International Journal of Medical Sciences And Clinical Research

VOLUME Vol.06 Issue04 2026
PAGE NO. 27-31
DOI 10.37547/ijmscr/Volume06Issue04-04

Determination of The Antiradical Properties of Quince Seeds Using the DPPH Method

Asqarov Ibrohim Rahmonovich
Doctor of Chemical Sciences, Professor, Department of Chemistry, Andijan State University, Andijan, Uzbekistan

Khojiqulov Azizbek Sobirovich
PhD in Chemical Sciences, Associate Professor, Department of Chemistry, Andijan State University, Andijan, Uzbekistan

Yuldasheva Maftuna Lutfullo kizi
Master's Student in Chemistry, Andijan State University, Andijan, Uzbekistan

Received: 25 February 2026; **Accepted:** 21 March 2026; **Published:** 10 April 2026

Abstract: This article is dedicated to the comprehensive evaluation of the antioxidant properties of quince seeds and the determination of their antiradical capacity through the application of the DPPH (2,2-diphenyl-1-picrylhydrazyl) method. Within the scope of this study, the primary active components contained within quince seeds, specifically phytochemical compounds and natural antioxidants, have been thoroughly examined. The antioxidant characteristics of the seeds were quantitatively assessed using the DPPH assay. The experimental findings clearly demonstrate that quince seeds possess a high level of antiradical activity. Furthermore, the results indicate that these seeds hold significant potential as a natural source of antioxidants, offering substantial benefits for human health and pharmacological applications.

Keywords: Antiradical activity, Quince seed, DPPH method, Polyphenols, Antioxidant, Quince seed extract.

Introduction: The seeds of the quince plant (Cydonia oblonga) are distinguished by their potent antiradical and antioxidant properties, which are primarily attributed to their rich composition of active chemical compounds. Quince seeds contain a diverse array of polyphenols, terpenoids, and numerous other bioactive substances that serve as vital secondary metabolites [1, 2]. Antioxidants are critical compounds that shield cells and biological tissues from oxidative processes, specifically mitigating the deleterious effects caused by free radicals. Although free radicals are generated naturally within the human body, an excessive concentration leads to oxidative stress, which serves as a fundamental catalyst for various pathologies, including cardiovascular diseases, malignancies, the acceleration of aging processes, and neurodegenerative disorders [3, 4]. Consequently, antioxidants derived from natural sources are of paramount importance within both the pharmaceutical and food industries due to their low toxicity and high efficacy [5].

Quince fruits and their respective seeds have been widely recognised as a significant source of bioactive constituents, particularly phenolic compounds, flavonoids, and essential vitamins. These molecules facilitate the neutralisation of free radicals within the organism, thereby providing robust antioxidant protection. In recent years, the biological properties of quince seeds, especially their antioxidant potential, have become a focal point of scientific inquiry [6]. Researchers are increasingly investigating the feasibility of utilising these seeds as pharmacological agents and functional food supplements to combat metabolic disorders [7].

Various methodologies are available for the assessment of antioxidant activity, the most prevalent of which is the spectrophotometric technique involving

the neutralisation of the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical. The DPPH radical is notable for its exceptional stability; its antioxidant activity within a sample can be assessed accurately and rapidly by monitoring absorbance levels or changes in colouration [8]. The primary advantages of this method include its simplicity, reproducibility, and high sensitivity, making it an ideal tool for determining the antioxidant potential of natural products. In this context, the objective of the present study is to evaluate the antioxidant and antiradical properties of quince seeds using the DPPH method and to ascertain their potential biological activity. This research aims to provide a systematic exploration of the bioactive compounds in quince seeds and their antioxidant capacity, thereby uncovering opportunities for their effective application as natural resources [9].

The DPPH (2,2-diphenyl-1-picrylhydrazyl) method is an extensively utilised technique for measuring antiradical activity. It is based on the principle that substances capable of neutralising the stable free radical (DPPH) demonstrate their specific antioxidant characteristics through this chemical interaction. Detailed information regarding the DPPH method was first introduced in the 1950s by the Japanese scientist M.S. Blois. In a seminal article published in 1958 titled "Antioxidant determinations by the use of a stable free radical", Blois elucidated the methodology for measuring antioxidant activity using the DPPH free radical. The fundamental principle of this method is as follows:

– The DPPH free radical initially possesses a deep violet colour in solution.

– When this radical reacts with an antioxidant substance, it undergoes a chemical reduction.

– This reaction results in a distinct colour change, typically shifting from violet to pale yellow or light green.

– The alteration in colour signifies the neutralisation of the free radical and a subsequent decrease in its radical activity.

– By measuring the intensity of this colour change via spectrophotometry, the degree to which a substance neutralises free radicals can be precisely determined [10].

Currently, the DPPH method is widely implemented across numerous scientific investigations due to its rapid execution, simplicity, and cost-effectiveness. This method serves as a primary analytical tool for studying the antioxidant and antiradical properties of various plant extracts, food products, pharmaceutical compounds, and cosmetic formulations. In our research conducted at the "Folk Medicine and Chemistry of Goods" laboratory at Andijan State University, we determined the antiradical properties of quince seed extract using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method.

Experimental Part

Evaluation of the Antiradical Capacity of the Sample Using the DPPH Method. The decolorisation of the violet-coloured 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution facilitates the identification of specific pure antioxidant compounds possessing hydrogen-atom or electron-donating properties. Stable DPPH• is a primary reagent utilised in spectrophotometric analysis. In this experiment, the method established by Blois for evaluating the inhibition of DPPH• free radicals was employed with minor modifications [7,8,9].

A 7.92 mM solution of DPPH• was prepared in ethanol within a 100 ml volumetric flask. To prevent photodegradation, the flask was wrapped in aluminium foil and stored in a dark place at room temperature for 30 minutes. To investigate the antioxidant potential of quince seeds, an alcoholic extract was prepared. The extraction process involved placing 1 g of the plant sample into 25 ml of 96% ethanol and subjecting it to ultrasonic extraction in an ultrasonic bath for 20 minutes. The resulting extract was then passed through a 0.45 µm syringe filter to ensure clarity before analysis.

To determine the antiradical properties of the samples, 3 ml of the DPPH solution and 100 µl of ethanol (serving as the blank sample) were added to a 4 ml quartz cuvette. This was placed in the spectrophotometer, and the absorbance (D_1) was measured at a wavelength of 517 nm every 5 minutes over a total duration of 30 minutes using a K7000 spectrophotometer manufactured by YOKE (China). To evaluate the antiradical activity of the sample, volumes of 25, 50, 75, and 100 µl of the extract were mixed with 3 ml of the DPPH solution, and the absorbance (D_2) at 517 nm was measured following the same procedure. Ethanol was added to the remaining volume to ensure that the total volume of the solution in the cuvette reached 3.1 ml. The antiradical capacity of the samples was calculated using the following formula:

$$ARF\% = \frac{D_1 - D_2}{D_1} \cdot 100\% \qquad (1)$$

The results obtained are given in the following table:

Table 1. Measured light absorption and calculated antiradical activity values of blank and tested Bexi seed alcohol extracted samples added to DPPH solution.

Volume, µl	Time, min.	Sample					
		Abs, D	ARF %	Volume, µl	Time, min.	Abs, D	ARF %
25	0	0.919	0.00	75	0	0.919	0.00
	5	0.906	1.41		5	0.845	8.05
	10	0.896	2.50		10	0.839	8.71
	15	0.893	2.83		15	0.831	9.58
	20	0.889	3.26		20	0.828	9.90
	25	0.881	4.13		25	0.824	10.34
	30	0.876	4.68		30	0.819	10.88
50	0	0.919	0.00	100	0	0.919	0.00
	5	0.884	3.81		5	0.813	11.53
	10	0.872	5.11		10	0.808	12.08
	15	0.863	6.09		15	0.805	12.40
	20	0.858	6.64		20	0.802	12.73
	25	0.849	7.62		25	0.801	12.84
	30	0.845	8.05		30	0.799	13.06

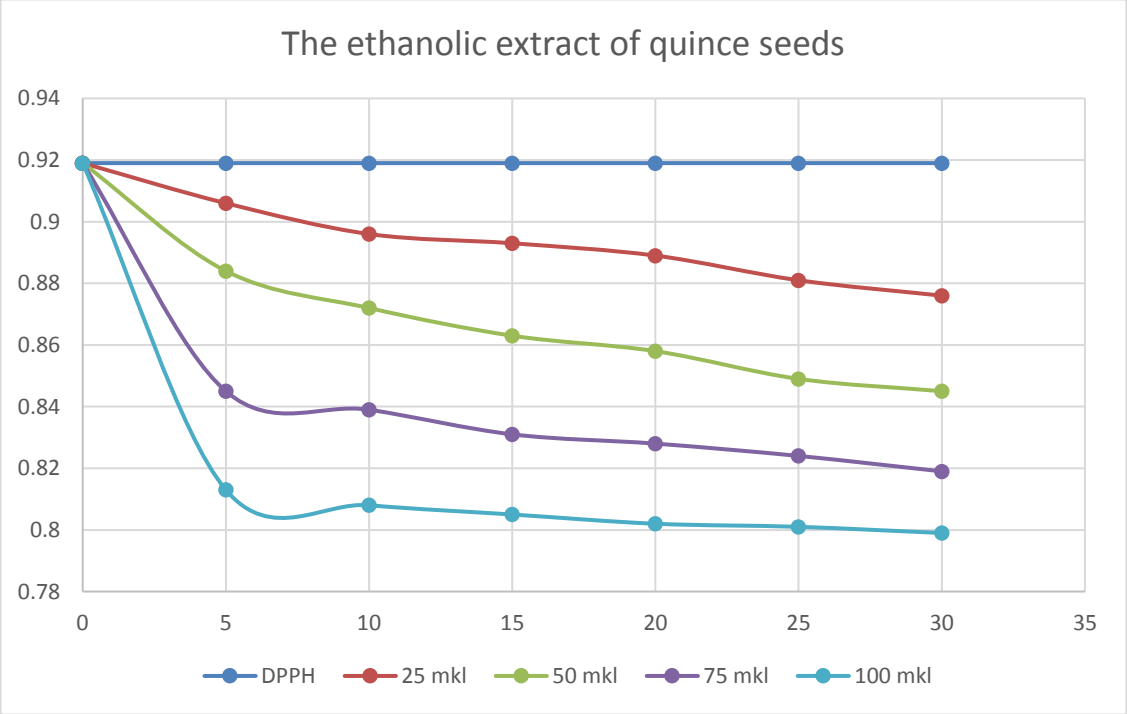

Figure 1. Graphical representation of the measured light absorption of blank and tested alcohol extracted sample solutions added to DPPH solution.

The values of antiradical activity (RSA%) at the 30-minute mark of each experiment, based on the volume of the added ethanolic samples, were utilised to construct the following graph. To calculate the IC50 of the samples—defined as the concentration required to inhibit 50% of the DPPH solution—a linear regression analysis was performed, and the value was derived using the resulting trendline function.

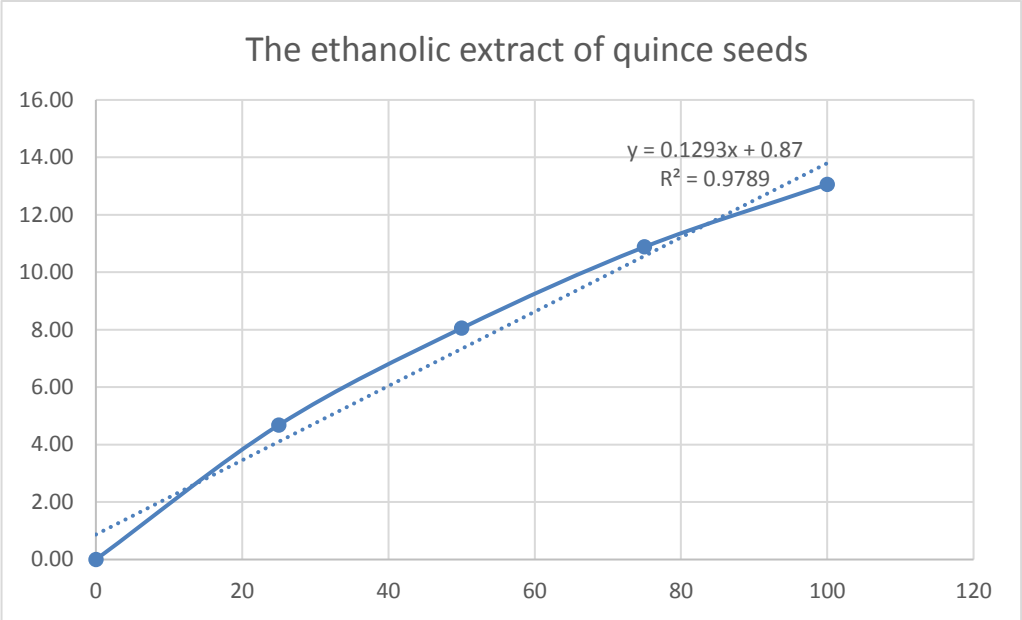

Figure 2. Correlation graph between the volumes of the ethanolic extract and the $RSA\%$ values determined at the 10-minute interval.

The volume exhibiting 50% RSA(IC50) was calculated using the formula x=(y-b)/m, derived from the linear regression trendline equation y=mx+b:

$$IC_{50} = \frac{(50 - 0.87)}{0.1293} = 379.97\, mkl \qquad (2)$$

RESULTS AND DISCUSSION

The experimental results obtained above demonstrate the systematic application of spectral analysis to evaluate antioxidant activity when interacting with a DPPH solution, illustrating the dynamic variations in the observed data.

The DPPH (2,2-diphenyl-1-picrylhydrazyl) solution undergoes a distinct chromatic alteration upon the acceptance of a hydrogen atom or an electron, which serves as a fundamental indicator for determining antioxidant efficacy. DPPH exists as a stable free radical; its decolorisation signifies the neutralisation of its radical state, confirming that the antioxidant has successfully scavenged the radical. Throughout the control phase of the experiment, the absorbance of the DPPH solution exhibited minimal fluctuations, ranging from 0.958 to 0.959, which confirms the inherent stability of the free radical prior to its interaction with the antioxidant compounds [10].

Following the introduction of the quince seed extract, the absorbance of the DPPH solution decreased significantly. The following observations were recorded:

– Upon the addition of 100 µl of the sample, the absorbance decreased from 0.919 to 0.799, resulting in an RSA% of 13.06%. This indicates a robust level of antiradical activity, demonstrating that a 100 µl volume of the sample neutralises the DPPH free radical with high efficiency.

– With a 75 µl sample volume, the absorbance fell from 0.919 to 0.819, achieving an RSA% of 10.88%. This confirms that the antioxidant remains effective at this concentration, albeit with slightly lower activity compared to the 100 µl dosage.

– For the 50 µl sample, the absorbance was reduced from 0.919 to 0.845, corresponding to an RSA% of 8.05%. This suggests comparatively lower antioxidant potency, yet it still retains a quantifiable capacity for radical inhibition.

The data clearly indicates a direct correlation between the sample volume and the antiradical activity (RSA%); as the volume of the extract increases, the scavenging percentage rises accordingly. The 100 µl sample exhibited the peak antiradical performance, implying that increasing the quantity of the sample enhances the overall efficiency of the radical neutralisation process. Conversely, while the 75 µl sample showed less activity, it nonetheless maintained a significant capacity to inhibit the DPPH free radical [10].

CONCLUSION

The experimental findings demonstrate a direct correlation between the sample volume and its impact on antioxidant activity when evaluated using the DPPH assay. Following the administration of the sample, the absorbance of the DPPH solution decreased significantly, reflecting the efficacy of the extract in neutralising free radicals. The peak antiradical activity was observed at a sample volume of 100 µl, indicating

highly efficient neutralisation of the DPPH free radical. A substantial level of activity was also recorded for the 75 µl sample, although its potency was slightly lower than that of the 100 µl dosage. Specifically, the results of the ethanolic extraction revealed an $RSA\%$ of 13.06% at the 30-minute mark. Furthermore, the IC_{50} value was determined to be 379.97 µl, confirming a measurable antiradical capacity.

These results validate that as the sample volume increases, the antioxidant activity enhances accordingly. This study further underscores the efficacy and utility of the DPPH method as a reliable tool for quantifying the activity of antioxidants. Based on the antioxidant potential observed in the extract derived from quince seeds (Cydonia oblonga), we conclude that it holds significant promise for use in traditional medicine, particularly as a therapeutic agent for the treatment of gastric ulcers.

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