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Investigation of fractions derived from the alcoholic extract of the *Acacia* root plant on LDL oxidation in vitro

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Abstract

Low-density lipoprotein (LDL) is formed during the oxidation process, and the damages resulting from these oxidative reactions lead to the development of cardiovascular diseases. Paraoxonase 1, a calcium-dependent enzyme bound to high-density lipoprotein (HDL). The aim of this study was to isolate LDL and investigate the fractions obtained from the alcoholic extract of Aqaqia root to assess their impact on oxidation LDL and paraoxonase 1 activity under in vitro conditions. Blood samples were obtained from healthy individuals after an overnight fast and their serum was separated using ultracentrifugation with a medium gradient. LDL samples were subjected to copper sulfate oxidation, and the kinetics related to LDL oxidation, inhibition of conjugated DNA formation, as well as paraoxonase enzyme activity in the presence of polyphenolic components from gel filtration chromatography of Aqaqia root extract, were examined. The oxidation of LDL under the influence of polyphenolic components derived from gel filtration chromatography of Aqaqia root extract resulted in a significant reduction and also lead to the inhibition of conjugated DNA formation, a secondary product of lipid peroxidation. However, it did not show a significant increase in paraoxonase enzyme activity. Findings indicated that phenolic compounds obtained from gel filtration chromatography of Aqaqia root extract were effective in reducing the LDL oxidation process and diminishing the production of conjugated DNA compared to the control sample. These compounds have the potential to be effective in preventing oxidative-related diseases including atherosclerosis.

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Introduction

Plants have been used for medicinal purposes for over 100 years (Vitalini *et al.*, 2013). Examining the history of herbal medicine usage from ancient times to the mid-20th century indicates a decrease in the consumption of medicinal plants until the 1940s and a subsequent resurgence in their use until the 1980s (Bauer, 2012). The presence of bioactive compounds among plants such as antioxidants, antimicrobial agents, and anti-tumor substances has enabled their utilization as medicinal herbs, preservatives, and dietary supplements (Samtiya *et al.*, 2021). One of the challenges faced by humans today is dealing with certain chronic and life-threatening diseases such as cancer, cardiovascular diseases, and respiratory disorders (Koene *et al.*, 2016). Free radicals and oxidative substances are continuously generated in living organisms due to various metabolic reactions (Chaudhary *et al.*, 2023). Considering the recognized role of free radicals and oxidants in the onset and progression of these diseases, the significance of antioxidants in the diet as neutralizers of the detrimental effects of free radicals becomes highly sensitive (Jena *et al.*, 2023). Aqaqia, commonly known as Black Locust, is a flowering tree or shrub belonging to the Fabaceae family, which grows in most regions of Iran. This tree is deciduous and its leaves are compound, with a color ranging from green to bluish-green (Uzelac *et al.*, 2023). Aqaqia (Robinia pseudoacacia) is among the resilient trees that have adapted to various climates and is utilized for beautifying green spaces, controlling soil erosion, and reclaiming cultivated lands. In the past, people used Aqaqia for the treatment of various diseases, and it still plays a significant role in modern pharmaceutical and nutritional sciences (Vítková *et al.*, 2017). A study conducted on Aqaqia root in India has demonstrated that many polyphenolic compounds obtained from the dietary intake of antioxidant plants are effective in laboratory conditions, similar to vitamins E and C (Bouayed *et al.*, 2010). Consequently, they may significantly contribute to the body's protective effects (Ansari *et al.*, 2023). Numerous studies have confirmed the high antioxidant activity of compounds isolated from *Robinia pseudoacacia* bark extract (Ostolski *et al.*, 2021). Studies have shown that the extract of this plant has blood sugar-lowering effects, which are related to the presence of flavonoids in the plant. This plant is used in the treatment of diabetes along with other herbs (Yen *et al.*, 2021). Its hepatoprotective effects have also been demonstrated. Phenolic compounds constitute an essential part of phytochemicals with diverse biological activities (Madrigal *et al.*, 2014). These include properties such as antioxidant, anti-tumor, anti-inflammatory, antibacterial, and more (Cuevas-Cianca *et al.*, 2023). LDL is the most significant cholesterol-carrying lipoprotein in the blood, delivering cholesterol from the liver to peripheral tissues (Genovesi *et al.*, 2023). LDL enters peripheral cells via endocytosis, transferring its contents to them (Siddiqui *et al.*, 2022). HDL2 plays a role in transporting cholesterol back to the liver, removing excess cholesterol from peripheral tissues in this process (Ouimet *et al.*, 2019). Increasing the production of free radicals or reducing the levels of antioxidants may lead to oxidative damage to polyunsaturated fatty acids present in the cell membrane structure (Lobo *et al.*, 2010). This phenomenon, recognized as lipid peroxidation, initiates a chain reaction, ultimately resulting in the formation of Malondialdehyde (Ayala *et al.*, 2014). If this oxidative damage initiates, it can progress in a chain reaction leading to cellular death, accompanied by widespread symptoms of disease (Singh *et al.*, 2019). Lipids are among the most important molecules targeted by free radicals' attacks (Phaniendra *et al.*, 2015). This process leads to lipid peroxidation, ultimately resulting in reduced cell viability and cell death (Li *et al.*, 2020). Among these, cell membranes rich in polyunsaturated fatty acids are more sensitive to peroxidation compared to other cellular components (Mortensen *et al.*, 2023). Lipid peroxidation causes membrane fluidity reduction and disrupts its structure and functionality, implicating its involvement in the pathogenesis of many diseases (Gaschler *et al.*, 2017). Free radicals, such as reactive oxygen species commonly referred to as oxidative agents, reduce the activity of the peroxidase enzyme and increase the rate of LDL oxidation (Phaniendra *et al.*, 2015).

LDL oxidation can create a conducive environment for atherosclerosis development (Poznyak *et al.*, 2020). Paraoxonase (PON1) is a key component of HDL and can deactivate toxic products generated from the oxidation of LDL lipid components (Durrington *et al.*, 2023). There is ample evidence supporting the potential protective effect of PON-1 in the atherogenic process (Longo *et al.*, 2021). The aim of this study was to isolate LDL and investigate the fractions obtained from the alcoholic extract of Aqaqia root to assess their impact on oxidation LDL and paraoxonase 1 activity under in vitro conditions.

Materials and methods

Preparing the extract

The process for preparing the extract from the Aqaqia plant involved soaking. To this end, 10 grams of finely ground Aqaqia plant material were weighed, and the resulting measurement was transferred into a 100-milliliter Erlan flask. Subsequently, 70% ethanol was added as the solvent. The Erlan flask, containing the plant powder and 70% ethanol, was placed in the refrigerator for 72 hours to ensure complete soaking and extraction of the plant's compounds. Following this, the contents inside the Erlan flask were filtered through filter paper to strain out impurities. The filtrate was then incubated for 48 hours. After the incubation period, the material was dried and subsequently frozen.

Column chromatography

Column Chromatography with Sephadex LH-20. In this method, gel permeation chromatography with Sephadex LH-20 was employed according to the Hagman procedure. In this chromatographic technique, molecules with different molecular weights are separated from each other at different time points.

LDL separation

The LDL in the serum was separated at the Cardiovascular Research Center of Isfahan University of Medical Sciences using the discontinuous gradient density method with the help of an ultracentrifuge. This technique, introduced by Bronzert in 1977, involved adding bromide sodium to the serum, increasing its density to 1.182 g/cm³. The separation was carried out using an ultracentrifuge Beckman at 16 degrees Celsius. This experiment proceeded through a two-stage process with durations of 6 and 12 hours, respectively, at 16,000 rotations. Following the protocol established by the HIMAC system, at the end of the first stage, chylomicrons and VLDL were separated as a yellow layer**.** Then, TaliGene Pars Company has provided a rapid measurement kit for Paraoxonase 1 activity using only one substrate. In this method, Paraoxonase 1 catalyzes paraxon to produce paranitrophenol. The amount of paranitrophenol produced was calculated by measuring the increased absorption at a temperature of 25 degrees Celsius and a wavelength of 412 nm. Then, the results obtained in each experiment were analyzed using SPSS 20 software and presented using Excel 2014. Statistical tests were applied to the data.

Results and Discussion

The fraction obtained from gel filtration chromatography, determined by a spectrophotometer at three wavelengths, 280, 365, and 520, is shown in Figs 1 to 3.

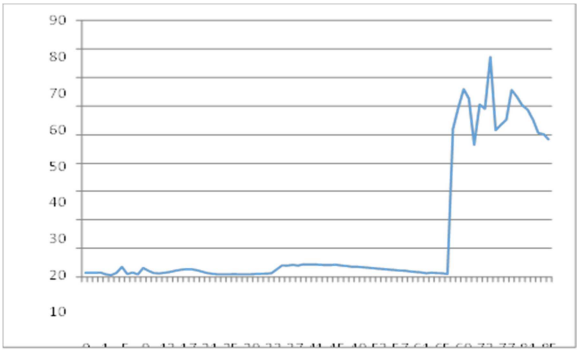


Fig. 1. Absorption Levels of Fractions Obtained from Aqaqia Root Plant at 280 Nanometers Wavelength

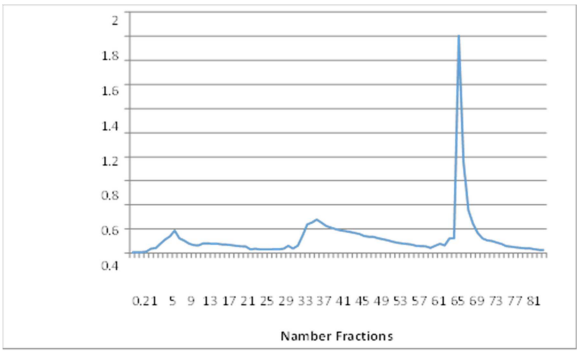


Fig. 2. Absorption Levels of Fractions Obtained from Aqaqia Root Plant at 365 Nanometers Wavelength.

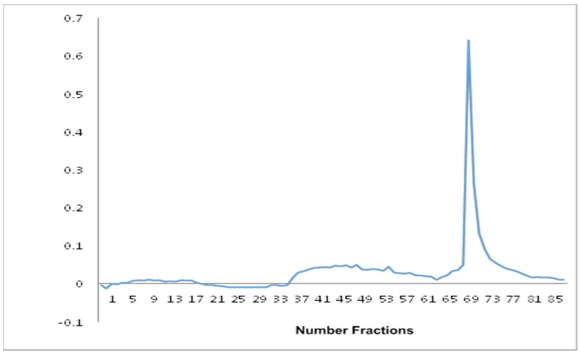


Fig. 3. Absorption Levels of Fractions Obtained from Aqaqia Root Plant at 520 Nanometers Wavelength.

Then considering the Figs 4-10 fractions numbered 10, 42, and 70 were selected in these experiments, possessing the highest concentrations of polyphenolic compounds and absorption. Their effects were evaluated in three different quantities. Furthermore, comparing the levels of conjugated DNA resulting from the oxidation process under the influence of different volumes, it was observed that fractions number 10 at 1 microliter, 2 microliters, 7 microliters significantly reduced the levels of conjugated DNA due to the phenolic compounds present in this fraction.

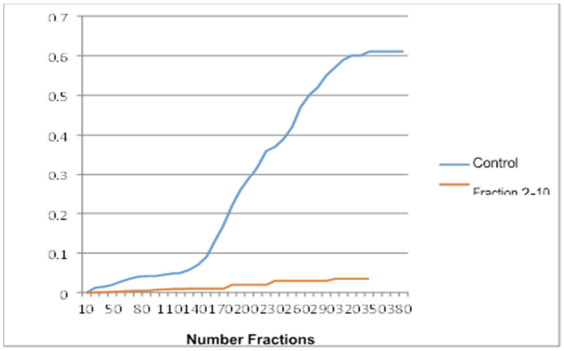


Fig. 4. Comparing the LDL Oxidation Graph Resulting from Fraction Number 10's Effect with 2 µL to the Oxidation Graph of the Control Sample.

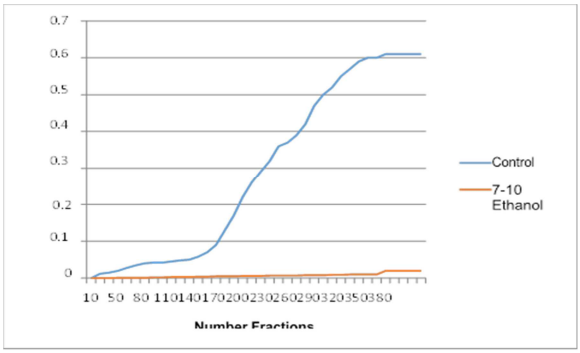


Fig. 5. Comparing the LDL Oxidation Graph Resulting from the Effect of Fraction Number 10 with 7 µl to the Oxidation Graph of the Control Sample.

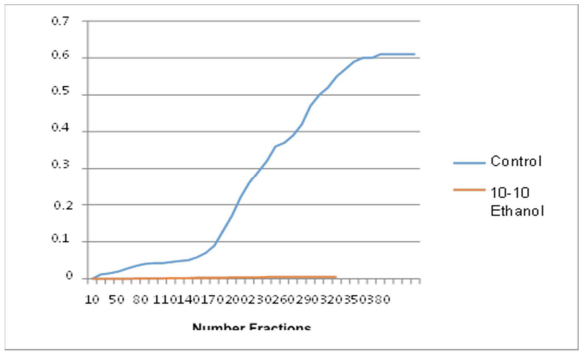


Fig. 6. Comparison of the LDL Oxidation Graph Resulting from the Effect of Fraction Number 10 with 10 µl to the Oxidation Graph of the Control Sample.

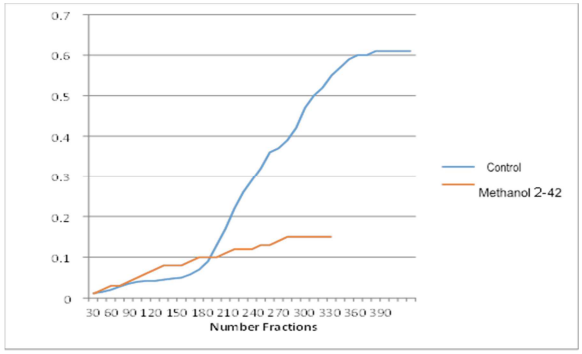


Fig. 7. Compare the LDL oxidation resulting from the effect of Fraction Number 42 at a concentration of 2 microliters with the oxidation graph of the control sample

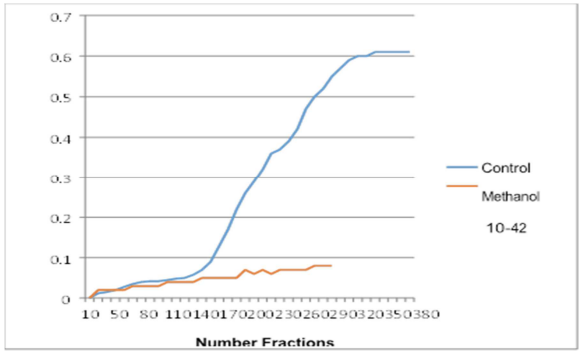


Fig. 8. Comparison of the LDL Oxidation Graph Resulting from the Effect of Fraction Number 42 with 10 Micro Liters to the Oxidation Graph of the Control Sample

Recent studies and research on medicinal plants, focusing on their antioxidant properties and active compounds, which play a role as factors in human health and well-being, serve as indicators of researchers' attention and interest in this important issue (Vaou *et al.*, 2021). Plants containing polyphenolic compounds have a high antioxidant potential.

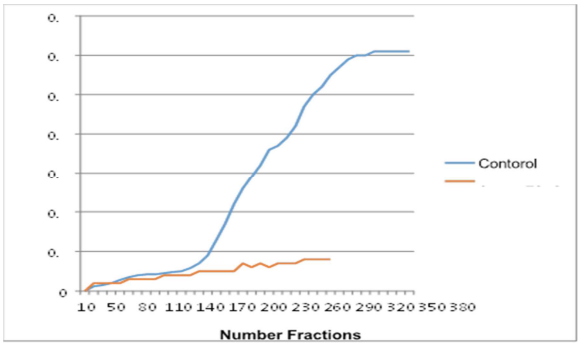


Fig. 9. Comparison of LDL Oxidation Graph Resulting from the Effect of Fraction Number 70 with 2 µl to the Oxidation Graph of the Control Sample.

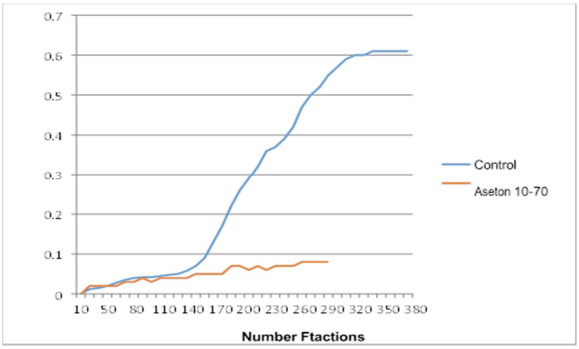
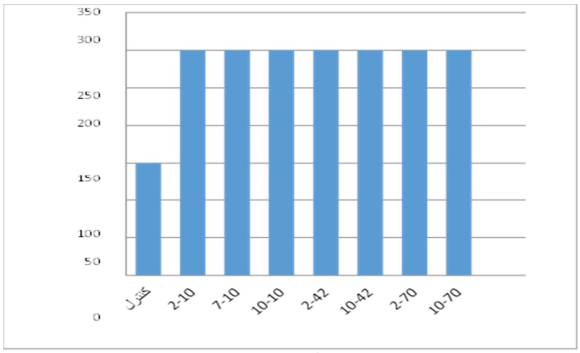


Fig. 10. Compare the LDL oxidation graph resulting from the effect of Fraction Number 70 at a concentration of 10 microliters with the oxidation graph of the control sample



Control=کنترول

Fig. 11. Comparison chart of long-time LDL oxidation.

Since the antioxidant activity of natural compounds and extracts has been identified by a wide range of methods, the question of which of these natural antioxidants have higher efficiency has been raised (Stagos, 2020). The conducted studies on the roots of the Aqaqia plant have indicated that this plant contains substantial amounts of flavonoids, anthocyanins, tannins, phenolic compounds, choline, syringin, starch, simple sugars, and water-soluble proteins (Saeidnia *et al.*, 2011). These compounds have transformed Aqaqia into a medicinal plant with anti-inflammatory, antiviral, antibacterial, and anticancer properties (Frański *et al.*, 2023). Since ancient times, these compounds have been used for the treatment and reduction of coronary heart diseases due to their high capability to absorb free radicals present in the body (Khurana *et al.*, 2013). Therefore, in this study, considering the conducted research on Aqaqia roots and the confirmation of the high capacity of tannins and anthocyanins, as well as the presence of phenolic and flavonoid compounds confirmed, and due to the high antioxidant properties of this plant, we selected it as a rich source of antioxidant compounds and an effective substance in reducing lipid oxidation and preventing atherosclerosis (Kaczorová *et al.*, 2021). The experiments conducted on this plant have shown that the methanolic extract of Aqaqia roots contains the highest antioxidant compounds (Czech *et al.*, 2023). In this study, Due to its significant amount of phenolic and flavonoid compounds, it exhibits superior antioxidant activity compared to its aqueous and alcoholic extracts. The results obtained from the effects of each fraction derived from the gel filtration chromatography of Aqaqia root extract demonstrate inhibition of the oxidation process. Considering the concentration of phenolic compounds in each fraction (Table 1), Fraction 10 exhibited the highest level of phenolic antioxidant compounds. Its impact on isolated LDL particles was investigated at specified quantities of 2, 7, and 10 microliters. It was found that phenolic antioxidant compounds possessed significant capabilities in delaying the process of LDL oxidation. Comparison of the obtained time lag points from the oxidation graph and the results from the time lag diagram (Fig. 11) indicated a significant difference at $p < 0.001$, suggesting a 100% inhibition of the LDL particle oxidation process. This justifies the hypothesis that the phenolic compounds present in Aqaqia root extract are capable of inhibiting free radicals generated from copper sulfate-induced LDL oxidation in vitro conditions. The time lag extended the oxidation curve to approximately 150 minutes compared to the control sample, which is acceptable based on the conducted experiments under in vitro conditions.

Table 1. The level of phenolic compounds in fractions obtained from the Aqaqia root plant.

Absorption	Phenolic compounds (± SD)	Fraction number
0.08	8.625 ± 0.05	1
0.13	12.528 ± 0.02	2
0.098	9.48 ± 0.06	3
0.111	10.689 ± 0.03	4
0.11	10.65 ± 0.05	5
0.18	17.582 ± 0.04	6
0.38	37.033 ± 0.02	7
0.814	78.472 ± 0.03	8
1.256	121.101 ± 0.06	9
1.838	177.25 ± 0.05	10
1.345	129.713 ± 0.08	11
1.18	114.348 ± 0.07	12
0.89	86.666 ± 0.06	13
0.61	58.93 ± 0.04	14
0.45	43.728 ± 0.02	15
0.43	42.051 ± 0.05	16
0.59	57.2 ± 0.06	17
0.66	64.521 ± 0.03	18
0.744	71.729 ± 0.04	19
0.75	72.638 ± 0.0	20
1.184	114.164 ± 0.04	21
0.7	67.484 ± 0.07	22
0.582	56.084 ± 0.06	23
0.474	45.666 ± 0.04	24
0.375	36.199 ± 0.02	25
0.306	29.503 ± 0.03	26
0.31	30.408 ± 0.04	27
0.31	30.638 ± 0.02	28
0.302	29.156 ± 0.05	29
0.33	31.828 ± 0.0	30

Gallic acid of mili gram in the fraction The results are presented as mean ± standard deviation

The past results from the studies conducted indicated that the phenolic compounds present in rosemary extract, at concentrations of 50, 100, and 200 micromolar, significantly reduced LDL oxidation (Afonso *et al.*, 2013). Moreover, the level of conjugated dienes resulting from LDL oxidation was significantly reduced under the influence of these extracts compared to the control sample. The past study conducted on the effect of antioxidant compounds from Aqaqia extract on LDL oxidation revealed that the phenolic compounds present in this plant extract led to a reduction in the oxidation process (Bagheri *et al.*, 2013). The results obtained from our research align with the findings of other studies conducted by researchers on the impact of plant phenolic compounds on LDL oxidation. It can be concluded that the phenolic compounds present in plants act as natural antioxidants, delaying the process of lipid particle oxidation. This is a crucial factor in preventing the onset of atherosclerosis. The results from our experiments (Figs 7&8) demonstrate that the effect of flavonoid compounds present in Fraction 42 significantly reduced the oxidation process ($p<0.001$). In such a way that, under the influence of volumes of 10 and 2 microliters used, the time lag of the oxidation process was extended by approximately 150 minutes compared to the control sample under laboratory conditions. This indicates the ability of these polyphenolic compounds present in the fraction obtained from the chromatography of Aqaqia root extract to completely inhibit LDL particle oxidation. In the present study, based on the conducted research on the effect of flavonoid compounds on LDL oxidation, it can be asserted that due to their high antioxidant capacity in trapping and inhibiting free radicals generated from copper sulfate in vitro and under similar conditions in vivo, flavonoids serve as potent antioxidants in the treatment of atherosclerosis and reduction of lipid peroxidation. Our experiments, using two volumes of 2 and 10 microliters of the fraction containing separated flavonoids from Aqaqia root extract (Fraction number 45), revealed that these compounds can completely inhibit LDL oxidation. This effect was observed in a dose-dependent manner, as an optimal concentration of flavonoids in Fraction number 45, with a volume of 10 microliters, significantly delayed the time lag and reduced the levels of conjugated dienes resulting from LDL oxidation. Increasing the concentration of flavonoids in Fraction number 45 had a significant impact on delaying the oxidation process and lowering the levels of conjugated dienes. In the present study, the antioxidant activity of fractions obtained from gel filtration chromatography of Aqaqia root extract was investigated, and the inhibitory percentage of each sample was determined. Based on the experimental results, it was observed that the inhibitory percentage of the antioxidant compounds in Aqaqia root extract depended on the type and concentration of the antioxidant compounds present in the separated fractions. Until now, studies have focused on investigating the impact of antioxidant compounds on the activity of the enzyme paraoxonase 1. The results have indicated the positive effects of these compounds on the enzyme activity, as

well as their increased protective effects against LDL oxidation. Therefore, in the current study, due to the presence of antioxidant compounds with high anti oxidative properties in the extract obtained from gel filtration chromatography of Aqaqia root, we examined the influence of these fractions on paraoxonase 1 enzyme activity. The past results obtained from the study conducted indicated the positive impact of pomegranate extract on paraoxonase enzyme activity (Parsaeyan *et al.*, 2012). The antioxidant compounds present in the extract of this plant significantly increased the activity of this enzyme. In our research, although the fraction containing flavonoid compounds (fraction 42) did not exhibit a significant reduction in enzyme activity compared to other fractions and antioxidant compounds, it is possible that due to the presence of other biochemical factors in our serum samples and their interaction with the polyphenolic compounds present in the fractions obtained from the extract, interference occurred in the activity of paraoxonase 1 enzyme.

Conclusion

Due to its diverse polyphenolic compounds and strong antioxidants such as phenols, tannins, flavonoids, and anthocyanins, Aqaqia plant exhibits potent antioxidant activity. By exerting its antioxidant effects, it can influence the oxidation system, reduce oxidative stress, and inhibit the atherogenic effects resulting from the accumulation of oxidized fatty molecules, thereby preventing atherosclerosis, which is now the leading cause of heart diseases worldwide.

References

Ansari P, Samia J, Khan J, Rahman M, Rahman A, Abdel-Wahabi Y. 2023. Protective Effects of Medicinal Plant-Based Foods against Diabetes: A Review on Pharmacology, Phytochemistry, and Molecular Mechanisms. Journal of Nutrients **15,** 32-66.
http://dx. doi.org. /10.3390/nu15143266.

Ayala A, Muñoz M, Argüelles S. 2014. Lipid Peroxidation: Production, Metabolism, and Signaling Mechanisms of Malondialdehyde and 4-Hydroxy-2-Nonenal. Journal of Oxidative Medicine and Cellular Longevity **2**, 36-38.
http://dx.doi.org/ 10.1155/2014/360438.

Afonso M, Silva A, Carvalho E, Rivelli D, Barros S, Rogero M. 2013. Phenolic compounds from Rosemary (*Rosmarinus officinalis* L.) attenuate oxidative stress and reduce blood cholesterol concentrations in diet-induced hypercholesterolemic rats. Journal of Nutrition & Metabolism **10**, 70-75.
http://dx.doi.org/10.1186/1743-7075-10-19.

Bauer Petrovska B. 2012. Historical review of medicinal plants' usage. Journal of Pharmacognosy Reviews **6**, 1-5.
http://dx.doi.org/10.4103/0973-7847.95849

Bouayed J, Bohn T. 2010. Exogenous antioxidants—Double-edged swords in cellular redox state. Journal of Oxidative Medicine and Cellular Longevity **3**, 228-237.
http://dx.doi.org/10.4161/oxim.3.4.12858

Bagheri S, Ahmadvand H, Khosrowbeygi A, Ghazanfari F, Jafari N, Nazem H. 2013. Antioxidant properties and inhibitory effects of *Satureja khozestanica* essential oil on LDL oxidation induced-CuSO4 in vitro. Asian Pacific Journal of Tropical Biomedicine **3,** 22-27.
http://dx.doi.org/10.1016/S2221-1691(13)60018-0.

Chaudhary P, Janmeda P, Docea A, Yeskaliyeva B, Razis A. 2023. Oxidative stress, free radicals and antioxidants: potential crosstalk in the pathophysiology of human diseases. Journal of Frontiers in Chemistry **11**, 115-8198.
http://dx.doi.org/10.3389/fchem.2023.1158198

Cuevas-Cianca S, Romero-Castillo C, Gálvez-Romero J, Juárez Z, Hernández L. 2023. Antioxidant and Anti-Inflammatory Compounds from Edible Plants with Anti-Cancer Activity and Their Potential Use as Drugs. Journal of Molecules **28**,14-88.
http://dx.doi.org/10.3390/molecules28031488.

Czech K, Gaweł-Bęben K, Szopa A, Kukula-Koch W, Hussain S. 2023. Phytochemical Profiling, Antioxidant and Tyrosinase Regulatory Activities of Extracts from Herb, Leaf and *In vitro* Culture of *Achillea millefolium* (Yarrow). Journal of Molecules **28**, 47-91.
http://dx.doi.org/10.3390/molecules28124791

Durrington P, Bashir B, Soran H. 2023. Paraoxonase 1 and atherosclerosis. Journal of Frontiers in Cardiovascular Medicine **10**, 106-5967. http://dx.doi.org/:10.3389/fcvm.2023.1065967

Frański R, Beszterda-Buszczak M. 2023. Comment on Villalva *et al.* Antioxidant, Anti-Inflammatory, and Antibacterial Properties of an *Achillea millefolium* L. Extract and Its Fractions Obtained by Supercritical Anti-Solvent Fractionation against Helicobacter pylori. Journal of Antioxidants **12,** 12-26. http://dx.doi.org/: 10.3390/antiox12061226

Genovesi S, Giussani M, Lieti G, Orlando A, Patti I. 2023. Evidence and Uncertainties on Lipoprotein(a) as a Marker of Cardiovascular Health Risk in Children and Adolescents. Journal of Biomedicines **11**, 16-61. http://dx.doi.org/10.3390/biomedicines11061661

Gaschler M, Stockwell B. 2017. Lipid peroxidation in cell death. Journal of Biochemical and Biophysical Research Communications **3**, 419-425. http://dx.doi.org/10.1016/j.bbrc.2016.10.086.

Jena A, Samal R, Bhol N, Duttaroy A. 2023. Cellular Red-Ox system in health and disease: The latest update. Journal of Biomedicine & Pharmacotherapy **2**, 114-606. http://dx.doi.org/10.1016/j.biopha.2023.114606

Koene R, Prizment A, Blaes A, Konety S. 2016. Shared Risk Factors in Cardiovascular Disease and Cancer. Journal of Circulation, **3**, 1104-1114. http://dx.doi.org/10.1161/CIRCULATIONAHA.115.020406

Khurana S, Venkataraman K, Hollingsworth A, Piche M, Ta T. 2013. Polyphenols: Benefits to the Cardiovascular System in Health and in Aging. Journal of Nutrients, **5**, 3779-3827. http://dx.doi.org/10.3390/nu5103779

Kaczorová D, Karalija E, Dahija S, Kubica P. 2021. Influence of Extraction Solvent on the Phenolic Profile and Bioactivity of Two Achillea Species. Journal of Molecules **6**, 16-11. http://dx.doi.org/10.3390/molecules26061601

Lobo V, Patil A, Phatak A, Chandra N. 2010. Free radicals, antioxidants and functional foods: Impact on human health. Journal of Pharmacognosy Reviews **4**,118-126. http://dx.doi.org/10.4103/0973-7847.70902

Li J, Cao F, Yin H, Huang Z, Lin Z, Mao N. 2020. Ferroptosis: past, present and future. Journal of Cell Death & Disease **8,** 38-3. http://dx.doi.org/10.1038/s41419-020-2295-8

Longo A, Veiga G, Cousen M, Karpinski C, Schneider A, Weber B. 2021. Factors associated with serum paraoxonase 1 activity in patients with cardiovascular disease. Journal of Endocrinology and Metabolism **6**, 676-683. http://dx.doi.org/10.20945/2359-3997000000354.

Madrigal E, Madrigal-Bujaidar E, Álvarez-González I, Sumaya-Martínez M, Gutierrez-Salinas J. 2014. Review of natural products with hepatoprotective effects. Journal of Gastroenterology **20,** 14787-14804. http://dx.doi.org/10.3748/wjg.v20.i40.14787

Mortensen M, Ruiz J, Watts J. 2023. Polyunsaturated Fatty Acids Drive Lipid Peroxidation during Ferroptosis. Journal of Cells **5**, 80-84. http://dx.doi.org/10.3390/cells12050804

Ostolski M, Adamczak M, Brzozowski B, Wiczkowski W. 2021. Antioxidant Activity and Chemical Characteristics of Supercritical CO2 and Water Extracts from Willow and Poplar. Journal of Molecules **3**, 54-55. http://dx.doi.org/10.3390/molecules26030545

Ouimet M, Barrett T, Fisher E. 2019. HDL and Reverse Cholesterol Transport: Basic Mechanisms and their Roles in Vascular Health and Disease. Journal of Circulation Research **10**, 1505-1518. http://dx.doi.org/10.1161/CIRCRESAHA.119.312617.

Phaniendra A, Jestadi D, Periyasamy L. 2015. Free Radicals: Properties, Sources, Targets, and Their Implication in Various Diseases Indian. Journal of Clinical Biochemistry **1**, 11-26. http://dx.doi.org/: 10.1007/s12291-014-0446-0.

Poznyak A, Nikiforov N, Markin A, Kashirskikh D, Myasoedova V, Gerasimova E. 2020. Overview of OxLDL and Its Impact on Cardiovascular Health: Focus on Atherosclerosis. Journal of Frontiers in Pharmacology **11**, 613-780. http://dx.doi.org/10.3389/fphar.2020.613780

Parsaeyan N, Mozaffari-Khosravi H, Mozayan M. 2012. Effect of pomegranate juice on paraoxonase enzyme activity in patients with type 2 diabetes. Journal of Diabetes & Metabolic Disorders **11**, 11-19. http://dx.doi.org/: 10.1186/2251-65

Samtiya M, Aluko R, Dhewa T, Moreno-Rojas J. 2021. Potential Health Benefits of Plant Food-Derived Bioactive Components: An Overview. Journal of Foods **4,** 83-89. http://dx.doi.org/10.3390/molecules24081583

Siddiqui H, Yevstigneyev N, Madani G, McCormick S. 2022. Approaches to Visualising Endocytosis of LDL-Related Lipoproteins. Journal of Biomolecules **2**, 15-58.

Singh A, Kukreti R, Saso L, Kukreti S. 2019. Oxidative Stress: A Key Modulator in Neurodegenerative Diseases. Journal of Molecules **8,** 15-83.

Stagos D. 2020. Antioxidant Activity of Polyphenolic Plant Extracts. Journal of Antioxidants **1,**19-26. http://dx.doi.org/10.3390/antiox9010019

Saeidnia S, Gohari A, Mokhber-Dezfuli N, Kiuchi F. 2011. A review on phytochemistry and medicinal properties of the genus Achillea. Journal of Daru **3**, 173-186. http://dx.doi.org/ 10.3390/biom12020158

Uzelac M, Sladonja B, Šola I, Dudaš S, Bilić J, Famuyide I. 2023. Invasive Alien Species as a Potential Source of Phytopharmaceuticals: Phenolic Composition and Antimicrobial and Cytotoxic Activity of *Robinia pseudoacacia* L. Leaf and Flower Extracts. Journal of Plants (Basel), **14,** 27-15. http://dx.doi.org/10.3390/plants12142715

Vitalini S, Iriti M, Puricelli C, Ciuchi D, Segale A. 2013. Traditional knowledge on medicinal and food plants used in Val San Giacomo (Sondrio, Italy) an alpine ethnobotanical study. Journal of Ethnopharmacology **2**, 517-529. http://dx.doi.org/10.1016/j.jep.2012.11.024

Vítková M, Müllerová J, Sádlo J, Pergl J, Pyšek P. 2017. Black locust (*Robinia pseudoacacia*) beloved and despised: A story of an invasive tree in Central Europe. Journal of Forest Ecology and Management **4**, 287-302. http://dx.doi.org/10.1016/j.foreco.2016.10.057

Vaou N, Stavropoulou E, Voidarou C, Tsigalou C, Bezirtzoglou E. 2021. Towards Advances in Medicinal Plant Antimicrobial Activity: A Review Study on Challenges and Future Perspectives. Journal of Microorganisms **9**, 20-4. http:dx.doi.org/10.3390/microorganisms9102041

Yen F, Qin C, Xuan S, Ying P, Le H, Darmarajan T. 2021. Hypoglycemic Effects of Plant Flavonoids: A Review. Journal of Evidence-Based Complementary and Alternative Medicine **6**, 57-53. http://dx.doi.org/10.1155/2021/2057333