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PHYTOCHEMICAL PROFILING AND EVALUATION OF IN-VITRO ANTIDIABETIC EFFECTS OF PYRACANTHA CRENULATA (D. DON) M. ROEM. FRUIT EXTRACTS

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ABSTRACT

Background: *Pyracantha crenulata* (D. Don) M. Roem. is a species of flowering plant, commonly known as Nepalese or Himalayan firethorn, and belongs to the Rosaceae family. Various species of the genus Pyracantha have been reported to reveal antidiabetic activity. The literature suggests that the fruits are conventionally used to reduce blood sugar levels, warranting further research. Therefore, this research provides valuable insights into phytochemical profiling and antidiabetic potential of different extracts of *Pyracantha crenulata* (D. Don) M. Roem fruits with in vitro models via alpha (α)-amylase and alpha (α)-glucosidase enzyme inhibition assay. **Methodology:** The successive extraction of *Pyracantha crenulata* (D. Don) M. Roem fruit was carried out by using different solvents with increasing polarity, including n-hexane, ethyl acetate, ethanol, and distilled water (dH2O) via the Soxhlet extraction technique. The distinct unprocessed extracts were then subjected to a qualitative/quantitative phytochemical investigation of phytonutrients, followed by an assessment of their in vitro antidiabetic potential. **Results and Discussion:** The results concluded that phytocompounds, including flavonoids, phenols, steroids, and tannins, were tentatively identified in this plant. The extracts inhibit alpha (α)-glucosidase and alpha (α)-amylase enzymes in a dose-dependent way. Among all the extracts, the ethanolic extract exhibited potent anti-diabetic activity, with an IC50 of 44.79 µg/ml at 100 µg/ml in the α-amylase inhibitory assay and 30.09 µg/ml at 100 µg/ml in the α-glucosidase inhibitory assay, which is comparable to that of the standard acarbose. **Conclusion:** The study demonstrates that ethanolic fruit extract ameliorates hyperglycemia by releasing bioactive substances, providing a rationale for its traditional use as a natural hypoglycemic agent.

INTRODUCTION

As stated by the World Health Organization (WHO), diabetes is among the non-communicable diseases (NCDs) that concern people [1]. The Greek vernacular word "diabetes" means "to pass through," while the Latin word "mellitus" means "sweet" [2].

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Diabetes mellitus (DM) is a metabolic syndrome caused by insufficiency or ineffective production of insulin by beta (β) cells of the islets of Langerhans in the pancreas, which results in hyperglycemia or hypoglycemia, resulting in macro- and microvascular complications [3]. Concretely, insulin is a crucial anabolic hormone that influences the breakdown and utilization of dietary carbohydrates, triglycerides, and proteins [4]. In 1997, the American Diabetes Association (ADA) classified diabetes as type 1 DM, type 2 DM, gestational diabetes mellitus (GDM), and other types [5].

The common symptoms of DM are unexplained weight loss, polydipsia, frequent urination, increased appetite, sleepiness, non-healing wounds, tingling in hands and feet, and fuzzy vision [6]. Chronic hyperglycemia can lead to damage to vital organs by heightening the likelihood of small vessel (microvascular) complications, including diabetic eye disease (retinopathy), kidney damage (nephropathy), and nerve disorders (neuropathy), as well as atherosclerotic macrovascular complications, including stroke, cerebrovascular, and various peripheral vascular diseases (PVD) [7]. TIDM, formerly known as insulin-dependent diabetes or autoimmune diabetes, is a destructive condition that involves the destruction of β-cells by activated $CD4^+$ T cells, $CD8^+$ T lymphocytes, and macrophages that invade the pancreatic islets of Langerhans. T2DM is characterized by inadequate insulin production and secretion, which result from insulin resistance (IR) [8]. Diabetes is supposed to be affected by a variety of factors; these include a sedentary lifestyle, a poor diet, and consumption of tobacco and alcohol [9].

Medications are mainly administered to preserve life and diminish symptoms. Another goal is to avert long-term complications associated with diabetes and, by addressing different risk factors, to enhance lifespan. Insulin management is essential for individuals with T1DM, whereas dietary changes and lifestyle adjustments are fundamental for managing type 2 DM [3]. There is currently no treatment that can eliminate diabetes, and managing the condition involves much more than just taking medications [10]. The foremost conventional classes of hypoglycemic medicines for the management of DM comprise biguanides, sulfonylureas, α-glucosidase inhibitors, and agonists of the peroxisome proliferator-activated receptor-γ (PPARγ). Insulin, a polypeptide hormone that is released by the pancreatic β cells. Its function is to lower plasma glucose levels by either preventing the liver from generating glucose ($C_6H_{12}O_6$) through glycogenolysis and gluconeogenesis or by enhancing glucose absorption in hepatic, muscular, and adipose tissues [11].

Given that type 2 diabetes mellitus (T2DM) largely stems from lifestyle choices, non-drug interventions for prevention show significant potential. High caloric intake and reduced physical activity contribute to the pathogenesis of T2DM. Following a healthy lifestyle, which includes eating healthy foods and exercising, is a natural approach to preventing type 2 diabetes mellitus (T2DM). Since type 1 diabetes mellitus (T1DM) is an autoimmune disease that causes insulin deficiency, insulin injections are the primary treatment. When oral hypoglycemic medications fail to stabilize glucose levels and HbA1c in T2DM, insulin can be delivered either alone or in combination with oral hypoglycemic drugs [12]. Since both the root and management of T2DM are correlated with a healthy lifestyle and a balanced diet, the ancient medicinal practice of Ayurveda offers a promising conventional tool for managing T2DM [13]. One intervention strategy for controlling post-meal hyperglycemia in T2DM is to impede the absorption of ingested carbohydrates. Pancreatic alpha (α)-amylase is an important biological enzyme that has the function of reducing ingested carbohydrates (starch) into simple sugars in the gastrointestinal tract (GIT). These simpler sugars are then additionally degraded by alpha (α)-glucosidases to form glucose, which moves into the systemic circulation upon absorption. As a result, the inhibition of alpha (α)-amylase and alpha (α)-glucosidase enzymes can hinder carbohydrate digestion, slow down glucose absorption, and ultimately lower plasma glucose levels. While medications such as acarbose, voglibose, and miglitol effectively inhibit alpha (α)-glucosidase and alpha (α)-amylase [14]. Medicinal plants are crucial for the development of preventive and therapeutic remedies for humans, particularly for impoverished patients. Additionally, herbal remedies are gaining popularity in both developing and developed nations due to their safety, effectiveness, high quality, minimal adverse effects, and easy accessibility [15].

As a result, over 80% of individuals have resorted to traditional medicine to treat various ailments, according to the WHO. *Pyracantha crenulata* (D. Don), M. Roem., syn. *Crataegus crenulata* Roxb., *Cotoneaster crenulata*, and *Mespilus crenulata*, belonging to the family Rosaceae, are often called

Himalayan, Indian, Nepalese, Nepal Hawthorn, Ghingharu, and Chota Saeb [16]. In India, it is grown at an altitude of 1000–2600 m [17]. The various components of the plant are rich in numerous bioactive compounds such as vitexin, vitexin rhamnoside, leucocyanidin, leucoanthocyanidins, and various flavonoids, including flavonol, kaempferol, and quercetin, along with glycosides, beta-sitosterol, and oligomeric saponins, among others. According to the literature survey, it has several pharmacological activities. It is used in the treatment of ailments such as hypertension, antispasmodic, sedative, diuretic, arteriosclerosis, stones, myocardial weakness, cardiac failure, and paroxysmal tachycardia [18]. On the other hand, no previous studies have investigated the in vitro antidiabetic properties of this plant. Given the assertions and available evidence, it was considered valuable to explore *Pyracantha crenulata* in relation to diabetes.

MATERIALS AND METHODS

Chemicals

All the chemicals and reagents used in this study were of analytical grade and possessed the highest purity available.

Collection of Plant Materials

The *Pyracantha crenulata* M. Roem fruits were gathered from Kosiya Kutoli, District Nainital, India, in the month of August. *Pyracantha crenulata* (D. Don) M. Roem was recognized by its regional name and later confirmed by the Botanical Survey of India (BSI), Dehradun, and a voucher specimen (number 1592) was deposited at the BSI, Dehradun.

Crude extraction

The fruits from the plant of *Pyracantha crenulata* were washed, dried in the dark, and crushed into a coarse material. Successive extractions of *Pyracantha crenulata* fruits were carried out using solvents of varying polarity, such as n-hexane, ethyl acetate, ethanol, and distilled water (dH_2O), at their boiling points, using the Soxhlet extraction technique to obtain crude plant extracts. The different extracts were kept at room temperature until a consistent mass was attained. The extracts were maintained in a dry environment and stored in desiccators containing calcium chloride ($CaCl_2$). The different extract was preserved at 4°C and used in various experiments to assess its bioactivity. The percentage yield (%) was demonstrated using the equation below [19].

$$\textbf{Percentage yield} = \frac{\textbf{Weight of the extract after evaporing solvent and drying}}{\textbf{Dry weight of plant material}} \textbf{ X 100}$$

Phytochemical profiling of fruit extract of *Pyracantha crenulata* (D. Don) M. Roem

Detection of bioactive plant constituents

All crude extracts were subjected to phytochemical profiling of bioactive compounds such as alkaloids, flavonoids, tannins, saponins, and steroids using standard procedures [20, 21].

Quantitative Estimation of Phytoconstituents

Estimation of total Phenolic content (TPC)

The total phenolic content (TPC) of each fruit extract was demonstrated by the Folin-Phenol assay. 1 ml of each fruit extract was dissolved by using 9-10 ml of deionized water. Then, the mixture was treated with Folin-Phenol reagent (1 ml), agitated carefully, and allowed to stand for 5 minutes. The previously prepared mixture was then treated with 10 ml of 7% w/v sodium carbonate, and then diluted with water to the final volume (25 ml). Gallic acid (GA) was used as a reference standard. The GA solutions were diluted using the same procedure as for the test solution, without the extract. After 90 minutes of incubation at 25°C for the test and the standard, the test absorbance was measured against the blank at 550 nm using an ultraviolet-visible spectrophotometer. The TPC of each fruit extract was assessed using the calibration plot of standard GA and represented as mg of GA equivalent/gram of dry material [22].

Estimation of total flavonoid content (TFC)

Standard Solution Preparation: Quercetin (QCT) was utilized to create the standard calibration curve. A stock solution of QCT (1000 ppm) was prepared. Then, serial dilutions of methanol were prepared (100–1000 µg/ml).

Test Solution Preparation

A stock test solution (1000 ppm) was prepared.

Procedure: 1 ml of the aliquot was withdrawn from the test solution, and each dilution of the standard was delivered into the container. Add deionized water (4 ml), followed by the subsequent addition of 5% sodium nitrite (0.3 ml) and 10% $AlCl_3$ (0.3 ml) to each test tube. The sample was incubated for 5 minutes at 25°C, after which 1 M sodium hydroxide (2 mL) was added to the above mixture. It waited till a yellowish-orange color appeared. Then, finally, make up the volume to 10 ml with

distilled water. Absorbance was measured at 510nm using a colorimeter. The test sample's TFC was represented as mg quercetin equivalent (QE) / gram [23].

Estimation of Total Tannins Content (TTC)

The total tannin content (TTC) was determined using the Folin-Denis method. Each fruit extract (0.1 mL) was deposited in a 10 mL graduated flask with deionized water (7.5 mL) and 0.5 mL of Folin-Ciocalteu reagent (FCR). 35% sodium carbonate solution (1 ml) and dilute to 10 ml with deionized water. The combination was uniformly blended and allowed to stand at 25°C for 30 minutes. The standard solutions of GA (20-100 µg/ml) were ready as previously published. A UV/visible spectrophotometer was employed to assess the absorbance of both test and standard solutions at a wavelength of 725 nm against the blank. The TTC was calculated as mg of GAE/g of extract [24].

Estimation of steroidal content

The steroidal content was determined by spectrophotometry. Stock solutions of each fruit extract (5 mg/mL) were prepared. To each extract (1 ml), 2 ml of 2 M sulfuric acid (H_2SO_4), 0.5% ferric chloride ($FeCl_3$), and 0.5 ml (0.5% w/v) potassium ferricyanide ($C_6N_6FeK_3$) solution were added, followed by heating for 30 minutes in an electric immersion bath at 70°C with periodic shaking. The final volume of solution was made up to 10 ml using deionized water. A series of standard solutions of cycloartenol was made using the same procedure as the test solution (without extracts). The absorbance of both the test solution and the standard was recorded at 780 nm using a UV-visible spectrophotometer, with the blank serving as a reference. The steroidal content of each fruit extract was computed using the standard (cycloartenol) calibration curve and represented as cycloartenol equivalents (mg CA/g of dried material) [22].

In vitro inhibitory assay of key carbolytic enzymes associated with diabetes mellitus

Inhibition of alpha (α)-amylase Enzyme

The suppression of alpha (α)-amylase function of each fruit extract of the *Pyracantha crenulata* was carried out by suspending 2 mg of potato starch in 0.2 ml w/v of 0.5M. Tris-hydrochloride at pH 6.9, containing 0.01 M calcium chloride, serves as the assay substrate. The tube containing the substrate mixture was heated in an electric water bath for 5 minutes, followed by pre-incubation for 5 minutes at 37°C. The fruit extract of *Pyracantha crenulata* was dissolved in the solvent DMSO (dimethyl sulfoxide), and a series of concentrations (10-100 µg/mL) was obtained. After adding 0.2 ml of each fruit extract to each tube containing a substrate solution, 0.1 ml of α-amylase (0.5 mg/ml) enzyme made in tris-hydrochloride buffer was incorporated. After 10 minutes of incubation at 37°C, 50% w/v acetic acid (CH_3COOH) (0.5 ml) was added to each test tube to stop the reaction. After that, the mixture was then spun at 3000-5000 rpm at 40°C for 5 minutes. The supernatant was separated, and absorbance was measured at 595 nm using a UV-visible spectrophotometer. Acarbose served as the standard drug, and the absorbance of the solutions was measured against a blank prepared in the same manner as the test solution, without extract. The reading was collected in three replicates. The following formula was used to estimate the enzyme's inhibitory activity.

$$\boldsymbol{\alpha - amylase \quad inhibitory \quad activity} = \frac{\boldsymbol{Abs.(control) - Abs.(std/test)}}{\boldsymbol{Abs.(control)}} \times \mathbf{100}$$

After calculating the percentage α-amylase inhibitory potential of each extract for a singular concentration, the IC50 values (concentration of extract/ standard required to inhibit 50% of α-amylase enzyme) were also determined for each extract and the standard drug (acarbose) [25,26].

Inhibition of α-Glucosidase Enzyme

About 50 µl of each extract from different concentrations (10-100 µg/ml) was treated with 10 µl of 1 unit/ml of alpha-glucosidase (maltase) solution, prepared in 0.1 M w/v of phosphate buffer (pH 6.8), and incubated (37°C) for 20 min. After incubation, the reaction was initiated by adding 20 µl of the substrate solution, i.e., 1 M pNPG (p-nitrophenyl glucopyranoside), and incubated for 30 minutes at 37°C. To the reaction mixture, 50 µL of 0.1 N w/v sodium bicarbonate (Na_2CO_3) was added to terminate the reaction, and the absorbance was recorded at 405 nm using a UV-visible spectrophotometer. Acarbose was used as a reference drug, and the absorbance of the test solutions was measured against a blank prepared in the same manner as the test solution, without adding extracts. The readings were taken in triplicate, and the alpha-glucosidase inhibitory activity was computed by using the following formula:

$$\boldsymbol{\alpha - glucosidase \quad inhibitory \quad activity} = \frac{\mathbf{Xa - Xb}}{\mathbf{Xa}} \times \mathbf{100}$$

Where Xa and Xb are the absorbance of the control and standard. After calculating the inhibitory activity for alpha-glucosidase of each extract for different concentrations, the

IC50 values (conc of extract (test)/standard required to inhibit 50% of α-glucosidase enzyme) were also determined for each extract and the standard drug (acarbose) [27,28,29].

Statistical Analysis

All the data presented in the study were analyzed in triplicate using Microsoft Excel & Graph Pad Prism 8.0, and the values are computed as Mean ± SD. The IC_{50} values of the standard drug and test extracts were calculated from the mean inhibitory values using logarithmic regression analysis.

RESULTS AND DISCUSSION

Yield efficiency

Yield of the *Pyracantha crenulata* fruit extracts was prepared using alternative solvents, namely n-hexane, ethyl acetate, ethanol, and distilled water (dH_2O). Table 1 displayed the impact of several solvents on the extraction yield.

Table 1: Percentage yield of *Pyracantha crenulata* (D. Don) M. Roem Fruit extract

Extracts	Colour	Consistency	% (w/w) Yield
n- Hexane	Brownish yellow	Thick	4.47±0.262
Ethyl acetate (EtOAc)	Blackish Green	Thick	3.48±0.256
Ethanolic (EtOH)	Dark Brown	Sticky semi-solid	15.41±0.183
Aqueous (AQE)	Dark Brown	non sticky	11.45±0.171

Investigations indicated that extraction yields with various solvents varied significantly. Among the solvents examined, the maximum extraction yield was obtained with ethanol (15.41%), followed by aq. (11.45%), n-hexane (4.47%) & ethyl acetate (3.48%).

Figure 1: Different fruit extracts of *Pyracantha crenulata* (D. Don) M. Roem.

Preliminary phytochemical profiling

The current research was conducted on four different crude extracts obtained from *Pyracantha crenulata* (D. Don) M. Roem fruit extract. The results confirmed that *Pyracantha crenulata* fruit extract contains flavonoids, saponins, steroids, tannins, and phenolic compounds. Complete details of phytochemicals appearing in various extracts are displayed in Table 2.

Quantitative Phytochemical Estimation by Spectrophotometry

The outcome of the quantitative examination of the secondary metabolites of selected fruit extracts of *Pyracantha crenulata* (D. Don) M. Roem is represented in Table 3.

Total Phenolic content (TPC)

Phenolic chemicals are important and extensively distributed bioactive compounds in the kingdom Plantae. Plant phenolics are primarily classed as phenolic acids and flavonoids. Several literature surveys have shown a strong relationship between TPC and antioxidant properties. These chemicals contribute to plant development and reproduction, as well as protection against UV radiation and infections. Therefore, quantifying phenolic compounds is of paramount importance. In the current study, the total phenol content of *Pyracantha crenulata* crude extracts was determined using the FCR method. In light of the findings from this investigation, it is confirmed that all extracts of *Pyracantha crenulata* possess considerable levels of phenols. The TPC of different extracts was expressed as GA equivalents (mg/g dry extract), according to the calibration curve equation y = 0.0101x + 0.2193 (R2 = 0.9901). Among the other extracts, the high phenolic content was observed in ethanolic (77.24±0.539 mgGA/gm) fruit extract, followed by aqueous extract (65.16±0.565 mgGA/gm), n-hexane extract (50.59 ± 0.735 mg GA/gm), and ethyl acetate extract (42.19 ± 0.568 mg GA/gm).

Flavonoid content

The TFC for ethanol, ethyl acetate, aqueous, and n-hexane crude extracts was determined using the aluminum chloride spectrophotometric method with quercetin as the standard, which forms stable complexes with aluminum chloride through interactions with flavonoid functional groups. Flavonoids are a group of phytoconstituents, well known as plant pigments, with

similar chemical structures. The calibration equation for quercetin is y = 0.0004x + 0.1994, with an R^2 of 0.9983. Total flavonoid content in ethanol extract is (81.28±0.238) mg QE/g dry extract, followed by aqueous (69.05±0.467) mg QE/g dry extract, hexane extract (45.28±0.377) mg QE/g dry extract, and ethyl acetate extract (40.89±0.564) mg QE/g dry extract. Based on the current research, it is confirmed that all *Pyracantha crenulata* extracts contain considerable levels of flavonoids; among them, the ethanol extract exhibited the highest flavonoid content.

Total Tannin content

GA was used as the standard for comparison, and the TTC was expressed as mg GAE/g of extract. Absorbance was measured spectrophotometrically at 725 nm. With a concentration of 29.33±0.416 mg GA/g, n-hexane extracts show the lowest tannin content. Among all the extracts, the aqueous extract had the highest tannin content (52.81±0.399 mgGA/gm), followed by the ethanol extract (41.19±0.547 mgGA/gm) and the ethyl acetate extract (33.11±0.520 mgGA/gm).

Steroidal content

A Standard curve was found using cycloartenol; the total steroid equivalence content was found to be (27.51±0.604 mgCA/gm) in the ethyl acetate extract of *Pyracantha crenulata*, followed by the n-hexane extract (18.46±0.332 mgCA/gm) and the ethanolic extract (16.82±0.291 mgCA/gm). Significant differences in the concentration of secondary metabolites were found in the phytochemical study of *Pyracantha crenulata* extracts based on the extraction solvent. Out of all the extracts that were examined, the ethanolic fraction had the highest quantities of flavonoids and phenols, while the aqueous extract had the highest tannin concentration. The ethyl acetate extract, on the other hand, exhibited a higher steroidal concentration than the different fractions. These outcomes align with earlier research, which has shown that phenolic and flavonoid compounds — well-known for their potent antioxidant and enzyme-inhibiting properties —are most readily extracted using polar solvents, such as ethanol and water. The IC_{50} values found in the α-amylase and α-glucosidase inhibition tests provided additional evidence of the extract's antidiabetic potential. The ethanolic extract showed the most substantial inhibitory potential, with the lowest IC_{50} values and the highest levels of flavonoids and phenolic compounds. Studies on *Momordica charantia* and *Gymnema sylvestre* have shown similar relationships between high polyphenolic content and enzyme-inhibitory activity.

The phytochemicals identified and quantified provided insight into those present in *Pyracantha crenulata* fruits, which may be responsible for its potential pharmacological effects and its use as a traditional and herbal medicine globally. The results of the quantitative and qualitative analysis indicated that the plant's ethanolic crude extract is rich in active phytoconstituents.

Further, the in vitro antidiabetic potential of the ethanolic extract was assessed to determine its pharmacological role in the treatment of diabetes mellitus. The significant concentration of different phytochemicals, such as steroids, phenolics, tannins, and flavonoids, identified in *Pyracantha crenulata* may have potential inhibitory effects on the alpha (α)-amylase enzyme because of their ability to interact with proteins, which subsequently aids in reducing postprandial hyperglycemia.

Assessment of alpha (α)-Amylase and alpha (α)-Glucosidase Enzyme Inhibition

Pyracantha crenulata inhibits α-amylase and α-glucosidase enzymes due to the presence of secondary metabolites, including phenols, flavonoids, tannins, and steroids. Plant-derived secondary metabolites interact with digestive enzymes via hydrogen bonding, hydrophobic interactions, and π-π stacking, influencing their catalytic activity. Plant-derived phenolic acids like gallic acid, vanillic acid, and cinnamic acid have been shown to inhibit α-amylase significantly. Their unique chemical structures largely determine the inhibitory activity of phenolic acids. Phenolic acids have been shown to inhibit enzymatic activity through hydrophobic and hydrogen bonding interactions with α-amylase. These interactions with α-amylase may alter the protein structure of the enzyme. According to Proenca et al. (2017), the flavonoids with the strongest α-glucosidase inhibitory action have a hydroxy group at C3 and two catechol groups at the A or B ring. In the absence of a double bond between C2 and C3 and a ketonic group at C4, flavonoids' ability to inhibit α-glucosidase is greatly diminished.

Flavonoids work by building complexes with enzymes through non-covalent interactions, which block α-glucosidase. By forming stable complexes with enzymes and acting as nonspecific protein binders, tannins reduce the catalytic efficiency of those enzymes. They have significant binding to

α-amylase and α-glucosidase due to their polymeric structure and numerous hydroxyl groups. In contrast to phenolics and flavonoids, the precise SAR of steroids with digestive enzymes remains unclear; however, they may act indirectly by modifying membrane-associated enzyme activity. The outcomes of the in vitro inhibitory assay of the different extracts of *Pyracantha crenulata* are presented in Tables 4 and 5, respectively. The potential antidiabetic effects of all the extracts were evaluated through in vitro assays for alpha (α)-amylase and alpha (α)-glucosidase, using the reference enzyme inhibitor (acarbose) for comparison. The IC50 value represents the concentration of extract required to reduce α-amylase activity by 50%. Extracts with lower IC50 values demonstrate a stronger antidiabetic activity, which was used to assess the antidiabetic properties of *Pyracantha crenulata* extracts. The findings from this study revealed that *Pyracantha crenulata* extract had a notable impact on alpha-glucosidase but exhibited a less pronounced effect on alpha-amylase across all tested concentrations.

In contrast to other extracts, the ethanol extract showed the strongest alpha (α)-amylase inhibitory action (IC50 = 44.79 µg/ml) at the highest concentration (100 µg/ml) examined. Still, it was less than that of the conventional medication, acarbose (IC50 = 33.34 µg/ml). The extracts, including ethanol, demonstrated dose-dependent anti-α-amylase action. At 95.57µg/ml, the ethyl acetate extract exhibited the least amount of inhibitory action. The effects of n-hexane, ethyl acetate, ethanol, and distilled water extracts of *Pyracantha crenulata* on α-glucosidase activity are shown in Table 5. Compared to water and n-hexane extracts, ethanol extracts demonstrated superior anti-α-glucosidase action.

The IC50 values of the ethanolic extract and the aqueous extract were 30.09 µg/ml and 52.59 µg/ml, respectively. The ethyl acetate extract, however, showed reduced α-glucosidase inhibitory action. Compared to all extracts, acarbose's IC50 (24.86 µg/ml) was lower than that of the positive control. The present findings substantiate the herbalist's assertion regarding the plant's antidiabetic properties and show that it functions as a natural inhibitor of these enzymes. The research investigation concluded that among the four extracts, the ethanolic fruit extract exhibits potent inhibitory activity against alpha-amylase and alpha-glucosidase.

Table 2: Results of Qualitative Phytochemical Analysis on *Pyracantha crenulata* M. Roem. Fruit extract

Test performed	n-Hexane Extract	Ethyl acetate Extract	Ethanol Extract	Aqueous Extract
Test for tannins				
Ferric chloride test	+	+	+	+
Lead acetate	+	+	+	+
Test for alkaloids				
Hager's test	-	-	-	-
Mayer's test	-	-	-	-
Phenolic and flavonoids				
Ferric chloride test	+	+	+	+
Dil. Iodine sol.	+	+	+	+
Lead acetate	+	+	+	+
Shinoda test	+	+	+	+
Test for steroids				
Salkowski test	+	+	+	-
Test for Saponins				
Froth test	-	-	+	+

(+): present; (−): not detected

Table 3: Results of Quantitative Phytochemical Analysis

Extracts (*P. Crenulata*)	Tannin (mgGA/gm)	Phenols (mgGA/gm)	Flavonoids (mgQE/gm)	Steroids(mgCA/gm)
n- Hexane	29.33±0.416	50.59±0.735	45.28±0.377	18.46±0.332
Ethyl acetate (EtOAc)	33.11±0.520	42.19±0.568	40.89±0.564	27.51±0.604

Ethanolic (EtOH)	41.19±0.547	77.24±0.539	81.28±0.238	16.82±0.291
Aqueous (AQE)	52.81±0.399	65.16±0.565	69.05±0.467	-

(−): Absent

Table 4: In-vitro Alpha-amylase activity of different fruit extracts of *Pyracantha crenulata* (D. Don) M. Roem

Percentage (%) Inhibitory Concentration					
Conc. (µg/ml)	**Acarbose**	**n- Hexane**	**Ethyl acetate**	**Ethanolic**	**Aqueous**
10	35.08±0.946	20.40±0.638	13.46±0.575	30.12±0.580	26.55±0.739
20	48.07±0.615	25.80±0.355	20.66±1.27	43.27±1.108	35.26±0.521
40	53.43±0.640	32.51±0.784	27.68±1.66	49.53±0.859	41.17±0.536
60	62.17±0.597	43.46±0.823	34.20±1.93	56.49±0.351	50.48±0.771
80	71.40±0.639	46.53±0.845	42.80±0.817	65.42±0.638	58.29±0.712
100	83.86±0.519	54.99±0.435	52.68±1.40	73.22±0.561	65.04±0.390
IC_{50}	**33.34**	**85.41**	**95.57**	**44.79**	**61.04**

Each value is the mean of three analyses ± SEM.

Table 5: In-vitro Alpha-glucosidase activity of different fruit extracts of *Pyracantha crenulata* (D. Don) M. Roem

Percentage (%) Inhibitory Concentration					
Conc. (µg/ml)	**Acarbose**	**n- Hexane**	**Ethyl acetate**	**Ethanolic**	**Aqueous**
10	43.61±1.21	25.62±0.863	16.96±0.867	38.91±1.400	32.08±1.03
20	46.87±0.81	33.41±1.101	25.99±1.19	49.11±1.796	39.68±0.932
40	55.86±1.38	38.29±1.05	35.22±0.675	53.45±1.312	44.56±0.847
60	66.61±1.19	47.92±1.202	42.33±1.29	63.31±1.241	52.25±0.738
80	76.29±1.52	55.30±1.745	51.40±0.984	70.18±1.143	61.84±1.18
100	78.82±1.06	63.99±0.760	56.86±1.18	77.94±1.471	67.49±0.861
IC_{50}	**24.86**	**66.27**	**79.28**	**30.09**	**52.59**

Each value is the mean of three analyses ± SEM.

CONCLUSION

Pyracantha crenulata (D. Don) M. Roem., a well-known medicinal plant, has been shown to exhibit antimicrobial and antiurolithogenic properties and is also effective in treating renal disorders. However, this plant has not been explored for its anti-diabetic properties. Thus, in the present study, phytochemical screening and in vitro antidiabetic potential of *Pyracantha crenulata* (D. Don) M. Roem. were assessed. The results of the present investigation highlighted the hypoglycemic activity of *Pyracantha crenulata* (D. Don) M. Roem. In the current research, it was demonstrated that the majority of bioactive secondary metabolites were present in both ethanolic and aqueous extracts; however, the ethanolic extract contained a greater abundance of flavonoids and phenolic compounds. Alpha-amylase and alpha-glucosidase are two enzymes that may break down carbohydrates into glucose. Before being absorbed, the starch is hydrolyzed by alpha (α)- amylase, which converts it to glucose. Alpha (α)-amylase inhibition can lower postprandial glucose elevation. The small intestine contains the enzyme alpha (α)-glucosidase, which is responsible for breaking down disaccharides into glucose. Therapeutically active bioactive compounds from medicinal plants are suitable substitutes for DM treatment, as they reduce postprandial hyperglycemia. Numerous biological advantages are conferred by flavonoids and phenolic compounds, including hypolipidaemic, hypoglycaemic, antioxidant, anti-inflammatory, and organ-protective effects.

Flavonoids ameliorate DM by reducing oxidative stress, reducing insulin resistance, and regulating blood sugar. The results of this study showed that the ethanolic extract inhibited digestive enzymes, suggesting a link to its bioactive compounds. Thus, implying that *Pyracantha crenulata* (D. Don) M. Roem. can be a potential alternative for managing diabetes. In light of the rising global prevalence of diabetes and the adverse effects of long-term use of synthetic antidiabetic medicines, this study

provides a potential foundation for the development of plant-based, cost-effective, and safer diabetes management options. The incorporation of such natural therapies into mainstream healthcare could improve patient outcomes while reducing costs to public health systems. Further academic investigation is needed to clarify the isolation of specific phenolic compounds and to understand their contributions to the antidiabetic and antioxidant benefits. The safety and effectiveness of *Pyracantha crenulata* (D. Don) M. Roem for broader therapeutic uses, such as the treatment of diabetes and related disorders, may also be confirmed through in vivo models. The present study provides a preliminary understanding of the phytochemical content (qualitative and quantitative) and in vitro antidiabetic efficacy of plant extracts. However, further research is needed to validate these findings. Future research should focus on in vivo experiments with ethanolic extracts to establish therapeutic efficacy, as well as the isolation, purification, and structural elucidation of bioactive chemicals that may be responsible for their activity. Advanced analytical techniques such as liquid chromatography–mass spectrometry (LC-MS) and Nuclear Magnetic Resonance (NMR) can be used to accomplish this. These systematic approaches are likely to provide a deeper mechanistic understanding, which may aid in the development of novel plant-derived therapeutic agents.

FINANCIAL ASSISTANCE
NIL

CONFLICT OF INTEREST
The authors declare no conflict of interest.

AUTHOR CONTRIBUTION
All authors contributed equally to the conception and design of the study. Veerma Ram and Pranshu Tangri were primarily responsible for the critical review and intellectual input throughout the research. All authors collaborated on the development of the study concept and methodology. Himani Dumka carried out all experimental procedures, performed data analysis and interpretation, and drafted the manuscript. All authors have read and approved the final version of the manuscript for submission.

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