

Analysis of antiviral, antioxidant and photosynthetic parameters in relict species under stress factors

Sevil B. Dadashova

Gulnara Kh. Babayeva

Institute of Botany, Ministry of Science and Education of the Republic of Azerbaijan, A. Abbaszade str., entr. 99, AZ1004, Baku, Azerbaijan

Aytaj I. Askerova

Scientific-Research Institute of Fruit and Tea, Ministry of Agriculture of the Republic of Azerbaijan, Guba-Khachmaz road, Guba, AZ4035, Azerbaijan

Gulnar G. Sultanova

Rena A. Ganiyeva¹

Institute of Botany, Ministry of Science and Education of the Republic of Azerbaijan, A. Abbaszade str., entr. 99, AZ1004, Baku, Azerbaijan

Abstract: Relict plant species have evolved biochemical strategies to withstand environmental stress, including the accumulation of antioxidant compounds. This study evaluated the antioxidant and antiviral properties of leaf extracts from *Buxus sempervirens*, *Ruscus hyrcanus*, and *Parrotia persica*. Antioxidant activity was assessed using the DPPH radical scavenging method, with *B. sempervirens* showing the strongest effect at 0,04 µg/ml. Resistance of photosynthetic pigments (chlorophyll a and b) to photobleaching under high-intensity light exposure was also analyzed, revealing greater pigment stability in *B. sempervirens* compared to the other species. To assess antiviral activity, cherry rootstocks (Gizella 6) infected with PNRSV were treated with the extracts. By the sixth week, treated plants exhibited reduced symptom severity and increased healthy leaf count, particularly with *B. sempervirens* and *R. hyrcanus* extracts. These findings suggest that specific relict species possess antioxidant and antiviral properties that may contribute to stress tolerance through the protection of photosynthetic function and membrane integrity.

Keywords: antioxidant, *Buxus sempervirens*, chlorophyll, *Parrotia persica*, photoinhibition, photosynthesis, *Ruscus hyrcanus*, virus PNRSV

INTRODUCTION

Relict plant species, which originated during the Cretaceous period, serve as living indicators of past ecological conditions and environmental factors. Their

study offers valuable insights into the evolution and genesis of vegetation [Grossheim, 1940; Safarov, 1962]. These species exhibit diverse and rich phytochemical profiles, including flavonoids, alkaloids, tannins, saponins, and other bioactive compounds that contribute to their resilience against oxidative stress [Rodrigues et al., 2021; Heidari et al., 2021; Oulebsir-Mohandkaci et al., 2021]. Among these, *Buxus sempervirens* L., *Ruscus hyrcanus* Woronow, and *Parrotia persica* C.A. Mey are widely distributed across Europe, Asia, and the Caucasus, typically thriving in shady, moist, and well-drained soils [Sarı & Çelikel, 2021].

These species demonstrate notable antioxidant potential (Fig.1). For instance, extracts of *B. sempervirens* obtained with polar solvents (chloroform, ethanol, methanol) exhibit significant radical scavenging and iron-chelating capacities, while non-polar extracts show negligible activity [Orhana et al., 2012]. *R. hyrcanus* is rich in steroid saponins and membrane-stabilizing compounds, which support its stress tolerance under hypothermic conditions [Bahadori et al., 2019; Ganiyeva et al., 2024], and its methanolic root extracts have shown strong DPPH radical scavenging activity [Yousefbeyk et al., 2022]. Similarly, *P. persica*, well adapted to cold and drought, contains high levels of flavonoids, tannins, and saponins. Several secondary metabolites isolated from this species were evaluated for their inhibitory activity against prolyl endopeptidase, identifying quercetin as particularly potent ($IC_{50} = 37.12 \pm 2.2 \mu M$), compared to bacitracin ($IC_{50} = 125.00 \pm 1.5 \mu M$) [Ali et al., 2020]. Moreover, many relict plants demonstrate antiviral and antimicrobial properties that enhance their resistance to pathogens [Amtaghri & Eddouks, 2024; Saleem et al., 2022; Naidoo et al., 2024].

Plants are constantly exposed to environmental stressors that affect key physiological and biochemical processes, notably photosynthesis, with Photosystem II being particularly vulnerable to light-induced and pathogen-related damage [Momni et al., 2015]. Photosynthesis is triggered by light quanta and highly dependent on light quality and intensity; both excessive and insufficient light can impair pigment stability and

¹E-mail: rena.ganiyeva@gmail.com

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Figure 1. The figure shows relict plants A. *Buxus sempervirens* L., B. *Ruscus hyrcanus* Woronow, C. *Parrotia persica* C.A.Mey. used in this work.

chlorophyll biosynthesis [Kulchin et al., 2020; Banerjee, Roychoudhury, 2016; Zhang et al., 2022; Solhaug, 2018; Bumann, Oesterhelt, 1995]. Light-harvesting complexes in the thylakoid membrane absorb photons and initiate redox reactions crucial for plant vitality [Momni et al., 2015; Modarelli et al., 2020]. However, under stress, plants accumulate reactive oxygen species (ROS), which cause oxidative damage to chloroplast membranes and photosynthetic pigments [Ahmad et al., 2008; Foyer, 2018]. This leads to photoinhibition and rapid degradation of the D1 protein, reducing photosynthetic efficiency [Rantala et al., 2021]. To counteract these effects, plants rely on robust antioxidant defense systems. The antioxidant, antiviral, and antibacterial properties of several plant species have been widely investigated under diverse environmental and physical stressors, aiming to discover novel bioactive compounds [Habibah et al., 2023].

MATERIAL AND METHODS

Plant material. Leaves from adult relict plants – *B. sempervirens*, *R. hyrcanus*, and *P. persica* – growing in the Central Botanical Garden (CBG, Baku) were used. Leaf collection was carried out in May. For antiviral activity assays, leaves were also collected from 45-day-old Gizella 6 cherry rootstocks grown under laboratory conditions at the Scientific Research Institute of Fruit Growing and Tea Growing, Guba, Republic of Azerbaijan.

Chloroplast isolation. Chloroplasts were extracted from leaves following a standard method using 0.066 M phosphate buffer (pH 7.8) [Anderson, 1980]. To determine the resistance of chlorophyll (Chl) forms to photoinhibition, chloroplasts at a concentration of 20 mg/ml were illuminated in a mini reactor at a light intensity of 1.46×10^{-2} W/cm². This light intensity was selected to optimize the detection of longer-term

damage [Dadashova et al., 1990; Jafarova et al., 2019]. Photoinhibition was carried out in the range from 5 min to 4 hours. The concentration of Chl (20 µg/ml) was determined in 80% acetone using the Arnon formula based on the McKinney coefficients. The absorption spectra of chloroplasts in the range from 400 to 750 nm were recorded on a Furze Cary 50 Scan Varian spectrophotometer at room temperature. To determine the resistance to photoinhibition, the main forms of pigments Chl a absorbing at λ 680 nm and Chl b absorbing at λ 645 nm were taken.

Extract preparation. Leaf extracts from the studied relict species were prepared by soaking plant material in a hydroalcoholic solution under constant agitation for 10 days.

Determination of antioxidant (AO) activity. Antioxidant activity was evaluated using the DPPH radical scavenging assay according to Brand-Williams et al. [1995]. The absorbance of DPPH (2,2-diphenyl-1-picrylhydrazyl) in methanol was measured at λ = 518 nm after 20 minutes using a Jenway 7305 UV-Vis spectrophotometer. Trolox was used as a standard (1–10 µg/ml), and measurements were performed in cuvettes with a 10 mm optical path. Data analysis was conducted using Perella Scientific Inc. (Amherst, USA) software [Molyneux, 2004]. The scavenging activity was calculated using the formula:

$$\text{IC\%} = [1 - (\text{Abs}_{\text{sample}} / \text{Abs}_{\text{control}})] \times 100$$

Determination of antiviral activity. Antiviral activity of relict extracts was tested on the leaves of Gizella 6 cherry rootstock plants infected with PNRSV (*Prunus necrotic ringspot virus*, Plum Pox Virus) viruses causing ring brown spots on leaves, which negatively affects the state of photosynthetic pigments. The rootstocks were grown in laboratory conditions (26°C, humidity

80%, lighting $250 \mu\text{W}/\text{cm}^2$. The soil near the Gizella 6 cherry rootstock was irrigated with relict extracts once a week. The selection of the concentration for rootstock irrigation expressed the optimal concentration of $500 \mu\text{g}/\text{ml}$. Infected leaves were crushed and processed to the desired state with reagent and buffers (Elisa Bioreba kit, extraction buffer (10x/5x), coating buffer, conjugate buffer (10x), substrate buffer (5x), washing buffer, substrate (pNPP)/ and placed in microplates for detection of PNRSV virus on a BioTek Epoch 2 Microplate Reader [Prihodko et al., 2024]. Each buffer was used at a certain stage of the experiment.

Experiments were conducted over a 6-week period. Infected but untreated rootstocks served as controls. All samples were analyzed in five replicates.

RESULTS AND DISCUSSION

The obtained absorption spectra demonstrated changes in the activity of photosynthetic pigments depending on the action of high-intensity light. To determine the resistance to photoinhibition, the main energy harvesters were isolated – Chl a 680 nm and Chl b 645 nm. It was shown that in chloroplasts obtained from the relict *B. sempervirens*, the resistance of Chl a and Chl b to photoinhibition was more stable. Complete fading of Chl a and Chl b was determined after 220 min (Fig. 2).

In contrast, *R. hyrcanus* showed pigment degradation after 90 minutes (Fig. 3), and in *P. persica*, the pigments degraded within just 45 minutes (Fig. 4). These differences likely reflect variations in the structural integrity of the light-harvesting chlorophyll–protein complex (LHC) and the effectiveness of energy dissipation mechanisms through the proton gradient in thylakoids [Kurbanova et al., 1987; Vass, 2012; Dadashova et al., 1990]. Chlorophyll breakdown is considered a primary photodamage event linked to photosystem II inhibition [Bumann, Oesterhelt, 1995].

Determination of the AO activity of the extracts of the studied relict species showed high activity, and the 50% inhibition concentration ($\text{IC}_{50} \mu\text{g}/\text{ml}$) in the DPPH reaction was observed at a concentration of $37 \mu\text{g}/\text{ml}$ for *P. persica*, $42 \mu\text{g}/\text{ml}$ for *R. hyrcanus*. The extract of *B. sempervirens* exhibited the strongest AO activity, with an IC_{50} of $0,04 \mu\text{g}/\text{ml}$ (Fig. 5).

The antiviral effects of the extracts were evaluated on Gizella 6 cherry rootstocks infected with PNRSV. In plants treated with *P. persica* extract, leaf abscission increased after the second and third week, reaching nine diseased leaves compared to two in the control. No healthy leaf regrowth was observed by the end of the

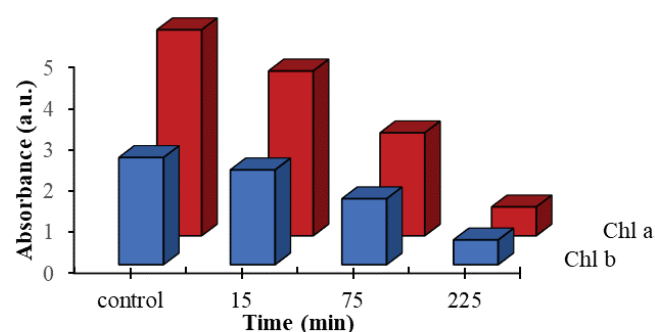


Figure 2. Dynamics of Chl a (680 nm) and Chl b (645 nm) degradation over 220 minutes in chloroplasts from *B. sempervirens* exposed to high-intensity light ($1.46 \times 10^{-2} \text{ W}/\text{cm}^2$).

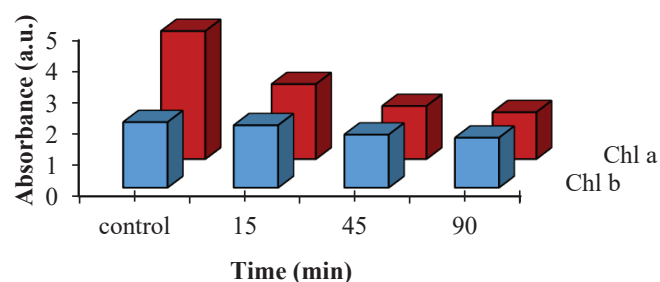


Figure 3. Dynamics of Chl a (680 nm) and Chl b (645 nm) degradation over 90 minutes in chloroplasts from *R. hyrcanus* under high-intensity light ($1.46 \times 10^{-2} \text{ W}/\text{cm}^2$).

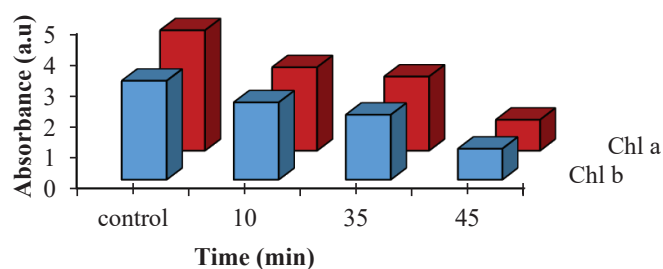


Figure 4. Dynamics of Chl a (680 nm) and Chl b (645 nm) over 45 minutes in chloroplasts from *P. persica* under high-intensity light ($1.46 \times 10^{-2} \text{ W}/\text{cm}^2$).

6-week experiment (Fig. 6). Treatment with *R. hyrcanus* extract led to the emergence of seven healthy leaves by week three, despite four diseased leaves in the control. For *B. sempervirens*, diseased leaves dropped after four weeks, and five of eight initially infected leaves were replaced by healthy ones by the experiment's end.

Relict plant species, with their long evolutionary history, have developed a broad range of adaptive mechanisms [Garayev, Sokolova, 2023]. Both viral

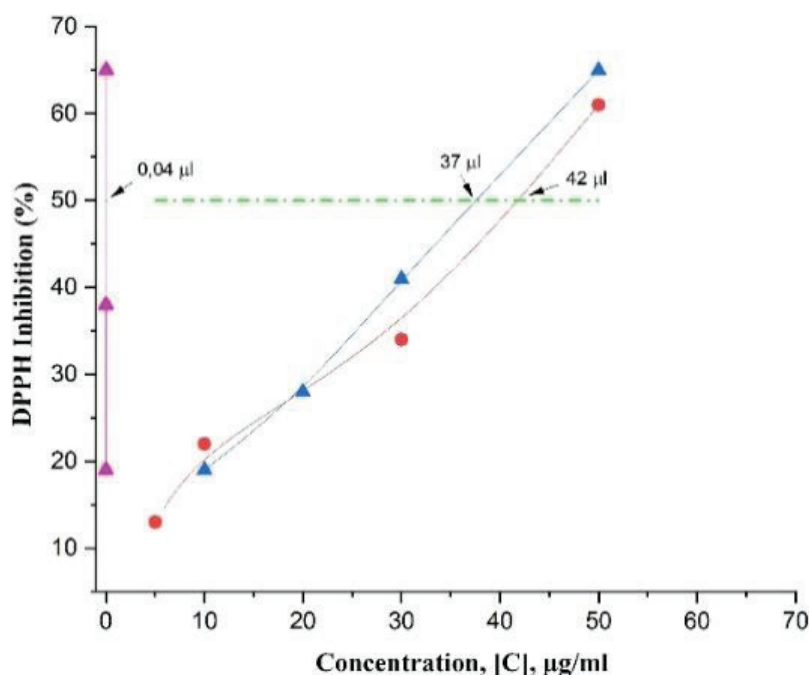


Figure 5. DPPH assay showing AO activity of extracts from *B. sempervirens* (▲), *R. hyrcanus* (▲), *P. persica* (●) with respective IC₅₀ values.

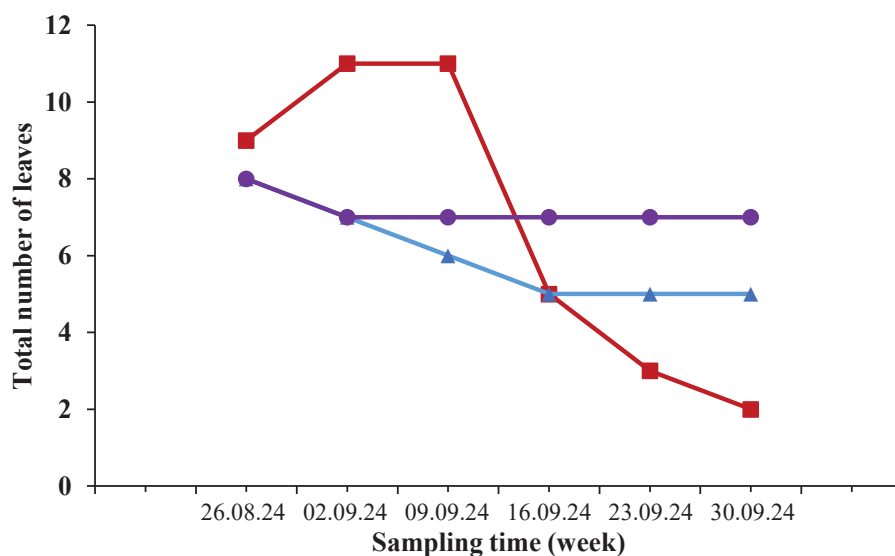


Figure 6. Effect of *P. persica* (■), *B. sempervirens* (▲) and *R. hyrcanus* (●) leaf extracts on PNRSV infected Gizella 6 rootstocks over six weeks (Aug 26 – Sep 30, 2024).

infections and photoinhibition compromise membrane integrity via the accumulation of reactive oxygen species (ROS) [Gill, Tuteja, 2010; Zhao et al., 2020; Ganiyeva et al., 2024]. The tested relict species showed antioxidant and antiviral properties, potentially protecting membranes and preventing viral entry into cells [Ganiyeva et al., 2018; 2021].

Our findings indicate that *B. sempervirens* has the strongest antioxidant and antiviral effects among the studied species, likely due to a higher content of low- and high-molecular-weight antioxidant compounds. The distinct antiviral responses observed suggest species-specific phytochemical profiles [Renzetti et al., 2025; Dias et al., 2021].

Similar studies in the literature confirm that many medicinal plants demonstrate robust antioxidant responses to abiotic and biotic stresses, promoting their potential use in therapeutic applications [Habibah et al., 2023; Iqbal et al., 2023; Mammadova et al., 2018]. The current findings highlight the importance of further research on the physiological and biochemical stress responses of relict species.

CONCLUSIONS

This study demonstrates the significant role of antioxidants in protecting plant cell membranes by neutralizing reactive oxygen species, such as superoxide (O_2^-) and hydroxyl radicals ($^{\bullet}OH$) [Nazemiyeha et al., 2020; Li et al., 2024; Walasek-Janusz et al., 2022]. Among the relict species analyzed, *B. sempervirens* exhibited the highest antioxidant activity, with an IC_{50} value of 0.04 $\mu g/ml$.

The extracts of *B. sempervirens* and *R. hyrcanus* showed notable antiviral effects on PNRSV-infected Gizella 6 cherry rootstocks, with *B. sempervirens* also displaying greater resistance of chlorophyll a and b to photobleaching under photoinhibitory stress.

These results suggest that the unique phytochemical profiles of these relict species enable them to effectively counteract oxidative stress and viral infections. This protective capacity likely contributes to their evolutionary resilience in harsh environmental conditions.

Further research is warranted to elucidate the mechanisms underlying the stabilization of photochemical reactions in photosystem II, a key component highly sensitive to environmental stress. Understanding these physiological and biochemical processes is essential to clarify the survival strategies of relict plants and assess their conservation status and potential applications in agriculture and medicine.

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Stress faktorları altında relikt növlərdə antiviral, antioksidant və fotosintetik parametrlərin analizi

Sevil B. Dadaşova

Gülənər X. Babayeva

Botanika İnstitutu, Azərbaycan Respublikası Elm və Təhsil Nazirliyi, A. Abbaszadə küç., giriş 99, Bakı, AZ1004, Azərbaycan

Aytac İ. Əsgərova

Meyvəçilik və Çayçılıq Elmi-Tədqiqat İnstitutu, Azərbaycan Respublikası Kənd Təsərrüfatı Nazirliyi, Quba-Xaçmaz yolu, Quba, AZ4035, Azərbaycan

Gülənər H. Sultanova

Rəna A. Qəniyeva

Botanika İnstitutu, Azərbaycan Respublikası Elm və Təhsil Nazirliyi, A. Abbaszadə küç., giriş 99, Bakı, AZ1004, Azərbaycan

Ətraf mühitin streslərinə qarşı relikt bitki növlərinin antioksidant birləşmələrinin akkumulyasiyası daxil olmaqla biokimyəvi strategiyaları işlənib hazırlanmışdır. Bu tədqiqatlarda *Buxus sempervirens*,

Ruscus hyrcanus və *Parrotia persica* ekstraktlarının antioksidant və antiviral xüsusiyyətləri qiymətləndirilib. Antioksidant aktivlik DPPH radikal təmizləmə metodu ilə qiymətləndirilib, bu zaman *B. sempervirens* 0.04 µg/ml miqdarında ən güclü təsir nümayiş etdirmişdir. Fotosintetik pigmentlərin (Xl a və b) yüksək intensivli işıq təsiri altında fotosolmaya qarşı müqaviməti də təhlil edilib və digər növlərlə müqayisədə *B. sempervirens* növündə daha yüksək pigment sabitliyi aşkar edilib. Antiviral fəaliyyəti qiymətləndirmək üçün PNRSV virusu ilə yoluxmuş albalı calaqaqları (*Gizella* 6) ekstraktlarla işlənmişdir. *B. sempervirens* və *R. hyrcanus* ekstraktları ilə işlənmiş bitkilərdə 6-cı həftədə simptomların şiddəti azalır və sağlam yarpaqların sayı artır. Əldə edilən məlumatlar göstərir ki, bəzi relikт növlər fotosintetik funksiyanı və membran bütövlüyünü qorumaqla stress müqavimətinin artmasına kömək edə bilən antioksidant və antiviral xüsusiyyətlərə malikdir.

Açar sözlər: antioksidant, *Buxus sempervirens*, xlorofil, *Parrotia persica*, fotoingibirləşmə, fotosintez, *Ruscus hyrcanus*, virus PNRSV

Анализ противовирусных, антиоксидантных и фотосинтетических параметров у реликтовых видов в условиях стрессовых факторов

Севиль Б. Дадашева

Гюльнара Х. Бабаева

Институт ботаники, Министерство Науки и Образования
Азербайджанской Республики, ул. А.Аббасзаде, подъезд 99, Баку,
AZ1004, Азербайджан

Айтадж И. Аскерова

Научно-исследовательский институт плодоводства и чаеводства,
Министерства сельского хозяйства Азербайджанской Республики, Губа-
Хачмазская дорога, Губа, AZ4035, Азербайджан

Гюльнар Г. Султанова

Рена А. Ганиева

Институт ботаники, Министерство Науки и Образования
Азербайджанской Республики, ул. А.Аббасзаде, подъезд 99, Баку,
AZ1004, Азербайджан

Реликтовые виды растений выработали биохимические стратегии, включая накопление антиоксидантных соединений, для противостояния стрессу окружающей среды. В этом исследовании оценивались антиоксидантные и противовирусные свойства экстрактов листьев *Buxus sempervirens*, *Ruscus hyrcanus* и *Parrotia persica*. Антиоксидантная активность оценивалась методом удаления радикалов DPPH, при этом *B. sempervirens* демонстрировал самый сильный эффект при 0,04 мкг/мл. Также была проанализирована устойчивость фотосинтетических пигментов (хлорофилла а и b) к фотообесцвечиванию при высокоинтенсивном световом воздействии, что выявило большую стабильность пигмента у *B. sempervirens* по сравнению с другими видами. Для оценки противовирусной активности подвой черешни (*Gizella* 6), инфицированные PNRSV, обрабатывались экстрактами. К шестой неделе обработанные растения демонстрировали снижение тяжести симптомов заболевания и увеличение количества здоровых листьев, особенно при использовании экстрактов *B. sempervirens* и *R. hyrcanus*. Полученные данные свидетельствуют о том, что определенные реликтовые виды обладают антиоксидантными и противовирусными свойствами, которые могут способствовать повышению устойчивости к стрессу за счет защиты фотосинтетической функции и целостности мембран.

Ключевые слова: антиоксидант, *Buxus sempervirens*, хлорофилл, *Parrotia persica*, фотоингибирование, фотосинтез, *Ruscus hyrcanus*, вирус PNRSV