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Extraction and Quantification of Flavonoids from Vietnamese *Dicranopteris linearis* Leaves Using Green Method and Determination of their *α*-Glucosidase Inhibitory Activity

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ABSTRACT

Flavonoids are one of the most promising agents in reducing post-prandial hyperglycaemia due to their *α*-glucosidase inhibitory activity. An investigation of the flavonoid-rich fraction from medicinal plants is imperative due to their potential health benefits. This study aims to develop a green and efficient method for extracting flavonoids from *Dicranopteris linearis* leaves, and to determine their *α*-glucosidase inhibitory activity. Air-dried and powdered *D. linearis* leaves were extracted with 70% ethanol by percolation at room temperature. The crude ethanol extract was subjected to macroporous resin (Diaion HP20) column chromatography to obtain flavonoid-rich fraction F as well as other non-flavonoid fractions (W and P). The total flavonoid content of the crude extract and fractions was determined using the aluminum chloride colorimetric assay. The *α*-glucosidase inhibitory activity was assessed using standard method. The quercitrin content of the crude extract and Fraction F was determined by high-performance liquid chromatography (HPLC). The HPLC method was validated following the ICH guidelines. Quercitrin was isolated from Fraction F by reverse phase preparative HPLC. A green method using Diaion HP20 resin produced a highly enriched flavonoid fraction from *Dicranopteris linearis* leaves that exhibited potent *α*-glucosidase inhibitory activity with IC_{50} value of 1.36 ± 0.03 µg/mL, 24-fold stronger than that of the crude extract (IC_{50} = 32.16 ± 0.22 µg/mL). A validated quantitative HPLC analysis revealed that quercitrin was the major constituent of the flavonoid-rich fraction. This study provides a sustainable method for obtaining flavonoids from *Dicranopteris linearis* leaves with potential application as natural remedy for type 2 diabetes.

Keywords: *Dicranopteris linearis*, Alpha-glucosidase, Diaion Hydrophobic-20, Quercitrin

Introduction

Flavonoids are a class of plant secondary metabolites characterized by their diverse polyphenolic structures and wide distribution across the plant kingdom.[1] Based on their carbon skeleton, these group of compounds are categorized into several subgroups, including flavones, flavonols, flavanones, flavanonols, flavanols, anthocyanins, and chalcones.[2] Flavonoids are found in various plant tissues and contribute to the colour and scent of flowers.[2] They also protect plants against biotic and abiotic stresses by serving as UV filters, detoxifying agents, and antimicrobial compounds, while playing a key role in plant acclimatization to extreme heat and cold conditions.[3] Flavonoids are valued for their many physiologically active members and are well known for their beneficial effects on human health, with numerous studies demonstrating their pharmacological and bioactive properties.[4,5]

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A key aspect of their significance lies in their ability to reduce and scavenge free radicals, demonstrating high antioxidant activity. Recent studies have shown that specific flavonoids, such as quercetin, isoquercetin, kaempferol, and quercitrin may play a key role in glucose metabolism by inhibiting *α*-glucosidase, with IC_{50} values of 0.017, 0.18, 8.97, and 74.5 µM respectively.[6] *α*-Glucosidase inhibitors can slow the breakdown and absorption of dietary carbohydrates, preventing glucose absorption into the bloodstream and thus suppressing postprandial hyperglycemia. Flavonoids are now regarded as indispensable components in various nutraceutical, pharmaceutical, medicinal, and cosmetic applications.[7,8]

Dicranopteris linearis (Burm.fil.) Underw. (Gleicheniaceae) is widely distributed in Vietnam and other parts of Asia and Africa.[9] It has been used in various traditional medicinal practices; in Malaysia, it is used to treat fever, in India it is used for the treatment of asthma and female infertility, and in Papua New Guinea, it is used for wound healing.[9] Phytochemical studies have identified various flavonol derivatives in the leaves of *D. linearis*. Quercetin, isoquercetin, kaempferol, and quercitrin are the primary active constituents responsible for its bioactivity.[9] Conventional solvent extraction of flavonoids from *D. linearis* leaves has typically used large amounts of organic solvents like methanol, *n*-hexane, and ethyl acetate.[9,10] However, these solvents are expensive, flammable, toxic, and non-biodegradable, posing significant environmental and health risks. Therefore, there is a critical need for a green extraction technique to increase the extraction yield of these bioactive compounds. In this study, we present an effective method using a nonionic polymer adsorption material, Diaion HP20 macroporous resin, for enriching the flavonoids in *D. linearis* leaf

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extract. This study focused on reducing the use of organic solvents, replacing them with a green and renewable alternative that would give a high extraction yield within a short time, and significantly increase *α*-glucosidase inhibitory effects.

Materials and Methods

Chemicals and reagents

Ethanol 96% was obtained from Cargill (Vietnam) and was diluted to 70% with deionized water. Macroporous resin Diaion HP20 was provided by Mitsubishi Chemicals Company (Japan). HPLC grade methanol was purchased from Fisher. Quercetin (98.86%), quercitrin (98.52%), and acarbose (97.85%) were obtained from the Institute of Drug Quality Control, Ho Chi Minh City, Vietnam. *α*-Glucosidase from *Saccharomyces cerevisiae*, *p*-nitrophenyl-*α*-D-glucopyranoside (pNPG), DMSO, $NaHPO_4$, and Na_2HPO_4 were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Plant material

The leaves of *Dicranopteris linearis* were collected in Lam Dong Province (Coordinates: 11°57′N 108°26′E), Vietnam, in December 2023. The plant material was identified by Prof. Dr. CL Tran at Tay Do University, Vietnam. An herbarium specimen with voucher number 2023612RTS was deposited at the Department of Traditional Pharmacy at the University of Medicine and Pharmacy in Ho Chi Minh City, Vietnam.

Extraction of plant material and preparation of flavonoid-rich fraction

Air-dried and powdered *D. linearis* leaves (2.5 kg) were extracted by percolation method with 70% ethanol (25 L) at room temperature. The extract was concentrated to dryness under reduced pressure to yield a crude extract residue (345 g) that was dispersed in deionized water (250 mL) and loaded onto the macroporous resin HP20 chromatography column. Selection of the Diaion HP20 resin was based on its adsorption and desorption capacities, as well as the ratio of desorption according to previous reports.[11,12] Deionized water (4 BV) was used to elute highly polar components until a colourless water fraction was obtained for which a negative result for flavonoids was confirmed by alkaline and Shinoda's tests. The water extracts were combined and evaporated on a water bath at 60ºC to produce fraction W (32.26 g). Then, 70% ethanol was used to elute the fraction containing flavonoids. Elution was continued until the fraction gave a negative result for flavonoids by alkaline and Shinoda's tests. The combined fractions were concentrated under reduced pressure at 40ºC to produce fraction F (76.58 g). Finally, isopropanol was used to elute the remaining constituents from the chromatography column, producing fraction P (184 g).

Determination of total flavonoid content

The total flavonoid content was determined using the aluminum chloride colorimetric assay. Various concentrations of standard quercetin (100, 80, 60, 40, and 20 µg/mL in methanol) were prepared. An aliquot of 1 mL quercetin solution at each concentration was added to a 10 mL volumetric flask containing 4 mL of double-distilled water. Then, 0.3 mL of 5% sodium nitrite was added. After 5 minutes, 0.3 mL of 10% aluminum chloride ($AlCl_3$) was introduced, followed by the addition of 2 mL of 1 M sodium hydroxide after 6 minutes. The total volume of the mixture was then adjusted to 10 mL with double-distilled water, and the mixture was thoroughly mixed. The absorbance of the resulting, pink-coloured solution was measured at 430 nm against a blank, which contained all the reagents except quercetin. A calibration curve was plotted based on the average absorbance values obtained at the different quercetin concentrations. The extracts were prepared at a concentration of 20 mg/mL and diluted to various concentrations (2.0, 1.0, 0.5, and 0.25 mg/mL). Following the procedure used for the standard, the absorbance of each extract concentration was recorded. The total flavonoid content of the extracts was expressed as mg quercetin equivalents per gram of dry extract (mg QE/g), calculated using the formula: X = cV/m, where X is the total flavonoid content in mg/g of dry extract, c is the concentration of quercetin obtained from the calibration curve (mg/mL), V is the volume of extract (mL), and m is the mass of the extract (g).

In vitro assay of α-glucosidase inhibitory activity

α-Glucosidase inhibitory activity was assessed using a previously described method.[14] An enzyme solution was prepared by dissolving 1 mg of *α*-glucosidase in 100 mL of phosphate buffer (pH 7) with 200 mg of bovine serum albumin, then diluted 25-fold with the same buffer.[15,16] In microplate wells, 25 µL of 10 mM *p*-nitrophenyl-D-glucopyranose (substrate) and 50 µL of 100 mM phosphate buffer (pH 7) were combined. Extract samples (10 µL) dissolved in DMSO were added to reach final concentrations of 50–1000 µg/mL. A 1% acarbose solution, mixed with 2 N HCl and centrifuged, was used as a control (at final concentrations of 0.1–10 µg/mL). Each sample, blank, and control was tested in triplicate. After incubating at 37°C for 5 minutes, 25 µL of enzyme solution was added, followed by a 15-minute incubation. The reaction was stopped with 100 µL of 200 mM Na_2CO_3, and absorbance at 400 nm was measured using a microplate reader. Enzyme inhibition (%) was calculated using the formula;

$$\%\ \text{of inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}}\ x\ 100$$

Quantitative HPLC analysis

HPLC analysis was performed using a Hitachi L2000 system equipped with two solvent delivery units, a diode array detector (DAD), an autosampler, a column oven, and EZChrom Elite software. Separation was achieved on a reversed-phase C18 column (Kinetex, 4.6 x 150 mm, 2.6 µm, Phenyl-Hexyl 100 Å, Phenomenex) using a mobile phase of methanol with 0.1% TFA (A) and water with 0.1% TFA (B) in a 48:52 ratio. The column temperature was set to 30°C, with a flow rate of 1 mL/min and detection at 254 nm. A 10 µL sample was injected for analysis. To prepare the quercitrin stock solution, quercitrin standard (7 mg) was dissolved in methanol (5 mL) with sonication for 10 minutes. Calibration standards were prepared by diluting the stock solution with methanol. For sample preparation, crude extract (150 mg) and fraction F (100 mg) were each dissolved in methanol (10 mL), sonicated for 10 minutes, and filled to the mark. The method was validated according to ICH guidelines for specificity, linearity, limit of detection (LOD), limit of quantification (LOQ), repeatability, precision, and accuracy.[17]

Isolation of quercitrin

Quercitrin was isolated using preparative high-performance liquid chromatography (HPLC) on a Waters Prep4000 system with a reversed-phase C18 column (19 × 300 mm, 5 µm, XBridge Phenyl, Waters). The mobile phase consisted of 60% water and 40% methanol, with compounds monitored at 254 nm. Fraction F was diluted in methanol, filtered (0.45 µm), and manually injected into the HPLC system. Peak fractions corresponding to quercitrin were collected, concentrated using a rotary evaporator, and freeze-dried.

Spectroscopic analysis

The NMR data were recorded on Bruker AV500, (500 MHz for ^{1}H, 125 MHz for ^{13}C) spectrophotometer. HRESIMS data were recorded on an Agilent Q-TOF spectrometer, and UV spectra were recorded on a Shimadzu UV-2450 spectrophotometer. All data were compared with reference quercitrin spectra.

Statistical analysis

Experiments were conducted with three independent test samples, and performed in duplicate. Data were analyzed using GraphPad Prism 5 and Excel 2019. Data were expressed as the mean ± standard deviation (SD) of three replicates. Differences between means were determined by Duncan's multiple range tests. Statistical significance was established at $p < 0.05$.

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Results and Discussion

Extraction efficiency of flavonoid-rich fraction

Three solvents comprising 40%, 70%, and 96% ethanol were investigated for the extraction of 100 g dry powdered of *D. linearis* leaves. The highest crude residue yield was obtained with 70% ethanol, which was then selected for the extraction process. Air-dried and powdered *D. linearis* leaves were extracted by percolation method with 70% ethanol with an extraction efficiency of 13.8%. To obtain the flavonoid-rich fraction, the crude extract was subjected to macroporous resin HP20 column chromatography, yielding three distinct fractions: W (32.26 g), F (76.58 g), and P (184 g). These fractions were characterized using thin layer chromatography (TLC). Fraction W showed no detectable spots, indicating that it likely contained water-soluble substances that did not bind to the HP20 resin. Fraction F contained flavonoid compounds, identified by a characteristic fluorescence under 366 nm UV light. After spraying with $FeCl_3$ reagent, the TLC plates revealed blue-black spots, and yellow spots were observed after spraying with 10% H_2SO_4 and heating at 105°C for 5 minutes. Fraction P contained chlorophyll and terpenoids, evidenced by the presence of green and purple spots after treatment with 10% H_2SO_4 and heating, and red spots under 365 nm UV light (Figure 1). This identification process allowed for the selection of the flavonoid-rich fraction (F), which was further studied for its bioactivity.

The choice of solvent significantly impacted flavonoid extraction efficiency. For the polar flavonoids, water-organic solvent mixtures are commonly used. While methanol is often used in analytical applications, its toxicity limits its use in food and pharmaceutical processing. Ethanol, a less toxic and environmentally friendly option, is usually preferred.[18] Adding a certain amount of water to ethanol can enhance extraction yields. Therefore, 70% ethanol was selected for percolating *D. linearis* in this study, considering its potential applications. Macroporous resins have proven effective in separating total flavonoids from natural products.[19] Eleven resins were evaluated for their adsorption and desorption properties using *Rosa setate* waste extracts. HP20 resin exhibited superior desorption capabilities, likely due to its pore size and steric effects.[11] This HP20 resin was chosen for further enrichment of the flavonoids in *D. linearis* leaf extract.

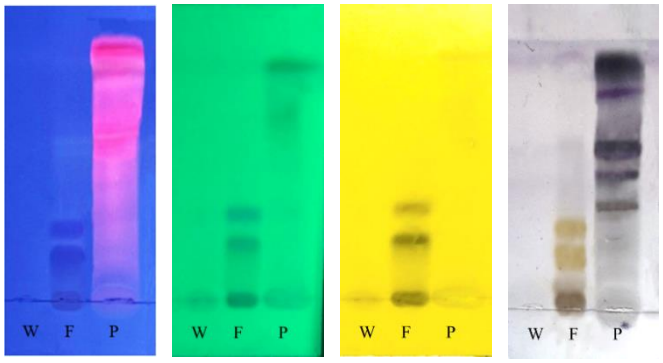

Figure 1: Thin Layer Chromatography (TLC) profile of three fractions W, F and P using silica gel F254 as stationary phase and chloroform:methanol 95:5 v/v as mobile phase; observed under (a) UV 365 nm; (b) UV 254 nm; (c) spray reagent $FeCl_3$; (d) spray reagent H_2SO_4 10% and heated at 105°C for 5 minutes.

Total flavonoids content

The total flavonoid content was quantified using the aluminum chloride colorimetric method, with quercetin as the standard. Results were expressed as milligrams of quercetin equivalents per gram (mg/g). A calibration curve was constructed using quercetin, yielding the linear regression equation $y = 0.0194x + 0.0217$ ($R^2 = 0.996$). This equation was used to calculate the total flavonoid content in various *D. linearis* extracts. After enrichment with HP20 resin, the 70% ethanol fraction displayed the highest total flavonoid content (83.71 ± 1.08 mg/g), approximately three times higher than that of the crude extract (27.2 ± 1.08 mg/g). The water and isopropanol fractions had significantly lower flavonoid contents (1.24 ± 0.08 mg/g, and 2.05 ± 0.05 mg/g, respectively) (Table 1).

Table 1: Total flavonoid contents of crude extract and fractions of *Dicranopteris linearis* leaves

Sample	Weight (mg)	Total flavonoid content (mg/g)[a]
Crude extract	0.1021	27.2 ± 1.08
Fraction W	0.1014	1.24 ± 0.08
Fraction F	0.1051	83.71 ± 1.08
Fraction P	0.1012	2.05 ± 0.05

[a] Values are mean ± standard deviation (SD) of three replicates.

α-Glucosidase inhibitory activity

The *in vitro* α-glucosidase inhibitory effect of the crude extract and three fractions W, F, and P was evaluated with acarbose used as a positive control (Table 2). Fraction F demonstrated the highest activity with an IC_{50} value of 1.36 ± 0.03 µg/mL. In comparison, the crude extract of *D. linearis* exhibited an inhibitory activity with an IC_{50} value of 32.16 ± 0.03 µg/mL. Fractions W and P showed minimal activity at the highest concentration (500 µg/mL) and no significant activity at lower concentrations. The positive control (acarbose) had an IC_{50} value of 122.2 ± 2.02 µg/mL.

Flavonoids are considered as the main bioactive constituents of many herbs. However, flavonoid content is rather low compared to that of other compounds like polysaccharides, cellulose, and other impurities, which impede the clinical use of the herb or its crude extracts. Therefore, flavonoid-rich extracts may have greater clinical utility.[20] In this study, the flavonoid content in 70% ethanol extract of *D. linearis* was increased 3-fold by the green and efficient chromatographic method. Moreover, the *in vitro* α-glucosidase inhibition assays indicated that the IC_{50} value decreased about 24-fold compared to the crude extract. The higher activity may be caused by the higher purity of the fraction after purification. Indeed, in the study of Jeong *et al*. (2023), highly purified extracts showed better antibacterial activity against food and dermatological pathogens, with narirutin and hesperidin concentration and purity possibly influencing the extracts' biological effects. Their study provides valuable insights into the improved extraction and purification of narirutin and hesperidin from *Citrus* x *junos* powder and their biological activities.[21] In this study, the higher quercitrin and other flavonoids contents, along with the higher purity of the fraction, could responsible for the improved α-glucosidase inhibitory effect. This report highlights the potential health benefits of the new extraction technique for the flavonoid-rich ingredients from the leaves of *D. linearis*.

Table 2: α-Glucosidase inhibitory activity of crude extract and fractions of *Dicranopteris linearis* leaves

Sample	IC_{50} (µg/mL)[a]
Crude extract	32.16 ± 0.22**
Fraction W	> 500
Fraction F	1.36 ± 0.03*
Fraction P	> 500
Acarbose[b]	122.2 ± 2.02*

[a] Values are mean ± standard deviation (SD) of three replicates. *p < 0.01; **p < 0.05; [b] Positive control.

Optimized HPLC method for the quantification of quercitrin

Quercitrin was the main flavonoid compound, with the highest concentration in the flavonoid-rich fraction F of the leaf extract of *D. linearis*. Therefore, quercitrin could be selected as a marker to quantify the active constituents in the crude extract of *D. linearis* leaves (Figure 2), and HPLC method could be used to determine its content in the 70% ethanol fraction (Figure 3). To develop and validate an efficient HPLC

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method for the analysis of quercitrin, different detection wavelengths, liquid chromatographic columns, and mobile phase compositions were explored. The results from the HPLC analysis identified mobile phase A and B in the ratio of 48:52 (v/v) as the optimal solvent system, where mobile phase A was 0.1% TFA in methanol and mobile phase B was 0.1% TFA in water (v/v). The column was a Kinetex C18 LC Column (150 x 4.6 mm, 2.6 µm Phenyl-Hexyl 100 Å). The column oven temperature was set at 30ºC, the flow rate was 1 mL/min, and the sample injection volume was 10 µL (Figure 4).

Validation parameters for the HPLC quantification method

Specificity: The retention time of quercitrin was suitable within the chromatographic method run time and the peak shape was symmetrical. The solvent peak was well separated and appeared before 5 min. The retention times for quercitrin in the standard and the samples were found to be the same, and no coeluting peaks were detected when adding the quercitrin standard to the sample solution. The highest absorption intensity of quercitrin was detected at 254 nm.

Linearity: The calibration curve was generated from five quercitrin standard concentrations (0.2, 0.26, 0.32, 0.4, and 0.7 mg/mL) and the corresponding peak areas. An excellent linearity was observed in a range 0.2−0.7 mg/mL of quercitrin. The linear equation for the calibration curve was y = 56307814x − 9051255 with a correlation coefficient (R^2) of 0.9993. Figure 5 displays the calibration curve for quercitrin at 254 nm.

Limit of detection and limit of quantification: The limit of detection (LOD) and the limit of quantification (LOQ) were determined from the calibration curves of the quercetin standard. LOD was calculated according to the expression SD x 3/a, where SD is the standard deviation of the response and a is the slope of the calibration curve. LOQ was calculated from the expression SD x 10/a. In this study, the LOD and LOQ were 0.010 and 0.033 mg/mL, respectively.

Repeatability and precision: The %RSD for six replicate injections of the quercitrin standard and measurement of peak areas was found to be 1.12%. Six sample solutions were prepared and analyzed by the developed method. The %RSD of 1.62% indicates that the method has an acceptable level of precision.

Accuracy: Three concentrations (80, 100, and 120%) of the standards were added to the sample to evaluate the accuracy of the quantitative method. The results showed high efficiency for extraction of quercitrin from the raw powder material. The recovery of quercitrin ranged from 95.68 to 98.56%. This confirms that the method can be used for the determination and quantification of quercitrin.

Determination of quercitrin content

The HPLC method developed is precise, specific, and accurate for the determination of quercitrin, with the advantages of speed and simplicity of sample preparation. Statistical analysis proved that the method is reproducible and selective for the analysis of quercitrin. The quantitative method was applied to determine the quercitrin content in the powdered extract of *D. linearis* and flavonoid-rich fraction F. The results showed that the quercitrin content in the dried 70% ethanol extract was 8.26 ± 0.41 mg/g. After chromatographic enrichment, the quercitrin concentration increased approximately by 3-fold to 24.51 ± 0.08 mg/g (Table 3).

The quantification method of quercitrin by RP HPLC-DAD was validated in terms of specificity, linearity, LOD, LOQ, repeatability, precision, and accuracy according to ICH guidelines. Both LOD and LOQ for quercitrin were comparable to those reported in previous works and the concentration of quercitrin in *D. linearis* leaves was approximately twice that in *Houttuynia cordata* Thunb. leaves.[24,25] Due to its wide distribution in humid, and tropical regions, as well as its propagation from spores which has been successfully developed in the last twenty years,[26] *D. linearis* may be a viable commercial source of flavonoids.

Isolation and spectra data of quercitrin

Quercitrin was successfully isolated from fraction F as a yellow powder which displayed an $[M-H]^-$ peak at *m/z* 447.0906 in negative electrospray ionization time of flight mass spectroscopy (ESI-TOF-MS) corresponding to the molecular formula $C_{21}H_{20}O_{11}$ (Figure 3).

The UV spectrum showed the presence of two absorption maxima at wavelengths of 250 and 350 nm, due to the C=C double bond of the aromatic structure.

^{1}H-NMR (500 MHz, DMSO-d_6) ppm: δ_H 7.31 (1H, d, J = 2.0 Hz, H-2′), 6.88 (1H, d, J = 8.4 Hz, H-5′), 7.27 (1H, dd, J = 8.4; 2.0 Hz, H-6′), 6.20 (1H, d, J = 1.8 Hz, H-6), 6.4 (1H, d, J = 1.8 Hz, H-8), δ 5.26 (1H, s, H-1′′), 0.83 (3H, d, J = 6.0 Hz, H-6′′), 3.17 (1H, m, H-5′′), 3.24 (1H, m, H-4′′), 3.51 (1, dd, J = 3.0 and 3.0 Hz, H-3′′), 3.99 (1H, m, H-2′′), 9.34 (1H, s, OH), 9.72 (1H, s, OH), 10.87 (1H, s, OH), and 12.68 (1H, s, OH).

^{13}C-NMR (125 MHz, DMSO-*d6*) ppm: δ_C 157.78 (C-2), 134.70 (C-3), 178.22 (C-4), 161.76 (C-5), 99.16 (C-6), 164.65 (C-7), 94.10 (C-8), 156.92 (C-9), 104.57 (C-10), 121.59 (C-1′), 115.94 (C-2′), 148.90 (C-3′), 145.67 (C-4′), 116.14 (C-5′), 121.22 (C-6′), 102.30 (C-1′′), 70.84 (C-2′′), 71.05 (C-3′′), 71.67 (C-4′′), 70.53 (C-5′′), 17.96 (C-6′′). The ^{1}H- and ^{13}C-NMR spectroscopic data were in agreement with that found in literature.[22,23]

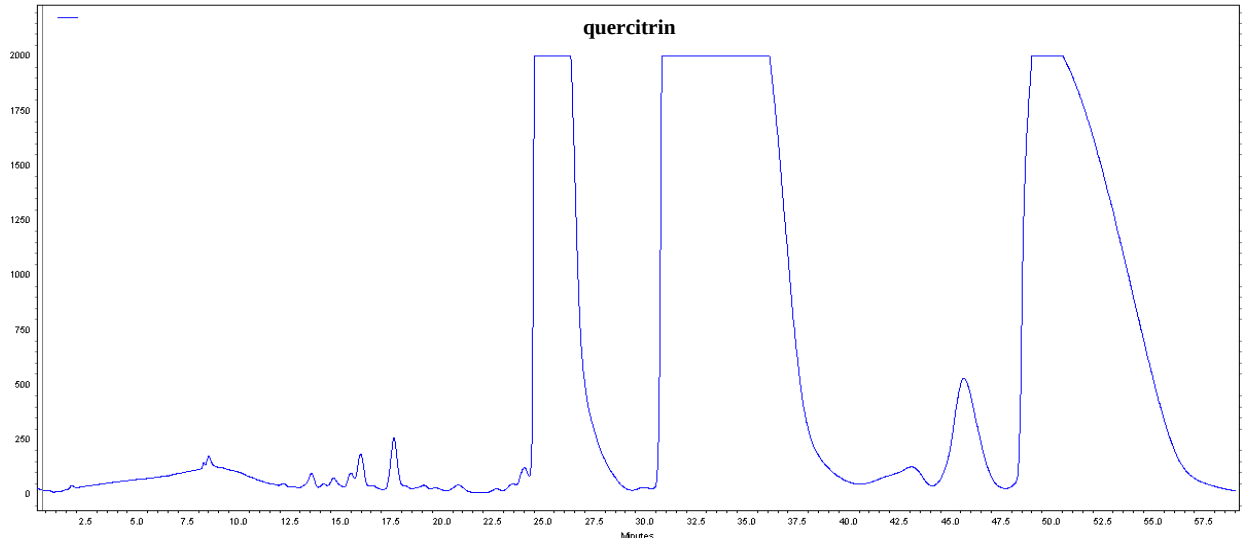

Figure 2: Preparative HPLC chromatogram of quercitrin

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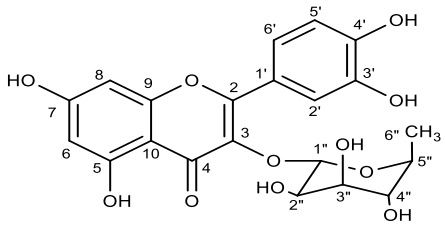

Figure 3: Molecular structure of quercitrin

Table 3: Quercitrin content of crude extract and fraction of *Dicranopteris linearis* leaves

Sample	Weight (mg)	Quercitrin (mg/g)[a]
Crude extract	150.2	8.26 ± 0.41
Fraction F	50.1	24.51 ± 0.08

[a] Values are mean ± standard deviation (SD) of three replicates.

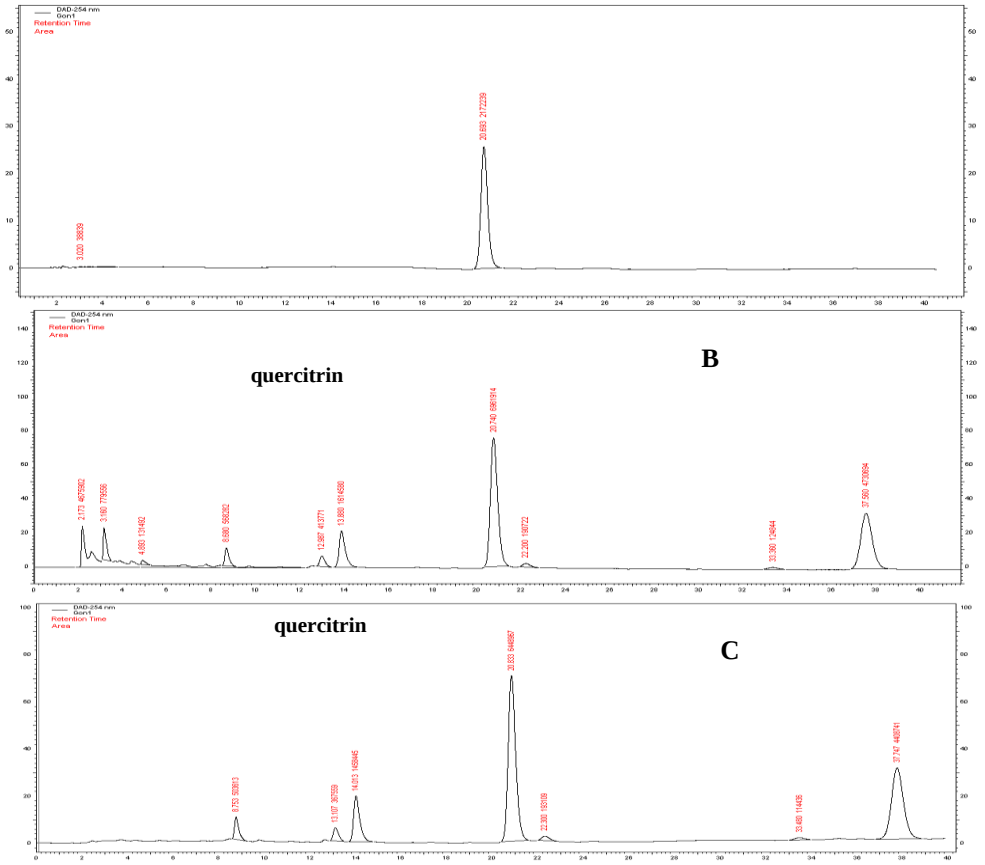

Figure 4: HPLC Chromatogram of quercitrin standard (A), crude extract (B) and fraction F (C)

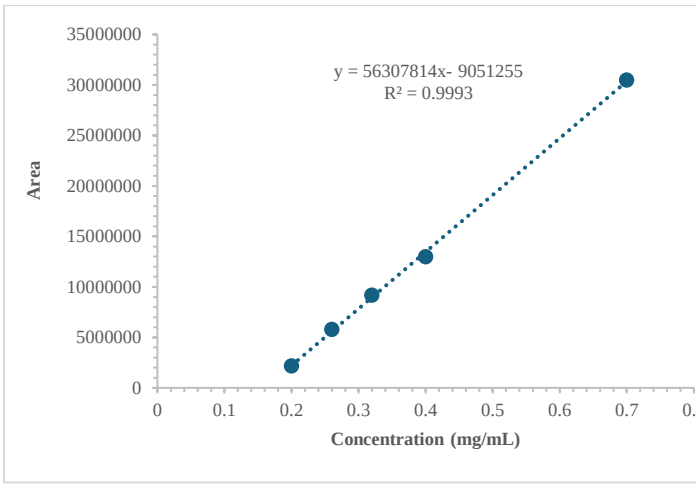

Figure 5: Calibration curve of quercitrin standard

Conclusion

This study presents an effective and sustainable method for extracting flavonoids from *D. linearis* leaves using an environmentally friendly method. The results demonstrated that a 70% ethanol solution is highly suitable for both the extraction of flavonoid compounds from the raw plant material and for desorbing flavonoid fractions from macroporous resin Diaion HP20. After a single enrichment step, the flavonoid content in the purified fraction increased approximately threefold, and the *α*-glucosidase inhibitory activity increased 24-fold. The RP-HPLC-DAD method used for quantifying quercitrin in *D. linearis* extract was validated as sensitive and reliable, successfully quantifying quercitrin in both the crude extract and the flavonoid-rich fraction. This analytical procedure is valuable for quality control in the extraction and purification of flavonoids from *D. linearis*. The high concentration of flavonoids in *D. linearis* and their high activity as *α*-glucosidase inhibitor suggest a promising potential application as a natural remedy for managing type 2 diabetes.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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