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Original Article

In vitro Elucidation of Antidiabetic and Antioxidant Activities with Phytochemical Profiling of Root Extract of *Paeonia emodi* Wall. ex Royle Traditionally Used Plant

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ABSTRACT

Background/Objectives: The present study explores the antioxidant and antidiabetic potential of *Paeonia emodi* Wall. ex Royle, known as Chandra which is the member of family Paeoniaceae, traditionally utilized by local inhabitants of the Chamoli district, Uttarakhand, for managing diabetes. The selection of this plant was based on ethnobotanical evidence indicating that its part roots are employed in folk medicine to control postprandial hyperglycemia. **Materials and Methods:** The ethanolic extract of *P. emodi* was subjected to phytochemical screening, which confirmed the presence of major bioactive constituents including phenolics and flavonoids. The antioxidant potential was evaluated using DPPH and H_2O_2 radical scavenging assays. Enzyme inhibition studies demonstrated marked Alpha-Amylase and Alpha-Glucosidase suppressive effects. **Results:** Where the extract exhibited strong free radical inhibition with IC_{50} values of 8.52 ±2.16 µg/mL and 92.85 ± 3.88 µg/mL, respectively. Also, the TPC and TFC content was 412.73± 2.04 mg GAE/g and 153.17 ± 0.13 mg QE/g of extract has been estimated. Enzyme inhibition studies demonstrated marked Alpha-Amylase and Alpha-Glucosidase suppressive effects, recording IC_{50} values of 2.38 ± 1.16 µg/mL for porcine Alpha-Amylase, 2.56±1.23 µg/mL for yeast Alpha-Glucosidase, and IC_{50} value 13.21±1.08 µg/mL for intestinal rat Alpha-Glucosidase. The extract exhibited significantly higher inhibitory efficacy ($p < 0.05$) than the standard drug Acarbose, indicating its strong potential in controlling carbohydrate digestion and glucose absorption. **Conclusion:** These findings highlight *P. emodi* as a novel source of natural antioxidant and antidiabetic compounds, demonstrating potent multi-target activity. This study provides a scientific basis for its traditional use and suggests that *P. emodi* could serve as a promising candidate for developing plant-based therapeutics for diabetes management.

Keywords: Alpha-Amylase, Alpha-Glucosidase, Antioxidant activity, Diabetes mellitus, *Paeonia emodi*, Uttarakhand.

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INTRODUCTION

Diabetes Mellitus (DM) is a long-term metabolic condition marked by elevated blood glucose levels resulting from impaired insulin secretion, reduced insulin sensitivity, or a combination of both factors. It represents one of the most widespread chronic non-communicable disorders globally, with its prevalence rising sharply across both industrialized and developing nations. As reported by the International Diabetes Federation (IDF), an estimated 537 million adults were affected by diabetes in 2021, and projections suggest this figure may reach 783 million by 2045. The disorder imposes a major health burden due to its association with serious complications, including heart disease, kidney failure, nerve damage, vision impairment, and delayed wound recovery. Diabetes is generally divided into two main categories: Type 1, caused by autoimmune destruction of pancreatic β-cells, and Type 2, which involves insulin resistance coupled with gradual β-cell dysfunction. Type 2 diabetes accounts for nearly 90-95% of total cases worldwide (Nisar *et al.*, 2022). Current therapeutic strategies aim to regulate blood glucose levels, prevent complications, and improve quality of life. Postprandial hyperglycemia, the rapid spike in blood glucose levels following a meal, is a key contributor to the development and progression of Type 2 diabetes and its complications. Digestive enzymes play a central role in this process. α-Amylase, secreted by the pancreas and salivary glands, catalyzes the hydrolysis of starch into maltose and dextrins (Krishnaiah *et al.*, 2009). α-Glucosidase, located in the intestinal brush border, further breaks down disaccharides into monosaccharides, such as glucose, facilitating absorption into the bloodstream (Saeed *et al.*, 2012). Inhibiting these enzymes delays carbohydrate digestion and absorption, thereby reducing postprandial glucose surges (Chang *et al.*, 2002). This

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mechanism is the basis for several clinically used α-glucosidase inhibitors; however, plant-derived inhibitors are emerging as promising alternatives with fewer side effects. Medicinal plants have been used for centuries in traditional medicine systems such as Ayurveda, Unani, and Traditional Chinese Medicine to manage diabetes. These plants often contain bioactive compounds (such as flavonoids, alkaloids, terpenoids, tannins, and phenolic acids) that exert antidiabetic effects through multiple mechanisms, including enzyme inhibition, insulin mimetic activity, antioxidant properties, and modulation of glucose transport (Brand-Williams *et al*., 1995). Ethnopharmacological research provides an evidence-based approach to validate these traditional claims and identify novel therapeutic agents for diabetes management. Efficient glucose uptake by peripheral tissues, especially skeletal muscle and adipose tissue, is essential for maintaining normal glycemia (Bozin *et al*., 2008).

Paeonia emodi Wall. ex Royle locally known as Chandra is an important member of family Paeoniaceae, which is largely distributed in North-West India (from Kashmir to Garhwal-Kumaun regions of Uttarakhand). In India, it is distributed in North-West Himalaya from Kashmir to Garhwal-Kumaun regions of Uttarakhand with an altitudinal range of 1800 to 2800 m. However, its population is scanty in higher- altitude regions. Apart from the literature studies relevant to our work, an extensive survey was conducted in different parts of the Chamoli district to gather valuable information. *Paeonia emodi* is locally known as Chandra in the regional dialect. After interacting with local inhabitants, especially with old age people and shepherds, valuable information was gathered. It was revealed that to cure diabetes, they took a half cup of plant extract a day, which was prepared by boiling plant roots. As per our knowledge, no prior reports on this plant's antidiabetic and antioxidant activities are available. Thereafter, the current study was examined to find out the antidiabetic efficacy of this plant through *in vitro* study. Therefore, our investigation's main objective was to test plant extract's antioxidant and antidiabetic potential. The total phenols, flavonoid content, and preliminary phytochemical analysis were also carried out. Besides their enzyme-inhibitory potential, plant-based bioactives contribute to oxidative stress reduction by neutralizing free radicals. Oxidative stress plays a major role in pancreatic β-cell dysfunction and insulin resistance. Therefore, antioxidant-rich plant extracts not only inhibit digestive enzymes but also offer dual protection by mitigating oxidative damage. Previous studies have established a positive correlation between total phenolic and flavonoid content and antioxidant potential. The present investigation was thus designed to explore both the antidiabetic and antioxidant capacities of *Paeonia emodi* collected from Uttarakhand, emphasizing the interrelationship between phytochemical content and bioactivity.

MATERIALS AND METHODS

Method of Extraction

Paeonia emodi Wall. ex Royle was collected from the Chamoli district of Uttarakhand during its blossoming season in May 2025. The plant was tentatively identified using morphological keys and taxonomic descriptions available in standard floras and monographs. The authentication of the plant specimen was carried out by comparison with authenticated herbarium samples preserved at the Herbarium of Panjab University Chandigarh (PAN), and a voucher specimen was deposited under the accession number 22989 for future reference. The freshly collected plant material was thoroughly washed with running water to remove dust and other impurities. The root were separated and air-dried under shade at ambient room temperature (27±2°C) for approximately two weeks to prevent degradation of phytoconstituents. The dried material was then pulverized using a mechanical grinder to obtain a fine homogeneous powder. For extraction, 20 g of the powdered sample was soaked in 50 mL of 90% ethanol and subjected to shaking on an orbital shaker for 22-24 hr to ensure complete solubilization of active compounds. The resulting mixture was filtered using Whatman No. 1 filter paper, and the filtrate was transferred to a clean Petri dish and allowed to evaporate at room temperature for 5-8 days until a concentrated residue was obtained. The extract was kept partially covered during concentration to prevent contamination. The dried ethanolic extract was subsequently stored in an airtight container and preserved at 4°C in a refrigerator until further use for phytochemical and bioassay evaluations.

Chemicals and Reagents

All chemicals and reagents used in the present study were of analytical grade and procured from reliable suppliers to ensure experimental accuracy. 2,2-Diphenyl-1-picrylhydrazyl (DPPH), porcine pancreatic α-amylase, α-glucosidase (from Saccharomyces cerevisiae), and p-nitrophenol-α-D-glucopyranoside were obtained from Sigma-Aldrich (USA). Other reagents, including dimethyl sulphonate, sodium chloride, soluble starch, sodium potassium tartrate, 3,5-dinitrosalicylic acid (DNSA), sodium carbonate, hydrogen peroxide (H_2O_2), ascorbic acid, Folin-Ciocalteu (F-C) reagent, gallic acid, aluminium chloride, quercetin, Hydrochloric Acid (HCl), Mercuric chloride, Sulphuric acid (H_2SO_4), Chloroform, Ferric chloride, Sodium Hydroxide (NaOH), ethanol, methanol, and glacial acetic acid, were purchased from GK enterprises' (Chandigarh, India).

Analysis of Phytoconstituents

The qualitative phytochemical screening of PE was carried out using ethanolic, methanolic, and aqueous extracts to identify the presence of various bioactive constituents. The analysis aimed to detect major secondary metabolites such as terpenoids, phenolic compounds, flavonoids, tannins, saponins, alkaloids, and

glycosides by employing established standard protocols (Harborne, 1998). Each extract was subjected to specific reagent-based tests to confirm the occurrence of these phytochemicals, which are known to play crucial roles in antioxidant, antimicrobial, and antidiabetic activities. The comparative solvent extraction ensured a broader recovery of compounds with varying polarities, thereby providing a comprehensive profile of the plant's phytoconstituents. The presence of these metabolites suggests that PE may possess significant therapeutic potential, supporting its traditional use and justifying its further evaluation through antioxidant and enzyme inhibitory assays.

TPC (Total Phenolics Content)

The Total Phenolic Content (TPC) of the ethanolic extract of PE was estimated using the Folin- Ciocalteu reagent method with slight modifications of the standard protocol. In brief, 0.5 mL of extract at varying concentrations (50-250 µg/mL) was mixed with 2.5 mL of diluted Folin-Ciocalteu reagent and allowed to stand for 5 minutes for initial reaction. Subsequently, 2.5 mL of 7.5% Sodium Carbonate (Na_2CO_3) solution was added, and the mixture was incubated at 45°C for 1 hour to facilitate color development. The absorbance of the resulting blue coloration was measured at 765 nm using a UV-visible spectrophotometer against a reagent blank. A calibration curve was constructed using gallic acid as the standard, and the TPC was expressed as milligrams of gallic acid equivalents mg GAE/g of extract. This assay provides an estimate of the total phenolic compounds, which are major contributors to the antioxidant potential of the plant extract (Kokate, 2017).

TFC (Total Flavonoid Content)

The Total Flavonoid Content (TFC) of PEwas quantified using the aluminum chloride colorimetric method with minor modifications. Different concentrations of the extract (50-250 µg/mL) were prepared in triplicate, and the final volume was adjusted to 0.5 mL with deionized water. To each test tube, 0.1 mL of Aluminum chloride ($AlCl_3$) solution was added, followed by 0.1 mL of potassium acetate. The total reaction volume was made up to 3.0 mL by adding 2.3 mL of 80% ethanol. The reaction mixtures were thoroughly mixed and incubated at room temperature for 30 minutes to allow complex formation. The absorbance of each sample was then measured at 420 nm against a reagent blank using a UV-vis spectrophotometer. The TFC was calculated from a quercetin standard calibration curve and expressed as milligrams of quercetin equivalents per gram of extract (mg QE/g). This method allows for precise estimation of flavonoids, which play a critical role in antioxidative defense, free radical scavenging, and enzyme inhibition mechanisms associated with antidiabetic potential (Quan *et al*., 2019).

Antioxidant Activity

By DPPH Method

The DPPH free radical scavenging activity of PE was assessed using the standard spectrophotometric method with slight modifications. Varying concentrations of the ethanolic extract (50-250 µg/mL) were prepared in Dimethyl Sulfoxide (DMSO) in triplicate. Each concentration was mixed with 2.5 mL of 0.1 mM DPPH solution in methanol, followed by incubation for 40 minutes at 28°C in the dark to prevent photodegradation. After incubation, the decrease in absorbance was measured at 517 nm against a reagent blank using a UV-vis spectrophotometer. Ascorbic acid was used as a reference antioxidant for comparison (Oboh *et al*., 2015).

The percentage of free radical inhibition was calculated using the formula:

$$\text{Antiradical activity (\%)} = [(Ab\text{^} - Ab\text{^^}) / Ab\text{^} \times 100]$$

where Ab^and Ab^^ are the optical density of the control and the test sample.

Hydrogen Peroxide Assay

The hydrogen peroxide scavenging activity of the ethanolic extract of PE was assessed according to the standard spectrophotometric method with slight modifications. Different concentrations of the extract (50-250 µg/mL) were prepared in triplicate, and 0.6 mL of H_2O_2 solution (40 mM in phosphate buffer, pH 7.4) was added to each test tube, except the blank. The reaction mixtures were incubated at room temperature for 10 minutes, and absorbance was measured at 230 nm against the reagent blank using a UV-visible spectrophotometer. Ascorbic acid served as the reference antioxidant (Kazeem *et al*., 2015).

The scavenging percentage of hydrogen peroxide was calculated using the formula:

$$\text{Scavenging (\%)} = [(Ab\text{^} - Ab\text{^^}) / Ab\text{^} \times 100]$$

where, Ab^ and Ab^^ are the optical density of the control and the test.

In vitro Antidiabetic Study

The α-amylase Inhibition Assay

The α-amylase inhibition assay of PE was performed according to the standard DNSA (3,5- dinitrosalicylic acid) colorimetric method with slight modifications. Plant extract of varying concentrations (50-250 µg/mL) were prepared in Dimethyl Sulfoxide (DMSO) in triplicate. To each test tube, 250 µL of α-amylase enzyme solution (0.001 g/mL in 20 mM phosphate buffer containing 6.7 mM NaCl, pH 6.9) was added, and the mixture was incubated at 37°C for 30 min. After incubation, 500 µL of 1% soluble starch solution was introduced as the substrate, followed by a second incubation for 12 min at 37°C to initiate enzymatic hydrolysis. The reaction

was terminated by adding 1 mL of DNSA reagent (prepared from 1 g DNSA, 30 g sodium potassium tartrate, and 20 mL of 0.5 M NaOH), and the mixture was heated in a boiling water bath for 5 min to develop a reddish-brown color. Tubes were then rapidly cooled in ice-cold water, and 5 mL of double-distilled water was added to each. The absorbance was measured at 540 nm using a UV-vis spectrophotometer against a reagent blank prepared without the enzyme. Acarbose served as the standard inhibitor, while the control containing only buffer and DMSO represented 100% enzyme activity (Tundis *et al*., 2010).

The percentage inhibition of α-amylase was calculated using the equation:

$$\% \text{ inhibition} = [(Ab^{\wedge} - Ab^{\wedge\wedge}) / Ab^{\wedge} \times 100]$$

where, Ab^ and Ab^^ are the optical density of control and the test. This assay provides a quantitative measure of the extract's potential to hinder carbohydrate hydrolysis, there by indicating its possible hypoglycemic activity.

The α-glucosidase Inhibition Assay (*Saccharomyces Cerevisiae*)

The α-glucosidase inhibitory potential of the ethanolic extract of PE was evaluated using the p-nitrophenyl-α-D-glucopyranoside (pNPG) assay with slight modifications to the standard method. Various concentrations of the extract (10-50 µg/mL) were prepared in triplicate using Dimethyl Sulfoxide (DMSO) as solvent. Each reaction tube contained 50 µL of extract (1 mg/mL) and 100 µL of α-glucosidase enzyme solution (0.1 U/mL in 0.1 M phosphate buffer, pH 6.9). The mixture was pre-incubated at 37°C for 10 min to allow enzyme-extract interaction. Following pre-incubation, 50 µL of 5 mM pNPG substrate (prepared in 0.1 M phosphate buffer, pH 6.9) was added to initiate the reaction. After incubation for 30 min at 37°C, the reaction was terminated by adding 2 mL of 0.1 M sodium carbonate (Na_2CO_3). The absorbance of the liberated p-nitrophenol was measured at 405 nm against a reagent blank using a UV-visible spectrophotometer (Sales *et al*., 2012). Acarbose, a standard α-glucosidase inhibitor, was used as the positive control, while the reaction mixture containing DMSO instead of extract served as the control (100% enzyme activity).

$$\% \text{ inhibition} = [(Ab^{\wedge} - Ab^{\wedge\wedge}) / Ab^{\wedge} \times 100]$$

where, Ab^and Ab^^ are the optical density of the control and the test. The assay provides insight into the extract's potential to delay carbohydrate hydrolysis and glucose absorption, supporting its possible role in postprandial hyperglycemia management.

Suppression of Rat Intestinal α-glucosidase

The rat intestinal α-glucosidase inhibition assay was performed using an enzyme source freshly prepared from the small intestine of male Wistar rats, following ethical guidelines approved by the Ethical Committee Central Animal House Panjab University (Reg. No. 45/99/CPCSEA). After sacrificing the animals under anesthesia, the small intestine was carefully excised and immediately rinsed with 0.9% saline solution to remove intestinal contents. All experimental steps were carried out at 4°C to preserve enzyme activity. The intestinal tissue was homogenized in 5 mM phosphate buffer (pH 7.0) for 5 min using a homogenizer. The homogenate was then centrifuged at 4000 rpm for 10 min, and the resulting supernatant served as the enzyme source for the *in vitro* α-glucosidase inhibition assay. The inhibition protocol followed was identical to that of the yeast α-glucosidase assay, using p-nitrophenyl-α-D-glucopyranoside as the substrate. Although yeast α-glucosidase is frequently.

Employed in *in vitro* screening, rat intestinal α-glucosidase is considered a more physiologically relevant model, as it closely mimics the enzymatic system in mammals (Eichler *et al*., 1984). Therefore, both yeast and rat intestinal enzyme systems were evaluated in this study to provide a comparative understanding of inhibitory efficacy and to better simulate the *in vivo* mechanism of postprandial glucose regulation.

Statistical Study

All experimental results were expressed as mean ± Standard Error of the Mean (SEM), calculated from three independent replicates ($n = 3$) to ensure data reliability. The IC_{50} values for antioxidant and enzyme inhibition assays were determined using non-linear regression analysis plotted in Microsoft Excel. Statistical significance between groups was assessed using one-way Analysis of Variance (ANOVA), followed by the Tukey post hoc multiple comparison test to evaluate pairwise differences among the means. To examine possible associations between phytochemical content and antioxidant efficiency, the Pearson correlation coefficient (r) was applied to assess the relationship between Total Phenolic Content (TPC), Total Flavonoid Content (TFC), and radical scavenging activities. A p-value of ≤ 0.05 was considered statistically significant in all analyses, indicating a 95% confidence level. All assays were conducted in triplicates under identical experimental conditions, and data were presented as Mean ± SEM for consistency and reproducibility of results.

RESULTS AND DISCUSSION

Qualitative Investigation of Phytochemicals

The preliminary phytochemical screening of plant extract was performed on its ethanolic, methanolic, and aqueous extracts to identify the principal classes of bioactive secondary metabolites. These included alkaloids, terpenoids, phenols, flavonoids, tannins, saponins, and glycosides, using well-established qualitative methods (Mechchate *et al*., 2021). The occurrence and relative intensity of each phytochemical group were assessed based on the color intensity of reactions, categorized as weak (+), moderate (++), or strong (+++). The analysis revealed that the ethanolic extract possessed a strong presence of phenolic compounds

(+++), a moderate level of tannins (++), and a mild presence of terpenoids (+), whereas alkaloids were absent in all solvent systems (Table 1). The methanolic extract also exhibited the presence of flavonoids and saponins, while the aqueous extract showed only a weak reaction for glycosides. These results suggest that the ethanolic extract is particularly rich in polyphenolic and flavonoid compounds, which are likely responsible for its pronounced antioxidant and enzyme inhibitory activities observed in subsequent analyses (Poovitha & Parani, 2016).

DPPH Free Radical Scavenging Assay

The percentage inhibition increased from 58.02 ± 1.74% at 50 µg/mL to 64.75 ± 0.41% at 100 µg/mL and 86.69 ± 0.59% at 150 µg/mL. Higher concentrations resulted in stronger inhibition, with 89.12 ± 0.20% at 200 µg/mL and 93.67 ± 0.64% at 250 µg/mL. The IC_{50} value for the extract was calculated as 8.52 ± 2.16 µg/mL, while the standard ascorbic acid showed a comparable IC_{50} of 6.08 ± 0.72 µg/mL, indicating strong antioxidant potential of the extract relative to the standard.

Hydrogen Peroxide (H_2O_2) Radical Scavenging Assay

The hydrogen peroxide scavenging activity of plant ethanol extract was determined following the standard phosphate buffer method. The ethanolic extract demonstrated significant H_2O_2 scavenging activity, increasing with concentration. The inhibition percentage was 18.92 ± 1.21% at 50 µg/mL, 26.41 ± 0.39% at 100 µg/mL, and 73.30 ± 0.96% at 150 µg/mL. At higher concentrations, activity rose to 84.17 ± 2.69% at 200 µg/mL and peaked at 98.34 ± 0.42% at 250 µg/mL. The extract exhibited an IC_{50} of 92.85 ± 3.88 µg/mL, while ascorbic acid displayed 96.16 ± 10.58 µg/mL, demonstrating nearly equivalent scavenging efficacy between the extract and standard. The progressive increase in hydrogen peroxide scavenging activity with concentration indicates a strong dose-dependent antioxidant potential of the plant sample (Figure 1). This enhanced activity may be attributed to the high phenolic and flavonoid content, which act as effective hydrogen donors and free radical quenchers. The comparable IC_{50} values of the extract (92.85 ± 3.88 µg/mL) and the standard ascorbic acid (96.16 ± 10.58 µg/mL) suggest that PE possesses substantial peroxide-neutralizing capacity. Hydrogen peroxide, although not highly reactive itself, can generate hydroxyl radicals in the presence of transition metals, leading to oxidative damage of biomolecules. Therefore, the ability of the extract to scavenge H_2O_2 implies its potential to mitigate oxidative stress and cellular damage. Similar trends have been reported in other medicinal plants rich in polyphenolic compounds, where hydrogen atom transfer and metal-chelating mechanisms contribute to antioxidant activity (Kazeem *et al*., 2015; Ali *et al*., 2006; Shanghai *et al*., 2020).

Table 1: **Phytochemical constituents of *P. emodi*.**

Test	Ethanolic	Methanolic	Aqueous
Alkaloids	-	-	-
Terpenoids	+	+	+
Phenols	+++	+	+
Flavonoids	+	+	+
Tannins	++	+	+
Saponins	+	+	+ +
Glycosides	+	+	+

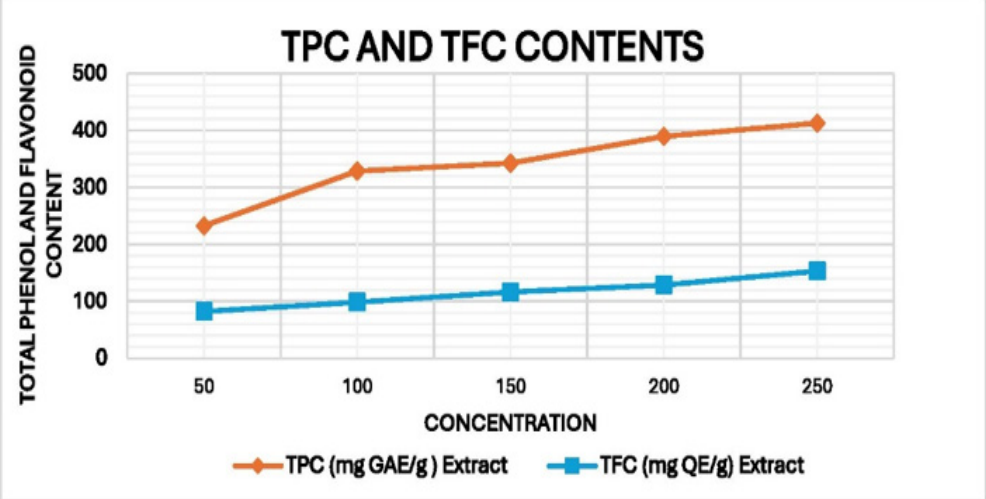

Figure 1: Graph of Total Phenol Content (TPC) and Total Flavonoid Content (TFC) of *P. emodi*.

Correlations

A strong positive correlation was observed between the Total Phenolic Content (TPC), Total Flavonoid Content (TFC), and the antioxidant activities measured by DPPH and H_2O_2 radical scavenging assays (Tables 2 and 3). As the concentration of the plant ethanol extract increased, both TPC and TFC values rose consistently, indicating an accumulation of bioactive compounds capable of donating hydrogen atoms and electrons to neutralize free radicals (Figure 2). Correspondingly, the percentage inhibition in both DPPH and H_2O_2 assays also increased with concentration, reflecting a direct relationship between phytochemical richness and antioxidant capacity (Nirja & Sharma, 2016). TPC 412.73 mg GAE/g of extract and TFC 153.17 mg QE/g of extract at 250 µg/mL, which aligned with maximum radical scavenging efficiencies in the DPPH (93.67%) and H_2O_2 (98.34%) assays. This correlation suggests that phenolic and flavonoid compounds are the major contributors to the observed antioxidant activity. Similar findings have been reported which is confirming that plants with higher polyphenolic content generally display stronger free radical scavenging and oxidative stress-reducing effects. Hence, (Ohta *et al*., 2002). The data collectively demonstrate that the antioxidant potential of PE is primarily dependent on its phenolic and flavonoid composition, establishing a clear phytochemical-bioactivity relationship.

In vitro Alpha-Amylase Inhibitory Assay

The α-amylase inhibition profile of plant ethanol extract is done. The ethanolic extract exhibited a concentration-dependent inhibition of α-amylase activity, with % inhibition ranging from 46.32 ± 0.24 at 50 µg/mL to 94.22 ± 0.18 at 250 µg/mL (Figure 3 and Table 4). The extract demonstrated a significantly lower IC_{50} value of 2.38 ± 1.16 µg/mL compared to the standard Acarbose (128.76 ± 1.52 µg/mL), suggesting approximately a 46-fold higher inhibitory potency. This indicates that PE contains potent α-amylase inhibitory constituents that could potentially delay carbohydrate digestion and glucose absorption. The inhibition of α-amylase is a well-established therapeutic

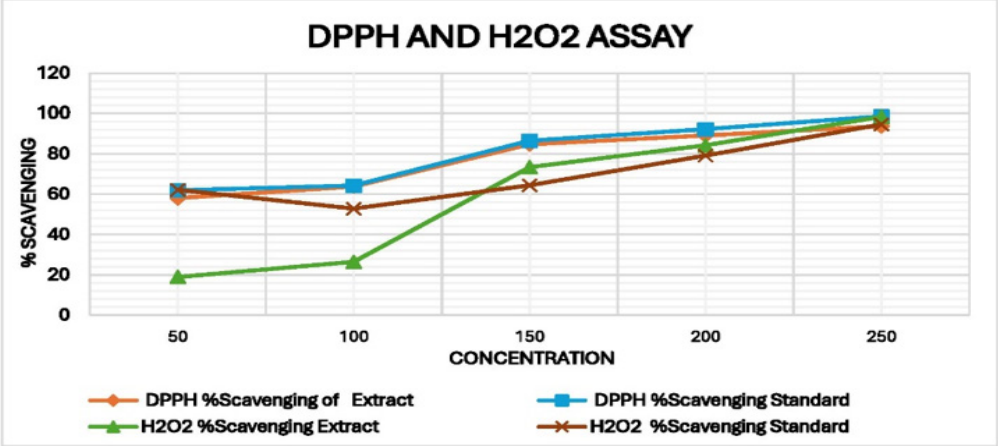

Figure 2: Graph of DPPH and H_2O_2 Assay of (*P. emodi*) and standard (Ascorbic Acid).

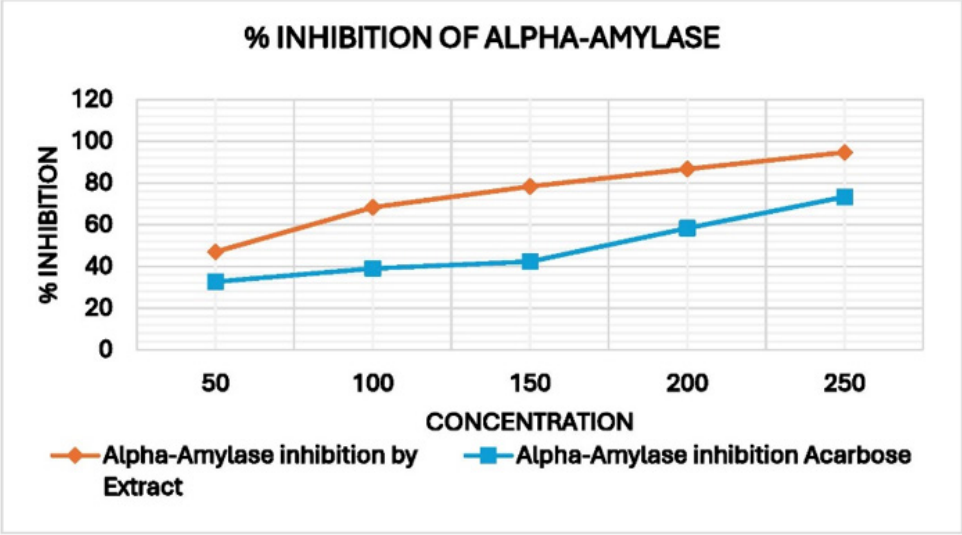

Figure 3: Graph of (%) inhibition of alpha-amylase by ethanolic extract of *P. emodi* and standard drug (Acarbose).

Table 2: Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) of Extract *P. emodi*.

TPC		TFC	
Conc (µg/mL)	TPC (Gallic acid (mg/g) of the extract)	Conc (µg/mL)	TFC (Quercetin (mg/g) of the extract)
50	232.55 ± 0.42[a]	50	82.14 ± 0.26[a]
100	328.73 ± 0.61[b]	100	98.53 ± 0.18[b]
150	342.36 ± 0.5[c]	150	116.29 ± 0.34[c]
200	389.24 ± 1.06[d]	200	128.31 ± 1.05[d]
250	412.73+ 2.04[e]	250	153.17 ± 0.13[e]

Different letters above the results in each column (a-e) indicate the significant changes at $p<0.05$; the value is shown as the Mean ± SEM of triplicates.

Table 3: Observation Table of DPPH and H_2O_2 Assay of *P. emodi*.

DPPH ASSAY			H_2O_2 ASSAY		
Conc. (µg/ml)	%Scavenging Extract	%Scavenging Standard	Conc. (µg/ml)	%Scavenging Extract	%Scavenging Standard
50	58.02±1.74[a]	61.88±0.13[a]	50	18.92±1.21[a]	62.14±0.67[a]
100	64.75±0.41[b]	64.27±0.14[b]	100	26.41±0.39[b]	52.82±0.86[b]
150	86.69±0.59[c]	86.46±1.06[c]	150	73.30±0.96[c]	64.41±0.91[c]
200	89.12±0.20[c,d]	92.23±0.42[d]	200	84.17±2.69[d]	79.16±0.63[d]
250	93.67±0.64[e]	98.59±0.04[e]	250	98.34±0.42[e]	94.59±0.35[e]
IC_{50} (µg/mL)	8.52 ± 2.16	6.08 ± 0.72	IC_{50} (µg/mL)	92.85 ± 3.88	96.16 ± 12.58

Different letters above the results in each column (a-e) indicate the significant changes at $p<0.05$; the value is shown as the Mean ± SEM of triplicates.

Table 4: Results of % inhibition of Alpha-Amylase, Alpha-Glucosidase (Yeast) and Alpha-Glucosidase (Rat) by ethanolic extract of *P. emodi* and standard drug Acarbose.

% inhibition of Alpha-Amylase			% inhibition of Alpha-Glucosidase (Yeast)			% inhibition of Alpha-Glucosidase (Rat)		
Conc (µg/mL)	Alpha-Amylase inhibition byExtract	Alpha-Amylase inhibition by Acarbose	Conc (µg/mL)	% Inhibition of Yeast alphaglucosidase byExtract	% Inhibition by Acarbose	Conc (µg/mL)	% Inhibition of Intestinal rat alpha glucosidase by Extract	% Inhibition by Acarbose
50	46.32 ± 0.24[a]	32.72 ± 0.19[a]	10	68.12±1.15[a]	36.43±1.23[a]	10	38.43±1.21[a]	29.65±1.26[a]
100	67.53 ± 0.31[b]	39.84 ± 1.28[b]	20	72.23±1.23[b]	42.36±1.36[b]	20	56.24±0.13[b]	48.54±2.13[b]
150	78.28 ± 0.17[c]	42.36 ± 0.52[c]	30	84.15±1.42[c]	68.03±2.41[c]	30	78.36±0.42[c]	54.13±2.64[c]
200	86.64 ± 0.40[d]	58.27 ± 0.36[d]	40	89.36±1.74[d]	74.52±2.78[d]	40	86.03±1.18[d]	78.26±1.36[d]
250	94.22 ± 0.18[e]	73.14 ± 0.73[e]	50	98.63±0.32[e]	81.32±2.29[e]	50	96.64±0.31[e]	83.34±1.09[e]
IC_{50} (µg/mL)	2.38 ± 1.16	128.76 ± 1.52	IC_{50} (µg/mL)	2.56±1.23	12.41±1.36	IC_{50} (µg/mL)	13.21±1.08	15.43±1.23

Different letters above the results in each column (a-e) denote the significant changes ($p<0.05$), IC_{50} value calculated as mean ± SEM of triplicates.

approach for controlling postprandial hyperglycemia in type 2 diabetes. α-Amylase inhibitors slow down starch hydrolysis, thereby reducing glucose availability and preventing sharp rises in blood sugar levels after meals (Tundis *et al.*, 2010; Alam *et al.*, 2017). The strong enzyme inhibition by PE aligns with reports on other polyphenol-rich plant extracts that suppress digestive enzymes by binding to their active sites and altering catalytic function. The high phenolic and flavonoid content of the extract may contribute to this mechanism, as these compounds are known to interact with α-amylase through hydrogen bonding and hydrophobic interactions, leading to conformational changes in the enzyme structure (Huang *et al.*, 2005). In conclusion, the ethanolic extract of PE displayed significant α-amylase inhibitory activity, surpassing that of Acarbose. Its markedly lower IC_{50} value indicates a potent inhibitory effect at minimal concentration, reinforcing its therapeutic potential in managing hyperglycemia

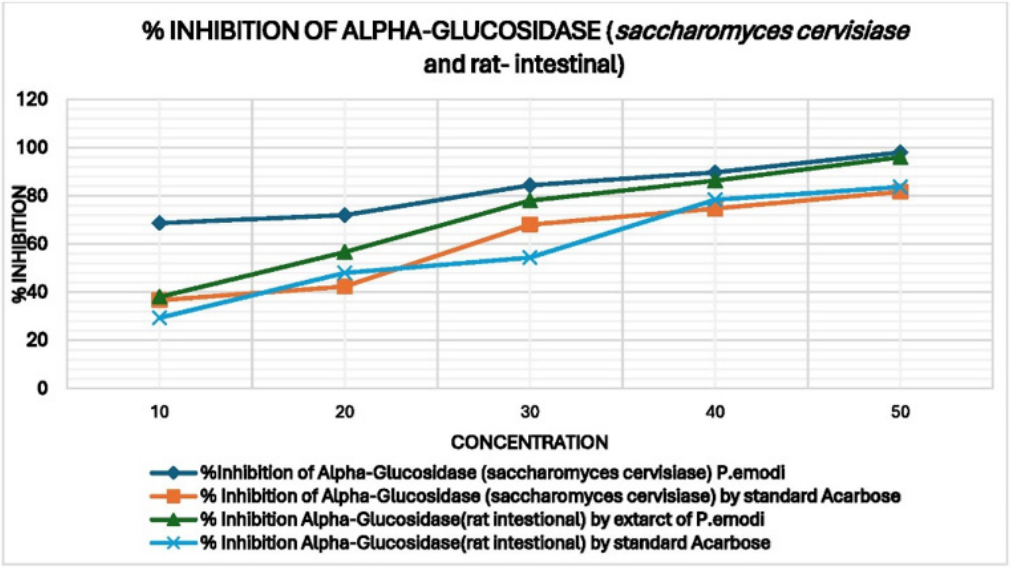

Figure 4: Graph of (%) inhibition of alpha-glucosidase (yeast) and alpha-glucosidase (rat) by ethanolic extract of *P. emodi* and standard drug (Acarbose).

and type 2 diabetes (Al-Owaisi *et al*., 2014). These findings are consistent with previous studies highlighting the antidiabetic potential of phytochemically rich plant extracts and support PE as a promising natural candidate for further pharmacological evaluation (Alam *et al*., 2017).

In vitro Alpha-Glucosidase Inhibitory Assay

The α-glucosidase enzyme, primarily located on the brush borders of the jejunal region of the small intestine, catalyzes the hydrolysis of disaccharides into absorbable monosaccharides — a critical step in carbohydrate digestion and glucose absorption. Inhibition of this enzyme plays a key therapeutic role in regulating postprandial hyperglycemia (Tundis *et al*., 2010). The ethanolic extract of PE demonstrated significant, concentration-dependent α-glucosidase inhibitory activity in both yeast and intestinal rat enzyme models, as presented in below (Figure 4). The yeast α-glucosidase assay revealed inhibition ranging from 68.12 ± 1.15% to 98.63 ± 0.32%, with an IC_{50} value of 2.56 ± 1.23 µg/mL, while the intestinal rat α-glucosidase inhibition ranged from 38.43 ± 1.21% to 96.64 ± 0.31%, showing an IC_{50} value of 13.21 ± 1.08 µg/mL. The standard drug Acarbose exhibited comparatively lower inhibition in both models, with IC_{50} values of 12.41 ± 1.36 µg/mL (yeast) and 15.43 ± 1.23 µg/mL (rat intestinal), confirming the superior inhibitory potency of plant ethanol extract. These results indicate that the extract effectively hinders enzymatic breakdown of carbohydrates, potentially delaying glucose absorption and reducing postprandial blood glucose spikes. Similar to the α-amylase inhibition assay, PE displayed a clear dose-dependent trend, highlighting its potential as a natural α-glucosidase inhibitor. Such dual enzyme inhibition supports its possible application in the formulation of plant-based antidiabetic therapeutics (Nirja & Sharma, 2016; Alam *et al*., 2017).

CONCLUSION

The present study concludes that the ethanolic extract of *Paeonia emodi* Wall. ex Royle, collected from the Chamoli district of Uttarakhand, exhibits remarkable *in vitro* antidiabetic and antioxidant potential. The extract demonstrated potent α-amylase inhibition and significant suppression of both yeast and rat intestinal α- glucosidase, indicating its strong ability to modulate carbohydrate metabolism and delay glucose absorption. Additionally, the extract showed high total phenolic and flavonoid content, which directly contributed to its DPPH and H_2O_2 radical scavenging activities, reflecting strong antioxidant efficiency. The dual inhibitory action against digestive enzymes, coupled with antioxidant potential. It scientifically validate its use in folk medicine suggests a multi-targeted mechanism in managing hyperglycemia. Such synergistic effects indicate that PE may help control postprandial blood glucose levels naturally and safely.

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ABBREVIATIONS

DM: Diabetes mellitus; **PE:** *Paeonia emodi*; **IDF:** International Diabetes Federation; **DPPH:** 2,2-Diphenyl-1-picrylhydrazyl; **TPC:** Total Phenolic Content; **TFC:** Total Flavonoid Content; **GAE:** Gallic acid equivalents; **QE:** Quercetin equivalents; **DMSO:** Dimethyl sulfoxide; **DNSA:** 3,5-dinitrosalicylic acid; **H_2O_2:** Hydrogen peroxide; **F-C:** Folin-Ciocalteu; **HCl:** Hydrochloric acid; **H_2SO_4:** Sulphuric acid; **NaOH:** Sodium hydroxide; **Na_2CO_3:** Sodium carbonate; **$AlCl_3$:** Aluminium chloride; **pNPG:**

p-nitrophenyl-α-D-glucopyranoside; **SEM:** Standard error of the mean; **ANOVA:** Analysis of variance.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

SUMMARY

The plant Extract represents a promising plant-based candidate for antidiabetic drug development. However, further *in vivo* studies and clinical evaluations are essential to confirm its efficacy, safety, and pharmacological relevance in therapeutic applications.

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