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ORIGINAL ARTICLE

Antimutagenic and Antigenotoxic Effects of *Ruscus aculeatus* L. Rhizomes and Roots

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ABSTRACT

Objectives: *Ruscus aculeatus* L. (butcher's broom) is widely used for its medicinal virtues, mainly attributed to steroidal saponins such as ruscogenin and neoruscogenin glycosides. Despite its long-standing use, data on its genotoxicity and safety remain limited. The present study aimed to assess the potential antimutagenic and antigenotoxic effects of extracts prepared from the underground parts of *R. aculeatus*.

Materials and Methods: Ethanolic (EtOH) extract and decoction (aqueous extract) were prepared from the underground parts of *R. aculeatus*. Ames, micronucleus (MN), and comet assays were conducted to evaluate (anti)mutagenic and (anti)genotoxic effects. The quantification of ruscogenins in the acid-hydrolyzed extract was performed using high-performance liquid chromatography (HPLC) to standardise the plant material.

Results: Total ruscogenin and neoruscogenin content was calculated as 0.71% in the plant material by HPLC analyses. Neither the ethanolic nor the aqueous extract exhibited mutagenicity, while the EtOH extract showed marked antimutagenic activity at the highest concentration in the Ames test. Likewise, no genotoxicity was observed in either of the investigated extracts in the Micronucleus and Comet assays. EtOH extract exhibited antigenotoxic activity in the micronucleus assay, whereas the aqueous extract (decoction) provided no significant protection. The comet assay revealed no antigenotoxic activity for either the EtOH extract or the decoction. Overall, the underground parts of *R. aculeatus* displayed neither intrinsic mutagenicity nor genotoxicity, whereas the EtOH extract exhibited selective genoprotective effects.

Conclusion: The EtOH extract showed remarkable antimutagenic and antigenotoxic effects. This study constitutes the first report on the genoprotective and antimutagenic effects of *R. aculeatus*. From a toxicological perspective, the use of the underground parts of *R. aculeatus* is considered safe.

Keywords: Butcher's broom, ames, micronucleus, comet, HPLC

INTRODUCTION

Türkiye, located at the intersection of three continents, harbors high botanical diversity, with over 11,000 identified taxa, including 3,649 endemic species.[1,2] This richness is largely attributed to the country's diverse edaphic, geological, and topographical conditions. The genus Ruscus (Asparagaceae) is represented in Türkiye by four species, of which *Ruscus aculeatus* L., commonly known as butcher's broom, is widespread in coastal forests. It is a small evergreen shrub, reaching up to 80 cm in height and characterized by spiny cladodes and bright red fruits. Its rhizomes, functioning as underground storage organs, give rise to adventitious roots. Each year, buds located between

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the nodes and internodes on the rhizomes produce shoots that typically mature within one growing season.[3] The underground parts of *R. aculeatus* consist of rhizomes and roots, which are rich in steroidal saponins, particularly glycosides of ruscogenin and neoruscogenin.[4] In accordance with this phytochemical profile, the European Pharmacopoeia defines "Rusci rhizome" as the dried, entire or cut underground organs of *R. aculeatus* and requires that they contain not less than 1% steroidal sapogenins, calculated as the sum of ruscogenin and neoruscogenin.[5] The aerial parts of *R. aculeatus* contain saponins, flavonoids (such as apigenin, quercetin, and kaempferol derivatives), and a variety of phenolic acids, mainly p-coumaric acid.[6,7] In the berries of *R. aculeatus*, anthocyanins, especially pelargonidin derivatives, were detected.[8]

Traditionally, the rhizomes of *R. aculeatus* have been widely used in ethnomedicine across various cultures. In Türkiye, infusions of the fruits are administered orally to treat hemorrhoids, whereas decoctions of the underground parts are used to remove kidney stones and alleviate eczema.[9,10] In Palestinian folk medicine, the underground parts are applied topically to treat skin diseases.[11] In Italy, decoctions prepared from the rhizomes have traditionally been used for the management of warts and chilblains.[12] Apart from its traditional use, *R. aculeatus* has been the subject of numerous studies investigating its antioxidant, anti-inflammatory, and anticancer activities.[6] Mimaki et al.[13] investigated the cytotoxic potential of saponins isolated from the underground parts of *R. aculeatus* in human promyelocytic leukemia HL-60 cells using the MTT assay. Among the isolated compounds, a furostanol saponin bearing a diglycoside moiety acylated with a (2S,3S)-2-hydroxy-3-methylpentanoic acid and an acetyl group, as well as its corresponding spirostanol saponin, exhibited pronounced cytotoxic activity, with IC_{50} values of approximately 3.5 and 3.0 µg/mL, respectively.[13] Zhao et al.[14] showed that ruscogenin exerts anti-apoptotic effects against lipopolysaccharide-induced pulmonary endothelial apoptosis by suppressing TLR4/MyD88/NF-κB signaling, suggesting that it could be a promising therapeutic agent for acute lung injury. In addition to the pharmacological activities previously investigated, its venotonic and vasoprotective properties have attracted particular attention, leading to the development of formulations to treat chronic venous insufficiency and hemorrhoids. Phenolic compounds and spirostanol saponins isolated from a methanolic extract of the underground parts of *R. aculeatus* were evaluated for their ability to inhibit thrombin-induced hyperpermeability in human microvascular endothelial cells and were compared with the aglycone neoruscogenin. Neoruscogenin exerted a mild concentration-dependent effect, reducing hyperpermeability to 71.8% at 100 µM. The highest activities were observed at 10 µM for the spirostanol saponins deglucoruscin and ruscin, and for esculin, resulting in reductions in thrombin-induced hyperpermeability to 41.9%, 42.6%, and 53.3%, respectively. These findings support the anti-edematous and vasoprotective role of *R. aculeatus* in chronic venous disorders.[15]

Despite the widespread use of *R. aculeatus* in both traditional and contemporary medicine, there are currently no reports on its potential genoprotective properties, including antimutagenic and antigenotoxic activities. Moreover, the European Medicines Agency 2018 report on the preclinical safety of *R. aculeatus* rhizome indicates that the available data address only general aspects of safety and that the lack of genotoxicity studies prevents a comprehensive safety evaluation.[16] Therefore, this study aimed to assess the potential (anti)mutagenic and (anti) genotoxic effects of the underground parts of *R. aculeatus*, addressing a gap in the literature. In addition, the plant material investigated in this study was standardized for ruscogenin and neoruscogenin using the HPLC method described in the European Pharmacopoeia.[5]

MATERIALS AND METHODS

Chemicals and solvents

HPLC-grade acetonitrile was purchased from Merck (Darmstadt, Germany). Analytical-grade methanol, dichloromethane and n-butanol were obtained from Sigma-Aldrich (Steinheim, Germany), while ethanol (EtOH) and hydrochloric acid (37%) were supplied by IsoLab (Eschau, Germany). Potassium hydroxide was also purchased from Sigma-Aldrich (Steinheim, Germany). A standard mixture containing ruscogenin and neoruscogenin in a ratio of 3:7 (w/w) was obtained from Bionorm (İzmir, Türkiye). *Salmonella typhimurium* strains and the post-mitochondrial fraction (S9) prepared from rat liver were supplied by Moltox Molecular Toxicology, Inc. (NC, USA). 2-aminofluorene was obtained from Merck (Hohenbrunn, Germany), and nutrient broth was supplied by Hi Media Laboratories Ltd. (Mumbai, India). Histidine was provided by Fluka (USA). Ethics committee approval and patient informed consent were not required for this study.

Plant material

The underground parts (roots and rhizomes) of *R. aculeatus* were collected in November 2023 in Şile, İstanbul, Türkiye (coordinates: 41.07624° N, 29.32419° E). The plant material was identified by Prof. Dr. Hasan Kırmızıbekmez, and the herbarium specimen (YEF 23014) has been deposited in the Herbarium of the Yeditepe University Faculty of Pharmacy, Department of Pharmacognosy, İstanbul, Türkiye.

Extraction of the plant material

To prepare the ethanolic (EtOH) extract, 50 g of dried and powdered plant material was macerated in 500 mL of ethanol overnight and then extracted at 45 °C for 4 h. After filtration, the solvent was removed under reduced pressure, and the resulting residue was dispersed in distilled water and then lyophilized (yield: 10.0%).

For the decoction, 50 g of dried and coarsely powdered plant material was mixed with 500 mL of distilled water, and the mixture was gradually heated to boiling. The mixture was then simmered over low heat for 30 min, filtered while hot, and lyophilized (yield: 10.4%).

The acid-hydrolyzed extract was prepared according to the European Pharmacopoeia to liberate aglycones.[5] The powdered herbal drug (2.000 g) was mixed with a solvent mixture (75 mL)

consisting of ethanol and distilled water (4:1, v/v) and potassium hydroxide (0.2 g), then extracted under a reflux condenser for 4 h. After cooling, the mixture was filtered into a 100 mL volumetric flask. The residue was rinsed with anhydrous ethanol (3 × 10 mL), and the rinsings were combined with the filtrate. The volume was then adjusted to 100 mL with anhydrous ethanol. Subsequently, a 25 mL aliquot of this solution was transferred to a round-bottom flask and evaporated to dryness under reduced pressure. The residue was dissolved in n-butanol (10 mL), followed by the addition of hydrochloric acid (3 mL) and distilled water (8 mL). The mixture was hydrolyzed under a reflux condenser for 1 h, and then cooled and transferred to a 100 mL volumetric flask. The round-bottom flask was rinsed with methanol (3 × 20 mL); the rinsings were combined, and the final volume was adjusted to 100 mL with methanol.

Thin-layer chromatography (TLC) analysis

TLC was performed on the EtOH extract and decoction to obtain preliminary compositional information and evaluate the presence of the sapogenins ruscogenin and neoruscogenin. The standard solution containing ruscogenin and neoruscogenin at a concentration of 2 mg/mL was used as the reference solution.

The sample test and the standard solutions were first applied to a silica gel plate and developed using dichloromethane-methanol-distilled water (80:20:2, v/v/v) as a mobile phase. After development, plates were derivatized with vanillin reagent, dried at 100–105 °C for 2 minutes, and evaluated under UV (366 nm) and white light.

High-performance liquid chromatography (HPLC) analysis

The Agilent 1260 Infinity HPLC system (Darmstadt, Germany) was used for the quantitative evaluation of ruscogenin and neoruscogenin in the sample test solutions. In accordance with the European Pharmacopoeia, the chromatographic separation of ruscogenin and neoruscogenin was achieved on a reverse-phase Agilent Zorbax Extend C_{18} column (4.6 mm x 250 mm, 5-µm particle size) using mobile phases A (distilled water) and B (acetonitrile). The mobile phase gradient was programmed as follows: 60% B (0–25 min), 60–100% B (25--27 min), 100% B (27–37 min). The flow rate was set to 1.2 mL/min, the injection volume was 20 µL, and detection was performed at 203 nm. Peaks were identified by comparing the retention times (t_R) of ruscogenin and neoruscogenin obtained from HPLC chromatograms of the standard solution (0.05 mg/mL) and sample test solutions. The percentage content of total sapogenins (ruscogenin and neoruscogenin) in the sample test solution was calculated using the following equation:

$$[(A_1 \times m_2 \times 4 \times p_1) / (A_2 \times m_1)] + [(A_3 \times m_2 \times 4 \times p_2) / (A_4 \times m_1)]$$

A_1 is the area of the peak belong to ruscogenin in the sample test solution obtained with the HPLC chromatogram. A_2 is the area of the peak of ruscogenin in the reference solution. A_3 is the area of the peak belong to neoruscogenin in the sample test solution obtained with the HPLC chromatogram. A_4 is the area of the peak of neoruscogenin in the reference solution. m_1 is the mass of the herbal drug in the test solution. m_2 is the mass of the ruscogenins in reference solution. p is the percentage content of ruscogenin and neoruscogenin.

Ames mutagenicity/antimutagenicity assay

Stock solutions of the EtOH and aqueous extracts were prepared by dissolving 150 mg of each in 1.5 mL of dimethyl sulfoxide (DMSO), followed by serial dilutions to obtain final concentrations of 10, 100, 1000, and 5000 µg/plate. *Salmonella typhimurium* strains were cultured at 37 °C and 120 rpm for 12–16 h to reach 1–2 × 10^9 cells/mL.

For the Ames assay, 0.05 mL of test sample, 0.5 mL of phosphate buffer (–S9) or S9 mix (+S9), and 0.1 mL of bacterial culture were mixed with top agar containing histidine–biotin (0.05 mM) and poured onto MGA plates. Plates were incubated at 37 °C for 48 h, after which the revertant colonies were counted and compared with controls. Antimutagenicity was evaluated by co-treating samples with standard mutagens in the presence or absence of S9 and was expressed as the percentage inhibition of revertant formation.[17]

Genotoxicity/antigenotoxicity assays

Cell culture and sample preparation

CHO cells (ATCC; CCL61) were cultured at 37 °C with 5% CO_2. The extract stock solutions (200 mg/mL in DMSO) were diluted in medium to final concentrations of 10, 50, 100, and 200 µg/mL.

Dose selection

Cytotoxicity was determined by MTT assay after a 24 h exposure, followed by a 3 h MTT incubation. Formazan crystals were solubilized in isopropanol and the absorbance was measured to calculate cell viability.

Micronucleus (MN) assay

The MN assay was carried out in an accredited laboratory (TÜRKAK TS EN ISO/IEC 17025, No: AB-1764-T) following the procedure described by Helvacioglu et al.[18] CHO-K1 cells (2 × 10^9 cells/well) were seeded in 6-well plates and treated with the extracts for 24 h. DMSO (0.5%) and EMS (6 mM) served as the negative and positive controls, respectively. After exposure, cells were incubated with cytochalasin B (2.4 µg/mL), then subjected to hypotonic treatment in 0.07 M KCl, fixed with methanol:acetic acid (3:1), and stained with 5% Giemsa solution. A total of 1,000 binucleated cells per group were analyzed microscopically (Zeiss Primo Star, 40×). MN frequency (MN%) and nuclear division index were determined using established formulas.[18]

Comet assay

CHO-K1 cells (3 × 10^9 cells/well) were seeded into 6-well plates, pre-incubated for 24 h, and then exposed to different extract concentrations. DMSO (0.5%) and EMS (5 mM) were used as negative and positive controls. Following 4 h of treatment, cells were harvested, mixed with low-melting-point agarose, applied to pre-coated slides, lysed, electrophoresed, and fixed. DNA was stained with ethidium bromide and observed under a fluorescence microscope (Carl Zeiss AG, Oberkochen, Germany). Tail parameters were evaluated using Bs 200

Pro software, with 100 nucleoids scored per treatment. DNA damage was expressed as the percentage of DNA in the tail.[18]

Antigenotoxic properties of the extracts were further assessed by co-treatment with EMS (6 mM for the MN assay and 5 mM for the comet assay). Exposure times were 24 h and 4 h, respectively, and the protective effects were evaluated following the same procedures.

Statistical analysis

Statistical analyses were performed using GraphPad Prism version 7.0 (GraphPad Software, La Jolla, CA, USA). Statistical significance was set at $p \leq 0.05$. Depending on the data distribution and experimental design, comparisons were performed using Student's t-test or the Kruskal-Wallis test. For the Ames test, Dunnett's multiple comparison test was used to compare treatment groups with the spontaneous control.

RESULTS

Chemical profiling of the R. aculeatus underground parts

The extracts prepared from the underground parts of *R. aculeatus* were evaluated for their antimutagenic and antigenotoxic potential using two extraction approaches. An acid hydrolysis step was applied to estimate the amounts of steroidal sapogenins, ruscogenin, and neoruscogenin. To perform chemical fingerprinting and check for the presence of reference compounds in the extracts, TLC analysis was performed. TLC chromatograms of the EtOH and aqueous extracts showed no bands co-migrating with the standards (ruscogenin/neoruscogenin mixture). By contrast, the acid hydrolyzed extract displayed distinct bands at the expected retardation factor (R_F) values for ruscogenin/neoruscogenin (data not shown), indicating liberation of the aglycones upon hydrolysis. Phytochemically, these patterns are consistent with ruscogenin/neoruscogenin being present predominantly as glycosidic forms (saponins) in the native matrix; decoction and, to a lesser extent, EtOH extraction under the conditions used yielded extracts largely depleted of free aglycones.

Further analyses were conducted with HPLC. As shown in Figure 1, the retention times (t_R) of neoruscogenin and ruscogenin were approximately 16.97 and 21.42 min, respectively. Peak identities were confirmed based on retention times. The EtOH extract (10 mg/mL) showed the presence of neoruscogenin, whereas ruscogenin was not observed (Figure 2). In contrast, the extract prepared by decoction (5 mg/mL) showed no peaks corresponding to either of the sapogenins (Figure 3). However, the acid-hydrolyzed extract contained detectable peaks for both neoruscogenin and ruscogenin (Figure 4), confirming that acid hydrolysis liberates sapogenins from saponins, as expected.

According to the pharmacopoeial (European Pharmacopoeia) specifications, the minimum required content of total sapogenins (neoruscogenin and ruscogenin) is 1%.[5] Based on the HPLC analysis of the acid-hydrolyzed extract, the total

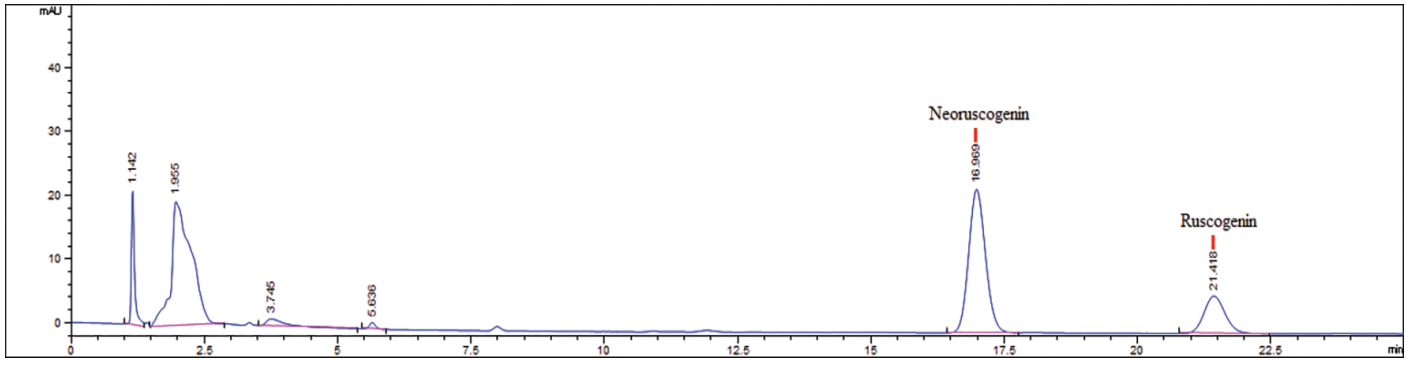

Figure 1. HPLC chromatogram of neoruscogenin and ruscogenin at 203 nm.
HPLC, high-performance liquid chromatography.

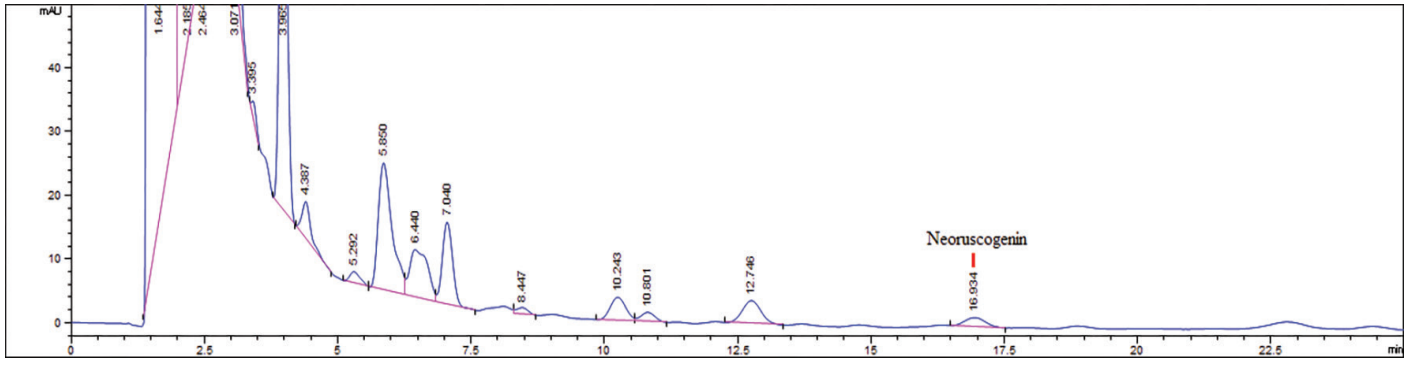

Figure 2. HPLC chromatogram of EtOH extract of *R. aculetaus* (10 mg/mL) at 203 nm.
EtOH, ethanolic; HPLC, high-performance liquid chromatography.

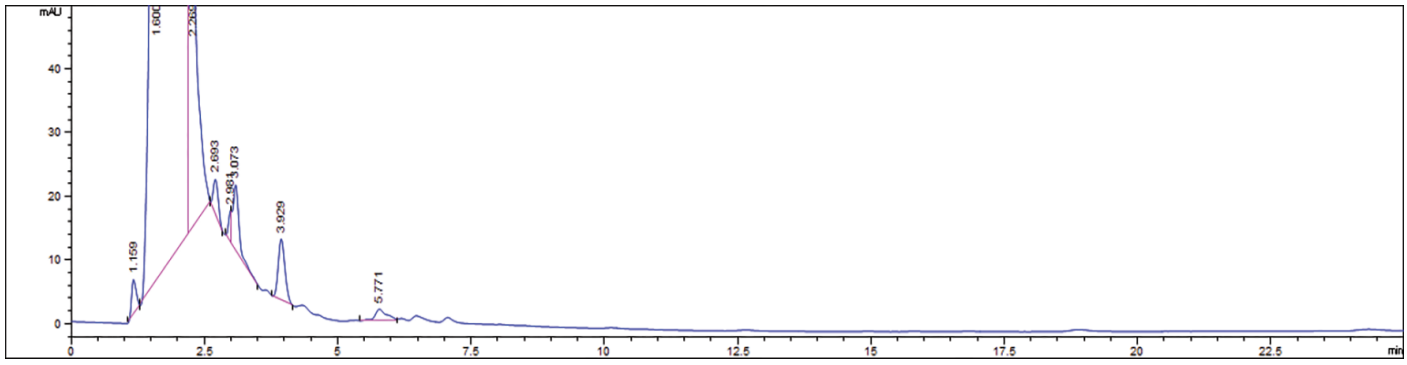

Figure 3. HPLC chromatogram of decoction of *R. aculeatus* (5 mg/mL) at 203 nm.
HPLC, high-performance liquid chromatography; *R. aculeatus*, *Ruscus aculeatus*.

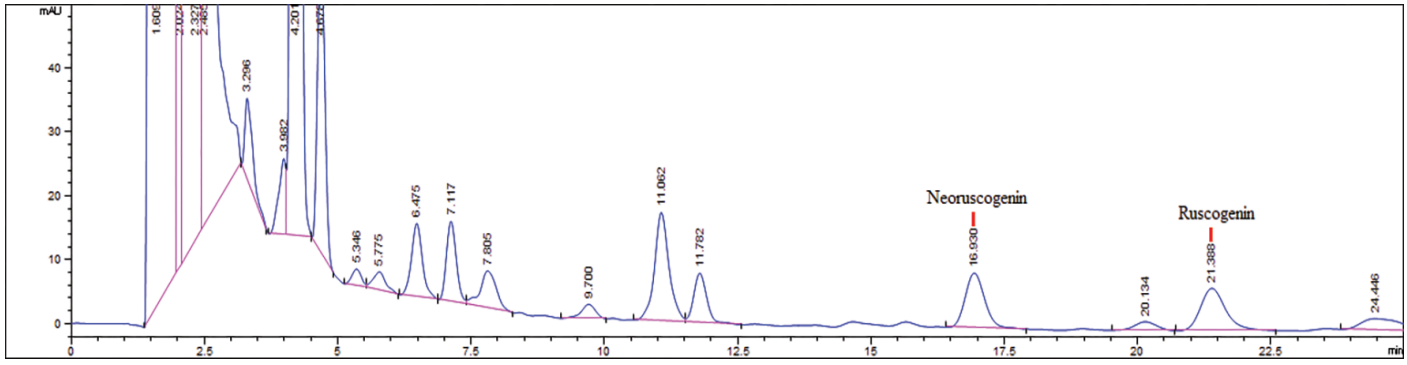

Figure 4. HPLC chromatogram of acid hydrolyzed extract of *R. aculeatus* at 203 nm.
HPLC, high-performance liquid chromatography; *R. aculeatus*, *Ruscus aculeatus*.

sapogenin content was 0.71%. As previously noted, HPLC analysis revealed no detectable sapogenin peaks in the extract prepared by decoction and only a minor peak in the EtOH extract, whereas clear peaks were observed following acid hydrolysis. These findings indicate that sapogenins are predominantly present in the raw material as glycosides, and hydrolysis is essential to meet pharmacopoeial compliance. Therefore, EP indicates an acid hydrolysis reaction prior to HPLC analysis.

Mutagenicity/antimutagenicity and genotoxicity/antigenotoxicity

The mutagenicity of the EtOH extract and decoction prepared from the underground parts of *R. aculeatus* was assessed in *Salmonella typhimurium* strains TA98 and TA100, and the mutagenic index (MI) was defined as (revertants in treated plates)/(revertants in the negative control). MI values remained < 2 for all tested concentrations, indicating the absence of a mutagenic effect.[19]

Consistent with these findings, the MN assay showed no significant increase in frequency of micronuclei (Figure 5), and the alkaline comet assay in CHO cells showed no significant change in % tail DNA relative to the negative control (Figure 6). Taken together, these results indicate that neither the EtOH extract nor the decoction exhibits mutagenic or genotoxic effects under the conditions tested, supporting the safety of *R. aculeatus* preparations in the context of traditional use and potential therapeutic applications.

Antimutagenic and antigenotoxic activities of the EtOH extract and the decoction from the underground parts of *R. aculeatus* were evaluated against known mutagenic/genotoxic agents. At the highest doses tested in the Ames assay (5000 µg/plate), the EtOH extract produced a marked inhibition of S9 activated mutagenicity in both Salmonella strains, meeting the criterion for strong antimutagenic activity (≥ 40% inhibition; Figure 7).

In the MN assay, EMS-induced increases in MN/1000 BN were significantly reduced by the EtOH extract ($p < 0.01$), demonstrating a clearer dose-dependent effect at 100–200 µg/mL, whereas the decoction produced no significant protection (Figure 8). In contrast, comet assay readouts (% tail DNA) did not differ significantly from the positive control for either extract, despite a downward trend at higher EtOH extract concentrations that was not statistically significant ($p > 0.05$) (Figure 9).

Taken together, these data suggest that, while the EtOH extract exhibits robust antimutagenic activity and antigenotoxic effects detectable at the chromosomal level (MN), it does not measurably protect against direct DNA strand breaks under the comet assay conditions used, whereas the decoction shows no detectable protection in either system.

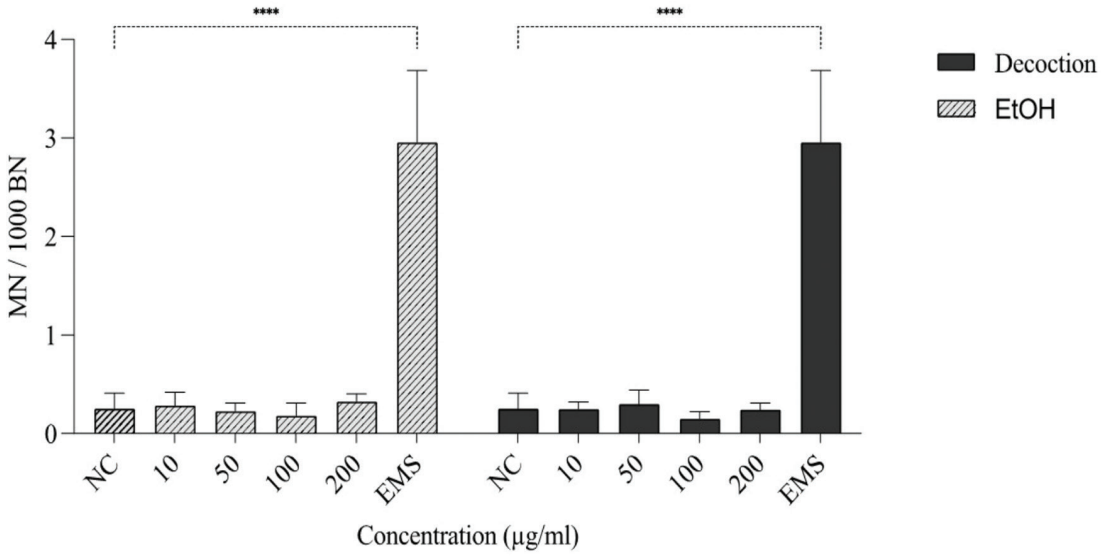

Figure 5. Cytokinesis-block MN assay results. The data represent the mean ± standard deviation from three independent experiments. Statistical analysis was performed using a one-way ANOVA followed by Dunnett's post-hoc test. ****$p < 0.0001$. The concentration of EMS used as a positive control was 6 mM.

ANOVA, analysis of variance; EMS, ethyl methanesulfonate; MN, micronucleus; NC: negative control.

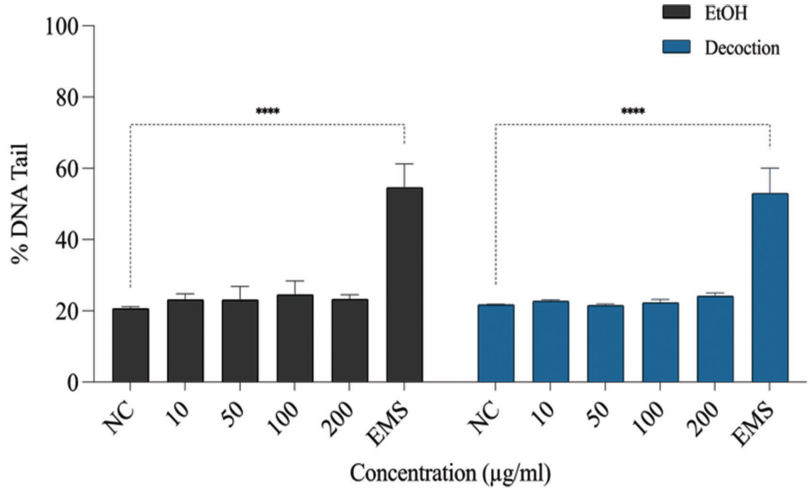

Figure 6. Comet assay results (% DNA in tail) in CHO cells. The data represent the mean ± standard deviation from three independent experiments. Statistical analysis was performed using one-way ANOVA followed by Dunnett's post-hoc test. ****$p < 0.0001$. The concentration of EMS used as a positive control was 5 mM.

ANOVA, analysis of variance; CHO: Chinese hamster ovary; EMS, ethyl methanesulfonate; EtOH, ethanol; MN, micronucleus; NC: negative control.

DISCUSSION

According to the HPLC findings, the acid-hydrolyzed extract exhibited markedly higher ruscogenin and neoruscogenin levels than the non-hydrolyzed extracts, as expected. Consistent with these results, Vlase et al.[20] reported that quantification of total ruscogenin and neoruscogenin (present mainly as saponins and only in small amounts as free aglycones) required a hydrolysis step achieved by heating samples with sulfuric acid, followed by extraction of the liberated sapogenins into an organic solvent. Comparable variability in sapogenin content has also been reported in the literature. Ozer et al.[21] determined the total sapogenin content of the underground parts of *R. aculeatus* collected from eighteen different locations in the Marmara region (Türkiye) using the HPLC method described in the European Pharmacopoeia, reporting values ranging from 0.5% to 1.5%, which were also in line with our findings. Similarly, Vlase et al.[20] reported ruscogenin and neoruscogenin contents of 0.020% and 0.046%, respectively, in roots of *R. aculeatus* collected in Romania in May by LC–MS/MS. In another study,

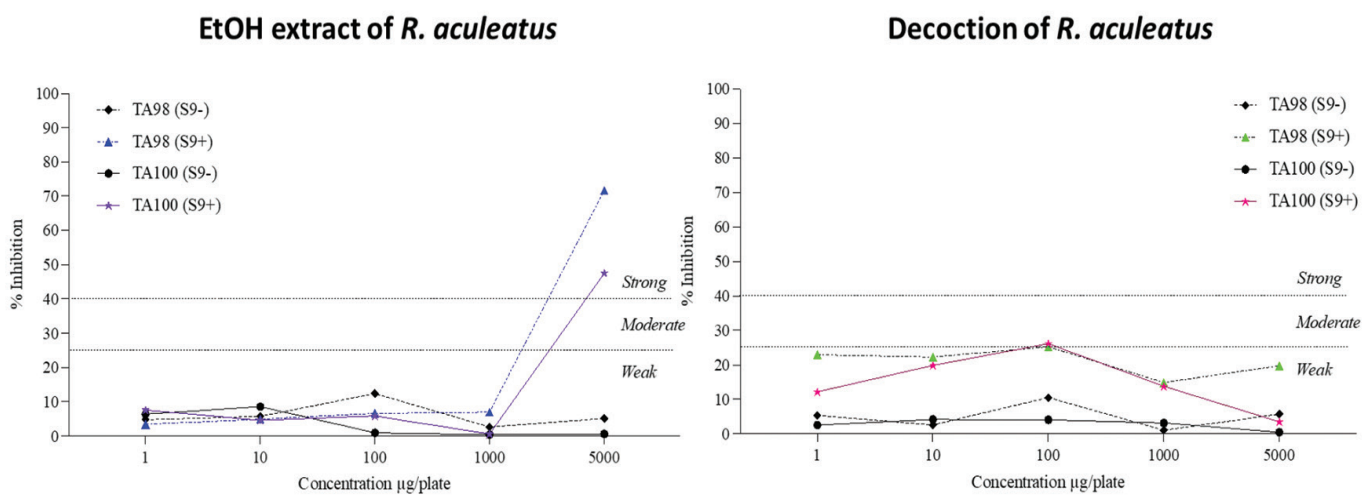

Figure 7. Antimutagenicity results of EtOH extract and decoction.
EtOH, ethanolic; *R. aculeatus, Ruscus aculeatus.*

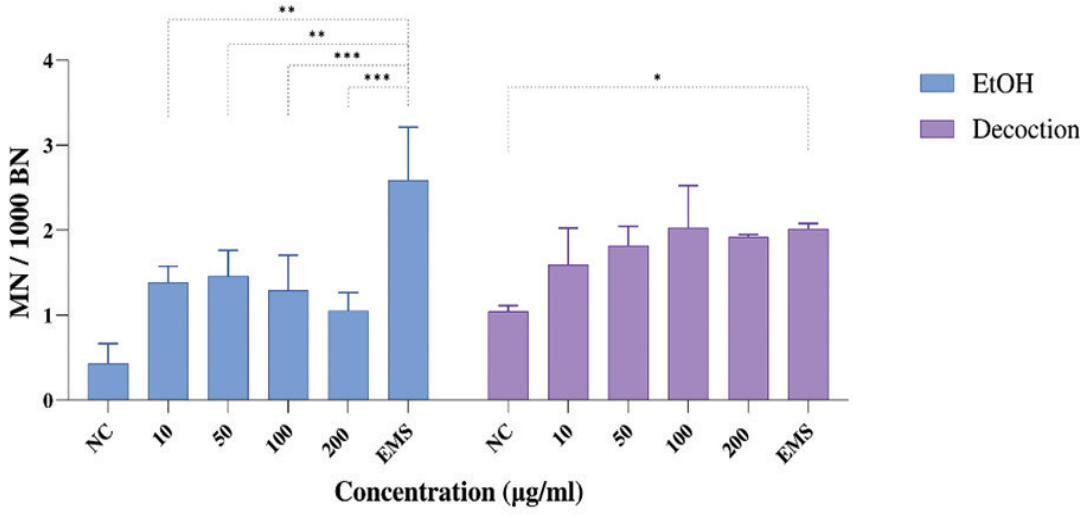

Figure 8. Cytokinesis-block MN assay results for antigenotoxicity assessment for extracts. The data represent the mean ± standard deviation from three independent experiments. Statistical analysis was performed using one-way ANOVA followed by Dunnett's post-hoc test. Comparisons were made between the positive control group and each treatment group. Statistical significance is indicated by asterisks: not significant ($p > 0.05$), *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$. The concentration of EMS used as a positive control was 6 mM.
ANOVA, analysis of variance; EMS, ethyl methanesulfonate; EtOH, ethanol; MN, micronucleus; NC: negative control.

Güvenç et al.[22] analyzed Ruscus taxa collected from different regions and seasons in Türkiye using ultra-performance liquid chromatography and reported free ruscogenin contents between 0.05% and 0.22%; however, following acid hydrolysis, total ruscogenin levels increased to between 0.7% and 1.5%. Collectively, these findings suggest that both analytical methodology and raw material variability, including collection site and season, play a critical role in determining sapogenin content.

Regarding the results of antimutagenic and antigenotoxic assays, the lack of antimutagenic and antigenotoxic activity of the decoction may be explained by its abundance of polysaccharides, resulting in relatively lower amounts of potential bioactive molecules (i.e., steroidal saponins and phenolic metabolites) compared with the EtOH extract. In line with our toxicological results, Verschaeve et al.[23] reported that aqueous leaf and fruit extracts of *Ruscus hypophyllum* L. exhibited no genotoxicity in the bacterial Vitotox test and induced effects only at high concentrations in the Comet assay performed on human C3A hepatocytes.

The phytochemical and toxicological studies were performed on plant material collected from only one location. Thus, the

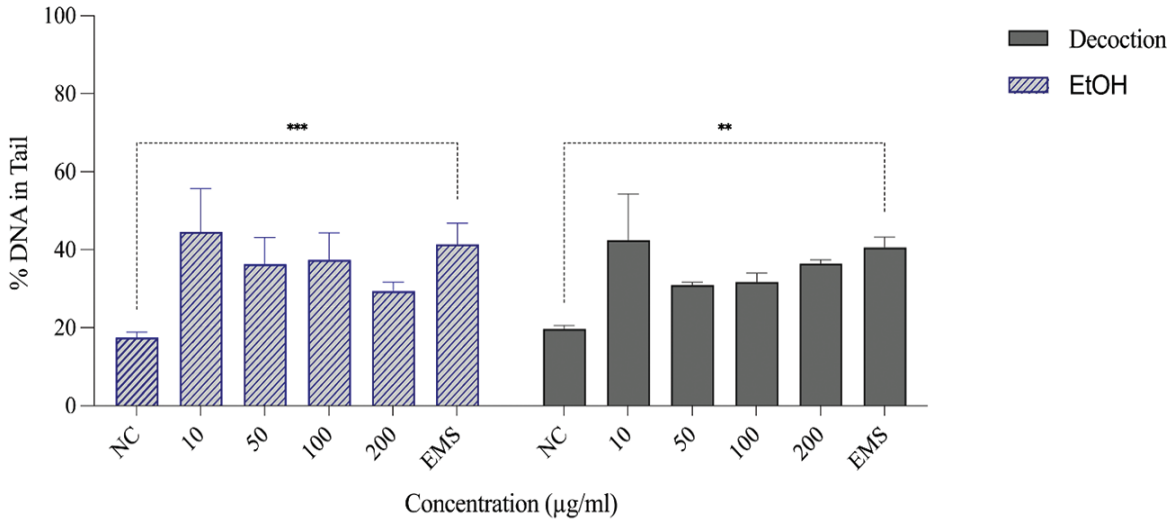

Figure 9. Comet assay results for the extracts (% DNA in tail) in CHO cells. The data represent the mean ± standard deviation from three independent experiments. Statistical analysis was performed using one-way ANOVA followed by Dunnett's post-hoc test. Comparisons were made between the positive control group and each treatment group. Statistical significance is indicated by asterisks: not significant ($p > 0.05$),*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$. The concentration of EMS used as a positive control was 6 mM.

ANOVA, analysis of variance; CHO: Chinese hamster ovary; EMS, ethyl methanesulfonate; EtOH, ethanol; MN, micronucleus; NC: negative control.

results cannot be extrapolated to all *R. aculeatus* specimens that grow wild in the flora of Türkiye.

CONCLUSION

This study constitutes the first report on the genoprotective and antimutagenic effects of *R. aculeatus* extracts. The plant material was also standardized for sapogenin content. The findings of this study will shed light on further comprehensive safety profile studies on *R. aculeatus*. Furthermore, the cancer chemopreventive potential of *R. aculeatus* extracts warrants attention. Besides, different extraction methods, such as Ultrasound-Assisted Extraction, Soxhlet Extraction, Pressurized Liquid Extraction, and Microwave-Assisted Extraction, can be used to prepare extracts and compare their bioactivities in future studies.

Ethics

Ethics Committee Approval: There is not required for ethical approval.

Informed Consent: There is not required for informed consent.

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Footnotes

Authorship Contributions

Concept: M.H., H.K., Design: E.G., M.H., H.K., Data Collection or Processing: G.E., D.T., H.Ko., E.G., M.H., Analysis or Interpretation: G.E., D.T., H.Ko., E.G., M.H., Literature Search: G.E., D.T., E.G., H.K., Writing: G.E., E.G., M.H., H.K.

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