h, cold lysis buffer), unwound in electrophoresis buffer (20 minutes, 4°C), and electrophoresed at 25 V and 300 mA for 20 minutes at 4°C. Slides were neutralized in 0.4 M Tris-HCl (pH 7.5), fixed with methanol, air-dried, and stained with ethidium bromide. DNA damage was scored under fluorescence microscope (BS 200 ProP, BAB Imaging System, Ankara, Türkiye) (17).

Cytokinesis-Block Micronucleus Assay

CHO-K1 cells (2 × 10^5 cells/well) were seeded in 6-well plates and treated with aqueous or ethanolic *A. unedo* extracts (10–500 µg/mL) for 24 hours. Dimethyl sulfoxide (0.5%) and 1 µM doxorubicin served as controls. After treatment, cells were rinsed and incubated with cytochalasin B (4.8 µg/mL) for 24 hours. Cells were then fixed in methanol:acetic acid (3:1), stained with 5% Giemsa, and examined microscopically (Nikon, Tokyo, Japan) at 40× magnification.

At least 1000 binucleated (BN) cells per condition were scored for micronucleus (MN) frequency and nuclear division index (NDI) using the following formula (Formula 2):

Formula 2= MN (%) = (Number of MN / Number of scored BN cells) × 100.

$$NDI = [M_1 + 2(M_2) + 3(M_3) + 4(M_4)] / N$$

where M_1 - M_4 represent the number of cells with one to four nuclei, and N is the total number of cells scored (16).

Statistical Analysis

Results were expressed as mean ± standard deviation (SD). Data were analyzed using one-way, two-way, or three-way analysis of variance (ANOVA) followed by Dunnett's post hoc test in GraphPad Prism version 10.0.0 (GraphPad Software, San Diego, CA, USA). A p value <0.05 was considered statistically significant.

RESULTS

Phytochemical Analysis

The chemical fingerprint profile of *A. unedo* fruit extracts was evaluated using HPTLC and compared to standard reference compounds, as shown in Figure 1. Among the standards used, no spots were observed that match with myricitrin and arbutin in the HPTLC chromatograms. However, a band matching the migration position of isoquercitrin (Rf ≈ 0.26) was observed in both ethanolic and aqueous extracts, indicating its presence in these samples. Similarly, a fluorescent band corresponding to quercitrin (Rf ≈ 0.42) was detected in both extracts.

To confirm the presence of isoquercitrin and quercitrin, the UV spectra of the reference spots and the corresponding spots were recorded using a TLC Scanner 3

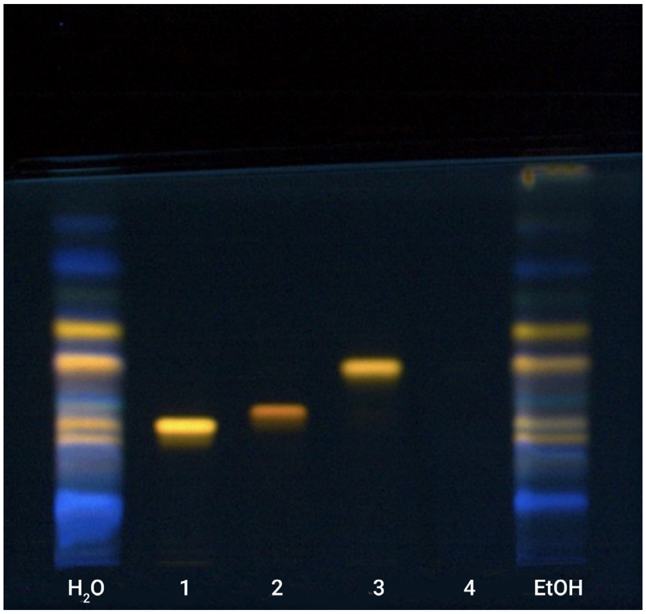

FIGURE 1. HPTLC chromatogram of aqueous and ethanolic extracts of *A. unedo* fruits and reference compounds. Mobile phase: ethyl acetate–dichloromethane–formic acid–acetic acid–water (100:25:10:10:11, v/v/v/v/v); detection at 366 nm. (1: isoquercitrin, 2: myricitrin, 3: quercitrin, 4: arbutin). Arbutin was detectable only at 254 nm.

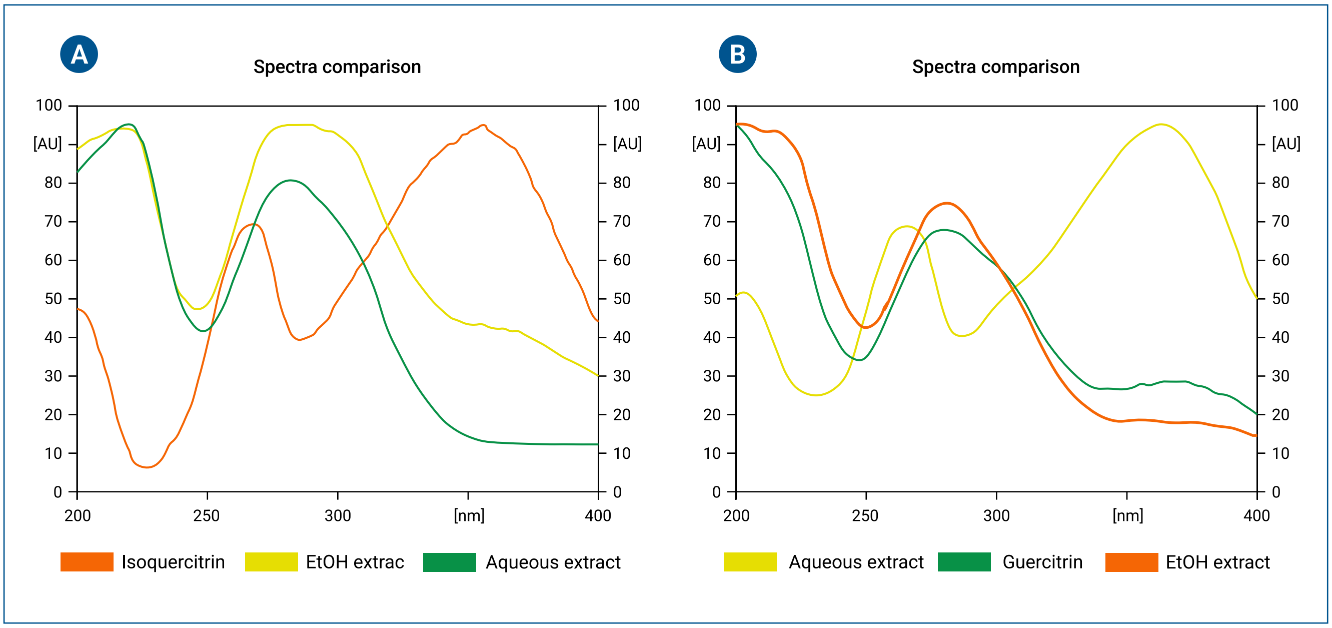

FIGURE 2. Overlay of UV spectra of isoquercitrin (A) and quercitrin (B) with the corresponding spots in the aqueous and ethanolic extracts of *A. unedo* fruits.

Table 1. Total phenolic content (TPC) and total flavonoid content (TFC) of *A. unedo* fruit extracts.

Sample	TPC (mg GAE/g)	TFC (mg CE/g)
Aqueous extracts (infusion)	33.7 ± 1.8	8.9 ± 0.2
Ethanolic extract	46.7 ± 0.6	14.2 ± 1.2

Values represent the mean ± standard deviation (SD) of three independent measurements (n = 3).

TPC: Total phenolic content, **TFC:** Total flavonoid content, **GAE:** Gallic acid equivalent, **CE:** Catechin equivalent.

(CAMAG, Muttenz, Switzerland). Interestingly, the UV spectra of the standard compounds did not overlay with those of the corresponding bands on the chromatogram of the extracts (Figure 2).

Accordingly, the combined evaluation of HPTLC chromatographic profiles and UV spectral overlay analyses indicated that none of the applied reference standards were detected in the *A. unedo* fruit samples collected from the university campus. High-performance liquid chromatography (HPLC) analysis may be more useful to confirm the occurrence of these compounds in the extract.

Total Phenolic and Flavonoid Content (TPC and TFC)

As shown in Table 1, the ethanolic extract of *A. unedo* fruits exhibited significantly higher TPC and TFC compared to the aqueous extract. Specifically, the TPC value of the ethanolic extract was 46.7 ± 0.6 mg GAE/g, whereas the aqueous extract (infusion) showed a lower value of 33.7 ± 1.8 mg GAE/g. Similarly, the TFC was 14.2 ± 1.2 mg CE/g in the ethanolic extract, which was markedly higher than the 8.9 ± 0.2 mg CE/g observed in the aqueous extract.

Antioxidant Activity of Extracts

The antioxidant capacity of A. unedo fruit extracts was assessed using DPPH, CUPRAC, and FRAP assays. In all tests, the ethanolic extract showed consistently higher activity than the aqueous extract (Table 2). The CUPRAC assay yielded the highest values: 114.9 ± 5.3 mg TE/g for the ethanolic and 94.4 ± 6.5 mg TE/g for the aqueous extract. In the DPPH assay, the ethanolic extract demonstrated stronger radical scavenging (89.9 ± 0.8 mg TE/g) than the aqueous extract (61.6 ± 7.3 mg TE/g). Ferric reducing antioxidant power results also favoured the ethanolic extract (47.5 ± 1.7 mg TE/g vs. 33.1 ± 2.3 mg TE/g).

Mutagenicity and Antimutagenicity Assays

As shown in Figure 3, the MI values for both the ethanolic and aqueous extracts remained below the threshold value of 2 in all tested conditions, indicating the absence of mutagenic effects. The reliability of the assay was confirmed by the significant responses of the positive controls— NPD, SA, and 2-AF.

The antimutagenic activity of *A. unedo* extracts was further evaluated using the same strains and conditions, in co-treatment with known mutagens. As illustrated in

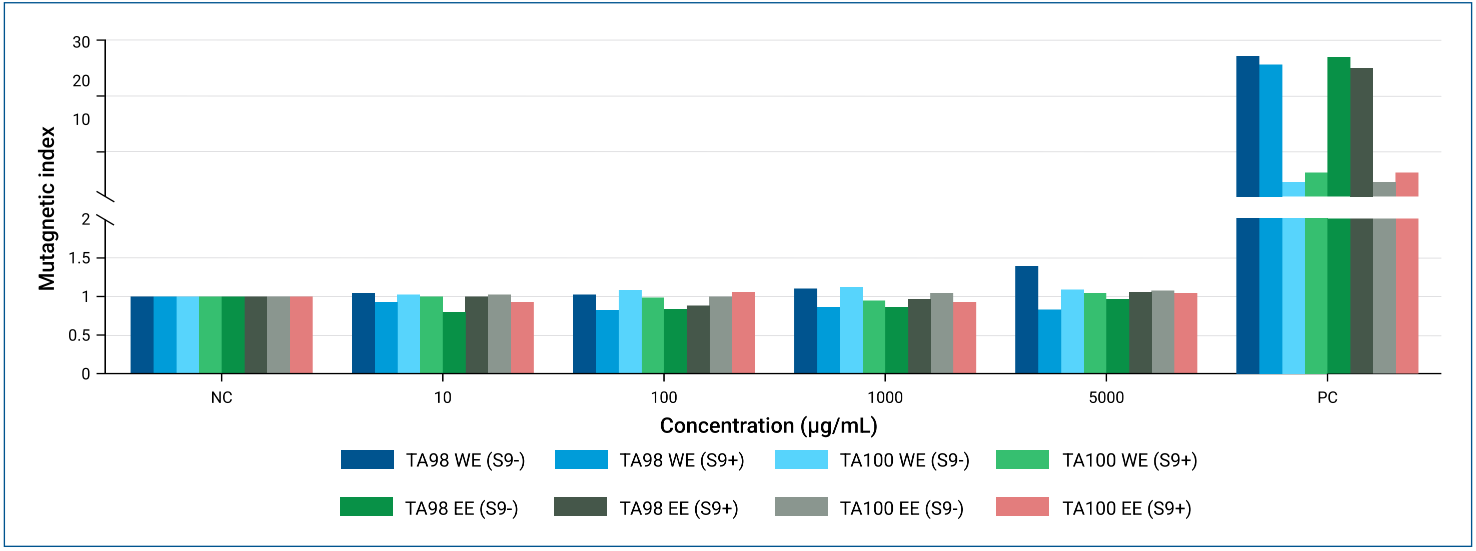

FIGURE 3. Mutagenic index (MI) values of ethanolic (EE) and aqueous (WE) extracts of *A. unedo* fruits at concentrations of 10–5000 µg/plate in *S. typhimurium* TA98 and TA100 strains, with and without S9 metabolic activation. Positive controls are included for comparison.

Table 2. Antioxidant capacity of *A. unedo* fruit extracts determined by CUPRAC, FRAP, and DPPH assays.

Sample	CUPRAC (mg TE/g)	FRAP (mg TE/g)	DPPH (mg TE/g)
Aqueous extracts	94.4 ± 6.5	33.1 ± 2.3	61.6 ± 7.3
Ethanolic extract	114.9 ± 5.3	47.5 ± 1.7	89.6 ± 0.8

Values represent the mean ± standard deviation (SD) of three independent measurements (n = 3).

CUPRAC: Cupric ion reducing antioxidant capacity, **FRAP:** Ferric reducing antioxidant power, **DPPH:** 2,2-diphenyl-1-picrylhydrazyl radical scavenging capacity, **TE:** Trolox equivalent.

Figure 4, the ethanolic extract exhibited notably higher inhibition percentages compared to the aqueous extract, indicating that ethanol-soluble phytochemicals may have stronger antimutagenic effects. Moderate antimutagenic activity was observed only at the highest concentration (5000 µg/plate) with metabolic activation, reaching 26.1% inhibition in TA98 and 39.1% in TA100. At all other tested concentrations, both extracts demonstrated weak antimutagenic activity in both strains, regardless of the presence or absence of metabolic activation.

Results of Alkaline Comet Assay

Figure 5 presents the genotoxicity and protective effects of *A. unedo* extracts in CHO-K1 cells using the alkaline comet assay. DNA damage was measured as the percentage of DNA in the comet tail.

Neither the aqueous nor the ethanolic extracts (up to 500 µg/mL) caused a significant increase in DNA strand breaks compared to the DMSO control, indicating no genotoxicity (Figure 5A).

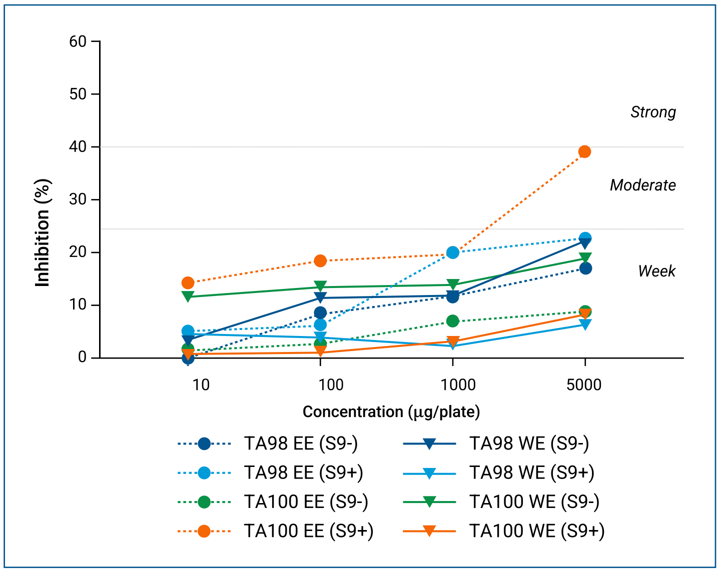

FIGURE 4. Percentage inhibition of mutagenicity by aqueous (WE) and ethanolic (EE) extracts of *A. unedo* fruits against known mutagens in *S. typhimurium* TA98 and TA100 strains, in the presence and absence of S9 metabolic activation.

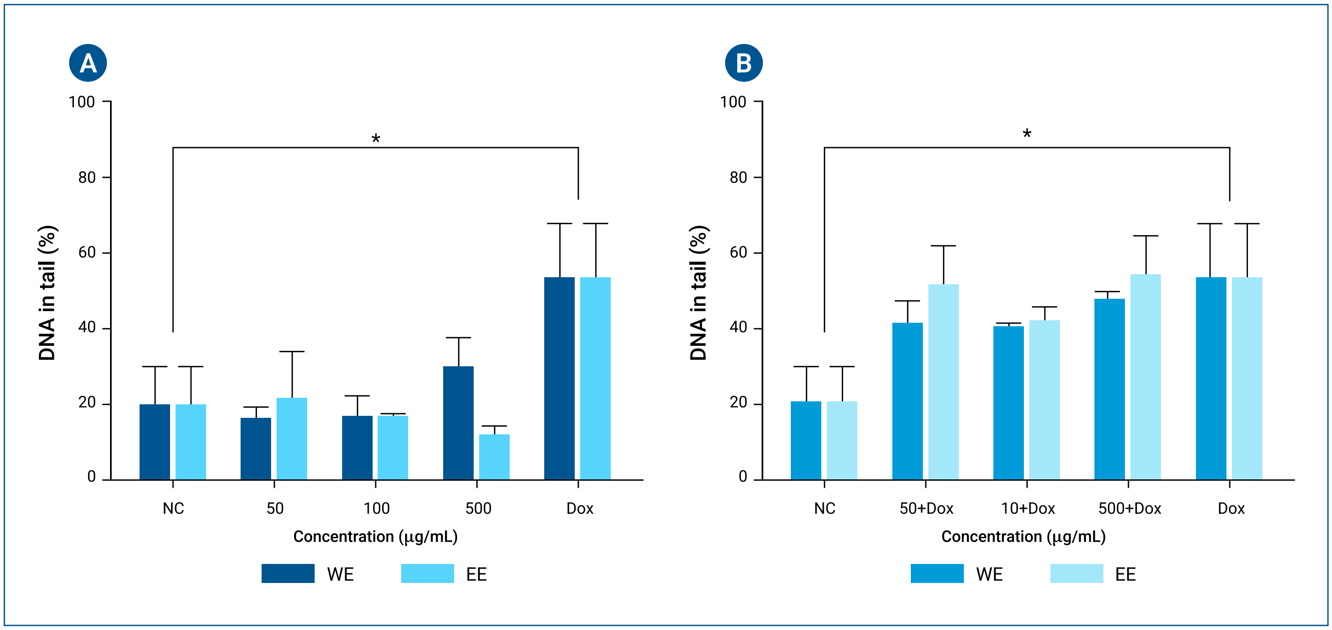

FIGURE 5. DNA strand breakage in CHO-K1 cells evaluated by the alkaline comet assay.

(A) DNA damage following treatment with *A. unedo* extracts alone.

(B) DNA damage after co-treatment with *A. unedo* extracts and doxorubicin.

Cells were treated with aqueous (WE) and ethanolic (EE) extracts of *A. unedo* at concentrations of 50, 100, and 500 µg/mL. Doxorubicin (Dox, 1 µM) was used as the positive control, and 0.5% (v/v) dimethyl sulfoxide (DMSO) served as the negative control. DNA damage is expressed as the percentage of DNA in the comet tail. Data are presented as mean ± standard error of the mean (SEM) from two independent experiments. $p<0.001$ versus the negative control group; for all other treatment groups, $p>0.05$.

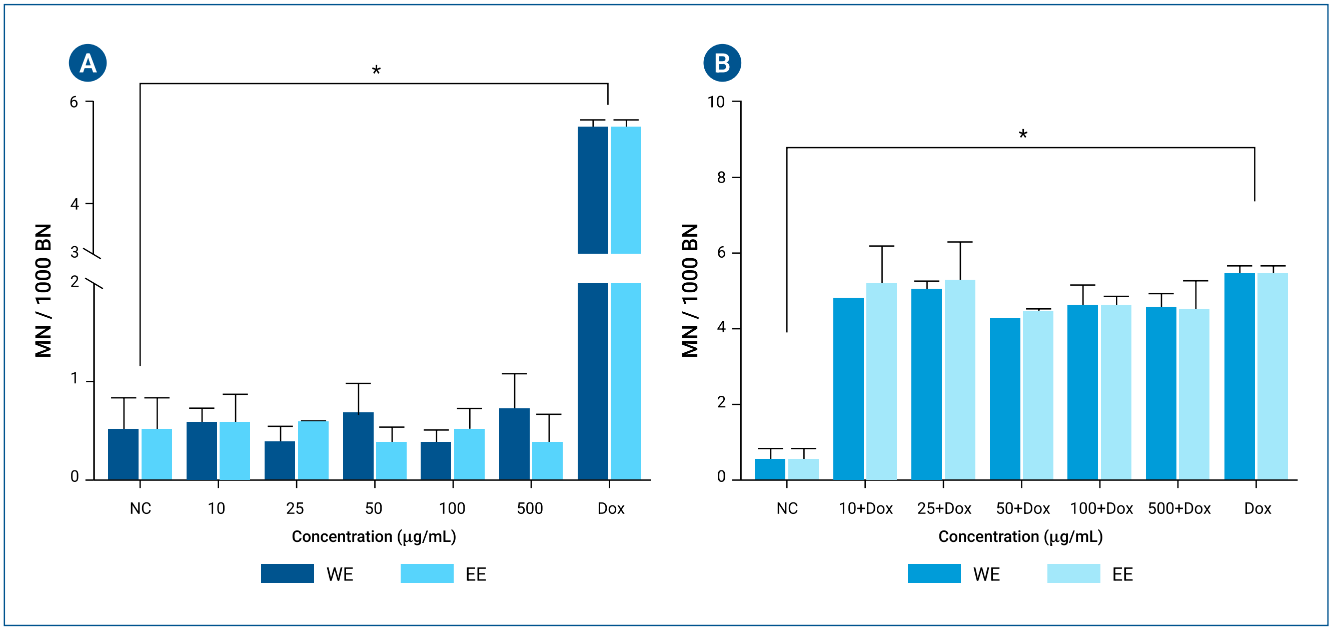

FIGURE 6. Frequency of micronucleus (MN) formation in CHO-K1 cells evaluated by the cytokinesis-block micronucleus (CBMN) assay.

(A) After treatment with *A. unedo* extracts alone.

(B) After co-treatment with *A. unedo* extracts and doxorubicin.

Cells were treated with aqueous (WE) and ethanolic (EE) extracts of *A. unedo* at concentrations of 10, 25, 50, 100, and 500 µg/mL. Doxorubicin (Dox, 1 µM) served as the positive control, and 0.5% (v/v) dimethyl sulfoxide (DMSO) as the negative control. Micronucleus frequency was determined by scoring 1,000 binucleated (BN) cells per sample. Results are expressed as mean ± standard error of the mean (SEM) from two independent experiments. $p<0.001$ versus the negative control group; for all other treatment groups, $p > 0.05$

In the co-treatment with doxorubicin (Figure 5B), neither extract significantly reduced DNA damage relative to the positive control, suggesting no notable protective effect against doxorubicin-induced genotoxicity under the tested conditions.

Results of Cytokinesis-block Micronucleus Assay

Figure 6 shows the genotoxic and antigenotoxic evaluation of *A. unedo* fruit extracts in CHO-K1 cells using the MN assay.

As shown in Figure 6A, neither the aqueous nor the ethanolic extracts (10–500 µg/mL) significantly increased micronuclei frequency compared to the DMSO control, indicating no clastogenic or aneugenic effects.

In co-treatment with doxorubicin (Figure 6B), the extracts did not significantly reduce micronuclei formation. All reductions were below 25%, suggesting no meaningful antigenotoxic activity under the tested conditions.

DISCUSSION

In this study, the biological activities of the ethanolic and aqueous extracts prepared from the ripe fruits of *A. unedo* were evaluated through antioxidant, antimutagenic, and antigenotoxic assays. The safety profiles of the extracts were also assessed using standard mutagenicity and genotoxicity tests. Phytochemical characterization was performed *via* TPC and TFC analyses, while HPTLC was employed to detect specific phenolic compounds.

Previous studies on *A. unedo* leaves have identified phenolic compounds such as quercetin 3-O-rhamnoside (quercitrin), quercetin 3-O-glucoside (isoquercitrin), myricitrin, and arbutin as major bioactive constituents (18). However, these compounds were not detected in our fruit extracts, suggesting that the chemical profile of the fruits may differ from that of the leaves. It is also worth noting that more sensitive techniques such as HPLC or LC-MS/MS may be necessary to confirm whether these compounds are present at trace levels. The phenolic composition of *A. unedo* fruits has been previously reported to vary by region. For example, fruits collected in Samsun (Türkiye) were found to contain gallic acid as the dominant phenolic compound, followed by protocatechuic acid, gentisic acid, *p*-hydroxybenzoic acid, vanillic acid, and m-anisic acid (19). In another study, wild fruits from northeastern Portugal were rich in flavan-3-ols and galloyl derivatives, followed by anthocyanins and flavanols (20). Spanish samples were shown to contain delphinidin 3-galactoside, cyanidin galactoside, cyanidin glucoside, and cyanidin arabinoside, with cyanidin galactoside being the predominant anthocyanin. Similarly, cyanidin 3-O-glucoside was reported as the major anthocyanin in fruits from Portugal (21). Taken together, these previously reported phenolic compounds, particularly anthocyanins and phenolic acids, may contribute to the antioxidant and antimutagenic activities observed in the ethanolic extract.

Both aqueous and ethanolic extracts contained notable levels of phenolics and flavonoids. The TPC was 33.7 ± 1.8 mg GAE/g for the aqueous extract and 46.5 ± 0.6 mg GAE/g for the ethanolic extract. Total flavonoid content values were 8.9 ± 0.2 mg CE/g and 14.2 ± 1.2 mg CE/g, respectively. These results align with those of Asmaa *et al.* (8), especially for the aqueous extract. The higher flavonoid content in the ethanolic extract may reflect ethanol's greater efficiency in extracting flavonoids or variations in fruit composition.

Both extracts showed notable radical scavenging activity, with the ethanol extract displaying superior antioxidant capacity, consistent with its higher phenolic and flavonoid content. This supports previous findings on ethanol's effectiveness in extracting phenolic compounds. In contrast, water primarily dissolves polysaccharides and other polar molecules, resulting in lower yields of phenolic and lipophilic constituents. Overall, these results suggest ethanol is a more efficient solvent than water for extracting antioxidant compounds from *A. unedo* fruits.

In the antimutagenicity assay, activity was observed only at the highest concentration in the TA100 strain with metabolic activation (S9). This suggests that the ethanolic extract may protect against base-pair substitutions rather than frameshift mutations. The requirement for S9 activation indicates that biotransformation products of the extract may be responsible for its antimutagenic effect. This aligns with previous reports showing that certain polyphenols and flavonoids inhibit cytochrome P450 enzymes (e.g., CYP1A1, CYP1A2), reducing the formation of mutagenic metabolites from procarcinogens (22).

On the other hand, no protective effects were observed in the comet assay against DNA strand breaks or in the MN against doxorubicin-induced clastogenic or aneugenic damage. While the Ames test showed moderate antimutagenic activity of the ethanolic extract at the highest concentration with S9 activation, no protective effects were observed in the comet or MN assays. This discrepancy may stem from fundamental differences between the assays. The Ames test is a bacterial system detecting point mutations, whereas the comet and MN assays are mammalian cell-based and detect DNA strand breaks or chromosomal damage. Notably, this is the first study to evaluate the genoprotective potential of *A. unedo* fruit extracts using both comet and MN assays.

Although scientific interest in *A. unedo* is growing, toxicological data on its fruits remain limited. Most previous genotoxicity assessments have focused on leaf extracts or isolated compounds rather than fruits. Jurica et al. (23, 24) investigated *A. unedo* leaf extracts and compounds such as arbutin and hydroquinone, reporting minimal cytotoxic and genotoxic effects in mammalian cell models. To our knowledge, this is the first study to comprehensively evaluate the safety of *A. unedo* fruit extracts using multiple genotoxicity bioassays.

In the Ames test, neither aqueous nor ethanol extracts exhibited mutagenicity in TA98 and TA100 strains, with or without metabolic activation (S9), indicating no direct or indirect mutagenic effects. These findings were supported by the MN assay, which showed no significant increase in micronuclei or change in the nuclear division index in CHO-K1 cells at any tested concentration, and by the comet assay, which revealed no significant DNA strand breaks compared to the positive control, doxorubicin. Collectively, these results indicate that *A. unedo* fruit extracts are non-genotoxic, in line with earlier reports on leaf extracts by Jurica et al. (23, 24).

This study presents the first comprehensive assessment of the phytochemical profile, antioxidant activity, and genotoxic/antigenotoxic potential of *A. unedo* fruits from Türkiye. The lack of mutagenic or genotoxic effects in both bacterial and mammalian systems supports their chemical safety. Moderate antimutagenic activity observed in the ethanolic extract at high concentrations, along with the absence of key reference compounds, points to a distinct fruit phytochemistry compared to the leaves. Overall, the findings confirm the non-genotoxic nature of *A. unedo* fruit extracts and suggest a limited, solvent-dependent chemopreventive potential.

However, the study is limited by the use of only two solvents for the extraction and a restricted set of chemical standards, which may overlook other active compounds. All tests were performed *in vitro*, which may not fully represent *in vivo* effects. Further research should include advanced techniques like HPLC, LC-MS/MS for broader phytochemical profiling and *in vivo* models to assess safety and efficacy. Seasonal, geographic, and dosage-related variations should also be explored to fully understand the biological potential of *A. unedo* fruits.

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REFERENCES

1 Miguel MG, Faleiro ML, Guerreiro AC, Antunes MD. *Arbutus unedo* L.: chemical and biological properties. Molecules. 2014;19(10):15799-823. [CrossRef]

2 Özcan MM, Hacıseferoğulları H. The strawberry (*Arbutus unedo* L.) fruits: Chemical composition, physical properties and mineral contents. J Food Eng. 2006;78(3):1022-8. [CrossRef]

3 Morgado S, Morgado M, Plácido AI, Roque F, Duarte AP. *Arbutus unedo* L.: From traditional medicine to potential uses in modern pharmacotherapy. J Ethnopharmacol. 2018;225:90-102. [CrossRef]

4 Bento I, Pereira JA. *Arbutus unedo* L. and its benefits on human health. J Food Nutr Res. 2011;50(2):73-85.

5 Carcache-Blanco EJ, Cuendet M, Park EJ, Su BN, Rivero-Cruz JF, Farnsworth NR, et al. Potential cancer chemopreventive agents from *Arbutus unedo*. Nat Prod Res. 2006;20(4):327-34. [CrossRef]

6 Mrabti HN, Bouyahya A, Ed-Dra A, Kachmar MR, Mrabti NN, Benali T, et al. Polyphenolic profile and biological properties of *Arbutus unedo* root extracts. Eur J Integr Med. 2021;42:101266. [CrossRef]

7 Güzelmeriç E, Ugurlu P, Çelik C, Şen NB, Helvacıoğlu S, Charehsaz M, et al. *Myrtus communis* L. (myrtle) plant parts: comparative assessment of their chemical compositions and antioxidant, anticancer, and antimutagenic activities. S Afr J Bot. 2022;150:711-8. [CrossRef]

8 Asmaa N, Abdelaziz G, Bakchiche B, Carbonell-Barrachina A, Cano-Lamadrid M, Noguera-Artiaga L. Chemical composition, antioxidant activity and mineral content of *Arbutus unedo* leaves and fruits. J Microbiol Biotechnol Food Sci. 2019;8:1335-9. [CrossRef]

9 Shraim AM, Ahmed TA, Rahman MM, Hijji YM. Determination of total flavonoid content by aluminum chloride assay: a critical evaluation. LWT. 2021;150:111932. [CrossRef]

10 Blois M. Antioxidant determinations by the use of a stable free radical. Nature. 1958;181:1199-200. [CrossRef]

11 Apak R, Güçlü K, Ozyürek M, Karademir SE. Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method. J Agric Food Chem. 2004;52(26):7970-81. [CrossRef]

12 Özyürek M, Güçlü K, Apak R. The main and modified CUPRAC methods of antioxidant measurement. TrAC Trends Anal Chem. 2011;30:652-64. [CrossRef]

13 Berker KI, Güçlü K, Demirata B, Apak R. A novel antioxidant assay of ferric reducing capacity measurement using ferrozine as the colour forming complexation reagent. Anal Methods. 2010;2:1770-6. [CrossRef]

14 Maron DM, Ames BN. Revised methods for the Salmonella mutagenicity test. Mutat Res. 1983;113(3-4):173-215. [CrossRef]

15 Zengin G, Uysal A, Gunes E, Aktumsek A. Survey of phytochemical composition and biological effects of three extracts from a wild plant (*Cotoneaster nummularia* Fisch. et Mey.): a potential source for functional food ingredients and drug formulations. PLoS One. 2014;9(11):e113527. [CrossRef]

16 Helvacioglu S, Charehsaz M, Bankoglu EE, Stopper H, Aydin A. The ameliorative effect of rosmarinic acid and epigallocatechin gallate against doxorubicin-induced genotoxicity. Drug Chem Toxicol. 2024;47(6):1087-99. [CrossRef]

17 Tice RR, Agurell E, Anderson D, Burlinson B, Hartmann A, Kobayashi H, et al. Single cell gel/comet assay: guidelines for *in vitro* and *in vivo* genetic toxicology testing. Environ Mol Mutagen. 2000;35(3):206-21. [CrossRef]

18 Oliveira I, Baptista P, Malheiro R, Casal S, Bento A, Pereira JA. Influence of strawberry tree (*Arbutus unedo* L.) fruit ripening stage on chemical composition and antioxidant activity. Food Res Int. 2011;44:1401-7. [CrossRef]

19 Ayaz FA, Kucukislamoglu M, Reunanen M. Sugar, non-volatile and phenolic acids composition of strawberry tree (*Arbutus unedo* L. var. ellipsoidea) Fruits. J. Food Compos. Anal. 2000;13:171-7. [CrossRef]

20 Guimarães R, Barros L, Dueñas M, Carvalho AM, Queiroz MJ, Santos-Buelga C, et al. Characterisation of phenolic compounds in wild fruits from Northeastern Portugal. Food Chem. 2013;141(4):3721-30. [CrossRef]

21 Pallauf K, Rivas-Gonzalo JC, del Castillo MD, Cano MP, de Pascual-Teresa S. Characterization of the antioxidant composition of strawberry tree (*Arbutus unedo* L.) fruits. J. Food Compos. Anal. 2008;21:273-81. [CrossRef]

22 Charehsaz M, Sipahi H, Giri AK, Aydin A. Antimutagenic and anticlastogenic effects of Turkish Black Tea on TA98 and TA100 strains of *Salmonella typhimurium* (*in vitro*) and mice (*in vivo*). Pharm Biol. 2017;55(1):1202-6. [CrossRef]

23 Jurica K, Brčić Karačonji I, Kopjar N, Shek-Vugrovečki A, Cikač T, Benković V. The effects of strawberry tree water leaf extract, arbutin and hydroquinone on haematological parameters and levels of primary DNA damage in white blood cells of rats. J Ethnopharmacol. 2018;215:83-90. [CrossRef]

24 Jurica K, Brčić Karačonji I, Mikolić A, Milojković-Opsenica D, Benković V, Kopjar N. *In vitro* safety assessment of the strawberry tree (*Arbutus unedo* L.) water leaf extract and arbutin in human peripheral blood lymphocytes. Cytotechnology. 2018;70(4):1261-18. [CrossRef]