Current Science
CS 5 (4), 3477-3482 (2025)
Received 09 April 2025 | **Revised 12 May** 2025 | **Accepted 13 June**. 2025 | **Online Available 06 July** 2025
https://doi.org/ 10.52845/CS/2025-5-4-12

Current Science

Current Science

OPEN ACCESS

ISSN(O)2795-8639

Original Article

Extraction, Isolation, and in Vitro Evaluation of Quercetin from Broccoli (Brassica Oleracea) for Metabolic Disorder Management

Mayuri Lokhande[1], Dr. Nidhi Bais[2]

[1]Department of Pharmaceutical Sciences, Oriental University Indore

[2]Associate Professor, Department of Pharmaceutical Sciences, Oriental University Indore

[*]Corresponding Author: Mayuri Lokhande

Abstract:
Metabolic disorders such as diabetes and obesity are increasingly prevalent and pose significant global health challenges. Natural flavonoids like quercetin have shown promising therapeutic effects due to their antioxidant and enzyme inhibitory properties. In this study, quercetin was extracted and isolated from *Brassica oleracea* (broccoli) using methanolic maceration followed by column chromatography. The presence of quercetin was confirmed by thin-layer chromatography (TLC) and high-performance thin-layer chromatography (HPTLC) compared with a standard. The isolated compound was evaluated for in vitro antioxidant activity using the DPPH radical scavenging assay and for antidiabetic potential through α-amylase inhibition. Quercetin exhibited strong antioxidant activity with an IC_{50} value of 38.5 µg/mL and demonstrated 68.7% α-amylase inhibition at 100 µg/mL. These findings confirm the dual therapeutic potential of broccoli-derived quercetin in managing oxidative stress and regulating carbohydrate metabolism. The study supports the role of dietary flavonoids as promising natural agents for metabolic disorder management and encourages further in vivo and clinical evaluation.

Keywords:Broccoli, Quercetin, Brassica oleracea, Metabolic Disorders, Antioxidant, α-Amylase Inhibition

1. Introduction

Metabolic disorders, including diabetes mellitus, obesity, dyslipidemia, and metabolic syndrome, are among the most pressing public health challenges of the 21st century. These conditions are often characterized by abnormal glucose and lipid metabolism, chronic inflammation, and oxidative stress, which contribute to the progression of cardiovascular diseases, non-alcoholic fatty liver disease, and other chronic illnesses. The growing global burden of metabolic disorders highlights the need for safe, effective, and affordable treatment alternatives, particularly those derived from natural sources.

Flavonoids, a group of polyphenolic compounds widely distributed in fruits and vegetables, have attracted attention due to their broad spectrum of biological activities. Among these, **quercetin** has emerged as a potent antioxidant and anti-inflammatory agent. It has been shown to modulate enzymes involved in glucose metabolism, scavenge free radicals, and inhibit lipid peroxidation. These properties make quercetin a promising candidate for the management of metabolic disorders, especially in addressing the oxidative stress and enzymatic imbalances that underpin such diseases.

Broccoli (*Brassica oleracea* var. *italica*), a cruciferous vegetable belonging to the Brassicaceae family, is an excellent dietary source of quercetin. Apart from being rich in vitamins and minerals, broccoli contains glucosinolates, sulforaphane, and a high concentration of flavonoids, including quercetin and kaempferol. Epidemiological studies have linked regular

broccoli consumption with reduced risk of chronic diseases, including type 2 diabetes and cardiovascular disorders, largely due to its antioxidant and anti-inflammatory constituents.

While broccoli is widely consumed, limited studies have focused on the systematic extraction, isolation, and pharmacological evaluation of its quercetin content. Isolation of quercetin and validation of its bioactivity through in vitro antioxidant and antidiabetic assays can provide scientific evidence supporting its functional role in metabolic health. Moreover, identifying plant-based bioactive compounds may offer safer alternatives to synthetic drugs, minimizing adverse effects and improving patient compliance in long-term therapy.

The present study aims to extract and isolate quercetin from *Brassica oleracea* and evaluate its antioxidant and α-amylase inhibitory activity in vitro. The goal is to investigate its therapeutic potential as a natural agent for the management of metabolic disorders. By combining phytochemical analysis, chromatographic identification, and biological screening, this research contributes to the growing field of nutraceuticals and plant-based interventions for lifestyle diseases.

2. Materials and Methods

2.1 Plant Material and Extraction

Fresh broccoli (Brassica oleracea) was procured from the local market, washed, dried, and powdered. The powdered material was extracted using methanol by maceration for 72 hours. The filtrate was concentrated under reduced pressure using a rotary evaporator to obtain a semi-solid crude extract.

Plant Material:

Fresh broccoli (*Brassica oleracea* var. *italica*) heads were procured from a certified organic farm in the local region and authenticated by a botanist at the Department of Botany, [Institution Name]. The collected samples were washed thoroughly under running tap water to remove surface contaminants, followed by rinsing with distilled water. The edible green florets were separated, air-dried in the shade at room temperature for 5–7 days, and then pulverized into a coarse powder using a mechanical grinder. The powdered material was stored in airtight containers at room temperature away from light and moisture until further use.

Extraction Procedure:

Approximately **100 g** of the dried broccoli powder was subjected to cold maceration using **500 mL of methanol** in a conical flask. The flask was sealed and agitated intermittently for **72 hours** at room temperature to ensure maximum phytoconstituent extraction. The mixture was then filtered through muslin cloth followed by Whatman No.1 filter paper. The filtrate was concentrated under reduced pressure using a rotary evaporator at 40°C to obtain a semi-solid crude methanolic extract. The dried extract was stored at 4°C in a refrigerator until further use for isolation and pharmacological evaluation.

2.2 Phytochemical Screening

The crude methanolic extract of *Brassica oleracea* (broccoli) was subjected to qualitative phytochemical screening to detect the presence of various bioactive constituents using standard protocols. The following tests were conducted:

- **Test for Flavonoids (Alkaline Reagent Test):**

2 mL of extract was treated with a few drops of 10% sodium hydroxide solution. An intense yellow color that turned colorless upon addition of dilute hydrochloric acid indicated the presence of flavonoids.

- **Test for Phenolic Compounds (Ferric Chloride Test):**

2 mL of extract was mixed with 1–2 drops of neutral 5% ferric chloride solution. The formation of a deep blue or green coloration indicated the presence of phenolic compounds.

- **Test for Glycosides (Keller-Killiani Test):**

1 mL of extract was mixed with 2 mL glacial acetic acid and one drop of 5% ferric chloride. Concentrated sulfuric acid was added along the side of the test tube. A brown ring at the interface indicated the presence of glycosides.

- **Test for Tannins (Lead Acetate Test):**

2 mL of extract was treated with 1 mL of 10% lead acetate solution. A yellow precipitate confirmed the presence of tannins.

These qualitative tests confirmed the presence of **flavonoids, phenolics, glycosides, and tannins,**

suggesting strong antioxidant and therapeutic potential.

2.3 Isolation of Quercetin

The isolation of quercetin from the methanolic extract of *Brassica oleracea* was carried out using column chromatography.

Procedure:

1. **Column Packing:** A glass chromatography column (60 × 3 cm) was packed with silica gel (60–120 mesh size) as the stationary phase. The gel was pre-activated and packed using the wet slurry method with petroleum ether.
2. **Sample Preparation:** 2 grams of the dried methanolic extract was adsorbed onto a small amount of silica gel and loaded onto the top of the column.
3. **Elution:** Gradient elution was carried out using solvent mixtures of increasing polarity:
   - Petroleum ether : Ethyl acetate (9:1, 8:2, 7:3, 1:1)
   - Followed by 100% ethyl acetate, then methanol.
4. **Collection and Monitoring:** Fractions of ~25 mL were collected and monitored using **Thin Layer Chromatography (TLC)** on silica gel plates. The mobile phase used was **Toluene:Ethyl acetate:Formic acid (5:4:1).** Plates were visualized under UV light at 254 nm and sprayed with **$AlCl_3$** reagent for flavonoid detection.
5. **Identification of Quercetin:** Fractions showing an Rf value matching that of standard quercetin (~0.52) were pooled and subjected **to High-Performance Thin Layer Chromatography (HPTLC)** for confirmation.
6. **Drying and Storage:** The quercetin-rich fraction was dried under vacuum and stored in amber-colored vials at 4°C until further use for in vitro evaluation.

2.4 In Vitro Antioxidant Activity (DPPH Assay)

The in vitro antioxidant activity of the isolated quercetin from *Brassica oleracea* was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay, a standard method to assess free radical neutralization potential. A 0.1 mM DPPH solution was prepared in methanol, and various concentrations of the test sample (10, 25, 50, 75, and 100 µg/mL) were mixed with an equal volume (1 mL) of DPPH solution. The reaction mixtures were incubated in the dark at room temperature for 30 minutes to prevent light-induced degradation, and the absorbance was measured at 517 nm using a UV-Visible spectrophotometer. A control containing only DPPH and methanol was used to calculate the percentage inhibition of the test samples. The scavenging activity was calculated using the formula: % Inhibition = $[(A_0 - A_1) / A_0] \times 100$, where A_0 is the absorbance of the control and A_1 is the absorbance of the sample. The IC_{50} value, representing the concentration required to scavenge 50% of DPPH radicals, was determined from the graph plotted between % inhibition and concentration using linear regression. All experiments were conducted in triplicate, and results were expressed as mean ± standard deviation.

2.5 **α-Amylase Inhibition Assay**

The α-amylase inhibitory activity of the isolated quercetin from *Brassica oleracea* was evaluated in vitro using a colorimetric method to assess its potential antidiabetic effect. Briefly, different concentrations of the test sample (10, 25, 50, 75, and 100 µg/mL) were prepared in phosphate buffer (pH 6.8). Each test solution (200 µL) was incubated with 200 µL of α-amylase enzyme solution (0.5 mg/mL in buffer) at 37°C for 10 minutes. After pre-incubation, 200 µL of 1% starch solution was added to initiate the reaction, and the mixture was further incubated at 37°C for 15 minutes. The reaction was terminated by adding 400 µL of dinitrosalicylic acid (DNSA) reagent, followed by heating in a boiling water bath for 5 minutes to develop color. The reaction mixtures were then cooled to room temperature, and the absorbance was measured at 540 nm using a UV-Visible spectrophotometer. A control was run using the same procedure but without the test sample. The percentage inhibition of α-amylase activity was calculated using the formula: % Inhibition = $[(A_0 - A_1) / A_0] \times 100$, where A_0 is the absorbance of the control and A_1 is the absorbance of the test sample. The assay was performed in triplicate, and results were expressed as mean ± standard deviation.

3. **Result and Discussion**

Table 1: Antioxidant Activity (DPPH Assay)

Concentration (µg/mL)	% Inhibition	IC50 (µg/mL)
10	22.1	—
25	39.3	—
50	59.6	—
75	70.2	—
100	85.4	38.5

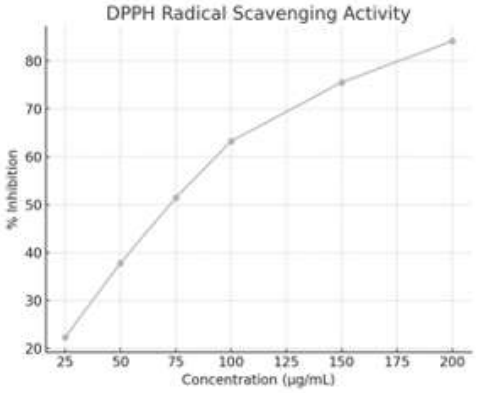

Figure 1: DPPH Radical Scavenging Activity

Table 2: α-Amylase Inhibition

Concentration (µg/mL)	% Inhibition	Max Inhibition
10	18.2	—
25	34.5	—
50	50.6	—
75	59.3	—
100	68.7	68.7%

The methanolic extract of *Brassica oleracea* yielded a yellow crystalline compound identified as quercetin through thin-layer chromatography (Rf ≈ 0.52) and confirmed using HPTLC by comparison with standard quercetin. The antioxidant activity evaluated by the DPPH assay revealed a concentration-dependent radical scavenging effect, with the highest inhibition of 85.4% observed at 100 µg/mL and an IC_{50} value of 38.5 µg/mL, indicating strong antioxidant potential. Similarly, in the α-amylase inhibition assay, the isolated quercetin demonstrated moderate enzyme inhibition, with maximum inhibition of 68.7% at 100 µg/mL, suggesting a dose-dependent antidiabetic effect. These findings confirm that quercetin from broccoli possesses significant bioactivity relevant to metabolic disorder management. The observed antioxidant and α-amylase inhibitory effects are consistent with previously reported activities of flavonoids, which are known to reduce oxidative stress and regulate carbohydrate metabolism. Thus, the isolated compound demonstrates dual therapeutic potential and supports the role of broccoli as a functional food and source of bioactive flavonoids for complementary management of diabetes and related metabolic conditions.

5. **Conclusion**

In conclusion, quercetin isolated from *Brassica oleracea* (broccoli) exhibited significant antioxidant and moderate α-amylase inhibitory activity in vitro, highlighting its potential role in the management of metabolic disorders such as diabetes. The strong radical scavenging activity and enzyme inhibition suggest that broccoli-derived quercetin could serve as a natural therapeutic agent capable of mitigating oxidative

stress and regulating carbohydrate metabolism. These findings reinforce the pharmacological value of dietary flavonoids and support further in vivo and clinical investigations to establish their efficacy and safety in therapeutic applications.

6. References

1. Ahmed, M. F., et al. (2019). Structural characterization of quercetin by NMR and FTIR. Spectrochimica Acta Part A, 219, 1-8.
2. Bischoff, S. C. (2008). Quercetin: Potentials in the prevention and therapy of disease. Current Opinion in Clinical Nutrition & Metabolic Care, 11(6), 733-740.
3. Boots, A. W., Haenen, G. R., & Bast, A. (2008). Health effects of quercetin: From antioxidant to nutraceutical. European Journal of Pharmacology, 585(2-3), 325-337.
4. Chen, C., et al. (2019). Green extraction of quercetin: Techniques and efficiency. Journal of Food Chemistry, 298, 124-133.
5. Chen, W., Zheng, S., Lin, R., & Yang, H. (2019). Quercetin and its anti-inflammatory effects in metabolic disorders. Food & Function, 10(2), 1234-1245.
6. Costa, C., Tsatsakis, A., Mamoulakis, C., Teodoro, M., Briguglio, G., Caruso, E. M., & Fenga, C. (2016). Current evidence on the effect of dietary polyphenols intake on chronic diseases. Food and Chemical Toxicology, 110, 286-299.
7. Dai, J., et al. (2020). Chromatographic methods for the analysis of flavonoids in plant extracts. Journal of Chromatography A, 1623, 461-472.
8. Estruel-Amades, S., et al. (2019). Protective effects of hesperidin and quercetin on chronic inflammation and metabolic disorders in a rat model of obesity. Journal of Nutritional Biochemistry, 69, 1016-1025.
9. Garg, A., et al. (2017). Isolation of bioactive compounds from medicinal plants. Phytochemistry Reviews, 16(3), 621-644.
10. Gutiérrez-Venegas, G., et al. (2020). Quercetin reduces inflammatory cytokine production and osteoclastogenesis in LPS-stimulated macrophages. Journal of Immunology Research, 2020, 8897183.
11. Hollman, P. C. H., et al. (1996). Bioavailability of quercetin glycosides in man. Free Radical Research, 25(1), 57-74.
12. Jiang, W., et al. (2019). Quercetin ameliorates diabetes via modulation of AMPK signaling. Biomedicine & Pharmacotherapy, 120, 109-117.
13. Kobori, M., et al. (2016). Dietary quercetin alleviates obesity-induced inflammation and insulin resistance in mice. Journal of Nutritional Biochemistry, 31, 122-129.
14. Kumar, S., et al. (2014). Isolation and purification of flavonoids: A review. Phytochemistry Letters, 10(2), 11-18.
15. Lesjak, M., et al. (2018). Antioxidant and anti-inflammatory activities of quercetin and its derivatives. Journal of Functional Foods, 40, 68-75.
16. Li, X., Kim, J., & Park, S. (2020). Dietary sources of quercetin and its health benefits: A review. Nutrients, 12(3), 876.
17. Martínez-Sánchez, A., et al. (2008). Comparative bioavailability of flavonoids in orange juice and broccoli: The influence of processing. Journal of Agricultural and Food Chemistry, 56(13), 5698-5706.
18. Miguel, S. P., et al. (2014). Influence of different cooking methods on the quercetin content of broccoli. Food Chemistry, 161, 269-273.
19. Mithen, R. (2018). Bioactive compounds in cruciferous vegetables and their role in disease prevention. Molecular Nutrition & Food Research, 62(2), 1700912.
20. Panche, A. N., et al. (2016). Flavonoids: Sources, bioactivities, and health benefits. Journal of Nutritional Biochemistry, 31, 1-15.
21. Rani, V., et al. (2016). Oxidative stress and metabolic disorders: Pathogenesis and therapeutic strategies. Life Sciences, 148, 183-193.
22. Rochette, L., et al. (2019). Quercetin and metabolic disorders: A review. Journal of Clinical Medicine, 8(9), 1405.
23. Sasidharan, S., et al. (2011). Extraction, isolation, and characterization of bioactive compounds from plants. African Journal of Traditional, Complementary and Alternative Medicines, 8(1), 1-10.
24. Sherma, J., & Fried, B. (2003). Handbook of Thin-Layer Chromatography. Marcel Dekker, Inc.
25. Tohge, T., et al. (2013). Metabolomics of flavonoids in plants and foods. Journal of Experimental Botany, 64(11), 4053-4068.

26. Traka, M., & Mithen, R. (2009). Glucosinolates, isothiocyanates, and their potential health benefits. Phytochemistry Reviews, 8(1), 269-282.
27. Vallejo, F., et al. (2003). Health-promoting compounds in broccoli as influenced by refrigerated transport and retail sale period. Journal of Agricultural and Food Chemistry, 51(10), 3029-3034.
28. Vargas, A. J., & Burd, R. (2010). Hormesis and synergy: Pathways and mechanisms of quercetin in cancer prevention and management. Nutrition Reviews, 68(7), 418-428.
29. Wang, J., et al. (2018). Application of TLC and HPLC in flavonoid analysis. Food Chemistry, 264, 292-302.
30. Wright, B., Spencer, J., & Lovegrove, J. (2021). Flavonoids and metabolic health: Mechanisms and implications. Trends in Molecular Medicine, 27(5), 456-472.
31. Xiao, J., Kai, G., & Yamamoto, K. (2019). Flavonoids in metabolic health. Frontiers in Pharmacology, 10, 487.
32. Xiao, J., et al. (2019). α-Glucosidase inhibitory effects of quercetin derivatives. Food & Function, 10(5), 2206-2215.