Dijlah Journal of Medical Sciences (DJMS) Special Issue, September, 2025, pp. 58-66
P-ISSN: 3078-3178, E-ISSN: 3078-8625, paper ID: 08

Determination of Quercetin Concentration Using Spectrophotometric Method Based on Diazotization Coupling Reaction

Fatma A. Khazaa, Farqid F. Mohammed* and Farah Al-Hammashi
Department of Chemistry, College of Science, University of Wasit, Kut, Iraq

Article Info

Article history:

Received May, 06, 2025
Revised June, 01, 2025
Accepted June, 20, 2025

Keywords:

Quercetin,
Sulphadiazin,
Spectrophotometry,
Diazotization coupling reaction

ABSTRACT

Quercetin is a natural organic compound and is the main component of plant pigments, which are responsible for the colours of many flowers, vegetables, and fruits. It has many therapeutic applications in human health. Spectroscopic methods (spectral analysis) were used to evaluate the pure form of quercetin. The interaction of quercetin (QUR) with sulfadiazine (SULF) led to the formation of an orange-colored complex, which was spectrophotometrically detected at a wavelength of 435 nanometres. The composition of the complex was determined using Job's method. The optimal method is influenced by several factors, including the volume of the reagent treated with diacetyl, the volume and type of the base medium, the order of addition, and the reaction time. The results showed that the linear range was from (10-100) µg/mL, with a determination coefficient (r^2 = 0.9982), and the limit of detection (LOD) was 0.1083 µg/mL, while the limit of quantification (LOQ) was 0.3943 µg/mL.

Corresponding Author:

* Farqid F. Mohammed
Department of Chemistry, College of Science, University of Wasit, Kut, Iraq
Email: : Farqed.Mohammed1205@sc.uobaghdad.edu.iq

1. INTRODUCTION

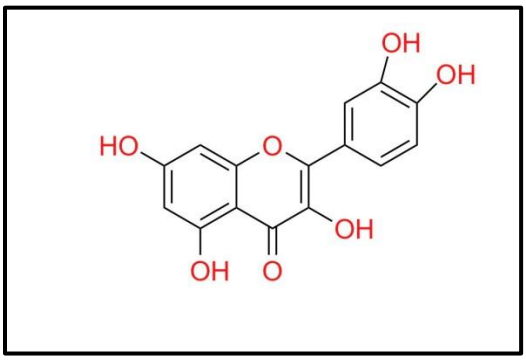
In the current stage of global population development, it is necessary to recognize the health benefits and potential of agricultural products that are regularly present in the human diet. Flavonoids are a group of polyphenolic compounds widely found in the plant kingdom and play an important role in human nutrition. They exhibit various biochemical and antioxidant properties, which have been linked to the prevention and management of diseases such as cancer, Alzheimer's disease, and atherosclerosis.

Quercetin is a flavonoid (plant pigment) commonly found in fruits and vegetables, particularly in onions, citrus fruits, and apples [1–4]. It is an important organic compound due to its anti-cancer and cardiovascular protective activities [5], [6]. Quercetin is one of the primary constituents of plant pigments classified as flavonoids [7]. Flavonoids encompass a large group of naturally occurring compounds, including flavones [8]. Most naturally occurring flavonoids are phenolic compounds responsible for the coloration of plants. These compounds are antioxidative organic materials formed through the phenylpropanoid metabolic pathway.

Structurally, flavonoids consist of one phenyl group attached to a benzo-γ-pyrone ring. They are biosynthesized through the shikimic acid pathway, resulting from the coupling of three acetate units with a phenylpropane moiety [7], [8]. Flavonoids are also present in mature plant tissues and cell sap and are commonly used as chemotaxonomic markers [9]. Various fruits and vegetables such as apples, tea, onions, nuts, berries, cauliflower, red cabbage, and numerous medicinal plants are rich sources of quercetin [10]. The dried peel of brown onions is considered a major source of free quercetin, whereas in other plant tissues, quercetin is commonly present in glycosidic forms [11].

Notably, quercetin content in the inedible parts of onions is approximately seventy times higher than in the edible parts [12]. Due to its health-promoting properties, quercetin has been incorporated into several dietary supplements and pharmaceutical products to enhance their effectiveness [10]. The primary application of quercetin lies in the healthcare field due to its notable therapeutic properties. It has been employed in the treatment of cardiovascular diseases, eye disorders, allergic conditions, arthritis, and certain types of cancer. Quercetin also exhibits antioxidant, anti-inflammatory, antimicrobial, antiviral, anti-allergic, cardioprotective, vasodilatory, and anti-cancer activities. Additionally, it is prescribed for managing hypertension due to its ability to reduce blood pressure [10], [11]. Structurally, quercetin displays amphiphilic behavior. It contains two phenyl rings forming the hydrophobic part of the molecule, while the hydroxyl (OH) groups (as shown in Figure 1) represent the polar, hydrophilic section [13–15]. As a result, quercetin is practically insoluble in water but is partially soluble in ethanol. In contrast, it is fully soluble in acetic acid and basic media [10], [15]. Quercetin can be extracted using solvents such as ethyl acetate and dimethylformamide (DMF), although the most commonly used solvents are methanol, ethanol, or aqueous mixtures of these alcohols [12]. It has been observed that at a constant temperature, quercetin solubility in ethanol/water and methanol/water binary mixtures increases with the concentration of the alcohol. Additionally, increasing the temperature further enhances quercetin's solubility. Among these, ethanol/water mixtures have been identified as the optimal solvents for achieving high solubility [13], [16]. Several analytical techniques are used to determine quercetin, including spectroscopy, chromatography, and electrochemical methods. Spectrophotometry and luminescence techniques are commonly employed for analyzing quercetin in simple matrices such as pharmaceuticals and food additives. In contrast, more sophisticated methods such as high-performance liquid chromatography (HPLC), gas chromatography-mass spectrometry (GC-MS), and capillary electrophoresis (CE) are used for its determination in natural products and biological samples. Recently, various electrochemical techniques have also been employed for quercetin detection [17], [18].

The aim of the current study is determining quercetin using simple spectroscopic methods. Spectroscopic methods are often preferred over HPLC and LC-MS due to their simplicity, speed, and cost-effectiveness. Unlike chromatographic techniques, spectroscopic methods typically require minimal sample preparation and consume less solvent, making them more environmentally friendly. Additionally, they offer rapid analysis and can be easily applied for routine quality control or real-time monitoring. While HPLC and LC-MS provide high sensitivity and specificity, they are often more expensive, require complex instrumentation, and demand skilled personnel. Therefore, for applications where ultra-high sensitivity is not essential, spectroscopic techniques such as UV-Vis, IR, or Raman spectroscopy provide a practical and efficient alternative.



Figure(1) Structure of Quercetin

2. METHOD

2.1 Chemicals: All chemicals used in this study were with analytical grade. Quercetin used in this study was purchased from chemical point, Germany. Sulphadiazin was obtained from the state company for drug industry and medical appliances (SDI) in Samarra, Iraq. All solvents were acquired from The HDL Company and utilized without extra purification.

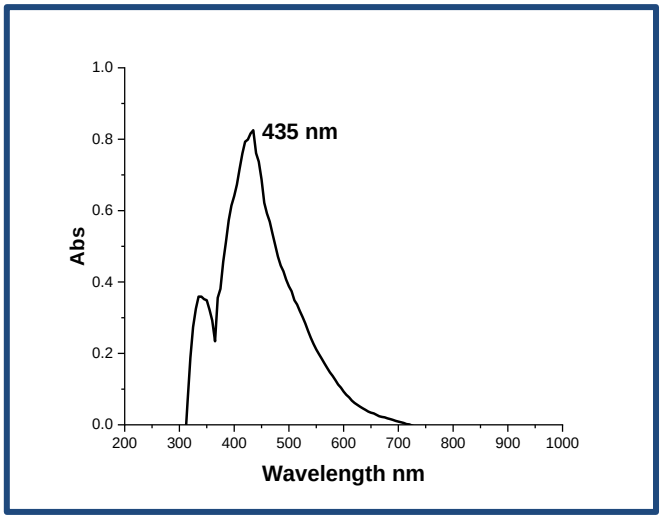
2.2 Apparatuses: Spectrophotometer Double Beam, PG 80+, Sensitive balance (SartoriusBL 210 S), Cold water bath (Haake, F3), Thermometer Mercury type.

2.3 Preparation of Reagents

The reagents used in this study were analytical reagent grade. QUR solution (100 µg/mL). 0.0100 g of reduced QUR was dissolved in 3 mL of ethanol to form the stock solution, which was then placed into a volumetric flask (100 mL). The mixture was then diluted to the desired concentration using deionized water. The working solution was prepared via the appropriate dilutions in water. SULF (0.019 g) was dissolved in 4 mL of HCl (1M) to obtain freshly synthesized diazotized reagent Sulfadiazine (SULF) solution (0.003 M), purity 99.0% which was kept cooled using an ice bath, to this solution , 0.005 g of sodium nitrite (Merck) was added with continuous stirring. After that, the mixture was transferred into a volumetric flask (25 mL) and top out the capacity with deionized water. A Simple dilution way using deionized water was followed to prepare several diluted solutions.

Standard operating procedures:

Following a series of QUR working solutions in concentrations ranging from 10 to 100 µg/mL, 50 µg/mL of QUR was combined with 0.003M SULF reagent. The resulting mixture was then combined with 0.9 M NaOH. The reddish orange products absorbance was measured at maximum absorption of 435 nm (Figure 2 a,b).



(a)

(b)

Figure 2 . Absorption spectra of the azo dyes when 50µg/mL of QUR coupled with SULF in alkaline medium

Table 1. lists the ideal experimental parameters for determining QUR.

parameter	Selected range	Optimum conditions
Wavelength (nm)	200 - 700	--
Effect of diazotized reagent Conc. (M)	- 0.009	0.003
Effect of (1 M) HCl volume (mL)	3 - 7	4
Type of basic medium	NaOH, KOH, Na_2CO3	NaOH
Effect of volume of base (mL)	1 - 5	4
Effect of order of addition	QUR, SULF, NaOH	SULF+ NaOH +QUR
Effect of the stability time (min)	1 - 60	45

3. RESULTS AND DISCUSSION

Through a series of studies, the key parameters, which are influencing the method efficacy were investigated. Every variable that could affect the colored product sensitivity and stability was optimized by gradually altering one while keeping other variables constant. For the optimization of all conditions, a solution of QUR with concentration of 100µg/mL was prepared. From this, another solution (50µg/10mL) in was then prepared and utilized. The absorbance was measured at 435 nm in comparison to the blank.

3.1Conditions of optimization

An investigation has been conducted on the chemical variables that impact the performance of the suggested spectrophotometric approach. Based on sensitivity, throughput and repeatability, the ideal factors for the selected procedure including Influence of reagent concentration, effect of acidic medium, effect of alkaline medium (base type, base concentration of base, base volume).

3.2Influence of reagent concentration

Various SULF concentrations ranged from 0.001 to 0.009M were tested. The obtained data showed that the highest absorbance was at a concentration of 0.003M. Above this point, nevertheless, the blank absorbance regularly increased as the concentration was increased, as shown in Fig (3). Hence 0.003M was designated as the finest concentration of the reagent.

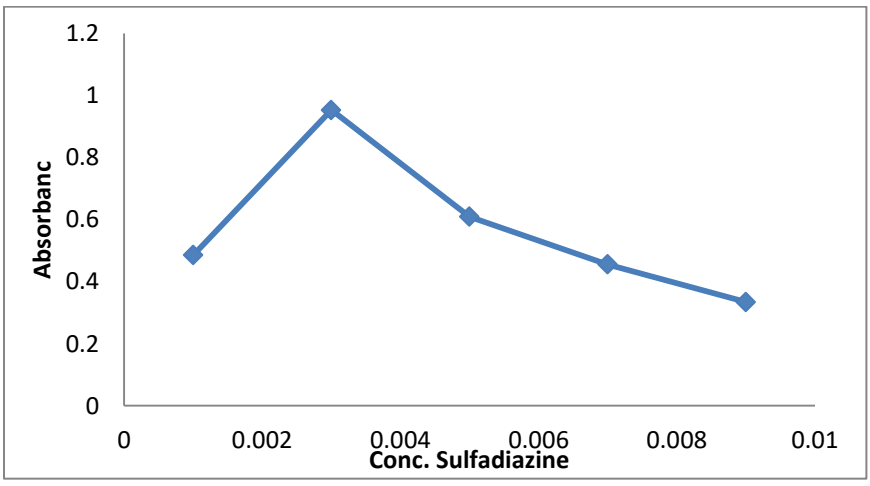


Figure. (3): Effect of the reagent concentration.

3.3 Effect of acidic medium

Because an acidic medium is required to complete the diazotization processes and generate nitrous acid, HCl was utilized. While the remaining variables were held constant, different amounts of HCl ranging from (3-7) mL were investigated and added $NaNO_2$ at a concentration equivalent to that of the reagent. With 1 mL of HCl (1M), a maximum color intensity was detected. The amine group in SULF was converted into ammonium ion, which did not couple in excess of acidic medium, resulting in a considerable drop in absorbance with increasing of acid volume. Therefore, a volume of 4 mL of (1 M) HCl was chosen for the subsequent trials as shown in Figure (4).

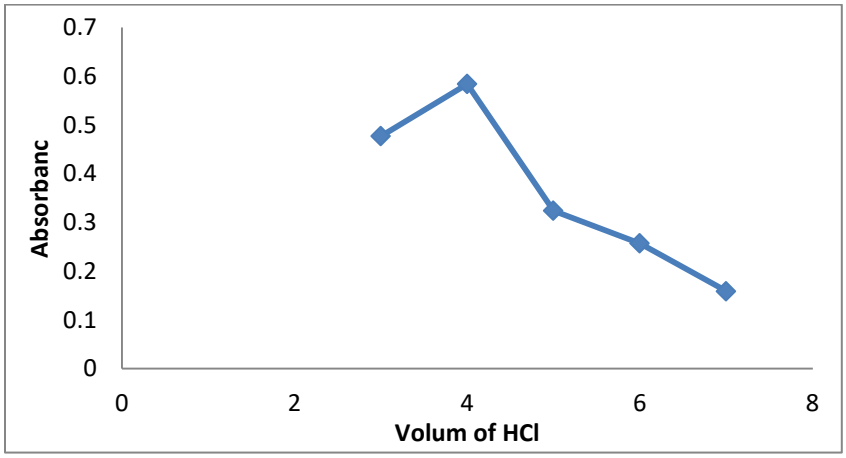
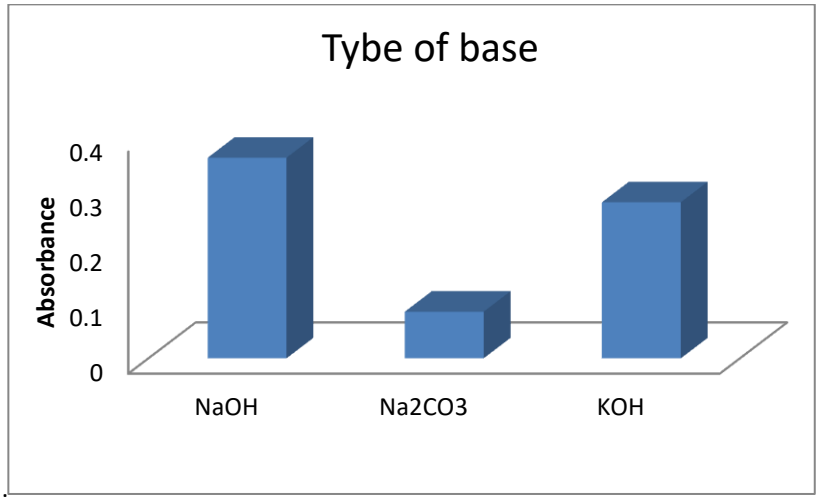


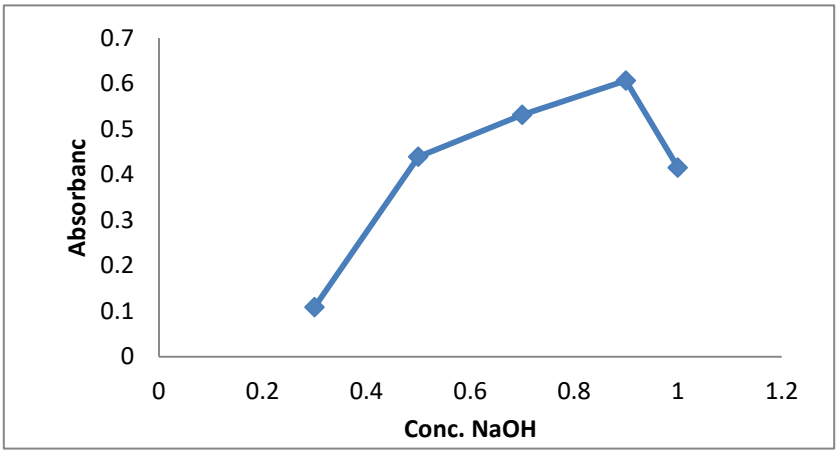
Fig. (4): Effect of acidic medium

3.4 Effect alkaline medium

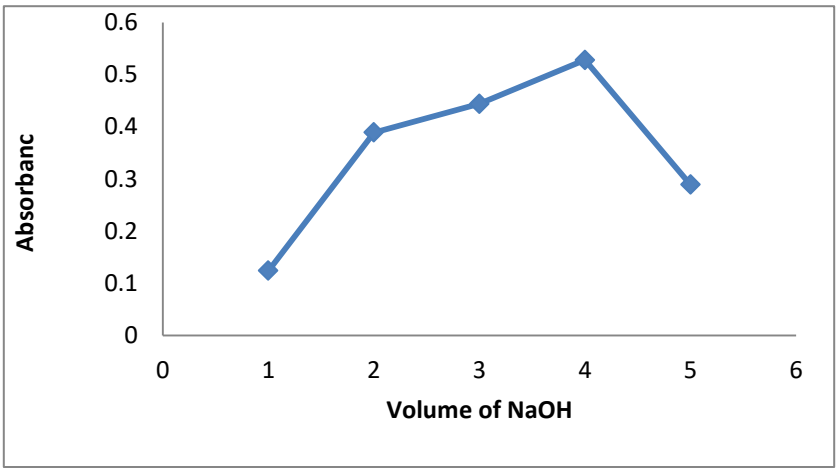
Following the primary experiments, which demonstrated the appearance of the orange coloration was significantly enhanced under basic conditions, various kinds of alkaline solutions were investigated as illustrated in Figure (2.a). The results showed that the highest absorbance, stability, and sensitivity were obtained when the concentration of NaOH is 0.9M. A range of NaOH solution with concentrations ranged from 0.3 to 1M were investigated. The effect of these solutions on the formation of colorful products were also examined. As a result, (0.9M and 4ml) of NaOH was selected and applied in the next experiments. Volume and concentration of bases are depicted in figure (4a, b, and c).



(a)



(b)



(c)

Figure (5): Effect of alkaline medium, a: type of base. b:concentration of base, c: volume of base

Table 1. lists the ideal experimental parameters for determining QUR.

parameter	Selected range	Optimum conditions
Wavelength (nm)	200 - 700	--
Effect of diazotized reagent Conc. (M)	- 0.009	0.003
Effect of (1 M) HCl volume (mL)	3 - 7	4
Type of basic medium	NaOH, KOH, Na_2CO3	NaOH
Effect of volume of base (mL)	1 - 5	4
Effect of order of addition	QUR, SULF, NaOH	SULF+ NaOH +QUR

Effect of the stability time (min)	1 - 60	45

3.5 Analytical Characteristics

The linear calibration graphs were produced via the appropriate diluting of the stock solution and were derived from QUR standard solutions under ideal circumstances. Plotting the calibration graphs between the absorption intensity and QUR concentrations, Figure (6) demonstrated that Beer's law was followed within the range of concentration of (10 - 100 µg/mL), and (0.9982) as a correlation coefficient.

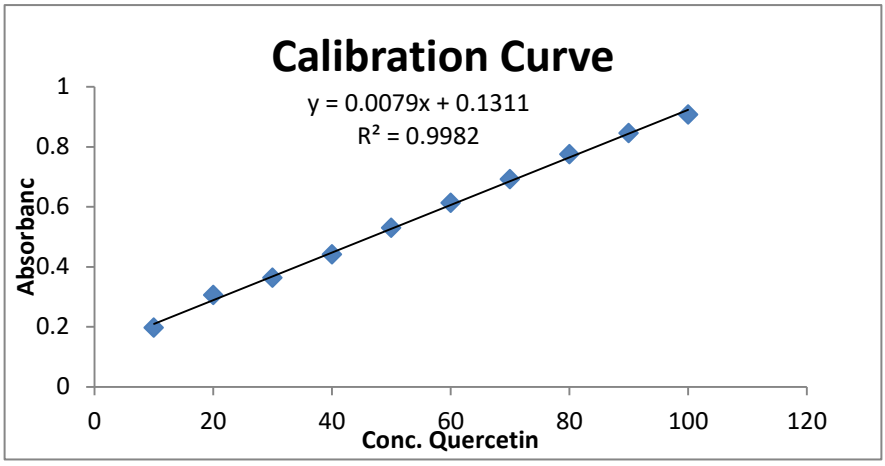


Figure (6). Calibration graph of QUR

Table 2. Analytical values of the techniques suggested for determinate QUR

Parameter	value
Regression equation	Y= 0.0079X + 0.1311
Correlation coefficient, r^2	0.9982
Linearity percentage ,R2 %	99.56
Slope, b (µg mL^{-1})	0.0079
Intercept, a	0.1311
Linearity range (µg mL^{-1})	10 – 100
Standard deviation of intercept (Sa)	0.012
Standard deviation of the slope, Sb	1.21×10^{-3}
Molar absorptivity , Ɛ (L mol-1 cm^{-1})	4.32×10^{4}
LOQ, (µg mL^{-1}), 10SDB/b	0.3943
LOD, (µg mL^{-1}), 3SDB/b	0.1083
Standard deviation of the residuals, Sy/x	3.74×10^{-2}
Sandell's sensitivity (µg cm^{-2})	0.0049

3.6 Precision and accuracy

Three sets of various concentrations of QUR solutions were examined through the suggested approach with five replicates in order to assess the method accuracy and precision. The process yielded analytical values, which are summarized in Table 3. The low percentages of RSD values for accuracy besides the accepted recovery percent values for precision show that the proposed method has appropriate reliability and correctness.

Table 3: The precision and accuracy.

Present	Found	Error	SD%	*Rec.%	*RSD%
20	20.125	0.63	0.002	100.63	0.62
30	29.13	-2.9	0.001	97.1	0.21

40	40.06	0.15	0.001	100.15	0.31

3.7 The applications of developed methods in real samples

Solutions of pharmaceutical formulations were prepared as in the prior section. The developed spectrophotometer system was successfully applied (Table 4) for (QUR) determination in capsules (Substitution Medicine Solutions, Inc.,500 mg) and three different concentrations of (QUR) in spectrophotometer under the optimum conditions. Table 5 shows a comparison of (QUR) determination in pharmaceutical applications.

Table 4: Determination of QUR in supplements using direct application

Sample (µg/mL)	Found (µg/mL)	RSD%	Error%	Rec.%
20	19.8	4	99.5	0.72
30	29.1	2.2	97.7	0.52
40	40.3	1	100.2	0.61

Table 5: Determination of QUR in supplements using standard addition

Sample (µg/mL)	Found (µg/mL)	SD%	Error%	RSD%
5	5.5	0.004	3	1.4
15	15.9	0.005	1.3	0.8
20	20.8	0.004	1	1.1

4. CONCLUSION

The study described the methods utilized to evaluate Sulphadiazin as a color reagent for development of simple, low-cost, quick-sensitive, and precise spectrophotometric techniques for the determination of Querectin. QUR can be accurately and precisely determined using the suggested spectrophotometric method to estimate its concentration in pure form. The method validation produced positive results, with good grades for sensitivity, repeatability, and linearity. The precision and simplicity of the proposed spectrophotometric method for determining QUR in pure form are advantages.

REFERENCES

[1] Uskoković-Marković, S., Milenković, M., & Pavun, L. (2020). Zinc-quercetin complex – from determination to bioactivity. Acta Agriculturae Serbica, 25(50), 113–120.

[2] Muhammed, F. F., & Abed, S. S. (2022). Flow injection spectrophotometric technique for determining genistein in pure and supplement formulations through diazotization coupling reaction. Iraqi Journal of Pharmaceutical Sciences, 31(1), 1–10.

[3] Muhammed, F. F., & Abed, S. S. (2023). Two spectrophotometric methods based on diazotization coupling reaction for determination of genistein in pure and supplement. Iraqi Journal of Science, 64(4), 1581–1591.

[4] Fadhil, G. (2025). Flow injection spectrophotometric method for determination of hesperidin in pure and supplement forms. Analytical & Bioanalytical Chemistry Research, 12(2), 159–167.

[5] D'Andrea, G. (2015). Quercetin: A flavonol with multifaceted therapeutic applications? Fitoterapia, 106, 256–271. https://doi.org/10.1016/j.fitote.2015.09.018

[6] European Food Safety Authority (EFSA). (2011). Scientific opinion on the substantiation of health claims related to quercetin and protection of DNA, proteins and lipids from oxidative damage (ID 1647), 'cardiovascular system' (ID 1844), 'mental state and performance' (ID 1845), and 'liver, kidneys' (ID 1846) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. EFSA Journal, 9(4), 2067. https://doi.org/10.2903/j.efsa.2011.2067

[7] Shashank, K., & Abhay, K. P. (2013). Chemistry and biological activities of flavonoids: An overview. The Scientific World Journal, 2013, Article 162750. https://doi.org/10.1155/2013/162750

[8] Mills, S., & Bone, K. (2013). Principles and practice of phytotherapy: Modern herbal medicine (Book review). Phytotherapy Research, 27(7), 1059–1060.

[9] Beecher, G. R. (2003). Overview of dietary flavonoids: Nomenclature, occurrence and intake. Journal of Nutrition, 133(10), 3248S–3254S. https://doi.org/10.1093/jn/133.10.3248S

[10] Dmitrienko, S. G., Kudrinskaya, V. A., & Apyari, V. V. (2012). Methods of extraction, preconcentration, and determination of quercetin. Journal of Analytical Chemistry, 67(3), 204–214. https://doi.org/10.1134/S106193481204003X

[11] Horbowicz, M. (2002). Method of quercetin extraction from dry scales of onion. Vegetable Crops Research Bulletin, 57(1), 53–58.

[12] Han, X., Shen, T., & Lou, H. (2007). Dietary polyphenols and their biological significance. International Journal of Molecular Sciences, 8(9), 950–988. https://doi.org/10.3390/i8090950

[13] Razmara, R. S., Daneshfar, A., & Sahraei, R. (2010). Solubility of quercetin in water + methanol and water + ethanol from (292.8 to 333.8) K. Journal of Chemical & Engineering Data, 55(9), 3934–3936. https://doi.org/10.1021/je9010757

[14] Kim, S. W., Ko, M. J., & Chung, M. S. (2019). Extraction of the flavonol quercetin from onion waste by combined treatment with intense pulsed light and subcritical water extraction. Journal of Cleaner Production, 231, 1192–1199. https://doi.org/10.1016/j.jclepro.2019.05.280

[15] Sayers, E. W., Beck, J., Bolton, E. E., Bourexis, D., Brister, J. R., Canese, K., et al. (2022). Database resources of the National Center for Biotechnology Information. Nucleic Acids Research, 50(D1), D20–D26. https://doi.org/10.1093/nar/gkab1112

[16] Ko, M. J., Cheigh, C. I., Cho, S. W., & Chung, M. S. (2011). Subcritical water extraction of flavonol quercetin from onion skin. Journal of Food Engineering, 102(4), 327–333. https://doi.org/10.1016/j.jfoodeng.2010.09.008

[17] Brachi, M., Colicchio, R., Sannino, F., Esposito, A., & Migliaccio, A. (2024). Advanced electroanalysis for electrosynthesis. ACS Organic & Inorganic Au, 4(1), 21–34. https://doi.org/10.1021/acsorginorgau.3c00051

[18] Welch, C. M., & Compton, R. G. (2006). The use of nanoparticles in electroanalysis: A review. Analytical and Bioanalytical Chemistry, 384(3), 601–619. https://doi.org/10.1007/s00216-005-0230-3