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Phytochemical Profiling and Antidiabetic Potential of an Unexplored Plant from the Lower Himalayas of Uttarakhand: *In vitro* Inhibition of α-Amylase and α-Glucosidase

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KEYWORDS

Diabetes mellitus, α-amylase inhibition, α-glucosidase inhibition, natural products

ABSTRACT:

Introduction: Diabetes mellitus (DM) poses a significant global health challenge, necessitating effective and safe treatments. Natural products, particularly plant extracts, are promising sources for anti-diabetic agents due to their diverse bioactive compounds.

Aim: This study aimed to characterise phyto-constituents and evaluate *in-virto* antidiabetic potential of *Asparagus curillus* Buch.-Ham. ex Roxb Root Extracts using α-Amylase and α-Glucosidase Inhibition method.

Materials and Methods: The extract of *A. curillus* Roots were prepared through successive extraction using Petroleum ether, Chloroform, and Ethanol by continuous soxhlation and water as solvent. The extracts were subjected to qualitative analysis of phytochemical. The *in- vitro* inhibitory activity against α-amylase and α-glucosidase enzymes were assessed using Spectrophotometer assay. Voglibose was used as standard drug.

Results and Discussion: Phytochemical analysis revealed distinct profiles across extracts. In inhibition assays, the ethanol extract demonstrated significant activity against α-amylase (IC_{50}: 16.41 mg/ml) and α-glucosidase (IC_{50}: 24.46 mg/ml), followed by the chloroform extract (α-amylase IC_{50}: 22.96 mg/ml; α-glucosidase IC_{50}: 58.09 mg/ml).

Conclusion: *A. curillus* root extracts exhibit promising anti-diabetic potential due to their rich phytochemical composition. These findings validate their traditional use and support further research aimed at isolating active compounds and elucidating their mechanisms of action for diabetes management.

Major Finding: The ethanol extract of *A. curillus* roots demonstrated notable *in-vitro* antidiabetic activity with significant inhibition of α-amylase and α-glucosidase enzymes. This supports its traditional use and highlights its potential for further development as an anti-diabetic agent.

1. Introduction

The consumption of junk food, sedentary lifestyle and excessive stress makes diabetes one of the most deadly metabolic disorders in modern life. At present, hundreds of prescription medications on the market control the sugar level in type 2 diabetic patients; no one drug cures diabetes totally specifically to restore β-cells to release sufficient insulin and normalize the body function for the glucose consumption. The available drugs, especially the synthetic ones, have several adverse effects include heartburn, nausea, stomach pain, bloating, constipation, diarrhea and obesity [1]. Recent investigations have revealed that numerous genes, hormones, enzymes, and proteins play a role in the mechanisms underlying diabetes, contributing to insulin resistance or the destruction of pancreatic beta cells [2]. Its high incidence, consisting increasing rate and its long-lasting associated effects have made diabetes mellitus (DM) a global concern [3]. Lack of an efficient therapy makes it more deadly as it gets worse with time. Though several treatments recognized for its management, medical science has advanced significantly, this condition remains incurable. In type 1 diabetes mellitus (T1DM) patients, insulin treatment is now the only method to control the glucose level.

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Patients with type 2 diabetes mellitus (T2DM) have access to a variety of oral medications for glycemic control that must be taken life-long to achieve normal blood glucose levels. Inhibiting the enzymes that hydrolyze carbohydrates- such as α-amylase and α-glucosidase- which postpone the absorption of glucose helps to reduce postprandial hyperglycemia in part. From that perspective, a lot of work has been invested into finding safer and more efficient α-glucosidase & α -amylase inhibitors from natural sources to produce physiologically functional foods that can be used to treat diabetes. However several conventional Indian plants have been considered for use with diabetes, only a few of these have been scientifically and medically examined to establish their efficacy [4]. This progressive condition has impacted almost the entire population, regardless of region, race, as well as sex. Type 2 diabetes mellitus represents the predominant form of diabetes, accounting for nearly 90% of all cases globally. The most recognized risk factors for T2DM include obesity, physical inactivity, and poor dietary habits, among others. In terms of the number of persons with diabetes, India was placed second among the top 10 countries in 2013. (20-79 years). In India, 65.1 million people (34.51 million men and 30.56 million women) were diagnosed with diabetes, compared to 98.4 million in China (1st place), with 1,065,053 deaths in India and 1,271,003 deaths in China. Diabetes affects 8.56 percent of the population in India [5].

The International Diabetes Federation (IDF) report from 2017 indicates that approximately 425 million individuals are living with diabetes, with 199 million of them being women. Diabetes affects 8.8% of the world's population. The IDF's South-East Asia region (Bangladesh, Nepal, India, Maldives, Mauritius, and Sri Lanka) accounts for one-fifth (approximately 84 million) of the world's diabetes patients. This area is known as the "Home of Diabetes." In the IDF areas, the North America and Caribbean (NAC) region has the highest prevalence of diabetes (13 percent). India is one of the IDF's South-East Asia Region's six countries. It is the world's second most popular IDF Region for diabetes. In the South-East Asia region, one out of every five adults has diabetes. The Diabetic Association of India, the Research Society for the Study of Diabetes, and the Diabetes Research Trust of India are all active organizations. In 2017, India's adult population (18-99 years) was 892,039,240 persons, with 74,047,266 diabetics. In 2017, India's diabetes prevalence increased by 8.3%, resulting in 1,123,804 diabetes-related deaths. In 2017, India's overall health expenditures for diabetes (20-79) were $8,713.0 million USD (119.4 USD per person) [2].

The Himalayas, a mountainous region in Asia encompassing China, India, Nepal, Pakistan, and Tibet, is acknowledged as a repository of medicinal plants, with over 8000 species documented, 1748 of which possess medicinal characteristics [6]. The indigenous populations of the Lower Himalayas utilize a diverse array of plants as medicines for their health needs [7]. A significant portion of the rural populations in India, China, Nepal, and Tibet utilize traditional medicine, including Ayurveda (India) and traditional Chinese medicine (China). Plants represent a unique source of traditional medicine; however, factors such as population growth, forest destruction, higher demand for traditional treatments, and unregulated collecting are leading to a significant depletion of herbal medicine sources [8]. As a result, there is a pressing need to find high-efficacy, low-side-effect replacements for currently used medicinal plants Approximately 800 medicinal plant species are recognized for their anti-diabetic properties, with various active compounds derived from these plants, such as alkaloids, terpenoids, flavonoids, steroids, and anthocyanidins, shown to be effective against hyperglycemia [9]. Usually, in the management of diabetes mellitus, herbal plants are considered safer than synthetic drugs. Many well-known plants for medicinal purposes that are included in our daily diet in the form of food or spice, such as cinnamon, coriander, ginger, black pepper, anise, cumin, turmeric, clove, fenugreek, mustard, garlic, and curry leaves, have been demonstrated to be useful in the treatment of diabetes [10]. The bioactive compounds present in these plants contribute to their anti-diabetic effects. It is important to recognize that various extraction procedures can yield distinct active principles. To date, alkaloids, terpenoids, steroids, xanthones, flavonoids, anthocyanidins, resins, and saponins have been investigated and identified as having activity against diabetes. Nonetheless, it is essential to consider various diabetic risk factors that

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need to be addressed prior to the use of these natural medications. Furthermore, a thorough understanding of the underlying mechanisms is essential for developing a more precise medication aimed at preventing or treating diabetes through an innovative pharmacological approach. Natural products should possess clear molecular targets, specific sites of action, and potential adverse responses [10].

Asparagus curillus Buch.-Ham. ex Roxb, is a much-branched, subscandent undershrub. The branchlets are grooved and minutely scaberulous. The plant features slender, recurved spines. Its cladodes are arranged in groups of 3-6, spreading or ascending, linear, flattened, and acute. The flowers are borne in short, few-flowered racemes, with minute bracts jointed below the middle. The berries turn red when ripe. The roots range from 7 to 18 inches in size, exhibit a light brown hue, are devoid of any odor, and possess a subtly sweet flavor [11, 12]. It grows between 600 and 1300 meters above sea level in open oak rhododendron woods in the subtropical Himalaya (Garhwal-Kumaon to Nepal) [11, 12, 13]. The roots are used as bitter tonic [13], appetizer, aphrodisiac, anti-inflammatory, stimulant, and galactagogue [11] and diuretic [14]. Its mature fruits are thought to induce abortion, and when mixed with honey, its tuberous roots are used to treat diabetes, diarrhoea, and dysuria [15]. In Uttarakhand, Himalaya, roots are used ethnomedicinally to treat gonorrhoea, piles, diabetes mellitus, coughing, dysentery, urinary disorders, as a revitalizing tonic and stomachache remedy [12,16]. The plant's roots are used in traditional medicine to treat diabetes; 10g of dried root powder combined with equal parts methi seed powder (Trigonella foenum-graecum) is administered once day for a month [12, 16]. Its chemical components include spirostanol glycosides, oligofurostanosides, oligospirostanosides, and steroidal saponins (asparagoside, curillosides, curillins, and sarsasapogenin) [17, 18]. Only anticancer and molluscicidal properties have been documented thus far, indicating that its pharmacological potential has not been as thoroughly investigated [12, 19-21]. According to ethnomedical data, *A. curillus* has been utilized for the treatment and prevention of diabetes. This study aims to assess the anti-diabetic efficacy of *A. curillus* root by clarifying its effects on α-glucosidase and α-amylase enzymes.

2. MATERIALS AND METHODS

Collection and Identification of Plant Material

The roots of *A. curillus* were collected from the Dhakrol village Nainbag area of Tehri District, Uttarakhand, India. The plant was verified by the Botanical Survey of India (BSI) in Dehradun, assigned accession number 753. A specimen of the plant has also been deposited at the BSI herbarium for future reference.

Preparation of Different Extracts

Freshly collected roots were carefully cleaned with plenty of tap water for removing contaminants and placed in the hot air oven at 50°C for 48 h. Successive extraction was conducted on the dried, coarsely powdered material to obtain extracts using solvents like Petroleum ether, Chloroform, and Ethanol through continuous soxhlation, along with an aqueous extract prepared via the decoction method [4]. Using vacuum rotatory evaporator (Equitron, India), the extracts were dried and kept at 4 °C for later use [22].

Qualitative Phytochemical Analysis of Extracts

In order to identify a variety of phytoconstituents, including glycosides, alkaloids, flavonoids, tannins and phenolic compounds, steroids, triterpenoids, saponins, carbohydrates, proteins, amino acids, fixed oils, and lipids, preliminary phytochemical screening was conducted on all four extracts [23, 24].

Thin Layer Chromatography (TLC) Analysis of Extracts

Preparation of TLC Plate- TLC plates were washed thoroughly and dried in an oven. The plates were prepared using the pouring method. Silica gel G was taken in a beaker and mixed with distilled water to form uniform slurry. The slurry was then poured and evenly spread over the glass plates by tipping them back and forth to ensure a uniform coating. The coated plates were first allowed to dry at room temperature, followed

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by activation in an oven at 110 °C for 30 minutes. Table 1 shows the specifications used for the preparation of TLC plates. Various solvent systems were employed for thin-layer chromatography to investigate different phytoconstituents present in the plant extracts [25].

Table 1- Specification for Preparation of TLC Plate

S. NO.	SPECIFICATION	
1.	Absorbent used	Silica gel G
2.	Vehicle used for preparation of slurry	Distilled water
3.	Method of preparation	Pour plate method
4.	Activation of plate	In hot air oven at 110 °C for 30 minutes
5.	Application of sample	About 10 to 15 µl of sample was applied with the help of glass capillary
6.	Mobile phase	Required quantity of solvents in TLC chamber, shaken well and utilized for chamber saturation
7.	Chamber saturation	30 minutes
8.	Parameters observed	Colour of the spot and R_f value

TLC Fingerprinting Analysis- The extracts were subjected to thin layer chromatographic studies to identify the number and nature of chemical constituents using different solvent systems. The R_f values of different phytoconstituents present in various extracts were determined [25, 26]. Specifications for chromatographic examination are shown in Table 2.

Table 2: Various Phyto-constituents, Detecting Agents and Solvent Systems Used in Chromatographic Examination

Phyto-Constituents	Detecting Reagent	Solvent System
Alkaloids	Dragendroff's reagent	Ethanol: Formic acid (8:2)
Tannins	Ferric chloride	Toluene : Chloroform : Acetone (40:25:35)
Flavonoids	Short UV 254 and Ferric chloride	Chloroform: Methanol (9:1)
Steroids	Long UV 364 and vanillin sulphuric acid	Chloroform : Methanol: Water (65:50:10)
Glycosides	Long UV 364 and sulphuric acid	Ethyl acetate : Formic acid : Glacial acetic acid : Water (100: 11: 11: 26)
Coumarins	UV	Butanol : Acetic acid : Water (4: 1: 5)

***In –Vitro* Anti-Diabetic Activity**

α-Glucosidase Inhibitory Activity

The inhibitory action against α-Glucosidase was evaluated using a 96-well microplate reader, specifically the Epoch Microplate Spectrophotometer from BioTek, USA. The stock solutions of yeast α-glucosidase enzyme (0.5 U/mL), test extract (200 mg/mL), and the positive control voglibose (20 mg/mL) were prepared using PBS (0.1 M/pH 6.8). In distinct microplate wells, 25 µL of the enzyme solution was combined with 25 µL of the test extract at concentrations of 1.562, 3.125, 6.25, 12.5, 25, 50, 100, and 200 mg/mL, along with the positive control at concentrations of 0.156, 0.312, 0.625, 1.25, 2.5, 5.0, 10.0, and 20.0 mg/mL. The microplate was subsequently incubated at 37° C for duration of 10 minutes. The test solutions were substituted with PBS (25 µL) to serve as a negative control. Subsequently, each well was treated with 25 µL of 0.5 mM p-NPG (p-nitrophenyl-α-D-glucopyranoside) solution, followed by incubation for an additional 30 minutes at 37° C. After the incubation period, 100 µL of a 0.2 M sodium carbonate solution was introduced to every well to halt the reaction. The value of the absorbance was measured at 405 nm using a spectrophotometer. The background readings for each test and standard sample were acquired using the substrate p-NPG along with test

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solutions that did not contain any enzyme. Three iterations of the experiment were conducted, and the mean data were taken into account when determining the inhibition. The % inhibition of α-glucosidase was determined using the following formula [22, 27-29].

$$\textbf{\textit{Inhibitory activity}}\ (\%) = \left[1 - \left(\frac{\textbf{\textit{At}} - \textbf{\textit{Ab}}}{\textbf{\textit{Ac}}}\right)\right] \textbf{\textit{X}}\ 100$$

At represents the absorbance of the test extract compared to the standard, ***Ab*** denotes the absorbance of the background, and ***Ac*** indicates the absorption of the control.

α- Amylase Inhibitory Activity

Stock solutions of the test extract and voglibose (standard drug) were prepared using PBS and serially diluted to achieve comparable quantities, adhering to the established procedures for the α-glucosidase inhibitory assay. In the wells, forty µL of 0.1 M Phosphate buffer Saline (PBS) and forty µL of starch solution (Two mg/mL, prepared with 0.4 M Sodium Hydroxide (NaOH) and the pH adjusted with two M HCl) were combined as a substrate, followed by the addition of five µL of each concentration of the test extract and the standard. The wells were subsequently incubated at 37° C for duration of one minute. Subsequently, each well was treated with forty µL of yeast alpha-amylase (two mg/mL, formulated in 0.1 M PBS), followed by a 15-minute incubation at 37° C. In the control wells, PBS (5 µL) was utilized instead of test samples. The reaction mixture was promptly halted following incubation by the addition of 100 µL of a 0.5 mM iodine solution (prepared with distilled water), followed by 20 µL of 0.1 M HCl to each well. A microplate spectrophotometer was utilized to measure the absorbance at 580 nm. The experiment was conducted in triplicate, and the inhibitory activity was assessed by calculating the mean value from each well. The inhibition percentage was calculated using the following formula [22, 27, 29, 30].

$$\textbf{\textit{Inhibitory activity}}\ (\%) = \left[1 - \left(\frac{\textbf{\textit{Ab}} - \textbf{\textit{Aa}}}{\textbf{\textit{Ad}} - \textbf{\textit{Ac}}}\right)\right] \textbf{\textit{X}}\ 100$$

Ab represents the absorbance of the test extract or standard combined with starch; ***Aa*** indicates the absorbance of the test or standard combined with starch and enzyme; ***Ad*** denotes the absorbance of starch alone; ***Ac*** refers to the absorbance of starch in the presence of enzyme.

3. Statistical Analysis

All the data presented in the study were analysed in triplicate using Microsoft Excel and value are computed as Mean±SD.IC_{50} value of standard drug and extracts were calculated from the mean inhibitory value by logarithmic regression analysis.

4. RESULTS

Qualitative Phytochemical Analysis

Major phyto components such flavonoids, phenolics, glycosides, and saponins were found in the petroleum ether (ACRPE), chloroform (ACRC), ethanol (ACRE), and aqueous (ACRW) extracts of *A. curillus*, according to phytochemical analysis. Steroids and lipids were the only substances found in ACRPE. Phenol was an alkaloid found in ACRC. Glycoside, saponin, and phenolic chemicals were found in ACRE, while protein, carbohydrates, and saponin were found in ACRW (Table 3).

Table 3: Phytochemical Analysis of *A. curillus* extracts

Phyto-constituents	**ACRPE**	**ACRC**	**ACRE**	**ACRW**
Alkaloid	-	+	-	-
Glycoside	-	-	+	-
Phenolic	-	+	+	-
Steroid	+	-	-	-
Protein	-	-	-	-
Carbohydrate	-	-	-	+
Saponin	-	-	+	+
Coumarin	-	-	-	-
Flavonoid	-	-	+	-
Fat and Fixed oil	+	-	-	-

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TLC Fingerprinting Analysis

The chromatographic evaluation of different extracts was performed in this study various classes of compounds were presented in the extract. They are identified on the basis of R_f value. The results are listed in Table 4.

Table 4: TLC Fingerprinting Analysis of Extracts**.**

S. No.	Extract	Solvent System	No. of spots	Rf values
1	**ACRPE**	Toluene : ethylacetate (4.5: .5)	4	0.2, 0.24, 0.4, 0.52
2	**ACRC**	Chloroform : ethylacetate (8:2)	4	0.14, 0.19, 0.28, 0.32
3	**ACRE**	Chloroform : methanol (8:2)	7	0.14, 0.25, 0.33, 0.44, 0.59, 0.70, 0.81
4	**ACRW**	Chloroform : Glacial acetic acid: Methanol : Water (64:32:12:8)	2	0.94,0.96

***In –Vitro* Anti-Diabetic Activity**

The inhibitory activity of α-amylase and α-glucosidase was evaluated for various extracts of *A. curillus* and compared with the standard drug Voglibose. For the α-glucosidase inhibitory activity, the petroleum ether extract (ACRPE) was not determined, while the chloroform extract (ACRC) showed an IC_{50} of 58.09 mg/ml. The ethanol extract (ACRE) had an IC_{50} of 24.46 mg/ml, and the water extract (ACRW) showed an IC_{50} of 32.27 mg/ml. In contrast, Voglibose exhibited a significantly lower IC_{50} of 2.1 mg/ml, indicating a much higher inhibitory potency (Table 5 and Figure 1)**.**

For the α-amylase inhibitory activity, the petroleum ether extract (ACRPE) had an IC_{50} of 64.84 mg/ml. The chloroform extract (ACRC) demonstrated an IC_{50} of 22.96 mg/ml, the ethanol extract (ACRE) showed an IC_{50} of 16.41 mg/ml, and the water extract (ACRW) had an IC_{50} of 23.13 mg/ml. Voglibose again showed superior inhibitory activity with an IC_{50} of 1.1 mg/ml. These results indicate that the ethanol extract (ACRE) showed the highest activity for both α-glucosidase and α-amylase inhibition among the extracts tested, although it was less effective compared to Voglibose (Table 5 and Figure 1)**.**

Table 5: α-glucosidase and α- amylase Inhibitory activity of extracts of *A. curillus*.

Groups	Treatment	α-Glucosidase (IC_{50}) mg/ml)	α-Amylase (IC_{50}) mg/ml
I	**ACRPE**	ND	64.84
II	**ACRC**	58.09	22.96
III	**ACRE**	24.46	16.41
IV	**ACRW**	32.27	23.13
V	**Voglibose**	2.1	1.1

ND- Not Determined; IC_{50}- Inhibitory Concentration

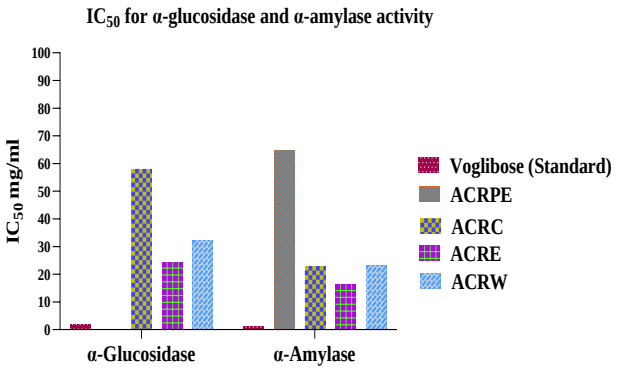

Figure 1- IC_{50} for α-glucosidase and α-amylase of Standard Drug and Tested Extract

5. DISCUSSION

Plants produce a wide variety of substances that help them grow, develop, and respond to their environment. These substances, called metabolites, come in many forms due to various chemical changes [31]. People have used plant metabolites for thousands of years, dating

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back to 2600 BC, mostly for medicine, poisons, and food. In 1806, scientists isolated morphine from the opium poppy, marking a significant milestone in understanding plant compounds. This discovery showed that the effects of plant extracts often come from a single, purifiable compound. Since then, research on natural products has expanded greatly. Today, over 30% of medicines are derived from natural sources, especially plants. Advances in technology over the past century have accelerated research, allowing scientists to identify and measure the beneficial compounds in plants more accurately. This research helps us understand the medicinal potential of different plants [32]. *A curillus* root extracts revealed various bioactive compounds. The petroleum ether extract contained steroids and fats, chloroform extract had alkaloids and phenols, ethanol extract was rich in glycosides, saponins, and phenolics, and the aqueous extract showed proteins, carbohydrates, and saponins. These diverse phytochemical profiles suggest significant pharmacological potential. TLC analysis revealed the presence of diverse phytoconstituents in different extracts of *A. curillus* roots, as indicated by varying numbers of spots and R_f values. The ethanolic extract (ACRE) showed the highest number of spots (7), suggesting a wide range of polar compounds. Petroleum ether (ACRPE) and chloroform extracts (ACRC) each showed 4 spots, indicating moderate phytochemical complexity. The aqueous extract (ACRW) displayed only 2 high R_f spots, reflecting the presence of highly polar compounds. These results highlight the influence of solvent polarity on the extraction of phytochemicals and support the need for further isolation and characterization studies.

Many therapeutic strategies have been developed in recent years to regulate high blood sugar; among them includes the inhibition of the enzymes α-glucosidase and α-amylase, which is thought to be very effective in lowering postprandial glucose levels in patients with Type 2 Diabetes Mellitus (T2DM) [9, 33, 34, 35]. Recent studies proved, natural products, especially plant derived products and phytoconstituents have a strong inhibitory effect on enzymes that hydrolyze carbohydrates [9]. The small intestine uses α-glucosidase to convert complex carbs into glucose. The American Association of Clinical Endocrinologists (AACE) and the International Diabetes Federation propose using inhibitors of this enzyme as first-line therapy, such as acarbose and miglitol. These inhibitors help T2DM patients' HbA1c levels in addition to helping with glycemic management. Similarly, α-amylase, responsible for hydrolyzing complex carbohydrates into glucose and maltose, can be targeted for diabetes treatment [34, 35]. The present study confirms the inhibitory activity of *A. curillus* root extracts, indicating their potential use as therapeutic agents for diabetes management.

6. CONCLUSION

Diabetes is one of the fastest-growing metabolic disorders worldwide, and while several synthetic drugs are available for its management, they often come with high costs and potential side effects. In contrast, natural therapies offer a safer alternative and have long been used across traditional medical systems. *A. curillus* root extracts demonstrated promising antidiabetic potential, likely due to their rich phytochemical content. TLC fingerprinting confirmed the presence of diverse compounds, particularly in the ethanolic extract. These bioactive constituents exhibited strong inhibitory activity against α-amylase and α-glucosidase, key enzymes involved in carbohydrate digestion and glucose regulation. The findings support the traditional use of *A. curillus* in managing diabetes and highlight the need for further studies to isolate, characterize, and understand the mechanisms of the active compounds. Such research may lead to the development of effective, plant-based antidiabetic therapies with fewer adverse effects.

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8. AUTHOR CONTRIBUTION

Mr. Anoop Singh Negi conducted research, prepared experimental studies, and drafted the initial manuscript, while Prof. (Dr.) Veerma Ram provided supervision, identified the research problems, and finalized the manuscript. All authors have given their approval for the final draft of the manuscript. All authors have reviewed and consented to the published version of the manuscript.

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