



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

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## 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 (H<sub>2</sub>O<sub>2</sub>) 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  $\alpha$ -amylase enzyme (132.38 $\pm$ 1.18  $\mu$ g/mL),  $\alpha$ -glucosidase enzyme (22.66 $\pm$ 0.11  $\mu$ g/mL), DPPH radical (103.03 $\pm$ 1.59  $\mu$ g/mL), ABTS radical (140.10 $\pm$ 3.40  $\mu$ g/mL) and H<sub>2</sub>O<sub>2</sub> radical (298.26 $\pm$ 4.37  $\mu$ 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.

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## 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

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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

$\alpha$ -Amylase (E.C. 3.2.1.1),  $\alpha$ -glucosidase (E.C. 3.2.1.20), acarbose, Folin-Ciocalteu reagent, *p*-nitrophenyl- $\alpha$ -D-glucopyranoside, DPPH, ABTS, and H<sub>2</sub>O<sub>2</sub> 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  $\mu$ 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<sup>-1</sup> with 1 cm<sup>-1</sup> 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 ( $\pm 0.02$ ) at 517 nm. About 3 mL DPPH radical solution was mixed with 100  $\mu$ 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 IC<sub>50</sub> 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<sub>control</sub> corresponds to the reaction mixture without extracts/standard drug and Abs<sub>sample</sub> 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  $\mu$ 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 ( $\pm 0.02$ ) absorbance at 734 nm. The radical working solution was prepared by extract/standard (10  $\mu$ 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 IC<sub>50</sub> values. The percent antioxidant activity was determined using the following formula:

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

#### H<sub>2</sub>O<sub>2</sub> radical scavenging activity

H<sub>2</sub>O<sub>2</sub> radical scavenging activity was determined by the reported method of Patel et al (22). H<sub>2</sub>O<sub>2</sub> radical solution was prepared in 40 mM phosphate buffer. About 50  $\mu$ L of samples were mixed with 100  $\mu$ L of 100 mM H<sub>2</sub>O<sub>2</sub> 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 IC<sub>50</sub> 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

#### $\alpha$ -Amylase inhibitory assay

$\alpha$ -Amylase inhibitory activity was evaluated by a previously modified method with minor modification

(23). A total 0.5 mL of extract was mixed with  $\alpha$ -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 IC<sub>50</sub> values. The % inhibition of  $\alpha$ -amylase was calculated using the following equation:

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

#### $\alpha$ -Glucosidase inhibitory assay

$\alpha$ -Glucosidase inhibitory activity was measured as described by Kim et al (24). Briefly, 50  $\mu$ L of the sample solutions and 100  $\mu$ L of the  $\alpha$ -glucosidase solution were incubated at 37°C for 10 minutes. After pre-incubation, 3 mM *p*-nitrophenyl- $\alpha$ -D-glucopyranoside (50  $\mu$ 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 Na<sub>2</sub>CO<sub>3</sub> (2 mL) was added to terminate the reaction and the final absorbance readings were recorded at 405 nm. The results were expressed as IC<sub>50</sub> 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. IC<sub>50</sub> 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 <sub>3</sub> H <sub>6</sub> O <sub>3</sub>                 | Carboxylic acid | Propanoic acid, 3-hydroxy-   |
| 10.797               | 1.25  | 144                      | C <sub>6</sub> H <sub>8</sub> O <sub>4</sub>                 | Ketone          | 3-Hydroxy-2,3-dihydromaltol  |
| 11.506               | 2.06  | 132                      | C <sub>9</sub> H <sub>8</sub> O                              | Aldehyde        | 3-Phenylacrylaldehyde  |
| 13.230               | 2.04  | 170                      | C <sub>9</sub> H <sub>14</sub> O <sub>3</sub>                | Ester           | Acetic acid, 3-methyl-6-oxo-hex-2-enyl ester   |
| 14.76                | 1.10  | 150                      | C <sub>9</sub> H <sub>10</sub> O <sub>2</sub>                | Phenolics       | 2-Methoxy-4-vinylphenol  |
| 18.659               | 1.33  | 164                      | C <sub>10</sub> H <sub>12</sub> O <sub>2</sub>               | Ketone          | Bicyclo[5.3.0]dec-1(7)-ene-2,5-dione   |
| 21.779               | 3.70  | 208                      | C <sub>8</sub> H <sub>16</sub> O <sub>6</sub>                | Carbohydrate    | Ethyl .alpha.-d-glucopyranoside  |
| 27.363               | 7.61  | 256                      | C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>               | Fatty acid      | Palmitic acid  |
| 29.611               | 5.38  | 296                      | C <sub>20</sub> H <sub>40</sub> O                            | Diterpene       | Phytol   |
| 30.033               | 1.94  | 280                      | C <sub>18</sub> H <sub>32</sub> O <sub>2</sub>               | Fatty acid      | 9,12-Octadecadienoic acid (Z,Z)-   |
| 30.136               | 2.92  | 210                      | C <sub>14</sub> H <sub>26</sub> O                            | Ester           | 7-Tetradecenal, (Z)-   |
| 30.452               | 1.30  | 157                      | C <sub>2</sub> H <sub>7</sub> NO <sub>3</sub> S <sub>2</sub> | Ester           | 2-Aminoethanethiol hydrogensulfate   |
| 39.209               | 1.32  | 410                      | C <sub>30</sub> H <sub>50</sub>                              | Phytosteroid    | Squalene   |
| 40.879               | 1.38  | 204                      | C <sub>13</sub> H <sub>16</sub> O <sub>2</sub>               | Ketone          | 2,4-Hexanedione, 5-methyl-1-phenyl-5-Methyl-1-phenyl-2,4-hexanedione                                 |
| 43.313               | 9.36  | 240                      | C <sub>14</sub> H <sub>24</sub> O <sub>3</sub>               | Ketone          | 3,6,6-Trimethylundecane-2,5,10-trione  |
| 44.220               | 1.00  | 430                      | C <sub>29</sub> H <sub>50</sub> O <sub>2</sub>               | Terpenoid       | Methanone, bis[4 (diethylamino)phenyl]   |
| 45.459               | 6.43  | 548                      | C <sub>28</sub> H <sub>36</sub> O <sub>11</sub>              | Triterpenoid    | Bruceantin   |
| 46.083               | 4.07  | 430                      | C <sub>29</sub> H <sub>50</sub> O <sub>2</sub>               | Terpenoid       | Vitamin E  |
| 47.036               | 6.08  | 410                      | C <sub>29</sub> H <sub>46</sub> O                            | Phytosterol     | Calysterol   |
| 48.295               | 10.33 | 398                      | C <sub>28</sub> H <sub>46</sub> O                            | Phytosterol     | Brassicasterol   |
| 51.328               | 6.79  | 334                      | C <sub>21</sub> H <sub>34</sub> O <sub>3</sub>               | 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 <sub>29</sub> H <sub>50</sub> O <sub>2</sub>               | Phytosterol     | Stigmasterol   |
| 59.630               | 1.49  | 244                      | C <sub>12</sub> H <sub>20</sub> O <sub>5</sub>               | 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 H<sub>2</sub>O<sub>2</sub> 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).

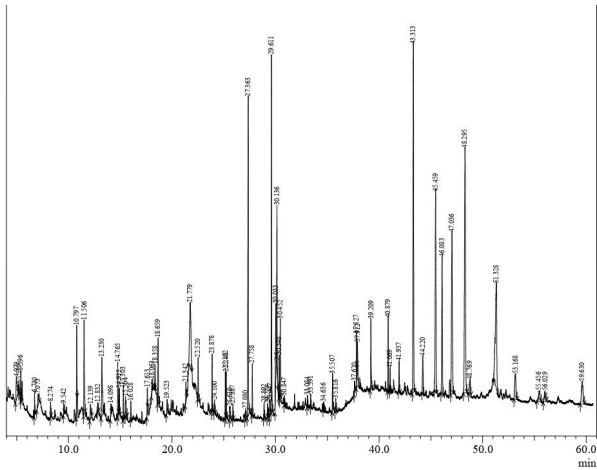
In DPPH scavenging assay, the IC<sub>50</sub> value for hydroalcoholic extract (103.03 ± 1.59 µg/mL) was close to that of standard drug ascorbic acid (90.72 ± 0.33 µg/mL) (Table 3). At the lower concentrations (25 and 50 µg/mL), shoot extract had better scavenging activity than ascorbic acid. Generally,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

indicates a higher antioxidant activity. Radical scavenging activity on ABTS radical exhibited excellent potency with respective IC<sub>50</sub> value of 140.10 ± 3.40 µg/mL. Further, *A. parviflora* was assessed for H<sub>2</sub>O<sub>2</sub> scavenging capacity. The results exhibited significant scavenging capacity with IC<sub>50</sub> 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 IC<sub>50</sub> 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

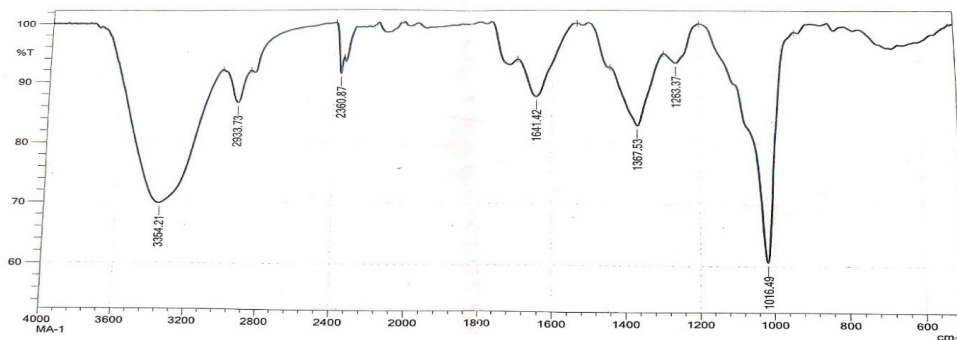


**Figure 1.** Gas chromatography–mass spectrometry (GC-MS) chromatogram of hydroalcoholic shoot extract of *Ajuga parviflora*.

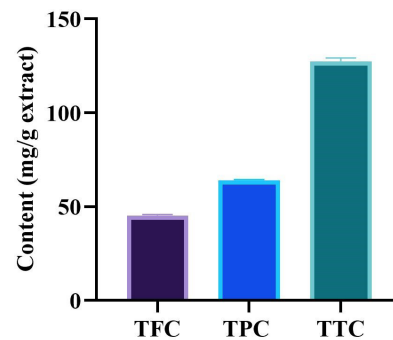
extract showed effective inhibition for  $\alpha$ -amylase with  $IC_{50}$  value being  $132.38 \pm 1.18 \mu\text{g/mL}$ , which is much lower than the standard drug acarbose ( $51.67 \pm 0.34 \mu\text{g/mL}$ ). A concentration-dependent in  $\alpha$ -glucosidase inhibition was observed in 6.25 to 200  $\mu\text{g/mL}$ . The extract showed excellent inhibition for  $\alpha$ -glucosidase with an  $IC_{50}$  value of  $22.66 \pm 0.11 \mu\text{g/mL}$ . The extract exhibited strong inhibition for  $\alpha$ -glucosidase and, the  $IC_{50}$  value was so close to the standard reference drug ( $19.55 \pm 0.09 \mu\text{g/mL}$ ). The shoot

**Table 2.** Fourier transform infrared spectrophotometer peak values and functional groups present in hydroalcoholic shoot extract of *Ajuga parviflora*

| Peak ( $\text{cm}^{-1}$ ) | Type        | Functional groups |
|---------------------------|-------------|-------------------|
| 3354.21                   | O-H Bending | Alcohol           |
| 2933.73                   | C-H Stretch | Alkane            |
| 2360.87                   | CN Stretch  | Nitrile           |
| 1641.42                   | C=C Stretch | Alkene            |
| 1367.53                   | C-F Stretch | Alkyl halide      |
| 1263.37                   | N-H Stretch | Amine             |
| 1016.49                   | C-O Stretch | Ether             |



**Figure 2.** Fourier transform infrared spectrophotometer spectrum of hydroalcoholic shoot extract of *Ajuga parviflora*.



**Figure 3.** Quantitative phytochemical analysis of *Ajuga parviflora* shoot. TFC: Total flavonoid content, TPC: Total phenolic content, TTC: Total tannin content.

extract exhibited strong inhibition with a significant  $IC_{50}$  value. No significant difference was observed in the enzymatic inhibition between standard (acarbose) and shoot samples.

### Discussion

In the present study, shoot extract of *Ajuga parviflora* was evaluated for biochemical profiling and total phytochemical contents (TPC, TFC, and TTC). The antidiabetic and antioxidant potential was also assessed. The GC-MS results revealed numerous biologically active compounds responsible for biological and pharmacological activities. Results showed that *A. parviflora* is rich in bioactive compounds, such as brassicasterol, palmitic acid, and phytol. Among these bioactive compounds, brassicasterol was found to be present in a higher percentage (10.33%). Brassicasterol is a phytosterol that is widely employed for its health benefits as an anti-obesity, cholesterol-lowering, and antidiabetic agent (25,26). Similarly, palmitic acid (7.61%) has been reported to exhibit pharmacological properties by acting as an antidiabetic and antioxidant agent (27). Moreover, phytol (5.38%) has been shown to possess antidiabetic and antioxidant activities (28). Therefore, the presence of these bioactive compounds validates the use of *A. parviflora* in the Himalayan folkloric medicine (29). The FTIR spectroscopy revealed the

**Table 3.** IC<sub>50</sub> value of DPPH, ABTS, H<sub>2</sub>O<sub>2</sub>, α-amylase and α-glucosidase

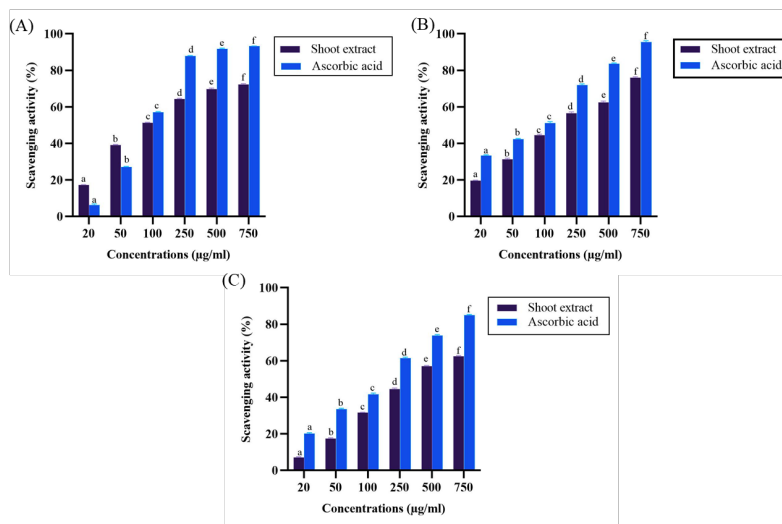
| Samples       | IC <sub>50</sub> (µg/mL±SEM)       |                                    |   |                                   |                                       |
|---------------|------------------------------------|------------------------------------|---|-----------------------------------|---------------------------------------|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

IC<sub>50</sub>: The half-maximal inhibitory concentration; DPPH: 2,2-diphenyl-2-picrylhydrazyl; ABTS: 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid); H<sub>2</sub>O<sub>2</sub>: hydrogen peroxide.

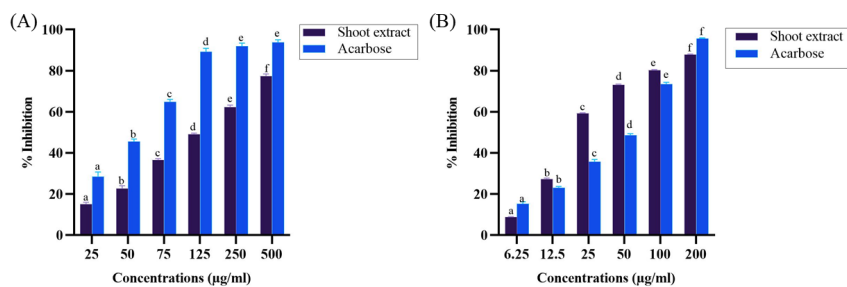
The superscript letters on each column represent significant differences between standard drug and extract as determined by Duncan's multiple range test at  $P=0.05$  ( $n=3$ ).

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) H<sub>2</sub>O<sub>2</sub> (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  $\alpha$ -amylase and  $\alpha$ -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  $\mu$ g/ml (37); however, there is no previous report for scavenging activity of *A. parviflora* shoot.

$\alpha$ -Amylase and  $\alpha$ -glucosidase enzymes play a major role in hydrocarbon degradation and intestinal absorption. It has been reported that  $\alpha$ -amylase and  $\alpha$ -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  $\alpha$ -amylase and  $\alpha$ -glucosidase (39). Plants having strong inhibition for  $\alpha$ -glucosidase and mild inhibition for  $\alpha$ -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  $\alpha$ -glucosidase inhibitory activity than  $\alpha$ -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.

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### 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.

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