scientific reports

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Modulation of aluminum-induced genotoxicity and oxidative stress in *Allium cepa* L. by *Ramalina pollinaria* (Westr.) Ach. extract

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Aluminum toxicity leads to oxidative stress and genetic instability in organisms, impairing growth and cellular function. This study investigated the protective potential of an aqueous extract obtained from the lichen species *Ramalina pollinaria* against aluminum-induced toxicity in *Allium cepa*, a widely used biological indicator in environmental monitoring. Onion bulbs were treated with aluminum chloride (50 mg L^{-1}), two concentrations (0.5 g L^{-1} and 1.0 g L^{-1}) of lichen extract and lichen extracts together with aluminum chloride. Physiological, cytogenetic, biochemical, and anatomical responses were evaluated following exposure. Aluminum exposure significantly reduced germination, root elongation, and weight gain, while increasing lipid peroxidation, proline accumulation, antioxidant enzyme activities, chromosomal abnormalities, and DNA fragmentation. Co-application of the lichen extract alleviated these effects in a dose-dependent manner. Higher concentrations of the extract led to greater improvements in growth, restoration of mitotic activity, stabilization of chlorophyll levels, and reduction in cellular and genetic damage. Cross-sectional analysis of root meristems confirmed a reduction in tissue injury when the extract was administered. Chemical analysis of the extract revealed a phenolic-rich composition, with compounds likely contributing to its antioxidant and protective efficacy. The findings suggest that *R. pollinaria* extract may mitigate aluminum toxicity in plants and could have potential applications in environmental protection and phytoremediation, although further studies are required.

Keywords *Allium cepa*, Antioxidant, Comet assay, Heavy metal, Lichen, Toxicity

Heavy metals are as elements with a density greater than 4 g cm^{-3}. They have started to accumulate significantly in terrestrial and aquatic environments because of their increasing utilization. Human activities like foundries, metal mining, and smelting contribute to heavy metal pollution in addition to natural factors including soil erosion, volcanic activity, metal corrosion, and geological weathering. Other possible reasons of heavy metal pollution include leaching of metals from landfills, automobiles, and roadworks. The agricultural use of pesticides, insecticides, and fertilizers are secondary causes of heavy metal pollution[1]. Due to their persistence and non-degradable nature, heavy metals are significant threats for the environment[2]. Heavy metals are also a major concern to human health because of their toxicity, persistence, abiotic breakdown, and bioaccumulation[3]. DNA damage and cell death caused by heavy metals are closely related to the fact that these elements cause oxidative stress by propagating the formation of reactive oxygen species (ROS) in living cells[4]. High levels of heavy metals can disrupt plant growth, seed germination, and biomass production in plants[5,6].

Aluminum (Al) is considered a heavy metal of particular concern due to its environmental abundance and biological toxicity. It serves as a flocculant in drinking water treatment procedures, an adjuvant in vaccine production, and an additive in beverages, desserts, cheeses, antiperspirants, and deodorants, in addition to its employment in various industrial sectors[7]. Soluble forms of Al are identified as genotoxic, and our daily exposure to these forms changes from subacute to chronic levels[8]. Al has no biological role in living organisms. Additionally, food packaging and kitchen utensils are particularly cited as a source of Al toxicity for humans[9]. Al has been identified as a causative agent of both cellular toxicity and genotoxicity[7]. Al stress is a critical factor that suppresses plant development and growth[10]. Research has demonstrated that Al stress can trigger oxidative stress and genetic damage in *Allium cepa*L[11]..

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The toxic and carcinogenic effects of synthetic antioxidants have given rise to interest in natural antioxidants[12]. Natural antioxidants can be obtained from various sources, including lichens. Lichen is defined as a stable, ecologically obligate, self-supporting ambient life form established between a mycobiont and unicellular or filamentous photobiont[13]. Some lichens are known to have various biological activities, such as antioxidant, antimicrobial, antigenotoxic, antidiabetic, neuroprotective, antiviral, analgesic, antitumor, anti-inflammatory, antiproliferative, antipyretic, and antiprotozoal[14]. Despite their promising antioxidant and anticarcinogenic capacities, there are very few studies on the antigenotoxic activities of lichens[15]. Phenolic compounds are the most important secondary metabolites synthesized by lichens and show strong antioxidant and antigenotoxic activities[16–18]. It has also been reported that lichens have the potential to bind metals and thus are important biosorbents for the removal of heavy metals[19]. *Ramalina pollinaria* (Westr.) Ach., a lichen species belonging to the *Ramalinaceae* family[20], is employed as a bioindicator for environmental toxicity detection, particularly concerning heavy metals, due to its economical and uncomplicated preparation[19].

Increasing industrialization leads to the accumulation of carcinogenic and mutagenic pollutants and serious exposures; however, it is not always possible to determine the effects of these substances on humans for ethnic, logistical, and practical reasons. Herbal testing systems are widely used to investigate both the toxic effects of pollutants and extracts that show potential to ameliorate or prevent these harms due to their low cost, high sensitivity, and lack of ethical approval compared to animal testing[21]. *A. cepa* (L.) is a prevalent plant testing system that has been utilized effectively since the 1930s. This system has facilitated significant discoveries, including phytotoxicity (measured by root length and germination percentage), cytotoxicity (measured by mitotic index (MI)), genotoxicity (measured by chromosomal aberrations (CAs)), and mutagenicity (measured by micronucleus (MN) formation)[22]. The Allium test exhibits a correlation rate in excess of 82% with mammalian test systems in comparison to rodents[23]. The popularity of Allium as a suitable model for the toxicity monitoring studies is due to the fact that it has a short life cycle in root meristem cells that come into close contact with contaminants. Moreover, it possesses a large but small (2n = 16) number of chromosomes that are easily visible under a microscope. Since Al has well-documented phytotoxic and genotoxic effects, using *A. cepa*, a widely accepted model for assessing heavy metal toxicity, is a sensitive and effective way to evaluate its biological effects.

The objective of this study was to examine the protective effects of a lichen species extract from *R. pollinaria* against Al toxicity, utilizing the *A. cepa* model plant as a subject. This study is the first to systematically assess the protective potential of *R. pollinaria* extract against Al-induced toxicity in *A. cepa*, contributing novel data on the antigenotoxic and physiological effects of lichen-derived phenolic compounds under heavy metal stress. Phenolic compounds were analyzed in lichen extracts. At the conclusion of the application period, a comprehensive investigation was conducted into the physiological parameters (germination percentage, root elongation, and weight gain), genetic parameters (MI, MN, CAs, and DNA damage), biochemical parameters (superoxide dismutase (SOD) and catalase (CAT) enzyme activities and malondialdehyde (MDA), proline, and chlorophyll pigment), and meristematic cell damage in *A. cepa* plants.

Materials and methods

Raw materials

A. cepa var. *aggregatum* (shallot) bulbs were obtained from a local market in Giresun, Türkiye. The identification of the bulbs was confirmed by Prof. Dr. Kadir Kınalıoğlu (Giresun University, Department of Biology, Head of Botany Division). The bulbs were chosen considering uniform size and weight. The old roots and the outermost brown leaves were removed. *R. pollinaria* samples were collected from Araklı, Trabzon-Türkiye (at a height of ca. 2317 m) in November 2022. The identification of lichens was conducted by Prof. Dr. Kadir Kınalıoğlu, using the floristic studies of Brodo et al.[24] and Smith et al.[25]. Experimental research and field studies on plant/lichen and plant/lichen parts (*A. cepa* bulbs, *R. pollinaria*), including the collection of plant material, comply with relevant institutional, national, and international guidelines and legislation. Lichens were thoroughly cleaned of any weeds and foreign plants, and then dried at room temperature and in the shade. *R. pollinaria* and *A. cepa* samples were deposited in the herbarium of the Department of Biology, Giresun University, under the voucher numbers RPL-2023 and ACA-28–2023, respectively.

Extraction conditions and experimental design

Lichen samples were left to air-dry in a dark room at ambient temperature for seven days. The dry materials were ground with a mechanical shredder until powdered. For extraction by maceration, 5 g of the powdered lichen were mixed with 100 mL of ethanol and stirred continuously for 48 h at 25 °C using a mechanical stirrer. The resulting mixture was filtered through Whatman No. 4 filter paper, and the filtrate was concentrated under reduced pressure using a rotary evaporator (Heidolph Hei-VAP ML, Germany). The final concentrations of the extract were adjusted by dilution with water. *A. cepa* L. bulbs were pre-tested by administration of these extracts. The bulbs were positioned within glass tubes, ensuring that the disk stems were in direct contact with the extract solutions. In the dark, the effects of the doses on the length of the adventitious roots that emerged after three mitotic stages were compared. Two of the extract doses (0.5 g L^{-1} and 1.0 g L^{-1}) were determined to be suitable for the main experiment. A comprehensive literature review was conducted to ascertain the optimal dosage of aluminum chloride ($AlCl_3$) solution[26]. A concentration of 50 mg L^{-1} $AlCl_3$ was selected for application to *A. cepa* based on its ability to induce sublethal yet distinct physiological and cytotoxic effects, allowing for measurable biological responses without causing complete cellular disruption.

A. cepa bulbs were divided into six groups ($n = 50$). The bulbs were placed in sterile beakers containing the appropriate solutions for 72 h at room temperature under dark conditions during the treatment period. A 72-hour exposure was selected as it encompasses multiple mitotic cycles in *A. cepa* root meristem cells, allowing for clear detection of cytogenetic and physiological effects. Tap water was used as the control to better simulate natural exposure conditions and to avoid osmotic stress that can result from the use of distilled water. The same

water source was applied across all experimental groups to maintain consistency. The contents of the beakers were replenished with new solutions on a daily basis (Table 1).

Assessment of physiological parameters

For the determination of physiological parameters[27], the bulbs were removed from the tubes at the end of the experimental period and washed with water. "Germination" was determined by taking into account the adventitious roots emerging from the *A. cepa* disk stem and having a length of at least 1 cm. Germination percentage ($n = 50$) was determined using the following equation (Eq. 1):

$$\text{Germination Percentage} = (\text{Number of Germinated Bulbs} / \text{Total Number of Bulbs}) \times 100 \quad (1)$$

For the determination of root elongation (cm), the length of 100 adventitious roots from randomly selected bulbs ($n = 10$) from each group was measured with the help of a ruler ($n = 10$). To determine the total weight gain, 10 onions randomly selected from each group were considered. Total weight gain (g) was determined by subtracting the weight of the onions at the end of the experiment from the weight at the beginning of the experiment ($n = 10$).

Assessment of genotoxicity parameters

The genotoxicity potential of the test chemicals was determined through the analyses of MI, MN, CAs, and DNA damage. Initially, 2-cm pieces were extracted from the roots of *A. cepa*. These pieces were then removed from the solutions, rinsed thoroughly, and decontaminated from any residual chemicals. The collected root tips were first fixed in Clarke's fluid (a mixture of 3-parts ethanol to 1-part acetic acid) for 2 h. Following fixation, they were hydrolyzed in 1 N hydrochloric acid at 60 °C for 15 min. After hydrolysis, the samples were stained with 1% acetocarmine and left at room temperature for 24 h. To evaluate the MI, CAs, and MN frequencies, slides were prepared using the squash method and analyzed under a research microscope at 400× magnification[28]. For each group, 1,000 cells were counted to assess MNs and CAs, while 10,000 cells per group were analyzed to calculate the MI. MI was determined using the following formula:

$$\text{MI}\ (\%) = (\text{Cells undergoing division} / \text{Total counted cells}) \times 100 \quad (2)$$

The quantification of DNA damage was performed by the Comet assay. The isolation of DNA from root tissue was conducted in compliance with the method proposed by Sharma et al.[29]. The comet assay was performed in accordance with the method initially proposed by Dikilitaş and Koçyiğit[30]. The Comet assay procedure was described in detail in the supplementary material. The images obtained from the Comet assay were analyzed using the TriTek 2.0.0.38 Automatic Comet Assay software. The DNA content in the head and tail regions of the fragments was quantified as a percentage. The extent of DNA damage was evaluated following the criteria outlined by Pereira et al.[31].

Assessment of biochemical parameters

The changes in the levels of MDA, proline, and chlorophyll pigments and the activities of SOD and CAT enzymes were assessed to monitor the impact of $AlCl_3$ and *R. pollinaria* extract treatments on the antioxidant system.

MDA concentration

MDA concentrations were assessed to evaluate lipid peroxidation levels in root cells. Fresh root samples were homogenized in 1 mL of 5% trichloroacetic acid, and the homogenates were centrifuged to obtain the supernatants. Equal volumes of the supernatant, 0.5% thiobarbituric acid, and 20% trichloroacetic acid were mixed thoroughly and incubated at 96 °C for 30 min to facilitate the reaction. The reaction mixtures were rapidly cooled on ice and then centrifuged at 10,000 g for 5 minutes[32]. The absorbance of the supernatants was recorded at 532 nm using a UV–Vis spectrophotometer[33].

Proline level

To evaluate the alterations in proline levels, 0.5 g of root sample was homogenized in 10 mL of 3% sulfosalicylic acid under chilled conditions. The homogenate was filtered and the clear filtrate was collected. A 2 mL of the filtrate was mixed thoroughly with 2 mL of glacial acetic acid and 2 mL of acid-ninhydrin solution in a glass tube. The mixture was kept at 100 °C for 1 h to promote the formation of the proline-ninhydrin complex. The reaction was then rapidly halted by cooling the tubes in an ice-water bath. Following this, 4 mL of toluene was

Groups	Test solution
Control	Tap water
RP 1	0.5 g L^{-1} *R. pollinaria* extract
RP 2	1.0 g L^{-1} *R. pollinaria* extract
Al	50 mg L^{-1} $AlCl_3$
AlRP 1	50 mg L^{-1} $AlCl_3$ + 0.5 g L^{-1} *R. pollinaria* extract
AlRP 2	50 mg L^{-1} $AlCl_3$ + 1.0 g L^{-1} *R. pollinaria* extract

Table 1. Solutions applied to the *A. cepa* groups.

introduced into each reaction tube, and the mixture was vigorously shaken for 20 s to obtain chromophore phase. The absorbance of the chromophore containing upper toluene layer was recorded at 520 nm using a spectrophotometer. Proline concentration in the tissue samples was calculated using a calibration curve drawn from known concentrations of L-proline standards. Proline level was determined on a fresh weight basis using Eq. 3:

$$\text{Proline}\ \left(\mu\text{mol g}^{-1}\text{FW}\right) = \left(\mu\text{g proline mL}^{-1} \times \text{mL toluene}\right) / \left(115.5 \times \text{g sample}\right) \quad (3)$$

Chlorophyll levels

A 0.2 g sample of *A. cepa* leaf sample was homogenized in 5 mL of 80% acetone using a chilled mortar and pestle to determine chlorophyll levels. The resulting extract was stored in the refrigerator in dark for one week. After this period, an additional 5 mL of 80% acetone was added, mixed thoroughly, and centrifuged at 1,000 g[34]. The measurement of the absorbances of the obtained supernatants was conducted at 645 nm and 663 nm, employing a UV/VIS spectrophotometer for the purpose. Pigment levels were calculated using the formula described by Witham et al.[35].

Enzyme activities

The extracts used for the evaluation of SOD and CAT activities, two enzymes that reduce oxidative stress and are affected by it, were prepared according to the method of Zou et al.[36]. Preparation of enzyme extracts for the evaluation of SOD and CAT activities was described in detail in the supplementary material.

To determine SOD enzyme activity, 0.01 mL of extract was added into a glass tube, containing sodium phosphate buffer, nitroblue tetrazolium chloride, methionine, riboflavin, EDTA-Na_2, polyvinylpyrrolidone, and deionized water. The reaction mixture was exposed to illumination under a 30 W fluorescent lamp for 10 min to induce the generation of superoxide radicals. Following illumination, the tubes were incubated in the dark for an additional 10 min to terminate the reaction. SOD activity levels were measured by determining the absorbance at 560 nm wavelength using a spectrophotometer[37].

To assess changes in CAT activity, hydrogen peroxide, deionized water, and sodium phosphate buffer were mixed with 0.2 mL of enzyme extract. The reduction of hydrogen peroxide was monitored by measuring the absorbance at 240 nm[38].

Assessment of meristematic cell damage

The effects of $AlCl_3$ and *R. pollinaria* extract treatments on *A. cepa* root meristem cells were evaluated by analyzing ten randomly selected cross-sections from each experimental group. The cross-sections, prepared manually, were stained with 1% methylene blue and examined under a light microscope at 400× magnification. Levels of meristematic cell damages were assessed and classified into four categories based on the severity of injury: uninjured, slightly injured, moderately injured, and severely injured[39].

Assessment of phenolic compounds in *R. pollinaria*

The phenolic compound profile of *R. pollinaria* was analyzed at HUBTUAM, Hitit University. The extraction was performed using a 4:1 methanol–dichloromethane solvent system, and the phenolic composition was determined by the means of liquid chromatography-tandem mass spectrometry (LC-MS/MS; Thermo Scientific). A detailed description of the methodology used for the assessment of phenolic compounds in *R. pollinaria* is provided in the supplementary material.

Statistical analysis

Data obtained from study were statistically analyzed using SPSS Statistics version 23.0. The normality of all data was checked ($p > 0.05$) using both the Kolmogorov–Smirnov and Shapiro–Wilk tests. Differences between group means were evaluated using ANOVA, followed by Duncan's test for post hoc comparisons. A p-value of less than 0.05 ($p < 0.05$) was accepted statistically significant. Results were expressed as mean ± standard deviation (SD) in the tables.

Results and discussion

Physiological parameters

The protective effect of *R. pollinaria* extract against physiological damage caused by $AlCl_3$ exposure in *A. cepa* root meristem cells was determined using germination percentage, root length, and weight gain parameters (Table 2). The control group, which was treated with tap water, exhibited 100% germination. The RP 1 and RP 2 groups exhibited values that were comparable to the control group, suggesting that the extract alone did not exert any adverse effects. In the Al group treated with $AlCl_3$, the germination percentage decreased to 55%. The mean values for root length and weight gain exhibited a decline of 80.49% and 76.56%, respectively, in comparison to the control group. The difference between the values was statistically significant ($p < 0.05$). In the AlRP 1 and AlRP 2 groups, where $AlCl_3$ and *R. pollinaria* extract were applied as a mixture, the germination percentages increased compared to the Al group and were found to be 66% in the AlRP 1 group and 75% in the AlRP 2 group. Additionally, the root length and weight gain values of these groups were significantly increased compared to the Al group ($p < 0.05$). The improvements were more pronounced at the higher extract dose. Indeed, the root length and weight gain values of the AlRP 2 group were 3.39 and 2.80 times those of the Al group, respectively.

In agreement with our findings, numerous studies have demonstrated that various heavy metals, including Al, can impede the germination and growth in plants[40,41]. For instance, Doğan et al.[42] reported that water samples collected from the Pazarsuyu stream in Giresun province led to a reduction in germination, root elongation, and

Groups	Germination (%)	Root elongation(cm)	Initial weight (g)	Final weight (g)	Weight gain (g)
Control	100[a]	7.74 ± 0.30[a]	11.3 ± 0.53	17.7 ± 0.45	+ 6.40[a]
RP 1	98[a]	7.64 ± 0.35[a]	11.2 ± 0.48	18.2 ± 0.49	+ 7.00[a]
RP 2	99[a]	7.82 ± 0.41[a]	11.0 ± 0.50	17.9 ± 0.42	+ 6.90[a]
Al	55[d]	1.51 ± 0.28[d]	11.0 ± 0.66	12.5 ± 0.42	+ 1.50[d]
AlRP 1	66[c]	3.15 ± 0.33[c]	11.2 ± 0.47	14.1 ± 0.48	+ 2.90[c]
AlRP 2	75[b]	5.12 ± 0.35[b]	11.4 ± 0.65	15.6 ± 0.49	+ 4.20[b]

Table 2. Protective efficacy of *R. pollinaria* extract in $AlCl_3$-mediated physiological toxicity. Control: Tap water, RP 1: 0.5 g L^{-1}*R. pollinaria* extract, RP 2: 1.0 g L^{-1}*R. pollinaria* extract, Al: 50 mg L^{-1} $AlCl_3$, AlRP 1: 50 mg L^{-1} $AlCl_3$ + 0.5 g L^{-1}*R. pollinaria* extract, AlRP 2: 50 mg L^{-1} $AlCl_3$ + 1.0 g L^{-1}*R. pollinaria* extract. Germination was assessed using 50 bulbs ($n = 50$), while root elongation and weight gain analyses were conducted on 10 bulbs ($n = 10$) per treatment. Means denoted by different letters ($^{a–d}$) within the same column are statistically significant ($p < 0.05$).

Damages	Control	RP 1	RP 2	Al	AlRP 1	AlRP 2
MI (%)	751 ± 18.7[a] (7.51)	757 ± 19.8[a] (7.57)	753 ± 20.5[a] (7.53)	415 ± 29.5[d] (4.15)	484 ± 19.7[c] (4.84)	553 ± 20.5[b] (5.53)
MN	0.20 ± 0.42[d]	0.30 ± 0.48[d]	0.00 ± 0.00[d]	60.8 ± 4.18[a]	50.3 ± 3.86[b]	38.9 ± 3.48[c]
VC	0.30 ± 0.48[d]	0.20 ± 0.42[d]	0.40 ± 0.52[d]	50.0 ± 4.16[a]	40.0 ± 4.06[b]	31.9 ± 3.51[c]
SC	0.50 ± 0.53[d]	0.40 ± 0.52[d]	0.30 ± 0.48[d]	41.1 ± 4.18[a]	31.4 ± 4.06[b]	23.5 ± 3.66[c]
FRG	0.00 ± 0.00[d]	0.00 ± 0.00[d]	0.00 ± 0.00[d]	33.5 ± 3.44[a]	24.1 ± 4.12[b]	16.5 ± 3.17[c]
UDC	0.20 ± 0.42[d]	0.30 ± 0.48[d]	0.10 ± 0.32[d]	28.4 ± 3.41[a]	21.4 ± 3.72[b]	16.9 ± 3.14[c]
B	0.00 ± 0.00[d]	0.00 ± 0.00[d]	0.00 ± 0.00[d]	21.4 ± 3.13[a]	15.6 ± 2.50[b]	10.0 ± 2.00[c]
D	0.00 ± 0.00[d]	0.00 ± 0.00[d]	0.00 ± 0.00[d]	16.9 ± 2.96[a]	10.6 ± 2.76[b]	6.90 ± 2.23[c]
BC	0.00 ± 0.00[d]	0.00 ± 0.00[d]	0.00 ± 0.00[d]	11.3 ± 2.06[a]	6.70 ± 1.77[b]	3.90 ± 1.79[c]

Table 3. Protective efficacy of *R. pollinaria* extract in $AlCl_3$-mediated genotoxicity. Control: Tap water, RP 1: 0.5 g L^{-1}*R. pollinaria* extract, RP 2: 1.0 g L^{-1}*R. pollinaria* extract, Al: 50 mg L^{-1} $AlCl_3$, AlRP 1: 50 mg L^{-1} $AlCl_3$ + 0.5 g L^{-1}*R. pollinaria* extract, AlRP 2: 50 mg L^{-1} $AlCl_3$ + 1.0 g L^{-1}*R. pollinaria* extract. MN and CAs were assessed using 1,000 cells, while MI analysis was performed on 10,000 cells per treatment. Means denoted by different letters ($^{a–d}$) within the same line are statistically significant ($p < 0.05$). MI: mitotic index, MN: micronucleus, VC: vagrant chromosome, SC: sticky chromosome, FRG: fragment, UDC: unequal distribution of chromatin, B: bridge, D: disorientation, BC: binuclear cell.

weight gain in *A. cepa*, resulting from the presence of heavy metals such as iron, strontium, barium, beryllium, molybdenum, and lithium. Furthermore, the presence of heavy metals such as uranium[43], manganese[44], cobalt[45], and nickel[46] has been observed to result in a decline in growth in *A. cepa*. Even micromolar Al concentrations cause a response at the tips of the roots, which are the most vulnerable portion of the plant root system. In this regard, two important markers of Al toxicity in plants are the quick suppression of root growth and the distortion of root shape[47]. According to Hajiboland et al.[48], the primary factor contributing to the restriction of cell proliferation and elongation within the root zone in the case of Al toxicity is the disruption of meristem cells in this region. A key factor in Al-affected root growth is variations in surface pH caused by the rate at which H^+ pumps across the root tip plasma membrane[49]. In addition to inhibiting germination, heavy metal ions can limit the absorption of water and essential nutrients required for cell division, leading to suppressed plant development[42,50]. Research has also shown that heavy metals alter grana structure, and inhibit chlorophyll synthesis, respiration, photosynthesis, and nitrogen metabolism. These disruptions collectively impair overall plant growth. A major factor underlying these toxic effects is the excessive production of ROS under heavy metal stress[43]. In addition, another heavy metal, manganese, has been shown to reduce growth by restricting auxin accumulation in newly developing roots[51].

Genotoxicity parameters

MI, a key indicator of cell proliferation, is widely used to assess the cytotoxic effects of environmental pollutants[52]. There were no statistically significant differences in the MI values or in the frequencies of MN and CAs between the RP groups and the control ($p > 0.05$) (Table 3). However, exposure to Al significantly reduced the MI in *A. cepa* root meristem cells and increased the frequency of MN and CAs (Table 3; Fig. 1). The decline in MI was consistent with reduced growth parameters, suggesting that growth inhibition is closely linked to suppressed mitotic activity in the meristematic region. The mean MI, MN, and CA values in the AlRP 1 and AlRP 2 groups were significantly different from those in the RP group ($p < 0.05$). Compared to the RP group, the MI value increased in these groups, while the MN and CA values decreased. The level of change increased with the dose of the extract in the Al-lichen extract mixture. Our findings were in agreement with other studies reporting

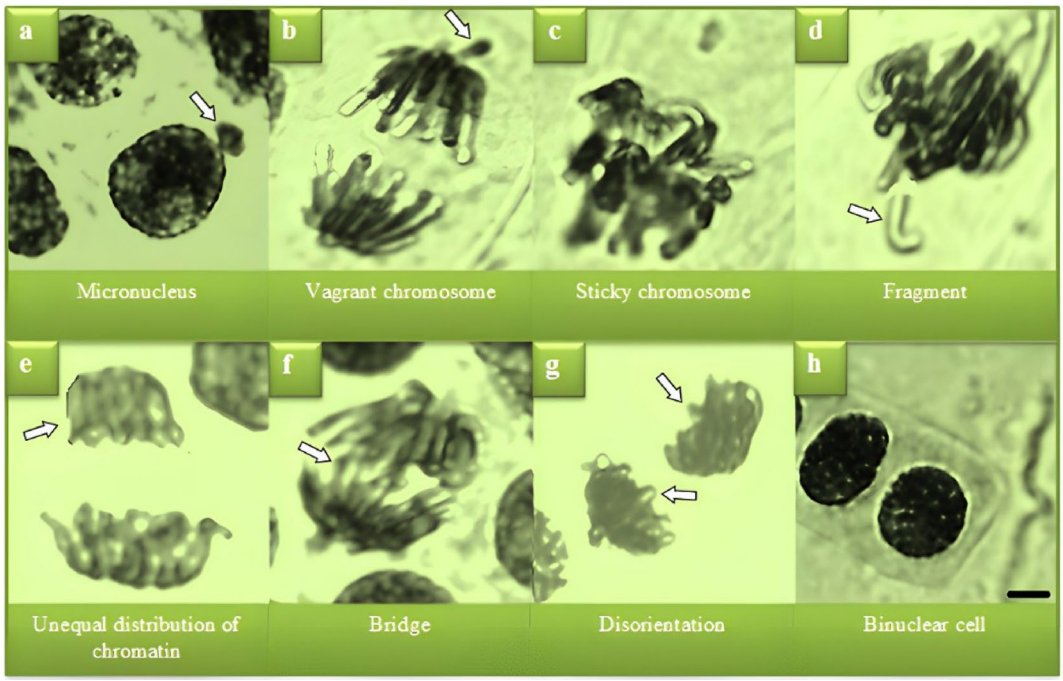

Fig. 1. $AlCl_3$-mediated CAs. Bar = 10 µm.

that heavy metal exposure decreases MI and leads to accumulation of MN and CAs[53,54]. Jaskowiak et al.[55] noted that Al restricts cell division by altering the composition and rigidity of the cell wall, which was confirmed by the Al-induced MI (Table 3) and growth reduction (Table 2) in this study. The MN test is a reliable method for determining the genotoxic potential of environmental agents[56]. MN (Fig. 1a) typically arises from chromosomal fragments or entire chromosomes excluded from daughter nuclei during telophase. Such formations can result from clastogenic or aneugenic exposure, oxidative stress, genetic disruptions in DNA repair or cell cycle regulation, deficiencies in cofactors critical for DNA metabolism, or errors in chromosomal segregation[57]. Heavy metals are known to cause DNA double-strand breaks, and if these are not repaired before DNA replication, structural CAs may occur[58]. Among the CAs observed, vagrant chromosomes were the most frequent (Table 3; Fig. 1b). These result from spindle apparatus dysfunction, causing improper segregation of chromosomes, often due to their premature movement toward the poles[59]. The second most frequent abnormality, sticky chromosomes (Table 3; Fig. 1c), indicates a lethal interaction between chemical agents and chromosomal DNA, leading to improper chromatin condensation and separation[60]. When telomeres are missing, chromosomes become "sticky" and can attach to other broken chromosomal ends[59]. According to Debnath et al.[61], chromosome stickiness is responsible for the development of chromosomal fragmentation (Fig. 1d) and bridge (Fig. 1f), and as noted by Barman and Ray[62], both fragmentation and bridge formation are direct effects of chemical-induced DNA damage. Bridge can result in structural chromosomal mutations and develop after translocation of unequal distribution of chromatin (Fig. 1e)[63]. According to Seth et al.[64], disorientation (Fig. 1g), bridge, and unequal chromatin distribution constitute key markers of a malfunctioning spindle thread system. The binuclear cell, which is evidence of blocked cytokinesis at any point in the cell cycle, was one of the most common CAs in the RP group (Table 3; Fig. 1h). Al is known to induce the formation of binuclear or polyploid cells by disrupting microtubule formation in plants[65]. The results of the present study are in agreement with those of Yi et al.[66] who showed that Al caused notable increases in the frequencies of MN and CAs resulting in a decrease in MI in *Vicia faba*. Similarly, da Cunha Neto et al.[67] reported that Al induced oxidative stress, caused chromosomal damage, reduced MI, and exhibited genotoxic effects in *A. cepa*. These findings reinforce the view that Al exerts cytotoxic and genotoxic effects across different plant species, likely through a combination of oxidative stress and disruption of the mitotic apparatus.

The Comet assay is a reliable, simple, reproducible, low-cost, and rapid system that can be used in a small number of cells of model organisms independent of mitosis[68]. One of the most notable features of the test is that it allows the evaluation of both DNA damage and DNA repair capacity at the single-cell level[69]. It was performed to determine DNA fragmentation resulted from $AlCl_3$ and *R. pollinaria* extract exposure in *A. cepa* cells. The percentage of tail DNA was used as a criterion for DNA damage. According to the findings of the control, RP 1, RP 2 groups, the DNA damage caused by tap water and *R. pollinaria* extracts in *A. cepa* cells was negligible or not detectable (Table 4). However, $AlCl_3$ caused high damage to the genetic material of *A. cepa* cells. The percentage of tail DNA from the AlRP 1 and AlRP 2 groups exhibited a statistically significant decrease compared to the Al group. This decline was more pronounced at higher concentrations of the extract in the mixture, and the DNA damage levels in both AlRP 1 and AlRP 2 groups were classified as "moderate damage." (Table 4).

Parameters	Control	RP 1	RP 2	Al	AlRP 1	AlRP 2
Head diameter (px)	28.000	30.000	26.000	20.000	18.000	28.000
Head density	158.783	212.129	174.962	155.369	116.038	180.387
Head DNA (%)	**96.2 ± 1.69^{b}**	**97.5 ± 1.43^{b}**	**99.5 ± 0.53^{a}**	**56.8 ± 1.75^{e}**	**67.5 ± 1.84^{d}**	**79.0 ± 1.49^{c}**
Tail length (px)	2.000	2.000	6.000	614.000	468.000	474.000
Tail density	6.217	5.365	851	118.283	55.743	48.068
Tail DNA (%)	**3.80 ± 1.69^{d}**	**2.50 ± 1.43^{d}**	**0.50 ± 0.53^{e}**	**43.2 ± 1.75^{a}**	**32.5 ± 1.84^{b}**	**21.0 ± 1.49^{c}**
Tail moment	0.075358	0.074002	0.019361	20.315	11.033	4.839

Table 4. Comet assay for DNA damage. Control: Tap water, RP 1: 0.5 g L^{-1}*R. pollinaria* extract, RP 2: 1.0 g L^{-1}*R. pollinaria* extract, Al: 50 mg L^{-1} $AlCl_3$, AlRP 1: 50 mg L^{-1} $AlCl_3$ + 0.5 g L^{-1}*R. pollinaria* extract, AlRP 2: 50 mg L^{-1} $AlCl_3$ + 1.0 g L^{-1}*R. pollinaria* extract. 1,000 cells were examined in each group. Means denoted by different letters ($^{a-e}$) within the same line are statistically significant ($p < 0.05$). The percentage of tail DNA was used to evaluate DNA damage based on the Comet scale. No or minimal (≤%5), low damage (%5–20), moderate damage (%20–40), high damage (%40–75), severe damage (≥%75).

Parameters	Control	RP 1	RP 2	Al	AlRP 1	AlRP 2
MDA (µM g^{-1}FW)	2.80 ± 0.46^{d}	2.82 ± 0.53^{d}	2.85 ± 0.49^{d}	8.14 ± 0.45^{a}	6.48 ± 0.41^{b}	5.14 ± 0.39^{c}
Proline (µmol g^{-1}FW)	11.3 ± 0.38^{d}	11.4 ± 0.41^{d}	11.7 ± 0.51^{d}	30.6 ± 0.59^{a}	23.5 ± 0.51^{b}	18.8 ± 0.60^{c}
Chlorophyll a (mg g^{-1}FW)	13.5 ± 0.44^{a}	13.3 ± 0.51^{a}	13.8 ± 0.55^{a}	3.93 ± 0.48^{d}	7.23 ± 0.48^{c}	9.31 ± 0.42^{b}
Chlorophyll b (mg g^{-1}FW)	7.97 ± 0.51^{a}	7.86 ± 0.47^{a}	7.92 ± 0.46^{a}	2.05 ± 0.41^{d}	3.31 ± 0.40^{c}	4.68 ± 0.38^{b}
SOD (U mg^{-1}FW)	103 ± 4.31^{d}	105 ± 6.30^{d}	104 ± 5.45^{d}	303 ± 5.46^{a}	260 ± 5.42^{b}	224 ± 4.98^{c}
CAT ($OD_{240\ nm}$min g^{-1}FW)	1.18 ± 0.05^{d}	1.16 ± 0.06^{d}	1.19 ± 0.07^{d}	2.95 ± 0.06^{a}	2.53 ± 0.07^{b}	2.13 ± 0.07^{c}

Table 5. Protective efficacy of *R. pollinaria* extract in $AlCl_3$-mediated biochemical toxicity. Control: Tap water, RP 1: 0.5 g L^{-1}*R. pollinaria* extract, RP 2: 1.0 g L^{-1}*R. pollinaria* extract, Al: 50 mg L^{-1} $AlCl_3$, AlRP 1: 50 mg L^{-1} $AlCl_3$ + 0.5 g L^{-1}*R. pollinaria* extract, AlRP 2: 50 mg L^{-1} $AlCl_3$ + 1.0 g L^{-1}*R. pollinaria* extract. Means denoted by different letters ($^{a-d}$) within the same line are statistically significant ($p < 0.05$). MDA: malondialdehyde, SOD: superoxide dismutase, CAT: catalase.

The correlation between Comet assay results and the presence of MN indicates that genomic stability is disrupted in *A. cepa* exposed to the toxic compound[70]. Similar to other studies[64,71], the present study confirmed that heavy metal exposure causes DNA damage in *A. cepa*, evidenced by Comet assay results. According to earlier research employing the Comet assay, growth on media containing Al causes DNA breakage in *Arabidopsis*[72]. Additionally, Lankoff et al.[73] reported that DNA damage caused by Al exposure in human peripheral blood lymphocytes is Al dose dependent. Meriga et al.[74] suggested that Al-induced DNA damage in *Oryza sativa* root cells is related to ROS generation, lipid peroxidation, or Al interaction with ions such as Mg^{2+} and Ca^{2+}, which play a crucial role in DNA integrity. Murali Achary and Panda[75] demonstrated using the Comet assay that Al-induced DNA damage in *A. cepa* root cells was primarily associated with the accumulation of hydrogen peroxide, and to a lesser extent superoxide radicals. The suppression of the DNA repair process by Al may also have increased DNA damage in *A. cepa* root cells exposed to $AlCl_3$. The unfolding of chromatin during S-phase to facilitate DNA replication renders DNA more susceptible to damage. It was also reported that Al metal has the capacity to completely dissolve DNA, thereby increasing its fragility, and that this process is irreversible[73]. On the other hand, Meriga et al.[74] suggested that toxic Al compounds increase the stiffness of the DNA double helix, form direct complexes with DNA, and repress transcription.

Biochemical parameters

To evaluate the biochemical toxicity induced by $AlCl_3$ and *R. pollinaria* extract in *A. cepa*, the levels of MDA, proline, chlorophyll a, and chlorophyll b, as well as the activities of SOD and CAT enzymes, were assessed (Table 5). The overproduction of ROS can interfere with essential cellular processes by compromising membrane stability, altering protein structures, and inducing DNA damage. One of the most commonly employed indicators of oxidative stress-induced membrane damage is MDA, which forms as a result of lipid peroxidation[76]. There was no statistically significant difference between the mean MDA values of the control, RP 1, and RP 2 groups ($p > 0.05$) (Table 5). In contrast, the MDA level in the group exposed to $AlCl_3$ alone (Al group) increased to approximately 2.9 times that of the control group. However, co-treatment with *R. pollinaria* lichen extract significantly reduced MDA levels, with decreases of 20.4% in the AlRP 1 group and 36.9% in the AlRP 2 group compared to the Al group. These results indicate that increasing the concentration of lichen extract in the mixture helped to alleviate $AlCl_3$-induced membrane damage. Similar to the findings determined in the MDA levels, the first three groups (the control, RP 1, and RP 2 groups) showed statistically similar results with regard to the activities of SOD and CAT enzymes ($p > 0.05$) (Table 5). On the other hand, SOD and CAT activities of the Al group was 2.9 and 2.5 times those of the control group, respectively. In the AlRP 1 and

AlRP 2 groups, the activities of antioxidant enzymes were significantly lower than those in the Al group. The AlRP 2 group exhibited a reduction of 26.1% in SOD activity and 27.8% in CAT activity compared to the Al group (Table 5). Our results align with those of prior studies, which demonstrated that Al exposure elevated the levels of MDA and the activity of SOD and CAT enzymes in sorghum[77] and tea plants[78]. Achary et al.[11] proved that Al exposure increased not only DNA damage but also MDA level and SOD activity in *A. cepa* as in our findings. Interestingly, however, their study also reported a suppression of CAT activity under Al stress, which contrasts with our results. The activation of antioxidant enzymes in plant tissues plays a crucial role in mitigating oxidative stress and supports the synthesis of growth-promoting compounds, thereby enhancing the plant's ability to cope with metal-induced stress. In their study, Achary et al.[11] observed that Al accumulation in *A. cepa* root tissues was accompanied by a sequential formation of ROS, starting with superoxide, followed by hydrogen peroxide, and finally hydroxyl radicals. The present study supports this finding by showing increased activities of SOD and CAT enzymes in the roots of the Al-treated group, which are responsible for scavenging superoxide and hydrogen peroxide radicals, respectively. However, the increased activities of SOD and CAT were not sufficient to prevent lipid peroxidation in cell membranes (Table 5). Al-induced ROS generation is likely mediated by multiple mechanisms, including rapid activation of membrane-bound NADPH oxidases, disruption of mitochondrial electron transport, apoplastic Fenton-type reactions, and disturbances in cellular pH and calcium signaling[79–81].

Under healthy conditions, antioxidant enzymes and non-enzymatic antioxidants, such as proline, function in a synergistic manner to produce and eliminate free radicals within the cell[78]. In this study, although there was no statistical difference between the proline content of the control, RP 1, and RP 2 groups, the proline level of the Al group was 2.7 times that of the control (Table 5). On the other hand, the proline levels of the AlRP 1 and AlRP 2 groups were significantly lower than those of the Al group. When AlRP 1 and AlRP 2 groups were compared, it was determined that the proline concentration of AlRP 2 group was lower than the other group. Indeed, the lichen extract administered to the AlRP 2 group reduced the proline accumulation caused by $AlCl_3$ alone by 38.6% in the Al group. There are many studies in the literature showing that Al alters proline concentration in plants. For instance, Al toxicity has been observed to induce proline accumulation in *Lactuca sativa*[82], *Secale cereale*[83], and *Pinus sylvestris*[84]. Proline is a crucial amino acid for the maintenance of normal metabolism and growth in plants subjected to abiotic stress. It functions as a molecular chaperone, a ROS-scavenging antioxidant, and a signal for the activation of stress tolerance genes under stress conditions, thanks to its metal-chelating properties[85]. This biomolecule also enhances the resistance of plants to abiotic stresses by increasing photosynthetic capacity and stimulating enzymatic and non-enzymatic antioxidant activity, regulating osmolyte accumulation, and stabilizing sodium and potassium homeostasis[86]. Proline has been shown to play a role in the maintenance of growth by increasing chlorophyll pigment in *Vigna unguiculata* exposed to cadmium toxicity[87]. The cellular damage observed under Al stress suggests that elevated proline accumulation, similar to enhanced antioxidant enzyme activity, was inadequate to provide effective protection.

Alterations in chlorophyll a and chlorophyll b levels content are sensitive and visible indicators of abiotic stress experienced by plants[88]. In the present study, the application of $AlCl_3$ resulted in a decline in the chlorophyll a and chlorophyll b level of the Al group. (Table 5). Although there was no significant difference in pigment content between the control, RP 1, and RP 2 groups ($p > 0.05$), chlorophyll a and chlorophyll b levels in the $AlCl_3$ group decreased by 70.9% and 74.3%, respectively, compared to the control group (Table 5). On the other hand, the chlorophyll a and chlorophyll b content in the AlRP 1 and AlRP 2 groups showed a significant increase compared to the Al group (Table 5). This change became more evident as the dose of *R. pollinaria* extract increased in the AlRP groups. Indeed, chlorophyll a and chlorophyll b levels in the AlRP 2 group were 2.4 and 2.3 times those of the Al group, respectively. Our findings align with previous studies showing that aluminum (Al) toxicity decreases chlorophyll levels in plants such as *Zea mays*[89], *Oryza sativa*[90], and *Vallisneria natans*[91]. Heavy metal stress can damage chloroplast membranes through lipid peroxidation and thus indirectly lead to chlorophyll degradation[92]. In addition, Yang et al.[93] suggested that some heavy metals accelerate chlorophyll breakdown by altering chlorophyllase activity. Furthermore, the replacement of magnesium in the center of the chlorophyll molecule with a heavy metal and suppression of δ-aminolevulinic acid dehydratase and protochlorophyllide reductase enzymes are also among the important causes of heavy metal-induced chlorophyll loss in plants[94]. Khan et al.[91] reported that vacuoles grew in Al-treated cells by storing Al as an intracellular detoxification mechanism. However, the presence of Al in the cell still disrupted the integrity of the chloroplast.

Meristematic cell damage

Table 6; Fig. 2 present the meristematic cell damage observed in *A. cepa* root meristem cells following exposure to $AlCl_3$ and treatment with *R. pollinaria* extract. In both the control group and the groups treated with *R. pollinaria* (RP 1 and RP 2), the cellular structures remained intact, with no visible abnormalities (Fig. 2a, c and e, and 2g). In contrast, treatment with 0.5 g L^{-1} $AlCl_3$ induced noticeable meristematic alterations, epidermis cell damage (Fig. 2b), flattened nucleus (Fig. 2d), an increase in nucleolus number (Fig. 2f), and cortex cell damage (Fig. 2h). On the contrary, the severity of all meristematic cell injury types decreased in a dose-dependent manner in the AlRP 1 and AlRP 2 groups (Table 6).

Al is known to be an important environmental stressor that negatively affects growth of plant and cellular organization, especially in root meristematic tissues where active cell division occurs[95]. Consistent with the elevated MDA levels and altered activities of SOD and CAT antioxidant enzymes, the damage to epidermal and cortex cells is likely associated with metabolic disturbances and membrane disruption. In addition, the presence of flattened nucleus and an increase in the number of nucleoli may serve as a hallmark of genetic damage, as evidenced by the genotoxicity findings of the present study (Table 3).

Conversely, treatment with *R. pollinaria* extract (AlRP 1 and AlRP 2) effectively protected *A. cepa* meristematic tissue from $AlCl_3$ induced toxicity. This suggests that the extract may contain bioactive phytochemicals such

Groups	ECD	FN	INN	KHÇK
Control	-	-	-	-
RP 1	-	-	-	-
RP 2	-	-	-	-
Al	+++	+++	++	+++
AlRP 1	++	++	++	++
AlRP 2	+	+	-	+

Table 6. Protective efficacy of *R. pollinaria* extract in $AlCl_3$-mediated meristematic cell damage. Control: Tap water, RP 1: 0.5 g L^{-1}*R. pollinaria* extract, RP 2: 1.0 g L^{-1}*R. pollinaria* extract, Al: 50 mg L^{-1} $AlCl_3$, AlRP 1: 50 mg L^{-1} $AlCl_3$ + 0.5 g L^{-1}*R. pollinaria* extract, AlRP 2: 50 mg L^{-1} $AlCl_3$ + 1.0 g L^{-1}*R. pollinaria* extract. ECD: epidermis cell damage, FN: flattened nucleus, INN: increase in the number of nucleoli, CCD: cortex cell damage. (-): no damage, (+): low damage, (++): moderate damage, (+++): severe damage. A total of 10 bulbs were used per group ($n = 10$). From each bulb, 10 root cross-sections were obtained, resulting in 100 sections per group. 0–5 damage: (–) No damage; 6–25 damage: (+) Low damage; 26–50 damage: (++) Moderate damage; 51 or more damage: (+++) Severe damage.

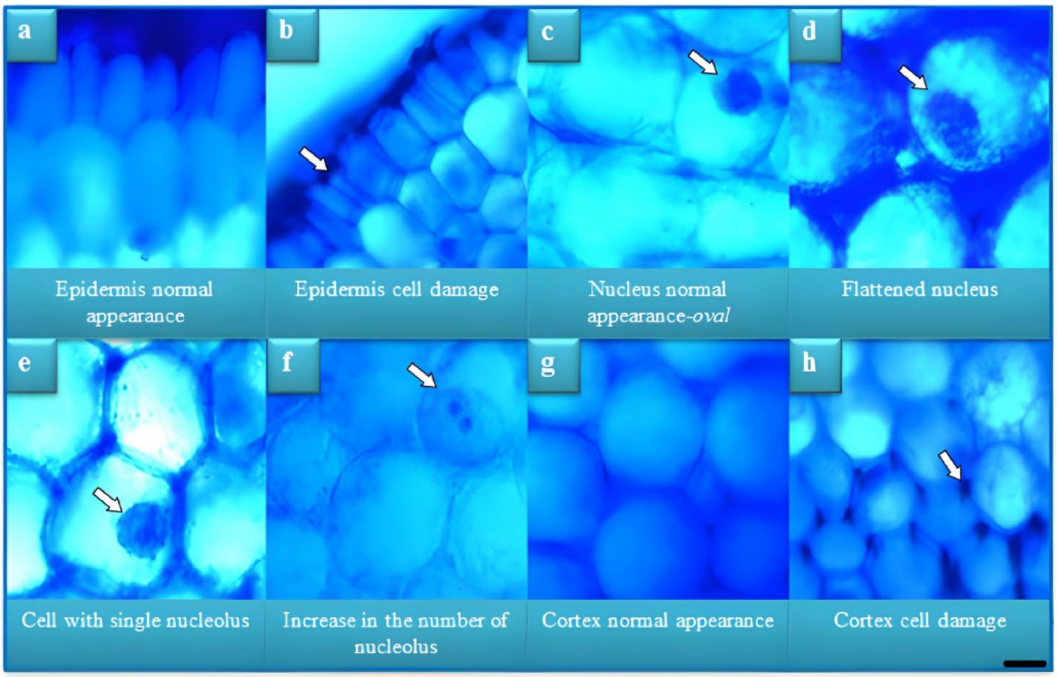

Fig. 2. $AlCl_3$-mediated meristematic cell damage. Bar = 10 µm.

as flavonoids, phenolic acids, or polysaccharides. By mitigating oxidative damage, the extract likely stabilizes membrane integrity, prevents nuclear distortion, and supports nucleolar homeostasis[96]. These protective effects align with previous studies demonstrating the role of plant-derived compounds in alleviating heavy metal-induced cytotoxicity in *A. cepa* through scavenging of free radicals and improvement of antioxidant enzyme activity[44,97].

Phenolic compounds in *R. pollinaria* extract

All of the secondary metabolites that lichens produce come from their fungal companion and have a variety of biological functions[98]. Species of the genus *Ramalina* are particularly notable for their bioactive secondary compounds, which are used in folk medicine and demonstrate a range of pharmacological properties, including antitumor, antifungal, antibacterial, antiviral, antioxidant, and anti-inflammatory effects[99]. In lichens, phenolic compounds make up a significant portion of secondary metabolites with a wide range of structural variations. Compared to the Al group, both the AlRP 1 and AlRP 2 groups showed impressive improvements in all parameters examined in this study. The investigation focused on the presence of 29 phenolic compounds in the *R. pollinaria* extract, utilizing various standards for analysis (Fig. 3). Retention times, R^2 and RSD values, LOD and LOQ values, as well as the quantitative data for all identified phenolic compounds were provided in Table 7. The presence of epicatechin, caffeic acid, sesamol, syringic acid, syringaldehyde, taxifolin, catechin, sinapic acid, salicylic acid, ferulic acid, protocatechualdehyde, rosmarinic acid, oleuropein, rutin, resveratrol, ellagic

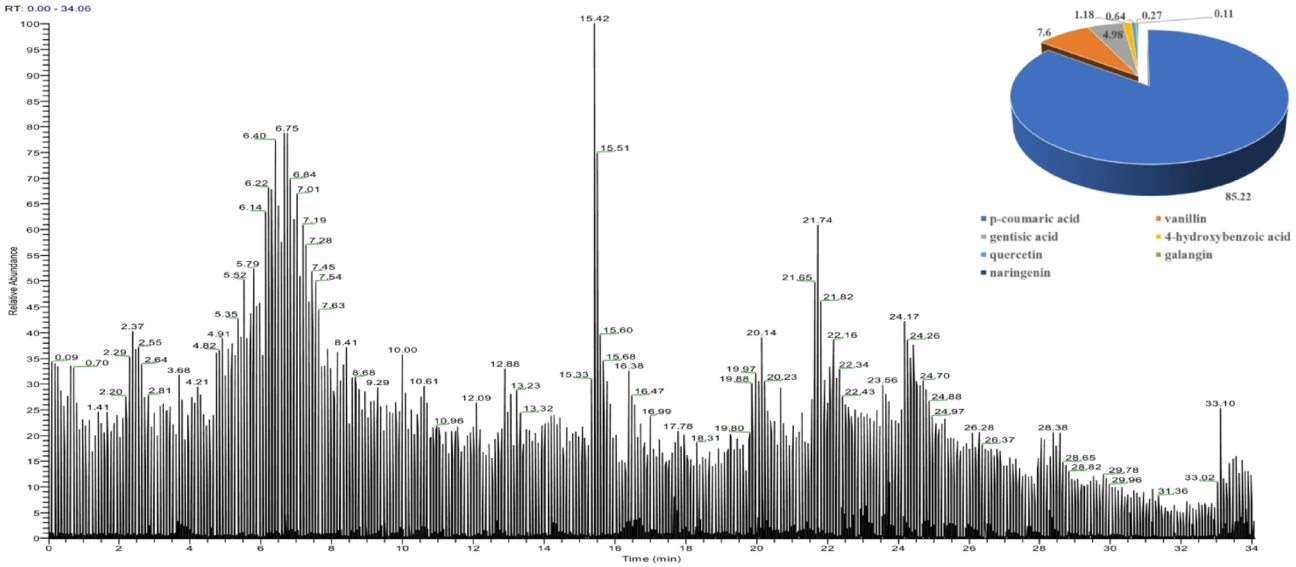

Fig. 3. LC-MS/MS chromatogram of *R. pollinaria* extract.

Rt	Phenolic substance	µg/g plant	LOD	LOQ	RSD%	R^2
8	Pyrogallol	NF	0,074	0,247	3,8	0,9982
9,68	Gallic acid	NF	0,061	0,203	4,5	0,9850
11,77	Protocatechuic Acid	NF	0,049	0,162	4,9	0,9983
12,64	Protocatechuic Aldehyde	NF	0,026	0,087	2,2	0,9984
12,65	Sesamol	NF	0,031	0,103	2,1	0,9961
12,91	Catechin	NF	0,010	0,032	3,1	0,9999
12,98	Gentisic acid	4.0	0,013	0,043	2,3	0,9985
14,38	Epicatechin	NF	0,003	0,006	2,5	0,9988
14,76	Caffeic Acid	NF	0,042	0,058	2,5	0,9991
15,27	Vannilin	6.11	0,023	0,076	3,7	0,9994
15,77	Syringic Asit	NF	0,194	0,647	4,6	0,9987
15,78	Syringic Aldehit	NF	0,127	0,423	3,2	0,9989
16,27	Taxifolin	NF	0,001	0,005	3,8	0,9994
16,42	p_Coumaric Acid	68.47	0,069	0,109	3,5	0,9985
16,87	Sinapic_acid	NF	0,091	0,303	2,8	0,9952
17,19	Salicylic Acid	NF	0,030	0,099	2,3	0,9989
17,31	Ferulic Acid	NF	0,063	0,118	4,5	0,9985
17,34	4_OH Benzoic Acid	0.95	0,243	0,809	4,1	0,9993
17,41	Rosmarinic Acid	NF	0,011	0,036	2,6	0,9999
17,51	Oleuropein	NF	0,003	0,005	2,0	0,9991
18,03	Rutin	NF	0,022	0,073	1,6	0,9950
18,05	Resveratrol	NF	0,019	0,062	4,1	0,9986
19,16	Ellagic acid	NF	0,087	0,289	4,9	0,9962
19,44	Sinammic Acid	NF	0,047	0,156	3,7	0,9954
19,71	Naringenin	0.09	0,005	0,017	3,9	0,9941
20,29	Quercetin	0.51	0,001	0,005	2,9	0,9983
21,55	Kaempherol	NF	0,092	0,306	3,9	0,9948
23,44	Galangin	0.22	0,034	0,113	5,0	0,9954
23,74	Flavone	NF	0,037	0,124	1,7	0,9995

Table 7. Amount of phenolics, Rt, R^2, LOD (mg/L), LOQ (mg/L) and RSD% values.

acid, cinnamic acid, kaempferol, gallic acid, protocatechuic acid, flavone, and pyrogallol was not found in the lichen extract. The composition of phenolic compounds produced by lichens is subject to variation according to the region and climatic conditions where the lichen is located. The phenolic compounds in the extract were listed as p-coumaric acid, vanillin, gentisic acid, 4-hydroxybenzoic acid, quercetin, galangin, and naringenin, in order of abundance (Fig. 3). There are various studies investigating the antioxidant and antibacterial effects of lichens belonging to the genus *Ramalina*[100–102]. However, to the best of our knowledge, there is no study in the extant literature that has focused on the phenolic compounds in the content of *R. pollinaria* extract to compare with the results of our own research. The antioxidant power of phenolic compounds is linked to their ability to scavenge free radicals, which is related to their reducing properties as hydrogen- or electron-donating agents. Additionally, their ability to chelate iron and copper allows them to suppress metal-catalyzed free radical formation[103]. Our study revealed that p-coumaric acid was the dominant phenolic compound in *R. pollinaria* lichen extract (Fig. 3). Navaneethan and Rasool[104] revealed that p-coumaric acid exhibited protective efficacy against cadmium-induced nephrotoxicity in rats. It has also been shown to have therapeutic effect on lung toxicity resulted from methotrexate exposure[105]. Kiliç and Yeşiloğlu[106] reported that p-coumaric acid exhibits effective scavenging activity against DPPH radical, ABTS radical, superoxide anion radical, and hydrogen peroxide, as well as reducing power against ferric ions and iron ions. Additionally, Nabi and Liu[107] demonstrated that p-coumaric acid can protect DNA from radical-induced oxidation. Vanillin, a prominent phenolic compound found in lichen extract, significantly reduced levels of MDA and hydrogen peroxide, as well as DNA damage, induced by maneb in the livers of albino mice[108]. Gentisic acid, which was found in high amounts in *R. pollinaria* extract, causes a restoration in antioxidant enzyme activity, remarkable decrease in MDA and glutathione levels, and decline in MN formation and DNA fragmentation[109]. Although p-coumaric acid was shown to be the predominant phenolic component, the presence of other bioactive phenolics implies a potential synergistic interaction that may improve the protective efficiency against Al-induced stress.

Conclusion

In the face of intensifying environmental contamination by heavy metals, the pursuit of safe, natural, and effective countermeasures has become increasingly vital. This study indicated that the lichen *R. pollinaria* shows protective activity against aluminum-induced toxicity under laboratory conditions. A comprehensive evaluation of the physiological, cytogenetic, and biochemical responses to Al-induced stress was conducted using the *A. cepa* test system, a well-established plant bioassay for toxicity monitoring. The findings revealed that the phenolic-rich extract of *R. pollinaria* effectively mitigates oxidative damage and genomic instability, likely due to its strong antioxidant capacity and metal-binding affinity.

These results emphasize the dual ecological and pharmacological potential of lichens, positioning *R. pollinaria* as a potential natural source of bioactive compounds, with possible relevance in phytoremediation and toxicity attenuation, pending confirmation in further studies. As the field of environmental toxicology advances toward integrative and sustainable strategies, *R. pollinaria* emerges as a cheap and biologically robust candidate for alleviating heavy metal-induced damage. The present study offers preliminary findings derived from controlled laboratory conditions with *A. cepa* and limited extract doses. Future research involving molecular-level analyses and broader biological models will be essential to further elucidate its protective mechanisms and application scope.

Data availability

This article provides all the data supporting the findings of the study. For further queries, the corresponding author was contacted.

Received: 18 June 2025; Accepted: 25 September 2025
Published online: 31 October 2025

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Acknowledgements

The authors would like to thank Giresun University Scientific Research Projects Office for its support of this study (FEN-BAP-A-240222-51).

Author contributions

T.K.M., O.M., E.Y. and K.Ç. designed the experiments and performed the analyses; K.K. carried out the collection of lichen samples and their identification; E.Y. and K.Ç. analyzed the biochemical parameters and the damage to meristematic cells; K.Ç. carried out the statistical analysis; T.K.M. and O.M. wrote the manuscript with the help of K.K., E.Y. and K.Ç.; T.K.M. edited the final version of the manuscript.

Funding

The consumables of this study were supplied by the Scientific Research Projects Office (FEN-BAP-A-240222-51), Giresun University.

Declarations

Competing interests

The authors declare no competing interests.

Statement regarding experimental research on plants

Experimental research and field studies on plant/lichen and plant/lichen parts (*A. cepa* bulbs, *R. pollinaria*), including the collection of plant material, comply with relevant institutional, national, and international guidelines and legislation.

Additional information

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-025-22036-9.

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