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Potential effect of fruit and flower extracts of *Arbutus unedo* L. on *Tetrahymena pyriformis* exposed to a cobalt-60 source

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Tetrahymena pyriformis

Aqueous extract

Ethanolic extract

Antioxidant

ABSTRACT

Exposure of *Tetrahymena pyriformis* cultures to cobalt-60 for 72 h significantly impacted the cells' growth, appearance, and physiology. This study aims to investigate the protective effects of *Arbutus unedo L* flowers and fruit extracts on *T. pyriformis* against gamma radiation. Initially, aqueous and 50% ethanolic extracts of the fruits and flowers were prepared, and their cytotoxicity on the ciliate was evaluated. The irradiated ciliate's cellular viability and morphological aspect improved when a non-toxic concentration of 25 µg/mL was added to the growth medium. The addition of extracts restored glyceraldehyde-3-phosphate dehydrogenase and succinate dehydrogenase activities to their initial levels, similar to non-irradiated cells. In addition, the extracts reduced oxidative stress markers, such as lipid peroxidation, and decreased the activities of antioxidant defence enzymes, catalase, and superoxide dismutase. This may be attributed to the antioxidant properties of the extracts. Results of this study revealed that the flower extracts exhibited better protective effects than the fruit extracts, with superior antioxidant activity in the in-vitro DPPH scavenging assay. These results suggest that *A. unedo* flower extracts may have potential as exogenous radioprotective agents.

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1 Introduction

The use of plant extracts for preventive and therapeutic purposes has attracted increasing research interest in recent years, as extracts are composed of complexes of plant metabolites, and their benefits have been recognized (Başaran et al. 2022). *Arbutus* species (Ericaceae) are traditionally used to treat various diseases and are known for their antioxidant, antiseptic, antidiabetic, diuretic, anti-inflammatory, and other properties (Mrabti et al. 2021). The strawberry tree (*Arbutus unedo* L.) is a small tree of the Ericaceae family native to the Mediterranean region that prefers regions with warm summers and temperate winters and is found in Europe (Portugal, Spain, France, Italy etc.), the Canary Islands, North Africa (Morocco, Algeria, Tunisia), and Western Asia (De Santis et al. 2023). *A. unedo* is a shrub with high resistance to difficult climatic and soil conditions, with rapid regeneration after forest fires, which is particularly important for faunal diversity and prevention of soil erosion (Helluy et al. 2021). The red and edible fruits of the strawberry tree, which generally coexist with the pinkish-white flowers that appear in winter, are part of the Mediterranean diet (Sanna et al. 2023). The plant's fruit has been used in several countries for therapeutic purposes in traditional medicine. In Morocco, different parts (fruit, root, leaves, and flowers) of *A. unedo*, also known as "sasnou", are often used traditionally to treat hypertension, diabetes, heart disease, high cholesterol, digestive problems, and as a diuretic, antidiarrheal, and anti-inflammatory (El Haouari et al. 2021). Because of its nutritional and medicinal values, several studies have investigated the composition of different extracts of *A. unedo*. The plant is an important source of phytochemicals, bioactive compounds (phenols, anthocyanins, triterpenes, iridoids, sterols), vitamins, minerals, essential fatty acids, and dietary fiber, which gives it interesting biological activities such as antioxidant, anti-inflammatory, antimicrobial and other activities (Wahabi et al. 2023).

Extracts of this plant are considered an important source of antioxidants, flavonoids, acid derivatives, anthocyanins, carotenoids, vitamins, etc. (Bajoub et al. 2023); it would be interesting to explore these properties against the prevention of reactive oxygen species (ROS) generated indirectly by ionizing radiation (IR). In fact, IR such as gamma radiation contributes indirectly, through the radiolysis of water, to the appearance of ROS such as superoxide ($O_2^{\bullet-}$), hydroxyl radicals ($HO^{\bullet}$), hydrogen radicals ($H^{\bullet}$), and hydrogen peroxide (H_2O_2) (Șolpan et al. 2022). An increase of these free radicals causes a cellular imbalance, leading to the appearance of oxidative stress by altering the activities of enzymes, membrane structure, and DNA, and consequently, the development of various pathologies (Ponnampalam et al. 2022; Pardillo-Díaz et al. 2022; Sadiq 2023).

Previous work has shown the damage caused by ROS generated after exposure to gamma radiation on a ciliated protozoan *Tetrahymena pyriformis* (Ziyadi et al. 2022b). The use of this ciliate as a model in various studies presented several advantages, such as being unicellular with a short life cycle, easy to culture under laboratory conditions, and the possibility of analyzing the toxic effects of substances over several generations (Lim 2022; Sabraoui et al. 2022). Previous study showed that IR affected the growth, morphology, and activities of metabolic enzymes, increased the production of ROS and their damage, and activated the antioxidant system of the protozoa after 72 h of exposure to a radioactive source of cobalt-60 (^{60}Co)(Ziyadi et al. 2022b).

In this context, protection against these IR-induced cellular perturbations by evaluating the radioprotective and antioxidant effects of natural plants known for their therapeutic virtues will be an interesting area of research (Ziyadi et al. 2022a). Therefore, this study aimed to evaluate the ability of aqueous and ethanolic extracts of the two plant parts of *A. unedo* (flowers and fruits) to reduce the effects observed in *T. pyriformis* after exposure to a ^{60}Co source. The study included growth (number and time of generation), morphological aspect, metabolic enzymes (glyceraldehyde-3-phosphate dehydrogenase-GAPDH and succinate dehydrogenase-SDH), as well as oxidative markers such as lipid peroxidation and antioxidant enzymes (catalase-CAT and superoxide dismutase-SOD).

2 Material and methods

2.1 Plant harvest and extract preparation

The flowers and the fruits of the strawberry tree (*A. unedo*) were collected in January 2022 (the tree produces flowers at the same time as the fruit ripens) from the "Forest of El Harcha" site in the "Rabat-Salé-Kénitra" region, Morocco (33°28'31.296" N latitude and 6°8'50.568" O longitude). Samples were immediately frozen at -20°C and then were dried using a lyophilization system (Labconco). Lyophilized samples were ground to a fine powder. The aqueous extract was obtained by decoction under reflux (30 min) of 25 g of powder with 250 mL of HPLC-grade water. After filtration and evaporation at 60°C, the extract obtained was frozen and finally dried by lyophilization. The 50% ethanolic extract was obtained by hydroalcoholic maceration (5 g of lyophilized powder /100 mL (w/v)) for 48 h. The mixture was filtered and evaporated at 65°C, dried through a desiccator, and stored at -20°C.

2.2 Antioxidant activity

The antioxidant activity of the extracts was evaluated according to the DPPH (2,2-diphenyl-1-picrylhydrazyl) test described by Tenuta et al. (2020). The *A. unedo* extracts were dissolved in methanol to obtain 25, 50, 100, 200, and 300 µg/mL concentrations. Subsequently, 200 µL of each prepared concentration was added to 800 µL of 100 µM DPPH solution. The mixture was incubated for 30 min in the dark at 25°C, and the

absorbances were determined by spectrophotometry at 517 nm. The percentage of antioxidant activity was calculated as $\% AA = \frac{(A0-A1)}{A0} \times 100$, where A0 is the absorbance of the control (methanol) and A1 is the absorbance of the extract. Plotting the curve of % AA as a function of extract concentration allowed the determination of the EC_{50} (concentration to obtain a 50% antioxidant effect).

2.3 Growth conditions of *T. pyriformis*

The culture medium for the ciliated protozoan *T. pyriformis* ATCC 30005 strain E was a PPYE medium consisting of 0.6% proteose peptone and 0.1% yeast extract (Rodrigues-Pousada et al. 1979). Culture was performed under aerobic conditions at 28°C for 72 h without shaking. Cell viability and morphology were assessed by optical microscope (Optika B-600Ti). Viability was estimated by counting the number of cells/mL using the Malassez counting chamber (0.0025 mm^2, 0.2 mm deep) after fixation with 10% glutaraldehyde in the PBS buffer at pH 7. Generation number (n) and generation time (g) were estimated according to Ziyadi et al. (2022b). The generation number was calculated using the formula: $n = \frac{\log N_1 - \log N_0}{\log 2}$, where N_1 is the cell number at 72 h and N_0 is the cell number at T_0. The generation time was calculated as: $g = \frac{\text{time of growt h}}{n}$. For morphology, photographs of cells in a microscope slide were taken with a camera (Optika M-699), and the ratio shortest (W)/longest (L) axis of the cell was calculated using Optika Vision 3.4 software.

2.4 Cytotoxicity of *A.unedo* extracts

The influence of aqueous and ethanolic extract of *A. unedo* fruits and flowers on the viability of *T. pyriformis* was evaluated at different concentrations of extracts ranging from (2 to 1000 µg/mL). The concentrations of extracts were prepared in 10 mL tubes of PPYE medium and inoculated with fresh pre-culture of the protozoa. Cells were counted in the exponential phase (72h). The probit analysis (Bliss 1935) determined the IC_{50} (inhibitory concentration at 50%).

2.5 Gamma exposure of cultures

The protozoan cultures (100 mL) were exposed to gamma radiation using a ^{60}Co source (North American Scientific, Chatsworth, CA) at 20 cGy/h for 72 h according to the protocol described by Ziyadi et al. (2022b). This exposure was performed in the absence of plant extracts (to evaluate radiation damage on *T. pyriformis*) and in the presence of extracts (to evaluate their protective effect on *T. pyriformis*).

2.6 Preparation of crude cell-free extract

T. pyriformis cells were harvested by centrifugation (5000 × g for 15 min at 4°C), washed twice with 20 mM Tris-HCl buffer (pH 7.5) and resuspended in 50 mM Tris-HCl buffer (pH 7.5) containing 2 m Methylenediamine tetraacetic acid (EDTA), 10 mM 2-mercaptoethanol, 2 mM phenylmethylsulfonyl fluoride (PMSF), 1 mM dithiothreitol and 1% (v/v) glycerol at a rate of 3 mL/g cell weight. The suspension was sonicated using a Bandelin Sonopuls sonicator (40 s, 90%, 10×) in an ice bath with 1 min rest between each sonication cycle, followed by centrifugation at 15,000 × g for 45 min at 4°C to obtain a soluble protein fraction. The Bradford method (Bradford 1976) with BSA as a standard was used to determine the protein concentration of the crude cell-free extract.

2.7 Lipid peroxidation assessment

Lipid peroxidation was estimated as described by Samokyszyn and Marnett (1990) by mixing 1 mL of the crude cell-free extract and 1 mL of thiobarbituric acid (TBA) prepared in 0.25 M HCl with 30% trichloroacetic acid. After incubation at 100°C for 15 min, the reaction was stopped by introduction into an ice bath and centrifuged at 1000 × g for 10 min. In this reaction, TBA reacts with the peroxidation product (malondialdehyde (MDA)) to form thiobarbituric acid reactive substances (TBARS). TBARS were measured by a spectrophotometric reading of the supernatant at 535 nm and converted to nmoles MDA/mg protein.

2.8 Enzymes activities

The activities of GAPDH, SDH, CAT, and SOD were measured by monitoring the reaction products with a spectrophotometer, and the conditions of the reactions are grouped in Table 1. The activities of the enzymes were expressed in µmole/min/mg of protein using the extinction coefficient of each substrate.

2.9 Statistical analysis

All assays were performed in three different experiments, and results were determined as mean ± standard deviation (SD). Statistical analyses were performed using one-way ANOVA analysis and Tukey's multiple comparison test using IBM SPSS Statistics (version 25). The 5% significance level was used.

3 Results

3.1 Extraction and cytotoxicity of extracts on *T. pyriformis*

Aqueous and 50% ethanol extractions were performed using freeze-dried fruits and flowers of the strawberry tree. The aqueous extract yielded 33.88 and 33% for fruits and flowers, respectively. In contrast, the yield of ethanolic extract was 63.4 and 46.6% for flowers and fruits, respectively. As a working solution, the dry extracts were taken up in distilled water at a 20 mg/mL rate.

The effect of aqueous and ethanolic extracts of fruits and flowers of *A. unedo* on the growth of *T. pyriformis* was studied by

Table 1 Enzymatic reaction conditions for GAPDH, SDH, CAT, and SOD

Enzyme	Reaction mixture	Absorbance length	According to
GAPDH	10 mM sodium arsenate, 1 mM NAD^+, 1 mM D-G3P in 50 mM tricine buffer (pH 8.5). The reaction is started by 10 µL of the crude extract.	340 nm	Iddar et al. 2002
SDH	0.053 mM dichloroindophenol, 0.3 mM EDTA, and 50 µg of protein in 100 mM potassium phosphate buffer (pH 7.4). The reaction starts with 50 µL of KCN Succinate (3.25 mg/mL of KCN in 0.5 M succinate).	625 nm	King 1967
CAT	7 mM H_2O_2 in 50 mM potassium phosphate buffer (pH 7.5). The reaction is started by 10 µL of the crude extract.	240 nm	Aebi 1984
SOD	3.5 mM 2-mercaptoethanol, 2.5 mM $MnCl_2$, 5 mM EDTA, and 10 µL of the crude extract in 50 mM potassium phosphate buffer (pH 7). The reaction was started by 0.27 mM of NADH.	340 nm	Paoletti et al. 1986

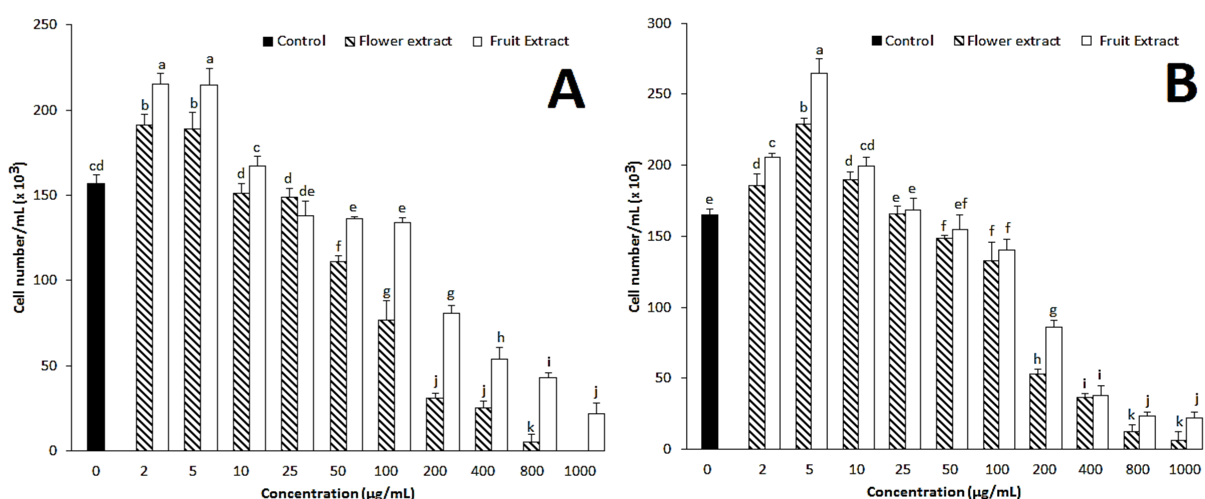

Figure 1 Cytotoxicity of flower and fruit extracts of *A. unedo* (A) Aqueous extracts, (B) Ethanolic extracts, Data are reported as mean ± SD of 3 independent experiments and 3 measurements, Letters indicate differences between values using the Tukey test at $P< 0.05$.

culturing the protozoan in the presence of each extract at different concentrations (2, 5, 10, 25, 50, 100, 200, 400, 800 and 1000 µg/mL). Cells were counted in the exponential growth phase (72 h) and compared to the control (*T. pyriformis* grown in PPYE medium without extracts). The cytotoxicity of the extracts is shown in Figure 1, and the result showed that at low concentrations, stimulation of growth compared to the control was observed with the addition of all the plant extracts, and it was up to 5 µg/mL for aqueous extracts and up to 10 µg/mL for ethanolic extracts. Inhibition of growth was observed above 50 µg/mL for the aqueous and ethanolic extracts, and it depends on the increasing concentration of the extracts, with a more pronounced inhibition for flower extracts compared to fruit extracts (Figure 1). Probit analysis was used to calculate the probable IC_{50} for the studied extracts, and the results show IC_{50} of 107.3, 321.6, 166.4, and 260.9 µg/mL for aqueous extract of flowers, fruits, ethanolic extract of flowers, and fruits, respectively. Moreover, the effect of *A. unedo* extracts on the appearance of *T. pyriformis* was determined by observations through an optical microscope, and no change in the appearance of the protozoan was observed at 50 µg/mL for aqueous extracts and 100 µg/mL for ethanolic extracts. Cell shrinkage was observed at high concentrations of the plant extracts (data not shown). For these reasons, 25 µg/mL concentration for aqueous and ethanolic extracts was chosen to study the protective effect against gamma radiation. These concentrations allowed for maintaining approximately the initial number of cells without affecting their appearance.

3.2 Assessment of the effect of *A. unedo* extracts against gamma radiation

3.2.1 influence of extracts on growth and morphology of irradiated cells

The effect of ^{60}Co source (20-cGy/h for 72 h) exposure on the growth of *T. pyriformis* was evaluated. The viability of *T. pyriformis* was estimated by measuring the generation number and time. The results, presented in Figure 2, showed a decrease in the generation number and an increase in the generation time, indicating a lower growth in the presence of a ^{60}Co source (the exposed cells completed 6.26 ± 0.15 cell generations, while the

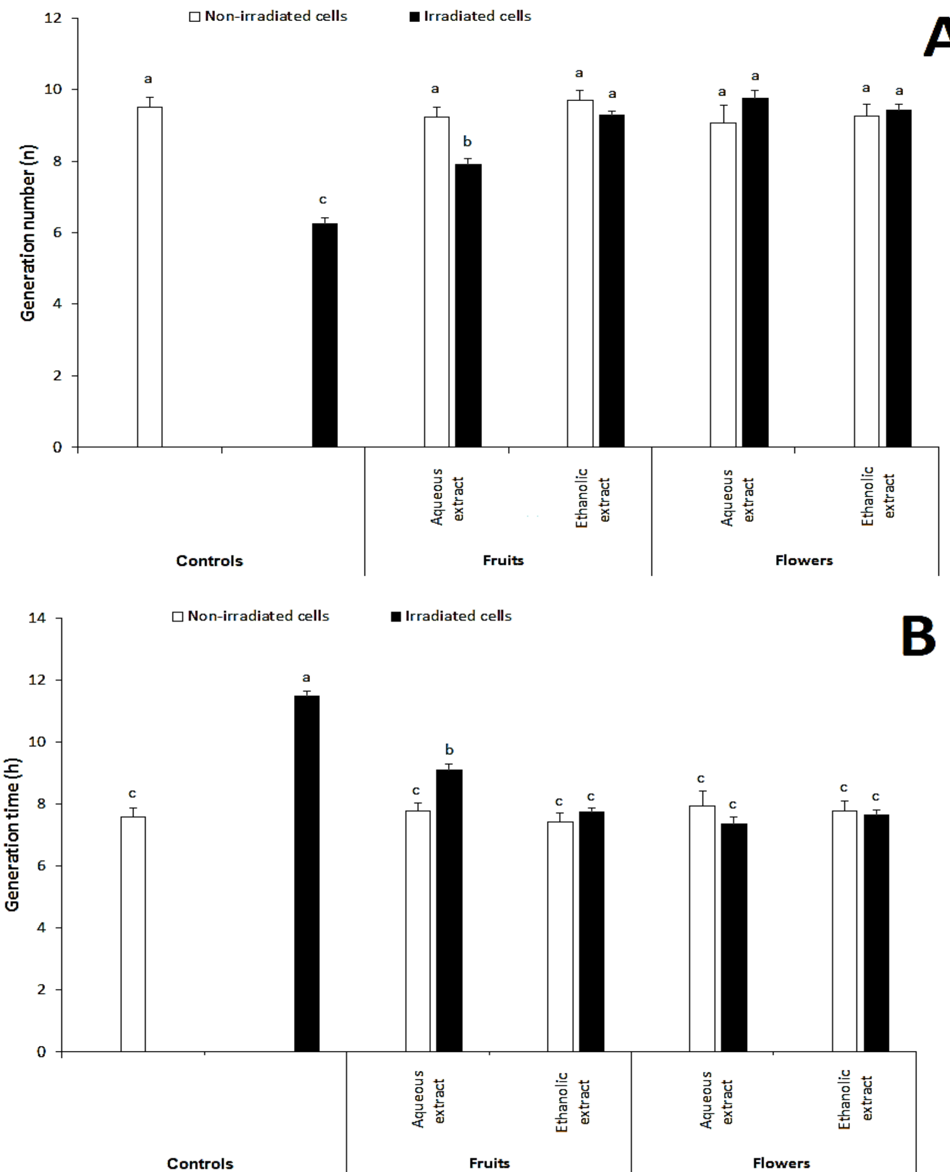

Figure 2 Effect of fruit and flower extracts of *A. unedo* on parameters of growth (A) Generation number, (B) Generation time; Data are reported as mean ± SD of 3 independent experiments and 3 measurements; letters indicate differences between values using Tukey test at $P < 0.05$.

control cells completed 9.5 ± 0.29 generations and the time required for one generation in the exposed cells was 11.5 ± 0.17 h, compared with 7.6 ± 0.22 h in the control cells). The exposed cells completed 6.26 ± 0.15 cell generations, while the control cells completed 9.5 ± 0.29 generations. Additionally, the time required for one generation in the exposed cells was 11.5 ± 0.17 h, compared with 7.6 ± 0.22 h in the control cells. On the other hand, the protective effect of aqueous and ethanolic extracts (25 µg/mL) of the flowers and fruits of the strawberry tree added to the ciliate culture medium against radiation growth inhibition was also evaluated.

Figure 2 shows the improvement in growth under the effect of plant extracts compared to the exposed and untreated cells. Adding flower and fruit extracts allowed the complete or partial recovery of growth. The ethanolic extracts were better than the aqueous extracts since both the ethanolic extracts of flowers and fruits made it possible to find the initial values of the generation time and the generation number when the protozoan was cultured under irradiation. Only the aqueous extract of the flower was able to restore normal growth, and the aqueous extract of the fruit restored about 80% of number and time generation values (generation number = 7.9 ± 0.18 and generation time = 9.11 ± 0.15 h) (Figure 2).

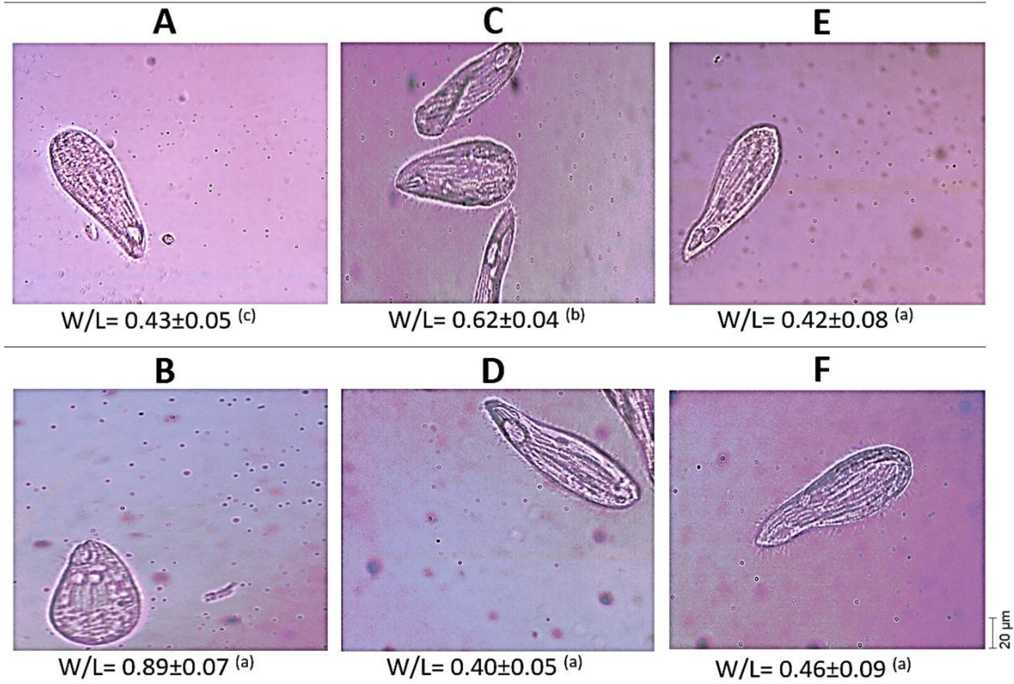

Figure 3 Effect of fruit and flower extracts of *A. unedo* on *T. pyriformis* morphology using microscope images with magnification × 40, (A) Control, (B) Exposure conditions without extracts, (C) Exposure conditions with aqueous fruits extract, (D) Exposure conditions with aqueous flowers extract, (E) Exposure conditions with ethanolic fruits extract, (F) Exposure conditions with aqueous flowers extract, Cell shape (W/L) was calculated for 50 cells in 3 independent experiments, Tukey test $P< 0.05$ was used to indicate significant differences between means (small letters in the figure).

For the morphology of *T. pyriformis*, photographs of cells in different conditions (irradiated cells with and without plant extracts) were taken with a microscope camera (Optika) and compared to the control (non-irradiated cells) (Figure 3). The shortest (W)/longest (L) ratio was calculated using Optika Vision 3.4 software. The W/L ratio was increased in *T. pyriformis* cells grown in a ^{60}Co source (W/L= 0.89±0.07 in irradiated cells compared to 0.43±0.05 in control cells) (Figure 3). The shape of irradiated cells grown with the ethanolic extracts was completely restored (W/L= 0.42±0.08 for fruits and 0.46±0.09 for flowers), compared to the shape of the irradiated cell without extracts (W/L= 0.89±0.07). The aqueous extract of the fruits also showed a potential protective effect against the effects of radiation on *T. pyriformis* cell morphology, significantly restoring part of the cell shape (W/L= 0.62±0.04), while the aqueous extract of the flowers made it possible to completely restore the initial shape of the exposed cells (W/L= 0.40±0.05).

3.2.2 influence of extracts on lipid peroxidation in the irradiated cells

As shown in Table 2, exposure to the ^{60}Co source significantly increased lipid peroxidation in *T. pyriformis* after 72 h of growth with an MDA production value of 3.55±0.12 nmol/mg compared to the control (0.17±0.05 nmol/mg). Adding the aqueous extract of the fruits at 25 µg/mL to the culture medium reduced the production of MDA in the irradiated cells to 1.75±0.09 nmol/mg. All the other extracts (aqueous extract of flowers and ethanolic extracts of flowers and fruits) made it possible to restore the initial value (control) of the MDA level of *T. pyriformis* cells under irradiation conditions (Table 2).

3.2.3 influence of extracts on some enzyme activities of the irradiated cells

The enzymatic activities of GAPDH, SDH, CAT, and SOD were determined in crude extracts of *T. pyriformis* grown under irradiation conditions with and without plant extracts and compared with non-irradiated cells with and without plant extracts. Table 2 summarizes the results of the analyses and shows that adding aqueous and ethanolic extracts of flowers and fruits of *A. unedo* did not affect the tested enzymatic activities when *T. pyriformis* was grown under normal conditions, and these activities were considered as controls. When *T. pyriformis* was grown in the presence of a ^{60}Co source, metabolic enzymes GAPDH and SDH activities were inhibited. Under irradiation conditions, GAPDH and SDH activities in the protozoa retained about 40 to 45% of the residual activities compared to the control (3.55±0.12 and 25.67±2.11 instead of 0.846±0.041 and 59.87±4.04 µmol/min/mg of protein for GAPDH and SDH, respectively). Adding aqueous

Table 2 Effect of irradiation with ^{60}Co source on lipid peroxidation (MDA production) and some enzyme activities (GAPDH, SDH, CAT, and SOD) of *T. pyriformis* grown with and without *A. unedo* extracts.

Growth conditions	MDA (nmol/mg)	GAPDH (µmol/min/mg)	SDH (µmol/min/mg)	CAT (µmol/min/mg)	SOD (µmol/min/mg)
Control*	0.17±0.05^{c}	0.846±0.041^{a}	59.87±4.04^{a}	0.354±0.037^{d}	0.288±0.036^{c}
Irradiation	3.55±0.12^{a}	0.376±0.027^{b}	25.67±2.11^{d}	1.965±0.191^{a}	1.99±0.097^{a}
Irradiation + fruit aqueous extract	1.75±0.09^{b}	0.814±0.030^{a}	41.27±1.01^{c}	1.017±0.093^{b}	0.482±0.055^{b}
Irradiation + flower aqueous extract	0.18±0.07^{c}	0.861±0.046^{a}	56.24±1.24^{a}	0.424±0.038^{cd}	0.281±0.031^{c}
Irradiation + fruit ethanolic extract	0.21±0.04^{c}	0.835±0.039^{a}	49.08±2.85^{b}	0.508±0.059^{c}	0.435±0.056^{b}
Irradiation + flower ethanolic extract	0.19±0.07^{c}	0.831±0.017^{a}	62.13±3.37^{a}	0.352±0.018^{d}	0.334±0.029^{c}

* No significant difference between MDA production and enzyme activities was observed in controls (non-irradiated *T. pyriformis*) without and with the plant extracts.

and ethanolic extracts of the flowers and fruits of *A. unedo* to the culture of irradiated cells of *T. pyriformis* made it possible to recover the totality of GAPDH activity (Table 2). For SDH, the activity was completely restored for *T. pyriformis* irradiated only in the presence of flower extracts (aqueous and ethanolic). The aqueous and ethanolic extracts enabled the recovery of 69 and 82%, respectively, compared to the initial activity. The control had an activity of 59.87±4.04 µmol/min/mg, while the aqueous and ethanolic extracts had activities of 41.27±1.01 and 49.08±2.85 µmol/min/mg, respectively (Table 2). CAT and SOD, involved in the antioxidant defence system, were also monitored in this study. Radiation increased the activity of these two enzymes, and their respective activities became 1.965±0.191 and 1.99±0.097 µmol/min/mg compared to the control with and without extract, which are 0.354±0.037 and 0.288±0.036 µmol/min/mg for CAT and SOD, respectively (Table 2). The initial activity found in the control was significantly recovered in *T. pyriformis* irradiated in the presence of aqueous and ethanolic extracts of flowers (CAT: 0.424±0.038 and 0.352±0.018; SOD: 0.281±0.031 and 0.334±0.029 µmol/min/mg for the aqueous and ethanolic extracts, respectively). The fruit extracts enabled the recovery of part of the activities compared to the control. The CAT activity was 1.017±0.093 µmol/min/mg, and SDH activity was 0.482±0.055 µmol/min/mg for the aqueous extract. For the ethanolic extract, CAT activity was 10.508±0.059 µmol/min/mg, and SDH activity was 0.435±0.056 µmol/min/mg (Table 2).

3.3 Evaluation of the *in vitro* antioxidant activity of *A. unedo* extracts

The antioxidant activity of aqueous and ethanolic extracts of *A. unedo* was evaluated in vitro using the DPPH scavenging test. The antioxidant activity of the flower extracts was better than that of the fruit extracts, with lower concentrations required to achieve a 50% antioxidant effect (EC_{50}) (Figure 4). The EC_{50} for the aqueous flower extract was significantly higher than that for the ethanolic

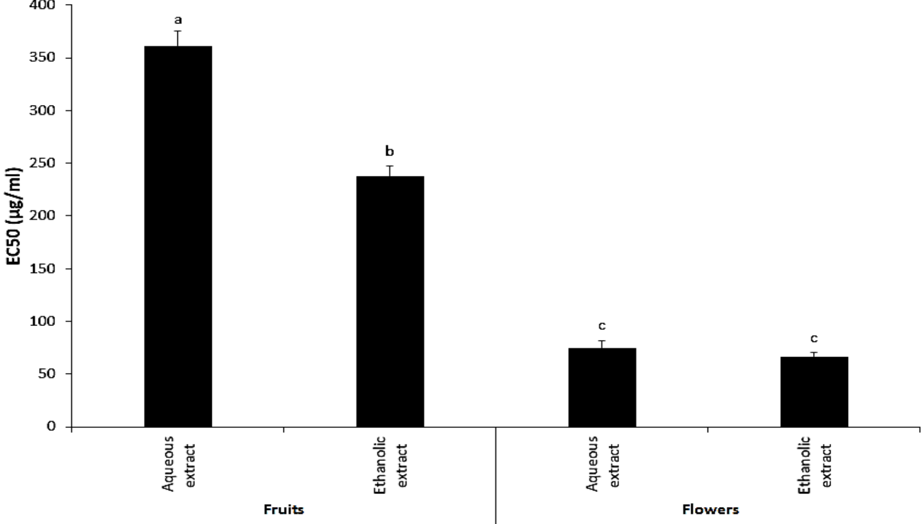

Figure 4 Effect of fruit and flower extracts of *A. unedo* on DPPH scavenging; Data are reported as mean ± SD of 3 independent experiments and 3 measurements; Letters indicate differences between values using the Tukey test at $P< 0.05$

flower extract, and the scavenging power of the aqueous and ethanolic flower extracts was similar. The EC_{50} values for aqueous fruit extract, ethanolic fruit extract, aqueous flower extract, and ethanolic flower extract were 360.85 ± 15.053, 237.43 ± 10.08, 71.13 ± 7.15, and 65.97 ± 4.13 µg/mL, respectively (Figure 4).

4 Discussion

In their previous work, Ziyadi et al. (2022b) investigated the impact of irradiation on the growth of the ciliate protozoa *T. pyriformis*. The results indicated that exposure to doses greater than 6 cGy/h using cobalt-60 (^{60}Co) or cesium-137 (^{137}Cs) sources had a negative effect on the cells, retarding their growth, inhibiting their metabolism, and altering their morphology. Our study found similar results, as exposing *T. piriformis* cultures to a ^{60}Co source at 20 cGy/h during the exponential phase reduced cell growth by decreasing the number of generations in 72 h and increasing the generation time. Additionally, radiation altered the morphology of the cells, causing them to become more rounded. This rounding aspect is a characteristic of *T. pyriformis* under various stress conditions (Suryanto et al. 2022). Radioactivity also affects certain metabolic enzymes, such as GAPDH and SDH, which are key enzymes in cellular metabolism and have important physiological roles (Wei et al. 2022; Wang et al. 2023). In addition, exposure to a ^{60}Co source indirectly affected cells by increasing the amount of ROS, thereby increasing lipid peroxidation, which is one of the damages caused by ROS. These free radicals can interact with biomolecules such as lipids, proteins, and DNA, leading to cellular dysfunction (Gebicki and Nauser 2021). Radiation exposure can cause an increase in ROS due to the radiolysis of water in the cell. The irradiated cells triggered the antioxidant defence system, resulting in increased activity of CAT and SOD in response to the increase of ROS in the cell. This phenomenon has been observed in microorganisms exposed to ROS and radiation (Iddar et al. 2022).

Various plant extracts have been tested for their antioxidant properties and ability to protect against the harmful effects of radiation (El-Shawi et al. 2023; Wu et al. 2023). In another study by Ziyadi et al. (2022a), the protective effects of certain pure essential oils (*Rosmarinus officinalis*, *Origanum compactum*, *Lavandula angustifolia,* and *Eucalyptus globulus*) on *T. pyriformis* against irradiation were evaluated. In this study, we assessed the ability of strawberry tree extracts from the El Harcha region of Morocco to protect *T. pyriformis* against the effects of radiation. We tested two types of extracts from the flowers and fruits of the tree: decoction and ethanol maceration. These methods are widely recognized as the simplest and most effective for extracting bioactive compounds from plant materials (Lezoul et al. 2020). The chemical defence compounds in plant extracts can be toxic to microorganisms (Zaynab et al. 2021). Therefore, we tested *A. unedo* extracts at a non-cytotoxic concentration of 25 µg/mL to evaluate their protective effect against radiation in *T. pyriformis*.

Adding the aqueous and ethanolic extracts of both flowers and fruits of *A. unedo* to the culture of irradiated cells resulted in the recovery of growth, similar to cells in normal conditions. However, the aqueous extract of the fruits only allowed for a partial recovery of growth at 80%. These extracts were also shown to protect the growth of *T. pyriformis* against the effects of radiation on metabolic enzymes essential for cell survival, such as GAPDH and SDH. For GAPDH, 100% of its activity was found in cells irradiated with all tested extracts (Table 2). As for SDH, only the aqueous and ethanolic extracts of flowers allowed for the recovery of 100% of its activity. The fruit extracts allowed for the recovery of 69 and 82% for aqueous and ethanolic extracts, respectively. Numerous studies have demonstrated the benefits of using specific plant extracts and natural products as radioprotectants in experimental models (Dowlath et al. 2021). In comparison, using non-toxic concentrations of pure essential oils (5 µg/mL for *R. officinalis* and *E. globulus* and 10 µg/mL for *O. compactum* and *L. angustifolia*) in the culture of *T. pyriformis* exposed to ^{60}Co at 20 cGy/h restored over 90% of the growth and metabolic enzyme activities (Ziyadi et al. 2022a).

Gamma radiation can cause radiolysis of water, resulting in the formation of ROS and cellular damage (Obrador and Montoro 2023). Irradiation-induced oxidative damage indirectly affects cellular proteins and lipids, leading to reactions such as thiolation and carbonylation under ROS (Dowlath et al. 2021). The *A. unedo* extracts effectively reduced lipid peroxidation in the irradiated protozoa, with the aqueous fruit extract having a slightly lesser effect (Table 2). This plant is rich in polyphenol and flavonoid compounds (Wahabi et al. 2023), which possess antioxidant and radioprotective properties (Dowlath et al. 2021). The DPPH test confirmed that the extracts had considerable scavenging activity, with the flower extracts exhibiting higher activity than the fruits. Finally, the antioxidant properties of the extracts also enabled the enzymes CAT and SOD, which are involved in the antioxidant defence system, to recover their initial levels before irradiation partially. The aqueous and ethanolic extracts of the flowers restored the enzymes to their initial activities, while the fruit extracts allowed for partial recovery of the activity compared to the control (Table 2). Previous studies have shown that adding exogenous antioxidants can support the enzymatic defense system by scavenging ROS (Supruniuk et al. 2023). The results of this work describe, for the first time, the use of *A. unedo* extracts as protective agents against the negative effects of irradiation in the *T. pyriformis* cell model.

5 Conclusion

Aqueous and ethanolic extracts of the strawberry tree (*A. unedo*) from the El Harcha region, Morocco, protected *T. pyriformis*

against the negative effects of exposure to a ^{60}Co radioactive source. Compared to the non-irradiated cells, the extracts completely or partially restored the protozoan's growth, morphology, and physiology in the irradiated cells. Flower extracts showed a higher protective potential and antioxidant activity (protection of cells against radiation-induced ROS) than fruit extracts. Also, ethanolic extracts proved more effective than aqueous extracts in protecting *T. pyriformis* against irradiation. These results may open prospects for using *A. unedo* flower extracts as exogenous radioprotective agents.

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Conflicts of interest

The authors reported no potential conflict of interest.

References

Aebi, H. (1984). Catalase in vitro. *Methods in enzymology*, *105*, 121-126. https://doi.org/10.1016/s0076-6879(84)05016-3.

Bajoub, A., Ennahli, N., Ouaabou, R., Chaji, S., et al. (2023). Investigation into Solar Drying of Moroccan Strawberry Tree (*Arbutus unedo* L.) Fruit: Effects on Drying Kinetics and Phenolic Composition. *Applied Sciences*, *13*(2), 769. https://doi.org/10.3390/app13020769.

Başaran, N., Paslı, D., & Başaran, A. A. (2022). Unpredictable adverse effects of herbal products. *Food and Chemical Toxicology*, *159*, 112762. https://doi.org/10.1016/j.fct.2021.112762.

Bliss, C. I. (1935). The calculation of the dosage-mortality curve. *Annals of Applied Biology*, *22*(1), 134-167. https:// doi. org/ 10. 1111/j.1744-7348. 1935. tb077 13.x.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical biochemistry*, *72*, 248-254. https://doi.org/10.1006/abio.1976.9999.

De Santis, S., Spada, F., & Magri, D. (2023). Geographic Range vs. Occurrence Records in Plant Distribution Mapping: The Case of *Arbutus* in the Old World. *Forests*, *14*(5), 1010. https://doi.org/10.3390/f14051010.

Dowlath, M. J. H., Karuppannan, S. K., Sinha, P., Dowlath, N. S., et al. (2021). Effects of radiation and role of plants in radioprotection: A critical review. *The Science of the total environment*, *779*, 146431. https://doi.org/10.1016/j.scitotenv.2021.146431.

El Haouari, M., Assem, N., Changan, S., Kumar, M., et al. (2021). An Insight into Phytochemical, Pharmacological, and Nutritional Properties of *Arbutus unedo* L. from Morocco. *Evidence-based complementary and alternative medicine :eCAM*, *2021*, 1794621. https://doi.org/10.1155/2021/1794621.

El-Shawi, O. E., El-Nashar, H. A., Abd El-Rahman, S. S., Eldahshan, O. A., et al. (2023). Protective effect of *Acrocarpus fraxinifolius* extract against hepatic fibrosis induced by gamma irradiation and carbon tetrachloride in albino rats. *International Journal of Radiation Biology*, *99*(2), 270-280.https://doi.org/10.1080/09553002.2022.2087926.

Gebicki, J. M., & Nauser, T. (2021). Initiation and Prevention of Biological Damage by Radiation-Generated Protein Radicals. *International journal of molecular sciences*, *23*(1), 396. https://doi.org/10.3390/ijms23010396.

Helluy, M., Gavinet, J., Prévosto, B., & Fernandez, C. (2021). Influence of light, water stress and shrub cover on sapling survival and height growth: the case of *A. unedo*, *F. ornusand S. domestica* under Mediterranean climate. *European Journal of Forest Research*, *140*(3), 635-647.https://doi.org/10.1007/s10342-021-01356-1.

Iddar, A., El Mzibri, M., & Moutaouakkil, A. (2022). Effects of the Cobalt-60 gamma radiation on *Pichia pastoris* glyceraldehyde-3-phosphate dehydrogenase. *International journal of radiation biology*, *98*(2), 244-252. https://doi.org/10.1080/09553002.2022.2009142.

Iddar, A., Serrano, A., & Soukri, A. (2002). A phosphate-stimulated NAD(P)+-dependent glyceraldehyde-3-phosphate dehydrogenase in Bacillus cereus. *FEMS microbiology letters*, *211*(1), 29-35. https://doi.org/10.1111/j.1574-6968.2002.tb11199.x.

King, T. E. (1967). Preparations of succinate—cytochrome c reductase and the cytochrome b-c1 particle, and reconstitution of succinate-cytochrome c reductase. *Methods in enzymology, 10*, 216-225).

Lezoul, N. E. H., Belkadi, M., Habibi, F., & Guillén, F. (2020). Extraction Processes with Several Solvents on Total Bioactive Compounds in Different Organs of Three Medicinal Plants. *Molecules (Basel, Switzerland)*, *25*(20), 4672. https://doi.org/10.3390/molecules25204672.

Lim, J. (2022). Broad toxicological effects of per-/poly-fluoroalkyl substances (PFAS) on the unicellular eukaryote, *Tetrahymena pyriformis*. *Environmental Toxicology and Pharmacology*, *95*, 103954. https://doi.org/10.1016/j.etap.2022.103954.

Mrabti, H. N., Bouyahya, A., Ed-Dra, A., Kachmar, M. R., et al. (2021). Polyphenolic profile and biological properties of *Arbutus unedo* root extracts. *European Journal of Integrative Medicine*, *42*, 101266.https://doi.org/10.1016/j.eujim.2020.101266.

Obrador, E., & Montoro, A. (2023). Ionizing Radiation, Antioxidant Response and Oxidative Damage: Radiomodulators. *Antioxidants*, *12*(6), 1219. https://doi.org/10.3390/antiox12061219.

Paoletti, F., Aldinucci, D., Mocali, A., & Caparrini, A. (1986). A sensitive spectrophotometric method for the determination of superoxide dismutase activity in tissue extracts. *Analytical biochemistry*, *154*(2), 536-541. https://doi.org/10.1016/0003-2697(86)90026-6.

Pardillo-Díaz, R., Pérez-García, P., Castro, C., Nunez-Abades, P., et al. (2022). Oxidative Stress as a Potential Mechanism Underlying Membrane Hyperexcitability in Neurodegenerative Diseases. *Antioxidants (Basel, Switzerland)*, *11*(8), 1511. https://doi.org/10.3390/antiox11081511.

Ponnampalam, E. N., Kiani, A., Santhiravel, S., Holman, B. W. B., et al. (2022). The Importance of Dietary Antioxidants on Oxidative Stress, Meat and Milk Production, and Their Preservative Aspects in Farm Animals: Antioxidant Action, Animal Health, and Product Quality-Invited Review. *Animals*, *12*(23), 3279. https://doi.org/10.3390/ani12233279.

Rodrigues-Pousada, C., Cyrne, M. L., & Hayes, D. (1979). Characterization of preribosomal ribonucleoprotein particles from Tetrahymena pyriformis. *European journal of biochemistry, 102*(2), 389-397. https://doi.org/10.1111/j.1432-1033.1979.tb04254.x.

Sabraoui, T., Grina, F., Khider, T., Nasser, B., et al. (2022). Alleviate effect of pomegranate peel extract in ameliorating fluoride-induced cytotoxicity, oxidative stress in *Tetrahymena pyriformis* model. *Biointerface Research in Applied Chemistry*, *12*(3), 3710-24.http://dx.doi.org/10.33263/BRIAC123.37103724.

Sadiq I. Z. (2023). Free Radicals and Oxidative Stress: Signaling Mechanisms, Redox Basis for Human Diseases, and Cell Cycle Regulation. *Current molecular medicine*, *23*(1), 13-35. https://doi.org/10.2174/1566524022666211222161637.

Samokyszyn, V. M., & Marnett, L. J. (1990). Inhibition of liver microsomal lipid peroxidation by 13-cis-retinoic acid. *Free radical biology & medicine*, *8*(5), 491-496. https://doi.org/10.1016/0891-5849(90)90063-o.

Sanna, C., Chiocchio, I., Mandrone, M., Bonvicini, F., et al. (2023). Metabolomic analysis and bioactivities of *Arbutus unedo* leaves harvested across the seasons in different natural habitats of Sardinia (Italy). *BMC Plant Biology*, *23*(1), 490.https://doi.org/10.1186/s12870-023-04497-0.

Şolpan, D., Ahmed Ibrahim, K. E., Elbashir, A. A., Mehrnia, M., et al. (2022). Radiolytic degradation of carbaryl in aqueous solution by gamma-irradiation/H_2O_2 process. *Applied radiation and isotopes*, *184*, 110210. https://doi.org/10.1016/j.apradiso.2022.110210.

Supruniuk, E., Górski, J., & Chabowski, A. (2023). Endogenous and Exogenous Antioxidants in Skeletal Muscle Fatigue Development during Exercise. *Antioxidants (Basel, Switzerland)*, *12*(2), 501. https://doi.org/10.3390/antiox12020501.

Suryanto, M. E., Vasquez, R. D., Roldan, M. J. M., Chen, K. H. C., et al. (2022). Establishing a High-Throughput Locomotion Tracking Method for Multiple Biological Assessments in *Tetrahymena*. *Cells*, *11*(15), 2326.https://doi.org/10.3390/cells11152326.

Tenuta, M. C., Deguin, B., Loizzo, M. R., Dugay, A., et al. (2020). Contribution of Flavonoids and Iridoids to the Hypoglycaemic, Antioxidant, and Nitric Oxide (NO) Inhibitory Activities of *Arbutus unedo* L. *Antioxidants (Basel, Switzerland)*, *9*(2), 184. https://doi.org/10.3390/antiox9020184.

Wahabi, S., Rtibi, K., Atouani, A., & Sebai, H. (2023). Anti-Obesity Actions of Two Separated Aqueous Extracts From *Arbutus* (*Arbutus unedo*) and *Hawthorn* (*Crataegus monogyna*) Fruits Against High-Fat Diet in Rats via Potent Antioxidant Target. *Dose-Response*, *21*(2), 15593258231179904. https://doi.org/10.1177/15593258231179904.

Wang, Q., Li, M., Zeng, N., Zhou, Y., et al. (2023). Succinate dehydrogenase complex subunit C: Role in cellular physiology and disease. *Experimental Biology and Medicine*, *248*(3), 263-270.https://doi.org/10.1177/15353702221147567.

Wei, H., Movahedi, A., Yang, J., Zhang, Y., et al. (2022). Characteristics and molecular identification of glyceraldehyde-3-phosphate dehydrogenases in poplar. *International Journal of Biological Macromolecules*, *219*, 185-198.https://doi.org/10.1016/j.ijbiomac.2022.08.001.

Wu, S., Tian, C., Tu, Z., Guo, J., et al. (2023). Protective effect of total flavonoids of *Engelhardia roxburghiana Wall.* leaves against radiation-induced intestinal injury in mice and its mechanism. *Journal of Ethnopharmacology*, *311*, 116428. https://doi.org/10.1016/j.jep.2023.116428.

Zaynab, M., Sharif, Y., Abbas, S., Afzal, M. Z., et al. (2021). Saponin toxicity as key player in plant defense against pathogens. *Toxicon* , *193*, 21-27. https://doi.org/10.1016/j.toxicon.2021.01.009.

Ziyadi, S., Iddar, A., Errafiy, N., Ridaoui, K., et al. (2022a). Protective Effect of Some Essential Oils Against Gamma-Radiation Damages in *Tetrahymena pyriformis* Exposed to Cobalt-60 source. *Current microbiology*, *79*(9), 279. https://doi.org/10.1007/s00284-022-02924-3.

Ziyadi, S., Iddar, A., Kabine, M., El Mzibri, M., et al. (2022b). Changes in Growth, Morphology, and Physiology of *Tetrahymena pyriformis* Exposed to Continuous Cesium-137 and Cobalt-60 Gamma-Radiation. *Current microbiology*, *79*(2), 61. https://doi.org/10.1007/s00284-021-02684-6.