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In vitro Antioxidant and Antidiabetic Activity of Solvent Extracts from Aerial Parts of *Polygonum plebeium* R. Br.

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This study investigated the aerial parts of *Polygonum plebeium* for their phytochemical composition, antioxidant potential, *in vitro* cytotoxicity and glucose uptake activity in yeast cells. Different solvent extracts, namely petroleum ether, chloroform, ethyl acetate, ethanol and aqueous, were evaluated. Antioxidant activity was assessed using DPPH, hydrogen peroxide scavenging and reducing power assays. HPLC analysis of the ethanol extract indicated the presence of several bioactive constituents with distinct retention times. Cytotoxicity studies on human pancreatic Min6 cells showed values of 59 ± 0.84% and 79 ± 0.78% for ethanol and ethyl acetate extracts, respectively, at 800 µg/mL. Among all tested extracts, the ethanol extract demonstrated the strongest antioxidant and antidiabetic potential. These findings suggest that the aerial parts of *P. plebeium* possess promising hypoglycemic activity and may serve as a potential natural source for diabetes management.

Keywords: Phytochemical, *In vitro*, Antioxidant, Cytotoxicity, Antidiabetic.

INTRODUCTION

Diabetes is increasing rapidly worldwide, particularly in developing countries. Currently, about 589 million people aged 20-79 years are living with diabetes, affecting nearly 1 in every 9 individuals. This number is projected to reach 853 million by 2050. In 2024, diabetes caused approximately 3.4 million deaths globally, equivalent to one death every 9 seconds and healthcare expenditure has exceeded USD 1 trillion, showing a 338% rise over the last 17 years [1,2].

Diabetes mellitus (DM) is a metabolic disorder characterized by persistent hyperglycaemia caused by impaired insulin secretion, insulin resistance or both. It affects carbohydrate, lipid and protein metabolism [3]. Type 1 diabetes accounts for about 5% of cases and requires insulin therapy. Other forms include secondary diabetes linked to chronic pancreatitis or long-term drug use and gestational diabetes caused by the pregnancy-related hormonal insulin resistance [4,5]. The common symptoms include polyuria, polydipsia, polyphagia, weight loss, fatigue, blurred vision, numbness and severe hyperglycaemia [6]. Although synthetic oral hypoglycaemic agents are widely used, they may cause adverse effects such as anorexia, gastric discomfort, drowsiness and weight gain [3]. Traditional medicine reports nearly 800 medicinal plants with antidiabetic potential, though only a limited number have been scientifically investigated for their phytochemicals and mechanisms [7].

Phytochemists have extensively explored medicinal plants to identify new antidiabetic agents capable of regulating blood glucose levels. Herbal remedies are widely preferred due to their safety, affordability and long-standing therapeutic use [8,9]. Many plant-derived phytochemicals have shown promise in the management of diabetes and related disorders [10]. Major bioactive groups such as flavonoids, alkaloids, tannins, phenolics, terpenoids, steroids and glycosides exhibit antidiabetic effects by stimulating insulin secretion, improving pancreatic function, reducing oxidative stress or inhibiting intestinal glucose absorption [11-14].

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Among these, flavonoids are particularly important polyphenolic compounds commonly present in edible and medicinal plants, including *Polygonum plebeium*. This plant has attracted attention as a potential source of antidiabetic agents due to its antioxidant, anti-inflammatory and glucose-lowering properties [15]. Quercetin, one of the best-studied flavonoids, modulates insulin signalling through PI3K pathways, inhibits GLUT2-mediated glucose absorption and reduces oxidative stress [16-18]. Other flavonoids such as rutin and kaempferol have also demonstrated beneficial effects on glycaemic control, highlighting their therapeutic value in diabetes management [19]. Recent studies by Nayak *et al.* [20] further reported that different parts of *P. plebeium* contain several phytochemicals with significant pharmacological potential, including antidiabetic activity.

Polygonum plebeium R. Br., commonly known as small knotweed, is a plant that belongs to the Polygonaceae family. An ethnomedical study indicates that rural populations use the powdered leaves to address menstrual irregularities and consume the entire plant as a food source. The aerial parts of the plant alone are employed in the treatment of various conditions, including pneumonia, inflammation, liver disorders, ringworm, dermatitis and digestive issues. Phytochemical investigations have shown that the aerial parts, roots, flowers, and leaves of *P. plebeium* possess diverse biological activities, including antioxidant, antibacterial, anti-inflammatory, cytoprotective effects and have traditionally been used in the management of dermatitis and pneumonia. However, available literature indicates limited scientific information on the leaves of this species and insufficient evaluation of its antidiabetic potential [20]. Therefore, the present study aimed to investigate the phytochemical constituents, antioxidant activity, antidiabetic potential and cytotoxicity of different solvent extracts obtained from the aerial parts of *P. plebeium*.

EXPERIMENTAL

Collection of plant material: The aerial part of *P. plebeium* was collected in April 2023 from Mulugaon Village, Jagatsinghpur, India (20.21º N, 86.27º E) and verified by Dr. K. Karthigeyan, Scientist-E at the Botanical Survey of India in Calcutta, India. A voucher specimen (CNH//2316Tech.II/2023/94) was submitted to the Pharmacology Department of the Faculty of Pharmacy at SOA University, Bhubaneswar. The aerial parts were thoroughly cleaned with normal tap water and dried in the shade and were kept at room temperature. A mechanical grinder was used to crush the dried plant material into coarse powder. The coarse powder from the plant material passed through mesh number 60. The plant material powder was kept in an airtight container for future use.

Crude extraction: In Soxhlet apparatus, 100 g of *P. plebeium* dry powder was placed in a thimble and subjected to a series of extractions using solvents of increasing polarity, namely petroleum ether, chloroform, ethyl acetate, ethanol and an aqueous phase. The process involves using petroleum ether at a temperature of 40-60 ºC, with a solvent-to-sample ratio of 5:1 to 10:1 (v/w) for a duration of 6 to 8 h, which corresponds to about 10 to 15 cycles. This initial phase aims to remove non-polar compounds such as fats and oils, from the petroleum ether extract.

Following this, chloroform (temperature 60-62 ºC) was employed under a similar solvent-to-sample ratio and extracted for an additional 6-8 h with 10-15 cycles to segregate slightly polar phytoconstituents. The next solvent used was ethyl acetate (temperature 77 ºC), extracted for 8-10 h with 12-18 cycles to collect moderately polar compounds. Subsequently, ethanol (temperature 78 ºC) was applied at a ratio of 8:12 (v/w) and extracted for 10-12 h with 15-20 cycles to obtain polar phytochemicals, including phenolics and glycosides.

Lastly, aqueous extraction was performed by using a Soxhlet apparatus through a reflux process by maintaining a temperature of 100 ºC, lasting for 6-8 h with 10-15 cycles, to recover highly polar phytoconstituents. During each extraction process, the solvent part was evaporated by using a rotary evaporator to yield the respective extract. The remaining aqueous fraction of each crude solvent extract was removed through freeze-drying and subsequently stored at 4 ºC for future use. The yields of petroleum ether, chloroform, ethyl acetate, ethanol and the aqueous extract of the crude extract from *P. plebeium* were 3.06%, 2.3%, 8.2%, 9.5% and 12.05% w/w, respectively. The final yield of solvent extract was placed in a desiccator for future purpose.

Phytochemical analysis

Qualitative analysis: A qualitative phytochemical analysis was performed on extracts of *P. plebeium* using petroleum ether, chloroform, ethyl acetate, ethanol and aqueous solvents. Standard procedures were employed to ascertain the presence or absence of organic compounds. These compounds include alkaloids, carbohydrates (Molisch's test), flavonoids, proteins (Biuret test), amino acids, saponins (Froth formation test), tannins, glycosides and steroids, as briefly described earlier [21].

Quantitative analysis of phytochemical

Quantification of total phenolic content: The total phenolic content (TPC) of each solvent extract obtained from the aerial part of *P. plebeium* was measured following the procedure described by Wolfe *et al.* [22] with minor modifications. Briefly, 1 mL plant extract sample of different dilution and Folin-Ciocalteu reagent were mixed. Afterwards, 2.8 mL of $NaHCO_3$ was added to the reaction solution. The resulting mixture was then incubated for 30 min. Absorbance was subsequently measured at 725 nm using a UV/VIS spectrophotometer (India) on the model SE 807. Gallic acid was used as the standard, while methanol acted as the negative control. The TPC results were expressed as mg GAE/g of extract (mg of gallic acid equivalent per g), with the average of three measurements recorded for each solvent extract.

Quantification of total flavonoid content: The total flavonoid content (TFC) of each solvent extract obtained from the aerial part of *P. plebeium* extracts was assessed using the method outlined by Ordonez *et al.* [23], with a minor modification. TFC was measured using the $AlCl_3$ procedure in all sample solutions. In this method, TFC was determined from calibration curve as rutin used as standard. A 1 mL of standard (rutin) or test solution was mixed with an equal amount of 2% $AlCl_3$. Absorption was measured at 415 nm using a UV-Visible spectrophotometer. Methanol was used as a negative

control or blank. Using the absorbance, the calibration equation was used to estimate the total flavonoid content (TFC). The TFC was then given as mg of rutin equivalent per gram (mg RE/g).

In vitro antioxidant assay

Free radical scavenging activities: The free-radical scavenging activities of various solvent extracts from the aerial parts of *P. plebeium* were evaluated using several *in vitro* assays *viz*., 2,2-diphenyl-1-picryl-hydrazyl (DPPH) radical scavenging assay, hydrogen peroxide assay and reducing power assay.

DPPH radical scavenging assay: The DPPH radical scavenging analysis of the aerial part of *P. plebeium* was conducted using the established method by Shahzad *et al.* [24]. In this method, 100 µM DPPH was dissolved in methanol to prepare the reaction mixture. Various concentrations of solvent extracts, specifically 31.2, 62.5, 125, 250 and 500 µg/mL, were introduced into the prepared mixture, which was subsequently incubated for 30 min at room temperature. The absorbance of the reaction mixture was then measured at 517 nm using a UV-Visible spectrophotometer. The data obtained from the scavenging activity were calculated as the percentage decrease in radical concentration. All experiments were performed in triplicate and the final results were expressed as the mean of three independent determinations.

Hydrogen peroxide assay: The hydrogen peroxide scavenging activity of different solvent extracts of *P. plebeium* aerial parts was evaluated according to the methods of Basit *et al.* [25] and Ethalsha *et al*. [26]. Briefly, 3.4 mL of phosphate buffer (0.2 M, pH 7.4) containing hydrogen peroxide and 0.6 mL of 40 mM H_2O_2 solution were mixed with various concentrations of the extracts. The decrease in absorbance was measured at 230 nm to determine hydrogen peroxide scavenging activity.

Reducing power assay: The various concentrations of *P. plebeium* extract (31.2, 62.5, 125, 250 and 500 µg/mL) were mixed with a phosphate buffer (0.5 mL of 0.2 M, pH 6.6). To this mixture, 0.5 mL of a 1% $K_3[Fe(CN)_6]$ solution was added and then the mixture was then incubated at 60 ºC for 30 min, followed by centrifugation with 0.5 mL of 10% trichloroacetic acid. The resulting supernatant was combined with 1 mL of distilled water and 0.2 mL of 0.1% $FeCl_3 \cdot 6H_2O$. The colour change was observed at 700 nm after 10 min. Standard ascorbic acid was used [27,28].

HPLC analysis of plant extract (ethanol): HPLC analysis of the ethanol extract of *P. plebeium* and standard compounds (quercetin and gallic acid) was carried out using a Shimadzu LC-20AD HPLC system (Shimadzu, Japan). The delivery system comprises a binary solvent system (LC-20AD), a Rheodyne-type injector featuring a 20 µL sample loop and a DAD detector (SPD-M 20 A). The Capcell Pack C-18, MGII, 5 µm, 250 mm × 4.6 mm mechanism was used for RP-HPLC with an extended guard column. The mobile phase consists of a mixture of methanol, acetonitrile and aqueous in a ratio of 40:15:45 (v/v/v), with the addition of 1.0% acetic acid and was subjected to isocratic elution for 30 min. The range of the diode array detector was set between 240 and 800 nm. Both the sample and reference solution volumes were 20 µL, with a flow rate of 1 mL/min. The peaks were identified by comparing retention times and monitoring UV spectra at λ_{max} 310 nm and were further confirmed by co-injecting the samples with small amounts of reference standards. Each diluted extract was injected into the HPLC at least 3 times and the average peak area was taken for quantification [29].

Cell culture: The Min6 mouse pancreatic beta cell line was acquired from National Centre for Cell Science (NCCS) Pune. Min6 cells undergo conversion *via* SV40 T antigen. The collected cells were cultured in a mixture of sterilised Dulbecco's modified Eagle medium (DMEM) with 15% fetal bovine serum. Subsequently, the cells were placed in an incubator that maintained a specific temperature of 37 ºC with 5% CO_2 and later kept under another specific condition of 95% air in CO_2. When microbial growth in the culture media reached 80-90%, the time was noted for subsequent subculturing conditions for further investigation [30].

Preparation of plant material: The stock solution of *P. plebeium* was prepared by mixing the extract with Tween 80 and then diluted with sterile distilled water**.**

Assessment of cell viability by MTT assay: The MTT assay is specifically used to measure the study of cell viability (Min6) and cytotoxicity. The cells were cultured overnight in 96-well plates. The density of the culture medium was regulated at 3×10^4. All the solvent extracts of *P. plebeium* were inoculated into the culture medium and placed in the incubator for 48 h. Concentrations of 50, 100, 200, 400 and 800 µg/mL of *P. plebeium* from all solvent extracts were evaluated for this assay. Subsequently, the medium containing the material was discarded and fresh cells were added before incubating with 1 mg/mL of MTT reagent. The medium was placed in a CO_2 incubator for 4 h at a regulated temperature of 37 ºC. After 4 h of incubation, the supernatant of the culture medium was carefully discarded. In this process, 100 µL of DMSO solution was added until the formazan crystals dissolved. The absorbance of the culture medium was measured at 570 nm using a microplate reader (Erba Lisa Scan EM-Transasia Bio-Medicals Ltd., Pune) [31]. The results were then analysed to determine the cytotoxic effects of the various concentrations on cell viability.

Glucose uptake in yeast cells: The yeast, *Saccharomyces cerevisiae*, was selected for the glucose uptake method. In this study, the yeast was kept in distilled water and subjected to repeated centrifugation (3000 ×g, 5 min) until clear supernatant fluids were obtained. A 10% (v/v) suspension was then prepared in distilled water. To the suspension were added various concentrations of all solvent *P. plebeium* extracts, ranging from 31.2 to 500 µg/mL. Following this, 1 mL of 5 mM glucose solution was added and the mixture was placed in an incubator for 10 min at 37 ºC. The reaction process continued with the addition of another 100 µL of the yeast suspension, followed by vigorous mixing. The mixture was then placed in an incubator for a duration of 60 min at 37 ºC. The amount of glucose was estimated after the completion of incubation period, during which the tubes were centrifuged at 2500 ×g for 5 min. The commercial drug metronidazole was used as the standard compound [32].

Statistical analysis: Data were expressed as mean ± SEM (standard error of mean), with n = 3. Statistical significance was determined using one-way ANOVA, with Tukey's post-hoc test employed for multiple comparisons. The software GraphPad Prism V9.1 (GraphPad Software, San Diego, USA) was used for statistical analysis to determine the significance of the data. A *p*-value of < 0.05 was regarded as significant in the context of the data analysis. The symbol (*) indicates statistically significant differences when compared to the standard group.

RESULTS AND DISCUSSION

Phenolic and flavonoid compounds primarily contribute to the majority of biological activity [33]. Phenolic compounds represent a significant group of phytochemicals showing anti-diabetic effects [34]. During the examination of all solvent extracts of *P. plebeium*, it was found that the petroleum ether extract does not trace flavonoids and phenolic constituents. Consequently, the total phenolic and flavonoid contents of the petroleum ether extracts were not analysed further for this study. The quantitative phytochemical analysis of all extracts, excluding petroleum ether, revealed elevated levels of phenolic and flavonoid compounds. The ethanol extract exhibited a higher concentration of flavonoids and phenolic constituents compared to other solvents, including chloroform, ethyl acetate and aqueous extracts. The order of flavonoids and phenolic constituents present in *P. plebeium*, arranged from highest to lowest, is as follows: ethanol > ethyl acetate > aqueous > chloroform extract. The ethanol extract contains 95.45 ± 0.04 mg of gallic acid equivalent per gram phenolic content. Table-1 provides a quantitative analysis of the phenolic compounds and flavonoids. The phenolic content for the ethyl acetate, aqueous and chloroform extracts is recorded as 92.12 ± 0.12 mg GAE/g, 36.25 ± 0.02 mg GAE/g and 27.33 ± 0.91 mg GAE/g, respectively. The flavonoid content for the ethanol, ethyl acetate, aqueous and chloroform extracts is observed as 70.12 ± 0.07 mg RE/g, 51.46 ± 0.01 mg RE/g, 34.01 ± 0.03 mg RE/g and 28.12 ± 0.05 mg RE/g, respectively. Therefore, phenolic compounds and flavonoids serve as effective agents for antioxidant action through electron donation.

Inhibition of DPPH radical: The antioxidant activity of *P plebeium* extracts was evaluated using DPPH (Table-2) displays the part of DPPH radical inhibition. The ethanol extract demonstrated superior efficacy in suppressing free radicals compared to extracts obtained from petroleum ether, ethyl acetate, chloroform and aqueous sources. It exhibited a significant inhibition rate of approximately 85.27 ± 0.17 at a concentration of 500 µg/mL, with an IC_{50} value of 71.52 ± 0.02. The IC_{50} values were established as follows: 157.4 ± 0.03 for petroleum ether, 190.5 ± 0.12 for chloroform, 121.8 ± 0.14 for ethyl acetate and 140.8 ± 0.01 for the aqueous extract. The reduced IC_{50} values signify enhanced inhibition of 50% of free radicals. Thus, the IC_{50} value of the ethanol extract was significantly lower than that of the other solvent extracts. Subsequently, *P. plebeium* effectively activates as a free radical scavenger at low concentrations. With increasing concentration, the inhibition of the ethanol extract was shown to be nearly comparable to that of the standard (ascorbic acid).

TABLE-1
TOTAL PHENOLIC AND FLAVONOIDS OF *Polygonum plebeium* OF DIFFERENT EXTRACTS

P. plebeium extract	Total phenolic content in mg GAE/g of extract	Total flavonoid content in mg RE/g of extract
Petroleum ether	–	–
Chloroform	27.33 ± 0.91	28.12 ± 0.05
Ethyl acetate	92.12 ± 0.12	51.46 ± 0.01
Ethanol	95.45 ± 0.04	70.12 ± 0.07
Aqueous	36.25 ± 0.02	34.01 ± 0.03

Hydrogen peroxide assay: The antioxidant activity of *P. plebeium* extracts was evaluated using hydrogen peroxide (Table-3), which displays the part of free radical inhibition. The ethanol extract demonstrated superior efficacy in reducing free radicals compared to petroleum ether, chloroform, ethyl acetate and aqueous extracts. It exhibits a strong inhibition level of approximately 80.64 ± 0.61 at a concentration of 500 µg/mL, with an IC_{50} value of 31.40 ± 0.24. The IC_{50} values were determined as follows: 65.51 ± 0.42 for petroleum ether, 93.45 ± 0.05 for chloroform, 32.81 ± 0.81 for ethyl acetate and 39.46 ± 0.32 for the aqueous extract.

Reducing power assay: The ethanol extracts had higher reduction capacities than other *P. plebeium* extracts. The test was performed at different concentrations of extracts: 31.2, 62.5, 125, 250 and 500 µg/mL. The reducing capacity of the petroleum ether extract at a low concentration (31.2 µg/mL) was minimal. Both the petroleum ether and chloroform extracts showed diminished reducing capacity against Fe(III). The colour of the solution in the reduction power assay transitions from yellow to various shades of blue and green, which is attributed to the presence of several phytochemicals in the extracts. The IC_{50} values were determined as follows: 45.51 ±

TABLE-2
ESTIMATION OF DPPH SCAVENGING ACTIVITY OF *Polygonum plebeium* EXTRACT AND STANDARD IN PERCENTAGE (%) INHIBITION

Conc. (µg/mL)	Ascorbic acid	Petroleum ether	Chloroform	Ethyl acetate	Ethanol	Aqueous
31.2	37.68 ± 0.04	12.25 ± 0.01*	7.59 ± 0.42*	22.31 ± 0.02*	30.46 ± 0.12*	17.24 ± 0.02*
62.5	51.77 ± 0.12	18.39 ± 0.04*	13.45 ± 0.14*	29.12 ± 0.01*	43.57 ± 0.14*	24.66 ± 0.23*
125	73.48 ± 0.01	41.34 ± 0.02*	36.25 ± 0.04*	51.67 ± 0.11*	63.58 ± 0.22*	47.59 ± 0.54*
250	79.77 ± 0.03	50.12 ± 0.22*	45.92 ± 0.19*	66.37 ± 0.03*	74.63 ± 0.16*	58.64 ± 0.12*
500	95.47 ± 0.02	62.53 ± 0.31*	57.12 ± 0.02*	77.89 ± 0.12*	85.27 ± 0.17*	72.59 ± 0.04*
IC_{50}	58.89 ± 0.42	157.4 ± 0.03	190.5 ± 0.12	121.8 ± 0.14	71.52 ± 0.02	140.8 ± 0.01

The values are expressed as the mean ± SEM (standard error mean) for n = 3, * denoting significance at $p < 0.05$. compared to the standard.

TABLE-3
HYDROGEN PEROXIDE ASSAY ACTIVITY DATA OF *Polygonum plebeium* OF DIFFERENT EXTRACT AND STANDARD IN PERCENTAGE (%) INHIBITION

Conc. (µg/mL)	Ascorbic acid	Petroleum ether	Chloroform	Ethyl acetate	Ethanol	Aqueous
31.2	46.9 ± 0.51	21.05 ± 0.19*	19.29 ± 0.16*	35.97 ± 0.03*	44.05 ± 0.13*	33.27 ± 0.29*
62.5	55.25 ± 0.61	31.59 ± 0.24*	29.14 ± 0.52*	51.05 ± 0.51*	54.21 ± 0.21*	44.05 ± 0.51*
125	67.75 ± 0.21	51.33 ± 0.05*	37.91 ± 0.42*	59.29 ± 0.15*	65.92 ± 0.04*	58.12 ± 0.24*
250	76.31 ± 0.18	60.41 ± 0.02*	42.52 ± 0.61*	66.05 ± 0.23*	73.66 ± 0.51*	62.25 ± 0.61*
500	82.01 ± 0.26	66.34 ± 0.33*	60.25 ± 0.51*	71.44 ± 0.42*	80.64 ± 0.61*	69.32 ± 0.71*
IC_{50}	29.47 ± 0.12	65.51 ± 0.42	93.45 ± 0.05	32.81 ± 0.81	31.40 ± 0.24	39.46 ± 0.32

The values are expressed as the mean ± SEM (standard error mean) for n = 3, * denoting significance at $p < 0.05$. compared to the standard.

0.45 for petroleum ether, 62.92 ± 0.25 for chloroform, 36.95 ± 0.61 for aqueous, 34.24 ± 0.43 for ethyl acetate and 33.78 ± 0.52 for ethanol extracts (Table-4). The reducing power test assessed the capacity of *P. plebeium* extract to reduce Fe(III) to an Fe(II) complex.

The phytochemicals present in *P. plebeium* contribute to its antioxidant activity by scavenging free radicals, which are linked to many chronic diseases. Unlike synthetic antioxidants that may cause side effects, natural plant antioxidants are generally safer [35,36]. All extracts showed the concentration-dependent antioxidant activity, with increasing reducing power at higher doses. Among them, the ethanol extract exhibited the strongest antioxidant effect. These results highlight *P. plebeium* as a promising natural source of antioxidants and support further studies on its phytochemical constituents and therapeutic potential [37,38].

HPLC analysis of ethanol extract of *P. plebeium*: The phytochemical constituents of the ethanol extract from *P. plebeium* were analysed *via* HPLC by comparing their retention time to those of reference standards. The amounts of quercetin and gallic acid in the ethanol extract of *P. plebeium* were assessed *via* RP-HPLC analysis. The retention times of quercetin and gallic acid in the extract (Fig. 1a) were found to be 4.059 and 4.557 min, respectively, which corresponded closely to the retention time of standard quercetin and gallic acid (Fig. 1b). The calibration curves for quercetin and gallic acid exhibited a linear correlation between peak areas and the concentrations of standard quercetin and gallic acid, with a correlation coefficient (r^2) of 0.9958. The concentrations of quercetin and gallic acid in the ethanol extract of *P. plebeium* were measured at 0.5 and 0.6 µg/mL, respectively.

***In vitro* cytotoxicity test:** The ethanol and ethyl acetate extracts show higher cell viability compared to the petroleum ether, chloroform and aqueous extracts of *P. plebeium*. The MTT assay was performed under various culture conditions, including a control and concentrations of 50, 100, 200, 400 and 800 µg/mL. The petroleum ether, chloroform and aqueous extracts of *P. plebeium* had no effect on cell viability. Table-5

TABLE-4
REDUCING POWER ASSAY DATA OF *Polygonum plebeium* OF DIFFERENT EXTRACTS AND STANDARD IN PERCENTAGE (%) INHIBITION

Conc. (µg/mL)	Ascorbic acid	Petroleum ether	Chloroform	Ethyl acetate	Ethanol	Aqueous
31.2	43.12 ± 0.11	29.51 ± 0.23*	26.05 ± 0.62*	40.25 ± 0.21*	41.06 ± 0.32*	35.25 ± 0.18*
62.5	60.01 ± 0.24	46.18 ± 0.28*	33.95 ± 0.72*	54.72 ± 0.33*	56.23 ± 0.04*	48.36 ± 0.15*
125	70.41 ± 0.16	60.11 ± 0.21*	58.18 ± 0.14*	66.91 ± 0.29*	68.04 ± 0.02*	62.95 ± 0.26*
250	74.62 ± 0.19	63.48 ± 0.35*	61.35 ± 0.05*	70.12 ± 0.18*	72.91 ± 0.51*	66.53 ± 0.21*
500	86.21 ± 0.28	72.13 ± 0.12*	68.15 ± 0.91*	81.35 ± 0.02*	82.12 ± 0.41*	72.81 ± 0.49*
IC_{50}	32.39 ± 0.27	45.51 ± 0.45	62.92 ± 0.25	34.24 ± 0.43	33.78 ± 0.52	36.95 ± 0.61

The values are expressed as the mean ± SEM (standard error mean) for n = 3, * denoting significance at $p < 0.05$. compared to the standard.

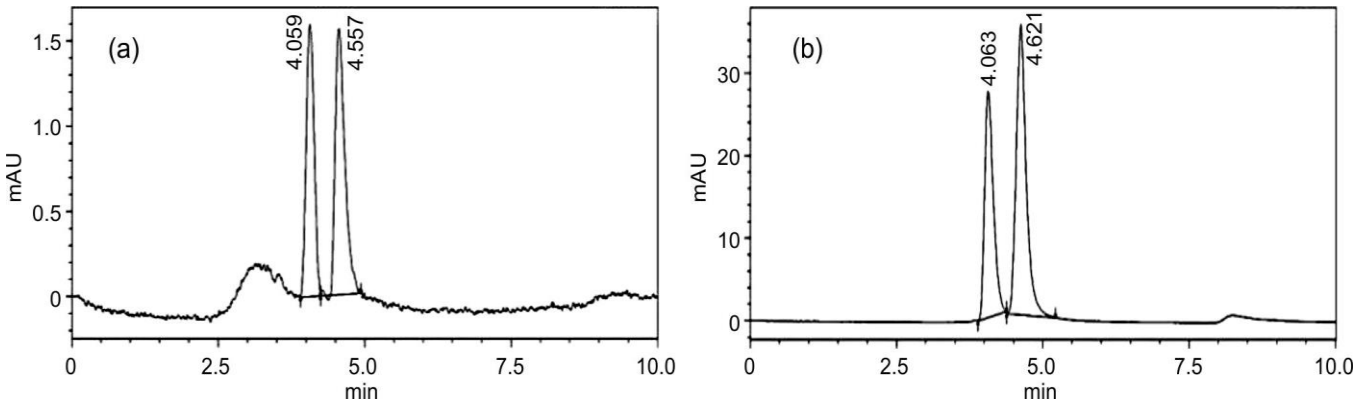

Fig. 1. High performance liquid chromatography chromatogram of (a) ethanol extract of *P. plebeium* and (b) standard quercetin and gallic acid

indicated that an increase in extract concentration was associated with more deceased cells. Furthermore, the treatment of Min6 cells with the ethanol extract resulted in a significant decrease in cell viability in a dose-dependent manner over a 48 h incubation period.

The cytotoxic effects of various solvent (petroleum ether, chloroform, ethyl acetate, ethanol and aqueous) extracts of *P. plebeium* were evaluated on Min6 pancreatic beta cells (Fig. 2) and morphological observations were recorded alongside assessments of cell viability. Control cells exhibited normal morphology with 100% viability. Among the tested extracts, both ethanol and ethyl acetate extracts show a concentration-dependent decrease in cell viability. The ethyl acetate extract showed a gradual reduction in viability from 94 ± 0.92% at 50 μg/mL to 79 ± 0.78% at 800 μg/mL, with mild to moderate morphological alterations such as cell shrinkage, rounding and partial detachment. In contrast, the ethanol extract exhibited a more significant cytotoxic effect, with viability rate decreasing from 89 ± 0.28% at 50 μg/mL to 59 ± 0.84% at 800 μg/mL, along with severe morphological changes that included extensive cell detachment and a loss of cell structural integrity. The standard drug, metformin (100 μg/mL), maintained high cell viability (96.53 ± 0.95%), indicating minimal cytotoxicity under the tested conditions. These findings indicated that the ethanol extract possesses a higher cytotoxic potential in comparison to the ethyl acetate extract. The higher significant value of ethanol extract may be the content of polar phytochemicals such as phenolics and glycosides. On the contrary, significant data were not acquired during the petroleum ether, chloroform and aqueous extracts; therefore, morphological characterisation could not be determined. The results indicate that both ethanol and ethyl acetate extracts exhibit dose-dependent cytotoxic effects on Min6 pancreatic beta cells [39].

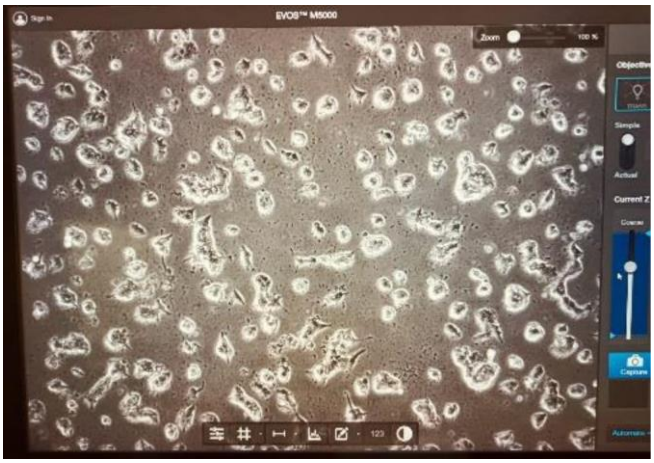

Fig. 2. Image of normal Min6 pancreatic beta cells

Glucose uptake in yeast cells: The effects of different doses of *P. plebeium* aerial parts in solvent extracts on glucose absorption are examined *in vitro* using yeast as a model organism. The extract's effectiveness in enhancing glucose absorption by yeast cells was evaluated against that of the widely-used antibiotic metronidazole. The ethanol extract had higher efficacy than the other evaluated solvent extracts. With the increase in *P. plebeium* extract, the percentage of glucose absorption similarly elevated (Table-6). The results confirmed that *P. plebeium* was almost as effective as the traditional antibiotic metronidazole in promoting glucose uptake by yeast cells.

Conclusion

The present investigation demonstrates that the aerial parts of *Polygonum plebeium* are a valuable source of bioactive phytochemicals with significant antioxidant and antidiabetic potential. The quantitative analysis revealed that the ethanol extract contained the highest levels of phenolic and flavonoid compounds, which strongly correlated with its superior free radical scavenging activity in DPPH, hydrogen peroxide and reducing power assays. HPLC analysis further confirmed the presence of important antioxidant constituents such as quercetin

TABLE-5
CELL VIABILITY EFFECTS OF *Polygonum plebeium* OF DIFFERENT EXTRACTS IN Min6 CELL LINE

Culture conditions (μg/mL)	Petroleum ether	Chloroform	Ethyl acetate	Ethanol	Aqueous
Control	100.00 ± 00	100.00 ± 00	100.00 ± 00	100.00 ± 00	100.00 ± 00
50	–	–	94 ± 0.92	89 ± 0.28	–
100	–	–	89 ± 1.01	85 ± 0.48	–
200	–	–	87 ± 0.72	84 ± 0.38	–
400	–	–	83 ± 0.46	82 ± 0.62	–
800	–	–	79 ± 0.78	59 ± 0.84	–
Metformin (100 μg/mL)	96.53 ± 0.95	96.53 ± 0.95	96.53 ± 0.95	96.53 ± 0.95	96.53 ± 0.95

TABLE-6
THE PERCENTAGE OF GLUCOSE UPTAKE BY YEAST CELLS TREATED WITH *Polygonum plebeium* OF DIFFERENT EXTRACTS

Conc. (μg/mL)	Metronidazole	Petroleum ether	Chloroform	Ethyl acetate	Ethanol	Aqueous
31.2	45.47 ± 0.68	26.17 ± 0.88*	19.20 ± 0.76*	35.65 ± 0.91*	43.20 ± 0.37*	28.02 ± 0.66*
62.5	57.22 ± 0.49	28.05 ± 0.34*	22.64 ± 0.94*	42.55 ± 0.54*	49.05 ± 0.58*	32.05 ± 0.58*
125	61.48 ± 0.96	34.12 ± 0.75*	27.04 ± 0.58*	47.51 ± 0.62*	56.54 ± 0.93*	35.44 ± 0.77*
250	66.50 ± 0.72	41.62 ± 0.49*	33.63 ± 0.93*	53.45 ± 0.48*	59.31 ± 0.72*	43.05 ± 0.91*
500	73.65 ± 0.59	54.26 ± 0.67*	47.87 ± 0.85*	67.45 ± 0.39*	70.12 ± 0.29*	57.45 ± 0.57*
IC_{50}	19.83 ± 0.04	57.17 ± 0.54	96.55 ± 0.73	33.04 ± 0.46	20.00 ± 0.82	48.43 ± 0.56

The data are expressed as the mean ± SEM (standard error mean) for n = 3. * Indicates significance at $p < 0.05$ compared to the standard.

and gallic acid. Biological evaluation showed that the ethanol extract markedly enhanced glucose uptake in yeast cells, indicating promising hypoglycaemic potential. Cytotoxicity studies on Min6 pancreatic beta cells demonstrated dose-dependent effects, with ethanol and ethyl acetate extracts showing moderate activity, while the standard drug maintained high cell viability. These findings suggest that *P. plebeium*, particularly its ethanol extract, may serve as a promising natural candidate for the development of antioxidant and antidiabetic formulations. The observed activities are likely associated with its rich phenolic and flavonoid composition. Further studies focused on isolation of active constituents, mechanistic evaluation, and *in vivo* validation are required to establish its therapeutic applicability and safety profile.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

DECLARATION OF AI-ASSISTED TECHNOLOGIES

During the preparation of this manuscript, the authors used an AI-assisted tool(s) to improve the language. The authors reviewed and edited the content and take full responsibility for the published work.

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