JOURNAL OF NATURAL REMEDIES
DOI: 10.18311/jnr/2025/45179

RESEARCH ARTICLE

Phytochemical Investigation and *In Vitro* Antidiabetic Potential of Leaf Extracts of Himalayan Herb: *Allium stracheyi* Baker

Lata Bisht[1,2], Neeraj Kumar[1*] and Veerma Ram[2]

[1]Department of Pharmacology, School of Pharmaceutical Sciences and Technology, Shree Guru Ram Rai University, Patel Nagar, Dehradun – 248001, Uttarakhand, India; neeraj_sidana@rediffmail.com

[2]Department of Pharmacology, School of Pharmaceutical Sciences and Technology, Sardar Bhagwan Singh University, Balawala, Dehradun – 248161, Uttarakhand, India

Abstract

Background: Various species of the genus *Allium* have been reported to exhibit antidiabetic potential. Among various species, *Allium stracheyi* Baker is one of the most popular and important ethnobotanical traditional perennial Himalayan seasoning herbs belonging to the family Alliaceae. Since no scientific studies have been carried out to assess the potential of species *Allium stracheyi* Baker in reducing blood sugar levels. Therefore, the present research work is aimed to screen different leaf extracts for the presence of bioactive phytochemicals and evaluate the *in vitro* antidiabetic potential of each extract. **Aim:** To ascertain phytochemical composition in *Allium stracheyi* Baker leaf extracts by using preliminary qualitative and quantitative screening methods and assessment of antidiabetic potential by *in vitro* enzymatic assays. **Methods:** The successive extraction of *Allium stracheyi* Baker leaf was carried out by using a sequence of solvents with increasing polarity including petroleum ether, methanol, hydroalcoholic and water by soxhlation apparatus. The concentrated extracts were subjected to preliminary phytochemical analysis for the presence or absence of active phytochemicals and their quantity in extract followed by determination of antidiabetic potential by application of standard *in vitro* enzymatic assays. **Results:** Phytochemical screening on each leaf extract confirmed the presence of various bioactive phytochemicals. Results of quantitative screening showed that the methanolic extract possesses the highest amount of phenolic and flavonoids which may contribute to the antidiabetic potential of the plant. All the extracts demonstrated marked antidiabetic potential on *in vitro* enzymatic (α-amylase and α-glucosidase) assays. Additionally, methanolic extract was the only one of the tested extracts with a significant antidiabetic potential. **Conclusion:** Research findings concluded that methanol leaf extract exhibited significant antidiabetic potential because of the existence of phenolic compounds as well as flavonoids in the majority. Further additional studies on plant phytochemicals are strongly suggested to isolate and purify the major bioactive phenolics and flavonoids from methanolic extract to confirm its antidiabetic potential in *in vivo* studies.

Major Findings: This study's main finding is that, out of all the extracts, the methanolic extract of *Allium stracheyi* Baker was the most effective at inhibiting the activity of the alpha-glucosidase enzyme (IC_{50}=32.92 µg/ml at 100 µg/ml) and the alpha-amylase enzyme (IC_{50}=50.51 µg/m at 100 µg/ml), which suggests that it may be used to treat diabetes. The high concentration of polyphenolic chemicals, which are followed by tannins, steroids, alkaloids, and saponins, is what causes this activity.

Keywords: *Allium stracheyi,* Antidiabetic, Ethnobotincal, Phytochemicals, Soxhlation

1. Introduction

Diabetes Mellitus (DM) is a chronic, moderate and multifactorial problem in which specifically metabolism of carbohydrates, lipids and proteins is inappropriately regulated by insulin, a pancreatic hormone released by the beta-cells of the pancreas, resulting in hyperglycemia, polyuria, polydipsia,

*Author for correspondence

Article Received on: 29.07.2024 Revised on: 24.10.2024 Accepted on: 03.12.2024

polyphagia, glycosuria, negative nitrogen balance and ketonemia (WHO). If left untreated, chronic hyperglycemia may lead to dysfunction of kidneys, nerves, eyes, heart, blood vessels and many other severe complications. Complications of diabetes are mostly associated with altered lipid profile, oxidative stress, mitochondrial dysfunction and glycation of protein[1,2]. Currently, diabetes mellitus is considered one of the most dangerous killer complications, rising globally in both developing and developed nations[3,4]. Over the past two to three decades, the number of population with diabetes has dramatically increased. As per the World Health Organization, 1.6 million people die each year from diabetes, which affects 422 million people in low- and middle-economic nations[5-8].

In addition to the insulin preparations, various oral anti-diabetic drugs are as of now used to treat diabetes along with a healthy balanced diet and regular exercise. However due to their prominent adverse effects and occasionally diminished response on prolonged usage; none can be regarded as the ideal treatment for diabetes[9,10]. These restrictions have mostly driven the investigation of management techniques involving the use of crude herbs and their extracts which are documented to be less expensive anti-diabetic medications with the least side effects[2,11]. In developing nations, herbs and herbal products are still a common and major source of medicine and treatment. Moreover, numerous bioactive compounds were isolated from the medicinal plants, which act as main active compounds or therapeutic agents[12]. The identification, isolation, and characterization of target molecules in the ethnomedicinal drug discovery process has been made easier by the development of contemporary analytical techniques. Literature survey proven that approximately more than 400 plants exhibit hypoglycaemic potential[13].

Allium stracheyi Baker (family *Alliaceae*) normally known as Pharan, is a small, perennial Himalayan seasoning herb of the genus *Allium*, is one of the popular, approved and important ethnobotanical traditional plants and has been widely used for centuries as a spice, flavouring agents and folk medicine for various physiological and pathological disorders in Himalayan states of India (especially in Uttarakhand)[14]. This species of *Allium* is widely distributed and cultivated in Afghanistan, Pakistan, Tajikistan, Serbria, India (Jammu Kashmir, Himachal Pradesh, Uttarakhand), Nepal, Tibet and its neighbouring borders within Uttarakhand[15]. Ethnopharmacologically it is popular for its stomachic, antioxidant, anxiolytic, analgesic and anti-inflammatory, haemolytic, antimicrobial, hypoglycaemic and hypolipidemic properties. *Allium stracheyi* Baker contains a rich amount of fibre and proteins with low fat and carbohydrates. Preliminary phytochemical screening reveals the presence of hydrocarbons, terpenoids, sulfur-containing compounds (dimethyl disulphide, dimethyl trisulfide, 1,2-bis(methylthio) ethene, 2,4-dimethylthiophene) phytosterols, phenolics, and flavonoids as a major phytoconstituents due to which it has been proven as a therapeutically active plant[16].

To verify the traditional and pharmacological uses of plant *Allium stracheyi* Baker various pre-clinical studies (*in vitro* and *in vivo)* have been done on various plant extracts using animal models. The *Allium cepa, A. sativum, A. tuberosum,* and *A. ampeloprasum* are some species belonging to the genus *Allium* that possess similar phytoconstituents and are ecologically related and were assessed for different pharmacological applications[17].

Moreover, inhibition of various carbolytic enzymes including alpha-glucosidase and alpha-amylase is one of the potential approaches for the management and treatment of diet-dependent rise in blood glucose level. Alpha-amylase and alpha-glucosidase are two pivotal digestive enzymes, that assume a significant part in the induction of diabetes mellitus. Alpha-glucosidase is particularly found in the brush border of the small intestine, while alpha-amylase is primarily present in saliva and pancreatic juice. They are strongly responsible for the hydrolysis of polysaccharides into monomers, thereby increasing majorly postprandial blood sugar levels. Inhibition of the effect of these two digestive enzymes particularly slows down the process of digestion and absorption of carbohydrates after a meal. This maintains the post-prandial blood glucose level in the body, which subsequently decreases the demand for insulin. Therefore, inhibition of these two digestive enzymes is regarded as a potential therapeutic approach to treat diet-dependent diabetes mellitus. Also, by understanding the functioning of carbolytic enzymes, a new natural therapeutic agent for diabetes can be identified and developed. A literature survey suggests that among all the species of the genus Allium,

only a little data is available on *A. stracheyi.* Also, different species of the genus *Allium* were analysed for their *in vitro* and *in vivo* antidiabetic activity, suggesting their significant modulatory role in diabetes mellitus while there is no information on the therapeutic potential of *A. stracheyi* Baker on diabetes mellitus in particular. As a result, the main purpose of this research was to demonstrate the scientific basis for the use of *A. stracheyi* in diabetes management and treatment[18,19].

2. Materials and Methods

2.1 Chemicals

Reagents, chemicals and enzymatic kits used in the research work were of analytical grade.

2.2 Collection and Identification of Plant Material

Allium stracheyi Baker leaves were obtained from the Mana village, Joshimath, district Chamoli, Uttarakhand (UK) in August 2022. The taxonomy of the herbal plant was identified from the Botanical Survey of India (BSI), Kaulagarh road, Dehradun (UK) (Accession No.: 1161) and the specimen of the plant was preserved.

2.3 Preparation of Plant *Allium stracheyi* Baker Leaves Extract

The leaves of the plant were washed under regular tap water and permitted to dry at room temperature for 2 weeks. After, complete dryness the leaves were pulverized using a mechanical grinder to obtain a powder material. The powdered material was extracted with solvent petroleum ether for defatting and then subjected to successive extraction by using different solvents according to the polarity (methanol, 70% methanol and water). The extracts obtained from different solvents were concentrated on a rotatory vacuum evaporator (40°C) and stored at 4°C in air-tight plastic for further analysis. The extractive yields of each extract were calculated[20].

2.4 Phytochemical Investigation of Leaf Extracts of *Allium stracheyi* Baker

2.4.1 Qualitative Phytochemical Screening

All the extracts were tested for phytochemical analysis to determine the presence of active phytoconstituents or secondary plant metabolites[21,22].

2.4.2 Quantitative Phytochemical Screening

2.4.2.1 Total Tannin Content

The total tannin content of each extract was measured by the method recommended by Mital and Jha[21]. To the 1 ml of each extract, Folin's phenol reagent (0.5 ml) was added and saturated with 1 ml of sodium bicarbonate. The final volume of mixtures was made up of 8ml of distilled water and incubated for 30 min. The incubated solution was then centrifuged, the supernatant collected and was perused at 725 nm in a UV-visible spectrophotometer. Gallic acid was used as a reference and a blank that had been prepared in the same way as the test solution but without extract was used to measure the absorbance of the solutions. The total tannin content of extracts was expressed as mg of tannic acid equivalent (TAE)/g[23,24]. The calibration curve equation for the standard compound (gallic acid) was used to calculate total tannin concentration, *i.e.*, y = 0.0101x + 0.2193; R2 = 0.999[24]. Each extract was examined in triplicates.

2.4.2.2 Total Alkaloid Content

The alkaloidal content of each extract was obtained using the Harborne methodology[24]. The 5gm of each leaf extract was dissolved in 200 ml of 10%v/v of acetic acid in ethanol. The mixture was enclosed, permitted to stand for 4-5 h, and filtered afterwards. The filtrated herbal extract was then concentrated to 1/4th of the original volume by evaporating a solvent in a water bath. Further, 15-20 drops of ammonium hydroxide (NH_4OH) were added dropwise to the concentrated extract till the formation of complete precipitation. The mixture was permitted to sediment and the precipitate was collected, washed in 0.1 M of ammonium hydroxide, and afterwards filtered. By using an oven, the residue was air dried, weighed in electronic balance and the percentage of total alkaloidal content was calculated using the following formula:

$$\% \text{ Alkaloidal content} = \frac{\text{weight of mass obtained (alkaloid)}}{\text{Weight of herbal extract}} X\,100$$

Each extract was examined in triplicates.

2.4.2.3 Total Phenolic Content

The phenolic content in each extract was evaluated by Folin-Ciocalteu assay. 9-10 ml of distilled water

was used to dissolve 1 ml of each herbal extract. The mixture was then treated with 1ml Folin-Ciocalteu reagent, shaken well and permitted to stand (5 min). Then, the mixture of an extract with reagent was further treated with 10ml of 7%w/v sodium carbonate (Na_2CO_3) and the final volume (25 ml) of the mixture was made up with water. Without adding any extract, a series of solutions of standard gallic acid were prepared in the same way as the test solution. After 90 min. of incubation of test as well as standard at 25ºC the absorbance was recorded at 550 nm against the blank using a UV-visible spectrophotometer. The total phenolic content of each herbal extract was evaluated from the calibration plot of standard gallic acid (y = 0.0101x + 0.2193; R2 = 0.999) and expressed as mg of gallic acid equivalent/gm dry material[24,25].

2.4.2.4 Total Flavonoid Content

The flavonoid content in each extract was determined by an aluminium chloride colorimetric assay. 1 ml of each extract was separately dissolved in 4 ml of distilled water in a volumetric flask (10 ml). Then, each extract was treated with 0.30 ml of 5% w/v of sodium nitrite ($NaNO_2$) solution and leftover for 5-10 min followed by further addition of 0.3 ml of 10% w/v solution of aluminium chloride ($AlCl_3$). After 5min, the mixture was treated with 1 M sodium hydroxide (NaOH (2 ml)) and diluted with 10 ml distilled water. The absorbance of each extract (test) and quercetin (standard) was measured at 510 nm against the blank using a spectrophotometer (UV-visible). The absolute flavonoid content of each extract was communicated as mg of QE/g of concentrate material and the adjustment bend condition as mg of QE/g of extract material[25,26] and the calibration curve of quercetin was used to estimate total flavonoid concentration, *i.e.*, y = 0.0004x + 0.1994; R2 = 0.9983[24].

2.4.2.5 Total Saponin Content

The Total saponin content of extracts was calculated by double extract gravimetric assay suggested by Harborne[27]. About 5 gm of each extract was weighed and dissolved in 50 ml of 20% v/v ethanol in a volumetric flask. The mixture was continuously heated for 4 h at a temperature of 50ºC in a water bath with continuous stirring before being filtered by What-man filter paper. The filtrate obtained from each extract was re-extracted with 50 ml of 20% v/v of ethanol. Both the filtrates obtained from each extract were concentrated by evaporating the solvent at a temperature of 90ºC to reduce the original volume up to 40 ml by using a water bath. To the concentrated filtrate, 20 ml of di-ethyl ether was added and shaken vigorously in a separating funnel. Then, the mixture was separated into two distinct layers. The complete process of extraction was repeated in triplicates until the aqueous layer became clear. The aqueous layer was again extracted with 60 ml of n-butanol at least three times and washed with 10 ml of 5% w/v sodium chloride (NaCl). The final extract was heated at 50ºC using a water bath to evaporate the solvent n-butanol. The obtained residue was dried in a hot oven at 60ºC and weight was measured in electronic balance and the percentage for total saponin content was computed with the following formula[28,29].

$$\% \text{ Saponin content} = \frac{\text{final weight of obtained residue}}{\text{Weight of extract}} \times 100$$

The procedure was repeated three times.

2.4.2.6 Total Steroidal Content

To the 1 ml of each extract (5 mg/ml), 2 ml of 2 M sulphuric acid (H_2SO_4), 0.5% ferric chloride ($FeCl_3$) and 0.5 ml (0.5%w/v) of potassium ferricyanide ($C_6N_6FeK_3$) solution were added pursued by heating for 30 min in water bath at temperature of 70ºC with periodic shaking and the final volume of solution was made up to 10ml using distilled water. A sequence of standard solutions of cycloartenol was made similar to the test solution (without extracts). The absorbance of the test and standard was recorded at 780 nm against the blank using a UV-visible spectrophotometer. The steroidal content of each extract was computed from the standard (cycloartenol) calibration curve, *i.e.*, Y = 0.0029x + 0.107 with regression R^2 = 0.9989 and expressed as cycloartenol equivalents (mg CA/g of dried material)[24,30].

2.5 *In Vitro* Inhibitory Assay of Key Carbolytic Enzymes Associated with Diabetes Mellitus

2.5.1 Alpha (α)-amylase Inhibition Assay

The alpha (α)-amylase inhibitory activity of each extract of the leaves of *A. stracheyi* was carried out by suspending 2 mg of potato starch in 0.2 ml w/v of 0.5

M of Tris-hydrochloride at pH 6.9 consisting of 0.01 M calcium chloride ($CaCl_2$), serve as a substrate for assay. The tube that contained the substrate solution was boiled in a water bath for 5 min, followed by pre-incubation for 5 min at a temperature of 37°C. The extracts were dissolved separately in the solvent DMSO and a sequence of concentrations, *i.e.*, 10-100 µg/mL was obtained. 0.2 ml of each extract was further added to each tube having a substrate solution and treated with 0.1 ml of alpha-amylase (0.5 mg/ml) enzyme prepared in the tris-hydrochloride buffer. After 10 min of incubation (37°C), 0.5 ml of 50% w/v of acetic acid was added to each cylindrical tube to stop the reaction. The reacted mixture was then centrifuged (3000-5000 rpm) at 40°C for 5 min and the supernatant was obtained. A spectrophotometer (UV-visible) was used to measure the absorbance at 595 nm. The absorbance of the supernatant was measured against a blank that had been prepared in the same pattern as the test solution without using extract. Acarbose was used as the reference drug[31,32]. The reading was taken in triplicate, and the enzyme inhibition was computed using the following formula:

$$\alpha - \text{amylase} \quad \text{inhibitory} \quad \text{activity} = \frac{\text{Xa} - \text{Xb}}{\text{Xa}} \times 100$$

where, Xa, is the absorbance of control and Xb, is the absorbance of standard and test. After calculating the percentage inhibitory activity of extracts for different concentrations, the IC_{50} values (concentration of extract/standard required to inhibit 50% of alpha-amylase enzyme) were also determined for each extract and the standard drug (acarbose).

2.5.2 Alpha (α)-Glucosidase Inhibition Assay

About 50 µl of each extract from different concentrations (10-100 µg/ml) were 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 between the mixtures was allowed to start by adding 20 µl of the substrate solution, *i.e.*, 1 M p-nitrophenyl-β-D-glucopyranoside, and incubated for thirty minutes at a temperature of 37°C. To the reaction mixture, 50 µl of 0.1 N w/v sodium bicarbonate (Na_2CO_3) was added to terminate the final reaction and absorbance was recorded at 405 nm using a UV-visible spectrophotometer. Acarbose was used as a reference drug and the absorbance of obtained test solutions was measured against a blank, prepared in the same pattern 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:

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

where, Xa, is the absorbance of control and Xb, is the absorbance of standard and test. After calculating the inhibitory activity for alpha-glucosidase of each extract for different concentrations, the IC_{50} values (concentration of extract (test)/standard requires to inhibit 50% of α-glucosidase enzyme) were also determined for each extract and the standard drug (acarbose)[33-35].

3. Statistical Analysis

All the data presented in the study were analysed in triplicates using Microsoft Excel and graph pad prism 8.0 and the values are computed as Mean±SD. IC_{50} value of standard drug and test extracts were calculated from the mean inhibitory value by logarithmic regression analysis.

4. Results and Discussion

4.1 Percentage Yield

Different leaf extracts of *A. stracheyi* Baker were obtained from the different solvents (methanolic, hydroalcoholic and aqueous) using the Soxhlet apparatus. The percentage yield for different extracts is shown in Table 1 and the yield was obtained by using the following formula:

$$\text{Percentage yield} = \frac{\text{weight of extract obtained}}{\text{weight of crude plant}} \times 100$$

The highest percentage yield was observed for the methanolic extract and was found to be 13.84±0.506% followed by hydroalcoholic extracts at 10.06±0.938% whereas the for aqueous extract it was comparatively low at 9.14±0.482%.

4.2 Preliminary Phytochemical Estimation of Leaf Extracts of *Allium stracheyi Baker*

4.2.1 Qualitative Phytochemical Estimation

The preliminary phytochemical screening for the bioactive phytoconstituents was performed for different extracts of leaf of *A. stracheyi*. The observation and interference attained from the qualitative identification tests are shown in Table 2. The findings of the qualitative chemical analysis show the presence of various phytoconstituents including tannin, alkaloids, polyphenols, flavonoids, saponin and steroids. The maximum phytochemicals were present in methanolic and hydroalcoholic extracts as compared to the aqueous extract of the leaf of *A. stracheyi*.

4.2.2 Quantitative Phytochemical Estimation

Phytoconstituents whose presence was observed in methanolic, hydroalcoholic and aqueous extract by qualitative photochemical analysis were subjected to further investigation to quantity the total tannin, alkaloids, phenolic, flavonoids, saponin and steroids content by quantitative phytochemical analysis. The results of quantitative testing of plant extracts are given in Table 3.

From the results, it has been observed that the methanolic and hydroalcoholic extract of the leaf of *A. stracheyi* usually had a maximum phytochemical content. The total tannin content in methanolic, hydroalcoholic and aqueous extract of *A. stracheyi* **Baker** leaf were 43.07±0.56, 46.03±0.526 and 51.54±0.396 mg GA/gm, respectively whereas the total alkaloidal content in the extract was 16.99±0.503%, 23.10±0.14% and 34.41±0.687 % respectively. A maximum amount of phenols and flavonoids were detected in the methanolic extract, *i.e.*, 86.68±0.887 mg GA/gm and 75.66±0.833 mg QE/gm, respectively as compared to hydroalcoholic (69.02±0.89 mg GA/gm and 67.33±0.833 mg QE/gm, respectively) and aqueous extract (57.68±0.967 mgGA/gm and 50.667±0.833mg GA/gm). The amount of saponin in the methanolic

Table 1. Percentage (% w/w) yield of different extracts of leaves of *A. straychei* Baker

Extracts	Colour	Consistency	% (w/w) Yield
Methanolic (MeOH)	Dark Green	Semi-solid	13.84±0.506%
Hydro alcoholic (HAE)	Brown	Semi-solid	10.06±0.938 %
Aqueous (AQE)	Dark Brown	Solid	9.14±0.482 %

The values are taken in triplicates (Mean±SD)

Table 2. Qualitative screening of methanolic, hydroalcoholic and aqueous extract of leaf of *A. stracheyi*

Tests	Methanolic Extract	Hydroalcoholic Extract	Aqueous Extract
Tannins			
Braymer's test	+	+	-
Lead acetate	+	+	+
Alkaloids			
Mayer's test	+	+	+
Hager's test	+	+	+
Phenolic and flavonoids			
Ferric chloride test	+	+	-
Dil. Iodine sol.	+	+	+
Lead acetate	+	+	+
Alkaline Reagent Test	+	+	+
Shinoda test	+	+	+
Steroids			
Salkowski test	+	+	-
Test for Saponins			
Froth test	+	+	+

(+): present; (–): not detected.

Table 3. Quantitative screening of methanolic, hydroalcoholic and aqueous extract of leaf of *A. straychei*

Extracts *stracheyi*)	Tannin (mgGA/gm)	Phenols (mgGA/gm)	Flavonoids (mgQE/gm)	Steroids (mgCA/gm)	Alkaloids (%)	Saponin (%)
MeOH	43.07±0.56	86.68±0.887	75.66±0.833	34.823±0.347	16.99±0.503	14.8±0.333
HAE	46.03±0.526	69.02±0.89	67.33±0.833	16.43±0.35	23.10±0.14	25.86±0.266
AQE	51.54±0.396	57.68±0.967	50.67±0.833	-	34.41±0.687	50.54±0.693

The values are taken in triplicates (Mean±SD)

extract of the plant was slightly lower, *i.e.*, 14.68±0.33% whereas in hydroalcoholic extract it was detected in moderate amounts of 25.86±0.266% and a higher amount in aqueous extract, *i.e.*, 50.54±0.693%. The steroidal content of methanolic and hydroalcoholic extract was 34.823±0.347 mgCA/gm and16.43±0.35 mgCA/gm. The phytochemicals identified and quantified gave an idea about the phytochemicals present in the leaf of *A. stracheyi*, which may be accountable for its potential pharmacological effects and the reason for their use as a traditional and herbal medicine globally. As the results of the qualitative and quantitative analysis suggested that the methanolic extract of the plant is rich in active phytoconstituents, further *in vitro* antidiabetic activity was carried out on methanolic extract to find out its pharmacological role in diabetes mellitus.

4.3 *In Vitro* α-Amylase and α-Glucosidase Inhibitory Assay

The findings of alpha (α)-amylase and alpha (α)-glucosidase inhibitory activity of the acarbose (standard drug) and the various leaf extracts (methanolic, hydroalcoholic and aqueous) of *Allium stracheyi* Baker at a sequent concentration (10, 20,40, 60, 80 and 100 µg/ml) is shown in Tables 4 and 5. Dose-dependent inhibitory activity enzyme was observed with each extract. The results revealed that out of three extracts methanolic extract exhibited the highest inhibitory activity whereas hydroalcoholic and aqueous extract showed marked and least enzyme inhibitory activity.

In alpha-amylase assay, the inhibition of the methanolic, hydroalcoholic and aqueous extract was found to be 67.28±2.732% 55.383±3.666% and 51.48±2.537%, respectively at 100 µg/ml with an IC_{50}

Table 4. *In vitro* alpha (α)-amylase inhibitory activity of leaf extracts of *A. stracheyi*

Percentage (%) Inhibitory Activity				
Conc. (µg/ml)	**Acarbose**	**MeOH**	**HAE**	**AQE**
10	38.33±3.453	34.203±4.474	20.54±3.508	13.51±3.666
20	44.47±3.817	39.40±3.506	26.25±3.166	21.50±2.583
40	52.71±4.416	46.64±3.333	33.75±2.543	28.91±2.456
60	60.37±3.360	53.46±1.416	43.19±2.779	35.45±4.830
80	68.41±2.866	61.54±2.373	49.183±1.373	44.14±3.163
100	75.26±2.799	67.28±2.732	55.383±3.666	51.48±2.537
$I_{C5}0$	35.35	50.51	82.61	95.05

The values are taken in triplicates (Mean±SD)
The alpha (α)-amylase and IC_{50} value of each extract at different concentrations (10-100 µg/ml) compared with standard drug (acarbose). The results of the present study were shown in percentage (%) inhibition±SD.

Table 5. *In vitro* alpha (α)-glucosidase inhibitory activity of leaf extracts of *A. stracheyi*

Percentage (%) alpha-glucosidase Inhibitory Activity				
Conc. (µg/ml)	**Acarbose**	**MeOH**	**HAE**	**AQE**
10	42.18±1.90	38.78±3.417	27.6±2.355	18.50±2.363
20	48.87±4.730	45.35±2.32	32.32±3.356	26.24±4.16
40	56.48±3.0723	53±2.12	39.78±1.720	34.61±3.366
60	66.58±3.600	63.21±1.801	47.59±2.366	41.94±3.24
80	74.71±2.283	70.35±1.200	57.13±2.400	49.99±2.435
100	84.05±0.76	75.41±2.545	63.5±1.94	55.12±2.94
IC_{50}	24.99	32.92	64.96	82.45

The values are taken in triplicates (Mean±SD)
The alpha (α)-glucosidase and IC50 value of each extract at different concentrations (10-100 µg/ml) compared with standard drug (acarbose). The results of the present study were shown in percentage (%) inhibition±SD.

(concentration that inhibits 50% of enzyme) values of 50.51, 82.61 and 95.05 µg/ml respectively. Among all the extracts, methanolic extract showed maximum inhibition. The Acarbose showed α-amylase inhibitory activity of 75.26±2.799% at 100 µg/ml with IC_{50} of 35.35 µg/ml.

Like α-amylase inhibitory activity, the methanolic extract exhibited the maximum alpha-glucosidase inhibitory activity of 75.41±2.545% at 100 *µg*/ml with IC_{50} of 32.92 *µg*/ml while for the hydroalcoholic and aqueous extract the α-glucosidase inhibitory activity was 63.5±1.94% (IC_{50} of 64.96 *µg*/ml) and 55.12±2.94% at a dose of 100 *µg*/ml (IC_{50} of 82.*45* *µg*/ml). The standard reference drug acarbose showed α-glucosidase inhibitory activity of 84.05±0.76% at 100 *µg/ml* with IC_{50} of 24.99 *µg/ml*.

Findings of the study concluded that among all three extracts, the methanolic leaf extract of *A. stracheyi* efficiently inhibited both alpha-amylase and alpha-glucosidase enzymes. The results of the present research work also suggested that *A. stracheyi* Baker might be valuable in the management and treatment of postprandial hyperglycaemia.

5. Conclusion

In the present research, the phytochemical composition and antidiabetic potential of the leaf extracts of *A. stracheyi* were screened by using preliminary phytochemical and *in vitro* biological assays. Outcomes concluded that the majority of bioactive phytochemicals were present in MeOH and HA extracts. The AQ extract showed the presence of almost similar phytochemicals. Moreover, MeOH extract possessed the highest quantity of flavonoids and phenolic compounds as compared to other phytochemicals and extracts. Dietary polyphenols, in addition to their antioxidant effects, have been reported to exert anti-hyperglycemic effects by binding to glucose transporters and competitive inhibition of digestive enzymes.

α-amylase and αglucosidase are carbohydrate-digesting enzymes. They are required to hydrolyze complex polysaccharides and disaccharides respectively to release glucose into the general circulation. Inhibition of these enzymes has proven to be an efficient approach to controlling postprandial sugar. In this present study. The results manifested that MeOH extract of plant possessed inhibitory effect on digestive enzymes, imputing its significant antidiabetic potential and might be useful in treating and slowing down the progress of diabetes and related complications. This activity may be linked to some class of compounds-phenolics, tannins and flavonoids which were higher in the MeOH extract.

The present finding would be useful for future research directions on the application of traditional medicinal plants in the development of nutraceuticals and pharmaceuticals. Further analysis of plant phytochemicals is strongly suggested to isolate and purify the major bioactive flavonoids and phenolics responsible for antidiabetic activity.

6. Acknowledgements

We would like to acknowledge Shri Guru Ram Rai University, Dehradun and Sardar Bhagwan Singh University, Dehradun for providing all the facilities for the successful conduction of this study.

7. Author Contribution

All authors have equal contributions to the design and conception of the study. Neeraj Kumar, Veerma Ram have reviewed the study. Concept and methodology were set by all the authors. Lata Bisht carried out all the experimental procedures, analysed and interpreted the data, manuscript writing. All authors have read and agreed to the published version of the manuscript.

7. References

1. Al-Hourani HM, Atoum MF. Body composition, nutrient intake and physical activity patterns in young women during Ramadan. Singap Med J. 2007; 48(10):906.
2. Sagbo IJ, van de Venter M, Koekemoer T, Bradley G. *In vitro* antidiabetic activity and mechanism of action of *Brachylaena elliptica* (Thunb.) DC. J Evid Based Complementary Altern Med. 2018; 2018:4170372. https://doi.org/10.1155/2018/4170372 PMid:30108655 PMCid:PMC6077518
3. Al-Ani IM, Santosa RI, Yankuzo MH, Saxena AK, Alazzawi KS. The antidiabetic activity of curry leaf *Murraya Koenigii* on the glucose levels, kidneys, and islets of Langerhans of rats with Streptozotocin induced diabetes. Makara J Health Res. 2017. p. 54-60. https://doi.org/10.7454/msk.v21i2.7393

4. Bhat M, Zinjarde SS, Bhargava SY, Kumar AR, Joshi BN. Antidiabetic Indian plants: A good source of potent amylase inhibitors. J Evid Based Complementary Altern Med. 2011. https://doi.org/10.1093/ecam/nen040 PMid:18955350 PMCid:PMC3137644
5. Lin X, Xu Y, Pan X, Xu J, Ding Y, Sun X, *et al*. Global, regional, and national burden and trend of diabetes in 195 countries and territories: An analysis from 1990 to 2025. Sci Rep. 2020; 10(1):14790. https://doi.org/10.1038/s41598-020-71908-9 PMid:32901098 PMCid:PMC7478957
6. Rajkumar G, Vinotha S. A review of anti-hyperglycemic effects of curry leaf tree (*Murraya koenigii*). Borneo J Pharm. 2022; 5(2):104-114. https://doi.org/10.33084/bjop.v5i2.3300
7. Kifle ZD, Yesuf JS, Atnafie SA. Evaluation of *in vitro* and *in vivo* anti-diabetic, anti-hyperlipidemic and anti-oxidant activity of flower crude extract and solvent fractions of *Hagenia abyssinica* (Rosaceae). J Exp Pharmacol. 2020. p. 151-67. https://doi.org/10.2147/JEP.S249964 PMid:32607006 PMCid:PMC7293407
8. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diab Care. 2014; 37(1):S81-S90. https://doi.org/10.2337/dc14-S081 PMid:24357215
9. Chattopadhyay RR. A comparative evaluation of some blood sugar lowering agents of plant origin. J Ethnopharmacol. 1999; 67(3):367-372. https://doi.org/10.1016/S0378-8741(99)00095-1 PMid:10617074
10. Bhutkar MA, Bhinge SD, Randive DS, Wadkar GH, Todkar SS. Cribado de la actividadhipoglucémica *in vitro* de *Murraya koenigii* y *Catharanthus roseu*. Ars Pharmaceutica. 2018; 59(3):145-151. https://doi.org/10.30827/ars.v59i3.7413
11. Atanasov AG, Waltenberger B, Pferschy-Wenzig EM, Linder T, Wawrosch C, Uhrin P, *et al*. Discovery and resupply of pharmacologically active plant-derived natural products: A review. Biotechnol Adv. 2015; 33(8):1582-1614. https://doi.org/10.1016/j.biotechadv.2015.08.001 PMid:26281720 PMCid:PMC4748402
12. Tafesse TB, Hymete A, Mekonnen Y, Tadesse M. Antidiabetic activity and phytochemical screening of extracts of the leaf of *Ajuga remota* Benth on alloxan-induced diabetic mice. BMC Complement Altern Med. 2017; 17:1-9. https://doi.org/10.1186/s12906-017-1757-5 PMid:28464813 PMCid:PMC5414132
13. Mahnashi MH, Alqahtani YS, Alyami BA, Alqarni AO, Ayaz M, Ghufran M, *et al*. Phytochemical analysis, α-glucosidase and amylase inhibitory, and molecular docking studies on *Persicaria hydropiper* L. leaf essential oils. J Evid Based Complementary Altern Med. 2022; 2022. https://doi.org/10.1155/2022/7924171 PMid:35096118 PMCid:PMC8791729
14. Majumder S, Singh S, Singh J. First report of garlic virus D in *Allium stracheyi* from India. J Plant Pathol. 2020; 102(4):1347. https://doi.org/10.1007/s42161-020-00632-0
15. Semwal DP, Saradhi PP, Nautiyal BP, Bhatt AB. Current status, distribution and conservation of rare and endangered medicinal plants of Kedarnath Wildlife Sanctuary, Central Himalayas, India. Curr Sci. 2007; 25:1733-1738.
16. Suresh KS, Senthi KB, Rajesh KS, Nangalingam M. Antihyperglycemic and antioxidant effect of *Allium sativum* aqueous bulb extract against alloxan-induced diabetic male albino rats. Drug Invent Today. 2019; 12(9):1965-1968.
17. Najeebullah S, Shinwari ZK, Jan SA, Khan I, Ali M. Ethno medicinal and phytochemical properties of genus *Allium*: A review of recent advances. Pak J Bot. 2021; 53(1):135-144. https://doi.org/10.30848/PJB2021-1(34)
18. Alqahtani AS, Hidayathulla S, Rehman MT, ElGamal AA, Al-Massarani S, Razmovski-Naumovski V, *et al*. Alpha-amylase and alpha-glucosidase enzyme inhibition and antioxidant potential of 3-oxolupenal and katononic acid isolated from *Nuxia oppositifolia*. Biomolecules. 2019; 10(1):61. https://doi.org/10.3390/biom10010061 PMid:31905962 PMCid:PMC7022278
19. Poovitha S, Parani M. *In vitro* and *in vivo* α-amylase and α-glucosidase inhibiting activities of the protein extracts from two varieties of bitter gourd (*Momordica charantia* L.). BMC Complement Altern Med. 2016; 16:1-8. https://doi.org/10.1186/s12906-016-1085-1 PMid:27454418 PMCid:PMC4959359
20. Halder S, Dutta S, Khaled KL. Evaluation of phytochemical content and *in vitro* antioxidant properties of methanol extract of *Allium cepa, Carica papaya* and *Cucurbita maxima* blossoms. Food Chem Adv. 2022; 1:100104. https://doi.org/10.1016/j.focha.2022.100104
21. Shaikh JR, Patil M. Qualitative tests for preliminary phytochemical screening: An overview. Int J Chem Stud. 2020; 8(2):603-608.
22. Kapoor M, Rani J. Qualitative and quantitative analysis of phytochemicals by GC-MS and antioxidant activity of *Catharanthus roseus* (L.) G. Don. Journal of Pharmacogn Phytochem. 2019; 8(1S):378-385.
23. BakirÇilesizoğlu N, Yalçin E, Çavuşoğlu K, SipahiKuloğlu S. Qualitative and quantitative phytochemical screening of *Nerium oleander* L. extracts associated with toxicity profile. Sci Rep. 2022; 12(1):21421. https://doi.org/10.1038/s41598-022-26087-0 PMid:36504046 PMCid:PMC9742154
24. Dumka H, Ram V, Bisht L, Negi AS, Kumar A, Semwal DK. Anticonvulsant activity of Rudraksha (*Elaeocarpus angustifolius* Blume) bark against picrotoxin-induced convulsions in rats. Indian J Nat Prod Resour. 2024; 15(1):104-112.
25. Harborne AJ. Phytochemical methods a guide to modern techniques of plant analysis. Springer Science and Business Media; 1998.
26. Tambe VD, Bhambar RS. Estimation of total phenol, tannin, alkaloid and flavonoid in *Hibiscus tiliaceus* Linn. wood extracts. J Pharmacogn Phytochem. 2014; 2(4):41-47

27. Harborne JB. Phytochemical methods. 1st ed. Chapman and Hall, London; 1973. https://doi.org/10.1007/978-94-009-5921-7_1
28. Ezeabara CA, Okeke CU, Aziagba BO, Ilodibia CV, Emeka AN. Determination of saponin content of various parts of six *Citrus* species. Int Res J Pure Appl Chem. 2013; 4(1):137-143. https://doi.org/10.9734/IRJPAC/2014/5831
29. Edewor TI, Owa SO, Ologan AO, Akinfemi F. Quantitative determination of the saponin content and GC-MS study of the medicinal plant *Cassytha filiformis* (Linn.) leaf. J Coast Life Med. 2016; 4(2):154-156. https://doi.org/10.12980/jclm.4.2016j5-210
30. Abd'quadri-Abojukoro AN, Nkadimeng SM, McGaw LJ, Nsahlai IV. Phytochemical composition and cytotoxicity of ethanolic extracts of some selected plants. J Appl Anim Res. 2022; 50(1):656-665. https://doi.org/10.1080/09712119.2022.2119979
31. Tamil IG, Dineshkumar B, Nandhakumar M, Senthilkumar M, Mitra A. *In vitro* study on α-amylase inhibitory activity of an Indian medicinal plant, *Phyllanthus amarus*. Indian J Pharmacol. 2010; 42(5):280. https://doi.org/10.4103/0253-7613.70107 PMid:21206618 PMCid:PMC2959209
32. Rao PS, Mohan GK. *In vitro* alpha-amylase inhibition and *in vivo* antioxidant potential of *Momordica dioica* seeds in streptozotocin-induced oxidative stress in diabetic rats. Saudi J Biol Sci. 2017; 24(6):1262-1267. https://doi.org/10.1016/j.sjbs.2016.01.010 PMid:28855820 PMCid:PMC5562449
33. Niaz A, Adnan A, Bashir R, Mumtaz MW, Raza SA, Rashid U, *et al*. The *in vitro* α-glucosidase inhibition activity of various solvent fractions of *Tamarix dioica* and 1H-NMR based metabolite identification and molecular docking analysis. Plants. 2021; 10(6):1128. https://doi.org/10.3390/plants10061128 PMid:34199333 PMCid:PMC8227178
34. Mechchate H, Es-Safi I, Louba A, Alqahtani AS, Nasr FA, Noman OM, *et al*. *In vitro* alpha-amylase and alpha-glucosidase inhibitory activity and *in vivo* antidiabetic activity of *Withania frutescens* L. foliar extract. Molecules. 2021; 26(2):293. https://doi.org/10.3390/molecules26020293 PMid:33430115 PMCid:PMC7826620
35. Pistia-Brueggeman G, Hollingsworth RI. A preparation and screening strategy for glycosidase inhibitors. Tetrahedron. 2001; 57(42):8773-8778. https://doi.org/10.1016/S0040-4020(01)00877-8