Volume 9 No 2 December, 2025

MINISTRY OF SCIENCE AND EDUCATION OF THE REPUBLIC OF AZERBAIJAN

TRANSACTIONS

OF THE INSTITUTE OF MOLECULAR BIOLOGY AND BIOTECHNOLOGIES

MINISTRY OF SCIENCE AND EDUCATION OF THE REPUBLIC OF AZERBAIJAN

TRANSACTIONS

OF THE INSTITUTE OF MOLECULAR BIOLOGY AND BIOTECHNOLOGIES

TRANSACTIONS
INSTITUTE OF MOLECULAR BIOLOGY & BIOTECHNOLOGIES

MINISTRY OF SCIENCE AND EDUCATION OF THE REPUBLIC OF AZERBAIJAN

2025 Volume IX No 2

The journal has published according to the decision of the Scientific Council of the Institute of Molecular Biology & Biotechnologies, ANAS on May 19, 2017 (protocol No 2). The journal was registered by the decision of the Ministry of Justice of the Republic of Azerbaijan No 4282 of January 10, 2020.

Binding to proteins and their fragmentation by resveratrol in the presence of copper ions

Aamir Ahmad[1,2*], Mohd Farhan[3,4], Tariq Al-Qirim[5], Hatem Zayed[6], Salman Akhtar[2], Alvina Farooqui[2], S.M.Hadi[7]

[1]Dermatology Institute and Translational Research Institute, Academic Health System, Hamad Medical Corporation, 3050, Doha, Qatar
[2]Department of Bioengineering, Integral University, UP, 226026, Lucknow, India
[3]Department of Chemistry, College of Science, King Faisal University, 31982, Al Ahsa, Saudi Arabia
[4]Department of Basic Sciences, Preparatory Year, King Faisal University, 31982, Al Ahsa, Saudi Arabia
[5]Faculty of Pharmacy, Al-Zaytoonah University of Jordan, 11733, Amman, Jordan
[6]Department of Biomedical Science, College of Health Sciences, Qatar University, Doha, Qatar
[7]Department of Biochemistry, Faculty of Life Sciences, AMU, UP, 202002, Aligarh, India

[*]***For correspondence:*** *aahmad9@hamad.qa*

Received: November 18, 2025; Reviewed: December 11, 2025; Accepted: December 18, 2025

Resveratrol is a natural polyphenol with promising anticancer properties. We have earlier reported its DNA-damaging ability in the presence of Cu(II) with the underlying prooxidant mechanism involving the generation of free radicals. Here, we report protein fragmentation by resveratrol in the presence of Cu(II), which further characterizes its prooxidant activity in the presence of copper ions, leading to macromolecular damage. Studies involving bathocuproine and scavengers of oxygen free radicals suggest that the fragmentation of BSA by resveratrol-Cu(II) involves a pathway similar to that responsible for the DNA cleavage activity, and is predominantly mediated by hydrogen peroxide and superoxide free radicals. We also studied the interaction of resveratrol with proteins. Fluorescence absorption studies show binding of resveratrol to BSA and to other proteins. A comparison of the pattern of binding of resveratrol to proteins with different L-tryptophan content indicates that resveratrol, in addition to tryptophan, may bind to other amino acids and also, non-specifically to the proteins. Given the recent interest in protein conformational changes and protein damage induced by anticancer agents with resulting altered sensitivity to therapies, our work proposes a closer examination of such activity of putative anticancer drugs.

Keywords*: Resveratrol, polyphenols, copper, pro-oxidant, oxidative protein damage*

INTRODUCTION

Phytoalexins, the low molecular weight antimicrobial organic metabolites, are produced by plants as the first line of defense against fungal infection (Liu et al., 2023). Small polysaccharides and proteins, produced by the invading pathogens, act as elicitors triggering the synthesis of phytoalexins in the tissues surrounding the infection site. In grapevines, resveratrol (3,4',5-trihydroxystilbene) is produced in response to fungal infection by *Botrytis cinerea* and UV irradiation (Jeandet et al., 1992). Resveratrol is a phytoalexin and a polyphenolic compound present in human dietary material such as peanuts, mulberries, grapes and red wine (Xu et al., 2024). It is widely considered to possess cardiovascular protective properties (Jojima et al., 2023) and has also been shown to be chemopreventive against various stages of chemically induced carcinogenesis (Ren et al., 2021). The initial spurt in studies on resveratrol was due to its association with the phenomenon of `French paradox', which refers to the paradoxical finding that the incidence of coronary heart disease in the population of Southern France is relatively low despite a high intake of saturated fats in the diet. Over the past few decades, a lot of data has been reported on its potential anticancer activity, among several other therapeutic properties (Radeva and Yoncheva, 2025; Farhan et al., 2019; Zhang et al., 2021).

Our earlier studies have established that several classes of plant-derived polyphenolic compounds, including resveratrol, are themselves capable of causing oxidative DNA damage in the presence of transition metal ions (Ahmad et al., 2005; Hadi et al., 2000; Ahmad et al., 2000). Although considerable evidence suggests that the antioxidant activities of

these compounds may not fully account for the observed chemopreventive effects of several such anticancer agents (Hadi et al., 2000), it appears that prooxidant rather than the antioxidant properties of polyphenolic compounds may be responsible for their observed anticancer and apoptosis-inducing ability (Farhan, Rizvi, 2022). While the interactions of resveratrol with nucleic acids are well documented, as reported by us as well as others (Ahmad et al., 2000; Subramaniam et al., 2015), the interactions of resveratrol with protein, if any, are not well known. The only knowledge on the subject of interactions of resveratrol with proteins is that resveratrol can stabilize protein-substrate interactions (Hou et al., 2016) and its phosphorylated form can suppress protein aggregation (Mehringer et al., 2022). Therefore, in this study, we sought to fill this gap in our knowledge by first using BSA and evaluating its fragmentation by resveratrol, followed by more characterization of resveratrol-protein interactions using proteins with different tryptophan content. Anticancer drugs bring about cell death by inducing protein damage, and therefore, the evaluation of such activity is important. Protein damage response plays an important role in overcoming such damage and therefore, suppressing such response has been advocated to increase the efficacy of anticancer drugs, and overcome resistance against therapy (Shao et al., 2025), thus further underlying the importance of our work.

MATERIALS AND METHODS

Fragmentation of proteins by resveratrol in the presence of Cu(II): *trans*-Resveratrol (Pharma Science Inc. Canada) was freshly dissolved in 3mM NaOH every time to prepare a stock solution of 1mM. Upon addition to reaction mixtures, in the presence of buffers and at the concentrations used, resveratrol remained in solution. The volumes of stock solutions added did not lead to any appreciable change in the pH of reaction mixtures. The reaction mixture (1.0 ml) contained 10 mM potassium phosphate buffer (pH 7.5), bovine serum albumin (2 mg/ml) and varying concentrations of resveratrol and Cu(II). In some experiments, bathocuproine or scavengers of oxygen-free radicals were added. The reactions were started by the addition of Cu(II). After incubation at 37^0C for specified time intervals, the reactions were stopped by adding 1 mM EDTA and were precipitated with 5% TCA. The tubes were immediately transferred to ice for 1 hour before centrifugation at 2000 rpm for 10 minutes at room temperature to remove the unfragmented protein. The generation of material soluble in TCA was assessed by estimating free amino groups using trinitrobenzenesulphonic acid (TNBS), 25 µl of 30 mM aqueous TNBS was added to 100 µl of the sample and 1.0 ml of disodium tetrahydroborate buffer (pH 9.5), vortexed to ensure complete mixing and allowed to stand at room temperature for 30 minutes. The absorbance was read at 420 nm against a blank, which consisted of 25 µl of 30 mM TNBS in 1.1 ml of borate buffer (pH 9.5). The reaction was calibrated with glycine and the principle of this assay is the formation of a highly absorbing (A_{420}) chromophore of picrylsulphonamides formed by condensation of the reagent with free amino groups.

The fragmentation products of BSA were also analyzed by SDS-PAGE. Samples were mixed with 5X sample loading buffer (125 mM Tris, 960 mM glycine, 0.1% SDS, pH 8.3) and heated at 90^0C for 10 minutes before loading on 10% (w/v) acrylamide gel containing 0.1% SDS. To optimize the detection of any fragments formed during exposure of BSA to resveratrol-Cu(II), lanes were heavily loaded (10 µg of BSA/lane) and the gel was visualized by silver staining, a sensitive staining procedure for visualizing proteins in polyacrylamide gels.

Absorption studies: The absorption spectra were obtained by using a Beckman DU-40 spectrophotometer (USA) equipped with a plotter.

Fluorescence studies: The fluorescence studies were performed on a Shimadzu spectrofluorophotometer RF-5000 (Japan) equipped with a calculator and a plotter. Resveratrol was excited at its absorption maximum (λ_{max}) of 308 nm, while L-tryptophan and all the proteins were excited at 280 nm. Emission spectra were recorded in the range mentioned in the legends to the figures.

Fluorescence quench titration and determination of binding of resveratrol to proteins: The fluorescence titration and the calculations of binding constant/binding capacity of resveratrol for proteins were performed exactly as described by Levine (Levine, 1977). The titration consisted of adding aliquots of resveratrol to the tryptophan/protein solutions and measuring the decrease in tryptophan fluorescence after each addition. In almost all cases, the region of interest was the resveratrol/(tryptophan/protein) ratio of 0 to 5.0. At least 40 points were obtained in the region of interest. The fluorescence quench curves were plotted with the molar ratio of resveratrol to tryptophan/protein on the abscissa and the fluorescence on the ordinate. Fractional quench (Q) was calculated from these curves by the relation, $Q=(F_o-F)/m$, where F is the fluorescence at the molar

ratio (resveratrol: tryptophan/protein) of 1, F_o is the fluorescence at zero ratio and m is the maximal quench of fluorescence. The fluorescence quench curves were used to generate scatchard plots and an analysis of the scatchard plots was done to determine the binding constant and the number of moles of resveratrol bound per mole of tryptophan/protein.

RESULTS

Protein fragmentation by resveratrol in the presence of Cu(II)

To investigate any potential protein-damaging property of resveratrol, we started with an evaluation of protein fragmentation by resveratrol. This is logical because the quercetin-Cu(II) system, which cleaves DNA, is also capable of protein fragmentation (Ahmed et al., 1994). Our own earlier work has established DNA cleavage activity of resveratrol (Ahmad et al., 2000; Ahmad et al., 2005). It was, therefore, of interest to examine whether the resveratrol-Cu(II) system also possesses the protein fragmentation property. Figure 1 shows the fragmentation of BSA (2 mg/ml) by resveratrol in the presence of Cu(II). The method determines the formation of acid-soluble amino groups by TNBS. The time-dependent fragmentation of BSA in the presence of 100 μM resveratrol and 50 μM Cu(II) (Figure 1**a**), as evidenced by an increase in the amount of acid-soluble amino acid groups, was found to be roughly linear up to 6 hours of incubation at 37ºC. Increasing times of incubation, over 6 hours, did not result in any appreciable increase in the fragmentation of BSA and therefore, 6 hours of incubation was used for studying the dependency of the BSA fragmentation reaction on resveratrol concentration and Cu(II) concentration. Increasing concentrations of resveratrol, at a fixed concentration (50 μM) of Cu(II), increased the BSA fragmentation (Figure 1b). An increase in the BSA degradation was also observed with increasing concentrations of Cu(II) (Figure 1c) in the presence of 100 μM resveratrol.

The degradation products of BSA, formed as a result of resveratrol-Cu(II) treatment, were analyzed on a 10% SDS-polyacrylamide gel. The results shown in Figure 2 confirm the fragmentation of BSA. Large aggregates of BSA are present in the commercial sample that migrate near the top of the gel. These aggregates seem to get degraded on exposure to resveratrol and Cu(II). At the 68 kDa BSA monomer position, there is an impression of broadening of the stained band (smearing) after treatment with increasing concentrations of resveratrol (lanes d-g) in the presence of 50 μM Cu(II). This is possibly due to a combination of overloading the staining system and monomer fragmentation to slightly smaller peptides. 200 μM resveratrol alone (lane b) and 50 μM Cu(II) alone (lane c) did not result in any BSA fragmentation, as no change, with respect to the control sample, was observed. The increasing concentrations of resveratrol increased the smearing, thus verifying the fact that BSA is degraded by resveratrol in the presence of Cu(II).

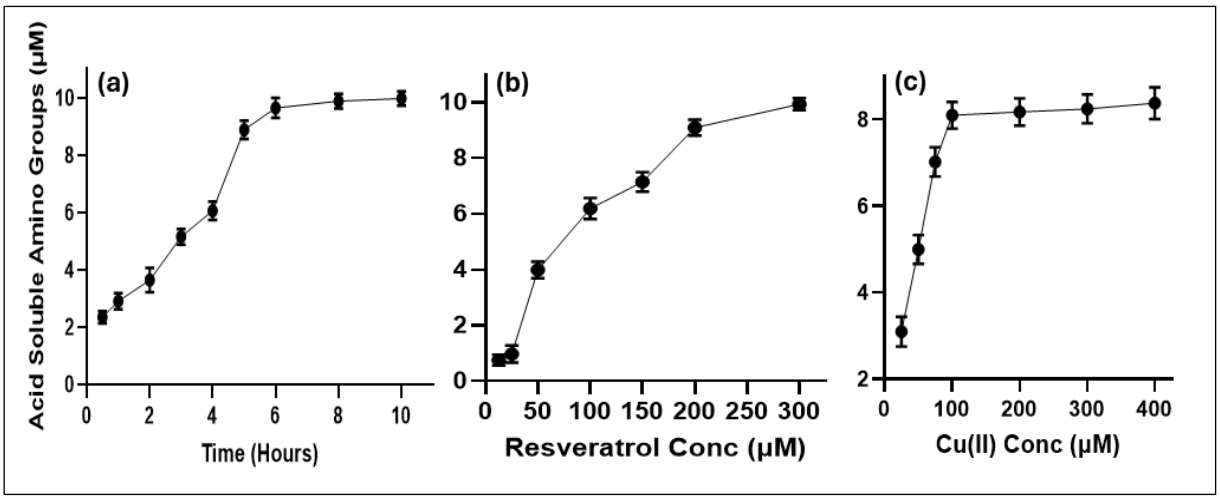


Fig. 1. Kinetics of protein fragmentation by resveratrol in the presence of Cu(II). BSA (2 mg/ml) was incubated with indicated concentrations of resveratrol and Cu(II) in a total reaction volume of 1.0 ml containing 10 mM potassium phosphate (pH 7.5) at 37^0C. **(a)** Effect of increasing times of incubation in the presence of 100 μM resveratrol and 50 μM Cu(II). **(b)** Effect of increasing concentrations of resveratrol in the presence of 50 μM Cu(II) after 6 hours of incubation. **(c)** Effect of increasing Cu(II) concentrations in the presence of 100 μM resveratrol after 6 hours of incubation. Acid-soluble amino groups were estimated as described in "Methods". All points represent triplicate samples and mean values have been plotted.

Mechanism of BSA fragmentation by resveratrol

To study the mechanism of fragmentation of BSA by resveratrol, bathocuproine and scavengers of reactive oxygen species were added to the reaction mixture prior to the addition of resveratrol (100μM) and Cu(II) (50 μM). Increasing concentrations of bathocuproine caused an inhibition of the resveratrol-Cu(II) mediated BSA fragmentation (Figure 3), confirming the essential role of Cu(I) in the reaction. The reaction was inhibited to an extent of almost 90% by 100 μM bathocuproine. Scavengers of reactive oxygen species were also found to inhibit the fragmentation of BSA (Table 1).

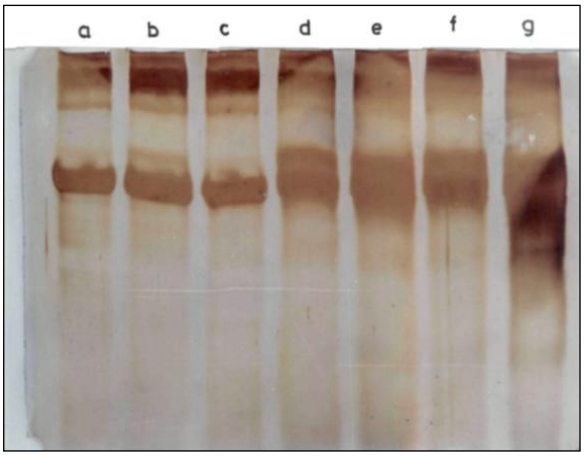


Fig. 2. SDS/polyacrylamide gel electrophoretic pattern of BSA after treatment with increasing concentrations of resveratrol in the presence of Cu(II). The reaction mixtures contained 20 μg BSA, 10 mM potassium phosphate (pH 7.5), 50 μM Cu(II) and increasing concentrations of resveratrol in a total reaction volume of 100 μl. Incubation was carried out at 37^0C for 6 hours. Lane a: BSA alone, Lane b: BSA + 200 μM resveratrol, Lane c: BSA + Cu(II), Lanes d - g: [BSA + Cu(II)] + 25 μM, 50 μM, 100 μM and 200 μM resveratrol, respectively.

Catalase caused the maximum inhibition, while sodium azide and SOD also significantly inhibited the fragmentation. Sodium benzoate was found to inhibit the fragmentation to some extent, whereas thiourea caused only negligible inhibition. These results suggest that BSA fragmentation by resveratrol in the presence of Cu(II) is mediated by the reduction of Cu(II) to Cu(I) and that Cu(I) is an essential intermediate in the reaction since sequestering of Cu(I) by bathocuproine almost completely inhibits the reaction. Further, the redox recycling of copper ions results in the generation of reactive oxygen species, particularly hydrogen peroxide, singlet oxygen and superoxide anion, which serve as the proximal protein fragmenting species. A comparison of these results with those of DNA damage (Ahmad et al., 2000) suggests that the mechanism of protein fragmentation by resveratrol-Cu(II) is identical to the DNA breakage reaction and is therefore biologically relevant.

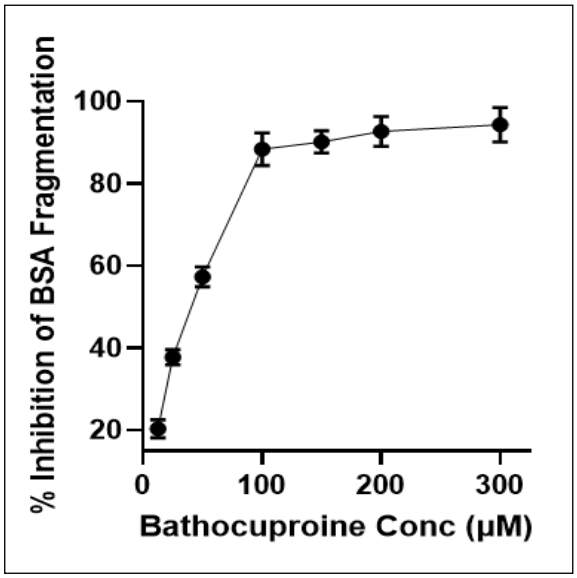


Fig. 3. Inhibition by bathocuproine of resveratrol-Cu(II) mediated fragmentation of BSA. 2 mg/ml BSA was incubated with 100 μM resveratrol, 50 μM Cu(II) and increasing concentrations of bathocuproine at 37^0C for 6 hours in a total volume of 1.0 ml containing 10 mM potassium phosphate (pH 7.5). Acid-soluble amino groups were estimated as described in "Methods". All the points represent triplicate samples and mean values have been plotted.

Table 1. Percent inhibition of BSA fragmentation after treatment with resveratrol (100 μM) and Cu(II) (50 μM) in the presence of scavengers of ROS.

Conditions	Inhibition (%)
Catalase (100μg/ml)	80.23±1.76
Boiled catalase (100μg/ml)	5.21±0.23
Sodium Azide (50mM)	48.56±2.34
Sodium Benzoate (50mM)	20.43±0.19
Thiourea (50mM)	2.00±0.05
SOD (100μg/ml)	41.54±2.76

SOD - Superoxide Dismutase. All values are expressed asMean±SE for three different experiments.

Binding of resveratrol to BSA

Figure 4a shows the changes in the fluorescence emission spectrum of BSA in the presence of increasing molar ratios of resveratrol. When a solution of BSA was excited at its absorption maximum of 280 nm, a fluorescence emission spectrum with maxima at 356 nm was observed (uppermost trace in the figure). The addition of increasing molar ratios of resveratrol to BSA resulted in a progressive quenching of the fluorescence emission. The results are suggestive of the binding of resveratrol to BSA.

The effect of increasing molar ratios of BSA on the fluorescence emission spectrum of resveratrol was also studied and the results are shown in Figure 4b. When excited at its absorption maximum of 308 nm, resveratrol exhibited a fluorescence emission with a peak at around 459 nm. Addition of BSA at molar ratios of 1, 10, 20 and 30 resulted in a dose-dependent quenching of the fluorescence peak of resveratrol, thus showing binding of BSA to resveratrol.

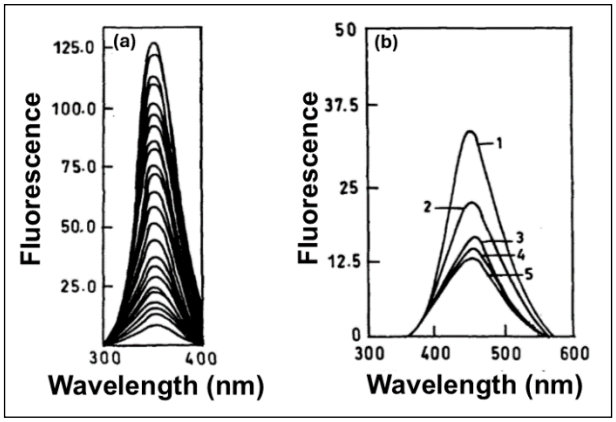


Fig. 4. Effects of increasing concentrations of resveratrol on the fluorescence emission spectra of BSA and vice versa. (a) BSA (in Tris-HCl, pH 7.5) was excited at 280 nm and the emission spectra were recorded between 300 and 400 nm. Traces, from top to bottom, are – BSA alone (1 μM), BSA: resveratrol molar ratio 1:0.1, 1:0.2, 1:0.4, 1:0.6, 1:0.8, 1:1, 1:1.2, 1:5, 1:7.5, 1:2, 1:2.5, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:10, 1:11, 1:12, 1:13, 1:14 and 1:15, respectively. (b) Resveratrol (in Tris-HCl, pH 7.5) was excited at its λmax (absorption) of 308 nm and the emission spectra were recorded between 360 and 580 nm. Trace 1: resveratrol alone (5 μM); Trace 2: resveratrol: BSA molar ratio 1:1; Trace 3: resveratrol: BSA molar ratio 1:10; Trace 4: resveratrol: BSA molar ratio 1:20; Trace 5: resveratrol: BSA molar ratio 1:30.

Binding of resveratrol to proteins with different tryptophan content

L-tryptophan is one of the major chromophores among the naturally occurring amino acids and is responsible for the absorbance shown by proteins at 280 nm. When excited at its absorption maximum of 280 nm, tryptophan was found to exhibit a fluorescence emission spectrum with peak at a 375 nm. This peak was quenched by the increasing molar ratios of resveratrol (data not shown) in the same pattern as that observed with BSA. An analysis of the quenching of tryptophan fluorescence by resveratrol revealed a linear relationship at lower concentrations of resveratrol. However, as additional resveratrol was added, the concentration of unbound resveratrol increased in a manner predicted by the mass-action equation and the affinity constant. The effect of about 40 increasing molar ratios of resveratrol on the tryptophan fluorescence was studied. The fractional quench was calculated as described in "Methods". This data was utilized to construct scatchard plots for determining the binding capacity and affinity of resveratrol for tryptophan. The scatchard plot for the binding of resveratrol to tryptophan is shown in Figure 5a. An analysis of this plot revealed that 2.19 moles of resveratrol are able to bind to one mole of tryptophan and the association constant of such binding is 4.7 x 10^7 liter/mol (Table 2).

Similar fluorescence quenching experiments were performed with 3 different proteins with varying numbers of tryptophan residues. Chymotrypsin has 1 tryptophan; BSA has 2 tryptophan residues while lysozyme has 6 tryptophan residues. Fluorescence quenching titration of these proteins was performed by adding increasing molar ratios of resveratrol in the same way as done with tryptophan and the subsequent scatchard plots are shown in Figure 5. The analysis of scatchard plots, as tabulated in Table 2 revealed that 5.58 moles of resveratrol bind to one mole of chymotrypsin, 2.89 moles of resveratrol bind to one mole of BSA and 2.82 moles of resveratrol bind to one mole of lysozyme. The number of moles of resveratrol bound per mole of BSA and lysozyme was quite similar, although there is a great difference in the number of tryptophan residues. Lysozyme contains three times as many tryptophan residues as BSA. This would suggest that the tryptophan residues in lysozyme are buried inside and are possibly not available for binding to resveratrol. Chymotrypsin contains only one tryptophan residue and still the number of moles of resveratrol bound to one mole of it was quite high. This may presumably be due to the binding of resveratrol to some other amino acids or may be a result of some non-specific binding which is not quite easy to characterize.

An analysis of the association constants of resveratrol for these proteins (Table 2) also showed a variation that does not appear to be related to their tryptophan content. The interesting result is that the association constant for tryptophan alone and the single tryptophan-containing chymotrypsin was quite similar [4.7 and 5.0 (x 10^7 liter/mol), respectively]. However, even the association constant for 6 tryptophan residue-containing lysozyme was not very high (5.7 x 10^7 liter/mol), whereas the association constant for BSA was the lowest (1.4 x 10^7 liter/mol). Taken together, these results seem to suggest a complex mode of binding of resveratrol to proteins, which might involve, in addition to tryptophan residues, some non-specific interactions.

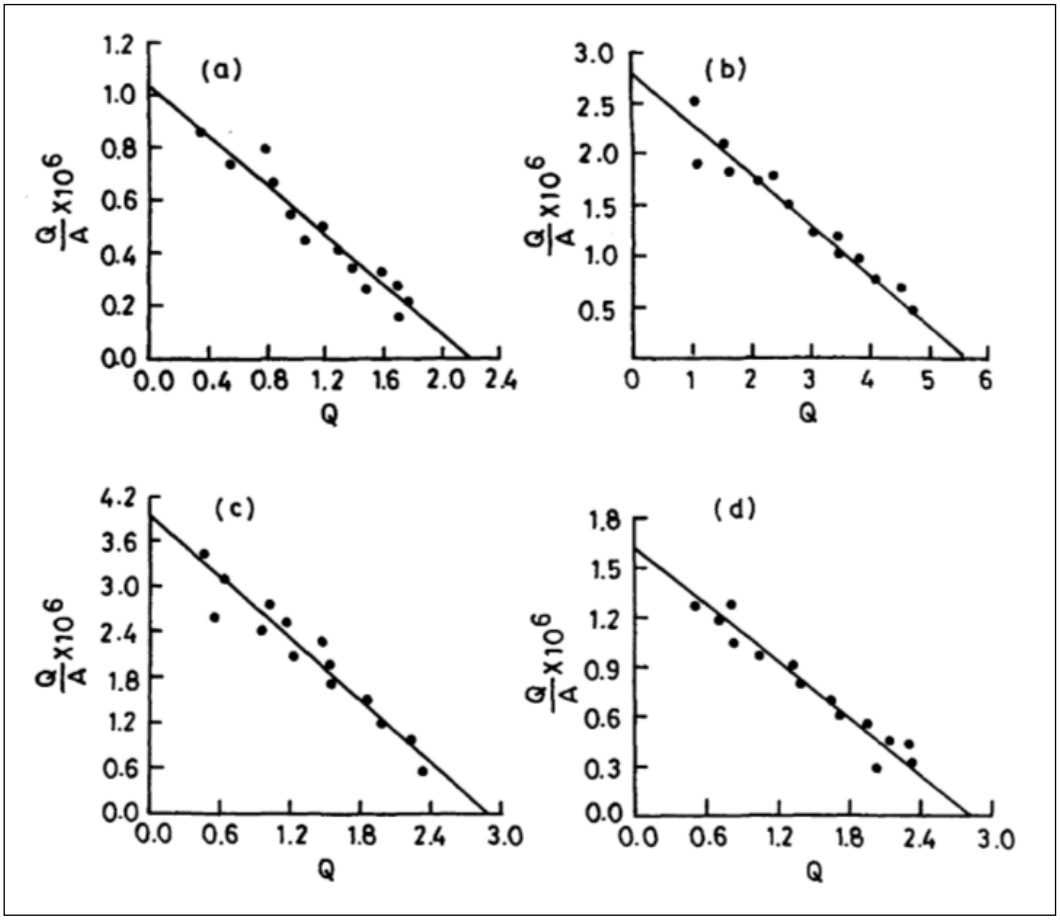


Fig. 5. Scatchard plots for the binding of resveratrol to proteins with different L-tryptophan content. Fluorescence titration of proteins was performed by adding increasing amounts of resveratrol to L-tryptophan/protein solutions. This data was utilized to generate scatchard plots for the binding of resveratrol to **(a)** L-tryptophan, **(b)** Chymotrypsin, **(c)** BSA and **(d)** Lysozyme. A detailed methodology is given in "Methods".

To further substantiate the above findings, the effect of increasing molar ratios of resveratrol on a few more proteins was compared with the proteins described above. The results are given in Table 3. The quenching effect of resveratrol molar concentration (5 times) on the fluorescence emission of tryptophan alone (58.3%) was quite similar to that on single tryptophan-containing RNase T1 (60.1%), whereas it was significantly different (78.0%) in the case of other single tryptophan-containing protein, chymotrypsin. However, the quenching effect of resveratrol at a molar ratio of 2 was quite similar in the case of RNase T1 and chymotrypsin (83.4 and 83.0%, respectively) and was significantly different from that observed with tryptophan alone (73.3%). The proteins containing six tryptophan residues-lysozyme, catalase and pepsin also showed a different pattern of quenching on the addition of increasing molar ratios of resveratrol. The quenching in the case of catalase was less as compared to tryptophan alone. All this data, taken together, suggests a complex mode of binding of resveratrol to proteins, probably involving some non-specific interactions and confirms the results of Table 2. An interesting observation was the maximal rate of quenching observed with BSA. This could possibly be because BSA has a relatively less complicated tertiary structure and the tryptophan residues are comparatively easily available for binding.

DISCUSSION

Oxygen radicals are believed to be generated by several physiological processes and such radicals have been proposed as the agents responsible for the destruction of model peptides via labile iminopeptides (Davies et al., 1987). The sensitivity of proteins to oxygen radical attack is well established. Oxidative modifications, such as those involving oxygen radicals, can disrupt protein folding and function (Kim et al., 2024). Such radicals can be formed from γ-irradiation and by metal ions and H_2O_2, Cu(II) and ascorbate and glucose in the presence of oxygen and Cu(II).

Table 2: Binding constants of resveratrol for proteins with varying numbers of L-tryptophan residues.

Amino Acid/Protein	Number of L-tryptophan residues	Association constant (K_a) (liter mol^{-1})	Moles of resveratrol/mole of protein
Tryptophan	1	4.7×10^7	2.19
Chymotrypsin	1	5.0×10^7	5.58
BSA	2	1.4×10^7	2.89
Lysozyme	6	5.7×10^7	2.82

All the values were calculated from the scatchard plots provided in Figure 5.

Table 3: Effect of resveratrol on fluorescence of different proteins with varying numbers of L-tryptophan residues.

AminoAcid/ Protein	Number of Tryptophans	Ratio of Resveratrol/ Protein	λmax	% Fluorescence
Tryptophan	1	0	375	100.0
		1		80.0
		2		73.3
		5		58.3
Chymotrypsin	1	0	368	100.0
		1		94.3
		2		83.0
		5		78.0
RNase T1	1	0	325	100.0
		1		98.3
		2		83.4
		5		60.1
BSA	2	0	356	100.0
		1		74.8
		2		56.8
		5		36.1
Lysozyme	6	0	358	100.0
		1		74.8
		2		73.7
		5		52.8
Catalase	6	0	356	100.0
		1		93.2
		2		88.4
		5		61.9
Pepsin	6	0	361	100.0
		1		89.1
		2		82.6
		5		49
				3

The last reaction is important since it appears to contribute, by the Amadori rearrangement, to the glycation of proteins during diabetes and ageing. Fragmentation of BSA by radiolytically generated hydroxyl radicals has been reported and hydroxyl radicals can release amino acids from proteins and peptides via oxidation (Liu et al., 2017). Additionally, free radicals can influence and therefore regulate protein-protein interactions (Iliadis and Papanikolaou, 2024), alter protein conformation (Orru et al., 2010) and even induce protein denaturation (Ruzza et al., 2021). Excess production of free radicals can be the cause of damage to proteins (Nakamura and Takada, 2021) and this can be envisioned in cancer cells that have elevated copper ion levels (Tang et al., 2023) and such copper can be mobilized by resveratrol, leading to the production of free radicals.

Our results clearly indicate the fragmentation of BSA by resveratrol in the presence of Cu(II). Cu(I), which is formed as a result of the reduction of Cu(II) by resveratrol (Ahmad et al., 2000), is shown to be an essential intermediate in this fragmentation reaction (Figure 3). This finding, together with that involving scavengers of oxygen- free radicals (Table 1), suggests that the fragmentation of BSA by resveratrol-Cu(II) possibly involves a pathway similar to that responsible for the DNA cleavage activity. We also report binding of resveratrol to L-tryptophan and various proteins with different numbers of tryptophan residues, as suggested by fluorescence quenching experiments.

The association constant of resveratrol for various proteins is of the order of 10^7 liter/mol (Table 2). Traditionally, therapeutic drugs (synthetic organic anions) have an association constant of approximately 10^4 liter/mol for physiological proteins (Meyer and Guttman, 1968) while the same of antibodies for their antigens is of the order of 10^9 liter/mol. This would suggest that binding of resveratrol to proteins might not be stereospecific, as in the case of antibodies, but it is still stronger than that observed for non-specific, electrostatic interactions. This, in part, accounts for the binding of resveratrol to L-tryptophan. A detailed study, involving proteins with varying tryptophan content, was carried out to investigate the site-specific binding of resveratrol to proteins. The results, however, are not very suggestive of tryptophan-specific binding of resveratrol to proteins. Instead, it appears that resveratrol, in addition to tryptophan, may bind to other amino acids and also, non-specifically to the proteins. Further, the inconclusive data of proteins with varying tryptophan content could be attributed to the fact that the tryptophan residues in many proteins are buried inside the tertiary structure and are not readily available for interaction with resveratrol.

As reported earlier (Ahmad et al., 2000), resveratrol can bind to Cu(II) and to proteins. Thus, a protein-resveratrol-Cu(II) complex can be envisaged, similar to a DNA-resveratrol-Cu(II) complex. Such a complex probably accounts for the protein fragmentation ability of resveratrol via the generation of oxygen-free radicals, which are known to cause damage in the close vicinity of their production site. It has been realized for a long time that proteins, damaged by free radicals, contain significant amounts of protein peroxides and a protein-bound reducing moiety which can reduce copper ions (Gieseg et al., 1993). Such a radical damage might play a significant role in the

propagation of pathological radical damage; firstly, by being stable enough to diffuse from the site of the initial radical generating event and thus transmitting the radical damage to new locations and secondly, by being able to promote further radical generating reactions by replenishing the levels of reduced metals available to carry out Haber-Weiss type reactions. In conclusion, the data presented here confirms that the free radicals generated by resveratrol and Cu(II) do cause fragmentation of BSA. The spectrofluorimetric evidence for the binding of resveratrol to proteins relates to some non-specific binding and, in part, to changes in fluorescence of tryptophan residues. Future experiments on other proteins with different structural features, in particular the number and environments of tryptophan and other amino acids containing conjugated side chains (phenylalanine, tyrosine and histidine) will establish the generality of the process. The positions of fragmentation have not been established in this work, but it seems that the primary sites may be histidine and proline residues, as these are the primary targets for free radicals generated by Cu(II)/H_2O_2 and also by γ-radiolysis (Dean et al., 1989). In a study that evaluated RNAse A lacking tryptophans, amino acids histidine, tyrosine and methionine were hypothesized to be affected by oxidation (Leinisch et al., 2018), thus potentially increasing the repertoire of putative protein sites that can be involved. Further, resveratrol is a stilbene, i.e., an aromatic compound with two benzene moieties which are prone to hydrophobic interactions, besides binding to specific receptors. In a native protein, the tryptophan moiety can be expected to be engaged in hydrophobic interactions. It would be interesting to test proteins that do not contain tryptophan (such as RNAse A) and model peptides that do not contain any aromatic amino acid. Such studies will provide more mechanistic insights, but our current study should be a trigger for the planning of these studies, as the topic of protein damage by potential anticancer drugs remains an under studied area of research.

ACKNOWLEDGEMENTS

trans-Resveratrol was kindly provided by Pharma Science Inc., Canada.

AUTHOR CONTRIBUTIONS

AA and MF performed experiments; AA, TA, HZ, SA and AF analyzed results; SMH supervised the study; AA and SMH prepared the first draft; All authors edited and proofread the manuscript.

ETHICAL CONSIDERATIONS

Not applicable.

FUNDING

No funding was received for this study.

CONFLICT OF INTEREST

None.

REFERENCES

Ahmad A., Farhan Asad S., Singh S., Hadi S.M. (2000) DNA breakage by resveratrol and Cu(II): reaction mechanism and bacteriophage inactivation. *Cancer Lett.*, **154:** 29-37.

Ahmad A., Syed F.A., Singh S., Hadi S.M. (2005) Prooxidant activity of resveratrol in the presence of copper ions: mutagenicity in plasmid DNA. *Toxicol Lett*, **159:** 1-12.

Ahmed M.S., Ainley K., Parish J.H., Hadi S.M. (1994) Free radical-induced fragmentation of proteins by quercetin. *Carcinogenesis*, **15:** 1627-30.

Davies K.J., Lin S.W., Pacifici R.E. (1987) Protein damage and degradation by oxygen radicals. IV. Degradation of denatured protein. *J. Biol. Chem.*, **262:** 9914-20.

Dean R.T., Wolff S.P., McElligott M.A. (1989) Histidine and proline are important sites of free radical damage to proteins. *Free Radic. Res. Commun.*, **7:** 97-103.

Farhan M., Rizvi A. (2022) Understanding the prooxidant action of plant polyphenols in the cellular microenvironment of malignant cells: Role of copper and therapeutic implications. *Front. Pharmacol.*, **13:** 929853.

Farhan M., Ullah M.F., Faisal M., Farooqi A.A., Sabitaliyevich U.Y., Biersack B., Ahmad A. (2019) Differential methylation and acetylation as the epigenetic basis of resveratrol's anticancer activity. *Medicines (Basel)*, **6(1):** 24.

Gieseg S.P., Simpson J.A., Charlton T.S., Duncan M.W., Dean R.T. (1993) Protein-bound 3,4-dihydroxyphenylalanine is a major reductant formed during hydroxyl radical damage to proteins. *Biochemistry*, **32:** 4780-4786.

Hadi S.M., Asad S.F., Singh S., Ahmad A. (2000) Putative mechanism for anticancer and apoptosis-inducing properties of plant-derived polyphenolic compounds. *IUBMB Life*, **50:** 167-171.

Hou X., Rooklin D., Fang H., Zhang Y. (2016) Resveratrol serves as a protein-substrate interaction stabilizer in human SIRT1 activation. *Sci. Rep.*, **6:** 38186.

Iliadis S., Papanikolaou N.A. (2024) Reactive oxygen species mechanisms that regulate protein-protein interactions in cancer. *Int. J. Mol. Sci.*, **25(17):** 9255.

Jeandet P., Sbaghi M., Bessis R. (1992) The production of resveratrol (3,5,4'-trihydroxy-

stilbene) by grapevine in vitro cultures, and its application to screening for grey mould resistance. *Journal of Wine Research*, **3:** 47-57.

Jojima K., Tanaka A., Node K. (2023) Resveratrol supplementation: a therapeutic potential for cardiac remodeling in hypertensive heart disease. *Hypertens. Res.*, **46:** 1596-98.

Kim S., Kim E., Park M., Kim S.H., Kim B.G., Na S., Sadongo V.W., Wijesinghe W.C.B., Eom Y.G., Yoon G., Jeong H., Hwang E., Lee C., Myung K., Kim C.U., Choi J.M., Min S.K., Kwon T.H., Min D. (2024) Hidden route of protein damage through oxygen-confined photooxidation. *Nat. Commun.*, **15:** 10873.

Leinisch F., Mariotti M., Hagglund P., Davies M.J. (2018) Structural and functional changes in RNAse A originating from tyrosine and histidine cross-linking and oxidation induced by singlet oxygen and peroxyl radicals. *Free Radic. Biol. Med.*, **126:** 73-86.

Levine R.L. (1977) Fluorescence-quenching studies of the binding of bilirubin to albumin. *Clin. Chem.*, **23:** 2292-2301.

Liu F., Lai S., Tong H.,. Lakey P.S.J, Shiraiwa M., Weller M.G., Poschl U., Kampf C.J. (2017) Release of free amino acids upon oxidation of peptides and proteins by hydroxyl radicals. *Anal. Bioanal. Chem.*, **409:** 2411-2420.

Liu J.C., Yang J., Lei S.X., Wang M.F., Ma Y.N., Yang R. (2023) Natural phytoalexins inspired the discovery of new biphenyls as potent antifungal agents for treatment of invasive fungal infections. *Eur. J. Med. Chem.*, **261:** 115842.

Mehringer J., Navarro J.A., Touraud D., Schneuwly S., Kunz W. (2022) Phosphorylated resveratrol as a protein aggregation suppressor *in vitro* and *in vivo*. *RSC Chem. Biol.*, **3:** 250-260.

Meyer M.C., Guttman D.E. (1968) The binding of drugs by plasma proteins. *J. Pharm. Sci.*, **57:** 895-918.

Nakamura H., Takada K. (2021) Reactive oxygen species in cancer: Current findings and future directions. *Cancer Sci.*, **112:** 3945-52.

Orru R., Pazmino D.E., Fraaije M.W., Mattevi A. (2010) Joint functions of protein residues and NADP(H) in oxygen activation by flavin-containing monooxygenase. *J. Biol. Chem.*, **285:** 35021-35028.

Radeva L., Yoncheva K. (2025) Resveratrol - a promising therapeutic agent with problematic properties. *Pharmaceutics*, **17(1):**134.

Ren B., Kwah M.X., Liu C., Ma Z., Shanmugam M.K., Ding L., Xiang X., Ho P.C., Wang L., Ong P.S., Goh B.C. (2021) Resveratrol for cancer therapy: Challenges and future perspectives. *Cancer Lett.*, **515:** 63-72.

Ruzza, P., Honisch C., Hussain R., Siligardi G. (2021) Free radicals and ROS induce protein denaturation by UV photostability assay. *Int. J. Mol. Sci.*, **22(12):** 6512.

Shao F., Li Z., Xiao H., Chen Y., Zhang Y., Li L., Peng Y., Li X., Hou Y., Li B., Chu X., Ran M., Tang D., Han X., Yao J., Zhang C., Wang L., Li H., Shao N., Miao K., Xu X., Shi, Y. Zhang C., Yan J., Lin Y., Deng C.X. (2025) Suppressing protein damage response to overcome multidrug resistance in cancer therapy. *Cell Discov.*, **11:** 80.

Subramaniam S., Vohra I., Iyer A., Nair N.K., Mittra I. (2015) A paradoxical relationship between Resveratrol and copper (II) with respect to degradation of DNA and RNA. *F1000Res*, **4:** 1145.

Tang X., Yan Z., Miao Y., Ha W., Li Z., Yang L., Mi D. (2023) Copper in cancer: from limiting nutrient to therapeutic target. *Front. Oncol.*, **13:** 1209156.

Xu Y., Fang M., Li X., Wang D., Yu L., Ma F., Jiang J., Zhang L., Li P. (2024) Contributions of common foods to resveratrol intake in the Chinese diet. *Foods*, **13 (8):** 1267.

Zhang L.X., Li C.X., Kakar M.U., Khan M.S., Wu P.F., Amir R.M., Dai D.F., Naveed M., Li Q.Y., Saeed M., Shen J.Q., Rajput S.A., Li. J.H. (2021) Resveratrol (RV): A pharmacological review and call for further research. *Biomed/ Pharmacother*, **143:** 112164.

ORCIDs:

Aamir Ahmad: https://orcid.org/0000-0003-1784-5723
Mohd Farhan: https://orcid.org/0000-0002-1519-9644
Tariq Al-Qirim: https://orcid.org/0000-0001-5124-6797
Hatem Zayed: https://orcid.org/0000-0001-8838-6638
Salman Akhtar: https://orcid.org/0000-0003-2921-3950
Alvina Farooqui: https://orcid.org/0000-0002-7260-0414
S.M.Hadi: https://orcid.org/0009-0000-9245-340X

Anti-VEGF agents in brain tumor therapy: analysis of current clinical trials

Ilgiz Gareev[1,2], Hongli Zhang[3], Elena Zharova[4], Elmar Musaev[5], Ozal Beylerli[1*]

[1]Educational and Scientific Institute of Neurosurgery, Peoples' Friendship University of Russia (RUDN University), 6 Miklukho-Maklay Str., 117198, Moscow, Russia
[2]Pirogov Russian National Research Medical University, Ministry of Healthcare of Russian Federation, 1 Ostrovityanov Str., 117997, Moscow, Russia
[3]Department of Neurosurgery, First Affiliated Hospital of Harbin Medical University, Heilongjiang Province, 150001, Harbin, P.R. China
[4]Moscow Hertsen Research Institute of Oncology, Branch of the National Medical Radiology Research Center, Ministry of Health of Russian Federation, 125284, Moscow, Russia
[5]Sechenov First Moscow State Medical University, 2 Bol'shaya Pirogovskaya Str., 119435, Moscow, Russia

[*]***For correspondence:*** *obeylerli@mail.ru*

Received: November 05, 2025; Received in revised form: December 13, 2025; Accepted: December 18, 2025

Brain tumor therapy with anti-vascular endothelial cell growth factor (VEGF) agents/drugs is a therapeutic approach aimed at inhibiting the growth of new blood vessels that feed the tumor. This method, often referred to as targeted therapy, uses drugs that block the action of VEGF, which slows tumor growth and neovascularization. This study analyzes existing clinical trials registered on the ClinicalTrials.gov website on the therapeutic use of anti-VEGF agents in the treatment of brain tumors. As of December 2025, approximately 65 registered clinical trials on the use of anti-VEGF agents in the treatment of brain tumors, including gliomas, meningiomas, schwannomas, medulloblastomas (adult and pediatric), ependymomas, and metastatic brain tumors, were posted on ClinicalTrials.gov. Furthermore, recurrent tumors were also studied. However, full results have been published for only 16 clinical trials demonstrating the safety and efficacy of anti-VEGF agents. The results of these clinical trials open new horizons for the latest methods of targeted therapy for brain tumors.

Keywords: *Brain tumors, vascular endothelial cell growth factor, clinical trials, targeted therapy, personalized medicine, complications*

INTRODUCTION

Antiangiogenic agents are among the most commonly used antitumor agents/drugs in brain tumor therapy in clinical practice today. The most commonly used antiangiogenic drugs are those that target vascular endothelial cell growth factor (VEGF) (Chekhonin et al., 2013; Beylerli et al., 2025). The question of whether certain anti-VEGF drugs can be used clinically largely depends on the approval of certain regulatory authorities. It is known that the possibility of using certain drugs in clinical practice usually comes from approval by regulatory agencies such as the U.S. Food and Drug Administration (FDA) or the European Medicines Agency (EMEA) (Kang et al., 2024). It should be noted that rigorous regulatory review is typically limited to large Phase 3 clinical trials. The adoption of modern efficacy endpoints such as survival (OS), progression-free survival (PFS), or response rate (RR) can be complicated by many factors. These include access to the same or similar treatment protocol, in which case the question may not simply be whether a new drug should be used earlier or later than a standard comparator. Clinical efficacy and toxicity may also depend on the use of concomitant chemotherapy or radiation therapy or a combination of both the patient's tumor and non-cancerous conditions that the patient also has, particularly in older patients (Niazi SK., 2024; Pisarska et al., 2019; Roda et al., 2024). Despite this complexity, anti-VEGF therapy has established itself as one of the most important classes of drugs for the treatment of oncological diseases, including brain tumors. The study aims to analyze the current possibilities of using anti-VEGF drugs in the treatment of brain tumors in clinical settings, as well as the possible side effects of use.

MATERIALS AND METHODS

Search strategy: We conducted a comprehensive search for clinical trials demonstrating the use of anti-VEGF agents in the treatment of brain tumors, alone or in combination with chemoradiation.

 DOI: https://doi.org/10.62088/timbb/9.2.2

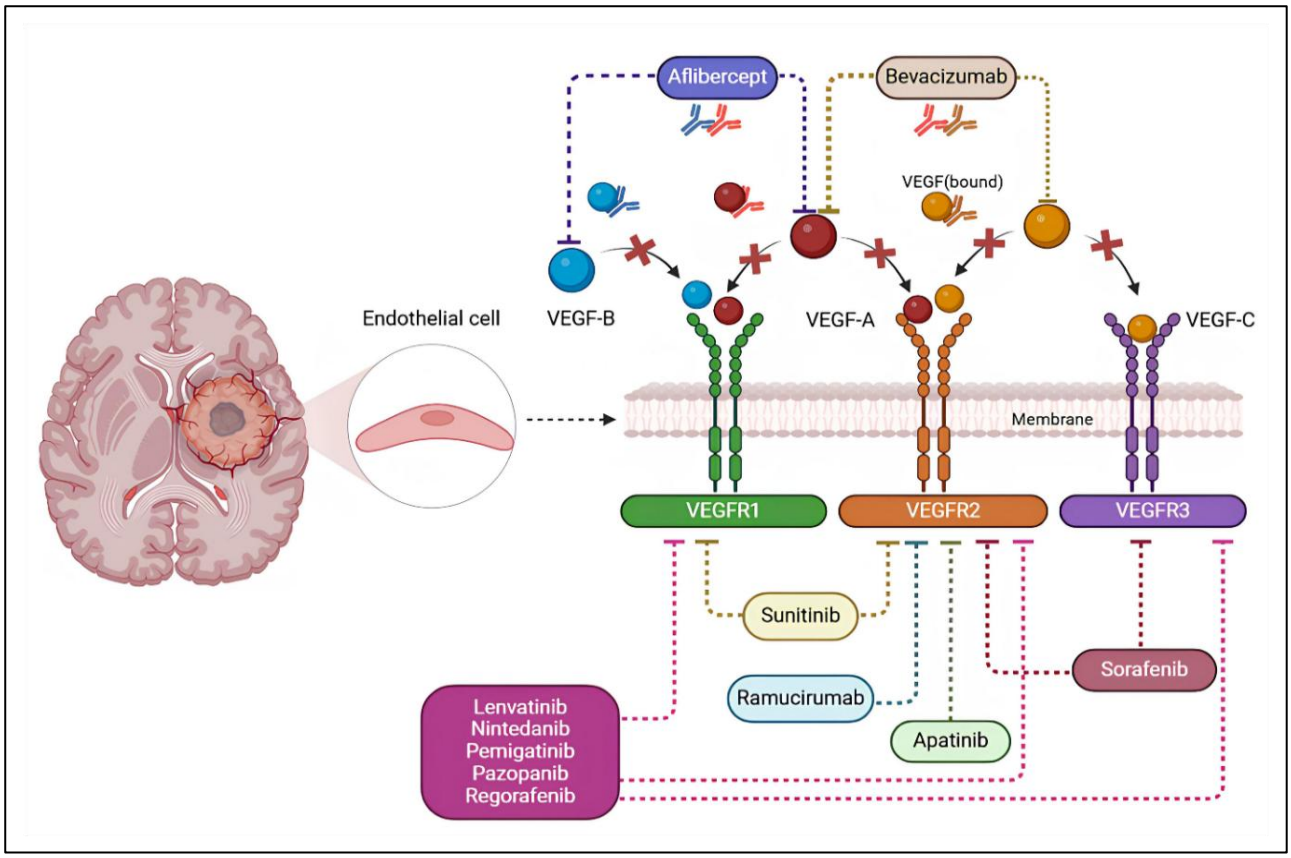


Fig. 1. Main anti-vascular endothelial growth factor (VEGF) agents in brain tumor treatment. The mechanism of action of some (e.g., bevacizumab) is based on binding to VEGF-A/B/C ligands and preventing their interaction with their receptors (VEGFR-1/2/3) on the surface of endothelial cells (ECs) of the GBM vessels. Others (e.g., ramucirumab) specifically bind to VEGF receptors and block the binding of the VEGF receptor to the VEGF-A/B/C ligands. Adapted from Beylerli et al. (Beylerli et al., 2025).

The clinicaltrials.gov database was used to retrieve all relevant information on ongoing clinical trials (Guelfi et al., 2024). The search was based on key words, specifically, in the Condition/disease search section, we used "brain tumors," "glioma," "glioblastoma," "meningioma," "medulloblastoma," "schwannoma," "ependymoma," and "brain metastasis," and in the Intervention/treatment search section, we used "vascular endothelial growth factor" and "VEGF." The following inclusion and exclusion criteria were used to select studies, divided into two stages (Figure 2).

Statistical Analysis: The t-test, ANOVA, chi-square analysis, or Mann-Whitney test were used. A p-value of < 0.05 (*), < 0.01 (**), or < 0.001 (**) was considered statistically significant. Statistical analysis was performed using IBM SPSS 22.0 software, and graphs were generated using Graphpad Prism 8.0.

RESULTS AND DISCUSSION

The use of anti-VEGF agents is a relatively new area with great potential to address many serious problems in neurosurgery and oncology (Beylerli et al., 2025; Pellerino et al., 2023; Pan et al., 2024). While further research may be required to fully exploit this potential, significant progress has already been made in studying the efficacy of anti-VEGF agents (Figure 3).

At the time of this study (December 2025), 65 clinical trials using anti-VEGF agents in the treatment of brain tumors were registered worldwide (note: some studies examined two or more tumor types) (Figure 4).

The number of registered clinical trials has increased significantly since the first official use of the anti-VEGF agent bevacizumab (recombinant hyperchimeric monoclonal IgG1 antibody) in the treatment of recurrent glioblastoma was reported on Clinicaltrials.gov on January 2, 2006 (Figure 5).
After applying the inclusion and exclusion criteria in Stage 1 of our study, a total of 23 clinical trials were excluded because they were either in early Phase 1, had no Phase specified, had an inapplicable Phase, or had been withdrawn (Figure 6A). We analyzed the remaining 42 clinical trials by their field of study, Phase, status, and location (Figures 6B-C).

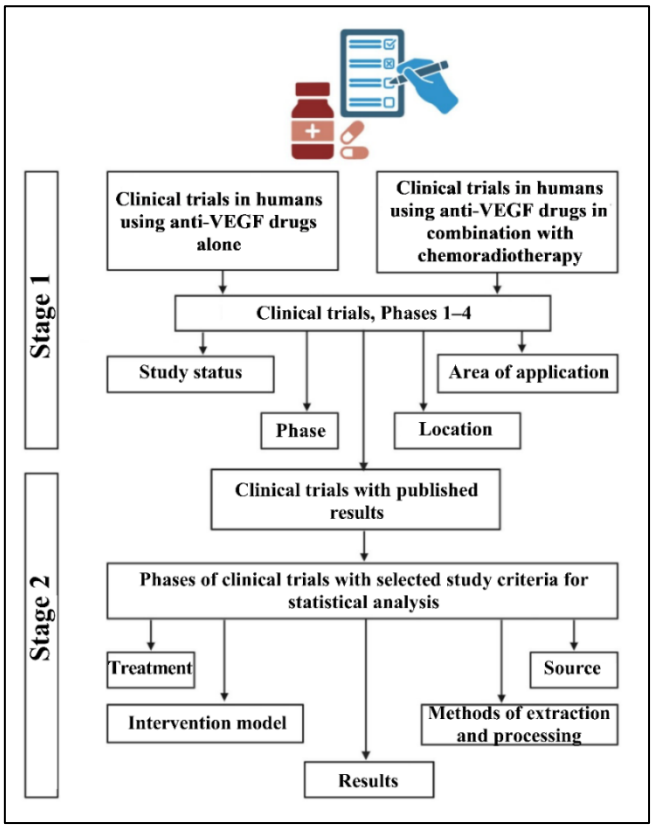


Fig. 2. Flow chart of study design.

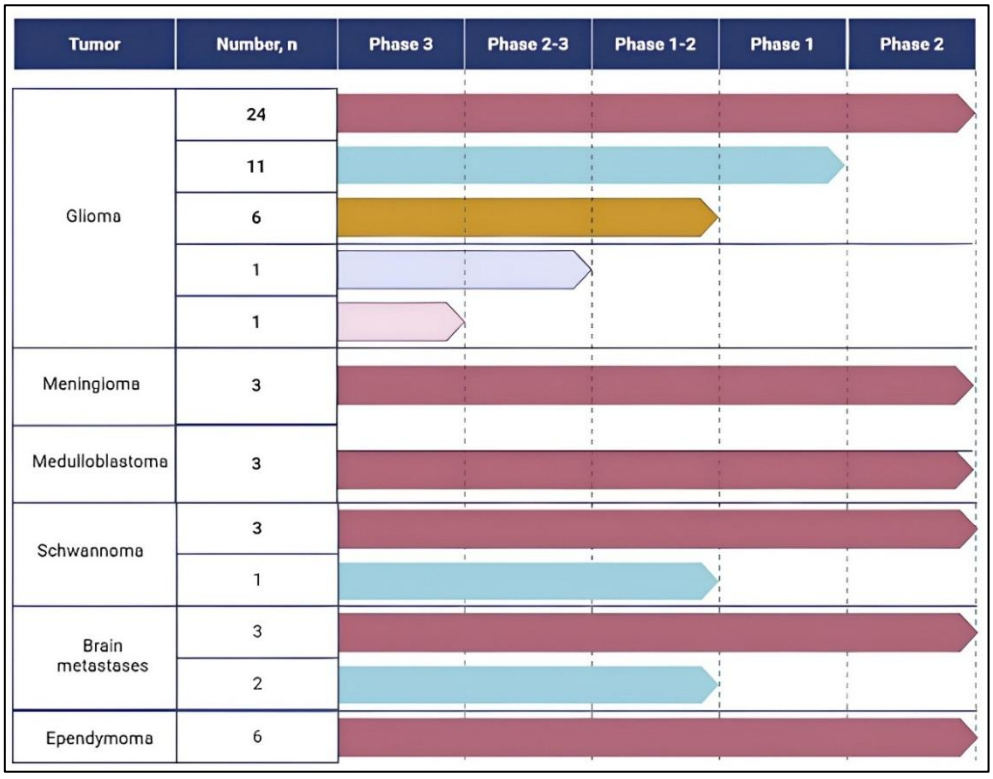


Fig. 3. Distribution of clinical trials by main brain tumors studied and by clinical Phases.

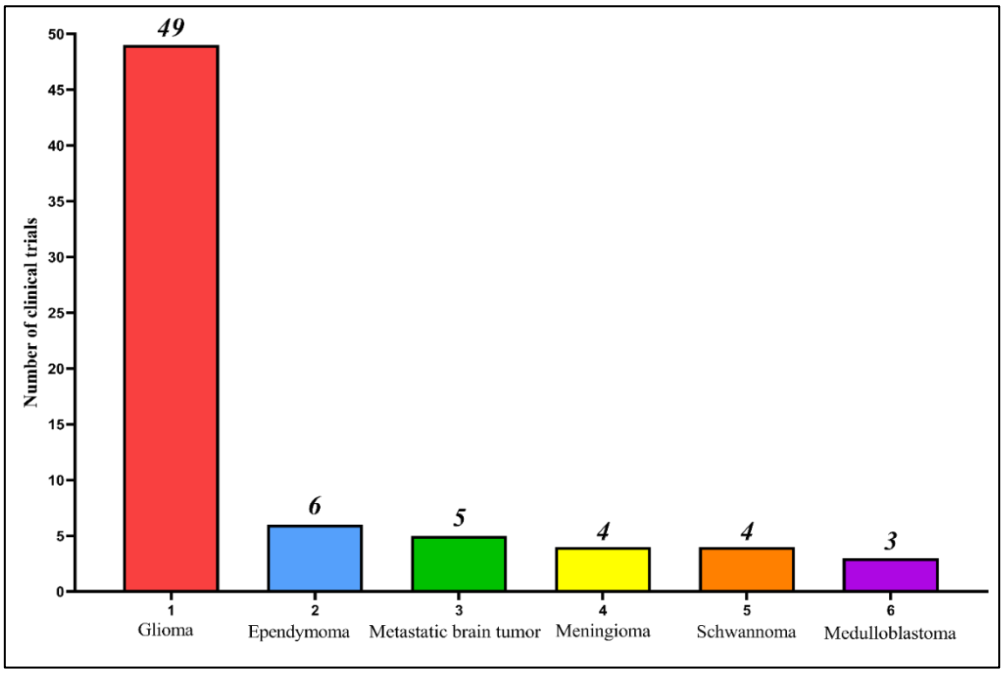


Fig. 4. Distribution of the number of clinical trials in descending order.

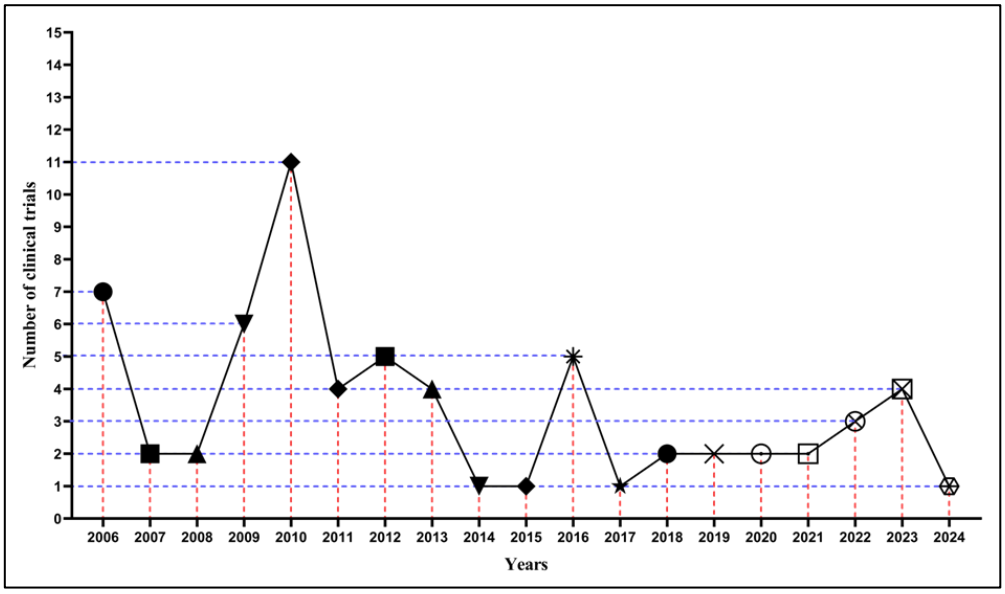


Fig. 5. Statistics of registered clinical trials for testing clinical potential in accordance with the dynamics of temporary (annual) registrations from the start and registration of the first clinical trial, based on data from the Clinicaltrials.gov website.

One clinical trial, NCT00985036, included a study of gliomas and meningiomas that did not specify a Phase and had a withdrawn status. The 42 clinical trials using anti-VEGF agents in our analysis are currently ongoing in 18 countries (note: some studies included treatment or research centers/institutions in two or more countries) (Figure 7). The United States, Canada, China, Germany, Israel, and Australia led the number of clinical trials.

Only 19 clinical trials using anti-VEGF agents alone or in combination with chemoradiation were selected for Phase 2 because they met the stated criteria for this study, namely, having a "Completed" status and published results. All of these clinical trials were primarily in Phase 2, primarily using bevacizumab alone or in combination with chemoradiation. Furthermore, glioma studies accounted for the largest number of clinical trials. The clinical trials showed positive results without serious side effects (Table 1).

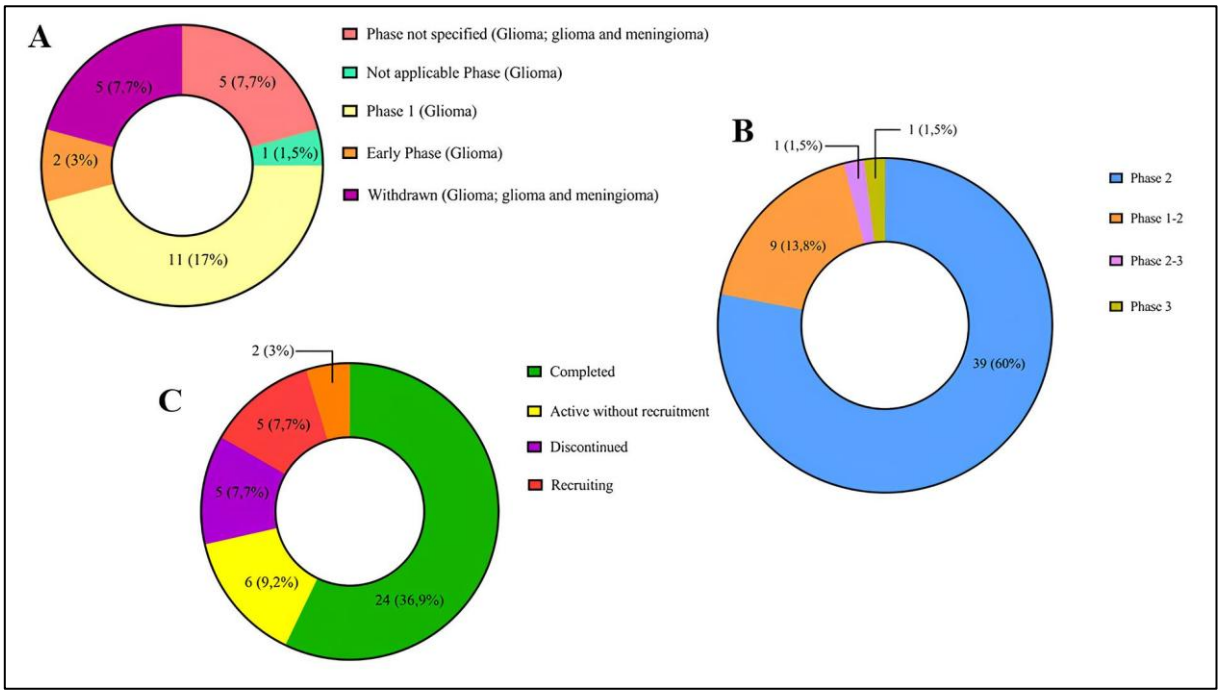


Fig. 6. Distribution of clinical trials. (A) Proportion of excluded clinical trials by Phase and Status. (B) Proportion of included clinical trials by Phase and (C) Proportion of included clinical trials by Status.

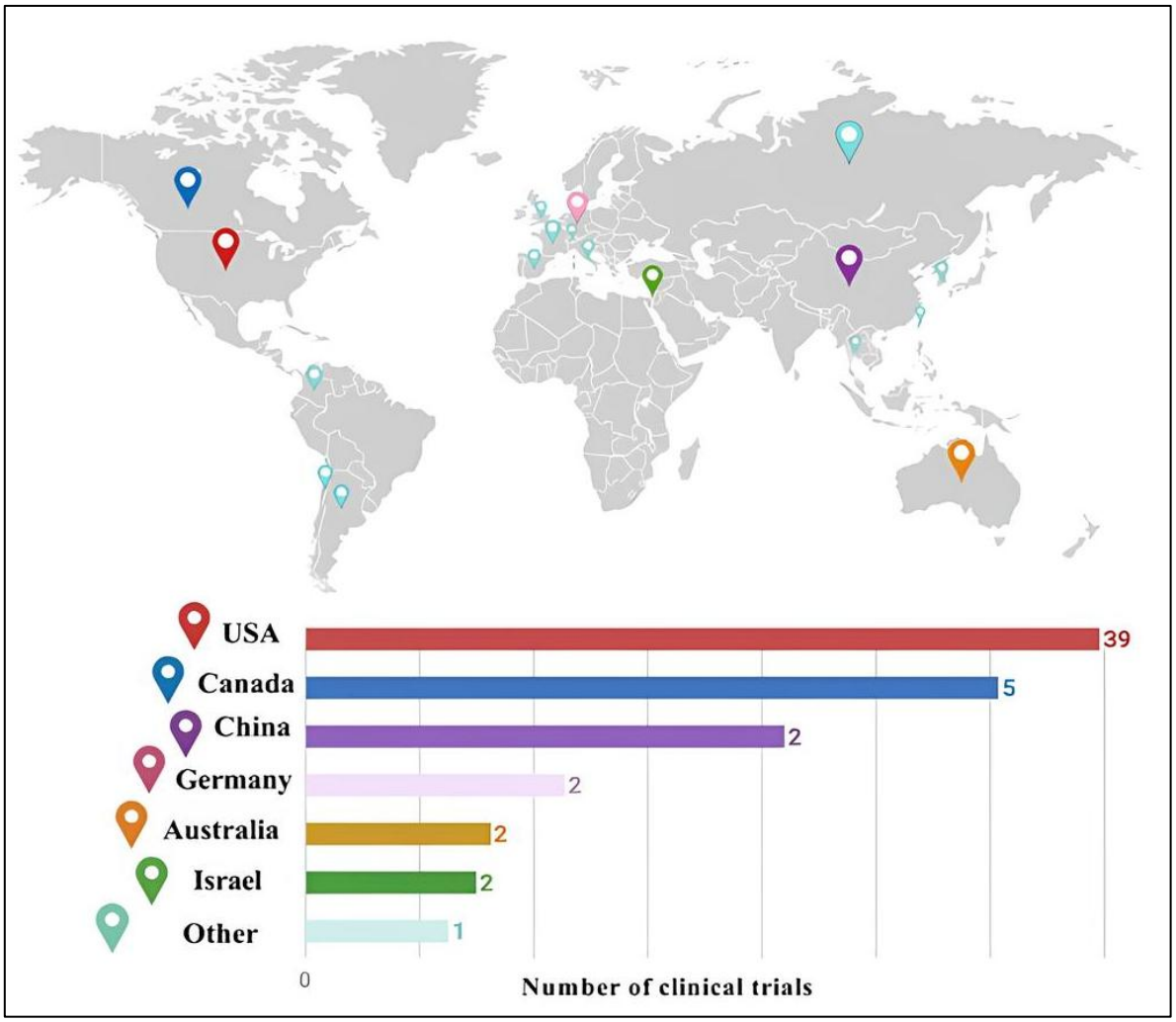


Fig. 7. Geographical distribution.

Table 1. Distribution of the most effective clinical trials (with a status of "Completed" and with results) registered on Clinicaltrial.gov as of December 2025 using vascular endothelial growth factor (VEGF) blocking drugs for the treatment of brain tumors.

NCT number	Tumor type	Therapy
NCT00369590 (Phase 2)	Glioma	Ziv-aflibercept
NCT00271609 (Phase 2)	Glioma	Bevacizumab
NCT00381797 (Phase 2)	Glioma	Bevacizumab + Fludeoxyglucose F-18 + Irinotecan hydrochloride
NCT01648348 (Phase 1–2)	Glioma	Anti-endoglin chimeric monoclonal antibody TRC105 + Bevacizumab
NCT01067469 (Phase 2)	Glioma	Bevacizumab (standard dose and low dose) + Lomustine
NCT00329719 (Phase 1–2)	Glioma	Sorafenib tosylate + Temsirolimus + Traditional surgery
NCT00433381 (Phase 2)	Glioma	Bevacizumab + Irinotecan hydrochloride + Temozolomide
NCT01236560 (Phase 2–3)	Glioma	Bevacizumab + Temozolomide + Vorinostat
NCT01730950 (Phase 2)	Glioma	Bevacizumab + Radiation Therapy
NCT01609790 (Phase 2)	Glioma	Bevacizumab + Trebananib
NCT00884741 (Phase 3)	Glioma	3-dimensional conformal radiation therapy + Bevacizumab + Intensity-modulated radiation therapy + Temozolomide
NCT00492089 (Phase 2)	Glioma, ependymoma and meningioma	Bevacizumab
NCT01753713 (Phase 2)	Glioma	Dovitinib
NCT01125046 (Phase 2)	Meningioma, schwannoma and ependymoma	Bevacizumab
NCT00883688 (Phase 2)	Ependymoma	Bevacizumab + Lapatinib
NCT01217437 (Phase 2)	Medulloblastoma	Bevacizumab + Irinotecan hydrochloride + Temozolomide
NCT01898130 (Phase 2)	Metastases	Bevacizumab
NCT01767792 (Phase 2)	Schwannoma	Bevacizumab
NCT01125046 (Phase 2)	Schwannoma	Bevacizumab

Anti-VEGF agents are widely used in the treatment of tumors but are associated with a characteristic set of complications due to the blockade of angiogenesis. The most common and clinically significant side effect is hypertension, which develops due to decreased nitric oxide production and increased vascular resistance. Anti-VEGF agents also damage the renal glomeruli, which is manifested by proteinuria and, in rare cases, nephrotic syndrome.

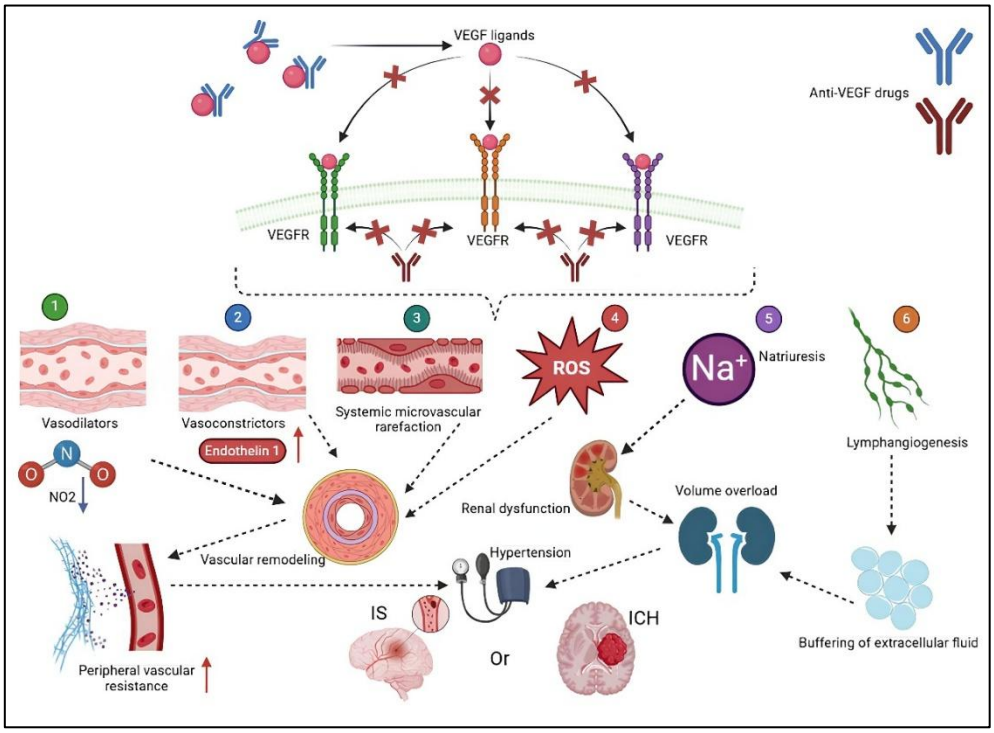


Fig. 8. Schematic illustration of the mechanism of development of arterial hypertension after the use of anti-vascular endothelial growth factor (VEGF) therapy in brain tumors. Adapted from Beylerli et al. (Beylerli et al., 2025).

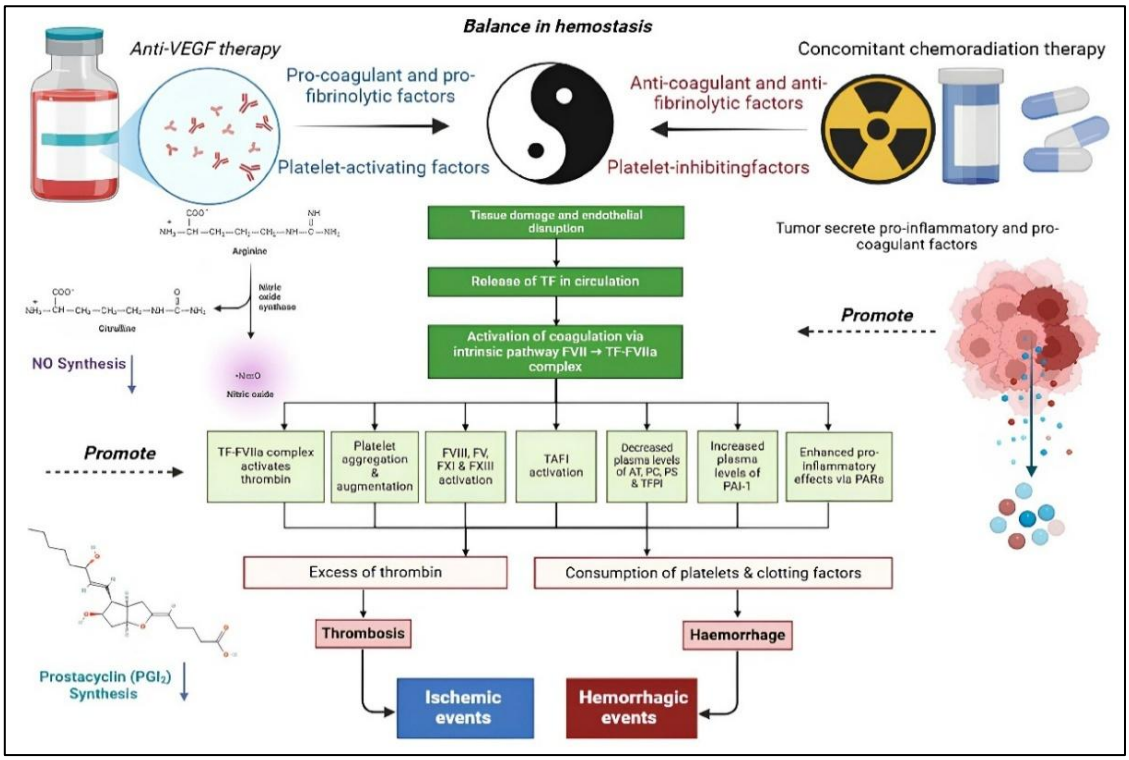


Fig. 9. Mechanism of influence of anti-vascular endothelial growth factor (VEGF) agents on hemostasis. Adapted from Beylerli et al. (Beylerli et al., 2025).

Table 2. The presence of the most serious adverse events that were detected as a result of anti- vascular endothelial growth factor (VEGF) therapy.

NCT number	Serious adverse events
NCT00369590 (Phase 2)	Oral hemorrhage and brain ischemia
NCT00271609 (Phase 2)	brain ischemia and hemorrhage, seizure proteinuria and thrombosis/thrombus/embolism
NCT00381797 (Phase 2)	Alanine aminotransferase increased, neutrophil and platelet count decreased, hypoalbuminemia, hypocalcemia, hyponatremia, hydrocephalus, seizure, hypertension and intracranial hemorrhage
NCT01648348 (Phase 1–2)	Thrombosis
NCT01067469 (Phase 2)	Fracture, muscle weakness – generalized, brain ischemia, confusion and headache
NCT00329719 (Phase 1–2)	Lymphocyte count, neutrophil and platelet decreased, serum triglycerides increased, leukoencephalopathy, peripheral motor neuropathy and seizure
NCT00433381 (Phase 2)	Hemoglobin, hemorrhagic stroke, leukopenia, lymphopenia, neutrophil and platelet count decreased, abdominal pain, colonic perforation, constipation, chest pain, fatigue, aspartate aminotransferase increased, convulsions and thrombosis
NCT01236560 (Phase 2–3)	Hydrocephalus
NCT01730950 (Phase 2)	Encephalopathy, seizure, hematoma and thromboembolic event
NCT01609790 (Phase 2)	Cognitive disturbance, seizure and stroke
NCT00884741 (Phase 3)	Lymphopenia, leukopenia, retinal detachment, blurred vision, abdominal pain, constipation, nausea, fever, neutrophil and platelet count decreased, hyperglycemia, muscle weakness, cognitive disturbance, intracranial hemorrhage, ischemic cerebrovascular, peripheral motor neuropathy, seizure
NCT00492089 (Phase 2)	Thrombosis
NCT01753713 (Phase 2)	Confusion and thrombosis
NCT01125046 (Phase 2)	Seizure
NCT00883688 (Phase 2)	Increase serum glutamic pyruvic transaminase (ALT, SGPT), cranial neuropathy CN IX, motor apnea and dyspnea
NCT01217437 (Phase 2)	Sepsis and hypotension
NCT01898130 (Phase 2)	Seizure
NCT01767792 (Phase 2)	Headache
NCT01125046 (Phase 2)	Seizure

VEGF inhibitors are associated with an increased risk of vascular events, including ischemic and hemorrhagic stroke, due to endothelial dysfunction, increased hypertension, and thrombotic complications (with some drugs, the risk of arterial thrombosis is higher than venous)

(Figure 8 and Figure 9) (Beylerli et al., 2025; Katsi et al., 2014; Tonooka et al., 2022; Soffietti et al., 2012; Ferroni et al., 2010). Hemorrhagic strokes are less common, but the risk increases with the combination of anticoagulants or coagulation disorders (Zaborowska-Szmit et al., 2020). The risk of complications is highest in patients with pre-existing cardiovascular disease, uncontrolled hypertension, older age, or diabetes, so regular monitoring of blood pressure and risk factors is necessary.

One of the serious, albeit relatively rare, complications is gastrointestinal perforation and fistula development, especially in tumors that invade the intestinal wall. Anti-VEGF agents significantly delay wound healing, so they are often discontinued well before surgery and resumed only after complete healing (Min et al., 2021; Schiffmann et al., 2019). The frequency and severity of complications depend on the specific drug, dose, concomitant therapy, and the patient's comorbidities. Their prevention and management require regular blood pressure monitoring, urine protein testing, cardiac function assessment, and close monitoring for bleeding symptoms and abdominal pain. Early discontinuation or dose adjustment if serious toxicities occur, a multidisciplinary approach, and patient education about signs requiring immediate medical attention are essential (Table 2).

Clinical trials of anti-VEGF therapies for brain tumors are actively underway. The leading drug in this field is Bevacizumab, which blocks VEGF-A and prevents the formation of new blood vessels. It has demonstrated efficacy in reducing tumor volume and improving symptoms in patients with recurrent gliomas. In randomized trials, Bevacizumab improves short-term survival rates and quality of life. However, the long-term benefits of this treatment remain debated, as the effect is often short-lived. Many patients receive a combination of anti-VEGF agents and chemotherapy to enhance the effectiveness of the therapy. Studies are currently underway to combine anti-VEGF therapies with immunotherapy and other new treatments. The use of such combinations helps reduce the risk of brain tumor resistance to monotherapy, a significant advantage. The combined use of anti-VEGF agents with chemotherapy or immunotherapy enhances the antitumor effect and promotes longer-term stabilization of the patient's condition. This approach not only improves quality of life but also increases overall survival. Combination regimens allow for more precise tailoring of treatment to the individual patient and tumor characteristics. Furthermore, appropriately selected combination agents can reduce dosages and reduce side effects, improving treatment safety. Research shows that combinations of anti-VEGF agents with newer drugs can promote more effective destruction of cancer cells and prevent their recurrence. However, additional clinical trials are needed to identify optimal regimens, doses, and timing of such combinations. In the future, the combination of anti-VEGF agents with other drugs may become a standard in brain tumor therapy, providing longer-term disease control. It is important to continue to study and improve these methods to improve their effectiveness and safety.

Despite the significant advantages of anti-VEGF drugs in the treatment of brain tumors, they also have a number of limitations. One of the main ones is the development of therapy resistance, when the tumor adapts to anti-VEGF agents and continues to grow. Other disadvantages include a short period of effectiveness, followed by disease progression. Many patients experience side effects such as hypertension, bleeding, thrombosis, and vascular damage. Treatment sometimes causes a deterioration in general condition or complications associated with impaired wound healing and vascular problems. Furthermore, anti-VEGF agents can reduce quality of life due to their side effects and the need for long-term treatment. Another limitation is their cost, which often makes them unaffordable for some patients. There are also limitations in their effectiveness for certain tumor types or in certain patient groups. Finally, the use of anti-VEGF agents in combination with other treatments may increase the risk of adverse reactions and complications.

Overall, anti-VEGF agents are an important part of the treatment of brain tumors, but they are not intended to be curative. Research is ongoing to develop more effective drugs and improve treatment outcomes. New combinations and personalized approaches to treating brain tumors are expected in the future.

CONCLUSION

Angiogenesis is a complex molecular process that remains incompletely understood. These clinical studies have demonstrated that anti-VEGF agents, both alone and in combination with chemoradiation therapy, may be effective in the treatment of malignant brain tumors. Based on the results of 19 completed clinical trials, anti-VEGF agents are associated with prolonged PFS and a reduction in vasogenic cerebral edema. Larger multicenter prospective studies are underway to confirm these results and assess their impact on patient survival. These studies are being conducted

in more than ten countries, including Russia (NCT03797326). Unfortunately, treatment failure inevitably occurs in the majority of patients. Further research is needed to identify other pro-angiogenic signaling pathways. Furthermore, combining anti-VEGF agents with immunotherapy may prevent the development of treatment resistance and maximize survival. New neuroimaging methods are needed to accurately assess tumor response to anti-VEGF therapy. However, inhibition of angiogenesis is a promising therapeutic approach that may have a significant impact on the treatment of both primary and metastatic brain tumors.

ACKNOWLEDGEMENTS

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

No datasets were generated or analyzed during the current study.

AUTHOR CONTRIBUTIONS

Conceptualization, writing - original draft, and writing - review & editing, I.G.; Data curation, formal analysis, investigation, and methodology, H.Z. and E.Z.; Software, validation and visualization, I.G. and O.B.; Project administration, conceptualization and supervision, O.B. and E.M. All authors agreed on the journal to which the article would be submitted, gave final approval for the version to be published, and agreed to be accountable for all aspects of the work.

ETHICAL CONSIDERATIONS

This study is based exclusively on the analysis of publicly available data from clinical trials registered on the ClinicalTrials.gov database. No new clinical interventions were performed, and no individual patient data were collected or analyzed. Therefore, ethical approval and informed consent were not required for this study. The analysis was conducted in accordance with internationally accepted principles of research integrity, transparency, and responsible use of publicly accessible scientific data.

FUNDING

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest, financial or otherwise, related to this study. The authors have no affiliations or involvement with any organization or entity with a financial or non-financial interest in the subject matter discussed in this manuscript.

REFERENCES

Chekhonin V.P., Shein S.A., Korchagina A.A., Gurina O.I. (2013) VEGF in tumor progression and targeted therapy. *Curr. Cancer Drug. Targets*, **13(4):** 423-443; doi: 10.2174/15680096113139990074.

Beylerli O., Gareev I., Kaprin A., Ahmad A., Chekhonin V., Yang S., Yang G. (2025) Hemorrhagic and ischemic risks of anti-VEGF therapies in glioblastoma. *Cancer Gene Ther*., **32(7):** 762-777; doi: 10.1038/s41417-025-00914-8.

Kang Y., Li H., Liu Y., Li Z. (2024) Regulation of VEGF-A expression and VEGF-A-targeted therapy in malignant tumors. *J. Cancer Res. Clin. Oncol*., **150(5):** 221; doi: 10.1007/s00432-024-05714-5.

Niazi S.K. (2024) The United States Food and Drug Administration's Platform Technology Designation to Expedite the Development of Drugs. *Pharmaceutics*, **16(7):** 918; doi: 10.3390/pharmaceutics16070918.

Pisarska M.D., Chan J.L., Lawrenson K., Gonzalez T.L., Wang E.T. (2019) Genetics and epigenetics of infertility and treatments on outcomes. *J. Clin. Endocrinol. Metab.*, **104(6):** 1871-1886; doi: 10.1210/jc.2018-01869.

Roda D., Veiga P., Melo J.B., Carreira I.M., Ribeiro I.P. (2024) Principles in the management of glioblastoma. *Genes (Basel),* **15(4):** 501; doi: 10.3390/genes15040501.

Guelfi S., Hodivala-Dilke K., Bergers G. (2024) Targeting the tumour vasculature: from vessel destruction to promotion. *Nat Rev Cancer*., **24(10):** 655-675; doi: 10.1038/s41568-024-00736-0.

Beylerli O., Gareev I., Musaev E., Ilyasova T., Roumiantsev S., Chekhonin V. (2025) Angiogenesis and resistance mechanisms in glioblastoma: targeting alternative vascularization pathways to overcome therapy resistance. *Curr. Pharm. Des*., Jul 22; doi: 10.2174/0113816128367551250703122830.

Pellerino A., Bruno F., Soffietti R., Rudà R. (2023) Antiangiogenic therapy for malignant brain tumors: Does it still matter? *Curr. Oncol. Rep*., **25(7):** 777-785; doi: 10.1007/s11912-023-

01417-1.

Pan X., Li X., Dong L., Liu T., Zhang M., Zhang L., Zhang X., Huang L., Shi W., Sun H., Fang Z., Sun J., Huang Y., Shao H., Wang Y., Yin M. (2024) Tumour vasculature at single-cell resolution. *Nature*, **632(8024):** 429-436; doi: 10.1038/s41586-024-07698-1.

Katsi V., Zerdes I., Manolakou S., Makris T., Nihoyannopoulos P., Tousoulis D., Kallikazaros I. (2014) Anti-VEGF anticancer drugs: Mind the hypertension. *Recent Adv. Cardiovasc. Drug Discov.*, **9(2):** 63-72; doi: 10.2174/1574890110999150604114127.

Tonooka A, Ohashi R. (2022) Current trends in anti-cancer molecular targeted therapies: Renal complications and their histological features. *J. Nippon. Med. Sch.*, **89(2):** 128-138; doi: 10.1272/jnms.JNMS.2022_89-221.

Soffietti R., Trevisan E., Rudà R. (2012) Targeted therapy in brain metastasis. *Curr. Opin. Oncol.*, **24(6):** 679-686. doi: 10.1097/CCO.0b013e3283571a1c.

Ferroni P., Formica V., Roselli M., Guadagni F. (2010) Thromboembolic events in patients treated with anti-angiogenic drugs. *Curr. Vasc. Pharmacol.*, **8(1):** 102-113. doi: 10.2174/157016110790226660.

Zaborowska-Szmit M, Krzakowski M, Kowalski DM, Szmit S. (2020) Cardiovascular complications of systemic therapy in non-small-cell lung cancer. *J. Clin. Med.*, **9(5):** 1268; doi: 10.3390/jcm9051268.

Min A.K.T., Mimura K., Nakajima S., Okayama H., Saito K., Sakamoto W., Fujita S., Endo H., Saito M., Saze Z., Momma T., Ohki S., Kono K. (2021) Therapeutic potential of anti-VEGF receptor 2 therapy targeting for M2-tumor-associated macrophages in colorectal cancer. *Cancer Immunol. Immunother.*, **70(2):** 289-298; doi: 10.1007/s00262-020-02676-8.

Schiffmann L.M., Fritsch M., Gebauer F., Günther S.D., Stair N.R., Seeger J.M., Thangarajah F., Dieplinger G., Bludau M., Alakus H., Göbel H., Quaas A., Zander T., Hilberg F., Bruns C.J., Kashkar H., Coutelle O. (2019) Tumour-infiltrating neutrophils counteract anti-VEGF therapy in metastatic colorectal cancer. *Br. J. Cancer*, **120(1):** 69-78; doi: 10.1038/s41416-018-0198-3.

ORCIDs:

Ilgiz Gareev: https://orcid.org/0000-0002-4965-0835
Hongli Zhang: https://orcid.org/0009-0001-4036-519X
Elena Zharova: https://orcid.org/0009-0005-6466-5348
Elmar Musaev: https://orcid.org/0000-0002-1241-3019
Ozal Beylerli: https://orcid.org/0000-0002-6149-5460

Association of the IL1RN gene VNTR polymorphism (rs2234663) with chronic inflammation-associated cancer

Nurmammad Mustafayev[1,2*], Lala Akhundova[1], Shalala Majidova[1], Nigar Mammadli[2], Ahliman Amiraslanov[3], Irada Huseynova[1,2]

[1]Population Genomica Laboratory, "Institute of Molecular Biology" Public Legal Entity, Ministry of Science and Education of the Republic of Azerbaijan, 11 Izzat Nabiyev Str., AZ1073, Baku, Azerbaijan
[2]Baku State University, Ministry of Science and Education of the Republic of Azerbaijan, 23 Academician Zahid Khalilov Str., AZ1148, Baku, Azerbaijan
[3]Oncology Clinic, Azerbaijan Medical University, Ministry of Health of the Republic of Azerbaijan, 208 S.Vurgun Str., AZ1022, Baku, Azerbaijan

[*]***For correspondence:*** *mustafayevn02@yahoo.co.uk*

Received: November 05, 2025; Reviewed: December 15, 2025; Accepted: December 24, 2025

One of the main tasks of modern medicine is to identify genetic predisposition to common diseases using molecular markers. This plays a crucial role in enabling early diagnosis and timely prevention. Currently, diseases that are either hereditary or non-hereditary, and which are caused by endogenous and exogenous factors, mutagenesis, and acute or chronic inflammatory processes, are the leading cause of both incidence and mortality. Of particular importance among these are various forms of cancer, which are associated not only with genetic factors but also with chronic inflammation. It is well established that proinflammatory cytokines, their biosynthesis and the proper functioning of signalling pathway components play a key role in the development and regulation of inflammatory processes, particularly chronic ones. In this context, along with agonists of interleukin-1 (IL-1), the interleukin-1 receptor antagonist (IL-1RA) and the gene encoding it (IL1RN) are critically involved in modulating IL-1 activity. The aim of the present study was to determine the association between the VNTR polymorphism (rs2234663) located in the second intron of the IL1RN gene and the risk of cancer presumably associated with chronic inflammation. The study material consisted of genomic DNA isolated from peripheral blood samples of cancer patients (experimental group, EG, n=80) and conditionally healthy individuals (control group, CG, n=84). Genotyping of the IL1RN VNTR polymorphism (rs2234663) was performed using the polymerase chain reaction (PCR) method with specific primers. Allele and genotype frequencies were calculated for both groups. Although all known alleles of the IL1RN gene were detected in the studied cohorts, several genotypes (*2*5, *2*6, *3*4, *3*5, *3*6, *4*5, *4*6, and *5*6) were not observed in either group. In the experimental group, the frequency of the normal allele *1 was approximately 1.4-fold lower, whereas the frequency of the mutant allele *2 was about 1.6-fold higher compared with the control group. Overall, the homozygous mutant genotype (*2*2) occurred approximately 2.1 times more frequently in cancer patients than in controls. To evaluate the strength of association between the IL1RN polymorphism and cancer susceptibility, odds ratios (OR), relative risks (RR), 95% confidence intervals (CI), Z-test statistics, and corresponding P values were calculated. The association between the risk allele *2 and cancer predisposition was statistically significant (OR≈2.2, RR≈1.53, P≈0.001). A pronounced association was also observed for the homozygous genotype *2*2 (OR≈2.84, RR≈2.18, P≈0.004). Notably, compared with heterozygous carriers (*1*2), individuals homozygous for the *2 allele (*2*2) exhibited approximately 2.4-fold higher odds (OR_{*2*2}/OR_{*1*2}) and about 2.0-fold higher relative risk (RR^{*2*2}/RR_{*1*2}) of developing cancer associated with chronic inflammation. Of the analysed genetic models, only the dominant model (*2*2 vs. *1*1 + *1*2) showed a statistically significant association with cancer risk (OR≈2.97, RR≈2.10, P=0.003).

Keywords: *Inflammation, cytokine, interleukin-1, agonist, antagonist, risk allele, cancer, association*

INTRODUCTION

A continuous rise is currently being observed in the incidence of both hereditary and non-hereditary cancers, which are linked to ongoing mutagenic processes in the human body. This rise is occurring alongside an increase in autoimmune, allergic and inflammatory diseases. Analysis of global epidemiological data shows that around six in ten disease-related deaths are due to chronic

 DOI: https://doi.org/10.62088/timbb/9.2.3

inflammatory conditions, such as stroke, chronic cardiovascular and respiratory diseases, allergies, various types of cancer, obesity, diabetes and related disorders (Pahwa et al., 2022; WHO Statistics, 2024). Cancer, particularly inflammation- and infection-associated malignancies, represents a growing global health burden, with steadily increasing incidence and mortality rates. Contemporary global cancer incidence and mortality statistics (Worldwide Cancer Data, 2024; Siegel et al., 2025) provide a comprehensive overview of these trends, as do reports from the American Cancer Association, which provides one of the most systematic and representative cancer surveillance datasets worldwide (Global Cancer Statistics, 2024).

The concept of linking malignant cellular transformation to chronic inflammatory diseases was first proposed in the nineteenth century. Since then, epidemiological evidence has demonstrated that inflammatory processes arising from various types of tissue injury, physical, chemical and biological, contribute to the development of at least 15% of all cancer types. The association between chronic inflammation and cancer is particularly evident in gastrointestinal diseases such as chronic hepatitis B and C, B+C co-infections, Barrett's oesophagus, gastric infections, chronic pancreatitis, ulcerative colitis and Crohn's disease. Similar relationships are also evident in the respiratory system, for example, in asbestosis, tuberculosis, chronic bronchitis, and pneumonias caused by exposure to wood and fur dust, as well as by airborne pathogens (e.g. coronaviruses). Furthermore, chronic inflammatory conditions of the genitourinary system, including chronic cervicitis and prostatitis, as well as sexually transmitted infections, exemplify the close interplay between persistent inflammation and carcinogenesis (Furukawa et al., 2011; Brovkina et al., 2022; Dinarello, 2023; Albini et al., 2025; Lee et al., 2025; Wu et al., 2025; Xu et al., 2025).

The molecular mechanisms underlying the bidirectional interplay between carcinogenesis and inflammation are based, firstly, on the expression of receptors for cytokines, chemokines, growth factors and immunoregulatory molecules by normal epithelial cells and, secondly, on the constitutive expression and activation-induced secretion of cytokines, eicosanoids, endothelins, defensins, nitric oxide and mediators of cell–cell interactions by these same cells. Elucidating the role of the highly complex, multifunctional and multicomponent immune system, which encompasses both innate and adaptive immunity and is governed by sophisticated regulatory mechanisms, is critical in this context. Specifically, pathogenic stimuli in the cytosol give rise to pathogen-associated molecular patterns (PAMPs), damage-associated molecular patterns (DAMPs), or lifestyle-associated molecular patterns (LAMPs). These patterns collectively trigger the assembly of multiprotein complexes known as inflammasomes. These pattern-recognition receptor-based platforms are a key part of the inflammatory immune response, ultimately leading to the activation of pro-inflammatory cytokines, including interleukins such as IL-1 and IL-18, as well as TNF and IFNs. The resulting cytokine cascade drives the induction and execution of inflammation-associated immune responses (Weber et al., 2010; Gong et al., 2020; Zindel & Kubes, 2020; Liu et al., 2022; Tokarz-Deptuła et al., 2024; Gartland et al., 2025).

Disruptions to this tightly regulated process, which occurs at any level, may lead to chronic and systemic inflammation. This inflammation can then promote the emergence of cancer stem cells. The dysregulation of signalling pathways that mediate the activity of pro-inflammatory and inflammatory cytokines, which are secreted by immune cells involved in inflammatory responses and their resolution, including natural killer (NK) cells, T helper cells, macrophages and related immune cell populations, can facilitate tumour initiation and progression. Once established, cancer stem cells may evade recognition by NK cells of the immune system and undergo unchecked expansion. Within this regulatory network, the pro-inflammatory cytokine interleukin-1 (IL-1) and its receptor (IL-1R) play a pivotal role (Weber et al., 2010). The IL-1 signalling axis comprises two endogenous agonists (IL-1α and IL-1β) and a naturally occurring antagonist (IL-1RA). Genetic alterations affecting these components, including VNTR-type polymorphisms in the IL1RN gene that encodes IL-1RA, particularly the IL1RN*2 mutant allele, can disrupt the normal function of the IL-1 signalling pathway. This pathway is a critical mediator of immune responses and its disruption can contribute to the development of a range of diseases, including multiple cancer types (Jaiswal et al., 2012; Sousa et al., 2013; Hashemi et al., 2015; Nedumpun et al., 2017; Saad et al., 2020).

Comprehensive insights into the relationship between tumour development and inflammation, particularly about the involvement of reactive oxygen, nitrogen and sulphur species (ROS, RNS and RSS) in this process, as well as the role of other key molecular and cellular factors, can be found in the works of F.R.Greten and S.I.Grivennikov (2019) and F.Okada et al. (2021). Additionally, H.Zhao et al. (2021) have provided generalised schematic representations of the signalling pathways involved in inflammation-driven tumourigenesis, together with their principal

components (including proteins and immune cell populations).

A multiprotein oligomeric complex known as the inflammasome initiates the cascade of inflammatory responses. The activation of inflammasomes promotes the maturation and secretion of the pro-inflammatory cytokines IL-1β and IL-18. These cytokines then trigger pyroptosis, which is a distinct form of programmed cell death. It is widely hypothesised that inflammasome dysregulation is one of the fundamental mechanisms underlying the development of numerous inflammation-associated diseases. Several key studies (Broz and Dixit, 2016; Jin and Yin, 2019; C.Zhao and W.Zhao, 2020; Seok et al., 2021; Inflammasomes, 2022) provide detailed descriptions of canonical and non-canonical inflammasomes, as well as their therapeutic regulatory roles in inflammatory processes.

The interleukin-1 (IL-1) family is a group of proteins that play central roles in both innate and adaptive immune responses, some of which are pro-inflammatory and some of which are anti-inflammatory. The most extensively studied and biologically significant member of this family is IL-1β. As a key mediator of inflammation, IL-1β induces fever and promotes immune activation by binding to IL-1 receptor type 1 (IL-1R1). Its production and secretion are tightly regulated and dependent on inflammatory stimuli. Initially, transcription of the biologically inactive precursor form, pro-IL-1β, is induced by the activation of Toll-like receptors (TLRs), tumour necrosis factor (TNF) signaling, or the engagement of IL-1 receptors by mature IL-1α or IL-1β (Yazdi and Ghoreschi, 2016).

Only upon the activation of caspase-1 are both IL-1 receptors (IL-1R1 and IL-1R2) released from their associated cytokines (IL-1β and IL-1α). This allows the conversion of the precursor cytokines into their mature forms, which are then secreted. Interestingly, IL-1R2 appears to regulate IL-1α activation differently during necrotic cell death, independently of inflammasome signalling. In this context, the IL-1 receptor antagonist (IL-1RA) acts as a natural inhibitor of IL-1 receptor-mediated signalling (Yazdi and Ghoreschi, 2016; Fields et al., 2019).

Cellular senescence acts as a critical barrier against oncogenic transformation. It is characterised by irreversible cell-cycle arrest and elevated levels of pro-inflammatory cytokines, such as IL-1, IL-6, IL-8 and TNF-α. The IL-1 cytokine family comprises 11 members that play a key role in regulating inflammation, including IL-1α, IL-1β, IL-1RA, IL-18, IL-33, IL-36RA, IL-36α, IL-36β, IL-36γ, IL-37 and IL-38.

Signaling cascades initiated by IL-1α, IL-1β, IL-18, IL-33, IL-36α, IL-36β and IL-36γ converge on the activation of the MAPK and NF-κB pathways. This ultimately leads to the transcriptional upregulation of pro-inflammatory cytokines, chemokines and secondary mediators of inflammation. Furthermore, mounting evidence suggests that various IL-1 family members play a role in regulating T-helper cell differentiation and effector functions.

Several members of the interleukin-1 (IL-1) family function as endogenous antagonists of IL-1 and IL-36 signaling, thereby exerting potent anti-inflammatory effects. The IL-1 receptor antagonist protein (IL-1RA) negatively regulates IL-1 signaling by competitively binding to IL-1 receptor type 1 (IL-1R1), thus preventing its interaction with the pro-inflammatory agonists IL-1α and IL-1β. Analogously, IL-36 receptor antagonist (IL-36RA) binds to IL-1 receptor–related protein 2 (IL-1Rrp2) and inhibits IL-36-mediated signal transduction. In addition, it has been proposed that IL-37 (IL-1F7) and IL-38 (IL-1F10), either individually or as part of regulatory complexes, also exert anti-inflammatory and immunosuppressive functions (for a comprehensive review, see: *IL-1 (Interleukin-1) Family*, 2025).

IL-1RA is a key anti-inflammatory regulator within the IL-1 cytokine family. It modulates the biological activity of IL-1 cytokines, thereby limiting their pro-inflammatory potential. It is produced by a variety of cell types, particularly macrophages, monocytes and epithelial cells. It is encoded by the IL1RN gene located on chromosome 2q14.1 and is also known by several alternative names, including DIRA, ICIL-1RA, IL-1RN, IL-1ra, IL-1ra3, IL1F3, IL1RA, IRAP and MVCD4.

IL-1RA was first isolated from human leukaemia monocyte cell lines (THP-1 cells) by Bienkowski et al. in 1990. This protein shares approximately 30% amino acid sequence homology with IL-1β, and can bind to IL-1 receptors even when there is no overt cellular activation. In vitro, IL-1RA effectively blocks IL-1-mediated stimulation of thymocytes (T lymphocytes), fibroblasts, endothelial cells and osteogenic cells, while in vivo it acts as a potent inhibitor of IL-1-driven inflammatory responses.

The gene encoding the interleukin-1 receptor antagonist protein (IL-1RA) was first cloned and characterised in terms of its chromosomal localisation and expression by D. B. Carter and colleagues in 1990, a discovery that was followed shortly thereafter by that of A. C. Lennard and colleagues in 1992. A comprehensive and critical review of the biology, regulation and functional

relevance of IL-1RA was later published by A.C.Lennard (2017). Detailed genomic and functional information on the IL1RN gene is available through public databases, including the NCBI Gene repository (Gene ID: 3557; https://www.ncbi.nlm.nih.gov/gene/3557).

A wide range of studies have addressed the association between the variable number of tandem repeats (VNTR) polymorphism of the IL1RN gene and susceptibility to inflammatory and inflammation-related diseases. These studies have been reported in both review articles and original research publications, and are discussed in the relevant sections of this manuscript. Our previous studies investigated the links between the A→G single nucleotide polymorphism (SNP) (2758 A>G, rs696) of the NFKBIA (or NF-κBIA) gene and diabetes mellitus and cancer development (Akhundova et al., 2022), and between the VNTR polymorphism (rs223466) of the IL1RN gene and susceptibility to and severity of coronavirus infection (Naghiyeva et al., 2023).

The primary objective of this study is therefore to evaluate the association between the VNTR polymorphism of the IL1RN gene (rs223466) and inflammation-driven carcinogenesis.

MATERIALS AND METHODS

The material of the study: The main material of the study was DNA isolated from blood samples collected from unrelated patients of different ages, diagnosed with various forms of cancer (experimental group (EG) - 80 people (44 women, 36 men) and conditionally healthy individuals (control group (CG) - 84 people (47 women, 37 men)) working in different occupational fields. Samples were collected on a voluntary basis in accordance with the Helsinki Declaration and international bioethical norms. Prior to the collection of samples, a letter was sent to the Oncology Clinic of the Azerbaijan Medical University, Ministry of Health of the Republic of Azerbaijan, and official consent was obtained.

It should be noted that the age range of patients in the studied EG was wide, from 45 (1980) to 73 (1952). The age range of the conditionally healthy control samples (CG) was from 43 (1982) to 75 (1950). Blood samples and information about the patients and the course of the disease were provided by the hospital where the patients in the experimental group (EG) were treated. According to the study, patients with stages II (33 people), III (40 people) and IV (seven people receiving intensive chemotherapy) of cancer were involved. The study also involved conditionally healthy individuals who did not exhibit any symptoms or complaints relating to the disease under study during the study period.

DNA isolation: The DNA from collected blood samples (200 μl) was extracted with a reagent kit, "DiatomTMDNA Prep 200" (Isogen, Moscow, RF), based on the manufacturer's protocol. The amount of DNA isolated and the degree of purity were measured with a NanoDrop 2000 spectrophotometer. The isolated DNA was stored at -20°C (or for a long time at -80°C).

Detection of rs2234663 polymorphism of IL1RN gene: The IL1RN gene rs223466 polymorphism was determined by polymerase chain reaction (PCR) using pairs of specific primers: Forward: 5' CTCAGCAACACTCCTAT-3'; Reverse: 5' TCCTGGTCTGCAGGTAA-3' (Arnalich et al., 2022; Kayar et al., 2015; Saad et al., 202; Swellam et al., 2013). The PCR conditions were determined by the gradient PCR. The gradient PCR was performed at 12 temperatures in the range of 45-60ºC, divided equally, and 48ºC was taken as the best annealing temperature of the primers. PCR reaction conditions were as follows: initial denaturation at 95ºC for 5 min before the first cycle; 60 sec at 94ºC (denaturation), 60 sec at 48ºC (annealing), 60 sec at 72ºC (elongation) - 35 cycles; and final elongation - 10 min. The synthesized fragments were electrophoresed in a 1.8% agarose gel using a 100 b.p. ladder (MBI Fermentas), visualized using ethidium bromide staining and documented by UVITES Gel Documentation System (CS Ltd. UK).

As a result of PCR, the following fragments were synthesized:

IL-1RN1 (allele *1), R=4, 410 b.p. fragment;
IL-1RN2 (allele *2), R=2, 240 b.p. fragment;
IL-1RN3 (allele *3), R=3, 325 b.p. fragment;
IL-1RN4 (allele *4), R=5, 500 b.p. fragment;
IL-1RN5 (allele *5), R=6, 585 b.p. fragment;
IL-1RN6 (allele *6), R=1, 155 b.p. fragment.

Statistical analyses: The obtained results were statistically analyzed using the Microsoft Excel software. Odds ratio (OR), relative risk (RR), etc., parameters of alleles, genotypes and genetic models associated with susceptibility to infection/course of the disease were calculated using online calculators [https://www.medcalc.org/calc/; http://vassarstats.net/odds2x2.html].

RESULTS AND DISCUSSION

A total of 164 people were included in the study, and genetic profiles were obtained for 164 of them (73 men and 91 women). The following genotypes were observed by gender in the experimental and control groups (Table 1). It should be noted that no statistically significant

associations were observed between the *2 allele or the genotypes involving this allele and disease susceptibility with respect to sex or age (in both cases p>0.05).

Some of the detected genotypes are shown as an example in the Figure, and the frequencies of alleles and genotypes are given in Table 2.

In summary, the 4-repeat (410 bp, IL1RN*1 – normal allele (*1)) and 2-repeat (240 bp, IL1RN*2 – major variant allele (*2)) alleles of the gene are more common (~90%), while the frequency of other alleles is generally ~10%.

Numerous studies have investigated the association between various mutations of the IL1RN gene, which encodes the interleukin-1 receptor antagonist (IL-1RA), as well as its variable number tandem repeat (VNTR) polymorphism (rs2234663) located in intron 2 and consisting of ~86 bp repeats, and a broad spectrum of inflammatory and inflammation-related diseases.

Table 1. Distribution of genotypes detected in the study groups (EG and CG) by gender

Genotypes	Experimental group			Control group		
	Total	Women	Men	Total	Women	Men
*1*1	20	10	10	31	17	14
*1*2	17	10	7	22	13	9
*1*3	2	1	1	4	2	2
*1*4	1	0	1	2	1	1
*1*5	3	2	1	3	2	1
*1*6	1	1	0	2	1	1
*2*2	29	16	13	14	8	6
*2*3	2	2	0	2	1	1
*2*4	3	2	1	2	1	1
*5*5	2	0	2	2	1	1

Table 2. The amounts and frequencies of alleles/genotypes detected in the experimental and control groups

GROUPS	Alleles (amount (%))					
	*1	*2	*3	*4	*5	*6
Experimental group (cancer patients with various diagnoses, n=80)	64 (40.0%)	80 (50.0%)	4 (2.5%)	4 (2.5%)	7 (4.4%)	1 (0.6)
Control group (conditionally healthy individuals, n=84)	95 (56.5%)	54 (32.1%)	6 (3.6%)	4 (2.4%)	7 (4.2%)	2 (1.2%)
By population (n=164)	159 (48.5%)	134 (40.9%)	10 (3.0%)	8 (2.4%)	14 (4.3%)	3 (0.9%)

Genotypes (amount (%))									
*1*1	*1*2	*1*3	*1*4	*1*5	*1*6	*2*2	*2*3	*2*4	*5*5
Experimental group (cancer patients)									
20 (25.00)	17 (21.25)	2 (2.50)	1 (1.25)	3 (3.75)	1 (1.25)	29 (36.25)	2 (2.50)	3 (3.75)	2 (2.50)
Control group (conditionally healthy individuals)									
31 (36.91)	22 (26.19)	4 (4.76)	2 (2.38)	3 (3.57)	2 (2.38)	14 (16.67)	2 (2.38)	2 (2.38)	2 (2.38)
By population									
51 (31.10)	39 (23.78)	6 (3.66)	3 (1.83)	6 (3.66)	3 (1.83)	43 (26.22)	4 (2.44)	5 (3.04)	4 (2.44)

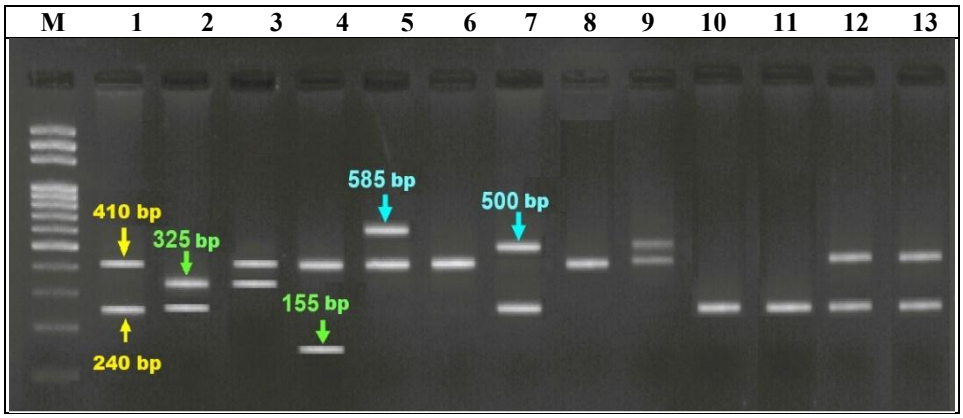


Fig. Electrophoresis of samples belonging to individuals in the experimental (samples 2, 3, 5, 7, 9, 11 and 13) and control (samples 1, 4, 6, 8, 10 and 12) groups. M – 100 bp ladder.

These include, but are not limited to, osteoarthritis, type 1 diabetes mellitus in children, type 2 diabetes mellitus, thrombocytopenia, ischemic stroke, hepatitis E virus infection, endometriosis, childhood melanoma, breast cancer, skin cancer, gastric cancer, and colorectal cancer (Attur et al., 2020; Bent et al., 2018; Broer et al., 2017; Clark et al., 2017; Dorling et al., 2022; El-Serag et al., 2006; Ibrahimi et al., 2022; Mier-Cabrera et al., 2022; Pesmatzoglou et al., 2012; Tripathy et al., 2024; Yang et al., 2018; see also references in Table 3).

In most of these studies, the 2 allele, which is a tandem repeat approximately 240 bp in length, is reported as a potential risk allele. Furthermore, several genotypes involving this allele have been associated with an increased risk of disease. However, contradictory findings have also been reported, which are most likely due to differences in allele frequencies and the strength of associations between inflammation-driven diseases and the IL1RN alleles and genotypes being investigated, which vary between populations.

The distribution and frequency of the two most prevalent IL1RN alleles (*1 and *2) across different populations is of particular interest overall. Table 3 summarises the frequencies of these alleles in selected populations, based on data derived from population-based studies conducted worldwide.

As shown in Table 3, the frequency of the *2 allele varies considerably between populations. In East and Southeast Asian populations (including most regions of China and Korea, except the Han population), African populations (e.g., Sudan) and certain Russian populations, the frequency of the *2 allele is below 10%.

Table 3. The IL1RN gene *1 and *2 alleles frequencies in different populations, %.

No	Population	Population size (n) and/or studied disease	*1 allele	*2 allele	References
1	Azerbaijan*	>460 (~240 healthy, ~220 unhealthy)	62.1	30.3	Naghiyeva et al., 2023; Mustafayev et al., 2025
2	UK (United Kingdom)	70	73.6	21.4	Tarlow et al., 1993
3	China, 19 population	1352	91.3	6.4	Jiang et al., 2010
4	China, Han	256	83.0	16.2	Xu et al., 2011
5	China, She	252	93.3	6.7	Xu et al., 2011
6	Italy	515 (382 healthy, 133 cutaneous melanoma)	73.3	24.0	Cauci et al., 2019
7	USA, 3 population	Total 782 (480 cases of ischemic stroke and 302 controls	76.5	24.9	Worral et al., 2007
8	USA (N.Jersey and North Carolina, Caucasians)	Total 896 (516 ischemic stroke and 380 control)	67.8	27.1	Peddareddygari et al., 2014
9	Türkiye	198 (94 rheumatoid arthritis, 104 control)	76.6	20.7	Arman et al., 2006
10	Türkiye	133 (33 cancer, 100 healthy)	51.5	44.7	Gümüşay et al., 2019
11	Korea	640	91.7	6.0	Um and Kim, 2003
12	Iran	515 (265 pulmonary tuberculosis and 250 healthy)	~81.0	~15.0	Hashemi et al., 2015
13	Iran	223 (126 cancer, 97healthy control)	77.4	17.0	Abbasian et al., 2018
14	Iran	275 (123 CRC, 152 control)	58.9	35.6	İbrahimi et al., 2019
15	Iran	356 (120 NHL, 50 HL, 186 control)	51.4	30.5	Sarani et al., 2021
16	India	336 (119 recurrent pregnancy loss and 200 healthy control women)	62.5	35.3	Nair et al., 2014
17	India	190 (86 kidney damage, 104 normal kidney function)	82.4	17.6	Bhaskar and Pattnaik, 2023
18	India	689 (331 male infertility, 358 healthy fertile men)	58.6	40.8	Jaiswal et al., 2012
19	Northeast Brazil	153 (39 osteomyelitis, 114 healthy)	84.3	15.00	Alves de Souza et al., 2017
20	Mexica	630 (frailty syndrome: prefrail 237, frail 72 and non-frail 319)	80.0-87.5	12.5-20.0	Pérez-Suáre et al., 2016
21	Mexica	486 (230 with CRC and 256 healthy)	54.5	41.8	Gallegos-Arreola et al., 2024
22	Poland	90 (from among 402 premature infants)	68.9	29.4	Szpecht et al., 2020
23	Poland	795 (366 gastric cancer cases and 429 controls)	57.0	33.6	El-Omar et al., 2000
24	Portugal	58	48.3	10.3	Sampaio-Fernandesa et al., 2015
25	Portugal	545 (112 patients with the NPS and 433 healthy)	66.2	31.7	Sousa et al., 2013
26	Egypt	200 (120 T1DM, 80 control)	71.0	23.0	Abed et al., 2022
27	Egypt	140 (80 ASD, 60 control)	70.4	29.3	Saad et al., 2020
28	Egypt	185 RA pacients	65.8	34.2	Swellam et al., 2013
29	Russia	196	72.2	9.9	Udina et al., 2022
30	Africa, Sudan	114 *54 cancer, 60 healthy)	89.3	9.4	Abeer et al., 2019
31	Sweden	259 (125 CC, 134 control)	68.5	28.8	Viet et al., 2005

Note: *In many cases, allele frequencies were calculated by us based on data provided in references.*

By contrast, most populations in South Asia (India), Europe, the Caucasus and the Middle East, including the Azerbaijani population, have allele frequencies ranging from approximately 10% to 35%. Interestingly, in populations from Turkey, India and Mexico, the frequency of the *2 allele exceeds 40%.

The Azerbaijani population exhibits an intermediate distribution comparable to that of neighbouring regions. Interestingly, a high frequency of the 2 allele is often seen in groups of people with diseases, which further supports its potential role as a genetic risk factor.

The VNTR polymorphism (rs2234663) under investigation has also been examined in the context of SARS-CoV-2 infection and disease progression (Naghiyeva et al., 2023). In that study, a non-specific allele of approximately 1,100 bp was reported in two cases, but this was not detected in our experimental cohort. Conversely, the smallest allele described in the previous study, at around 155 bp, was not present in their analysis, but was detected in our study, in both the experimental group (one case) and the control group (two cases). This discrepancy is likely due to the larger sample size used in the present study.

Analysis of the literature indicates that the combined frequencies of the normal (*1) and more common mutant (*2) alleles of the IL1RN gene account for approximately 90–93% of all alleles. The remaining rare (*3, *4, *5 and *6) alleles collectively comprise ~5–7%. In both the experimental (EG) and control (CG) groups, as well as in the overall population sample, there was no significant difference in the cumulative frequencies of alleles 1 and 2, which represent the predominant fraction of total alleles (~90% in EG, ~89% in CG and a similar proportion in the population sample).

In our study, a slight decrease in the frequencies of the major alleles (*1 and *2) and a modest increase in the minor alleles (*3, *4, *5, and *6) (~3–4% each) were observed. Due to the low prevalence of the minor alleles and the difficulty of establishing statistically significant correlations or associations, subsequent analyses and interpretations focused primarily on alleles *1 and *2 and the genotypes they form. Notably, the frequency of the *1 allele was approximately 1.4-fold lower and the frequency of the *2 allele was approximately 1.6-fold higher in the experimental group than in the control group.

The distribution of genotypes observed in the study groups exhibited some notable differences. Certain genotypes, including *2*5, *2*6, *3*4, *3*5, *3*6, *4*5, *4*6, and *5*6 were not detected in either the experimental (EG) or control (CG) groups. Interestingly, the genotypes *1*6 and *2*3 were observed exclusively in the EG, and carriers of these genotypes represented the youngest cancer patients in terms of age. Furthermore, in the total cohort (EG), the *1*4 and *5*5 genotypes were absent among female patients, whereas the *1*6 and *2*3 genotypes were not observed among male patients.

There were pronounced differences in the frequencies of the homozygous *2*2 genotype between groups. Overall, the prevalence of this genotype, which is associated with an increased risk, was higher than in our previous study (Naghiyeva et al., 2023): ~26.22% vs. 11.2%. Notably, the frequency of the *2*2 genotype in the experimental group (EG) was approximately 2.1-fold higher (36.25%) than in the control group (CG) (16.67%).

The frequencies of the homozygous *1*1 and heterozygous *1*2 genotypes were as follows: *1*1 25.00% in EG, 36.91% in CG, and 21.25% in the overall studied population; *1*2: 21.25% in EG, 26.19% in CG, and 23.78% in the overall population. Accordingly, the frequency of the *1*1 genotype in EG was approximately 1.5-fold lower, while that of the *1*2 genotype was approximately 1.2-fold lower compared to CG.

To evaluate the association between cancer susceptibility and the presence of the mutant *2 allele of the IL1RN gene, which encodes the natural antagonist of the pro-inflammatory cytokine IL-1 receptor, statistical parameters including odds ratios (ORs) and relative risks (RRs) with 95% confidence intervals (CIs), Z-test statistics reflecting the distribution coefficient, and significance levels (P) were calculated for the study groups and the overall population using online calculators (https://www.medcalc.org/calc/; http://vassarstats.net/odds2x2.html).

Calculations were performed relative to the control group, and only genotypes carrying the mutant *2 allele were included in the analysis. The results of the statistical evaluation of the risk allele (*2) and the genotypes formed with its participation (*2*1 and *2*2) in the studied groups are summarized in Table 4.

As shown in Table 4, the odds ratio (OR = 2.1991) and relative risk (RR = 1.5329) for the association between the *2 risk allele and cancer are both greater than one and statistically significant ($P < 0.001$), indicating that this allele meaningfully contributes to cancer susceptibility. A different pattern was observed for genotypes containing the *2 allele. Although the odds ratio (OR=1.1977) and relative risk (RR=1.1069) for the heterozygous *1*2 genotype were slightly greater than unity, no statistically significant association with cancer was detected (P=0.419).

Table 4. Statistical data of the risk allele (*2) and genotypes (*1*2 and *2*2) detected in the studied groups.

Allee/Genotype	Odds ratio (OR, CI=95%)	Relative risk (RR)	P-value	Z-statistics
Allelles				
*1	-	-	-	-
*2	2.1991 (1.3763-3.5137)	1.5329 (1.1840-1.9846)	0.001	1.96
Genotypes				
*1*1	-	-	-	-
*1*2	1.1977 (0.5137-2.7925)	1.1069 (0.6893-1.7774)	0.419	0.418
*2*2	2.8431 (1.3662-5.9166)	2.1750 (1.2429-3.8061)	0.0036	2.795

Table 5. Results of statistical analysis of genetic models in the studied group.

Genetic models	Odds ratio (OR, CI=95%)	Relative risk (RR)	P-value	Z-statistics
Dominant *2*2 vs (*1*1+*1*2)	2.97 (1.38-6.37)	2.10 (1.23-3.61)	0.003	2.70
Recessive (*2*2+*1*2) vs *1*1	1.98 (0.97-4.03)	1.30 (0.99-1.70)	0.062	1.87
Overdominant (*1*1+*2*2) vs *1*2	1.41 (0.66-2.99)	1.11 (0.89-1.38)	0.240	0.894

This suggests that the normal *1 allele may offset the harmful effects of the *2 allele, thereby reducing the risk of inflammation-associated cancer. In contrast, the homozygous *2*2 genotype demonstrated a pronounced and statistically significant association with cancer. In contrast, the homozygous *2*2 genotype demonstrated a pronounced and statistically significant association with cancer. The corresponding values (OR=2.8431, RR=2.1750, P=0.0036) indicate a strong correlation between this genotype and disease susceptibility. Specifically, compared with heterozygous carriers (*1*2), individuals homozygous for the *2 allele (*2*2) exhibited approximately a 2.4-fold increase in odds (OR_{*2*2}:OR_{*1*2}) and about a 2.0-fold increase in relative risk (RR_{*2*2}:RR_{*1*2}) of developing cancer associated with chronic inflammation.

In this context, it is particularly interesting to analyse the associations between the possible genetic models formed by the *1 and *2 alleles and cancer that is presumed to develop as a result of chronic inflammation. This approach also allows us to indirectly assess the potential functional capacity of the immune system, depending on the presence of the mutant risk allele, which can lead to acute and severe chronic inflammatory responses. As shown in Table 5, a statistically significant association with cancer was detected only for the dominant genetic model (P=0.003). This finding is fully consistent with the results obtained for the individual analysis of the *2 risk allele and the homozygous *2*2 genotype, both of which demonstrated a significant association with cancer presumably induced by chronic (acute and severe) inflammation (Table 4).

Let us consider the genotype ratios corresponding to the dominant, recessive, and overdominant (codominant) genetic models formed by the presence of the *2 risk allele, analyzed separately within each study group (experimental group, EG; control group, CG) as well as in the total population sample. Under the dominant model of the *2 allele, the genotype ratios were as follows: in the experimental group, *2*2/(*1*1+*1*2)=0.78; in the control group, *2*2/(*1*1+*1*2)=0.26; and in the overall population sample, *2*2/(*1*1+*1*2)=0.48. These values suggest that the association between the dominance of the *2 risk allele and cancer (inflammation-related pathology) is approximately 3.0 times higher in the experimental group than in the control group, and around 1.6 times higher in the entire population.

Assuming a recessive effect of the *2 allele in the genetic model, the genotype ratios were as follows: in the experimental group, (*2*2+*1*2)/*1*1=2.30; in the control group, (*2*2+*1*2)/*1*1=1.17; and in the overall population sample, (*2*2+*1*2)/*1*1=1.61. Thus, although the presumed association between the *2 allele and inflammation-related cancer under the recessive model is less pronounced than that observed in the dominant model, a measurable disease risk remains. Specifically, this ratio is approximately 2.0-fold higher in the experimental group than in the control group and about 1.4-fold higher than in the population sample as a whole.

According to the overdominant (codominant) genetic model, the ratio of homozygous genotypes (*2/*2+*1/*1) to the heterozygous genotype (*1/*2) was 2.88 in the experimental group, 2.05 in the control group, and 2.41 in the overall population. Within this model, the ratio reflecting the presumed association between the presence of the *2 allele and cancer (inflammation-related

pathology) was modestly elevated in the experimental group. In quantitative terms, this indicator was approximately 1.4-fold higher in the experimental group compared with the control group and about 1.2-fold higher than that observed in the population sample overall.

A comparative analysis of genotype ratios across all three genetic models demonstrates that, despite the relatively lower numerical values observed in the dominant model involving only the homozygous *2*2 genotype, the effect of this allele/genotype on disease susceptibility and progression is substantial. This observation is fully consistent with the results obtained for the corresponding alleles and genotypes in Table 4, thereby reinforcing the evidence for the significant contribution of the *2 allele to cancer risk, presumably mediated by chronic inflammation.

CONCLUSION

In order to elucidate the potential genetic associations involved in the development of cancer, the VNTR-type polymorphism (rs2234663) of the IL1RN gene was investigated. This gene encodes the interleukin-1 receptor antagonist (IL-1RA), which is a key regulator of the pro-inflammatory cytokine interleukin-1. The study population comprised a representative sample of 164 individuals from the Azerbaijani population. The study population comprised an experimental group (EG) of patients diagnosed with various forms of cancer and a control group (CG) of individuals in good health. The frequencies of all known alleles and genotypes of the IL1RN gene were determined within the framework of this study.

According to the results obtained, the frequency of the common *1 allele, reported as the most prevalent in many populations worldwide, was 40.0% in the EG and 56.5% in the CG. Conversely, the frequency of the *2 allele, considered a potential risk allele, was notably higher among cancer patients (50.0%) than in the control group (32.0%).

A comparative analysis of the observed allele frequencies with published data from 30 populations across different geographical regions revealed that the frequency of the *2 allele in the Azerbaijani population is relatively high, comparable to that reported for Caucasian, Middle Eastern and certain American populations. Notably, numerous studies have documented an increased prevalence of the *2 allele in cohorts affected by disease, further supporting its proposed role as a genetic risk factor in the development of cancer and other inflammation-associated pathologies.

Association analyses of the IL1RN*2 risk allele, genotypes containing this allele (i.e., *1*2 and *2*2), and the corresponding genetic models demonstrated that the *2 allele is significantly associated with cancer that is presumed to develop as a result of chronic inflammation. Notably, the odds ratio (OR ≈ 2.2) and relative risk (RR ≈ 1.53) for the association between the *2 allele and cancer were greater than one and statistically significant (P ≈ 0.001), suggesting a substantial role for this allele in disease susceptibility.

Stronger association parameters were observed for the homozygous *2*2 genotype (OR ≈ 2.84, RR ≈ 2.18, P ≈ 0.004), suggesting the presence of an allele-dose effect. Further comparative analysis showed that individuals homozygous for the *2 allele had approximately 2.4 times the odds and 2.0 times the relative risk of developing cancer associated with chronic inflammation compared to heterozygous carriers (*1*2).

Of the evaluated genetic models, only the dominant model (*2*2 vs. *1*1 + *1*2) showed a statistically significant association with cancer risk (OR ≈ 2.97; RR ≈ 2.10; P = 0.003), which is consistent with the allele- and genotype-based analyses.

Taken together, the results obtained indicate a potential association between the IL1RN gene *2 allele and genetic susceptibility to cancer and other inflammation-related diseases. However, before considering this allele as a reliable clinical prognostic marker, further large-scale, long-term studies across diverse populations are required. The relatively high frequency of the *2 allele in the population (>30%) underscores the importance of evaluating its role in predisposing individuals to inflammatory diseases. Nevertheless, therapeutic interventions targeting the IL-1 signalling pathway, such as the use of the recombinant interleukin-1 receptor antagonist anakinra (Kineret®), should only be considered within established clinical indications and under appropriate medical supervision.

ACKNOWLEDGMENTS

We are thankful to all the patients and volunteers for providing blood samples. We are also grateful to the staff of the Oncology Clinic of Azerbaijan Medical University for their assistance in collecting samples and providing information about patients.

FUNDING

This study did not receive any specific grant from the state, commercial or non-commercial organizations.

AUTHOR CONTRIBUTION STATEMENT

Nurmammad Mustafayev: scientific idea, conceptualization, study design, experimental procedures, discussion, and writing of the first draft.

Lala Akhundova, Shalala Majidova, and Nigar Mammadli: sample collection, DNA isolation, genotyping, statistical analysis, and other experimental procedures.

Ahliman Amiraslanov and Irada Huseynova: discussion, reviewing, and editing of the manuscript.

Ahliman Amiraslanov: expert recommendations and comments related to cancer disease.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest related to the publication of this manuscript.

ETHICAL ISSUES

The research followed the ethical principles of the Declaration of Helsinki. This study was approved by the ethics committees of the Institute of Molecular Biology and Biotecnologies, and the Oncology Clinic of the Azerbaijan Medical University, Ministry of Health of the Republic of Azerbaijan. Informed written consent was obtained from all the participants (patients and volunteers) included in the study. Besides, ethical issues including plagiarism, data fabrication and double publication were completely avoided by the authors.

REFERENCES

Abbasian M.H., Abbasi B., Ansarinejad N. et al. (2018) Association of interleukin-1 gene polymorphism with risk of gastric and colorectal cancers in an Iranian population. *Iran. J. Immunol*., **15(4):** 321-328; doi: 10.22034/IJI.2018.39401.

Abed N.T., Ramadan I.A., Mohammed S.A., El-Shanawany E.M. (2022) Genetic polymorphism of interleukin-1 receptor antagonist in Type 1 diabetic children. *Pediatr. Res*., **91(6):** 1536-1541; doi: 10.1038/s41390-021-01569-5.

Abeer B.I., Amany E.A., Sulafa M.E. et al. (2019) Independently carriage of IL-1RN*2 allele associated with increased risk of gastric cancer in the Sudanese population. *medRxiv,* 27 p.**;** doi: https://doi.org/10.1101/19013573

Akhundova L.A., Hamidova E.T., Mustafayev N.Sh., Amiraslanov A.T., Huseynova I.M. (2022) The initial study of NF-κBIA gene polymorphism in a small group of patients with cancer and diabetes mellitus. *Transactions of the Institute of Molecular Biology & Biotechnologies,* **6(1):** 03-11; doi: 10.30546/2709-0752.6.1.2022.3

Albini A., Paola L.Di, Mei G. et al. (2025) Inflammation and cancer cell survival: TRAF2 as a key player. *Cell Death Dis*., **16(1), article ID 292:** 1-13; doi: 10.1038/s41419-025-07609-w.

Alves De Souza C., Queiroz Alves De Souza A., Queiroz Alves De Souza M.D.S., Dias Leite J.A., Silva De Morais M., Barem Rabenhorst S.H. (2017) A link between osteomyelitis and IL1RN and IL1B polymorphisms-a study in patients from Northeast Brazil. *Acta Orthop*., **88(5):** 556-561; doi: 10.1080/17453674.2017.1348439.

Arman A., Yilmaz B., Coker A., Inanc N., Direskeneli H. (2006) Interleukin-1 receptor antagonist (IL-1RN) and interleukin-1B gene polymorphisms in Turkish patients with rheumatoid arthritis. *Clin. Exp. Rheumatol*., **24(6):** 643-648; PMID: 17207379.

Arnalich F., López-Maderuelo D., Codoceo R., Lopez J., Solis-Garrido L.M., Capiscol C., Fernandez-Capitán C., Madero R., Montiel C. (2002) Interleukin-1 receptor antagonist gene polymorphism and mortality in patients with severe sepsis. Clin. Exp. Immunol., **127(2):** 331 336; doi: 10.1046/j.1365-2249.2002.01743.x.

Attur M., Zhou H., Samuels J., Krasnokutsky S. et al. (2020) Interleukin 1 receptor antagonist (IL1RN) gene variants predict radiographic severity of knee osteoarthritis and risk of incident disease. *Ann. Rheum. Dis.*, **79(3):** 400-407; doi: 10.1136/annrheumdis-2019-216055.

Bent R., Moll L., Grabbe S., Bros M. (2018) Interleukin-1 beta - A friend or foe in malignancies?. *Int. J. Mol. Sci*., **19(8), Article ID 2155:** 1-34; doi: 10.3390/ijms19082155.

Bhaskar L.V.K.S., Pattnaik S. (2023) IL1RN VNTR Polymorphism and kidney damage in sickle cell anemia patients. *J. Nephropharmacol*., **12(1):** e10437; doi: 10.34172/npj.2022.10437.

Bienkowski M.J., Eessalu T.E., Berger A.E., Truesdell S.E. et al. (1990) Purification and characterization of interleukin 1 receptor level antagonist proteins from THP-1 cells. *J. Biol. Chem*., **265(24):** 14505-14511; PMID: 2143761.

Broer P.N., Aung T., Heidekrueger P.I., Prantl L., Narayan D. (2017) Divisive influence of interleukin-1 receptor antagonist polymorphisms in melanoma patients. *Clin. Hemorheol. Microcirc*., **67(3-4):** 319-326; doi: 10.3233/CH-179212.

Brovkina O.I., Pronina I.V., Burdennyy A.M. et al. (2022) The role of long non-coding RNA CCAT1 and SNHG14 in activation of some protein-coding genes associated with the development of ovarian cancer. *Bull. Exp. Biol. Med.*, **172(6):** 760-764; doi: 10.1007/s10517-022-05473-8.

Broz P., Dixit V.M. (2016) Inflammasomes: mechanism of assembly, regulation and signaling. *Nat. Rev. Immun.*, **16 (7):** 407-420; doi:10.1038/nri.2016.58.

Carter D.B., Deibel M.R.Jr., Dunn C.J. et al. (1990) Purification, cloning, expression and biological characterization of an interleukin-1 receptor antagonist protein. *Nature*, **344(6267):** 633-638; doi: 10.1038/344633a0.

Cauci S., Buligan C., Rocchi F., Salvador I., Xodo L., Stinco G. (2019) Interleukin 1 receptor antagonist gene variable number of tandem repeats polymorphism and cutaneous melanoma. *Oncol. Lett*., **18(6):** 5759-5768; doi: 10.3892/ol.2019.10923.

Clark M., Kroger C.J., Tisch R.M. (2017) Type 1 diabetes: A chronic anti-self-inflammatory response. *Front. Immunol*., **8, Article ID 1898:** 1-10; doi: 10.3389/fimmu.2017.01898.

Dinarello C.A. (2023) Interleukin-1 in inflammation and cancer: The expanding role of IL-1 family cytokines. *Nature Reviews Immunology*, **23(2):** 87-101; doi: 10.1038/s41577-022-00779-4.

Dorling L., Carvalho S., Allen J. et al., NBCS Collaborators: Collée J.M., Czene K., Dennis J. et al.; kConFab Investigators; SGBCC Investigators: Jakubowska A., Jung A., Khusnutdinova E. et al. (2022) Breast cancer risks associated with missense variants in breast cancer susceptibility genes. *Genome Med.*, **14(1):** 51; doi: 10.1186/s13073-022-01052-8.

El-Omar E.M., Carrington M., Chow W.H. et al. (2000) Interleukin-1 polymorphisms associated with increased risk of gastric cancer. *Nature*, **404:** 398-402; doi: 10.1038/35006081, Erratum: Nature 2001, v. 412, p 99.

El-Serag H.B., Hampel H., Javadi F. (2006) The association between diabetes and hepatocellular carcinoma: a systematic review of epidemiologic evidence. *Clinical Gastroenterology and Hepatology*, **4:** 369–380; doi: 10.1016/j.cgh.2005.12.007.

Fields J.K., Günther S., Sundberg E.J. (2019) Structural basis of IL-1 family cytokine signaling. *Front. Immunol*., **10:** 1412; doi: 10.3389/fimmu.2019.01412.

Free statistical calculators (2025) MedCalc Software Ltd., 2025; URL: https://www.medcalc.org/calc/

Furukawa T., Kuboki Y., Tanji E. et al. (2011) Whole-exome sequencing uncovers frequent GNAS mutations in intraductal papillary mucinous neoplasms of the pancreas. *Sci. Rep*., **1, Article ID 161:** 1-7; doi: 10.1038/srep00161.

Gallegos-Arreola M.P., Garibaldi-Ríos A.F., Gutiérrez-Hurtado I.A. (2024) Association of variants in IL-1RN (rs2234663) and IL-1β (rs1143627, rs16944) and interleukin-1β levels with colorectal cancer: experimental study and in silico analysis. *Genes (Basel)*, **15(12), Article ID 1528:** 1-17; doi: 10.3390/genes15121528.

Garlanda C., Di Ceglie I., Jaillon S. (2025) IL-1 family cytokines in inflammation and immunity. *Cell Mol. Immunol.*, **22(11):** 1345-1362; doi: 10.1038/s41423-025-01358-8.

Gene IL1RN interleukin 1 receptor antagonist [*Homo sapiens* (human)] [Electronic resource] (2025) NCBI National Library of Medicine, USA, April 7, 2025. URL: https://www.ncbi.nlm.nih.gov/gene/3557

Global Cancer Statistics 2024 (2024) American Cancer Society, April 4, 2024; URL: https://pressroom.cancer.org/GlobalCancerStatistics2024.

Gong T., Liu L., Jiang W., Zhou R. (2020) DAMP-sensing receptors in sterile inflammation and inflammatory diseases. *Nat. Rev. Immunol*., **20(2):** 95-112; doi: 10.1038/s41577-019-0215-7.

Greten F.R., Grivennikov S.I. (2019) Inflammation and cancer: Triggers, mechanisms, and consequences. *Immunity*, **51(1):** 27-41; doi: 10.1016/j.immuni.2019.06.025.

Gümüşay Ö., Nursal A.F., Yiğit S., Tekcan A., Öz T. (2019) Impact of the functional VNTR variants of the interleukin-1 receptor antagonist and interleukin-4 genes on oral squamous cell carcinoma. *Istanbul Med. J*., **20(3):** 202-207; doi: 10.4274/imj.galenos.2018.82195

Hashemi M., Naderi M., Ebrahimi M. et al. (2015) Association between Interleukin-1 receptor antagonist (IL1RN) variable number of tandem repeats (VNTR) polymorphism and pulmonary tuberculosis. *Iran. J. Allergy, Asthma Immunol*., **14(1):** 55-99; PMID: 25530139.

Ibrahimi M., Moossavi M., Mojarad E.N. (2019) Positive correlation between interleukin-1 receptor antagonist gene 86bp VNTR polymorphism and colorectal cancer susceptibility: a case-control study. *Immunol Res*., **67(1):** 151-156; doi: 10.1007/s12026-018-9034-3.

Ibrahimi R., Ibrahimi M., Jamalzei B., Akbari M.E. et al. (2022) Association between interleukin-1 receptor antagonist (IL-1RA) VNTR, gene polymorphism and breast cancer susceptibility in the Iranian population: Experimental and web-based analysis. *Int. J.*

Immunogenet., **49(4):** 254-259; doi: 10.1111/iji.12584.

IL-1 (Interlekine 1) Family (2025) SinoBiological Inc., URL: https://www.sinobiological.com/research/cytokines/il1-family

Inflammasomes (2022) InvioveGen infocus: Practical guide, 20 p. (https://www.invivogen.com/)

Jaiswal D., Trivedi S., Singh R. et al. (2012) Association of the IL1RN gene VNTR polymorphism with human male infertility. *PLoS One*, **7(12), Article ID e51899:** 1-5; doi: 10.1371/journal.pone.0051899.

Jiang J., Zhang X., Sun D., Jin Y., Bai J., Chen F., Fu S. (2010) Study on VNTR polymorphism of gene IL-1RA in 19 Chinese populations. *Int. J. Immunogenet*., **37(2):** 73-77; doi: 10.1111/j.1744-313X.2009.00891.x

Jin T., Yin Q. (Eds.) (2019) Structural Immunology. *Advances in Experimental Medicine and Biology*, **1172:** 443 p.; doi:10.1007/978-981-13-9367-9.

Kayar N.A., Alptekin N.Ö., Erdal M.E. (2015) Interleukin-1 receptor antagonist gene polymorphism, adverse pregnancy outcome and periodontitis in Turkish women. *Arch. Oral. Biol*., **60(12):** 1777-1783; 10.1016/j.archoralbio.2015.09.013.

Khazim K., Azulay E.E., Kristal B., Cohen I. (2018) Interleukin 1 gene polymorphism and susceptibility to disease. *Immunol. Rev.*, **281(1):** 40-56; doi: 10.1111/imr.12620. PMID: 29247999.

Lee C.L., Riya I.J., Piya I.J. et al. (2025) Immune checkpoint inhibitor-induced pancreatic injury (ICI-PI) in adult cancer patients: A systematic review and meta-analysis. *Cancers (Basel)*, **17(7), Article ID 1080:** 1-17; doi: 10.3390/cancers17071080.

Lennard A., Gorman P., Carrier M. et al. (1992) Cloning and chromosome mapping of the human interleukin-1 receptor antagonist gene. *Cytokine*, **4:** 83-89; doi: 10.1007/BF01991137.

Lennard A.C. (2017) Interleukin-1 receptor antagonist. *Crit. Rev. Immunol*., **37(2-6):** 531-559; doi: 10.1615/CritRevImmunol.v37.i2-6.160.

Liu Z., Ma Y., Yang J., Li H. (2022) IL1RN VNTR polymorphism and cancer susceptibility: A meta-analysis of 20 case-control studies. *Journal of Cancer Research and Clinical Oncology*, **148(1):** 11-22; doi: 10.1007/s00432-021-03761-5.

Mier-Cabrera J., Cruz-Orozco O., de la Jara-Díaz J. et al. (2022) Polymorphisms of TNF-alpha (- 308), IL-1beta (+ 3954) and IL1-Ra (VNTR) are associated to severe stage of endometriosis in Mexican women: a case control study. *BMC Women's Health*, **22(1), Article ID 356:** 1-10; doi: 10.1186/s12905-022-01941-5.

Naghiyeva B., Akhundova L., Majidova Sh., Mustafayev N., Huseynova I. (2023) Study of VNTR type polymorphism (rs2234663) of the IL1RN gene encoding interleukin-1 receptor antagonist (IL1RA) in patients infected with coronavirus. *Transactions of the Institute of Molecular Biology & Biotechnologies,* **7(1):** 09-18; doi: 10.5281/zenodo.8079714.

Nair R.R., Khanna A., Singh K. (2014) Association of interleukin 1 receptor antagonist (IL1RN) gene polymorphism with recurrent pregnancy loss risk in the North Indian population and a meta-analysis. *Mol. Biol. Rep*., **41(9):** 5719-5727; doi: 10.1007/s11033-014-3443-8.

Nedumpun T., Wongyanin P., Sirisereewan C. et al. (2017) Interleukin-1 receptor antagonist: an early immunomodulatory cytokine induced by porcine reproductive and respiratory syndrome virus. *J. Gen. Virol*., **98(1):** 77-88; doi: 10.1099/jgv.0.000665.

Okada F., Izutsu R., Goto K., Osaki M. (2021) Inflammation-related carcinogenesis: Lessons from animal models to clinical aspects. *Cancers (Basel)*, **13(4), Article ID 921:** 1-38; doi: 10.3390/cancers13040921.

Pahwa R., Goyal A., Jialal I. (2022) Chronic inflammation. In: StatPearls, 2022 [Internet], Treasure Island (FL): StatPearls Publishing, 2023, PMID: 29630225; Bookshelf ID: NBK493173.

Peddareddygari L.R., Sen S,. Pahwa A., Levenstien M.A., Grewal R.P. (2014) Analysis of the interleukin-1 receptor antagonist gene variable number tandem repeats in ischemic stroke. *J. Stroke Cerebrovasc. Dis*., **23(6):** 1599-1603; doi: 10.1016/j.jstrokecerebrovasdis.2013.12.045.

Pérez-Suárez T.G., Gutiérrez-Robledo L.M., Ávila-Funes J.A., Acosta J.L., Escamilla-Tilch M., Padilla-Gutiérrez J.R., Torres-Carrillo N., Torres-Castro S., López-Ortega M., Muñoz-Valle J.F., Torres-Carrillo N.M. (2016) VNTR polymorphisms of the IL-4 and IL-1RN genes and their relationship with frailty syndrome in Mexican community-dwelling elderly. *Aging Clin. Exp. Res*., **28(5):** 823-832; doi: 10.1007/s40520-015-0503-4.

Pesmatzoglou M., Lourou M., Goulielmos G.N., Stiakaki E. (2012) DNA methyltransferase 3B gene promoter and interleukin-1 receptor antagonist polymorphisms in childhood immune thrombocytopenia. *Clin. Dev. Immunol*., **2012, Article ID 352059:** 1-6; doi: 10.1155/2012/352059.

Saad K., Abdallah A.M., Abdel-Rahman A.A. et al. (2020) Polymorphism of interleukin-1β and interleukin-1 receptor antagonist genes in children

with autism spectrum disorders. *Prog. Neuropsychopharmacol. Biol. Psychiatry*, **103, Article ID 109999:** 1-7; doi: 10.1016/j.pnpbp.2020.109999.

Saad K., Abdallah A.M., Abdel-Rahman A.A. et al. (2020) Polymorphism of interleukin-1β and interleukin-1 receptor antagonist genes in children with autism spectrum disorders. *Prog. Neuropsychopharmacol. Biol. Psychiatry*, **103, Article ID 109999:** 1-7; doi: 10.1016/j.pnpbp.2020.109999.

Sampaio-Fernandesa M., Vaza P.C., Bragab A.C., Figueirala M.H. IL1RN gene polymorphism in a Portuguese population with implant-supported overdentures – An observational study. *Revista Portuguesa de Estomatologia, Medicina Dentária e Cirurgia Maxilofacial*, **56(4):** 207-214.

Sarani H., Molashahi B., Taheri M. et al. (2021) Association between the interleukin-1 receptor antagonist (IL1RN) variable number of tandem repeats (VNTR) polymorphism and lymphoma. *Int. J. Hematol. Oncol. Stem Cell Res.*, **15(2):** 90-95; doi: 10.18502/ijhoscr.v15i2.6039.

Seok J.K., Kang H.C., Cho Y.Y. et al. (2021) Therapeutic regulation of the NLRP3 inflammasome in chronic inflammatory diseases. *Arch. Pharm. Res*., **4(1):** 16-35; doi: 10.1007/s12272-021-01307-9.

Siegel R.L., Kratzer T.B., Giaquinto A.N. et.al. (2025) Cancer statistics, 2025. *CA Cancer J. Clin*., **75(1):** 10-45; doi: 10.3322/caac.21871.

Sousa H., Breda E., Santos A.M. et al. (2013) IL-1RN VNTR polymorphism as a susceptibility marker for nasopharyngeal carcinoma in Portugal. *Arch. Oral Biol*., **58(8):** 1040-1046; doi: 10.1016/j.archoralbio.2013.02.004.

Swellam M., Gabal K.M., Youssef S.S. (2013) Interleukin-1 receptor antagonist gene polymorphism and hepcidin in rheumatoid arthritis: Correlations with clinical and laboratory indices of disease activity. *IUBMB Life*, **65(10):** 883-888; doi: 10.1002/iub.1205.

Szpecht D., Chmielarz-CzarnociŃska A., Gadzinowski J., Seremak-Mrozikiewicz A., KurzawiŃska G., Szymankiewicz M., Drews K., Gotz-WiĘckowska A. (2020) Inflammation-associated gene polymorphisms and clinical variables in the incidence and progression of retinopathy of prematurity. *Cent. Eur. J. Immunol*., **45(3):** 283-293; doi: 10.5114/ceji.2020.94789.

Tarlow J.K., Blakemore A.I.F., Lennard A. et al. (1993) Polymorphism in human IL-1 receptor antagonist gene intron 2 is caused by variable numbers of an 86-bp tandem repeat. *Hum. Genet*., **91:** 403-404; doi: 10.1007/BF00217368.

Tokarz-Deptuła B., Baraniecki Ł., Palma J., Stosik M., Deptuła W. (2024) Characterization of platelet receptors and their involvement in immune activation of these cells. *Int. J. Mol. Sci.*, **25(23):** 12611; doi: 10.3390/ijms252312611.

Tripathy A.S., Wagh P., Shahapure G. et al. (2024) Association of IL1RN VNTR and NKG2A polymorphisms with hepatitis E infection, a case study from western India. *Arch. Virol*., **169(12), Article ID 250**; doi: 10.1007/s00705-024-06179-0.

Udina I., Vasiliev Y., Volobuyev V., Gulenko O., Gracheva A. (2022) Molecular genetic study on VNTR-polymorphism of two cytokine genes antagonist of the receptor of interleukin 1 (rs2234663) and interleukin 4 (rs8179190) associated with dental caries in children. *OBM Genetics,* **6(2):** 158; doi:10.21926/obm.genet.2202158.

Um J.Y., Kim H.M. (2003) Frequencies of interleukin 1 gene polymorphisms in Koreans. *Clin. Chem*., **49(12):** 2101-2102; doi: 10.1373/clinchem.2003.022855

VassarStats: Website for Statistical computation (2025). Richard Lowry, USA, 2001-2023: URL: http://vassarstats.net/odds2x2.html.

Viet H.T., Wågsäter D., Hugander A., Dimberg J. (2005) Interleukin-1 receptor antagonist gene polymorphism in human colorectal cancer. *Oncol. Rep.*, **14(4):** 915-918; PMID: 16142351.

Weber A., Wasiliew P., Kracht M. (2010) Interleukin-1 (IL-1) pathway. *Sci. Signal*., **3(105), Article ID cm2:** 1-2; doi: 10.1126/scisignal.3105cm1.

WHO World Health Statistics 2024 (2024): Monitoring health for the SDGs, Sustainable Development Goals. WHO: Electronic version, May 24, 2024, 85 p.

Worldwide Cancer Data (2024) World Cancer Research Fund, Great Britain, London, May, 2024. URL: https://www.wcrf.org/preventing-cancer/cancer-statistics/worldwide-cancer-data/

Worrall B.B., Brott T.G., Brown R.D.Jr., Brown W.M., Rich S.S., Arepalli S., Wavrant-De Vrièze F., Duckworth J., Singleton A.B., Hardy J., Meschia J.F.; SWISS, ISGS, and MSGD Investigators. (2007) IL1RN VNTR polymorphism in ischemic stroke: analysis in 3 populations. *Stroke*, **38(4):** 1189-1196; doi: 10.1161/01.STR.0000260099.42744.b0.

Wu B., Liu J., Shao C. et al. (2025) Integrating inflammation, nutrition, and immunity: the CALLY index as a prognostic tool in digestive system cancers - a systematic review and meta-analysis. *BMC Cancer*, **25(1), Article ID 672:** 1-12; doi: 10.1186/s12885-025-14074-3.

Xu D.P., Ruan Y.Y., Pan Y.Q., Lin A., Li M., Yan W.H. (2011) VNTR polymorphism of

human IL1RN in Chinese Han and She ethnic populations. *Int. J. Immunogenet*., **38(1):** 13-16; doi: 10.1111/j.1744-313X.2010.00975.x.

Xu X., Xu J., Gao H. et al. (2025) From obesity to inflammation: emerging frontiers in prostate cancer and metabolic syndrome studies. *Transl. Androl. Urol*., **14(3):** 553-566; doi: 10.21037/tau-2024-671.

Yang Y., Wu W., Wang L., Ding Y. (2018) Lack of association between interleukin-1 receptor antagonist gene 86-bp VNTR polymorphism and ischemic stroke: A meta-analysis. *Medicine (Baltimore)*, **97(31), Article ID e11750:** 1-8; doi: 10.1097/MD.0000000000011750.

Yazdi A.S., Ghoreschi K. (2016) The Interleukin-1 family. *Adv. Exp. Med. Biol.*, **941:** 21-29; doi: 10.1007/978-94-024-0921-5_2.

Zhao C., Zhao W. (2020) NLRP3 Inflammasome - a key player in antiviral responses. *Front. Immun*., **11, Article ID 211:** 1-8; doi: 10.3389/fimmu.2020.00211.

Zhao H., Wu L., Yan G. et al. (2021) Inflammation and tumor progression: signaling pathways and targeted intervention. *Signal Transduct Target Ther.*, **6(1), Article ID 263:** 1-46; doi: 10.1038/s41392-021-00658-5.

Zindel J., Kubes P. (2020) DAMPs, PAMPs, and LAMPs in immunity and sterile inflammation. *Annu. Rev. Pathol*., 2020, **15:** 493-518; doi: 10.1146/annurev-pathmechdis-012419-032847.

ORCIDs:

Nurmammad Mustafayev: https://orcid.org/0000-0002-8493-0429
Lala Akhundova: https://orcid.org/0000-0003-0553-3706
Shalala Majidova: https://orcid.org/0009-0009-2679-8333
Nigar Mammadli: https://orcid.org/0009-0007-5786-9654
Ahliman Amiraslanov: https://orcid.org/0009-0002-2504-9532
Irada Huseynova: https://orcid.org/0000-0003-3336-2203

Using modern biotechnologies for conservation of the genepool of commercial and wild grapes

Mirakbar Yakubov[1], Fayzulla Abdullaev[2]

[1]Center for Advanced Technologies, Almazar District, 3a Talabalar shakharchasi, 100174, Tashkent, Uzbekistan
[2]LLC "Center for the Implementation of Innovations and Technologies", Yukori-Chirchik District, Surnkent, Sakhovat Str., 111910, Tashkent Region, Uzbekistan

For correspondence: *mirakbardan@yahoo.com*, *f-abdullaev@yahoo.com*

Received: November 01, 2025; Reviewed: November 30, 2025; Accepted: December 10, 2025

Plant genetic resources represent a fundamental basis for biodiversity and the sustainable development of agriculture. Their conservation is critically important for breeding, adaptation to climate change, and ensuring food security. One of the most effective approaches for the preservation and regeneration of rare and endangered plant forms is the use of *in vitro* technologies. This study aimed to develop an *in vitro* initiation protocol for commercial grape cultivars «Rizamat» and «Taifi Rozoviy», as well as the wild subspecies *Vitis vinifera* ssp. *silvestris*. Thimerosal was used as a sterilizing agent at different concentrations. The most effective treatment was 0.1 mg/L for 5-7 minutes, which ensured complete sterility and 100.0% explant survival. Two hormone-free nutrient media, MS and WPM, were tested. The MS medium demonstrated superior performance, with 90.0% bud break and up to 80.0% shoot induction. Shoots developed strong morphology and normal structure. In contrast, the WPM medium resulted in slower development and thinner shoots. Rooting was performed on a modified MS medium supplemented with 0.2 mg/L NAA. Optimal rooting occurred in shoots measuring 2.5-3.0 cm in height and bearing 2-3 well-developed leaves. These shoots developed complete and functional root systems. The developed protocol enables efficient cultivation of both cultivated and wild grape forms. It provides a valuable foundation for the production of healthy planting material and for the long-term conservation of grapevine germplasm. The results are applicable in the practice of micropropagation, grapevine biotechnology, and breeding programs in Uzbekistan and other countries with similar agro-climatic conditions.

Keywords: *Grape, micropropagation, in vitro, production of healthy mother plants*

INTRODUCTION

Plant genetic resources form the foundation of biodiversity and sustainable agricultural development. Their conservation is essential for crop breeding, adaptation to climate change, and ensuring food security.

Global climate change, agroecosystem degradation, and the expansion of viticulture into stress-prone regions require a re-evaluation of strategies for conserving and regenerating the genepool of both cultivated and wild grapevine (*Vitis* spp.). The genetic diversity of this crop determines its adaptability, resistance to diseases and pests, and underpins effective breeding and the development of competitive planting material (Abdullaev et al., 2025).

Modern viticulture in Uzbekistan, despite its scale and export potential, relies largely on grape cultivars developed in the mid-20th Century. These cultivars show limited resistance to major pathogens and abiotic stresses. At the same time, wild grapevine forms with high resistance and valuable adaptive traits remain underexplored and insufficiently conserved (The bouquet of Uzbek winemaking: Grapes and their features, 2025).

Field collections are the traditional method for preserving vegetatively propagated grape germplasm, but they are subject to biological risks (*infections, mutations, plant senescence*) and organizational risks (*limited funding, natural disasters, loss of accessions*). These limitations emphasize the need for alternative conservation strategies (Zlenko et al., 2003).

In vitro technologies are among the most effective methods for conserving and restoring rare and endangered plant species. Cell and tissue cultures make it possible to maintain genetic material in the form of callus lines, microclones, or cell suspensions. This approach is particularly

 DOI: https://doi.org/10.62088/timbb/9.2.4

relevant for species under threat of extinction, with narrow geographic distribution or low seed productivity. Methods such as *in vitro* regeneration, cryopreservation, and micropropagation allow not only for maintaining collections in laboratory conditions but also for restoring populations in natural habitats if needed (Abdullaev et al., 2025; Zlenko et al., 2003; Murashige and Skoog, 1962).

Modern biotechnological approaches, including in vitro culture, offer new opportunities for the sanitation of grapevine plants, the production of planting material, and the long-term conservation of genetic diversity. *In vitro* micropropagation accelerates the production of healthy mother plants suitable for vegetative propagation and the establishment of high-yielding, disease-resistant vineyards (The bouquet of Uzbek winemaking: Grapes and their features 2025; Uzbek National Encyclopedia, 2000). These methods are also used in grape breeding to rapidly develop hybrids with valuable traits, including tolerance to biotic and abiotic stresses (Zlenko et al., 2003).

The development of effective biotechnological protocols for *in vitro* introduction of both industrial cultivars and wild grapevine forms makes it possible to:

- produce sterile and genetically uniform plants;
- accelerate the sanitation and mass propagation process;
- preserve unique genotypes in both laboratory and natural conditions;
- ensure genetic stability and integrity of collections;
- facilitate breeding progress under controlled environmental conditions (Zlenko et al., 2003; Uzbek National Encyclopedia, 2000).

Thus, integrating classical approaches with advanced biotechnological methods, particularly *in vitro* technologies, is a strategic direction in the conservation and sustainable use of plant genetic resources.

Among the fruit crops widely cultivated worldwide, grapevine (*Vitis* spp.) holds a leading position. Since ancient times, it has been used as a food crop and as a raw material for the wine industry, which accounts for its high economic importance. The genetic resources of grapevine are of strategic value for breeding and adaptation to stress conditions such as drought, high temperatures, and pathogens. Wild subspecies such as *V.vinifera* subsp. *silvestris*, distributed in the mountainous regions of Uzbekistan, are especially valuable due to their potential for conferring resistance and adaptability in new cultivars (Abdullaev et al., 2025).

Despite this potential, systematic breeding programs for grapevine in Uzbekistan are nearly absent. Industrial production still relies on cultivars developed 40-50 years ago, which limits the sector's competitiveness. Moreover, the widespread presence of plant diseases-especially fungal infections-continues to cause substantial losses in both yield and quality. These challenges underscore the urgent need for biotechnological solutions in the country's viticulture sector. Grapevine was among the first woody species for which methods of plant biotechnology and cell engineering were developed (Abdullaev et al., 2025).

Currently, Uzbekistan lacks coordinated programs for implementing advanced biotechnological tools in grapevine conservation and breeding. Addressing this gap requires a shift toward an integrated biotechnology-based strategy (The bouquet of Uzbek winemaking: Grapes and their features, 2025).

One of the key directions is the application of *in vitro* methods for the propagation and preservation of both cultivated and wild grapevine forms. Wild accessions possess a high adaptive potential and may serve as sources for breeding cultivars with resistance to drought, frost, and pathogens. However, the success of *in vitro* techniques depends largely on the initial stage of introducing field material into culture. At this critical phase, obtaining sterile and viable primary explants is essential for ensuring the success of further regeneration and micropropagation (Murashige and Skoog, 1962).

In this context, the development of robust and reproducible *in vitro* protocols for both industrial and wild grapevine genotypes represent a relevant research priority. Such protocols contribute to biodiversity conservation, enhanced crop resilience, and the modernization of viticulture in Uzbekistan.

This study aims to assess the potential of biotechnological approaches for conserving and regenerating the genepool of industrial and wild grapevine accessions, with a focus on implementing highly efficient in vitro systems for micropropagation, sterilization, cultivation, and rooting.

Research Aim and Objectives:

Research aim: The aim of this study is to develop optimal conditions for *in vitro* culture initiation of commercial grapevine cultivars cultivated in Uzbekistan, as well as a wild species growing under natural conditions. Special emphasis is placed on evaluating the effect of different silver compounds on the efficiency of obtaining sterile and viable explant material suitable for subsequent micropropagation.

Objectives:

- To select representative commercial grape cultivars and wild *Vitis* species adapted to the environmental conditions of Uzbekistan;

- To conduct a comparative assessment of different sterilizing agents, including silver-based compounds (*silver nitrate, $AgNO_3$, and silver thiosulfate, $Ag_2S_2O_3$*), based on explant viability and contamination rates;
- To determine the optimal concentration and exposure time of silver compounds that ensure maximum sterility while maintaining the regenerative potential of plant tissues;
- To refine the technological parameters for *in vitro* culture initiation, including explant type, plant developmental stage, and duration of pre-treatment;
- To establish a foundation for the development of a micropropagation protocol based on the optimized *in vitro* initiation scheme;
- To evaluate the biological response of different grapevine genotypes to the tested conditions, highlighting differences between cultivated varieties and wild forms.

MATERIALS AND METHODS

Plant material. The study employed three grapevine genotypes as initial plant material:

- «Rizamat» cultivar (*Vitis vinifera* L.) - a table grape developed by Uzbek breeders in the 1970s through hybridization of local forms «Katta-Kurgan» and «Parkent» cultivars. It is known for its high productivity, large clusters, and stable market traits.
- «Taifi rozoviy» cultivar (*Vitis vinifera* L.)- a traditional table grape widely cultivated in Central Asia. Although its precise origin remains unknown, the cultivar demonstrates drought tolerance, rich flavor, and good postharvest keeping quality.
- Wild grapevine (*Vitis vinifera* ssp. *silvestris*)- accessions obtained from the genetic collection of the Research Institute of Plant Genetic Resources. According to morphological features and local records, the age of donor plants was at least 30 years. This subspecies is considered a potential donor of stress-tolerant traits.

1. Explant selection and preparation. Apical and sub-apical shoot meristems were used as explants for in vitro introduction. Sampling was carried out in spring during the active growth phase. Shoots were first washed under running water and then treated with a detergent solution followed by multistep surface disinfection.

2. Surface sterilization. Silver-based agents, including silver nitrate ($AgNO_3$), were used at concentrations of 5-20 mg/L with exposure times of 5-15 minutes. Conventional disinfectants such as sodium hypochlorite (NaOCl, 0.5-1.0%) and ethanol (C_2H_5OH, 70%) were also employed.

3. Culture Medium. The introduction was performed on a modified Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) supplemented with vitamins and growth regulators, including 0.2 mg/L naphthaleneacetic acid (NAA). For wild grape accessions, cysteine and activated charcoal were additionally included to reduce phenolic oxidation.

4. Culture Conditions. Explants were incubated at 25±1°C with a 16-hour photoperiod and a light intensity of 2500 lux. Viability, contamination, and signs of regeneration were assessed on days 3, 7, and 14.

5. Data Analysis. Experimental results were analyzed using one-way analysis of variance (*ANOVA*) via «MS Excel» and «Statistica 10» software packages. Statistical significance was determined by Student's t-test at a confidence level of $p < 0.05$.

Explants and Medium Composition. Nodal stem segments with a lateral (axillary) bud were used as explants. Material was harvested in the early morning during the period of active spring growth. Surface sterilization was carried out using aqueous solutions of thimerosal (sodium ethylmercurithiosalicylate, $C_9H_9HgNaO_2S$) at concentrations of 0.05-0.20% with exposure times ranging from 5 to 15 minutes, followed by rinsing in sterile distilled water. Explants were cultured on hormone-free agar-based MS media [4] containing: 1) standard macro- and micronutrients (as per original MS formulation); 2) 30.0 g/L sucrose ($C_{12}H_{22}O_{11}$); 3) 100.0 mg/L inositol ($C_6H_6O_6$); 4) pH adjusted to 5.6 before autoclaving.

Antimicrobial additives. To suppress endogenous microbial contamination, the culture medium was supplemented with: silver nitrate ($AgNO_3$) at concentrations of 1-5 mg/L, colloidal silver (Ag^0) at volumes of 0.5-2.0 ml/l. These treatments were designed to assess the impact of silver compounds on explant contamination rates and survival.

Culture conditions and monitoring. Explants were incubated in a climate chamber under the following conditions: 1) temperature: 24±2°C; 2) photoperiod: 16 hours light / 8 hours dark; 3) light intensity: 3000 lux. Contamination was assessed visually on days 3, 10, and 30 post-inoculations. Bacterial and fungal infections were recorded separately.

Replication. For each genotype and sterilization variant, 20 explants were used. All experiments were performed in triplicate. The data were statistically analyzed using ANOVA at a significance level of $p < 0.05$.

Table. Efficiency of grapevine *in vitro* introduction stages.

Stage	Parameter/Medium	Conditions	Result
Sterilization	Thimerosal	0.1 mg/ml, 5-7 min	100% sterile and viable explants
Culture medium	MS	Hormone-free	90.0% bud break, 80.0% - ≥2 shoots
	WPM	Hormone-free	84% bud break, low shoot proliferation
Shoot quality	MS	-	Vigorous shoots, green leaves, normal morphology
	WPM	-	Thin, weak shoots with reduced growth
Rooting	MS + NAA (0.2 mg/L)	Shoots 2.5-3.0 cm with 2-3 leaves	Well-developed root system formation

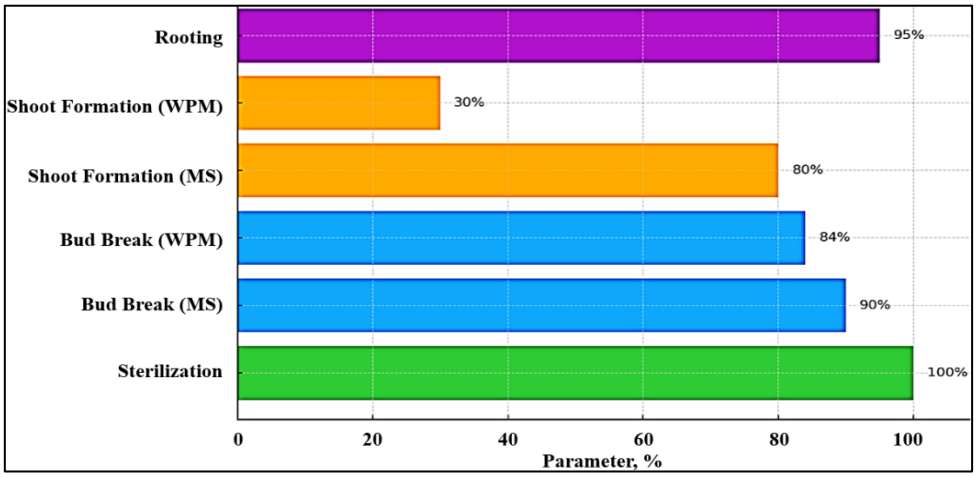


Fig. Efficiency of key stages in grapevine *in vitro* introduction.

RESULTS AND DISCUSSION

During the introduction of grapevine into *in vitro* culture, it was established that the optimal sterilization treatment consisted of using 0.1 mg/ml thimerosal for 5-7 minutes. This treatment ensured 100.0% sterility and viability of explants without signs of phytotoxicity.

A comparative analysis of MS and WPM media revealed the superiority of the MS medium. Explants cultured on MS exhibited:

- Higher bud break frequency (90.0% vs. 84.0% on WPM),
- Greater shoot proliferation rate (80.0% of explants formed two or more shoots),
- Formation of vigorous shoots with normal leaf development and internodes.

In contrast, explants grown on WPM showed slender, weak shoots with slower growth rates and signs of physiological weakness.

At the rooting stage, it was shown that using shoots of 2.5-3.0 cm in length with 2-3 well-developed leaves resulted in maximum rhizogenesis efficiency. Rooting was performed on MS medium. supplemented with 20 g/L sucrose, vitamins, and 0.2 mg/L α-naphthaleneacetic acid (NAA).

All key parameters of the *in vitro* introduction protocol are summarized in Table.

Visual representation of the main phases in the in vitro introduction of grapevine: (1) explant surface sterilization, (2) bud break and shoot formation on MS and WPM media, and (3) in vitro rooting of microshoots. The highest efficiency across all stages was achieved using MS medium supplemented with 0.2 mg/L NAA during the rooting phase (Figure).

The results demonstrated the high efficiency of thimerosal at a concentration of 0.1 mg/L for the surface sterilization of grape explants. This treatment ensured 100.0% sterile and viable explants, confirming its practical applicability for the initiation of in vitro culture of various *Vitis vinifera* cultivars as well as wild accessions. Comparable levels of sterility have been reported using other antiseptics; however, thimerosal proved to be less toxic and more tissue-friendly compared to many commonly used disinfectants (Abdullayev et al., 2025).

A comparative assessment of nutrient media revealed that the Murashige and Skoog (MS) medium was more effective than the Woody Plant Medium (*WPM*) for shoot induction. This finding is consistent with previous studies where MS medium was successfully used for organogenesis in grapes and other woody species. Shoots regenerated on MS MS medium exhibited favorable morphology, including normal internode length and bright green leaf coloration, indicating good physiological status of the regenerated plants.

Successful in vitro rooting was achieved by supplementing the culture medium with 0.2 mg/L of the auxin α-naphthaleneacetic acid (NAA). The highest rooting response was observed in shoots 2.5-3.0 cm in length with well-developed leaves. These results support previously established morphophysiological criteria for optimal rooting of grape microshoots. The use of MS medium with a reduced sucrose concentration (20 g/L) and a complete vitamin set promoted the formation of a strong root system, which is critical for subsequent ex vitro acclimatization.

Particular attention should be given to the successful *in vitro* establishment of the wild subspecies *V.vinifera* ssp. *silvestris*. This result highlights its potential use in breeding programs aimed at enhancing tolerance to abiotic stresses and resistance to pathogens. Wild grape genotypes are known to possess valuable traits, such as drought and disease resistance, and can serve as important parental components in hybridization schemes (Zlenko et al., 2003).

Therefore, the proposed protocol can be recommended for the mass production of pathogen-free planting material for both commercial grapevine cultivars and wild forms utilized in genetic improvement programs.

CONCLUSION

This study demonstrated the effectiveness of biotechnological approaches for the *in vitro* regeneration and preservation of both cultivated and wild grapevine genotypes. Experimental work included the optimization of sterilization parameters, selection of nutrient media, and evaluation of morphogenetic responses during shoot induction and rooting. The findings confirmed the feasibility and reproducibility of the proposed protocols. These conclusions provide scientific justification for the broader application of in vitro methods in grapevine sanitation, genetic resource conservation, and breeding.

1. A reproducible protocol for the *in vitro* culture initiation of industrial grape cultivars (*«Rizamat» and «Taifi rozoviy»*) and the wild subspecies *Vitis vinifera* ssp. *silvestris*, adapted to the mountainous regions of Uzbekistan, has been developed.
2. The optimal sterilization conditions- 0.1 mg/L of thimerosal for 5-7 minutes - ensured 100% explant survival and complete elimination of microbial contamination.
3. The hormone-free Murashige and Skoog (*MS*) medium demonstrated the highest efficiency in inducing bud break (*90.0%*) and shoot formation (*80.0%*) across all tested genotypes.
4. For *in vitro* rooting, the most effective condition was MS medium supplemented with 0.2 mg/L of NAA, using shoots 2.5-3.0 cm in length with 2-3 well-developed leaves, which resulted in consistent and stable root system development.
5. The wild subspecies *V.vinifera* ssp. *silvestris* exhibited a high regenerative capacity under *in vitro* conditions, confirming its potential as a valuable donor for breeding programs aimed at improving resistance to abiotic and biotic stresses.
6. The obtained results confirm the applicability of the developed technology for grapevine sanitation, rapid clonal propagation, establishment of stress-resilient vineyards, and integration of wild grape forms into biotechnology and breeding programs.

ACKNOWLEDGEMENTS

The authors express their sincere appreciation to the Center for Advanced Technologies (Tashkent, Uzbekistan) and the LLC “Center for the Implementation of Innovations and Technologies” (Tashkent Region, Uzbekistan) for providing institutional support, laboratory facilities, and technical assistance necessary for conducting this study. The authors also acknowledge the contributions of researchers and institutions whose previous work on grape biotechnology and genetic conservation informed this research.

ETHICAL CONSIDERATIONS

This study focuses on the application of modern biotechnological methods for plant genetic resource conservation and does not involve human participants, indigenous knowledge holders, or animal experimentation. All procedures related to plant materials were conducted in accordance with national and international guidelines for the conservation and sustainable use of plant genetic resources. Therefore, ethical approval and informed consent were not required for this research.

AUTHOR CONTRIBUTIONS

- Mirakbar Yakubov: Conceptualization of the study, methodological design, data interpretation, manuscript drafting, and correspondence.
- Fayzulla Abdullaev: Experimental design support, analysis of biotechnological approaches, critical revision of the manuscript, and validation of scientific content.

Both authors have read and approved the final version of the manuscript.

FUNDING

This research did not receive any specific funding from public, commercial, or non-profit funding agencies.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest related to this study. No financial, institutional, or personal relationships influenced the research design, data interpretation, or publication of results.

REFERENCES

Abdullaev F.Kh., Rustamov N.S., Yakubov M.D. (2025) Uzbekistan Genebank. *In*: J.M.Al-Khayri, K.F.M.Salem, S.M.Jain, H.C.Kourda (eds.) *Plant Gene Banks*. Springer, Singapore; doi: 10.1007/978-981-99-4236-7_57-1

The bouquet of Uzbek winemaking: Grapes and their features (2022) Retrieved June 29, 2025; URL: https://review.uz/post/buket-uzbekskogo-vinodeliya

Zlenko V.A., Kotikov I.V., Troshin L.P. (2003) Methods of *in vitro* propagation of sanitized grapevine planting material. *Vinodelie i Vinogradarstvo (Viticulture and Winemaking)*, **3:** 38-39 (in Russian).

Murashige T., Skoog F. (1962) A medium revised for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant*., **15:** 473-497.

Uzbek National Encyclopedia (2000) Ed. by M.Kh.Khasanov. 1st edition. In 12 vols., **Vol. 1.** Tashkent: UzEncyclopedia (in Russian).

ORCIDs:
Mirakbar Yakubov: https://orcid.org/0000-0003-2928-8805
Fayzulla Abdullaev: https://orcid.org/0000-0003-2162-291X

Anthropology and bioethics: dialogical universality, ethical relationality, and the transcendence of epistemic boundaries in contemporary human sciences

Tabib Noureddine, Drizi Aicha

University of Oran 2 Mohamed Ben Ahmed, Human Sciences Research Unit for Philosophical, Social and Humanistic Studies, Algeria

***For correspondence*:** *tebib.noureddine@univ-oran2.dz, drizi.aicha@univ-oran2.dz*

Received: November 02, 2025; Reviewed: November 30, 2025; Accepted: December 15, 2025

The accelerating transformations in biomedical and biotechnological research, ranging from genetic modification to artificial reproduction, have radically redefined the human condition. This article examines the intersections of anthropology and bioethics as a philosophical response to this reconfiguration, arguing that universality in bioethics cannot be conceived as an immutable or transcendent category. Rather, it is a dialogical construct arising within the interplay of cultural difference, recognition, and human vulnerability. By integrating anthropological reflection into the bioethical discourse, the paper highlights how the plurality of human experience demands an ethical model grounded in dialogue rather than domination. Anthropology, in its redefined form, transcends its classical descriptive mission to become a space of mediation between epistemic diversity and moral reflection. The study proposes a framework of "situated universality", where local ethical systems interact with global moral imperatives, producing a plural yet coherent understanding of human dignity. This shift challenges the technocratic reduction of life to biological or instrumental terms and restores the ontological centrality of the human being as both a cultural and ethical subject.

Keywords: *Anthropology, bioethics, universality, dialogical ethics, cultural plurality, human dignity, ontological fragility, transcendence, post-technological humanity*

METHODOLOGY

This article employs a qualitative, interpretive, and philosophical-analytical methodology, combining textual hermeneutics with comparative analysis of bioethical and anthropological paradigms. The research is not empirical in the conventional sense but instead relies on conceptual analysis of key terms such as "universality," "ethics," and "humanity" as treated in both Western and cross-cultural philosophical traditions. Critical reading of major anthropological and ethical theorists (e.g., Kant, Dumont, Lévi-Strauss, Foucault, Habermas) is used to trace the evolution of the "human question" within modern science. A dialogical synthesis juxtaposes local ethical perspectives (non-Western, communitarian, or culturally embedded frameworks) with universalist bioethical norms to explore areas of convergence and tension.

INTRODUCTION

At the dawn of the twenty-first century, humanity confronted itself with an unprecedented epistemic and ethical upheaval imposed by scientific breakthroughs in the biomedical and biotechnological fields: genetic engineering, organ transplantation, in vitro fertilisation, surrogacy, and other biomedical practices. These transformations are no longer merely technical achievements enriching medical knowledge; instead, they have become an ontological threat that touches the very essence of the human being, reconfiguring the boundaries between the natural and the artificial, between the body as a biological datum and the self as an existential value. It is precisely here that the onto-ethical question arises in its most profound form: what meaning do dignity, freedom, and justice retain when the body itself becomes a site of technology and experimentation?

In that case, its task becomes more pressing. Whereas the history of classical anthropology was preoccupied with the description and comparison of peoples, the present moment obliges it to transcend this narrow descriptive dimension, moving instead towards an engagement in a debate where the epistemic intersects with the ethical and the scientific with the ontological. From this perspective, our central problematic emerges: how can anthropology approach bioethical principles as a claim to universality without falling into the trap

 DOI: https://doi.org/10.62088/timbb/9.2.5

of ethnocentrism? Moreover, is it capable of articulating an alternative ethical discourse for contemporary humanity, one that transcends the boundaries of both the universal and the local?

ANTHROPOLOGY AND THE DISCOURSE OF THE HUMAN

Since its inception, anthropology has remained closely tied to the cultural and social patterns of traditional and local peoples, focusing initially on the natural dimensions of human beings, such as the study of bones, artefacts, and fossils, and adopting the methods of the natural sciences as a model to determine what is nonnatural in humans. The ambiguity surrounding Kant's question, *"What is the human being (Was ist der Mensch)?"* (Michel, n.d., p. 280), a question to which all others return, if not concealed behind every question, arises precisely from the attempt to understand humanity through what lies outside of it.

As a result, the discourse on the human being receded, particularly with the emergence of the human sciences, which approached the human as an alien "other." These sciences became anti-human rather than being in service to humanity, as their exclusively empirical treatment reduced the human being to an object among other objects in the world, effacing their dimension as a subject. Consequently, this led to a discourse *about* the human rather than a discourse *within* the human. In this way, anthropology itself became entangled in the paradox that marks the history of the modern Western human sciences, which appear, in fact, as a history of forgetting the human.

In this regard, the Canadian anthropologist Fernand Dumont (1927-1997) observes, *"The fact remains that anthropology exists elsewhere than the place in which we think we are. It is constructed in our absence"* (Dumont, 1981, p. 10). That is, throughout its history, anthropology has not been concerned with the human as much as it has circled them, despite its constant claim to undertake the task of understanding humanity through studying it across various domains, primarily the cultural domain. Dumont further considers that *"anthropology is a replica of culture, and that it seeks within culture a prehuman interpretation of the human, while simultaneously constructing an interpretation of the human that transcends culture"* (Dumont, 1981, p. 11), thereby enabling anthropology to place the very concept of the human in brackets.

Since culture constitutes the conceptual ground of the human as a cultural being, the task of anthropology today lies in its search for a conception of the human prior to such representations; that is, a search for humanity in its raw form, freed from the moulds that anthropology itself imposes. This would enable researchers to construct a clear understanding and vision of the human that transcends all preconceived notions. On this basis, Dumont affirms that anthropology, in essence, is not the study of the human or philosophy. The human being is not an object of study for either anthropology or philosophy (Dumont, 1981, p. 18) because both disciplines have approached humanity in a methodological manner that has failed to address the deeper meanings of inquiry into it, especially in light of today's world dominated by technology, which has altered the very concept of the human.

All these factors have led contemporary anthropology to depart from its traditional trajectory and to venture into new domains that were not historically its own in response to the pressing need for methodological tools capable of uncovering and understanding the realities of contemporary humanity. This shift is evident in the field of medicine, which has long treated the human being as a biological apparatus, overlooking the cultural, social, and religious components that form an essential part of human nature. Hence, there is an urgent need for the intervention of anthropology, particularly medical anthropology, to broaden the medical perspective from its narrowly biological focus to a broader sociocultural dimension, one capable of encompassing the full complexity of the human being.

Health and illness can thus be regarded as fundamental entry points for understanding contemporary humanity, as they constitute the connecting link between the anthropological and bioethical domains. Viewing the human being through the lens of fragility and vulnerability establishes a new paradigm of understanding, wherein existence and orientation in the world become contingent upon the extent to which one accepts this fragility. In this sense, weakness itself becomes a source of strength, or, as Nietzsche remarked, *"What does not kill me makes me stronger"* (Friedrich, 2006, p. 110). In contrast, any attempt to abolish such fragility amounts to an annihilation of the human being and an emptying of their essence, even if one assumes otherwise.

Indeed, the project of transhumanism represents nothing but a form of such an attempt. However, is it truly in the interest of humanity to prolong life indefinitely? Or to pursue immortality? Would this not negate the very condition of being human, transforming the individual into a mere machine devoid of spirit and sensibility? The voices that dream of futures are defined by endings, the end of the physician, the end of the school, and

other such proclaimed endings ultimately conceal only one conclusion: the end of humanity itself.

THE QUESTION OF THE HUMAN BETWEEN ANTHROPOLOGY AND BIOETHICS

Bioethics represents one of the manifestations of Enlightenment philosophy and Anglo-Saxon thought, presenting itself as an alternative to classical ethics by claiming both universality and absoluteness in the domain of morality. *"Under these conditions, for anthropologists, bioethics can only transmit and reproduce what Kleinman has termed the culture of medical centrality".* The neglect of cultural variables within the health paradigm is precisely what has granted medical anthropology both priority and legitimacy in its critique of bioethics. Anthropology underscores that the concepts of health and illness are not reducible to biological givens alone; instead, they are embedded in the cultural and social fabric, as in beliefs surrounding the evil eye, sorcery, spirit possession, and other symbolic representations.

In this context, the research of the French anthropologist Richard Pottier (1909-1994) is instructive, as it examines the anthropological factors underlying bioethical debate factors that touch upon human sanctity and extend to environmental issues, as well as attitudes toward vulnerable beings such as the foetus, those suffering from dementia, people with low incomes, and others (Richard, 2021, pp. 175-177). This opens the door to a range of questions concerning the boundaries of bioethics.

For Pottier, the central problem lies in the relationship between life and human dignity, which in turn reflects the human relationship with the body, as in the case of abortion: should the foetus be treated as a fully human being, or merely as a potentiality of one? Regardless of the ethical positions adopted, Western thought remains captive to a natural ontology (Richard, 2021). On this basis, pressing questions emerge: who determines these ethical standards? Is it the human being, religion, bioethics, or the law and political institutions? More critically and often unspoken is the question of whether these ethical principles are truly innocent. Do they genuinely arise in service of humanity and relieve existential suffering? Or do they, under the guise of universality, merely reinforce new forms of domination?

Pottier argues that Western debates linking respect for life and human dignity are, at their core, grounded primarily in a universal moral intuition shared across all societies, an intuition that determines the nature of one's relationship with the other (Richard, 2021). However, this conception raises several pressing questions: first, who defines this intuition in the first place? Second, is it truly possible to speak of a moral intuition common to all human beings? These questions compel us to question the Western promotion of ethical universality and the universality of bioethical principles. Although Western normative discourse has indeed extended the scope of ethics to areas of central anthropological concern, such as the universality of human rights, religious fundamentalism, and tolerance toward certain inherited customs (Masse, 2000, p. 105), bioethical ethics nonetheless remain tested when confronted with cultural particularity.

With its ostensibly universal principles, bioethics often marginalises the question of local specificity within non-Western societies, seeking instead to construct a universal vision of humanity while disregarding its sociocultural and symbolic uniqueness. For this reason, anthropologists have called for the integration of field-based anthropological research into the formulation of bioethical frameworks to render them more inclusive and scientifically credible. There can be no universal conception of humanity when humanity itself is founded on diversity and difference, whether at the biological, spiritual, or cultural level.

Accordingly, it becomes essential for anthropologists and bioethicists to collaborate in re-examining global ethical principles and in attempting to integrate the local sociocultural dimension into holistic visions of humanity. In this way, bioethics may be reformulated as an ethics that embraces universality without negating difference. Moreover, bioethics has traditionally focused on applying a set of principles to medical practices to guide medicine and biology towards ethical orientations. Anthropology, through its applied methodologies, demonstrates the necessity of delineating a new field for bioethics, one that incorporates both the universal and the local, through social, cultural, and historical contexts, thereby framing its discourse and regulating its practical applications.

Owing to this anthropological – bioethical encounter, openness to the cultural repertoire of local communities becomes indispensable through an appreciation of each society's distinctive value system. Only in this way can bioethical practices descend to the level of local realities, engaging with their specificities and thereby laying the foundation for a new ethical praxis in which the universal dimension is integrated with the local. This reformulation redefines the relationship between anthropology and bioethics, as *"ethics depends on*

the human being, on the image they form of themselves, and on the ideals, they emulate in their ethical representations" (Autres, p. 32). Consequently, anthropology, particularly medical anthropology, has come to concern itself with safeguarding humanity and reflecting on human agency in the field of life sciences (Gueh, 2013).

Whereas in Darwin's theory it was nature that selected, today it is the human who selects; survival is no longer reserved for the naturally fittest but for those able to adapt to universal bioethical values. From an anthropological perspective, *"bioethics is considered a cultural phenomenon grounded in Western philosophical and legal traditions, which prioritise the individual and insist on their particular rights of self-determination"* (Ana Marin, n.d., pp. 17-45). In this sense, bioethics itself becomes, in a manner of speaking, a branch of anthropology.

Since bioethics rests on three principal domains – clinical ethics, the ethics of scientific research, and issues of public care – anthropology is capable of encompassing these domains by its grounding in the human and sociocultural dimensions upon which it ultimately depends. This is evident in the interventions of anthropologists within these fields, guiding them toward directions overlooked by bioethics under the dominance of universality and global values in its principles. In essence, bioethics remains an incomplete discipline, as most of its practitioners originate from other fields, notably medicine, theology, law, and biology. This has led it to exaggerate the moral dimension to such an extent that the American philosopher Daniel Callahan (1930-2019) described it as *"moral mania"* (Marshall, March 1992, pp. 49-73). This mania has driven it to neglect cultural, social, political, and religious issues.

However, through collaboration between anthropologists and bioethicists, solutions may be found to the ethical and health dilemmas that continue to proliferate with the accelerating pace of contemporary scientific and technological developments. From this standpoint, suspicion of bioethics has emerged, particularly concerning the values and principles it claims to be universal. Anthropologists pose a critical question: What are the reasons and motivations that have led bioethicists to exclude cultural particularity from their ethical frameworks?

To address this problem, anthropology has begun to deconstruct the content of Western ethics and align it with the requirements of local contexts. Since the declared aim of bioethics is to serve humanity, the fundamental question arises: which human beings do we speak of? Does this concept encompass all humanity, or does it refer specifically to the Western human? From this critical perspective advanced by anthropologists toward bioethics, two principal approaches have emerged:

The first approach affirms the existence of fundamental moral values shared across cultures, despite their diversity. Among its representatives are Stephen Toulmin, Charles Taylor, Sissela Bok, and Martha Nussbaum.

The second approach rejects the very notion of moral universality, emphasising the locality of ethics, the heterogeneity of cultures, and the diversity of value frameworks. Its most notable proponents include Stanley Fish, Stanley Hauerwas, Alasdair MacIntyre, and H. Tristram Engelhardt. This debate compelled normative discourse to expand into key areas of anthropological concern, such as the universality of human rights, religious fundamentalism, and tolerance of inherited customs (Masse, 2000). With the encounter between anthropology and bioethics, the latter found itself obliged to redefine its identity in light of the cultural factor, which complicated the equation, particularly given that bioethics received little attention from anthropologists until the 1990s.

However, with the rise of bioethical claims to universality, anthropology has resisted such propositions. *"For anthropologists, bioethicists needed to realise that bioethics is a product of Western culture, shaped by elements with cultural and historical orientations linked to ideological events"* (Ana Marin, n.d., pp. 17-45). In other words, the principles on which bioethics rest are conditioned by temporal and spatial factors tied fundamentally to the crisis of values and the loss of meaning in Western existence, brought about by the decline of transcendence and the dominance of materialism. This dominance has transformed bioethics into a new form of technical theology, assuming the same function as the sacred, but through technological instruments. This situation has compelled scholars in the field of ethics to devise a new framework of morality. This onto-ethical system aspires to liberate contemporary humanity from its existential and biological crises.

We cannot deny that improvements in public health are directly linked to medical and biotechnological progress. However, this progress has not been merely a technical achievement but rather a profound transformation of social and cultural structures, particularly with respect to perceptions of the body. The body has become the locus of political, economic, and ethical stakes. At the same time, health care today stands at the forefront of state priorities within a global health paradigm that guides public policy and redefines the human relationship with both the self and the

world. Thus, we encounter a philosophical question open to interpretation in the space between anthropology and bioethics. Suppose that bioethics aspires to establish universal ethics that encompass all human beings. In that case, anthropology reminds us that humanity is plural in its biological and spiritual universality, oscillating between the universal and the local.

HEALTH AND ILLNESS FROM AN ANTHROPO-ETHICAL PERSPECTIVE

The promises heralded by contemporary biotechnology that humanity could achieve eternal youth, perfect health, strength, and beauty soon collided with a harsh reality dominated by lethal epidemics and intractable diseases such as AIDS, cancer, and diabetes, which exposed the illusory nature of such claims. This epistemological paradox has led to an internal shift within medical practice itself, as noted by Bernard Hours (1959), who argued that the newer generations of physicians, being more modest and less rigid, have recognised the importance of an anthropological perspective on health and illness (Bernard, 2004). Consequently, medical knowledge has moved beyond its narrow technical framework to a holistic perspective that integrates cultural and human dimensions. From this standpoint, Hours has called for the inclusion of anthropology in biomedical training, both at the level of knowledge and ethics, to broaden medical vision and render it more comprehensive, capable of grasping the human being in their entirety rather than as a mere biological body.

According to this perspective, several physicians have become aware of the gap between techno-medical applications and bioethical principles, realising that the actual problem does not lie in scientific or medical progress itself but rather in the universalist tendency of bioethical principles, which disregard the cultural, religious, and environmental contexts of human life. This neglect directly triggered debates of acceptance and rejection of bioethical practices within both Western and non-Western societies.

From this standpoint, these physicians sought solutions beyond the confines of traditional ethics and found in medical anthropology a suitable framework for addressing the problem. Medical anthropology, with its capacity to comprehend and integrate cultural, religious, and environmental diversity, offers the most appropriate ground for restoring balance not only to humanity itself but also to the reconfiguration of bioethics in a new form. They further argued that bioethics, as a product of a Western system founded on secular thought and universalist tendencies, has deliberately overlooked cultural and human particularities, thereby reinforcing the Western project of domination across the world from a health-related perspective.

In light of this reality, these physicians considered medical anthropology to be the refuge capable of redirecting bioethics to its proper course, transforming it into a comprehensive ethical foundation that addresses all human beings. In this way, medical practice fulfils its fundamental role in relieving human pain and suffering. Moreover, bioethics has expanded to include diverse human specificities in parallel with the field research offered by medical anthropology. Thus, a three-dimensional approach can be realised, encompassing medical, ethical, and cultural dimensions, enabling the transcendence of current crises and the orientation of practice towards its rightful direction.

In this context, philosophy emerges as an external factor capable of identifying and analysing the gaps between these domains, thereby enabling closer convergence among the different disciplines. Philosophy thus becomes a therapeutic practice that contributes to the healing of contemporary humanity from its existential suffering, working alongside the principles of bioethics and the field-based research of medical anthropology. Contemporary epidemics and diseases have raised philosophical questions that go beyond the familiar boundaries of medicine, opening possibilities for new preventive strategies aimed at containing worsening conditions and the spread of illness. This, in turn, has led to the integration of multiple fields within medicine, including anthropology, which has played a crucial role in highlighting the conceptual plurality of health and illness. These are no longer confined to the mechanical dimension of the body but extend to sociocultural and sociohistorical realities.

The same applies to anthropology, which, propelled by philosophical debates, has moved beyond its traditional preoccupations with dichotomies such as city/rural or primitive/modern. It has now been recognised that the issue far exceeds these binaries. On this basis, *"anthropology seeks to integrate the idea of respect for cultural diversity as one of the principles of bioethics"* (Ana Marin, n.d.). Respecting cultural diversity entails acknowledging the values, beliefs, and specificities of each society, particularly with respect to medical issues inherently marked by controversy and disagreement, such as organ transplantation, abortion, and euthanasia, among other bioethical practices. Thus, respect for cultural diversity in biomedical ethics lays the foundation for multicultural bioethics that is, at its core,

grounded in respect for humanity.

Anthropology has paved the way for bioethics to become entangled with everyday life by opening itself to bioethics through its applied methodologies. These methodologies, in turn, have enabled bioethics to engage with the cultural and social dimensions necessary for understanding the ethical tensions that arise when bioethical practices encounter cultural contexts. Therefore, anthropology views ethical problems as rooted primarily in cultural and social issues, maintaining that the solution lies not in the set of abstract principles advanced by bioethics but rather in comprehending the nature of the relationships among human beings, culture, health, and illness.

The American anthropologist Richard W.Lieban (1934) observed that *"reviews of medical anthropology between 1953 and 1983 did not mention the field of bioethics, reflecting the absence of medical ethics from bioethical literature"* (Marshall, March 1992, pp. 49-73). This absence can be attributed, on the one hand, to cultural relativism, which led bioethicists to reject recourse to anthropological research, and, on the other hand, to anthropology's adherence to its traditional concerns with the study of non-Western societies, neglecting the medical and biological innovations of the West. Moreover, ethical issues largely remained detached from the sociocultural contexts of societies, leaving bioethics at a distance from the realities of everyday life.

LIFE ETHICS AND THE POSITION OF THE HUMAN BETWEEN THE LOCAL AND THE UNIVERSAL

The presence of anthropology within bioethics has manifested through comparative anthropological studies and the analysis of medical systems that reveal how bioethics has emerged as a contemporary philosophical discipline while also highlighting the role of social power in directing bioethical decisions. In this context, the work of Kleinman demonstrates that different cultures hold divergent views on the issue of informing patients about their illnesses (Marshall, March 1992, pp. 49–73). In some cultural contexts, concealing the truth of a diagnosis such as cancer from the patient is considered a compassionate act aimed at preserving their psychological well-being, since disclosing reality is believed to ruin their life and cause them to die slowly.

Within this framework, medical anthropology contributes to clarifying the cultural meanings of death. For example, in some societies, patients prefer to die in their homes rather than in hospitals. This practice compels certain physicians to leave terminally ill patients in the care of their families.

Numerous practical cases highlight the tensions between the anthropological and bioethical domains. However, the COVID-19 pandemic represents the clearest example, as it exposed the limits of contemporary medical knowledge and revealed human fragility in the face of an invisible microscopic virus. This crisis brought forth multiple bioethical dilemmas, such as euthanasia and what might be called the prioritisation of the right to life. Health systems worldwide found themselves facing complex ethical quandaries: Who should be treated first? Who should be left to die in the absence of ventilators and the scarcity of oxygen? In this way, the matter became a political – ethical decision in which both the state and medical authority intervened. At the same time, the number of human beings has appeared to be reduced to mere numbers in daily statistics of infections and deaths.

In contrast, the anthropological role of the pandemic emerged in multiple forms. Local communities reinstated the value of traditional medicine, which conceives of illness not only as a biological dysfunction but also as a spiritual and sociocultural experience. At the same time, public trust in official medicine diminished, as it proved unable to curb the spread of the virus. This return to traditional medicine was not simply an act of nostalgia; rather, it constituted an anthropological outcry expressing the need for alternative modes of confronting human vulnerability and a desire to restore meaning to the life–death dichotomy at a time when the discourse of official medicine had been reduced to rigid technical language, stripped of meaning and bereft of humanity.

The COVID-19 crisis has shed light on the tension between the local and the universal. While the World Health Organisation and political authorities have attempted to impose standardised therapeutic protocols, many communities have resorted to their practices, thereby exposing the limits of medical universality in the face of sociocultural specificity. This conflict was not merely a confrontation between traditional and official medicine; at its core, it was a struggle over the discourse of authority in defining health and illness, life and death.

Accordingly, the pandemic was not a transient health event but rather an anthropo-ethical laboratory that revealed the fragility of the classical bioethical model founded on universalist ethics. This demonstrated the urgent need for new ethics of life that acknowledge the sociocultural dimensions of the health–illness and life–death dualities. The time has come for anthropologists and bioethicists to work towards establishing an ethics that

transcends the narrow confines of both bioethics and culture, embracing life in its entirety, which may be termed an *ethics of life* that encompasses both the internal components of the human being, such as cells and genes, and the external dimensions, including cultural and social systems. Through such ethics, a new human emerges, situated within the framework delineated jointly by anthropology and bioethics.

The concept of the anthropo-ethical human, as formulated by both anthropology and bioethics, can be realised only through an ethics of life. In this sense, the ethics of life constitutes a response to contemporary challenges that threaten human dignity by moving beyond a vertical relationship with the world towards a horizontal relationship with others free from all forms of authority imposed in the name of culture or ethics alike. It is, in effect, a call to dissolve differences, whether cultural or social, into a unifying humanity. From a philosophical standpoint, this vision resonates with Spinoza's assertion that *"whatever exists, exists in God, and nothing can exist or be conceived without God"* (Spinoza, 2009, p. 45). Here, God embodies the notion of unity, the unity of nature, cosmos, and humanity within a single totality, contrary to the dualisms upon which modern philosophies were built. From this perspective, the necessity of establishing a new relationship between humanity, the self, and the world becomes evident, as one is founded on participation and dialogue.

Thus, Kant's question concerning the human being is revived, a question through which he sought to liberate humanity from egoism and self-centredness by directing it towards a transcendent horizon that bridges the local and the universal. According to this conception, the human becomes both a thing among the things of nature and, at the same time, a centre of the cosmos by their capacity to encompass all changes and differences. Between objecthood and centrality, a new conception of the human emerges, one that reflects the constant tension between individuality and collectivity, between strength and fragility, and between universality and particularity. It is a conception that invites us to rethink the position of the human in the world.

On this basis, bioethics and anthropology together become tools for understanding human nature and existence through an awareness of fragility and an acceptance of vulnerability and an openness that paves the way for new horizons of spiritual and moral development. Humanity is not measured by strength alone but by the capacity to confront weakness and to embrace the challenges that stand in its path, by what practical wisdom requires, or what Aristotle referred to as *phronesis*.

CONCLUSION

The anthropological approach to bioethics reveals that universality is neither a ready-made datum nor a fixed essence nor an absolute truth. Instead, it is a horizon that continually takes shape within the dialectic of difference and recognition. The human being cannot be reduced to a single model but is disclosed in sociocultural plurality that foregrounds both human fragility and the limitations of biomedical knowledge. Thus, no contemporary bioethical project can remain captive to a closed normative model; it must open itself to other fields of knowledge, chief among them anthropology, with its capacity to provide field-based studies that seek to understand the human experience in its bodily and cultural multiplicity.

Thus, the present philosophical challenge lies in moving beyond a narrow reductionist conception of universality towards an open universality one formulated through intercultural dialogue and built within the dialectic of interaction between the local and the universal rather than through the imposition of a single normative hegemony. Only such an approach can render bioethics more capable of responding to contemporary health and human challenges, especially in the context of transboundary environmental and epidemic crises. By liberating bioethics from its centralising tendencies and grounding it in a genuinely human ethic that honours plurality and difference, it restores humanity's place as fragile and vulnerable rather than reducing it to a mere object of rigid technical standards.

ETHICAL CONSIDERATIONS

Although the study does not involve empirical data or human subjects, it adheres to the ethical norms of philosophical scholarship by ensuring intellectual integrity, accurate citation, and respect for the plurality of cultural and religious worldviews. The article consciously avoids cultural appropriation or moral relativism, striving to represent diverse ethical traditions with fairness and conceptual precision. It upholds the principles of transparency, academic honesty, and scholarly responsibility as outlined by COPE and international research ethics guidelines.

FINDINGS AND DISCUSSION

1. Reconceptualization of Universality: Universality in bioethics is not an abstract absolute but a dialogical construct born from intercultural engagement.

2. Anthropology's Ethical Turn: Anthropology must transcend its descriptive legacy and reorient itself toward ethical participation.
3. Human Fragility as Ethical Foundation: Fragility becomes a universal condition that calls for care, dialogue, and recognition.
4. Critique of Technological Reductionism: The dominance of the biomedical paradigm risks reducing the human to a manipulable biological entity.
5. Possibility of Transcendence: The interaction between anthropology and bioethics opens the possibility of ethical transcendence – a movement toward a shared human ethos rooted in dialogue and dignity.

ACKNOWLEDGEMENTS

The authors express their sincere gratitude to the Human Sciences Research Unit for Philosophical, Social, and Humanistic Studies at the University of Oran2 Mohamed Ben Ahmed for its continuous academic and institutional support. They also thank their colleagues and reviewers for insightful discussions that enriched the philosophical depth of this work.

FUNDING STATEMENT

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

CONFLICT OF INTEREST

The authors declare no conflict of interest. The views expressed are solely those of the authors and do not necessarily reflect the official policies of their institution.

REFERENCES

Arbaoui M. (2025) From paternalism to systemic bioethics: principles, characteristics, and contemporary relevance of applied ethics in medicine and society. *Science, Education and Innovations in the Context of Modern Problems*, **8(12):** 62-69; doi: 10.56352/sei/8.12.6

Beauchamp T.L., Childress J.F. (2019) Principles of biomedical ethics. 8th ed. Oxford University Press.

Benatar S.R., Brock, G. (2011) Global health and global health ethics. Cambridge University Press.

Borry P., Schotsmans P., Dierickx K. (2005) The birth of the empirical turn in bioethics. *Bioethics*, **19(1):** 49-71; doi: 10.1111/j.1467-8519.2005.00424.x

Callahan D. (2012) The roots of bioethics: Health, progress, technology, death. Oxford University Press.

Chadwick R., ten Have H., Meslin E.M. (Eds.). (2011) The SAGE handbook of health care ethics: Core and emerging issues. SAGE Publications.

Dalloz E.Z.G. (2013) Anthropologie biologique et bioéthique: Élément de dialogue. *Revue Africaine d'anthropologie, Nyansa*, (15). Côte d'Ivoire.

Dumont F. (1981) *L'anthropologie en l'absence de l'homme* (1st ed.). Presses Universitaires de France.

Engelhardt H.T.Jr. (2017) The foundations of bioethics. 2nd ed.. Oxford University Press.

Farmer P. (2005). Pathologies of power: Health, human rights, and the new war on the poor. University of California Press.

Folscheid D. et al. (1997). *Philosophie éthique et droit de la médecine*. Paris: Dalloz.

Foucault M. (2008) The birth of biopolitics: Lectures at the Collège de France, 1978-1979 (G.Burchell, Trans.). Palgrave Macmillan.

Foucault M. (n.d.). *Al-Kalimat wa al-ashyaʾ [The order of things]* (M. Safadi et al., Trans.). Markaz al-Inmaʾ al-Qawmi.

Fox R.C., Swazey J.P. (2008) Observing bioethics. Oxford University Press.

Hours B. (2004) Trois objets-étapes de la globalisation de la norme de santé. *Autre*, (29). Éditions Presses de Sciences Po.

Kass L.R. (2002) Life, liberty, and the defense of dignity: The challenge for bioethics. Encounter Books.

Kleinman A. (2006) What really matters: Living a moral life amidst uncertainty and danger. Oxford University Press.

Marin A., Bouffard C. (n.d.). La bioéthique à l'épreuve de la diversité socioculturelle: La portée du sens donné à un concept; pp. 17-45.

Marshall P.A. (1992) Anthropology and bioethics. *Medical Anthropology Quarterly, New Series,* **1(1):** 49-73.

Massé R. (2000) Présentation: L'anthropologie au défi de l'éthique. *Anthropologie et Sociétés,* **24(2):** 5-10. Université Laval.

Nietzsche F. (2006) *Qadiyat Fagnerīyah: Nietzsche didd Fagner [The case of Wagner; Nietzsche contra Wagner]* (ʿA. Misbah, Trans.). Al-Jamal Publications.

Pellegrino E.D., Thomasma D.C. (1993). The virtues in medical practice. Oxford University Press.

Pottier R. (2021) Voyage d'un anthropologue dans le monde de la bioéthique. *L'Homme,* **238(2):** 175-177.

Ragua A. (2025) The significance of bioethics as an ethical inquiry into science. *Science, Education*

and Innovations in the Context of Modern Problems, **8(4):** 600-606; doi: 10.56352/sei/8.4.65.

Singer P. (2011) Practical ethics. 3rd ed. Cambridge University Press.

Spinoza B. (2009) *ʿIlm al-akhlaq [Ethics]* (J. al-Din Saʿid, Trans.). The Arab Organisation for Translation.

Turner L. (2003) Bioethics in a multicultural world: Medicine and morality in pluralistic settings. *Health Care Analysis*, **11(2):** 99-117; doi: 10.1023/A:1025338514936

Zwart H. (2017) Psychiatry, subjectivity, and biological reductionism: Revisiting bioethical issues in the age of neuroscience. *Medicine, Health Care and Philosophy*, **20(3):** 329-339; doi: 10.1007/s11019-016-9725-2

ORCIDs:
Tabib Noureddine: https://orcid.org/0009-0004-8396-6413
Drizi Aicha: https://orcid.org/0009-0000-6463-9506

Overdiagnosis of peripartum cardiomyopathy in pregnancy: A prospective echocardiographic cohort study

Nigar Kamilova, Khatira Mirzoeva

Department of Obstetrics and Gynecology № I, Azerbaijan Medical University, 23 Bakikhanov Str., AZ1022, Baku, Azerbaijan

For correspondence: *nigar65@mail.ru, m.khatira@yahoo.com*

Received: November 12, 2025; Reviewed: November 30, 2025; Accepted: December 19, 2025

Peripartum cardiomyopathy (PPCM) is a rare but potentially life-threatening cause of heart failure occurring in late pregnancy or the early postpartum period. Physiological cardiovascular adaptation and pregnancy-related complications may mimic PPCM, leading to diagnostic overestimation. This prospective cohort study included 60 pregnant women with clinically and echocardiographically suspected PPCM, stratified by gestational trimester, and 15 healthy pregnant controls. All participants underwent transthoracic echocardiography with assessment of left ventricular ejection fraction (LVEF), chamber dimensions, and diastolic function. True PPCM was confirmed in 4 women (6.7%), predominantly in the third trimester. Compared with earlier gestational groups, women evaluated in the third trimester more frequently demonstrated symptoms of heart failure, significantly reduced LVEF, progressive ventricular dilatation, and marked diastolic dysfunction. In most cases, echocardiographic abnormalities were attributable to physiological cardiac adaptation, anaemia, or hypertensive pregnancy disorders rather than true PPCM. In healthy controls, changes in LVEF remained within physiological limits. These findings indicate that the majority of suspected PPCM cases represent reversible pregnancy-related conditions. Strict diagnostic criteria and dynamic echocardiographic monitoring are essential to prevent overdiagnosis and unnecessary treatment.

Keywords: *Peripartum cardiomyopathy, pregnancy, heart failure, echocardiography, left ventricular dysfunction, diastolic dysfunction, differential diagnosis*

INTRODUCTION

The aetiology of PPCM is multifactorial and involves oxidative stress, inflammatory activation, endothelial dysfunction, and cardiomyocyte apoptosis. Particular attention has been directed toward the prolactin pathway, whereby cathepsin D cleaves prolactin into a 16-kDa fragment with pro-apoptotic and anti-angiogenic effects, contributing to myocardial injury and delayed recovery of ventricular function (Hilfiker-Kleiner et al., 2007; Kodogo et al., 2023). Despite advances in imaging, PPCM remains diagnostically challenging. Several pregnancy-related conditions, including anaemia, hypertensive disorders, and myocarditis, may present with overlapping clinical and echocardiographic features. Previous studies suggest that up to 30% of women initially diagnosed with PPCM have alternative or transient causes of cardiac dysfunction (Bauersachs et al., 2019; Honigberg et al., 2019; Bello et al., 2019). This overlap underscores the risk of overdiagnosis and highlights the importance of a structured differential diagnostic approach.

Objective: To determine the true incidence of peripartum cardiomyopathy among pregnant women with suspected disease and to evaluate trimester-related changes in echocardiographic parameters compared with healthy controls in a prospective cohort study.

MATERIALS AND METHODS

This prospective observational study was conducted at the Research Institute of Cardiology of the Ministry of Health of Azerbaijan between 2023 and 2024.

Study groups:

- Group I (n=20): first trimester (8–12 weeks), with follow-up 6 months postpartum
- Group II (n=20): second trimester (24–28 weeks)
- Group III (n=20): third trimester (36 weeks)
- Control group (n=15): healthy pregnant women

Echocardiography was performed using a Mindray BeneHeart R12 system. Measurements

included LVEF, left ventricular end-diastolic and end-systolic dimensions, E/A ratio, and tissue Doppler indices.

Statistical analysis was performed using IBM SPSS 26.0. Continuous variables were compared using Student's *t*-test or one-way ANOVA, and categorical variables using the χ^2 test. A p-value <0.05 was considered statistically significant.

RESULTS

Clinical characteristics differed significantly across study groups. Women examined in the first and second trimesters reported predominantly mild symptoms, limited to increased fatigue and exertional dyspnoea. In contrast, patients evaluated in the third trimester exhibited more pronounced manifestations, including dyspnoea with minimal exertion, lower extremity oedema, and tachycardia, with a mean heart rate of 94±7 beats per minute. During the early postpartum period, hospitalisation for clinical deterioration was required in 16.7% of women in group I, 33.3% in group II, and 53.3% in group III (χ^2=10.7; p=0.041), indicating a progressive increase in the risk of decompensation with advancing gestation. Echocardiographic parameters demonstrated significant intergroup differences. In the control group, left ventricular ejection fraction (LVEF) showed a modest decline consistent with physiological adaptation to pregnancy, decreasing from 63.2±4.1% in the first trimester to 58.9±5.0% in the third trimester (p > 0.05). In contrast, LVEF was significantly reduced in all study groups: 42.8±4.9% in group I, 39.5±5.3% in group II, and 37.2±5.1% in group III (p<0.001 vs. controls). The difference between groups I and III was statistically significant (p=0.018), with the greatest systolic impairment observed in the third trimester (37.2% vs. 58.9% in controls; t=9.17; p<0.001). Analysis of cardiac chamber dimensions confirmed progressive left ventricular dilatation in women with suspected PPCM. Left ventricular end-diastolic diameter increased from 57.3±4.8 mm in group I to 61.5±5.4 mm in group III, significantly exceeding control values (50.2±3.9 mm; p<0.001). Similarly, end-systolic diameter was elevated across study groups, ranging from 41.2±4.1 mm to 46.5±5.0 mm, compared with 33.1±3.0 mm in healthy pregnant women (p<0.001). Assessment of diastolic function revealed a consistent trend toward impairment among women with suspected PPCM. While the control group demonstrated normal myocardial relaxation (E/A ratio 1.21±0.12), significantly lower E/A ratios were observed in group I (0.81±0.09), group II (0.79±0.07), and group III (0.72±0.08; all p<0.001 vs. controls). Diastolic dysfunction (E/A<1) was present in all women in group III, whereas no cases were identified in the control group (χ^2=35.6; p<0.001). True peripartum cardiomyopathy was confirmed in only four women (6.7% of the total cohort): one patient from group II with disease manifestation in the third trimester and three patients from group III (15%). In the remaining cases, clinical and echocardiographic abnormalities were attributed to alternative diagnoses, most commonly anaemia (20–40%), gestational hypertension or pre-eclampsia (25–35%), and myocarditis (10%). Thus, the majority of cases initially suspected as PPCM were ultimately explained by transient or potentially reversible conditions (Tables 1 and 2).

DISCUSSION

Our findings indicate that clinical symptoms of heart failure in pregnant women with suspected peripartum cardiomyopathy (PPCM) increased with advancing gestation; however, true PPCM was confirmed in only 6.7% of cases. This aligns with data from international registries (IPAC, ESC), which report a prevalence of 4–10% among women initially suspected of PPCM (McNamara et al., 2015; Regitz-Zagrosek et al., 2018; Bauersachs et al., 2019; Sliwa et al., 2020). Consequently, the potential for overdiagnosis remains substantial, underscoring the importance of a meticulous differential diagnosis. Left ventricular ejection fraction (LVEF) was significantly lower in the study groups compared with controls, with the most pronounced reduction observed in the third trimester (37.2% vs. 58.9%; p<0.001), confirming the key diagnostic criterion of LV systolic dysfunction (Honigberg and Givertz, 2019; Davis et al., 2023). Notably, some women exhibited reduced EF without typical clinical symptoms, reflecting physiological cardiac adaptations to pregnancy. Similar observations have been reported by Bello et al. (2019), where up to 30% of suspected PPCM cases were 'false positives' (Bello et al., 2019).

Diastolic dysfunction (E/A<1) was prevalent, particularly in group III (100%), significantly exceeding control values (χ^2=35.6; p<0.001). This may represent a combination of true myocardial involvement in PPCM and the hemodynamic changes associated with late pregnancy, consistent with findings by Ersbøll et al. (2022) (Ersbøll et al., 2022).

Table 1. Echocardiographic parameters in pregnant women with suspected PPCM and in the control group

Parameter	Control (n=15)	Group I (n=20)	Group II (n=20)	Group III (n=20)	F (ANOVA)	P
LVEF, %	63.2±4.1 → 58.9±5.0	42.8±4.9	39.5±5.3	37.2±5.1	42.6	<0.001
LVEDD, mm	50.2±3.9	57.3±4.8	59.1±5.0	61.5±5.4	31.4	<0.001
LVESD, mm	33.1±3.0	41.2±4.1	44.0±4.7	46.5±5.0	29.7	<0.001
E/A ratio	1.21±0.12	0.81±0.09	0.79±0.07	0.72±0.08	38.2	<0.001

Note: *Data are presented as mean±SD. Intergroup comparisons were performed using one-way analysis of variance (ANOVA). All differences vs. control are statistically significant*

Table 2. Clinical characteristics and outcomes during follow-up

Parameter	Group I (n=20)	Group II (n=20)	Group III (n=20)	Control (n=15)	χ^2	P
Heart failure symptoms (NYHA II–III)	3 (15%)	5 (25%)	11 (55%)	0	12.4	0.006
Hospitalizations (decompensation)	3 (16.7%)	7 (33.3%)	11 (53.3%)	0	10.7	0.041
Confirmed PPCM	0	1 (5%)	3 (15%)	0	4.6	0.032
Alternative diagnoses	Anemia 8 (40%); adaptation 5 (25%)	Anemia 6 (30%); GH/PE 7 (35%)	GH/PE 5 (25%); myocarditis 2 (10%)	---	---	---

Abbreviations: *PPCM — peripartum cardiomyopathy; GH/PE – gestational hypertension/preeclampsia.*

Alternative etiologies, including anaemia, gestational hypertension, pre-eclampsia, and viral myocarditis, were more frequently responsible for cardiovascular manifestations than PPCM. In resource-limited settings such as Azerbaijan, anaemia can induce hyperdynamic circulation that mimics heart failure, while hypertensive disorders of pregnancy can transiently impair diastolic function and produce clinical signs of heart failure (Regitz-Zagrosek et al., 2018; Bauersachs et al., 2019; Davis et al., 2023).

Taken together, our data suggest that a reliable diagnosis of PPCM requires the coexistence of severe LV systolic dysfunction (EF<45%), ventricular dilatation, and clinical signs of congestive heart failure. Otherwise, observed changes should be interpreted as physiological adaptations or manifestations of other pathologies. This approach aligns with the 2019–2020 European Society of Cardiology (ESC) recommendations (Regitz-Zagrosek et al., 2018; Sliwa et al., 2020), helping to minimize overdiagnosis, avoid unnecessary therapy, and focus on treating the underlying cause of decompensation. In summary, PPCM remains rare (≤10%), and most cases initially suspected are attributable to transient or reversible conditions. Rigorous differential diagnosis and dynamic echocardiographic monitoring are therefore essential.

CONCLUSION

1. Among 60 pregnant women with suspected peripartum cardiomyopathy (PPCM), only 4 cases (6.7%) were confirmed, one in group II (third-trimester onset) and three in group III (15%). The remaining abnormalities were attributable to anaemia (20–40%), gestational hypertension or pre-eclampsia (25–35%), and myocarditis (10%).
2. Clinical symptoms and risk of decompensation increased with gestational age. Heart failure signs were observed in 15% of group I, 25% of group II, and 55% of group III (χ^2=12.4; p=0.006), while hospitalisations rose from 16.7% to 53.3% (χ^2=10.7; p = 0.041).
3. Echocardiographic findings in the third trimester differed markedly from controls: mean LVEF was 37.2±5.1% versus 58.9±5.0% (p<0.001), end-diastolic diameter reached 61.5±5.4 mm versus 50.2±3.9 mm (p<0.001), and diastolic dysfunction (E/A <1) was observed in 100% versus 0% of controls (χ^2=35.6; p<0.001).
4. Most observed cardiac changes were transient, reflecting physiological adaptations to pregnancy, often compounded by anaemia or hypertensive disorders, highlighting the importance of a careful differential diagnosis.
5. True PPCM remains rare (≤10%), while the majority of cardiovascular abnormalities in pregnancy are reversible or secondary. Accurate diagnosis requires the combination of significant LV systolic dysfunction (EF <45%), ventricular dilatation, and clinical signs of congestive heart failure to avoid overdiagnosis and unnecessary interventions.

ETHICAL CONSIDERATIONS

This study was conducted in accordance with the ethical principles of the Declaration of Helsinki and relevant national regulations governing research involving human participants. Ethical approval was obtained from the Ethics

Committee of Azerbaijan Medical University before the initiation of the study. All participants were fully informed about the purpose, procedures, potential benefits, and risks of the study, and written informed consent was obtained from each participant before enrollment. Participation was voluntary, and participants were assured of their right to withdraw from the study at any stage without any impact on their medical care. All collected data were anonymized and handled with strict confidentiality.

ACKNOWLEDGEMENTS

The authors would like to express their sincere gratitude to the medical and technical staff of the Department of Obstetrics and Gynaecology No. I, Azerbaijan Medical University, for their invaluable assistance in patient recruitment, echocardiographic assessments, and data collection. Special appreciation is extended to all pregnant women who generously participated in this study.

FUNDING

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

CONFLICT OF INTEREST

The authors declare that they have no known competing financial or personal interests that could have appeared to influence the work reported in this paper.

REFERENCES

Bauersachs J., König T., van der Meer P., Hilfiker-Kleiner D., Mbakwem A., Hamdan R. et al. (2019) Pathophysiology, diagnosis and management of peripartum cardiomyopathy: A position statement from the ESC Working Group. *European Heart Journal,* **40(47):** 3828–3837; doi: 10.1093/eurheartj/ehz767

Bello N.A., Arany Z., Halkein J., Rauch B., Borlaug B.A., Colvin-Adams M. et al. (2019) The relationship between parity and peripartum cardiomyopathy. *Journal of the American College of Cardiology,* **73(5):** 463–472; doi: 10.1016/j.jacc.2018.10.065

Davis M.B., Arany Z., McNamara D.M., Goland S., Elkayam U. (2023) Peripartum cardiomyopathy: JACC state-of-the-art review. *Journal of the American College of Cardiology,* **81(4):** 412–434; doi: 10.1016/j.jacc.2022.10.023

Ersbøll A.S., Johansen M., Damm P., Rasmussen T.B., Gustafsson F., Køber L. et al. (2022) Peripartum cardiomyopathy: Epidemiology, risk factors and outcomes. *Nature Reviews Cardiology,* **19(6):** 363–379; doi: 10.1038/s41569-021-00660-7

Hilfiker-Kleiner D., Kaminski K., Podewski E., Bonda,T., Schaefer A., Sliwa K. et al. (2007) A cathepsin D–cleaved 16 kDa form of prolactin mediates postpartum cardiomyopathy. *Cell,* **128(3):**589–600; doi: 10.1016/j.cell.2006.12.036

Honigberg M.C., Givertz M.M. (2019) Peripartum cardiomyopathy. *BMJ,* **364*:*** k5287; doi: 10.1136/bmj.k5287

Kodogo V., Sliwa K., Ntusi N.A.B. (2023) Role of cathepsin D in the pathophysiology of peripartum cardiomyopathy. *Heart Failure Reviews,* **28:** 531–542; doi: 10.1007/s10741-022-10260-5

McNamara D.M., Elkayam U., Alharethi R., Damp J., Hsich E., Ewald G. et al. (2015) Clinical outcomes for peripartum cardiomyopathy in North America: Results of the IPAC study. *Journal of the American College of Cardiology,* **66(8):** 905–914; doi: 10.1016/j.jacc.2015.06.1309

Regitz-Zagrosek V., Roos-Hesselink J.W., Bauersachs J., Blomström-Lundqvist C., Cífková R., et al. (2018) ESC guidelines for the management of cardiovascular diseases during pregnancy. *European Heart Journal,* **39(34):** 3165–3241; doi: 10.1093/eurheartj/ehy340

Sliwa K., Hilfiker-Kleiner D., Petrie M.C., Mebazaa A., Pieske B., Buchmann E. et al. (2020) Current state of knowledge on aetiology, diagnosis, management, and therapy of peripartum cardiomyopathy: A position statement from the Heart Failure Association. *European Journal of Heart Failure,* **22(6):** 1270–1291; doi: 10.1002/ejhf.1906

ORCIDs:
Nigar Kamilova: https://orcid.org/0000-0002-7443-1503
Khatira Mirzoeva: https://orcid.org/0009-0007-5418-6030

Ethnobotanical overview of selected *Asteraceae* species

Salman Majeed[1*], Fakhra Bibi[1], Yusra Khan[1], Sehrish Rubab[1], Muhammad Zafar[2]

[1]*Department of Botany, University of Mianwali, 42200, Mianwali, Pakistan*
[2]*Department of Plant Sciences, Faculty of Biological Sciences, Quaid-i-Azam University, 45320, Islamabad, Pakistan*

[*]***For correspondence:*** *salmansunny61@gmail.com*

Received: November 18, 2025; Reviewed: December 15, 2025; Accepted: December 24, 2025

This study has reported the overview of the ethnobotanical value of a selected species of Asteraceae used for traditional plant-based medicines. The published ethnobotanical literature was compiled to compare the medicinal uses, the mode of administration and the therapeutic relevance of taxa that commonly occur. The species reviewed had been mostly applied in treating inflammatory diseases, infections, digestive diseases and skin-related diseases. The most common preparation methods were also found to be the decoction, infusion, and topical pastes, a fact that shows the availability and ease of use of traditional measures in health care. This overview proved that the representatives of the Asteraceae family have significant ethnomedicinal potential and remain essential in the rural health care systems. This study overviews the value of conservation of traditional knowledge and offers a scientific foundation for further phytochemical as well as pharmacological research of the medicinal plants.

Keywords: *Asteraceae, disorders, decoctions, ethnomedicine, medicinal plants*

INTRODUCTION

The family *Asteraceae (Compositae)* is one of the most diverse and large flowering plant families that contains over 32,000 species spread across the globe (Devkota, 2022). This family is ecologically diverse and is highly used in the traditional medical systems because of their good phytochemical content, such as flavonoids, sesquiterpene lactones, phenolics and essential oils. These compounds play a role in a diverse range of biological activities, which include anti-inflammatory, anti-microbial, anti-diabetic, hepatoprotectic, and anti-cancer (Kazeminia et al., 2022).

Knowledge about the medicinal usages of Asteraceae has been passed down through generations, especially in rural and indigenous communities where plant-based remedies continue to play a primary role in healthcare (Rolnik & Olas, 2022). The species of this family are usually utilized in the treatment of gastrointestinal diseases, skin infections, respiratory diseases, fever, and metabolic diseases. Nonetheless, regardless of the vast traditional application of these species, the ethnobotanical data on most species are dispersed, thus necessitating systematic documentation (Sharma et al., 2022).

The current research paper aimed to collect and review the ethnobotanical knowledge of selected species of the family Asteraceae, focusing on their traditional medicinal uses, growth, and therapeutic applications as described in earlier ethnobotanical studies.

MATERIALS AND METHODS

The present study was based on a comprehensive review of ethnobotanical literature related to selected Asteraceae species. Data were collected from peer-reviewed research articles, ethnobotanical surveys, floras, books, and online scientific databases, such as Google Scholar, Scopus, and PubMed. Keywords including *Asteraceae*, *ethnobotany*, *traditional medicine*, *medicinal uses*, and individual plant names were used to retrieve relevant literature. Information regarding plant habit, common names, and ethnomedicinal applications was extracted, compiled, and critically analyzed to ensure accuracy and consistency.

RESULTS

The ethnobotanical descriptions of the selected Asteraceae species revealed a wide range of medicinal applications across different therapeutic categories. The most frequently reported use was

for anti-inflammatory purposes, documented in 15 species (Figure 1). Antimicrobial and wound-healing uses were also common, each reported for nine species. They were used in digestive disorder treatment with seven species and four species of the genus were reported to have hepatoprotective, antioxidant, and diuretic uses. Fewer species were linked to the antidiabetic, sedative, anti-hemorrhagic, and hair-growth-promoting attributes. These data show that the plants of the Asteraceae family are widely used in traditional healthcare systems to treat inflammatory and infectious diseases and wounds.

The modes of administration (Table; Figure 2) were analyzed and found that the most frequently used form of preparation was decoction in 11 species, then paste or poultice use in eight species and infusion prep in seven species. Five species were being reported on extract-based preparations, and five to six were reported on juice, seed-based preparations, and whole-plant use. The prevalence of decoctions and topical pastes indicates a heavy dependence on water-based methods of extraction that are simple to use, indicating that there is a heavy dependence on knowledge systems based on the traditional that can be prepared using readily available and affordable means.

Table. Ethnobotanical overview of the selected Asteraceae species.

Species	Habit	Traditional Medicinal Uses	Mode of Preparation / Administration	Citation
Ageratum conyzoides L.	Annual herb	Wound healing, fever, antimicrobial	Leaf paste applied topically; decoction taken orally	Oyeniyi et al. (2025)
Bidens pilosa L.	Annual herb	Anti-inflammatory, antidiabetic	Decoction or infusion of whole plant	Yang, (2014)
Calendula officinalis L.	Annual herb	Skin disorders, ulcers	Flower ointment and infusion	Givol et al. (2019)
Calendula stellata Cav.	Annual herb	Antimicrobial, wound healing	Leaf paste and decoction	Patil et al. (2022)
Carthamus lanatus L.	Annual herb	Digestive ailments, inflammation	Seed infusion	Popov & Kang (2011)
Centaurea calcitrapa L.	Biennial herb	Antimicrobial, tonic	Aerial part decoction	Mekky et al. (2024)
Cichorium intybus L.	Perennial herb	Liver tonic, digestive aid	Root decoction	Das et al. (2016)
Cirsium arvense (L.) Scop.	Perennial herb	Diuretic, anti-inflammatory	Whole plant decoction	Aggarwal et al. (2022)
Dahlia pinnata Cav.	Perennial herb	Skin ailments, inflammation	Leaf paste	Lim, (2013)
Eclipta prostrata (L.) L.	Annual herb	Liver disorders, hair growth	Juice and paste	Tripathy et al. (2024)
Erigeron canadensis L.	Annual herb	Diuretic, anti-hemorrhagic	Infusion of aerial parts	Sharma et al. (2014)
Gazania linearis (Thunb.) Druce	Perennial herb	Anti-inflammatory	Decoction	El Kady et al. (2015)
Gazania rigens L.	Perennial herb	Anti-inflammatory	Decoction	Samy et al. (2025)
Helianthus annuus L.	Annual herb	Anti-inflammatory, diuretic	Seed and leaf decoction	Singh et al. (2022)
Lactuca serriola L.	Annual herb	Sedative, analgesic	Infusion	Abdul-Jalil, (2020)

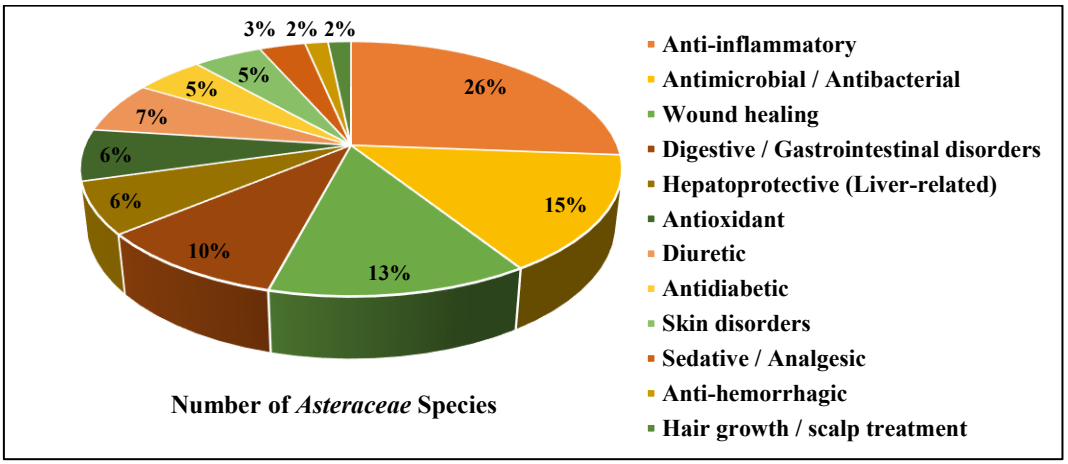


Fig. 1. Pie graph showing the overview of *Asteraceae* species used to treat different ailments.

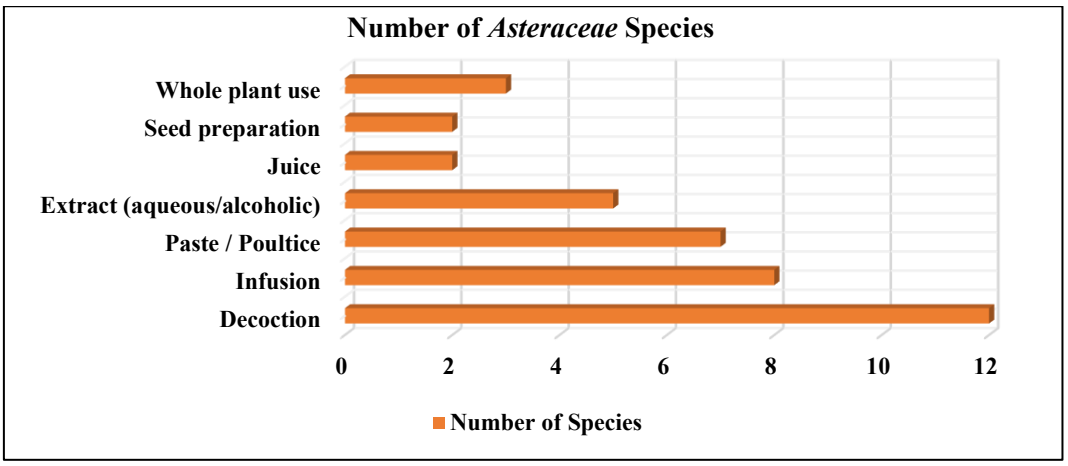


Fig. 2. Bar graph overview of the mode of administration utilized by selected Asteraceae species.

CONCLUSION

This study showed that the Asteraceae plants can be considered a good source of traditional medicine. The recorded species were highly utilized in the treatment of inflammatory diseases, infections, digestive ills and skin-related illnesses, and most of the treatment methods were based on decoction and topical application. The study described from the literature the high reliance on plant-based remedies since they are easily available and effective in treatment. The uniform ethnomedicinal application of such species justifies their future pharmacological validation as well as the isolation of bioactive compounds.

ACKNOWLEDGEMENTS

The authors express their sincere gratitude to the Department of Botany, University of Mianwali, Pakistan, and the Department of Plant Sciences, Quaid-i-Azam University, Islamabad, for providing academic support and access to scientific literature and research resources. The authors also acknowledge the contributions of earlier researchers whose ethnobotanical documentation formed the foundation of this review.

ETHICAL CONSIDERATIONS

The authors have carefully respected ethical standards related to the use of traditional knowledge by appropriately acknowledging all sources and avoiding misrepresentation or misuse of indigenous ethnobotanical information.

AUTHOR CONTRIBUTIONS

- *Salman Majeed*: Conceptualization, literature survey, data synthesis, manuscript drafting, and correspondence.
- *Fakhra Bibi*: Data compilation, analysis of ethnobotanical uses, and manuscript review.
- *Yusra Khan*: Interpretation of medicinal applications and preparation methods.
- *Sehrish Rubab*: Organization of reviewed taxa and contribution to methodological structure.
- *Muhammad Zafar*: Supervision, critical revision of the manuscript, and scientific validation.

All authors have read and approved the final version of the manuscript.

FUNDING

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest, whether financial, personal, or academic, that could have influenced the work reported in this paper.

REFERENCES

Abdul-Jalil T.Z. (2020) *Lactuca serriola*: short review of its phytochemical and pharmacological profiles. *International Journal of Drug Delivery Technology*, **10(3):** 505-508.

Aggarwal G., Kaur G., Bhardwaj G., Mutreja V., Sohal H.S., Nayik G.A., Sharma A. (2022) Traditional uses, phytochemical composition, pharmacological properties, and the biodiscovery potential of the genus Cirsium. *Chemistry*, **4(4):** 1161-1192.

Das S., Vasudeva N., Sharma S. (2016) Cichorium intybus: A concise report on its ethnomedicinal, botanical, and phytopharmacological aspects. *Drug Development and Therapeutics*, **7(1):** 1-1.

Devkota H.P., Aftab T. (2022) Medicinal plants of the *Asteraceae* family: Traditional uses, phytochemistry and pharmacological activities, 1st ed., 239 p.

El Kady W.M., Salama A.A., Desoukey S.Y., Hagag E.G., El-Shenawy S.M., El-Shanawany M.A. (2015) Comparative DNA profiling, botanical identification and biological evaluation of *Gazania longiscapa* DC and *Gazania rigens* L. *Bulletin of Faculty of Pharmacy, Cairo University*, **53(2):** 129-145.

Givol O., Kornhaber R., Visentin D., Cleary M., Haik J., Harats M. (2019) A systematic review of Calendula officinalis extract for wound healing. *Wound Repair and Regeneration*, **27(5):** 548-561.

Kazeminia M., Mehrabi A., Mahmoudi R. (2022) Chemical composition, biological activities, and nutritional application of Asteraceae family herbs: A systematic review. *Trends in Phytochemical Research*, **6(3):** 187-213.

Lim T.K. (2013) *Dahlia pinnata*. In: *Edible Medicinal and Non-Medicinal Plants:* **Volume 7:** *Flowers,* pp. 333-339. Dordrecht: Springer Netherlands.

Mekky A.E., Saied E., Abdelmouty E.S., Haggag M.I., Khedr M., Khalel A.F., Mahmoud N.N. (2024) Phytochemical analysis of *Centaurea calcitrapa* L. aerial flowering parts serial solvent extracts and its antibacterial and antioxidant activities. *Life*, **14(7):** 900.

Oyeniyi O., Aghedo O.G., Akwolu C.C., Uwaoma C.P.K. (2025) Ethnobotanical survey of medicinal plants used in the treatment of skin infections in ogun and oyo states, Nigeria. *Journal of Public Health*, **02(01-2025):** 1-9.

Patil K., Sanjay C.J., Doggalli N., Devi K.R., Harshitha N. (2022) A review *of Calendula officinalis* - magic in science. *Journal of Clinical & Diagnostic Research*, **16(2):** ZE23-ZE27.

Popov A.M., Kang D. (2011) Analgesic and other medicinal properties of Safflower (*Carthamus tinctorius* L.) seeds. In: *Nuts and seeds in health and disease prevention*. Academic Press: pp. 995-1002.

Rolnik A., Olas B. (2021) The plants of the *Asteraceae* family as agents in the protection of human health. *International journal of molecular sciences*, **22(6):** 3009.

Samy M.N., Kemelbek M., Gomaa A.A.R., Darwish A.G., Burasheva G.S., Ross S.A. (2025) Isolation and chemical characterization of secondary metabolites from *Gazania rigens* and their antimicrobial and cytotoxic activities. *Natural Product Research*, **39(19):** 5722-5726.

Sharma R.K., Verma N., Jha K.K., Singh N.K., Kumar B. (2014) Phytochemistry, pharmacological activity, traditional and medicinal uses of *Erigeron* species: A review. *IJARI*, **2:** 379-383.

Sharma M., Sharma M., Bithel N., Sharma M. (2022) Ethnobotany, phytochemistry, pharmacology and nutritional potential of medicinal plants from *Asteraceae* family. *Journal of Mountain Research*, **17(2):** 67-83.

Singh A., Pandey S., Srivastava R.P., Devkota H.P., Singh L., Saxena G. (2022) *Helianthus annuus* L.: traditional uses, phytochemistry, and pharmacological activities. In: *Medicinal Plants of the Asteraceae Family: Traditional Uses, Phytochemistry and Pharmacological Activities.* Singapore: Springer Nature Singapore: pp. 197-212.

Tripathy S., Singh J.P., Tripathi A., Srivastava S., Chaurasia V.K., Kumar R., Pandey S. (2024) A review on the pharmacological, biological, chemical and therapeutic value of Eclipta prostrate (Bhringraj plant). *Biochemical & Cellular Archives*, **24(2):** 3011-3026.

Yang W.C. (2014) Botanical, pharmacological, phytochemical, and toxicological aspects of the antidiabetic plant *Bidens pilosa* L. *Evidence-Based Complementary and Alternative Medicine*, **2014(1):** 698617.

ORCIDs

Salman Majeed: https://orcid.org/0000-0002-3143-4421
Fakhra Bibi: https://orcid.org/0009-0004-1155-9261
Yusra Khan: https://orcid.org/0009-0004-1533-1491
Sehrish Rubab: https://orcid.org/0009-0009-5119-3598
Muhammad Zafar: https://orcid.org/0000-0003-2002-3907

Biological characterization of thermophilic bacterial strains from hot springs in the Republic of Azerbaijan

Gunay Abbasli*, Farayat Ahmadova

Baku State University, 33 Academician Zahid Khalilov Str., AZ1148, Baku, Azerbaijan

***For correspondence:** gunay_a-va1995@mail.ru*

Received: September 29, 2025; Received in revised form: November 20, 2025; Accepted: December 11, 2025

The work is devoted to the study of the biological properties of strains of thermophilic bacteria isolated from hot springs of Azerbaijan for their taxonomic identification. Strain B1 was isolated from the Babazanan hot spring in the Salyan district, strains KA2 and KY2, from the Ashagi Istisu and Yukhari Istisu springs in the Kalbajar district, respectively. As a result of studying the morphology of the strains, it was established that the bacterial cells are gram-positive rods that form endospores. The cultural properties of the strains were generally similar, characterized by a milky color, round shape, raised surface, wavy edges and soft consistency. Physiological studies have shown that the strains efficiently utilize organic acids, alcohols, and sugars as carbon sources. They also demonstrate effective utilization of both organic and inorganic nitrogen sources. The optimal temperature for the growth of strains was 55-60°C, and the optimal pH value was 7.0-9.0. According to biochemical characteristics, the strains were catalase-positive, while oxidase activity was absent. Proteolytic activity was observed in strains KA2 and KY2 but not in strainB1.The absence of hydrogen sulfide synthesis indicates that these strains are safe for use in biotechnological purposes. As a result of a general analysis of morphological, cultural, physiological and biochemical characteristics, the isolates were attributed to the genus *Bacillus*. The adaptation of these bacteria to extremophilic conditions, along with their non-pathogenic nature and metabolic flexibility, forms a solid foundation for their application in biotechnology and nanobiotechnology – particularly in the environmentally safe synthesis of metal nanoparticles.

Keywords: *Genus Bacillus, morphological characterization, thermophilic enzymatic activity, physiological traits of thermophiles, biochemical characterization*

INTRODUCTION

Thermophilic bacteria are garnering increasing attention from researchers due to their remarkable ability to thrive in extreme environmental conditions. These microorganisms are predominantly found in hot springs and have a number of unique physiological and biochemical adaptations (Aanniz et al., 2015; Marzban & Tesei, 2025).

One of the most promising areas of application of thermophilic bacteria is the biological synthesis of metal nanoparticles. Unlike traditional chemical and physical methods, biological synthesis allows obtaining nanoparticles without the use of toxic reagents and harsh physical conditions (Iravani, 2014; Deljou & Goudarzi, 2016). Even though there are other methods of biological synthesis of nanoparticles, such as their accumulation in various components of ecosystems, including plants, fish, mollusks and parasites (Hadjiyeva et al., 2024; Rzayev et al., 2022), the use of bacteria for these purposes is more promising due to their availability and wide distribution, high growth rate and large amount of biomass they form (Gunashova, 2022; Sulaiman et al., 2018).

Azerbaijan has a rich reservoir of thermal water springs, which are located in various regions of the Republic. Thermal springs such as Ashagi Istisu and Yukhari Istisu in the Kalbajar district, as well as Babazanan in the Salyan district, are characterized by high temperatures and rich mineral composition, which makes them a favorable environment for the development of thermophilic microflora (Ahmadova, 2007; Kamal, 2012; Gunashova et al., 2021).

This work aims to conduct a detailed investigation of the morphological, cultural, physiological and selected biochemical characteristics of thermophilic bacterial strains isolated from the aforementioned water sources in Azerbaijan, to identify them at the genus level.

MATERIALS AND METHODS

This paper will examine the morphological, cultural, physiological and biochemical properties

of strains B1, KA2 and KY2. Methods for isolating pure cultures of these strains and assessing their ability to synthesize silver nanoparticles are described in detail in our previous publications (Gunashova et al., 2021; Gunashova et al., 2021; Gunashova, 2022).

Morphological characteristics. The morphological features of the studied strains were studied using light microscopy (XSP-30 series, China). The bacterial cells were observed at 100× magnification using oil immersion. Observations were made on fixed, Gram-stained slides, as well as using the hanging drop method to assess cell motility. Samples were prepared from fresh cultures grown on nutrient agar at 60°C for 24 hours.

Cultural characteristics. Cultural characteristics were observed on colonies on the surface of nutrient agar and in nutrient broth. Cultures were incubated at 60°C for 24–48 hours. The growth pattern, colony morphology and pigmentation, consistency, sediment formation, surface film, and turbidity of the medium were carefully assessed.

Physiological and biochemical characteristics. The ability of the strains to utilize various carbon and nitrogen sources was assessed using Smith medium, supplemented with different substrates as the sole carbon or nitrogen source.

The names of the sources are given in Tables 2 and 3. Smith's medium without the addition of any carbon or nitrogen source was used as a control to assess the baseline optical density (OD). After inoculation of the strains into the appropriate medium, incubation was carried out at 60°C for 24 hours. The OD of the bacterial suspensions was measured both before incubation, to determine the initial turbidity, and after incubation, to estimate biomass accumulation depending on the carbon and nitrogen sources. The measurements were carried out in triplicate using a Jenway 7315 spectrophotometer (UK) at a wavelength of 600 nm and the results are presented as mean values.

Catalase activity was tested by adding a drop of 10% hydrogen peroxide solution to the bacterial biomass. The appearance of oxygen bubbles indicated a positive reaction.

Oxidase activity was determined using test strips impregnated with tetramethyl-p-phenylenediamine. A color change to blue or violet within 30 seconds indicated a positive result.

Proteolytic activity was assessed by the ability to hydrolyze casein on milk-casein agar. After incubation at 60°C for 24 hours, the formation of transparent zones around the colonies proved the cleavage of casein.

Carbohydrate fermentation was also tested on triple sugar iron (TSI) agar containing glucose, lactose, sucrose, phenol red indicator and sodium thiosulfate. After inoculation and incubation at 60°C for 24-48 h, colour change, gas formation and formation of a black precipitate (H_2S) were recorded.

The ability to utilize L-rhamnose, D-xylose, and lysine was assessed using differential media containing each compound as the sole carbon source (L-rhamnose, D-xylose) or sole amino acid source (lysine). The criteria for a positive reaction were bacterial growth and a change in indicator color. To determine the optimal conditions for the strains, the temperature and pH range of growth were studied. To determine the pH range, the strains were inoculated into nutrient broths with different pH values (5.0-11.0) and incubated at 60°C for 24 hours. The temperature range was determined by inoculating the strains into a nutrient broth with a neutral pH of 7.0. Incubation was carried out at different temperatures in the range from 35°C to 75°C. The growth of strains at different temperatures and pH values was determined by spectrophotometric measurement of the OD of bacterial suspensions.

RESULTS

Morphological characteristics. Microscopic analyses of the morphology of bacterial strain cells showed that the cells of all strains were gram-positive rods, the sizes of which varied from 0.9 to 4.5 μm in length and from 0.6 to 1.2 μm in width. The cells were mostly single rods and were predominantly motile (Fig. 1, Table 1). Endospore formation was observed in older cultures or under conditions unfavorable for thermophilic bacteria.

Cultural characteristics. The cultural properties of the strains were studied both on nutrient agar and in nutrient broth. Milky, beige colonies were observed on nutrient agar with a round or irregular shape, slightly convex profile, wavy edges and soft consistency. In nutrient broth, growth was manifested as moderate turbidity and visible sediment formation (Table 1).

Physiological and biochemical studies. Among all the studied substrates, the greatest bacterial growth was observed when using organic acids. Pyruvic acid provided the highest OD values: 1.52 for strain KA2, 1.44 for KY2 and 1.12 for B1. High utilization was also observed with the addition of citric acid and oxalic acid. Among sugars, good utilization was observed for sucrose and glucose, while lactose was not assimilated by any of the strains. Strain KY2 demonstrated active utilization of galactose (OD - 1.00), which is characterized by the specific enzymatic activity of the strain. Interesting results were obtained in the analysis of alcohols: glycerol and ethanol were actively metabolized by

all strains, especially strain B1. This may be an indicator of the presence of thermostable dehydrogenase systems in these bacteria. The ability of strains to utilize various carbon-containing substrates is presented in Table 2.

As a result of studying the ability of three thermophilic bacterial strains KA2, KY2 and B1 to use various nitrogen sources, it was found that among all the studied compounds, the greatest bacterial growth was observed when using peptone. Peptone provided the maximum OD values: 1.88 for KA2, 1.44 for KY2 and 1.76 for B1. High nitrogen utilization rates were also recorded for ammonium sulphate and potassium nitrate, demonstrating efficient uptake of both inorganic and organic nitrogen sources. Moderate growth was observed with tryptone and asparagine, while methionine supported comparatively lower growth, indicating the strains' specific requirements for nitrogen sources. The control variant showed the lowest OD (0.32), indicating no growth in the absence of nitrogen sources. Data reflecting the response of the strains to various nitrogen sources are presented in Table 3.

Biochemical analysis of three bacterial strains (B1, KA2 and KY2) showed that all of them exhibited catalase-positive activity, while none of the strains exhibited oxidase activity, which was confirmed using a positive control (*Pseudomonas aeruginosa*) and a negative control (*Escherichia coli*). Protease activity, assessed using milk agar, was observed in strains KA2 and KY2, which was manifested by the formation of clear lysis zones around their colonies. In contrast, strain B1 did not have such zones, indicating the absence of proteolytic activity.

When cultured on triple sugar iron agar (TSI), all three strains produced yellow coloration at both the bottom and top of the agar, indicating fermentation of glucose and sucrose. No black precipitate was found in the tube, indicating a negative result for hydrogen sulfide (H_2S) production in all tested strains. Further testing on media containing D-xylose and L-rhamnose revealed no colour change, demonstrating the inability of the strains to ferment these sugars. This indicates the absence of the necessary enzymatic pathways to metabolise D-xylose and L-rhamnose and therefore the inability to use them as carbon sources.

Lysine decarboxylase activity was assessed using lysine broth initially stained purple. Strains B1 and KA2 retained the purple color, indicating a positive decarboxylation reaction, while KY2 showed a color change to yellow, indicating a negative result. The accuracy of these observations was confirmed using control microorganisms: *Escherichia coli* (positive control) and *Staphylococcus aureus* (negative control).

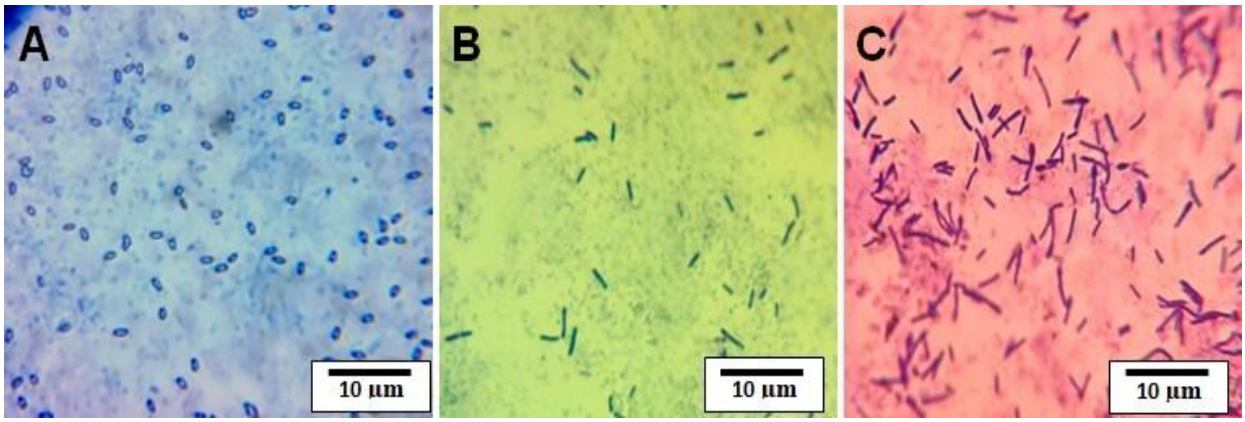


Fig. 1. Cells of 24-hour bacterial strains under a light microscope (scale bar=10 µm; 100×)
A) cells of strain B1 B) cells of strain KA2 C) cells of strain KY2.

Table 1. Morphological and cultural characteristics of thermophilic bacterial strains.

Morphological characteristics of thermophilic bacterial strains				
Bacterial strains	Cell shape	Cell size, µm	Cell motility	Types of flagella
B1	Rods	0.9-1.8x0.6-1	+	Peritrichous
KA2	Rods	1-2.8x0.5-0.8	-	-
KY2	Rods	1.7-3.8x0.6-0.9	+	Peritrichous

Cultural characteristics of thermophilic bacterial strains					
Bacterial strains	Colony color	Colony shape	Colony margin	Colony elevation	Colony consistency
B1	Milky	Round	Wavy	Raised	Soft
KA2	Milky	Round	Wavy	Raised	Soft
KY2	Milky	Round	Wavy	Flat	Soft

Table 2. Utilization of carbon sources by thermophilic bacterial strains isolated from hot springs of Kalbajar and Babazan.

Carbon source, 0.5%	Optical density of strains, UV spectrophotometer Jenway 7315, in (Smith) medium		
	KA2	KУ2	B1
Sugars			
Control variant	0.36	0.36	0.36
Glucose	0.64	0.76	0.92
Sucrose	0.76	0.84	0.92
Galactose	0.60	1.00	0.76
Lactose	0.36	0.36	0.36
Alcohols			
Ethanol	1.04	0.96	0.80
Mannitol	0.56	0.72	0.88
Glycerol	0.88	0.80	1.04
Salts of organic acids			
Oxalic acid	1.44	1.00	1.20
Malic acid	1.12	0.96	1.28
Citric acid	1.36	1.48	1.00
Pyruvic acid	1.52	1.44	1.12
Acetic acid	1.28	1.12	1.20

Table 3. Utilization of nitrogen sources by thermophilic bacterial strains isolated from hot springs of Kalbajar and Babazan.

Nitrogen source. 0.5%	Optical density of strains. UV spectrophotometer Jenway 7315, in (Smith) medium		
	KA2	KУ2	B1
Control (negative) variant	0.32	0.32	0.32
KNO_3	1.08	1.20	1.36
$(NH_4)_2SO_4$	1.60	1.52	1.68
Peptone	1.88	1.44	1.76
Methionine	0.80	0.96	1.24
Asparagine	1.00	1.16	1.44
Tryptone	1.20	1.28	1.52

Experimental data demonstrated a clear dependence of the growth of bacterial strains on the pH of the incubation medium. At extreme pH values of 5.0 and 11.0, the growth of all strains studied was significantly reduced or completely absent, indicating that these conditions are outside the physiologically acceptable range for maintaining the metabolic activity of thermophiles. However, when the pH increased to 10.0, growth decreased significantly (OD: B1 – 1.1, KY2 – 0.7), indicating the onset of the negative impact of excessive alkalinity. In contrast, strain KA2 showed maximum growth at pH 7.0 (OD – 1.75), although significant growth was also observed at pH 8.0 and 9.0 (1.4 and 1.1, respectively). At an acidic pH of 5.0, KA2 showed no growth, and at pH 6.0, only minimal growth (OD – 0.7) was observed, confirming its limited tolerance to acidic environments. The effect of pH on the growth of strains at a constant incubation temperature of 60°C, based on OD measurements, is shown in Fig. 2.

As a result of the analysis of the dependence of the OD of bacterial suspensions on temperature, it was established that the optimal temperature for the growth of all three strains was 60°C, at which the highest values of OD were observed. These results indicate that 60°C represents the optimal growth temperature for all three strains, with increased metabolic activity and biomass accumulation. At 35°C, only strain B1 showed moderate growth, while KY2 and KA2 showed no growth. At 75°C, growth was completely inhibited for strains B1 and KA2, and only minimal growth was detected for KY2, indicating that these temperatures are beyond the physiological capabilities of the strains. Significant growth was also observed at 55°C, especially for strain B1, although slightly lower growth values were recorded for KY2 and KA2 compared to their peak at 60°C. At intermediate temperatures (40–45°C), all strains showed moderate growth. Thus, the optimal growth temperature range of all thermophilic strains studied was between 55°C and 60°C, where the maximum growth rates were observed. Growth was significantly slower at both lower and higher temperatures, reflecting the temperature limits of their physiological adaptation (Fig.3).

The obtained results confirm that strains B1, KY2 and KA2 are true thermophiles, since their optimal growth and metabolic activity occur at

elevated temperatures, which is a distinctive feature of thermophilic microorganisms. True thermophiles are adapted to exist at temperatures significantly higher than those optimal for most other microorganisms. The highest growth rate of the studied strains was observed at a temperature of 60°C, which is typical for thermophilic organisms, since their enzymatic and biochemical processes occur most effectively in this temperature range. Thus, the conducted analysis of the physiological and biochemical properties of the isolated thermophilic bacteria showed that the identified strains have pronounced thermotolerance, the ability to utilize various carbohydrate substrates and specific enzymatic activity.

DISCUSSION

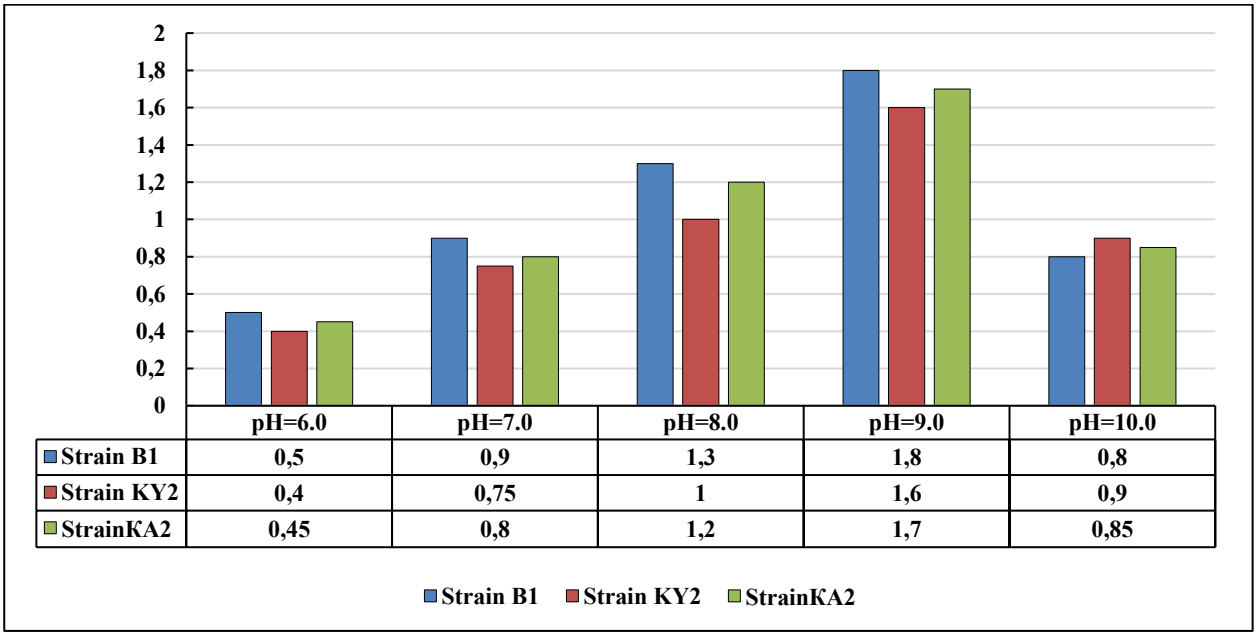
This paper presents the characteristics of three thermophilic bacterial strains – B1, KA2 and KY2, isolated from thermal springs in Azerbaijan. Previously, these strains had already been the objects of research, where the ability of these strains to synthesize silver nanoparticles was studied, but at this stage, it became possible to conduct a detailed physiological-biochemical and morphocultural study.

As a result of studying the morphology of the cells, typical features and characteristics of the genus *Bacillus* were identified: gram-positive staining, the ability to form spores, and rod-shaped cells. Colonies on nutrient agar were round in shape with a raised surface, soft in consistency and milky-cream in color, with distinctly wavy edges. Similar phenotypic characteristics have been described for thermophilic bacilli such as *Bacillus licheniformis* and *B. stearothermophilus* (Hoult et al., 1997; Logan & De Vos, 2009; Sonenshein et al., 1993).

Catalase activity, along with the absence of oxidase activity found in all strains, is consistent with the characteristics described by Logan (2012), indicating a typical enzymatic profile for most members of the genus *Bacillus*. Proteolytic activity was observed in strains KA2 and KY2, but was absent in B1, demonstrating interstrain differences typical for this genus. Similar differences were noted in lysine decarboxylase activity, which may reflect high metabolic flexibility and the ability to adapt to different ecological niches (Gudzenko et al., 2023).

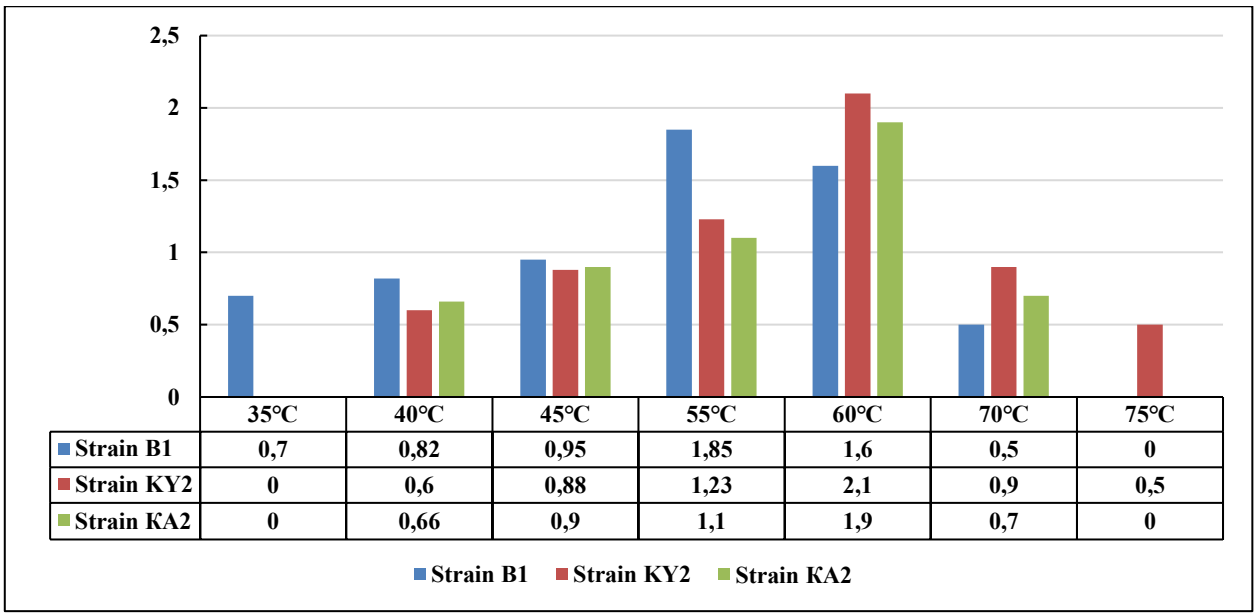
As a result of studying the ability of strains to utilize different sources of carbon and nitrogen, it was found that the greatest growth was observed when using organic acids such as pyruvic, citric and oxalic. This is consistent with existing knowledge of thermophilic bacteria that are adapted to efficiently utilize organic acids as carbon and energy sources (Brock, 1978; Libor et al., 1978).

The efficient use of sucrose and glucose, along with the absence of lactose, D-xylose and L-rhamnose fermentation in all strains, confirms the specificity of their enzymatic apparatus and suggests the absence or low activity of lactase, which is characteristic of many thermophilic bacteria.



	pH=6.0	pH=7.0	pH=8.0	pH=9.0	pH=10.0
Strain B1	0,5	0,9	1,3	1,8	0,8
Strain KY2	0,4	0,75	1	1,6	0,9
StrainKA2	0,45	0,8	1,2	1,7	0,85

Fig. 2. Optical density of strain suspensions measured at different pH values afterincubation at 60°C.



	35°C	40°C	45°C	55°C	60°C	70°C	75°C
Strain B1	0,7	0,82	0,95	1,85	1,6	0,5	0
Strain KY2	0	0,6	0,88	1,23	2,1	0,9	0,5
Strain KA2	0	0,66	0,9	1,1	1,9	0,7	0

Fig. 3. Optical density of strain suspensions at different temperatures with a nutrient medium pH of 7.0.

Interestingly, strain KY2 demonstrated active galactose metabolism, suggesting the presence of additional specific enzymes that expand the spectrum of its carbohydrate metabolism. However, the inability to utilize D-xylose and L-rhamnose indicates a limited capacity for pentose metabolism. Active metabolism of glycerol and ethanol, especially pronounced in strain B1, demonstrates metabolic flexibility, allowing the use of various alcohols as carbon sources. Similar abilities have also been described for thermophilic bacteria of the genus *Geobacillus*, which are adapted to various ecological niches (Brumm et al., 2010; Madigan et al., 2019).

Nitrogen source studies showed that peptone supported the highest growth of all strains, indicating a preference for organic nitrogen compounds. The high utilization of ammonium sulfate and potassium nitrate further indicates the ability to efficiently utilize both organic and inorganic forms of nitrogen, a characteristic of many thermophiles adapted to changing environmental conditions. Moderate growth on tryptone and asparagine, with relatively low growth on methionine, may indicate special biosynthetic requirements and methionine limitations in these strains (Sreekanth et al., 2013; Madigan et al., 2019; Liu et al., 2025). The control variant with the minimum OD confirms the necessity of available nitrogen sources for active growth and metabolism of thermophilic strains. It is also important to note the absence of hydrogen sulfide production, which may serve as a potential biosafety marker of these strains for future biotechnological and medical applications.

A study of the dependence of growth on temperature and pH of the environment confirmed that the isolated strains can develop in a wide range of conditions: at temperatures from 35°C to 75°C with an optimum of 55–60°C and at pH from 6.0 to 10.0 with an optimum of 7.0–9.0. This tolerance indicates a high degree of adaptation to extreme environmental conditions. Similar growth parameters and physiological resistance were found for thermophilic *Bacillus* species in the studies of Zeigler (2011). In conclusion, it should be noted that the combination of morphological, physiological and biochemical data convincingly confirms that strains B1, KA2 and KY2 belong to the genus *Bacillus*. Further study of the genomic and proteomic features of these microorganisms may contribute to a deeper understanding of the mechanisms of thermal tolerance and metal reduction, as well as optimization of their cultivation for applied purposes.

CONCLUSION

In the present study, three thermophilic bacterial strains, B1, KA2 and KY2, isolated from thermal springs in Kalbajar district, were taxonomically identified and comprehensively characterized. Detailed morphological, cultural and physiological-biochemical analysis allowed them to be classified as belonging to the genus *Bacillus*. All three isolates were gram-positive spore-forming rods, varying in size and forming milky in color, round colonies. Optimum growth was observed at temperatures from 55°C to 60°C and pH values from 7.0 to 9.0, although strains were tolerant over

a wider range of temperatures from 35°C to 75°C and pH from 6.0 to 10.0. Physiological studies have shown the ability of the strains to utilize a number of carbohydrates, including glucose, fructose, sucrose and maltose, whereas the enzymatic activity of lactose, D-xylose and L-rhamnose was not detected. The obtained data confirm that strains B1, KA2 and KY2 are thermophilic *Bacillus* species with a high degree of adaptation to extreme environmental conditions.

ACKNOWLEDGEMENTS

The authors would like to express their sincere appreciation to the staff of the Department of Biology, Baku State University, for their technical support and access to laboratory facilities.

ETHICAL CONSIDERATIONS

Sample collection was conducted in accordance with local environmental regulations and with respect for natural habitats. All laboratory procedures complied with standard microbiological safety and biosafety guidelines. The studied bacterial strains were confirmed to be non-pathogenic, and no genetically modified organisms were produced during the course of this research.

FUNDING

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest related to this study. The authors confirm that they have no financial or personal relationships that could have influenced the research outcomes or interpretation of the results.

REFERENCES

Aanniz T., Ouadghiri M., Melloul M., Swings J., Elfahime E., Ibijbijen J., Ismaili M., Amar M. (2015) Thermophilic bacteria in Moroccan hot springs, salt marshes and desert soils. *Braz. J. Microbiol*., **46(2):**443-453.

Ahmadova F.R. (2007) Some physiological and biochemical characteristics of *Bacillus* species distributed in thermal waters of the Republic of Azerbaijan. *Reports of the National Academy of Sciences of Azerbaijan*, **LXIII(2):** 79-83.

Brumm P.J., Budzik J.M., Krumholz L.R., Dees P.M., Bryant D.A. (2010) *C*arbohydrate-active enzymes from pigmented Bacilli: a genomic approach to assess carbohydrate utilization and degradation. *BMC Genomics,* **11:** 98.

Brock T.D. (1978) Thermophilic microorganisms and life at high temperatures. Springer-Verlag: 465 p.

Deljou A., Samad Goudarzi (2016) Green extracellular synthesis of the silver nanoparticles using thermophilic Bacillus sp. AZ1 and its antimicrobial activity against several human pathogenetic bacteria. *Iran. J. Biotech.*, **14(2):** e1259, 25-32.

Gudzenko O.V., Varbanets L.D., Avdiyuk K.V., Pasichnyk L.A. (2023) Proteolytic activity of Bacillus strains isolated from rice agrocenosis soil. *Mikrobiolohichnyi Zhurnal,* **85(6):** 41–47.

Gunashova G.Y., Ahmadova F.R., Khalilov R.I. (2021) Thermophilic bacteria of the hot springs "Ashagi Istisu" and "Yukhari Istisu" of the Kalbajar region of the Republic of Azerbaijan. *Journal of Life Sciences & Biomedicine*, **3(76), No 2:** 134-140.

Gunashova G.Y., Ahmadova F.R., Khalilov R.I. (2021). Biosynthesis of silver nanoparticles using thermophilic *Bacillus* sp. B1. *Advances in Biology & Earth Sciences*, **6(2):** 142-145.

Gunashova G.Y. (2022) Synthesis of silver nanoparticles using a thermophilic bacterium strain isolated from the spring Yukhari Istisu of the Kalbajar region (Azerbaijan*). Advances in Biology & Earth Sciences,***7(3):**198-204.

Hajiyeva A., Mamedov Ch., Gasimov E., Rzayev F., Isayev O., Khalilov R., Eftekhari A., Benis Kh.Z. (2024) Ultrastructural investigation of iron oxide nanoparticles accumulation in the liver of common carp (*Cyprinus carpio* Linnaeus, 1758). *Aquatic Toxicology*, **272:**106961; doi: 10.1016/j.aquatox.2024.106961

Holt J.G., Krieg N.R., Sneath P.H.A., Staley J.T., Williams S.T. (1994) *Bergey's manual of determinative bacteriology* (9th ed.). Williams & Wilkins. 787 p.

Iravani S., Korbekandi H., Mirmohammadi S. V., Zolfaghari B. (2014) Synthesis of silver nanoparticles: Chemical, physical and biological methods. *Research in Pharmaceutical Sciences,***9:** 385–406; PMID: 26339255

Kamal A. (2012) Kalbajar Battalion and the Legendary Regiment. Baku: Azernashr, 330p. (in Azerbaijani).

Lavrenchuk L.S., Ermoshin, A.A. (2019) Microbiology: Practical Course. Ministry of Science and Higher Education of the Russian Federation, Ural Federal University Publishing: Yekaterinburg, 107p.

Libor S.M., Sundaram T.K., Scrutton M.C. (1978) Pyruvate carboxylase from a thermophilic

Bacillus. Studies on the specificity of activation by acyl derivatives of coenzyme A and on the properties of catalysis in the absence of activator. *Biochemical Journal,* **169(3):** 543-558; doi: 10.1042/bj1690543

Liu X., Zhang H., Chen Y., Wang J. (2025) Optimization of the fermentation culture conditions of Bacillus amyloliquefaciens CK-05 using response surface methodology. *Frontiers in Microbiology,* **16:** Article 1552645.

Logan N.A. (2012) Bacillus and relatives in foodborne illness. *Journal of Applied Microbiology*, **112(3):** 417–429.

Logan N.A., De Vos P. (2009) Bacillus. In: *Bergey's Manual of Systematic Bacteriology*, 2nd ed. Springer, **3:** 21-128.

Madigan M.T., Martinko J.M., Bender K.S. (2019) Brock Biology of Microorganisms. 15th ed.). Pearson: 104 p.

Marzban G., Tesei D. (2025) The *Extremophiles*: Adaptation mechanisms and biotechnological applications. *Biology*, **14(4):** 412;doi: 10.3390/biology14040412

Rzayev F.H., Gasimov E.K., Agayeva N.J., Manafov A.A., Mamedov C.A., Ahmadov I.S., Khusro A., Arasu M.V., Sahibzada M.U.K., Al-Dhabi N.A., Choi K.C. (2022) Microscopic characterization of bioaccumulated aluminium nanoparticles in simplified food chain of aquatic ecosystem. *Journal of King Saud University – Science,***34(1):** 1-8 (Art. No 101666).

Sonenshein A.L., Losick R., Hoch, J.A. (Eds.). (1993) *Bacillus subtilis* and other Gram-positive bacteria: Biochemistry, physiology, and molecular genetics. American Society for Microbiology, 987 p.

Sreekanth M.S., Vijayendra S.V.N., Joshi G.J. (2013) Effect of carbon and nitrogen sources on simultaneous production of α-amylase and polyhydroxyalkanoates by *Bacill*us sp. CFR-67. *Journal of Food Science and Technology*, **50(2):** 278–286; doi: 10.1007/s13197-012-0639-6.

Sulaiman G.M., Tawfeeq A.T., Naji A.S. (2018) Extracellular biosynthesis of magnetic iron oxide nanoparticles by *Bacillus cereus* strain HMH1: characterization and in vitro cytotoxicity analysis on MCF-7 and 3T3 cell lines. *Artificial Cells, Nanomedicine, and Biotechnology,* **46(6):** 1215-1229; doi: 10.1080/21691401.2017.1366335

Zeigler D.R. (2011) The genus Bacillus: Agriculture and biotechnology. In: *Bacillus: Cellular and Molecular Biology*. Norfolk, UK: Horizon Scientific Press, pp. 1–18. doı:10.1201/9781420009330.ch24.

ORCIDs:
Gunay Abbasli: https://orcid.org/0000-0003-2015-4720
Farayat Ahmadova: https://orcid.org/0009-0003-5834-5386

Investigation of the impact of iron oxide (Fe_3O_4) nanoparticles on the ultrastructure of the intestine and on the embryonic development of common carp (*Cyprinus carpio* Linnaeus, 1758) reared under aquaculture conditions

Chingiz Mammadov*, Rovshan Khalilov

Baku State University, 23 Academician Zahid Khalilov Str., AZ1148, Baku, Azerbaijan

***For correspondence:** m_chingiz@yahoo.com*

Received: November 08, 2025; Reviewed: December 11, 2025; Accepted: December 18, 2025

The vast presence of metal nanoparticles within the global aquatic environment and the detrimental effect on human health have become issues of global concern. Therefore, the research focus of this project was to investigate the microscopic bioaccumulation and localization of magnetite (Fe_3O_4) nanoparticles within the cellular structures of *Cyprinus carpio* L. (Common Carp) in aqueous environments. A number of researchers have reported the accumulation of Fe_3O_4 nanoparticles by aquatic organisms, including fish and have also described the numerous pathological changes caused to the host organism by the presence of these particles. This study examined the bioaccumulation of iron oxide (Fe_3O_4) nanoparticles in the intestinal tissue of Cyprinus carpio L., which was raised in an aquaculture environment, as well as the effects of the presence of these nanoparticles on the early stages of embryonic development during artificial breeding. After being exposed to Fe_3O_4 nanoparticles (10 and 100 mg/10g of food) over 7 days in the current investigation using the Common Carp, it was noted that the intestinal tissue exhibited pronounced pathomorphological changes. These included: 1) loss of microvilli; 2) cytoplasmic edema; 3) damage to mitochondria; and 4) damage to vascular endothelium. At the lowest dose (10 mg) used in the study, clear indications of damage, such as villi breakdown in the intestine and pathology of cytoplasmic structure in enterocytes, were visible. Electron microscopy demonstrated the sequential entry and bioaccumulation of Fe_3O_4 nanoparticles through the enterocytes, beginning with the microvilli and progressing through various cellular organelles. The size of the nanoparticles found in the structural components of the fish intestine was consistent at up to 20 nm. The results demonstrate that Fe_3O_4 nanoparticles may accumulate in fish at all stages of breeding and can be used in practice in aquaculture. The use of nanoparticles as a result of studies on the effects of the nanoparticles on the embryonic development of fish resulted in an increase in the amount of viable free embryos and the number of fertilized eggs by approximately 12-14% when 0.001 grams of Fe_3O_4 was added to the sperm before it fertilized the eggs. These results can be important for determining the toxicity of the nanoparticles at different stages of fish reproduction and could have practical applications in aquaculture.

Keywords: *Common carp, Fe_3O_4 nanoparticles, small intestine, bioaccumulation, embryonic development, fertilization rate*

INTRODUCTION

With the large-scale increase in the production and application of nanomaterials over the last few years the risk of environmental pollution from these materials has grown. Due to this growing number of produced nanomaterials that end up in the water systems (lakes, rivers, seas) they are causing great effects on the ecosystems and its elements (Gupta et al., 2016). Nanoparticles have the potential to pollute the environment and the health of humans through contaminating the aquatic environment, which then accumulates within the bodies of living organism in the ecosystem, with examples being commercial fish (Sabeeh et al., 2021).

Therefore, based on the information provided, it is reasonable to consider these types of particles as fish biomodels, being consumed by humans, with an example being the Common Carp (Cyprinus Carpio Linnaeus, 1758). The Common Carp is an aquatic fish, with the advantages of having an easy way of reproducing commercially and being farmed in many countries around the world, including in Azerbaijan. These fish are also an excellent model for the study of animal ecology, developmental biology, and evolution (Bongers et al., 1998). More than 2,400 species are included in the Cyprinidae family (Nelson, 2006).

Nevertheless, although there are a number of species included in the Cyprinidae family, it is the Common Carp that represents 14% (3.4-4.0 million tons) of all freshwater fish (commercial) produced through aquaculture (Mammadov et al., 2016; Fiorino, 2018). Aquaculture farms use spring, river, and basin waters as their water source. To some degree, these basins have been impacted by human activities, resulting in pollution of various levels. In this regard, over the past several years, there has been increasing interest from many researchers concerning selecting fish as a biological, ecological, toxicological model (Burgos-Aceves, 2019).

Some experiments tested free-form nanoparticles or metal oxides in carp model fish (Lokka et al., 2013; D'Amico, 2005; Nelson, 2006; Gupta et al., 2016; Jha et al., 2022) and examined the absorption of these various nanoparticles into different organs of the host (stomach, intestines, liver, vascular-blood system), the toxicity of these nanoparticles and the morphological changes in carp fish caused by exposure to the nanoparticles under experimental conditions. Iron oxide has both catalytic and magnetic properties. Additionally, iron is a significant component in vertebrate animals and takes part in carrying oxygen and electrons (carriage of transport); in DNA synthesis; and in the development of the immune system (Abbaspour et al., 2014).

Although, as a result of an excess (beyond the normal limits), in an organism, it may be associated with some negative consequences (the weakening of motility and visual acuity, increasing hemoglobin, erythrocytes, hematocrit levels and decreasing the number of white blood cells, the damage to tissues) (Raji and Norouzi, 2013; Chen et al., 2013; Abbaspour, 2014; Valiyeva et al., 2022). In spite of that, little is known about how metal oxide nanoparticles affect fish (Karthikeyeni et al., 2018).

Currently, in Azerbaijan there are large scale scientific studies being conducted with respect to the synthesis of free nanoscale particles and their compounds, assessment of their biological properties and practical uses, and the accumulation of free nanoscale particles in a variety of components of an ecosystem (bacteria, soil, mollusks, fish, unicellular and multicellular organisms, water, etc.) as well as natural nanoparticles (ferritin) (Hajiyeva et al., 2019; Agayeva et al., 2020). Therefore, the primary goal of this research is to establish if magnetite nanoparticles (Fe_3O_4) can accumulate in the different parts of the small intestine and liver of the common carp (Cyprinus carpio Linnaeus, 1758) that have been raised under aquaculture conditions and to determine if pathological morphologic changes occur in the areas where the nanoparticles are accumulated by employing light and electron microscope techniques for visualization, and to assess how the exposure of the embryos of artificially bred carp to these nanoparticles affects development.

MATERIALS AND METHODS

In this study, 33 yearlings (0+) of common carp (*Cyprinus carpio* Linnaeus, 1758) were used as biomodels to investigate possible bioaccumulation of Fe_3O_4 nanoparticles in organs of organisms included in the food chain. These yearlings were raised at a fish farm in the Neftchala district of Azerbaijan, where they were initially fed granulated feed for sturgeons. In September 2022, the fry was transported to Baku, and experiments were conducted under laboratory conditions at the Department of Biophysics and Biochemistry. The experimental fish were divided into three groups of 11 individuals each and placed in three aquariums of equal volume (60 liters): I control group, II and III experimental groups. The average length (L) of the yearlings was 6.9 cm, and the average weight (P) was 4.9 g. In the experimental aquariums, constant conditions were maintained: water volume 30 liters, hydrochemical parameters temperature 22–24°C, oxygen content 8.2–8.6 mg/L, pH 7.4–7.6. Feeding ration 10 g of compound feed per day.

The experiment used Fe_3O_4 nanoparticles (98+%, 10–30 nm, product number: 3320DX), purchased from Skyspring Nanomaterials Inc., Houston, Texas, USA. Fish in the control group (I) received only compound feed, while fish in groups II and III were daily supplemented with 10 mg and 100 mg of Fe_3O_4 nanoparticles, respectively, along with feed. The duration of the experiment was 7 days, after which the internal organs (small intestine and liver) of yearlings from all three groups were extracted and fixed for further analysis. Fixation of extracted organs was carried out in a solution containing 2.5% glutaraldehyde, 2% paraformaldehyde, 4% sucrose, and 0.1% picric acid in phosphate buffer (pH = 7.4). After 24-hour fixation, samples were post-fixed in 1% osmium tetroxide solution in the same buffer for 2 hours. Then, Araldite-Epon blocks were prepared according to the standard electron microscopy method.

Using a Leica EM UC7 ultramicrotome (Leica, USA), semi-thin sections (1–2 μm) were obtained and stained with methylene blue, azure II, basic fuchsin, or toluidine blue. Sections were examined under a Primo Star microscope (Zeiss, Germany),

and images were recorded with an EOS D650 digital camera (Canon, Japan). From the same blocks, ultrathin sections (50–70 nm) were also obtained and studied using a JEM-1400 transmission electron microscope (JEOL, Japan) at 80–120 kV, producing electronograms (Hajiyeva et al., 2023; Mammadov et al., 2024; Rzayev et al., 2022).

When analyzing electronograms of ultrathin unstained sections using the computer program "Intensity profile," the horizontal axis displayed the length of the structure in nanometers, while the vertical axis showed grayscale levels. Image intensity depends on the number of shades of gray (from black to the lightest). The Veletta camera (Olympus, Germany) used in the study processes 14-bit information per pixel, allowing differentiation of 16,384 shades of gray, ensuring precise determination of nanoparticle localization in cells of living organisms.

Studies aimed at investigating the effects of iron oxide nanoparticles (Fe_3O_4) on embryonic development of common carp were initially conducted (Hajiyeva et al., 2022) in June–July 2021 at the "Samukh-fish" fish farm (Barda city, Azerbaijan Republic), but repeated experiments were carried out in June 2025 at LLC "Salyan Agropark Agribusiness" (Salyan city, Azerbaijan Republic). During the studies, mature males and females prepared for fish farming were preliminarily grouped according to morphological and physiological characteristics. Their length (L), weight (P), and Fulton's condition factor (Pravdin, 1966; Hajiyev et al., 2024) were determined. In the latter case (2025), the male measured 35 cm in length and weighed 2.0 kg, while the female measured 76 cm and weighed 4.0 kg.

Mature eggs were obtained in vivo by the "stripping" method from sexually mature breeders and placed in plastic containers with smooth surfaces. First, Fe_3O_4 nanoparticles were added to 1 ml of milt obtained by stripping from mature males, and then this mixture was added to 20 g of carp eggs obtained in the same way. Fertilization of eggs was carried out by the "dry" method (Dettlaff et al., 1981; Agayeva et al., 2020). In the course of the study, Fe_3O_4 nanoparticles at various concentrations (0.0001 g, 0.001 g, and 0.05 g) were added both to gametes (sperm, eggs) before fertilization and to already fertilized eggs. In accordance with the objectives, the obtained results were compared with the control group.

RESULTS

Before examining how nanoparticles accumulate within the carp organism via bioaccumulation the authors initially used both light and electron microscopy to assess the regular structural characteristics of the small intestine and all of its layers for comparative purposes (the small intestine has three distinct anatomical parts or segments- the anterior, the middle, and the posterior; each part has a different morphology; and among the digestive system's organs the longest segment is the middle portion of the small intestine which contains the majority of the body's intestinal digestion and absorption of nutrients); therefore the authors chose the middle portion of the small intestine as the subject of this investigation.

Between the muscle layers, elements of the myenteric nerve plexus are visible. In the center of the Schwann cell (neurolemmocyte) lies an oval nucleus with euchromatin and a well-defined nuclear envelope. The cytoplasm contains the Golgi apparatus, lysosomes, mitochondria, endoplasmic reticulum, and other organelles. Surrounding the plasmalemma of the neurolemmocyte are numerous axons containing neurofilaments and neurotubules, as well as regions of mitochondria and cisternae of agranular endoplasmic reticulum. Beneath the muscle layer lies the submucosa (SM), where elements of connective tissue are visible blood vessels, lymphocytes, macrophages, and other cells. The final layer is the mucosa, which is divided into three parts: epithelium (Ep), lamina propria (LP), and muscularis mucosae. The epithelial layer consists of columnar cells enterocytes (En) and goblet cells (GC). On the apical surface of enterocytes are microvilli. In the studied middle section of the small intestine, goblet cells occur more frequently than in other sections, and their secretion (mucus) protects the microvilli from damage caused by bacteria and toxins.

After 7 days of administering iron nanoparticles at doses of 10 mg and 100 mg together with feed to experimental fish raised under aquaculture conditions, the small intestine was examined by light and electron microscopy and compared with the normal structure. First, the effect of the 10 mg dose of nanoparticles on various layers of the intestinal wall was studied. It was observed semi-thin (1 μm) sections of the intestinal mucosa, including the epithelium. Pathomorphological changes were noted in the apical regions of epithelial cells facing the intestinal lumen. The microvilli of enterocytes (MV) located on the lateral parts of intestinal folds were preserved, but in the central part of the intestinal lumen, their structure was disrupted they were scattered, and organelles of cells, including fat droplets (FD), entered the lumen. These processes are clearly visible in electronograms. In the area marked with snowflakes, the microvilli in the apical

part of enterocytes were completely destroyed. Such changes impair the absorptive function of the intestine.

At higher magnification under the electron microscope, it was observed that the outer membranes of some organelles, including mitochondria (Mt), were damaged, the structure of cristae was destroyed, and edema was present in the cytoplasm (marked with an asterisk). Clear signs of cytoplasmic edema in enterocytes and disruption of the integrity of epithelial cells located at the intestinal lumen are visible, while cells farther from the lumen partially retained their structure. At the 10 mg dose, no serious changes were detected in other layers of the intestine.

Under exposure to a dose of 100 mg of Fe_3O_4 nanoparticles, more severe changes were observed in the structure of the small intestinal wall. Pathological alterations were noted in the serosal and muscular layers, as well as in neural elements, the submucosa, and blood vessels. In the electronogram, the serosal layer is completely destroyed, and in the muscular layer, vacuolization and edema are visible between muscle cells. In the cytoplasm of muscle cells, mitochondria are swollen and the structure of cristae is disrupted.

Damage to the integrity of neurolemmocyte membranes located between muscle layers leads to the formation of myelin-like bodies of various shapes, vacuolization of the cytoplasm, swelling of mitochondrial cristae, and thickening of the nuclear envelope of the neurolemmocyte. The integrity of the basal membrane of non-myelinated nerve fibers is disrupted. In axons, swelling of the granular endoplasmic reticulum is observed.

In the submucosa (SM), edematous fluid accumulates between connective tissue elements, and numerous macrophages are also observed. In this same layer, changes in the ultrastructure of blood vessels were detected. The endothelium lining the vessel lumen is deformed, forming finger-like protrusions into the lumen.

It is important to note that once nanoparticles enter erythrocytes, they begin to circulate throughout the organism via the vascular system. All observations were confirmed by electron micrographs and grayscale intensity profiles. Regardless of localization, grayscale values ranged between 5200–5400, indicating the presence of particles identical in composition Fe_3O_4 nanoparticles. The size of these particles was 10–20 nm. This proves that Fe_3O_4 nanoparticles with magnetic properties, when introduced into the carp intestine, travel from the microvilli to the erythrocytes and accumulate in various organelles.

As a result of studies conducted in 2025, it was established that when Fe_3O_4 nanoparticles (20–30 nm) were added to the sperm of common carp before egg fertilization in amounts of 0.0001 g, 0.001 g, and 0.05 g, the percentage of egg fertilization and the release of free embryos from the egg membrane were higher compared to other variants. Thus, at a concentration of Fe_3O_4 nanoparticles (20–30 nm) of 0.0001 g, egg fertilization was 66.6%; at 0.001 g 80.5%; and at 0.05 g 75.0% (Table 1).

DISCUSSION AND CONCLUSION

The study of the normal structure of the digestive organs, in particular the small intestine of the common carp, was carried out using histological and electron microscopic methods. As a result, it was established that the wall of the small intestine consists of four layers: serosa, muscular layer, submucosa, and mucosa. All of these layers were identified by different methods. These layers, in turn, are subdivided into components. For example, the mucosa is divided into the epithelium (enterocytes and goblet cells), lamina propria, and muscularis mucosae. A similar mucosal structure has been noted in other species of bony fish, such as *Catla catla*, *Anguilla anguilla*, *Clarius batrachus*, *Salmo salar*, *Oncorhynchus mykiss*, and *Serrasalmus nattereri*.

The muscular layer of the small intestine consists of circular and longitudinal muscle fibers, between which lies the myenteric nerve plexus an element of the enteric nervous system. During the study, neurolemmocytes (Schwann cells) and non-myelinated nerve fibers were detected, confirming similar findings by other researchers who studied the neural elements between the muscular layers of the carp intestine.

Table 1. Addition of iron oxide nanoparticles (Fe_3O_4) to the sperm of common carp before egg fertilization.

Fe_3O_4 nanoparticles (20-30 nm), amount (g)	Fish length L, cm	Fish weight P, g	Egg mass in experiment, g	Number of eggs per 1 gram, pcs.	Total number of eggs in experiment, pcs.	Free embryos output		Number of dead eggs	
						%	pcs.	%	pcs.
0,0001	76	4000	20	451	9020	66.6	6007	33.4	3013
0,001	76	4000	20	451	9020	80.5	7261	19.5	1759
0,05	76	4000	20	451	9020	75.0	6765	25.0	2255
Control	76	4000	20	451	9020	67.3	6070	32.7	2950

In addition to its detoxification function, the fish intestine also serves as an important indicator of the general condition of the organism and as a pathway for nanoparticle penetration. This makes it a convenient model for assessing the toxicity of various nanoparticles and the associated pathological changes. In other studies, Fe_3O_4 was shown to cause changes in the stomach, liver, skin, muscles, and scales. It has been established that iron nanoparticles can induce immunotoxicity, accumulating in the small intestine and liver. Depending on the dose and duration of exposure, pronounced pathological changes are observed: villus degeneration, disruption of wall integrity, reduction in villus number, thinning of the wall, epithelial edema, and rupture of intercellular junctions.

Despite prolonged administration of high doses, by the 21st day, the level of nanoparticles in the carp organism decreased significantly. Other authors have noted that disturbances in the mechanism of nanoparticle penetration into enterocytes (via endocytosis and subsequent exocytosis into the vascular system) are caused by the structural changes described above. In contrast, in the present study, at comparatively high doses (10 and 100 mg per 10 g of feed), alterations were already detected by day 7: destruction of enterocyte microvilli, release of fat droplets into the intestinal lumen, mitochondrial damage, and cytoplasmic edema. At the 100 mg dose, pathological processes affected all layers of the intestinal wall: complete destruction of the serosa, muscle vacuolization, intercellular edema, disruption of neural elements, submucosal swelling, endothelial deformation of blood vessels, and enterocyte destruction.

Based on literature analysis and our own observations, it can be stated that the severity of pathological changes in the host organism depends on both dose and duration of nanoparticle exposure. In particular, the study showed that changes in the nuclei of vascular erythrocytes varied depending on the Fe_3O_4 dose: in the lamina propria and vascular lumina of the submucosal layer, abnormalities in nuclear envelopes of erythrocytes, cytoplasmic vacuolization, and deformation of individual cells were recorded.

Accumulation of metallic nanoparticles in the organism depends on their size: 4 nm predominantly in the kidneys; 10–28 nm in the stomach wall and small intestine. Larger particles are retained in mucus secreted by goblet cells, without penetrating deeper into the tissue. According to transmission electron microscopy (TEM) data, nanoparticles sized 10–30 nm can pass through the lamina propria and reach connective tissues, endothelium, and erythrocytes, spreading to various organs. Although nanoparticles of 10–30 nm were used in this study, TEM visualized particles predominantly 10–20 nm in villi, enterocyte cytoplasm, organelles, endothelium, and erythrocytes, consistent with other results obtained in rainbow trout. Both literature and our data confirm that nanoparticle size plays a key role in cellular and tissue penetration.

Under aquaculture conditions, ultrastructural changes associated with the penetration and accumulation of iron oxide nanoparticles in the small intestine and liver of common carp were investigated. Exposure to different doses (10 and 100 mg) caused dose-dependent damage: from enterocyte destruction at the lower dose to total morphological alterations of all intestinal wall layers at the higher dose. Nanoparticles (especially those ≤20 nm) passed through microvilli, entered the cytoplasm, then penetrated the mucosa, lamina propria, endothelium, and erythrocytes spreading further to other organs (Hajiyeva et al., 2023; Mammadov et al., 2024).

Based on the results of studies on the effects of iron oxide (Fe_3O_4) nanoparticles on carp embryonic development, it was established that adding 0.001 g of iron nanoparticles to sperm before egg fertilization ensured the highest fertilization rate (80.5%). It is assumed that Fe_3O_4 nanoparticles (20–30 nm) at this concentration exert a catalytic effect on sperm acrosomes, enhance their energetic activity, and consequently increase sperm motility. The obtained results may be applied in the aquaculture of economically important fish species across various taxonomic groups, to reduce losses at embryonic developmental stages and increase overall productivity.

ACKNOWLEDGEMENTS

The authors express their sincere gratitude to the staff of the aquaculture and microscopy laboratories of Baku State University for their technical assistance and support during the experimental procedures. Special thanks are extended to colleagues who provided valuable consultations during the electron microscopy analyses and embryological observations.

ETHICAL CONSIDERATIONS

All experimental procedures involving live fish were conducted in strict accordance with internationally accepted guidelines for the care and use of aquatic organisms in scientific research. The study design complied with ethical principles aimed at minimizing stress, suffering, and the number of animals used. Handling, feeding, exposure to iron

oxide (Fe_3O_4) nanoparticles, sampling, and euthanasia procedures were performed following standard aquaculture and laboratory animal welfare protocols. The experimental procedures were approved by the relevant institutional ethics committee of Baku State University, and all efforts were made to ensure humane treatment of the experimental animals throughout the study.

AUTHOR CONTRIBUTIONS

Chingiz Mammadov conceived and designed the study, supervised the experimental work, conducted the histological and ultrastructural analyses, and drafted the original manuscript.

Rovshan Khalilov contributed to the experimental setup, aquaculture maintenance, nanoparticle exposure protocols, embryonic development assessments, and data interpretation.

Both authors critically reviewed the manuscript, approved the final version, and agreed to be accountable for all aspects of the work.

FUNDING

This scientific research was carried out during 2024–2025 with financial support from the Science Foundation of the Republic of Azerbaijan under Grant No. AEF-MGC-2024-2(50), awarded within the framework of the "Year of Solidarity for a Green World" Main Grant Competition.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

REFERENCES

Abbaspour N., Hurrell R., Kelishadi R. (2014) Review on iron and its importance for human health. *Journal of Research in Medicine Sciences*, **19(2):** 164-174.

Agayeva N.J., Rzayev F.H., Gasimov E.K., Mamedov Ch.A., Ahmadov I.S., Sadigova N.A., Khusro A., Al-Dhabi N.A., Arasu M.V. (2020) Exposure of rainbow trout (*Oncorhynchus mykiss*) to magnetite (Fe_3O_4) nanoparticles in simplified food chain: Study on ultra-structural characterization. *Saudi Journal of Biological Sciences*, **27(12):** 3258-3266; doi: 10.1016/j.sjbs.2020.09.032

Ahmadov I.S., Qasimov E.K., Sadiqova N.A., Agayeva N.J., Rzayev F.H., Manafov A.A. (2018) Transfer of nanoparticles in a simplified aquatic food chain: from water plant *Elodea canadensis* to molluscs *Lymnaea auricularia*. *Journal of Low Dimensional Systems*, **2(2):** 41-45.

Bongers A.B., Sukkel M., Gort G., Komen J., Richter C.J. (1998) Development and use of genetically uniform strains of common carp in experimental animal research. *Laboratory Animals*, **32:** 349-363; doi: 10.1258/002367798780599749

Burgos-Aceves M.A., Lionetti L., Faggio C. (2019) Multidisciplinary haematology as prognostic device in environmental and xenobiotic stress-induced response in fish. *Sci. Total Environ*., **670:** 1170-1183; doi: 10.1016/j.scitotenv.2019.03.275.

Chen P.J., Wu W.L., Wu K.C.W. (2013) The zerovalent iron nanoparticle causes higher developmental toxicity than its oxidation products in early life stages of medaka fish. *Water Res*., **47(12):** 3899-3909; doi: 10.1016/j.watres.2012.12.043

D'Amico F.A. (2005) polychromatic staining method for epoxy embedded tissue: a new combination of methylene blue and basic fuchsine for light microscopy. *Biotech Histochem*., **80(5–6):** 207-210; doi: 10.1080/10520290600560897

Dettlaf T.A., Ginzburg A.S., Shmalgauzen O.I. (1981) Development of sturgeon fishes. Moscow, Nauka, 222 p.

Fiorino E., Sehonova P., Plhalova L., Blahova J., Svobodova Z., Faggio C. (2018) Effects of glyphosate on early life stages: comparison between *Cyprinus carpio* and *Danio rerio*. *Environ. Sci. Pollut. Res*., **2:** 8542-8549; doi: 10.1007/s11356-017-1141-5.

Gupta, Y.R., Sellegounder, D., Kannan, M., Deepa, S., Senthilkumaran, B., Basavaraju, Y. (2016) Effect of copper nanoparticles exposure in the physiology of the common carp (*Cyprinus carpio*): Biochemical, histological and proteomic approaches. *Aquaculture and Fisheries*, **1(1):** 15-23; http://dx.doi.org/10.1016/j.aaf.2016.09.003

Hacıyev R.V., Akhundov M.M., Tagıyev A.N., Mammadov Ch.A. (2024) Ichthyology *(Textbook for the bachelor level of higher education)*. Baku, "Zardabi Naşr" LLC, 2024, 512 p.

Hajiyeva S., Hasanova U., Gakhramanova Z., Israyilova A., Ganbarov Kh., Gasimov E., Rzayev F., Eyvazova G., Huseynzada A., Aliyeva G., Hasanova I., Maharramov A. (2019) The role of diazacrown ether in the enhancement of the biological activity of silver nanoparticles. *Turkish Journal of Chemistry*, **43:** 1711–1721; doi: 10.3906/kim-1907-10

Hajiyeva A., Mamedov Ch., Gasimov E., Rzayev F., Khalilov R., Ahmadian E., Eftehari A., William C. (2023) Ultrastructural characteristics of the accumulation of iron nanoparticles in the intestine of *Cyprinus carpio* (Linnaeus, 1758) under aquaculture. *Ecotoxicology and Environmental Safety*, **264:** 115477; doi: 10.1016/j.ecoenv.2023.115477

Jha N., Annamalai A., Essakiraj P., Balamurugan R., Lakra A.K., Tilwani Y.M., Arul V. (2022) Effects of polysaccharide-based silver and selenium nanoparticles on growth perfor-mance, biochemical parameters, and immune response of *Cyprinus carpio*. *Fish and Shellfish Immunology Reports,* **3:** 100062; doi: 10.1016/j.fsirep.2022.100062

Karthikeyeni S., Siva Vijayakumar T., Vasanth S., Ganesh A., Manimegalai M., Subramanian P. (2013) Biosynthesis of iron oxide nanoparticles and it haematological effects on fresh water fish *Oreochromis mossambicus*. *J. Acad. Indus. Res*., **1(10):** 645-649.

Mammadov Ch.A., Qasımov R.Y., Hacıyev R.V. (2016) Aquaculture *(Textbook for higher education)*. Baku, "BST", 360 p.

Lokka G., Austbo L., Falk K., Bjerkas I., Koppang E.O. (2013) Intestinal morphology of the wild Atlantic salmon (*Salmo salar*). *J. Morphol*., **274:** 859-876; doi: 10.1002/jmor.20142

Nelson, J.S. (2006) Fishes of the World. 4th ed. Hoboken, NY, USA: John Wiley and Sons, Inc., pp: 135-165.

Pravdin I.F. (1966) Guide to the study of fish. Moscow: Food Industry, 375 p. (In Russian)

Raji A.R., Norouzi E. (2010) Histological and histochemical study on the alimentary canal in walking catfish (*Claris batrachus*) and piranha (*Serrasalmus nattereri*). *Iranian. J. Vet. Res*., **3:** 255-261; doi: 10.22099/IJVR.2010.130

Rzayev F.H., Gasimov E.K., Agayeva N.J., Manafov A.A., Mamedov C.A., Ahmadov I.S., Khusro A., Valan Arasu M., Sahibzada M.U.K., Al-Dhabi N.A., Choi K.C. (2022) Microscopic characterization of bioaccumulated aluminium nanoparticles in simplified food chain of aquatic ecosystem. *Journal of King Saud University Science*, **34(1)**: 101666 doi: 10.1016/j.jksus.2021.101666

Sabeeh H.T., Alshami I.J.J., Al-Tameemi R.A. (2021) Histochemical study of the intestine in the common carp *Cyprinus carpio* fingerlings to detect the impact of replacement of dietary fish and soybean meals with shrimp wastes. *Egyptian Journal of Aquatic Biology & Fisheries*., **25(2):** 721-734; doi: 10.21608/ejabf.2021.169190

Valiyeva G.G., Palma L.Di., Hajiyeva S.R., Ramazanov M.A., Hajiyeva F.V., Gasimov E.K., Rzayev F.H. (2022) Reuse of bimetallic nanoparticles for nitrate reduction. *IEEE Transactions on Nanotechnology*, **22:** 613-619; doi: 10.1109/TNANO.2022.3230284

ORCIDS:
Chingiz Mammadov: https://orcid.org/0000-0001-5785-2392
Rovshan Khalilov: https://orcid.org/0000-0002-8684-1390

The use of sumac (*Rhus coriaria*) plant in value-added cosmetic, health and food products

Musa Karadag[1,2*], Mehmet Firat Baran[3], Kadir Sinan Aslan[4], Mehmet Tevfik Adican[5]

[1]*Vocational School of Technical Sciences, Department of Chemistry and Chemical Processing Technologies, Igdir University, 76100, Igdir, Turkiye*
[2]*Research and Application Laboratory and Research Center, Igdir University, 76100, Igdir, Turkiye*
[3]*Department of Medical Services and Techniques, Vocational School of Health Services, Mardin Artuklu University, Mardin, Turkiye*
[4]*Dicle University, Science Faculty, Department of Biology, 21280, Diyarbakir, Turkiye*
[5]*Electricity and Energy Department, Vocational School, Mardin Artuklu University, Mardin, Turkiye*

[*]***For correspondence:*** *musa.karadag@igdir.edu.tr*

Received: November 18, 2025; Reviewed: December 11, 2025; Accepted: December 18, 2025

This study was conducted to determine the phenolic compound profile of *Rhus coriaria* L. (sumac) and to investigate the potential of these components for developing value-added products in the cosmetic, health, and food industries. Sumac, which holds an important place among medicinal and aromatic plants, possesses strong antioxidant, antimicrobial, and anti-inflammatory effects thanks to its rich content of phenolic acids, flavonoids, and organic compounds. In this study, the phenolic content of ethanol-water (80:20, v/v) extracts obtained from sumac fruit samples was analyzed using high-performance liquid chromatography (HPLC-DAD). A total of 10 phenolic compounds were identified as a result of the analysis. The compound with the highest concentration was gallic acid (5212.65 ng/µL), followed by ferulic acid (671.76 ng/µL), ascorbic acid (255.23 ng/µL), protocatechuic acid (111.67 ng/µL), and o-coumaric acid (81.76 ng/µL). Rutin, hesperidin, neohesperidin, vanillic acid, and p-coumaric acid were detected in lower concentrations. The results indicate that sumac extracts are among the natural antioxidant sources with high phenolic content. The presence of phenolic compounds reveals that the sumac plant plays an effective role in combating oxidative stress, cell regeneration, and suppressing microbial activity. Compounds such as gallic and ferulic acids, in particular, can be evaluated as natural active agents in the cosmetics industry due to their anti-aging, UV-protective, and collagen synthesis-supporting properties. Furthermore, sumac extracts can be used as natural preservatives and flavorings in food products; thanks to their antimicrobial properties, they extend shelf life and increase product stability. In the health sector, due to their antioxidant and metabolic balancing effects, they can be considered as a potential raw material in phytotherapeutic formulations. These findings demonstrate that *Rhus coriaria* is a rich source of phytochemicals in terms of biologically active components and can be a strategic raw material in sustainable production, natural cosmetic formulations, and functional food development processes. Considering Turkey's climatic advantages, the study also reveals the potential of sumac to create economic added value through domestic production.

Keywords: *Rhus coriaria*, *phenolic compounds, HPLC-DAD, antioxidant activity, cosmetic applications, functional foods*

INTRODUCTION

Medicinal and aromatic plants have been used for both nutritional and health purposes since the beginning of human history, and today they have strategic importance in terms of the global economy and industrial applications (Aras et al., 2024; Ekor et al., 2020). The demand for plant-based products is shaped by factors such as naturalness, sustainability, environmental awareness, and health consciousness. Accordingly, consumers prefer functional, natural, and safe products; this trend is increasingly highlighting the importance of plant-based raw materials in innovative product development processes, particularly in the cosmetics, health, and food sectors. (Başar et al., 2024; Bagheri et al., 2020). Sumac is a perennial plant belonging to the Anacardiaceae family and grows naturally in the Mediterranean, Middle Eastern, and Anatolian regions. Throughout history, sumac fruits have been used as a spice, a natural coloring agent, and for medicinal purposes.

DOI: https://doi.org/10.62088/timbb/9.2.10

Ethnobotanical research reveals that sumac has a wide range of uses in traditional medicine due to its antioxidant, antimicrobial, anti-inflammatory, and metabolic regulatory properties (Al, 2015; Mehdizadeh et al., 2016). Sumac, thanks to its rich content of phenolic compounds, flavonoids, organic acids, and essential oils, possesses remarkable potential not only in traditional uses but also in modern industrial applications. These components support the plant's functional properties, contributing to its antioxidant, antimicrobial, anti-inflammatory, and free radical scavenging effects. Therefore, sumac is considered an important raw material for the development of value-added natural products in the pharmaceutical, cosmetic, and food industries. Recent phytochemical and pharmacological studies have shown that sumac extracts contain high levels of phenolic compounds, and these components play a decisive role in biological activities (Karadag et al., 2024; Bagheri et al., 2020). These findings reveal that sumac can be a valuable natural resource not only in traditional medicine but also in modern biotechnological and industrial production processes. The aim of this study is to comprehensively examine the biologically active components of *Rhus coriaria* and their potential application areas in the pharmacological, cosmetic, health, and food industries. Furthermore, the potential for creating added value from sumac in terms of sustainable production, industrial application possibilities, and R&D studies is evaluated. In this context, the phenolic components of the sumac sample obtained in the study were determined using the HPLC-DAD method, the results were compared with existing literature data, and the industrial and biotechnological potential of the plant was revealed.

1.1. Chemical composition of sumac (*Rhus coriaria* L.): Sumac has a rich phytochemical profile, with its main components being phenolic acids, flavonoids, tannins, organic acids, and essential oils. The fruit and leaf parts of the plant contain particularly potent antioxidant compounds such as gallic acid, gallotannins, catechin, quercetin, myricetin, rutin, and naringin (Al, 2015; Mehdizadeh et al., 2016). Furthermore, organic acids such as malic acid, citric acid, and ascorbic acid support the plant's sour taste and antimicrobial effects. In the essential oil fraction, terpenoid compounds such as β-caryophyllene, limonene, α-pinene, and citral have been identified. This rich chemical composition gives sumac antioxidant, antimicrobial, anti-inflammatory, and free radical scavenging properties, thus making it a valuable natural ingredient in the health, food, and cosmetic industries.

1.2. Phenolic compounds and flavonoids of *Rhus coriaria* L.: Sumac is particularly rich in phenolic compounds and flavonoids. Fruit and leaf extracts contain compounds such as gallic acid, ellagic acid, tannins, quercetin, and kaempferol. These compounds are responsible for the plant's antioxidant, antimicrobial, and anti-inflammatory activities (Karadağ et al., 2021; Bagheri et al., 2020). The concentration of phenolic compounds varies depending on factors such as plant species, growing region, and harvest time.

Table 1. Uses of sumac in cosmetic, health and food products.

Industry	Application	Functional Effect	Application Area	References
Cosmetic	Skin care products	Antioxidant, anti-aging, skin regenerating, anti-inflammatory	Antioxidant creams, serums and lotions (1–3% sumac extract)	Bagheri et al, 2020; Mehdizadeh et al, 2016
	Hair care products	Prevents hair loss, strengthens the scalp, prevents dandruff	Shampoo and hair tonic (1–2% sumac extract)	Bagheri et al, 2020
	Natural colorants & preservatives	Increases product stability thanks to phenolic compounds	Creams and lotions with 1–3% extract	Mehdizadeh et al, 2016
Healty	Phytotherapeutic applications	Antioxidant, anti-inflammatory, analgesic, metabolic support (blood sugar and hypertension)	Tea, capsules, tablets, ointments	Al, 2015; Bagheri et al, 2020
	Topical and aromatherapy	Wound healing, skin regenerating, soothing	Ointments, lotions, aromatherapy products	Bagheri et al, 2020
Food	Spices and flavorings	Provides natural taste and aroma	Salads, sauces, meals (0.5–1% spice addition)	Mehdizadeh et al, 2016
	Natural preservatives	Phenolic compounds prevent microbial spoilage	Baked goods, snack bars, functional foods	Bagheri et al, 2020
	Functional food	Antioxidant and biological activity enhancer	Tea, herbal supplements, natural extract products	Mehdizadeh et al, 2016
	Food safety	Use in accordance with EFSA/FDA toxicological limits	Standardized with toxicological tests	Mehdizadeh et al, 2016

1.3. Essential oils and terpenes of *Rhus coriaria* L.: The essential oils found in sumac fruits and leaves give the plant its characteristic aroma and biological activity. α-pinene, limonene, and β-caryophyllene are the main terpene components. These essential oils are used both as flavoring agents in the food and cosmetics industries and as antimicrobial and anti-inflammatory agents (Koçak et al., 2021; Mehdizadeh et al., 2016).

1.4. Vitamin and mineral composition of *Rhus coriaria* L.: *Rhus coriaria* L. is also an important herb in terms of vitamins and minerals. It is especially rich in vitamin C and vitamin K. Among the minerals, calcium, magnesium, iron, and potassium stand out. These components both increase the nutritional value of food products and contribute to functional health products (Zhang et al., 2023; Al, 2015).

1.5. Organic acids of *Rhus coriaria* L.: *Rhus coriaria* L. fruits contain organic acids such as malic acid, citric acid, and acetic acid. These acids function as acidity regulators and preservatives in food and health products. Additionally, along with phenolic compounds, they support antioxidant capacity, extending the shelf life of products (Karadag ve Doğan, 2024; Bagheri et al., 2020).

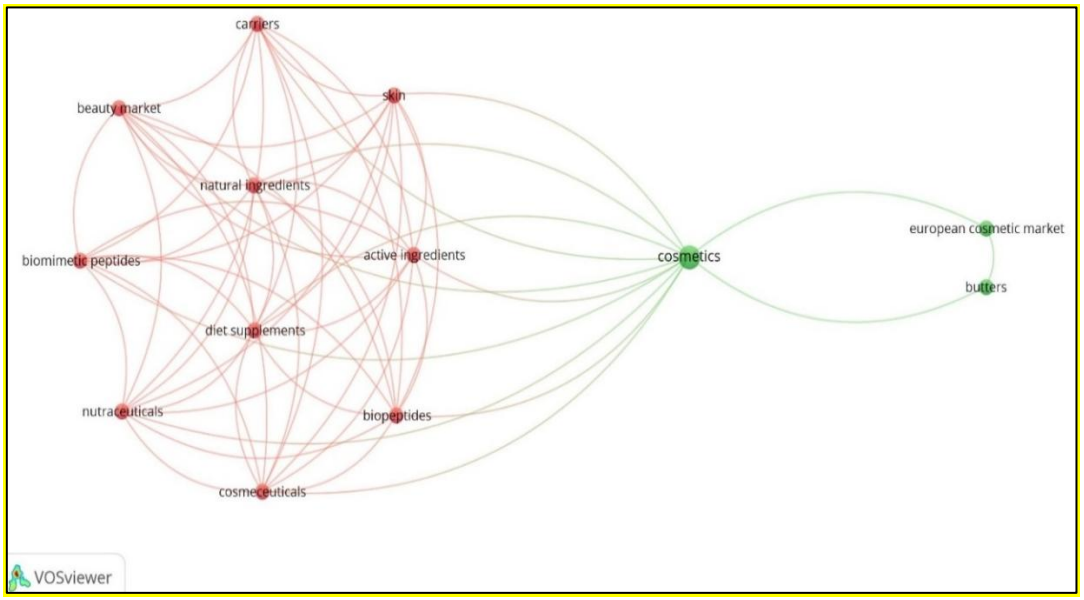


Fig. 1. Keywords used in academic studies regarding *Rhus coriaria* (Webof Sience) (vosviwer).

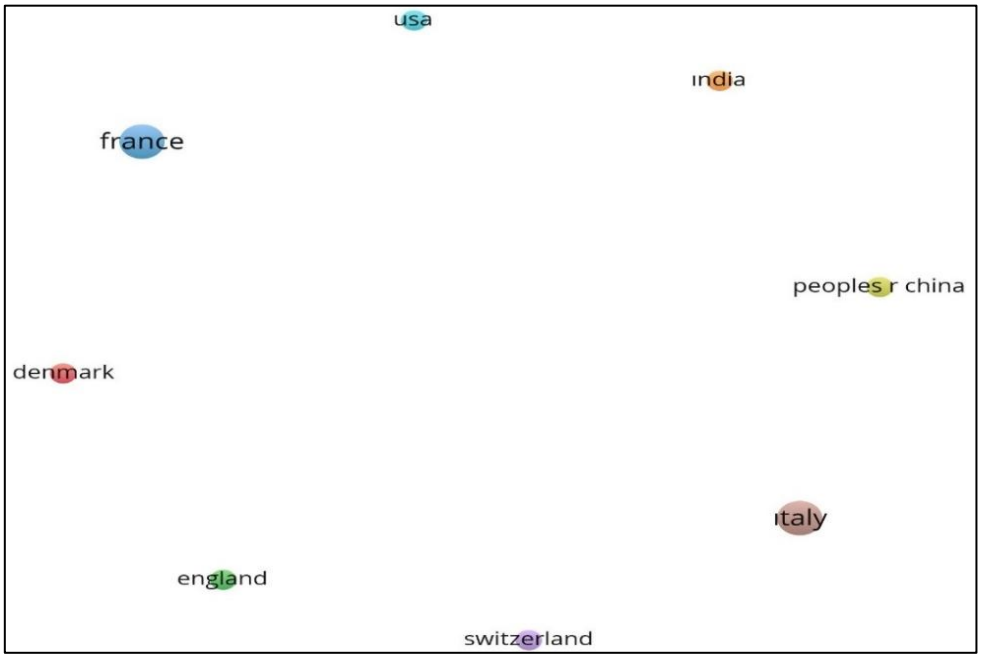


Fig. 2. Countries where *Rhus coriaria* is studied academically (Web of Science) (vosviewer).

Table 2. Moisturizing cream formula with *Rhus coriaria* L. extract.

Ingredients	% (w/w)	Function
Deionized Water	72.00	Solvent
Glycerin	5.00	Moisture retainer
Sumac Extract (EtOH–H_2O 80:20)	2.00	Antioxidant, skin regenerator
Stearyl Alcohol	3.00	Emulsifier and thickener
Cetyl Alcohol	2.00	Emulsifier, emollient
Glyceryl Stearate SE	4.00	Emulsifier
Caprylic/Capric Triglyceride	5.00	Carrier oil, emollient
Shea Butter	4.00	Skin nourishing oil
Dimethicone	1.00	Skin protector, emollient
Phenoxyethanol + Ethylhexylglycerin	0.80	Protective system
Fragrance	0.20	Fragrance
pH adjuster (Citric acid or NaOH)	1.00	pH regulator

1.6. Extraction methods of *Rhus coriaria* L.: The extraction of biologically active compounds from *Rhus coriaria* L. varies depending on the extraction method used. Common methods include ethanol, methanol, water, and supercritical CO_2 extraction. These methods affect both the phenolic and flavonoid yields and the stability of the resulting extract. Supercritical CO_2 extraction, in particular, allows for the production of a highly efficient and pure extract without leaving solvent residue (Karadağ ve Omarova, 2024; Mehdizadeh et al., 2016; Bagheri et al., 2020).

1.7. Diversity of chemical composition of *Rhus coriaria* L.: The chemical composition of *Rhus coriaria* L. is affected by environmental and agronomic factors such as species, ecological conditions, soil structure, climate, irrigation, and harvest time. Therefore, controlling the source material and determining quality parameters are critical for standardized extract production in industrial applications (Karadağ et al., 2025; Bagheri et al., 2020).

2. MATERIALS AND METHODS

2.1. *Rhus coriaria* plant material: The *Rhus coriaria* fruit sample used in this study was obtained from within the borders of Adıyaman province, Turkey. The collected fruits were separated under natural conditions, dried in the shade and in a well-ventilated environment at room temperature (20–25 °C), and ground into a homogeneous powder using a laboratory-type grinder. The ground sample was reduced to a particle size of 0.5–1 mm (or the sieve size used), and its moisture content was recorded using a moisture analyzer. The prepared powder sample was stored at -20°C in light-proof containers, protected from light and oxidation, before analysis (Karadağ, 2025).

2.2. Extract preparation: The solvent used in sample extraction was EtOH (ethanol). The extraction procedure was performed as follows: 2.00 g of ground sumac powder was taken and transferred to a 50 mL conical tube with 20.0 mL of 80% EtOH–H_2O (v/v) solution. The tube was extracted in an ultrasonic bath (35 kHz, 30 min), then centrifuged at 4,000 × g for 10 minutes, and the supernatant was passed through a 0.45 µm membrane filter to prepare it for HPLC analysis.

2.3. Preparation and Concentration: If necessary, the filtered extracts were slightly concentrated using a rotavapor (vacuum; <40 °C) to standardize the solvent volume of the solution. If the samples given for analysis were close to the saturation limit, they were diluted to the appropriate factor and homogenized before injection. All solutions were analyzed in dark glass bottles at +4 °C and within a maximum of 48 hours.

PRODUCTION STAGES

1. Phase A (water phase): Glycerin is dissolved in deionized water heated to 70–75 °C in a water bath.
2. Phase B (oil phase): In a separate container, stearyl alcohol, cetyl alcohol, glyceryl stearate SE, shea butter, and caprylic/capric triglyceride are mixed and melted at 70–75 °C.
3. Emulsification: The oil phase is slowly added to the water phase and mixed with a high-speed homogenizer (10,000 rpm, 5 min).
4. While the mixture is cooling to 40–45 °C, sumac extract (previously prepared with an 80% EtOH–H_2O mixture) is added.
5. Preservative, silicone, and perfume are added, and the pH is adjusted (pH 5.5).
6. When the cream reaches a homogenous structure after homogenization, it is packaged. Feature: High antioxidant effect, reduces free radical damage to the skin. It has a light, natural color (light pink tone due to sumac polyphenols).

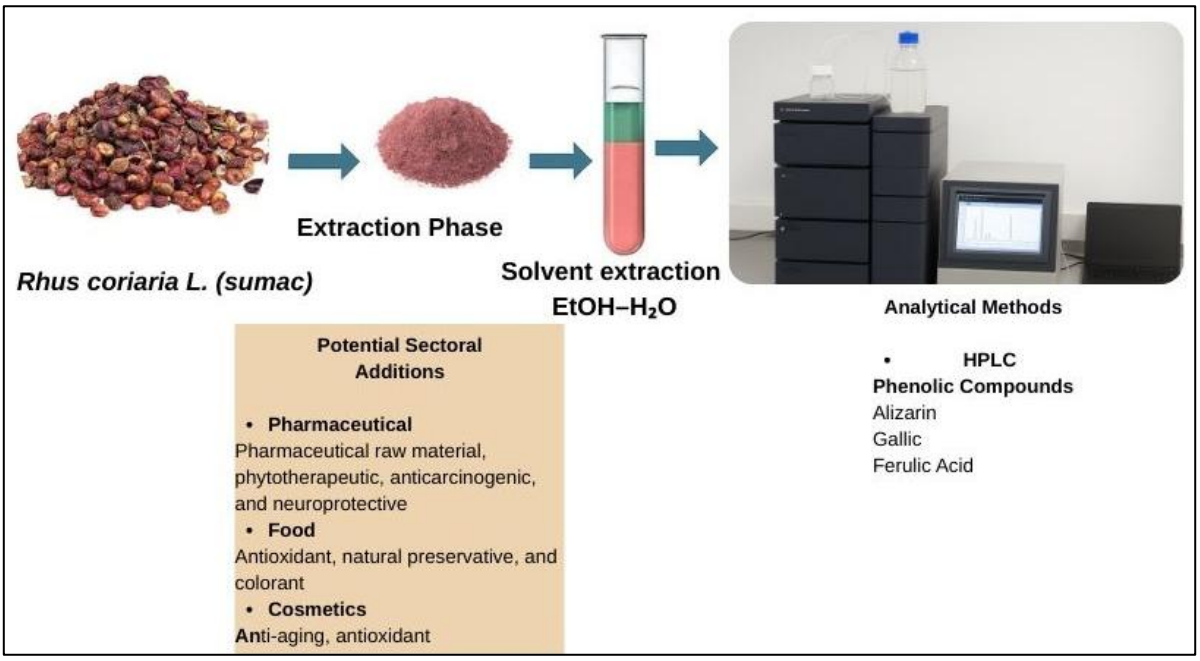


Fig. 3. The steps involved in preparing the cream from the sumac plant.

2.4. Analysis of phenolic compounds: The determination of phenolic compounds was performed using an Agilent brand HPLC system with a diode array detector (DAD). Detector settings were made at 300/200 nm wavelengths, with a reference value of 500/100 nm. Chromatographic analysis conditions were determined as follows: mobile phase: 83% (water containing 0.1% phosphoric acid) – 17% acetonitrile mixture, flow rate: 0.8 mL/min, column temperature: 30 °C, and injection volume: 10 µL. In the gradient system, the organic phase ratio was gradually increased between 0-40 minutes. Standards used for calibration included gallic acid, ferulic acid, rutin, hesperidin, o-coumaric acid, vanillic acid, protocatechuic acid, and ascorbic acid. The obtained data were evaluated using the external standard method, and the results were calculated in ng/µL (Koçak et al., 23021).

3. FINDINGS

According to HPLC-DAD analysis results, a total of ten different phenolic compounds were detected in the sumac sample. The compound with the highest concentration was gallic acid (5212.65 ng/µL), constituting approximately 81% of the total phenolic content. Gallic acid was followed by ferulic acid (671.76 ng/µL), ascorbic acid (255.23 ng/µL), protocatechuic acid (111.67 ng/µL), and ocoumaric acid (81.76 ng/µL), respectively. Lower concentrations of rutin (19.29 ng/µL), hesperidin (21.65 ng/µL), neohesperidin (14.72 ng/µL), vanillic acid (17.06 ng/µL), and p-coumaric acid (2.23 ng/µL) were also detected. The total phenolic content was calculated as 6414.70 ng/µL, indicating that sumac has a high antioxidant potential. The high levels of gallic and ferulic acids, in particular, support the idea that the plant may be effective in combating oxidative stress. The presence of these compounds reveals that sumac extracts are an important source of antioe.

Rhus coriaria L. extracts offer wound-healing, skin-renewing, and anti-aging effects in dermatological products. Furthermore, the aromatherapy properties of the essential oils are supported by stress-reducing and mentally relaxing effects. Therefore, sumac is preferred as a reliable and natural ingredient in both topical applications and aromatherapy products (Al, 2015; Mehdizadeh et al., 2016).

Rhus coriaria L. has the potential to develop high-value-added health products thanks to its biological activities and rich phytochemical profile. Phytotherapeutic and pharmaceutical products, formulated with naturally derived and functional compounds, can offer advantages in terms of consumer safety and efficacy. Sumac, thanks to both its biologically active components and versatile applications, has the potential to create high added value through an integrated chain encompassing agriculture, extraction, formulation, and marketing. The plant's economic and industrial potential stems from its use as a premium raw material in the cosmetics, health, and food sectors (Bagheri et al., 2020).

Table 3. Phenolic compounds detected in sumac (*Rhus coriaria* L.) sample.

No	Compound Name	Rejection Time (min)	Amount (ng/µl)	Functional Property
1	Ascorbic acid	3.38	255.23	Antioxidant, source of Vitamin C
2	Gallic acid	4.31	5212.65	Powerful antioxidant, antimicrobial
3	Protocatechuic acid	5.72	111.67	Cell protective, antioxidant
4	Vanillic acid	9.72	17.06	Anti-inflammatory
5	p-Coumaric acid	17.11	2.23	Free radical scavenger
6	Rutin	18.93	19.29	Capillary protective, antioxidant
7	Ferulic acid	19.98	671.76	UV protective, skin regenerating
8	o-Coumaric acid	28.81	81.76	Antimicrobial
9	Neohesperidin	29.52	14.72	Vascular protective, antioxidant
10	Hesperidin	36.76	21.65	Skin regenerating, anti-inflammatory

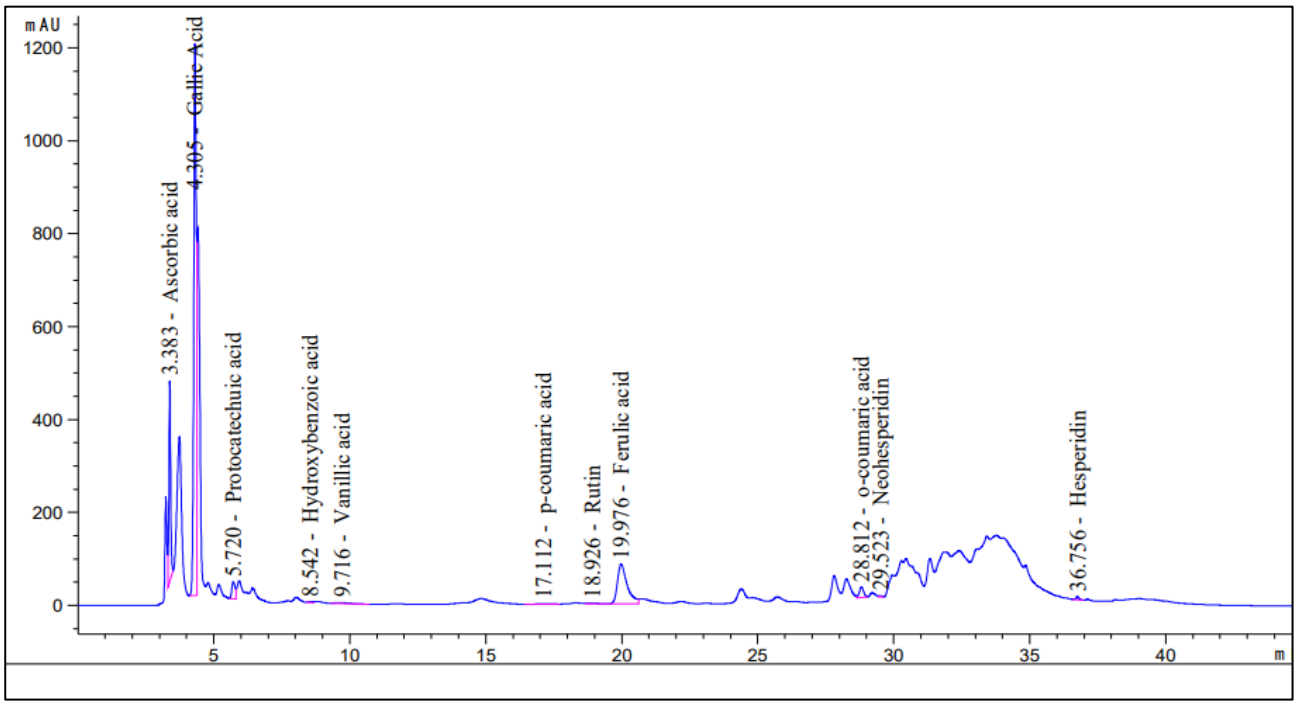


Fig. 4. Sumac (*Rhus coriaria*) HPLC phenolic chromatogram.

4. DISCUSSION

The results obtained are largely consistent with data reported in the literature (Bagheri et al, 2020; Mehdizadeh et al, 2016; Al-Snafi, 2015). Gallic acid, identified as the dominant compound in sumac extracts, is a potent antioxidant and antimicrobial agent, valued as a natural preservative in the food and cosmetic industries. Ferulic acid is a phenolic acid frequently used in cosmetic formulations due to its UV protection, collagen synthesis support, and skin elasticity enhancement properties. Furthermore, the presence of flavonoids such as rutin, hesperidin, and neohesperidin plays a role in the development of vascular protective, anti-inflammatory, and anti-aging effects. These compounds make significant contributions to maintaining skin health, supporting the circulatory system, and reducing free radical damage. The phenolic profile of sumac extracts demonstrates high antioxidant capacity and reveals its potential to reduce oxidative stress-induced cellular damage. Literature reports that *Rhus coriaria* extracts have antimicrobial effects against bacteria such as *Staphylococcus aureus*, *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella* spp. (Bagheri et al, 2020). This supports the use of sumac extracts as a food preservative, cosmetic anti-aging agent, and phytotherapeutic component. In conclusion, the high phenolic content biological profile of the sumac plant indicates that it can be evaluated as a sustainable raw material in the development of natural preservatives, antioxidants, and therapeutic products. Furthermore, flavonoids such as rutin and hesperidin play a supportive role in preventing metabolic diseases through their effects that increase vascular elasticity and suppress inflammation. The results obtained indicate that sumac extracts can be considered a safe, natural, and effective component in both topical and oral applications.

4.1. Use in Cosmetic products: Sumac extract can be used as a powerful antioxidant agent in skin care. When included in formulations at a rate of 1–3%, it provides anti-aging, skin barrier strengthening, and anti-inflammatory effects. It also offers anti-dandruff and hair loss reduction effects in hair care products (1–2%).

4.2. Use in health products: Sumac can be used as an antioxidant, analgesic, and metabolic regulator in phytotherapeutic preparations (tea, capsules, ointment). Clinical studies report that sumac extracts show positive effects in lowering blood sugar and cholesterol levels (Karadag et al, 2024; Al, 2015).

4.3. Use in the food ındustry: In addition to its natural aroma and flavor-enhancing properties, sumac extends product shelf life by preventing microbial spoilage. Thanks to its phenolic compounds, it reduces the need for synthetic preservatives in food products. In functional foods (tea, bars, baked goods), sumac extract can be considered as an antioxidant additive.

4.4. Sustainable production and R&D potential: By-products (leaves, stems, bark) obtained from sumac production can be utilized through extraction, providing economic and environmental benefits. Furthermore, the use of green technologies such as supercritical CO_2 extraction allows for the production of high-purity extract without solvent residue. This is a significant advantage in terms of sustainable production on an industrial scale.

5. CONCLUSION AND RECOMMENDATIONS

This study revealed that the *Rhus coriaria* L. plant is quite rich in phenolic compounds, and that gallic acid and ferulic acid components, in particular, play a decisive role in biological activity. The findings show that sumac extracts have high antioxidant, antimicrobial, anti-inflammatory, and photoprotective effects, and that these properties can be evaluated in the cosmetic, health, and food industries.

Thanks to their natural and non-toxic structure, sumac extracts are an ideal component for anti-aging cosmetic products, functional foods, phytotherapeutic preparations, and natural preservative agents. These results show that it is possible to transform the plant into economic value within the framework of sustainable agriculture and green chemistry principles.

• Standardization: The phenolic compound profile of sumac extracts to be used on an industrial scale should be standardized, and component fluctuations between production batches should be minimized.

• Green Extraction Techniques: Supercritical CO_2, ethanol, or water-based extraction methods should be popularized to support environmentally friendly production processes that do not leave solvent residues.

• Toxicological and Clinical Verification: The safety of sumac extracts for use in food and cosmetic products should be verified through toxicological tests and clinical studies. • University-Industry Collaboration: The development of innovative sumac-based products should be encouraged through collaboration between R&D centers and the cosmetic/food industry.

• Sustainable Agricultural Practices: Controlled production zones should be established, taking into account Turkey's climatic advantages, and sumac production should be planned in a way that contributes to the protection of agricultural biodiversity.

• Value-Added Product Development: The utilization of by-products derived from sumac extracts (e.g., phenolic concentrates, essential oils, colorants) in food supplements, cosmetic formulations, and natural preservative systems should be encouraged. Acknowledgments: I would like to express my gratitude to the YIP0723I08 project for providing financial support during the research and preparation of the data presented in this article.

ETHICAL CONSIDERATIONS

This study did not involve human participants or experimental animals. The plant material (*Rhus coriaria* L.) used in the research was obtained from commercially available sources and/or collected in compliance with national and institutional regulations governing the collection and use of plant resources in Turkey. All experimental procedures were conducted in accordance with internationally accepted ethical standards for laboratory research, ensuring scientific integrity, data accuracy, and reproducibility. The authors confirm that no ethical approval was required for this study.

ACKNOWLEDGEMENTS

The authors would like to express their sincere gratitude to Iğdır University Research Application and Analysis Laboratory (ALUM) for providing technical infrastructure and analytical support

during the HPLC-DAD analyses. The authors also thank Mardin Artuklu University and Dicle University for their institutional support and contributions to the successful completion of this research.

FUNDING

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

CONFLICT OF INTEREST

The authors declare that there are no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

REFERENCES

Al-Snafi A. E. (2015) Chemical constituents and pharmacological effects of *Rhus coriaria* – A review. *International Journal of Pharmacy and Pharmaceutical Sciences,* **7(9):** 12–20.

Aras A., Karadağ M. (2024) Bazı bitkilerin sekonder metabolit içeriklerinin HPLC ile analizi. *Journal of Agriculture*, **7(2):** 157-167.

Bagheri S. et al. (2020) Antioxidant and antimicrobial activities of *Rhus coriaria* extracts: A comprehensive review. *Food Science and Nutrition,* **8(4):** 1732–1745.

Başar Y., Gül F., Karadağ M., Alma M.H., Demirtas I., Tel A.Z. (2024) Determination of the volatile, phenolic and fatty acid contents of Helichrysum plicatum by chromatographic methods. *Turkish Journal of Biodiversity*, **7(2):** 83-94.

Ekor M. et al. (2020) Medicinal and aromatic plants: Industrial applications and global perspectives. *Journal of Herbal Medicine,* **22:** 100-110.

Karadag M., Doğan S. (2024) Mask for moisturizing skin and body made from cold-pressed paste of peanuts (*Arachis hypogaea* L.). *Adv. Biol. Earth. Sci.*, **9(1):** 155-60.

Karadag M., Baran A., Güneş Z. (2024) Production of value-added cosmetic products from cold-pressed *Helianthus annuus* L. oil and pulp. *Advances in Biology & Earth Sciences*, **9(Special Issue):** 35-39.

Karadağ M. (2025) *Mentha arvensis* L.(Kır Nanesi) örneklerinin fenolik bileşik profili. *Türkiye Teknik Bilimler ve İnovasyon Dergisi*, **1(1):** 12-23.

Karadağ M., Omarova S. (2024) Use of *Prunus armeniaca* L. seed oil and pulp in health and cosmetic products. *Adv. Biol. Earth. Sci.*, **9(1):** 105-10.

Karadağ M., Güneş Z., Çelik S. (2025) Iğdır'da yetişen biberiye (*Rosmarinus officinalis* L.) bitkisinden elde edilen uçucu yağın kimyasal kompozisyonu ve kozmetik alanındaki kullanımı. *Journal of Agriculture*, **8(1):** 53-64.

Karadağ M., Koyuncu M., Atalar M.N., Aras A. (2021) SPME/GC-MS analysis of *Artemisia campestris* subsp. *glutinosa*, *Lavandula angustifolia* Mill., and *Zingiber officinale* volatiles. *Erzincan Üniversitesi Fen Bilim Enstitüsü Derg.*, **14:** 41-49.

Koçak M.Z., Karadağ M., Çelikcan F. (2021) Essential oil composition of *Salvia officinalis* and *Rosmarinus officinalis*. *Journal of Agriculture*, **4(1):** 39-47.

Mehdizadeh L. et al. (2016) Phytochemical and pharmacological properties of sumac (*Rhus coriaria*): A review. *Journal of Medicinal Plants Research,* **10(11):** 155–163.

Zhang N., Baran A., Valioglu F., Teng L., Atalar M.N., Keskin C., ... Beilerli A. (2023) Antioxidant, AChE inhibitory, and anticancer effects of *Verbascum thapsus* extract. *Cellular and Molecular Biology*, **69(14):** 211-216.

ORCIDs:

Musa Karadag: https://orcid.org/0000-0003-2498-3403
Mehmet Firat Baran: https://orcid.org/0000-0001-8133-6670
Kadir Sinan Aslan: https://orcid.org/0000-0003-4564-1285
Mehmet Tevfik Adican: https://orcid.org/0000-0001-7733-9676

MINISTRY OF SCIENCE AND EDUCATION REPUBLIC OF AZERBAIJAN

TRANSACTIONS

INSTITUTE OF MOLECULAR BIOLOGY & BIOTECHNOLOGIES

Volume IX, No 2 2025

CONTENT

Ministry of Science and Education
of the Republic of Azerbaijan
Institute of Molecular Biology and Biotechnologies

11 Izzat Nabiyev Str.,
AZ1073 Baku, Azerbaijan
Tel: +994 12 538 1164
Fax: +994 12 510 2433
E-mail: imbb@science.az
www.timbb.az