



In vitro antidiabetic, antioxidant activities and chemical composition of *Ajuga parviflora* Benth. shoot

Amrita Suryavanshi¹, Suresh Kumar^{1*}, Dolly Kain¹, Atul Arya¹, Vandana²¹Medicinal Plants Research Laboratory, Department of Botany, Ramjas College, University of Delhi, Delhi-110007, India²Department of Chemistry, Dyal Singh College, University of Delhi, Delhi-110007, India

ARTICLE INFO

Article Type:
Original Article

Article History:
Received: 19 April 2021
Accepted: 27 August 2021

Keywords:
Medicinal plant
Functional groups
Bioactive compounds
Radical scavenging activities
 α -Amylase
 α -Glucosidase

ABSTRACT

Introduction: *Ajuga parviflora* Benth. (Lamiaceae) is an herbaceous plant that possesses ethnomedicinal values and is well known for its folkloric management of diabetes. This study was aimed to provide an experimental justification for its traditional antidiabetic use.

Methods: Hydroalcoholic extract of *A. parviflora* shoot was quantified for its total phenolic content (TPC), total flavonoid content (TFC), and total tannin content (TTC). Gas chromatography-mass spectrometry (GC-MS) and Fourier transform infrared spectrophotometer (FTIR) spectroscopy were also used for their chemical nature. Additionally, the extract was evaluated for its inhibitory potential against key enzymes linked with hyperglycemia by *in vitro* means. Subsequently, for estimation of the antioxidant capacities 2,2-diphenyl-2-picrylhydrazyl radical (DPPH), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) radical (ABTS), and hydrogen peroxide (H₂O₂) scavenging activities were determined.

Results: GC-MS analysis revealed numerous biologically active phytoconstituents including brassicasterol, phytol, and palmitic acid. The presence of different active functional groups such as alcohol, nitrile, amine, alkyl halide, alkene, and alkane was confirmed by FTIR analysis. The extract showed a significant ($P \leq 0.05$) dose-dependent inhibition for α -amylase enzyme (132.38 \pm 1.18 μ g/mL), α -glucosidase enzyme (22.66 \pm 0.11 μ g/mL), DPPH radical (103.03 \pm 1.59 μ g/mL), ABTS radical (140.10 \pm 3.40 μ g/mL) and H₂O₂ radical (298.26 \pm 4.37 μ g/mL). TPC, TFC, and TTC were found 64.06 \pm 0.35 mg/g of the gallic acid equivalent (GAE), 45.27 \pm 0.58 mg/g of the rutin equivalent (RE), and 127.42 \pm 1.82 mg/g of the tannic acid equivalent (TAE), respectively.

Conclusion: *A. parviflora* extract showed significant antioxidant and antidiabetic potentials. Thus, this plant might be served as a novel approach for discovering new and effective drug molecules against hyperglycemia.

Implication for health policy/practice/research/medical education:

The shoot extract of *Ajuga parviflora* Benth. possesses antidiabetic and antioxidant properties; therefore, it might be a potential source in the pharmacological industries after searching and isolating effective phyto-molecules against diabetes mellitus.

Please cite this paper as: Suryavanshi A, Kumar S, Kain D, Arya A, Vandana. *In vitro* antidiabetic, antioxidant activities and chemical composition of *Ajuga parviflora* Benth. shoot. J Herbmed Pharmacol. 2022;11(1):131-139. doi: 10.34172/jhp.2022.15.

Introduction

Since ancient times, people have been associated with medicinal plants for their primary and first line of health care. Medicinal plants contain an array of phytoconstituents such as alkaloids, phenolics, flavonoids, terpenes, which are the potential source of medicinal and pharmaceutical applications (1). Medicinal plants are not only used as medicines to take care of their health but are

also consumed as food by several tribes (2). Recently, herbal medicines have received considerable attention because of their diverse pharmacological properties, including antimicrobial, antioxidant, antidiabetic, anticancer, and antihypertensive activities (3). The main reason for the vast experimentation in natural herbal medicines is their inexpensiveness and novel properties to cure diseases (4). Therefore, the cure for these dreadful diseases through an

*Corresponding author: Suresh Kumar,
Email: suresh.kumar@ramjas.du.ac.in

eco-friendly approach of herbal medicinal plants is the need of the hour.

Globally, metabolic disorders are a worldwide plague, as declared by the World Health Organisation. According to the International Diabetes Federation (2019), diabetes affects 463 million people in developed and developing countries and may affect 578 million by 2030. Particularly, diabetes type 2 (T2DM) accounts for 90% of cases of diabetes (5). In T2DM, cells cannot metabolize sugar properly due to inability or irregularity in the action of insulin. The severity of this hyperglycemia results in various unhealthy and unfavorable symptoms such as polyuria, blurring of vision, and drastic weight loss in a short period (6). Various classes of approved oral hypoglycemic medications such as sulfonylureas, metformin, meglitinides, miglitol, voglibose, acarbose, nateglinide, and repaglinide are available for the treatment of T2DM. However, they are associated with adverse side-effects leading to life-threatening complications such as diarrhoea, kidney failure, liver problems, lactic acidosis, and multiple organ failure (7).

Oxidative stress plays a serious role in the pathogenesis of both micro and macrovascular diseases (8). The reactive oxygen and nitrogen species (ROS and RNS) create oxidative stress causing multifactorial health complications (9). Generally, the reducing properties are associated with antioxidants, which exert their action by interrupting the free radical chain reaction and subsequently reduce oxidative stress (10). Antioxidants are known to lower the risk of several diseases caused by free radicals like diabetes, cardiovascular diseases, and cancer. The pharmaceutical and food industries focus on natural antioxidants because synthetic antioxidant drugs have unwanted or adverse effects.

Ajuga parviflora Benth. is an annual and short-lived-perennial herb belonging to the Lamiaceae family, widely distributed in temperate regions of India, Pakistan, and Afghanistan. Several ethnomedicinal surveys have reported that *A. parviflora* is frequently used by tribal communities, i.e., Gaddi and Gujjar tribes (11), Bhotia tribe (12), and Jaunsari tribe (13), against diabetes. Previously this plant has been evaluated for its antiviral (14), antibacterial (4), and anti-hepatotoxic (15) activities. In the current study, antioxidant and antidiabetic properties were evaluated using different *in vitro* assays. Also, chemical composition was investigated by Gas chromatography-mass spectrometry (GC-MS) analysis and determination of total phenolic, flavonoid and tannin contents. To the best of our knowledge, this type of study has not yet been analyzed.

Materials and methods

Chemicals

α -Amylase (E.C. 3.2.1.1), α -glucosidase (E.C. 3.2.1.20), acarbose, Folin-Ciocalteu reagent, *p*-nitrophenyl- α -D-glucopyranoside, DPPH, ABTS, and H₂O₂ were

purchased from Sigma-Aldrich. Ascorbic acid, gallic acid, rutin, tannic acid, starch, dinitrosalicylic acid (DNSA), monobasic sodium phosphate, dibasic sodium phosphate, sodium carbonate, aluminum chloride, potassium persulfate, and sodium nitrite were purchased from Merck Company. All other solvents were of the highest purity and analytical grades.

Collection, identification, and extraction

The fresh plants of *A. parviflora* were collected from Dharmshala, Himachal Pradesh, with a GPS location of 32.2471° N and 76.3107° E. The plant was identified and authenticated by NISCAIR (Ref. NISCAIR/RHMD/Consult/2017/3083-32). A plant specimen was deposited in RHMD (Raw Materials Herbarium and Museum) of NISCAIR (National Institute of Science Communication and Information Resources) with reference number NISCAIR/RHMD/Consult/2017/3083-32.

The shoots were air-dried for 7 days and powered by an electric blender. Twenty-five grams of powdered sample was extracted with 125 mL of ethanol: water (90:10) solvent at the temperature of 50°C for 48 hours using a Soxhlet extractor. The recovered extract was filtered, and the solvent was concentrated using a rotary evaporator. Stock solution (1 mg/mL) was prepared and this stock solution was further diluted with the hydroalcoholic solvent to obtain different working concentrations.

GC-MS and FTIR analysis

One percent extract (1 μ L) was used to analyze the phytoconstituents in GC-MS analysis (Shimadzu QP-2010). The spectroscopic analysis involved a high electron ionization system (70 eV) with the constant flow of helium gas. The column (Omegawax 100) temperature was ranging from 50°C to 280°C with a gradual increase of 10°C/min. The total run time was 60 minutes. The phytoconstituents present in the extract were expressed as a percentage based on peak area produced in the chromatogram. For Fourier transform infrared spectrophotometer (FTIR) analysis, 1 mg semi-dried samples were used (Perkin Elmer FTIR Spectrometer). The frequency regions of spectrum analysis were from 4000 to 400 cm⁻¹ with 1 cm⁻¹ resolution. The mass spectra were identified and matched with the National Institute of Standard and Technology (NIST) library.

Quantification of phenolic, flavonoid and tannin content

Total phenolic content (TPC), total flavonoid content (TFC), and total tannin content (TTC) were estimated as reported by Slinkard and Singleton (16), Ahmed et al (17), and Kavitha Chandran and Indira (18), respectively. TPC, TFC, and TTC were calculated in the terms of milligrams of gallic acid equivalent per gram of extract (mg GAE/g extract), rutin equivalent per gram of extract (mg RE/g extract), and tannic acid equivalent per gram of extract (mg TAE/g extract), respectively.

Evaluation of *in vitro* antioxidant potential

DPPH radical scavenging activity

DPPH radical scavenging activity was measured according to the method reported by Brand-Williams et al (19), with some modifications (20). The stock solution was prepared by dissolving 2.4 mg DPPH with 10 mL methanol. After that, the prepared DPPH solution was further mixed with methanol to obtain an absorbance of 0.98 (± 0.02) at 517 nm. About 3 mL DPPH radical solution was mixed with 100 μ L of the sample/standard. This mixture was settled in the dark. Absorbance was then recorded at 517 nm. Ascorbic acid was taken as standard. The results were expressed as IC₅₀ values. The percent inhibition was calculated from the following formula:

$$\% \text{ Antioxidant activity} = [(A_{\text{control}} - A_{\text{standard/extract}} / A_{\text{control}})] \times 100$$

Where Abs_{control} corresponds to the reaction mixture without extracts/standard drug and Abs_{sample} corresponds to the reaction mixture with extract/standard drugs.

ABTS decolorization assay

ABTS decolorization assay was determined using a previously reported method (21). The stock solution of ABTS radical was prepared by 9.5 ml of ABTS (7 mM) along with 245 μ L of potassium persulfate (100 mM) by raising the volume up to 10 ml with distilled water. After that, this reaction mixture was stored in the dark at room temperature for 18 hours. ABTS solution was then diluted with potassium phosphate buffer (0.1M, pH 7.4) to obtain 0.70 (± 0.02) absorbance at 734 nm. The radical working solution was prepared by extract/standard (10 μ L) mixed with 2.90 mL ABTS. The absorbance was recorded at 734 nm. Ascorbic acid was taken as a standard. The results were expressed as IC₅₀ values. The percent antioxidant activity was determined using the following formula:

$$\% \text{ Antioxidant Activity} = [(A_{\text{control}} - A_{\text{standard/extract}} / A_{\text{control}})] \times 100$$

H₂O₂ radical scavenging activity

H₂O₂ radical scavenging activity was determined by the reported method of Patel et al (22). H₂O₂ radical solution was prepared in 40 mM phosphate buffer. About 50 μ L of samples were mixed with 100 μ L of 100 mM H₂O₂ at pH 7.4. After that, the reaction solution was incubated for 30 minutes in the dark and measured absorbance at 230 nm. Ascorbic acid was used as a standard. The results were expressed as IC₅₀ values. The percent antioxidant activity was calculated using the following formula:

$$\% \text{ Antioxidant activity} = [(A_{\text{control}} - A_{\text{standard/extract}} / A_{\text{control}})] \times 100$$

Evaluation of *in vitro* antidiabetic properties

α -Amylase inhibitory assay

α -Amylase inhibitory activity was evaluated by a previously modified method with minor modification

(23). A total 0.5 mL of extract was mixed with α -amylase (0.5 mg/mL) present in 0.02M sodium phosphate buffer (pH 6.9, 0.006M NaCl), which was incubated at 25°C for 10 minutes. 0.5 mL of 1% starch solution was added and placed at room temperature for 10 minutes. Afterward, the final reactions were terminated using 1 mL of DNSA reagent, placed in a water bath at 95°C for 10 minutes, and allowed to cool at room temperature. The reaction mixtures were then diluted with distilled water, and absorbance was recorded at 540 nm. Acarbose was used as a reference. The results were expressed as IC₅₀ values. The % inhibition of α -amylase was calculated using the following equation:

$$\text{Inhibition (\%)} = (Abs_{\text{control}} - Abs_{\text{sample}}) / (Abs_{\text{control}}) \times 100$$

α -Glucosidase inhibitory assay

α -Glucosidase inhibitory activity was measured as described by Kim et al (24). Briefly, 50 μ L of the sample solutions and 100 μ L of the α -glucosidase solution were incubated at 37°C for 10 minutes. After pre-incubation, 3 mM *p*-nitrophenyl- α -D-glucopyranoside (50 μ L), which was prepared in 20 mM phosphate buffer (pH 6.9) was added and then the reaction mixture was incubated for 20 minutes at 37°C. 0.1M Na₂CO₃ (2 mL) was added to terminate the reaction and the final absorbance readings were recorded at 405 nm. The results were expressed as IC₅₀ values. The % inhibition was calculated using the following equation:

$$\text{Inhibition (\%)} = (Abs_{\text{control}} - Abs_{\text{sample}}) / (Abs_{\text{control}}) \times 100$$

Statistical analysis

The experiments were performed in triplicates and expressed as mean \pm SEM (standard error of means). The data were subjected to analysis of variance (ANOVA) followed by Duncan's multiple comparison test ($P=0.05$) using SPSS version 21. IC₅₀ values were calculated using GraphPad Prism software version 8.3.0.

Results

GC-MS analysis

The chromatogram of GC-MS analysis has shown the presence of 65 different bioactive compounds including brassicasterol (10.33%), 3,6,6-trimethylundecane-2,5,10-trione (9.36%), palmitic acid (7.61%), 1H-naphtho[2,1-b]pyran-7-carboxylic acid, 3-ethenyldodecahydro-3,4a,7,10a-tetramethyl-,methyl ester (6.79%), bruceantin (6.43%), calysterol (6.08%), phytol (5.38%), vitamin E (4.07%), ethyl. alpha.-d-glucopyranoside (3.70%), 7-tetradecenal (2.92%), 3-phenylacrylaldehyde (2.06%), and acetic acid,3-methyl-6-oxo-hex-2-enyl ester (2.04%) (Table 1). The remaining compounds were less than two percent (Figure 1).

FTIR analysis

The FTIR spectrum of hydroalcoholic shoot extract of *A.*

Table 1. Compounds identified in the hydroalcoholic shoot extract from *Ajuga parviflora*

Retention time (min)	Area%	Molecular weight (g/mol)	Formula	Nature	Compound names
4.97	1.03	90	C ₃ H ₆ O ₃	Carboxylic acid	Propanoic acid, 3-hydroxy-
10.797	1.25	144	C ₆ H ₈ O ₄	Ketone	3-Hydroxy-2,3-dihydromaltol
11.506	2.06	132	C ₉ H ₈ O	Aldehyde	3-Phenylacrylaldehyde
13.230	2.04	170	C ₉ H ₁₄ O ₃	Ester	Acetic acid, 3-methyl-6-oxo-hex-2-enyl ester
14.76	1.10	150	C ₉ H ₁₀ O ₂	Phenolics	2-Methoxy-4-vinylphenol
18.659	1.33	164	C ₁₀ H ₁₂ O ₂	Ketone	Bicyclo[5.3.0]dec-1(7)-ene-2,5-dione
21.779	3.70	208	C ₈ H ₁₆ O ₆	Carbohydrate	Ethyl .alpha.-d-glucopyranoside
27.363	7.61	256	C ₁₆ H ₃₂ O ₂	Fatty acid	Palmitic acid
29.611	5.38	296	C ₂₀ H ₄₀ O	Diterpene	Phytol
30.033	1.94	280	C ₁₈ H ₃₂ O ₂	Fatty acid	9,12-Octadecadienoic acid (Z,Z)-
30.136	2.92	210	C ₁₄ H ₂₆ O	Ester	7-Tetradecenal, (Z)-
30.452	1.30	157	C ₂ H ₇ NO ₃ S ₂	Ester	2-Aminoethanethiol hydrogensulfate
39.209	1.32	410	C ₃₀ H ₅₀	Phytosteroid	Squalene
40.879	1.38	204	C ₁₃ H ₁₆ O ₂	Ketone	2,4-Hexanedione, 5-methyl-1-phenyl-5-Methyl-1-phenyl-2,4-hexanedione
43.313	9.36	240	C ₁₄ H ₂₄ O ₃	Ketone	3,6,6-Trimethylundecane-2,5,10-trione
44.220	1.00	430	C ₂₉ H ₅₀ O ₂	Terpenoid	Methanone, bis[4 (diethylamino)phenyl]
45.459	6.43	548	C ₂₈ H ₃₆ O ₁₁	Triterpenoid	Bruceantin
46.083	4.07	430	C ₂₉ H ₅₀ O ₂	Terpenoid	Vitamin E
47.036	6.08	410	C ₂₉ H ₄₆ O	Phytosterol	Calysterol
48.295	10.33	398	C ₂₈ H ₄₆ O	Phytosterol	Brassicasterol
51.328	6.79	334	C ₂₁ H ₃₄ O ₃	Carboxylic acid	1H-Naphtho[2,1-b]pyran-7-carboxylic acid, 3-ethenyldodecahydro-3,4a,7,10a-tetramethyl-, methyl ester
53.168	1.32	412	C ₂₉ H ₅₀ O ₂	Phytosterol	Stigmasterol
59.630	1.49	244	C ₁₂ H ₂₀ O ₅	Ester	Adipic acid, ethyl 3-oxobut-2-yl ester

parviflora confirmed the presence of various functional groups (Figure 2). The FTIR peak values and functional groups are presented in Table 2.

Quantitative of phenolics, flavonoids, and tannins

The total phenolic, flavonoid, and tannin contents were 64.06 ± 0.35 mg GAE/g, 45.27 ± 0.58 mg RE/g, and 127.42 ± 1.82 mg TAE/g, respectively in the hydroalcoholic extract of *A. parviflora* shoot. However, significant differences ($P \leq 0.05$) were observed among the different phytoconstituents (Figure 3).

DPPH, ABTS, and H₂O₂ radical scavenging activity

In the current investigation, the shoot extract of *A. parviflora* exhibited noticeable scavenging activity in a dose-dependent manner (20 µg/mL-750 µg/mL).

In DPPH scavenging assay, the IC₅₀ value for hydroalcoholic extract (103.03 ± 1.59 µg/mL) was close to that of standard drug ascorbic acid (90.72 ± 0.33 µg/mL) (Table 3). At the lower concentrations (25 and 50 µg/mL), shoot extract had better scavenging activity than ascorbic acid. Generally, the lower IC₅₀ value of the sample

indicates a higher antioxidant activity. Radical scavenging activity on ABTS radical exhibited excellent potency with respective IC₅₀ value of 140.10 ± 3.40 µg/mL. Further, *A. parviflora* was assessed for H₂O₂ scavenging capacity. The results exhibited significant scavenging capacity with IC₅₀ value of 298.26 ± 4.37 µg/mL. No significant difference ($P \leq 0.05$) was recorded between the scavenging activity of standard (ascorbic acid) and shoot samples (Figure 4).

Inhibition of α-amylase and α-glucosidase

The inhibition of α-amylase and α-glucosidase were evaluated to determine the antidiabetic activity of hydroalcoholic shoot extract of *A. parviflora*. Figure 5 shows the percentage inhibition against α-amylase and α-glucosidase and, the IC₅₀ values were also calculated (Table 3).

Ajuga parviflora showed significant inhibition of α-amylase and α-glucosidase enzyme. The hydroalcoholic shoot extract decreased the activity of the enzyme (α-amylase) in a concentration-dependent pattern and then increased significantly with increasing concentrations (25, 50, 75, 125, 250, and 500 µg/mL). The

Table 3. IC₅₀ value of DPPH, ABTS, H₂O₂, α-amylase and α-glucosidase

Samples	IC ₅₀ (µg/mL±SEM)				
	Scavenging ability on DPPH radical	Scavenging ability on ABTS radical	Scavenging ability on H ₂ O ₂ radical	Inhibition potential of α-amylase	Inhibition potential of α-glucosidase
Shoot extract	103.03 ± 1.59 ^a	140.10 ± 3.40 ^a	298.26 ± 4.37 ^a	132.38 ± 1.18 ^a	22.66 ± 0.11 ^a
Ascorbic acid	90.72 ± 0.33 ^b	88.77 ± 2.22 ^b	141.55 ± 1.01 ^b	—	—
Acarbose	—	—	—	51.67 ± 0.34 ^b	19.55 ± 0.09 ^b

IC₅₀: The half-maximal inhibitory concentration; DPPH: 2,2-diphenyl-2-picrylhydrazyl; ABTS: 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid); H₂O₂: hydrogen peroxide.

The superscript letters on each column represent significant differences between standard drug and extract as determined by Duncan's multiple range test at $P=0.05$ ($n=3$).

existence of different biologically active functional groups like amine, alcohol, and nitrile. The presence of lone pair of electrons in amine and nitrile functional groups could provide stability to the free radicals that further help in the prevention of oxidative stress in the cell. Wang et al also studied the antioxidant ability of the amine group and reported that introducing an amine group could improve the scavenging activity (30).

Secondary metabolites, like phenolic compounds, flavonoids, and tannins, have shown their potential in lowering blood sugar levels by inhibiting α-amylase and α-glucosidase activities and also for their antioxidant properties (31). Results depicted in Figure 3 showed that TTCs were higher than flavonoids and phenolics. Tannins include a large number of hydroxyl group associates that can interact with proteins in a nonspecific manner.

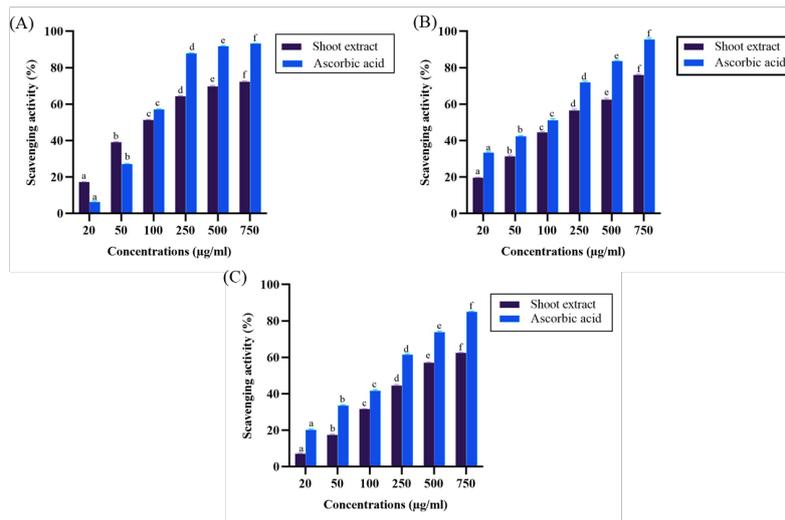


Figure 4. Radical scavenging activity against (A) DPPH (2,2-diphenyl-2-picrylhydrazyl radical); (B) ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) radical), and (C) H₂O₂ (hydrogen peroxide). The superscript letters on each column represent significant differences between standard drug and extract as determined by Duncan's multiple range test at $P=0.05$ ($n=3$).

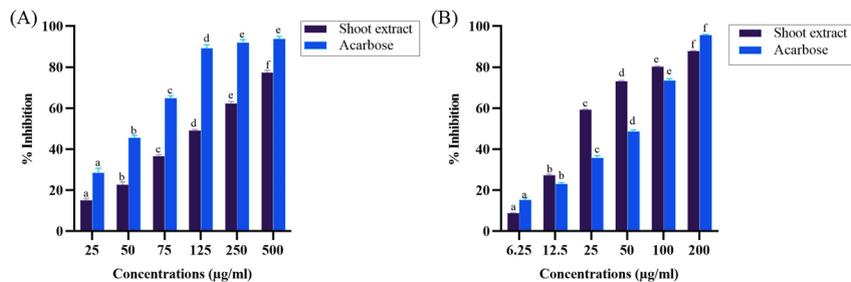


Figure 5. Inhibitory potential against α-amylase (A) and α-glucosidase (B). The superscript letters on each column represent significant differences between standard drug and extract as determined by Duncan's multiple range test at $P=0.05$ ($n=3$).

Recent investigations have shown that tannins are bound to α -amylase and α -glucosidase non-specifically and are important for optimising the inhibitor-protein interactions (32). In an important study, Gonçalves et al reported that the inhibition efficacy was strongly affected by the number, position, and protein binding interaction of hydroxyl substituents of tannins (33).

Free radical scavenging activity is the most common and relatively straightforward to determine the antioxidant capacity of plants and plant-based food products (34). In general, the radical scavenging capacity of an extract is directly proportional to its antioxidant properties (35). Hence, different radicals (DPPH, ABTS, and H_2O_2) were used as a substrate to determine the antioxidant capacity of *A. parviflora*. At lower concentrations, hydrogen peroxide (H_2O_2) acts as a signaling molecule, while higher concentrations of H_2O_2 in the cell may lead to the production of hydroxyl radicals that damages the biological membranes and sub-cellular organelles through oxidative stress. DPPH and ABTS are synthetic radicals that are commonly employed for the measurement of antioxidant activity (34). In this study, *A. parviflora* showed significant radical scavenging activity against DPPH, ABTS, and H_2O_2 radicals (Figure 4). Thus, *A. parviflora* shoot possesses noticeable antioxidant properties due to its free radical scavenging capacity. Maritim et al stated that oxidative stress causes lipid peroxidation, non-enzymatic protein glycation, and also increased insulin resistance in the physiological system of the body that leads to the genesis of diabetic mellitus (36). Thus, the searching for natural antioxidants that can prevent diabetes and ROS formation. Thakur et al reported 97.20% and 92.33% scavenging activity using *A. parviflora* whole plant extract at a concentration of 250 μ g/ml (37); however, there is no previous report for scavenging activity of *A. parviflora* shoot.

α -Amylase and α -glucosidase enzymes play a major role in hydrocarbon degradation and intestinal absorption. It has been reported that α -amylase and α -glucosidase inhibitors are beneficial in delaying glucose absorption after food consumption (38). Therefore, a significant approach to reduce and decrease postprandial hyperglycemia is to slow down carbohydrate-hydrolyzing digestion enzymes like α -amylase and α -glucosidase (39). Plants having strong inhibition for α -glucosidase and mild inhibition for α -amylase indicate good potency for the antidiabetic approach and can reduce or delay diabetes mellitus type II (40). A large number of studies have reported that these natural inhibitors could act as a safer alternative medicine for diabetes patients. *A. parviflora* shoot had stronger α -glucosidase inhibitory activity than α -amylase (Figure 5). The result supports the use of *A. parviflora* in traditional antidiabetic practices to cure diabetes. The present findings are in accordance with Rouzbehan et al results, where different species of Labiatae were reported to possess antidiabetic properties (41). Despite some

reports on bioactivities of *A. parviflora*, the present work is the first report regarding the chemical composition and the evaluation of antioxidant and antidiabetic activities of *A. parviflora* shoot.

Conclusion

In conclusion, our findings showed that *A. parviflora* Benth. is endowed with numerous pharmacologically active phytoconstituents and has significant antidiabetic and antioxidant activities. Therefore, this plant might be used as a natural source for the study of antidiabetic drugs and natural antioxidants. However, further studies are encouraged to isolate and screen the bioactive compounds to be a boon against oxidative stress and diabetes complications.

Acknowledgement

The authors are grateful to Prof. Suman Lakhanpaul, Head, Department of Botany, University of Delhi, and Dr. Manoj K. Khanna, Principal, Ramjas College, for encouragement and support.

Authors' contributions

AS performed the experiments, analyzed the data and drafted the manuscript. SK designed the experiments, monitored, and edited the manuscript. V, DK, and AA reviewed the manuscript and confirmed it for publication.

Conflict of interests

The authors declare no conflict of interest.

Ethical considerations

All ethical issues (such as plagiarism, misconduct, data fabrication, falsification, double publication or submission, redundancy) have been completely observed by the authors. The authors claim that no animal and human subjects were associated with this examination.

Funding/Support

The authors are indebted to University Grant Commission (UGC), India for financial support during the investigation (Grant Number:21/06/2015(i)EU-V).

References

1. Donkor S, Larbie C, Komlaga G, Emikpe BO. Phytochemical, antimicrobial, and antioxidant profiles of *Duranta erecta* L. parts. *Biochem Res Int.* 2019;2019:8731595. doi: 10.1155/2019/8731595.
2. Samyurai P, Thangapandian V, Aravinthan V. Wild habits of Kolli Hills being staple food of inhabitant tribes of Eastern Ghats, Tamil Nadu, India. *Indian J Nat Prod Resour.* 2012;3(3):432-7.
3. Kasote DM, Katyare SS, Hegde MV, Bae H. Significance of antioxidant potential of plants and its relevance to therapeutic applications. *Int J Biol Sci.* 2015;11(8):982-91. doi: 10.7150/ijbs.12096.
4. Chatterjee S, Khunti K, Davies MJ. Type 2 diabetes.

- Lancet. 2017;389(10085):2239-51. doi: 10.1016/s0140-6736(17)30058-2.
5. Shin HJ, Kim JH, Yi JH, Han SW, Kim HJ. Polyuria with the concurrent manifestation of central diabetes insipidus (CDI) & type 2 diabetes mellitus (DM). *Electrolyte Blood Press.* 2012;10(1):26-30. doi: 10.5049/ebp.2012.10.1.26.
 6. Alhadramy MS. Diabetes and oral therapies: a review of oral therapies for diabetes mellitus. *J Taibah Univ Med Sci.* 2016;11(4):317-29. doi: 10.1016/j.jtumed.2016.02.001.
 7. Türkan F, Atalar MN, Aras A, Gülçin İ, Bursal E. ICP-MS and HPLC analyses, enzyme inhibition and antioxidant potential of *Achillea schischkinii* Sosn. *Bioorg Chem.* 2020;94:103333. doi: 10.1016/j.bioorg.2019.103333.
 8. Oguntibeju OO. Type 2 diabetes mellitus, oxidative stress and inflammation: examining the links. *Int J Physiol Pathophysiol Pharmacol.* 2019;11(3):45-63.
 9. Kurutas EB. The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: current state. *Nutr J.* 2016;15(1):71. doi: 10.1186/s12937-016-0186-5.
 10. Thakur M, Asrani RK, Thakur S, Sharma PK, Patil RD, Lal B, et al. Observations on traditional usage of ethnomedicinal plants in humans and animals of Kangra and Chamba districts of Himachal Pradesh in North-Western Himalaya, India. *J Ethnopharmacol.* 2016;191:280-300. doi: 10.1016/j.jep.2016.06.033.
 11. Arya D, Goel S, Joshi GC, Sharma OR, Sharma SK. Medico-ethno botanical practices among Bhotia tribe of Kumaon Himalaya: a case study from Bageshwar district, Uttarakhand, India. *J Drug Res Ayurvedic Sci.* 2018;3(1):43-7. doi: 10.5005/jp-journals-10059-0034.
 12. Kumar A, Aswal S, Chauhan A, Semwal RB, Kumar A, Semwal DK. Ethnomedicinal investigation of medicinal plants of Chakrata region (Uttarakhand) used in the traditional medicine for diabetes by Jaunsari tribe. *Nat Prod Bioprospect.* 2019;9(3):175-200. doi: 10.1007/s13659-019-0202-5.
 13. Yousaf T, Rafique S, Wahid F, Rehman S, Nazir A, Rafique J, et al. Phytochemical profiling and antiviral activity of *Ajuga bracteosa*, *Ajuga parviflora*, *Berberis lycium* and *Citrus lemon* against hepatitis C virus. *Microb Pathog.* 2018;118:154-8. doi: 10.1016/j.micpath.2018.03.030.
 14. Suryavanshi A, Kumar S, Arya DK. Evaluation of phytochemical and antibacterial potential of *Ajuga parviflora* Benth. *Med Plants Int J Phytomed Relat Ind.* 2020;12(1):144-9. doi: 10.5958/0975-6892.2020.00019.2.
 15. Burki S, Burki ZG, Mehjabeen, Ahmed I, Jahan N, Owais F, et al. GC/MS assisted phytochemical analysis of *Ajuga parviflora* leaves extract along with anti-hepatotoxic effect against anti-tubercular drug induced liver toxicity in rat. *Pak J Pharm Sci.* 2020;33(1 Suppl):325-31.
 16. Slinkard K, Singleton VL. Total phenol analysis: automation and comparison with manual methods. *Am J Enol Vitic.* 1977;28(1):49-55.
 17. Ahmed D, Fatima K, Saeed R. Analysis of phenolic and flavonoid contents, and the anti-oxidative potential and lipid peroxidation inhibitory activity of methanolic extract of *Carissa opaca* roots and its fractions in different solvents. *Antioxidants (Basel).* 2014;3(4):671-83. doi: 10.3390/antiox3040671.
 18. Kavitha Chandran CI, Indira G. Quantitative estimation of total phenolic, flavonoids, tannin and chlorophyll content of leaves of *Strobilanthes kunthiana* (Neelakurinji). *J Med Plants Stud.* 2016;4(4):282-6.
 19. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. *LWT Food Sci Technol.* 1995;28(1):25-30. doi: 10.1016/S0023-6438(95)80008-5.
 20. Shah NA, Khan MR, Ahmad B, Noureen F, Rashid U, Khan RA. Investigation on flavonoid composition and anti free radical potential of *Sida cordata*. *BMC Complement Altern Med.* 2013;13:276. doi: 10.1186/1472-6882-13-276.
 21. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic Biol Med.* 1999;26(9-10):1231-7. doi: 10.1016/s0891-5849(98)00315-3.
 22. Patel A, Patel A, Patel A, Patel NM. Determination of polyphenols and free radical scavenging activity of *Tephrosia purpurea* linn leaves (Leguminosae). *Pharmacognosy Res.* 2010;2(3):152-8. doi: 10.4103/0974-8490.65509.
 23. Ranilla LG, Kwon YI, Genovese MI, Lajolo FM, Shetty K. Antidiabetes and antihypertension potential of commonly consumed carbohydrate sweeteners using in vitro models. *J Med Food.* 2008;11(2):337-48. doi: 10.1089/jmf.2007.689.
 24. Kim YM, Jeong YK, Wang MH, Lee WY, Rhee HI. Inhibitory effect of pine extract on alpha-glucosidase activity and postprandial hyperglycemia. *Nutrition.* 2005;21(6):756-61. doi: 10.1016/j.nut.2004.10.014.
 25. Mukherjee K, Biswas R, Chaudhary SK, Mukherjee PK. Botanicals as medicinal food and their effects against obesity. In: Mukherjee PK, ed. *Evidence-Based Validation of Herbal Medicine*. Boston: Elsevier; 2015. p. 373-403. doi: 10.1016/b978-0-12-800874-4.00018-0.
 26. Srigley CT, Haile EA. Quantification of plant sterols/stanols in foods and dietary supplements containing added phytosterols. *J Food Compos Anal.* 2015;40:163-76. doi: 10.1016/j.jfca.2015.01.008.
 27. Ponnamma SU, Manjunath K. GC-MS Analysis of phytochemicals in the methanolic extract of *Justicia wynaadensis* (Nees) T. Anders. *Int J Pharma Bio Sci.* 2012;3(3):570-6.
 28. Elmazar MM, El-Abhar HS, Schaalan MF, Farag NA. Phytol/Phytanic acid and insulin resistance: potential role of phytanic acid proven by docking simulation and modulation of biochemical alterations. *PLoS One.* 2013;8(1):e45638. doi: 10.1371/journal.pone.0045638.
 29. Sidhu MC, Thakur S. Documentation of antidiabetic medicinal plants in district Mandi of Himachal Pradesh (India). *Int J PharmTech Res.* 2015;8(8):164-69.
 30. Wang L, Yang F, Zhao X, Li Y. Effects of nitro- and amino-group on the antioxidant activity of genistein: a theoretical study. *Food Chem.* 2019;275:339-45. doi: 10.1016/j.foodchem.2018.09.108.
 31. Pieczykolan A, Pietrzak W, Gawlik-Dziki U, Nowak R. Antioxidant, anti-inflammatory, and anti-diabetic activity of phenolic acids fractions obtained from *Aerva lanata* (L.) Juss. *Molecules.* 2021;26(12):3486. doi: 10.3390/molecules26123486.
 32. da Silva SM, Koehnlein EA, Bracht A, Castoldi R, de Moraes

- GR, Baesso ML, et al. Inhibition of salivary and pancreatic α -amylases by a pinhão coat (*Araucaria angustifolia*) extract rich in condensed tannin. *Food Res Int.* 2014;56:1-8. doi: 10.1016/j.foodres.2013.12.004.
33. Gonçalves R, Mateus N, de Freitas V. Inhibition of α -amylase activity by condensed tannins. *Food Chem.* 2011;125(2):665-72. doi: 10.1016/j.foodchem.2010.09.061.
34. Köksal E, Gülçin I, Beyza S, Sarikaya O, Bursal E. In vitro antioxidant activity of silymarin. *J Enzyme Inhib Med Chem.* 2009;24(2):395-405. doi: 10.1080/14756360802188081.
35. Khan RA, Khan MR, Sahreen S, Ahmed M. Evaluation of phenolic contents and antioxidant activity of various solvent extracts of *Sonchus asper* (L.) Hill. *Chem Cent J.* 2012;6(1):12. doi: 10.1186/1752-153x-6-12.
36. Maritim AC, Sanders RA, Watkins JB 3rd. Diabetes, oxidative stress, and antioxidants: a review. *J Biochem Mol Toxicol.* 2003;17(1):24-38. doi: 10.1002/jbt.10058.
37. Thakur N, Richa, Sharma ML. Antidiabetic and antioxidant activity of ethanolic extract of *Ajuga parviflora* Benth. (Lamiaceae) vern. Neelkanthi, Neelbati. *Int J Pharm Sci Rev Res.* 2016;41(2):232-8.
38. Kim KY, Nam KA, Kurihara H, Kim SM. Potent α -glucosidase inhibitors purified from the red alga *Grateloupia elliptica*. *Phytochemistry.* 2008;69(16):2820-5. doi: 10.1016/j.phytochem.2008.09.007.
39. Hanhineva K, Törrönen R, Bondia-Pons I, Pekkinen J, Kolehmainen M, Mykkänen H, et al. Impact of dietary polyphenols on carbohydrate metabolism. *Int J Mol Sci.* 2010;11(4):1365-402. doi: 10.3390/ijms11041365.
40. Krentz AJ, Bailey CJ. Oral antidiabetic agents: current role in type 2 diabetes mellitus. *Drugs.* 2005;65(3):385-411. doi: 10.2165/00003495-200565030-00005.
41. Rouzbehan S, Moein S, Homaei A, Moein MR. Kinetics of α -glucosidase inhibition by different fractions of three species of Labiatae extracts: a new diabetes treatment model. *Pharm Biol.* 2017;55(1):1483-8. doi: 10.1080/13880209.2017.1306569.