IN VITRO ANTIDIABETIC ACTIVITY OF ETHANOLIC EXTRACT OF *PARMOTREMA PERLATUM* AND QUANTITATIVE ESTIMATION OF QUERCETIN BY HPTLC

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Abstract
The present study aimed to evaluate the in vitro antidiabetic activity of the ethanolic extract of *Parmotrema perlatum* and to quantify quercetin using High Performance Thin Layer Chromatography (HPTLC). The antidiabetic potential was assessed by enzyme inhibition assays using acarbose as the standard drug. The ethanolic extract exhibited concentration-dependent inhibitory activity in both assays. In the first assay, the extract showed an IC_{50} value of 48.41 µg/mL compared to 14.76 µg/mL for acarbose, while in the second assay the IC_{50} value of the extract was 60.74 µg/mL compared to 15.25 µg/mL for acarbose. Although the extract demonstrated lower potency than the standard, it showed significant enzyme inhibitory activity. HPTLC analysis confirmed the presence of quercetin in the extract, and quantitative estimation revealed a quercetin content of 0.1015% w/w. The presence of this bioactive flavonoid may contribute to the observed antidiabetic activity. The findings suggest that *Parmotrema perlatum* possesses promising natural antidiabetic potential and could serve as a source for the development of phytopharmaceutical agents.

Keywords
Parmotrema perlatum, In vitro antidiabetic activity, α-amylase inhibition, α-glucosidase inhibition, Acarbose, HPTLC, Quercetin, Flavonoids, Herbal standardization.

How to cite this article: Chandrakantrao GA, Dwivedi AK. In Vitro Antidiabetic Activity of Ethanolic Extract of Parmotrema perlatum and Quantitative Estimation of Quercetin by HPTLC. Int J Drug Deliv Technol. 2026;16(61s): 78-83. DOI: 10.25258/ijddt.16.61s.11

Introduction

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from defects in insulin secretion, insulin action, or both (Baynes; 2015). The global prevalence of diabetes has increased dramatically in recent decades, posing a major public health challenge. Prolonged hyperglycemia is associated with severe complications such as neuropathy, nephropathy, retinopathy, and cardiovascular diseases (Khunti et al., 2023). One of the major mechanisms involved in diabetic complications is oxidative stress caused by excessive production of reactive oxygen species (ROS), which leads to cellular damage and impaired glucose metabolism (Giacco and Brownlee; 2010). Therefore, the management of diabetes not only requires glycemic control but also the reduction of oxidative stress.

Medicinal plants and lichens have gained significant attention as potential sources of natural antidiabetic agents due to their rich content of bioactive secondary metabolites, including flavonoids, phenolics, terpenoids, and alkaloids (Thadhani and Karunaratne; 2017).

These phytoconstituents are known to exhibit antioxidant, enzyme inhibitory, and insulin-sensitizing properties. Among them, flavonoids such as quercetin have been extensively studied for their ability to inhibit carbohydrate-hydrolyzing enzymes like α-amylase and α-glucosidase, thereby reducing postprandial hyperglycemia (Pathak et al., 2017). Quercetin also demonstrates strong antioxidant activity, which may contribute to its protective role against diabetes-induced oxidative damage.

Parmotrema perlatum, a widely distributed lichen species, is traditionally used as a spice and in folk medicine. Previous studies have reported its antioxidant, antimicrobial, and anticancer properties, which are attributed to the presence of phenolic compounds and flavonoids. However, scientific evaluation of its antidiabetic potential, particularly in vitro enzyme inhibition studies, remains limited (Saha et al., 2021). Furthermore, standardization of herbal extracts through identification and quantification of marker compounds is essential to ensure quality, efficacy, and reproducibility.

High Performance Thin Layer Chromatography (HPTLC) is a reliable, rapid, and cost-effective analytical technique widely used for the qualitative and quantitative estimation of phytoconstituents in herbal formulations. It allows accurate identification and quantification of bioactive markers such as quercetin, thereby supporting the standardization of plant extracts (Mutha et al., 2025).

Therefore, the present study was undertaken to evaluate the in vitro antidiabetic activity of the

ethanolic extract of *Parmotrema perlatum* using enzyme inhibition assays and to perform quantitative estimation of quercetin by HPTLC for standardization of the extract. This study aims to explore the therapeutic potential of *Parmotrema perlatum* as a natural source of antidiabetic agents.

Material

The present investigation utilized dried and powdered thalli of *Parmotrema perlatum* for preparation of the ethanolic extract. Analytical grade ethanol was used as the extraction solvent. Acarbose was employed as the standard antidiabetic drug for enzyme inhibition studies. Reagents required for α-amylase and α-glucosidase inhibition assays were used for evaluating in vitro antidiabetic activity. For HPTLC analysis, standard quercetin, silica gel 60 F_{254} precoated TLC plates, and suitable mobile phase solvents were used. All chemicals and reagents were of analytical grade, and absorbance measurements were carried out using a UV–Visible spectrophotometer.

Methods

In vitro* anti-diabetic activity of ethanolic extract of *Parmotrema perlatum

Alpha amylase inhibition assay

The α-amylase inhibition assay was performed using the DNSA method as reported by Wickramaratne et al. (2016) and Miller (1959). The test samples were first dissolved in 2 mL of 10% DMSO and then diluted with phosphate buffer (Na_2HPO_4/NaH_2PO_4, 0.02 M; NaCl, 0.006 M; pH 6.9) to obtain concentrations ranging from 10–50 µg/mL. Acarbose was used as the standard drug. For the assay, 200 µL of α-amylase solution (2 U/mL) was mixed with 200 µL of the sample and incubated at 30 °C for 10 minutes. After incubation, 200 µL of 1% starch solution was added and the mixture was incubated for an additional 3 minutes. The reaction was stopped by adding 200 µL of DNSA reagent. The reaction mixture was then heated in a water bath at 85–90 °C for 10 minutes, followed by cooling to room temperature. After cooling, 5 mL of distilled water was added, and the absorbance was measured at 540 nm using a UV–Visible spectrophotometer. A control representing 100% enzyme activity was prepared by replacing the sample with buffer, and a separate blank was prepared for each sample concentration without the enzyme. The α-amylase inhibitory activity was calculated as percentage inhibition using the standard formula.

% inhibition = [(Control absorbance– Test absorbance)/Control absorbance] *100

The % α-amylase inhibition was plotted against the sample concentration and the IC_{50} values were obtained from the graph.

Alpha glucosidase inhibition assay

The α-glucosidase inhibitory activity was evaluated according to the method described by Apostolidis et al. (2007) with slight modifications. Stock solutions of the extracts were prepared by dissolving 10 mg of each extract in 10 mL of DMSO.

For the assay, 50 µL of extract solution (concentration range: 12.5–200µg/mL) was mixed with 100 µL of yeast α-glucosidase solution prepared in phosphate buffer (pH 6.9) and incubated at 25°C for 10 minutes. After incubation, 50 µL of p-nitrophenyl-α-D-glucopyranoside solution (5 mM in 0.1 M phosphate buffer, pH 6.9) was added as the substrate. The reaction mixture was further incubated at 25 °C for 5 minutes.

The reaction was terminated by adding 3 mL of sodium carbonate solution (100 mM). The absorbance of the released p-nitrophenol was measured at 405 nm using a UV–Visible spectrophotometer. Acarbose was used as the positive control. The inhibitory activities of α-glucosidase were expressed as percentage inhibition and calculated using the standard formula.

% Inhibition = [(Absorbance control-Absorbance extract)/Absorbance control] × 100

The IC_{50} values defined as the concentration of the extract that inhibited 50% of the enzyme activity were determined from plots of % inhibition versus log inhibitor concentration and calculated by logarithmic regression analysis from the mean inhibitory values.

HPTLC analysis

HPTLC analysis carried out for the quantitative estimation for quercetin present in flower extract of *Parmotrema perlatum* (Baghel et al., 2017).

Estimation of Quercetin using HPTLC method

A CAMAG HPTLC system (Switzerland) comprising CAMAG Linomat 5 applicator, CAMAG TLC scanner 3, CAMAG Wincats software, version 1.44, Hamilton syringe (100µl), CAMAG Reprostar 3, CAMAG TLC plate heater, CAMAG UV Cabinet were used for the study.

Preparation of the Standard

From this 1mg of Quercetin diluted with 1ml of methanol and hence the concentration of the standard was 1000µg/ml.

Preparation of the extract Sample

1mg/ml of the flower extract of *Parmotrema perlatum* was prepared with methanol seperately.

HPTLC Analysis

Table 1: Optimized HPTLC Method Parameters

Parameter	Details
Chamber type	Twin trough glass chamber (20 × 10 cm)
Mobile phase	Toluene : Ethyl acetate : Formic acid (5:4:1, v/v/v)
Solvent front position	70.0 mm
Mobile phase volume	10.0 mL
Drying device	Hot air oven
Drying temperature	60 °C
Drying time	5 minutes
Standard application volume	2, 4, 6, 8, and 10 µL
Sample application volume	8 µL per spot
Application position	8.0 mm
Instrument	CAMAG TLC Scanner 3 (S/N 020105; version 1.14.30)
Number of tracks	5
Position of first track	15.0 mm
Distance between tracks	11.6 mm
Scan start position (X)	5.0 mm
Scan end position (X)	75.0 mm
Slit dimensions	5.0 × 0.30 mm (Micro)
Optical system	Light
Scanning speed	20 mm/s
Data resolution	100 µm/step
Detection wavelength	254 nm
Lamp	Deuterium (D_2) & Tungsten (W)
Measurement type	Emission
Measurement mode	Absorption
Optical filter	Second order
Detector mode	Automatic
PM high voltage	429 V

Preparation of the plates

The plates used for HPTLC was silica gel 60 F 254 (E.MERCK KGaA). 100 µg/ml of the Standard was applied in the form of bands using LINOMAT IV applicator. The volumes applied were 2, 4, 6, 8 and 10µl.

The mobile step used was Toluene: ethyl acetate: formic acid (5:4:1v/v/v). Built the chromatograph For 15 minutes, dried at room temperature and scanned at 425nm. The normal maximum peak area was measured. Average peak area of the standard was calculated. The calibration curve of the standard drug concentration (X-axis) over the average peak height / area (Y-axis) was prepared to get a regression equation by Win Cats software.

Estimation of Quercetin in herbal extracts

Estimation of Quercetin in flower extract of *Parmotrema perlatum,* the mean peak area of the sample was calculated and the content of Quercetin was quantified using the regression equation obtained from the standard curve.

Results and Discussion

The in vitro antidiabetic activity of the ethanolic extract of *Parmotrema perlatum* was evaluated using enzyme inhibition assays and compared with the standard drug acarbose. The results presented in Table 2 demonstrated a concentration-dependent increase in percentage inhibition for both acarbose and the extract. Acarbose exhibited higher inhibitory activity with an IC_{50} value of 14.76 µg/mL, whereas the ethanolic extract showed moderate inhibition with an IC_{50} value of 48.41 µg/mL. Similarly, the results shown in Table 3 also indicated dose-dependent inhibition, where acarbose exhibited stronger activity (IC_{50} = 15.25 µg/mL) compared to the ethanolic extract (IC_{50} = 60.74 µg/mL). Although the extract was less potent than the standard, it demonstrated significant enzyme inhibitory activity, suggesting the presence of bioactive phytoconstituents responsible for antidiabetic potential.

Thin Layer Chromatography (TLC) analysis under normal light, short UV, and long UV (Figures 1, 2, and 3) revealed distinct spots corresponding to flavonoid compounds. The chromatogram of standard quercetin (Figure 4) showed a well-defined peak, which was comparable to the peak observed in the HPTLC chromatogram of the extract (Figure 5), confirming the presence of quercetin in the ethanolic extract. The quantitative estimation results presented in Table 4 showed that the extract contained 0.1015%

w/w of quercetin, with a corresponding peak area of 428.7.

The presence of quercetin, a well-known flavonoid with reported α-amylase and α-glucosidase inhibitory activity, may contribute significantly to the observed antidiabetic effect of the extract. Flavonoids are known to reduce postprandial hyperglycemia by inhibiting carbohydrate-hydrolyzing enzymes and by exerting antioxidant effects that protect pancreatic β-cells from oxidative stress. Therefore, the moderate enzyme inhibitory activity observed in Tables 2 and 3, along with the confirmed presence and quantification of quercetin in Table 4 and Figures 4–5, supports the potential of *Parmotrema perlatum* as a natural source of antidiabetic agents. Further in vivo and mechanistic studies are required to substantiate its therapeutic efficacy.

Table 2: Results of % Inhibition of Acarbose and ethanolic extract of *Parmotrema perlatum*

S. No.	Concentration (μg/ml)	% Inhibition	
		Acarbose	Extract
1.	10	43.35	13.50
2.	20	56.14	22.34
3.	30	65.89	32.69
4.	40	76.85	43.35
5.	50	81.22	50.56
IC_{50} Value (μg/ml)		**14.76**	**48.41**

Table 3: Results of % Inhibition of Acarbose and ethanolic extract of *Parmotrema perlatum*

S. No.	Concentration (μg/ml)	% Inhibition	
		Acarbose	Ethanolic extract
1.	12.5	37.50	21.63
2.	25	56.18	37.78
3.	50	59.97	53.37
4.	100	83.71	67.98
5.	200	88.06	74.02
IC_{50} Value (μg/ml)		**15.25**	**60.74**

Figure 1: TLC in Normal light
Figure 2: TLC in in Short U.V

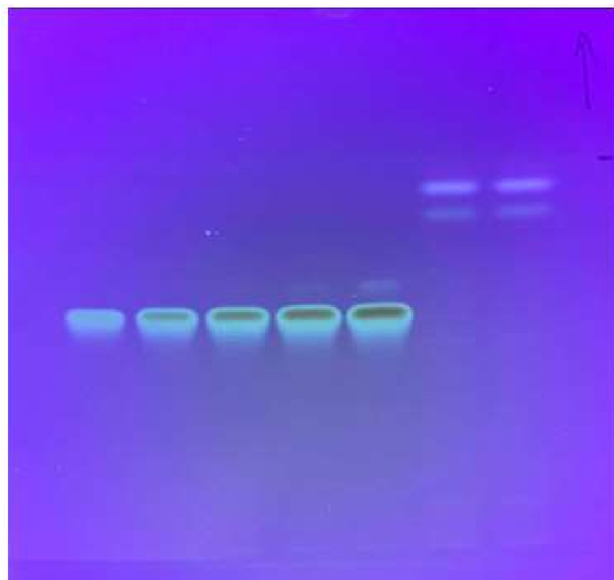

Figure 3: TLC in long U.V

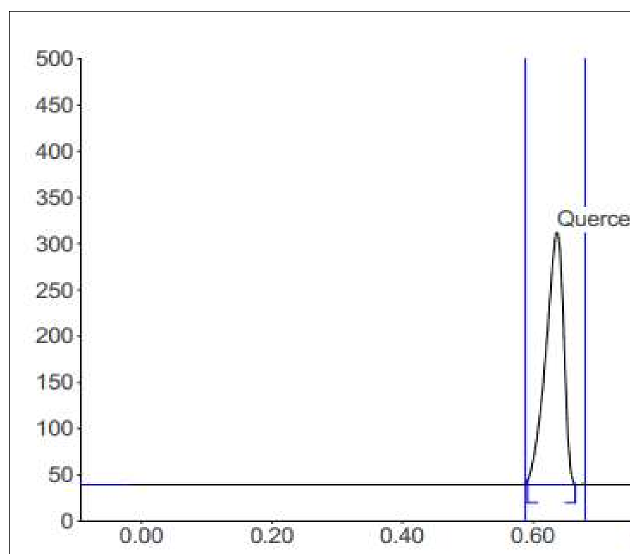

Figure 4: Chromatogram of standard Quercetin

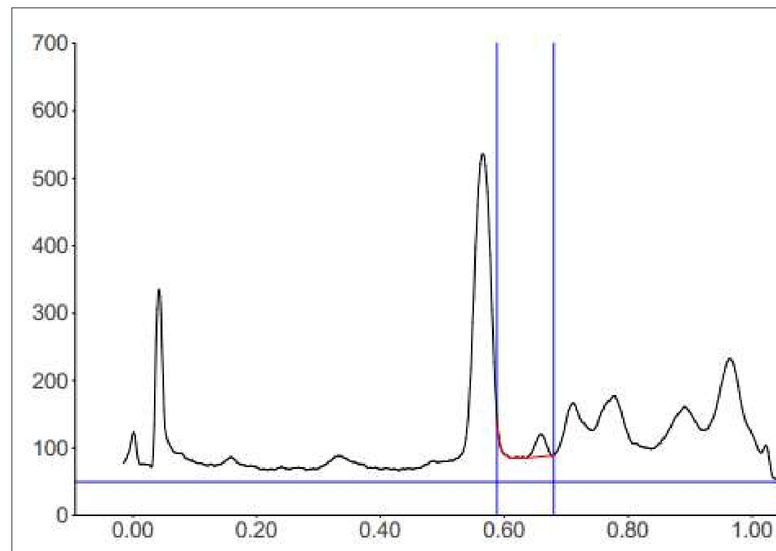

Figure 5: HPTLC chromatogram of flower extract of *Parmotrema perlatum*.

Table 4: Results of percentage content of Quercetin in extract

S. No.	Ethanolic extract	Area	% Found
1	Extract of *Parmotrema perlatum*.	428.7	0.1015

Conclusion

The present study demonstrates that the ethanolic extract of *Parmotrema perlatum* possesses significant in vitro antidiabetic activity, as evidenced by its concentration-dependent inhibition of carbohydrate-hydrolyzing enzymes. Although the extract showed lower potency compared to acarbose, it exhibited considerable inhibitory effects. HPTLC analysis confirmed the presence and quantification of quercetin (0.1015% w/w), which may contribute to the observed antidiabetic activity. These findings suggest that *Parmotrema perlatum* has promising potential as a natural source for antidiabetic agents and warrants further in vivo investigations.

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