INVESTIGATIONS ON THE PHARMACOLOGICAL PROPERTIES OF AJUGA BRACTEOSA EXTRACTS: ANTIOXIDANT ACTIVITY, GLUCOSE METABOLISM, NEUROPROTECTION AND PHYTOCHEMICAL EVALUATION

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ABSTRACT

The study evaluated the phytochemical profiles and pharmacological activities of *Ajuga bracteosa Wall ex Benth.* Leaf extracts, ABME-L (methanolic extract) and ABWE-L (aqueous extract), revealing their potential for therapeutic applications. While alkaloids, glycosides, and saponins were only found in ABME-L, demonstrating methanol's greater extraction effectiveness, phytochemical screening revealed the presence of phenols, carbohydrates, flavonoids, terpenoids, phytosterols, proteins, and tannins in both extracts. ABME-L exhibited higher total phenolic content (379.74 mg GAE/g) than ABWE-L (288.95 mg GAE/g), suggesting greater antioxidant potential. Glucose utilization and DPP-4 inhibition assays demonstrated the antidiabetic activity of both extracts, with ABME-L showing higher efficacy. Intracellular reactive oxygen species (ROS) levels were lowered by ABME-L and ABWE-L in a dose-dependent manner, with notable effects at higher concentrations. ABME-L showed greater neuroprotective potential by lowering ROS levels induced by H_2O_2. These findings indicate the antioxidant, antidiabetic, and neuroprotective properties of ABME-L and ABWE-L, supporting further exploration of their pharmacological applications and active constituents.

Keywords: Antidiabetic, Neuroprotective, *In vitro* studies, *Ajuga bracteosa*Wall ex Benth., Glucose utilization, DPP-4 inhibition,

INTRODUCTION

Phytochemicals, the bioactive compounds found in plants, have garnered significant attention in pharmaceutical research due to their diverse therapeutic properties. These natural components—which include flavonoids, phenols, terpenoids, alkaloids, and saponins—are essential for managing and preventing a number of illnesses, especially those linked to oxidative stress and metabolism. The growing interest in phytochemicals stems from their potential to serve as safer alternatives to synthetic drugs, offering fewer side effects and a wide array of biological activities. Among these, antioxidant and antidiabetic properties have emerged as key areas of research, given the rising global prevalence of chronic conditions such as diabetes and neurodegenerative diseases (1-5).

The startling rise in diabetes mellitus cases globally has made the hunt for efficient antidiabetic treatments more pressing. One of the main causes of illness and death worldwide is diabetes, which is typified by hyperglycemia brought on by insulin resistance or decreased insulin output. Conventional treatments, including insulin and oral hypoglycemic agents, often come with limitations such as adverse effects and inadequate glycemic control. Because of their multi-targeted modes of action, which include improving glucose absorption, modifying insulin sensitivity, and blocking important enzymes like dipeptidyl peptidase-4 (DPP-4), plant-based medicines have become attractive options in this regard (1, 2, 6, 7).

A serine protease called DPP-4 breaks down incretin hormones including glucagon-like peptide-1 (GLP-1), which is essential for glucose metabolism. Inhibiting DPP-4 prolongs the action of these hormones, enhancing insulin secretion and lowering blood glucose levels. While synthetic DPP-4 inhibitors are available, their long-term safety profile remains a concern, prompting interest in natural inhibitors derived from plant sources.

Phytochemicals like flavonoids and phenolic acids have shown potential as DPP-4 inhibitors, offering a natural alternative with additional antioxidant benefits (8-14). Chronic diseases such as diabetes, neurological disorders, and cardiovascular ailments are also significantly influenced by oxidative stress, which arises from an imbalance between the body's antioxidant defences and the creation of reactive oxygen species (ROS). ROS can damage cellular components such as DNA, lipids, and proteins, leading to cellular dysfunction and death. Plants rich in phenolic compounds are particularly valued for their ability to neutralize ROS, thereby mitigating oxidative stress and its associated complications (3-5, 10, 15-17).

The bioactive chemicals found in *Ajuga bracteosa* Wall ex Benth. and other medicinal plants have been the subject of much research due to their antidiabetic and antioxidant qualities. Methanolic (ABME-L) and aqueous (ABWE-L) extracts of *Ajuga bracteosa*are known for their phytochemical richness, offering a wide range of therapeutic effects. Methanol, being a polar solvent, often extracts a broader spectrum of bioactive compounds compared to water, including phenols, alkaloids, and saponins. This difference in extraction efficiency can significantly influence the biological activity of the extracts, as observed in studies comparing their antioxidant and antidiabetic effects (13, 14, 18).In addition to their antioxidant properties, plant extracts have shown promise in enhancing glucose metabolism. Cellular studies using L6 myoblasts provide a reliable model for evaluating glucose uptake, a key indicator of antidiabetic potential. Insulin-mimetic activity, as observed in plant extracts, can improve glucose uptake in muscle cells, a primary site of insulin action. Dose-dependent effects further highlight the therapeutic potential of these extracts in managing hyperglycemia(3-5). Another topic of increasing attention is the neuroprotective properties of plant extracts, especially in light

of neurodegeneration brought on by oxidative stress. Antioxidants are a key component of neuroprotective therapies since elevated ROS levels are a characteristic of neurodegenerative illnesses including Parkinson's and Alzheimer's. Plant extracts' potential to prevent or lessen neurodegenerative diseases is highlighted by their capacity to dose-dependently lower intracellular ROS levels (13, 14, 18).

The antioxidant, antidiabetic, and neuroprotective properties of Ajuga bracteosa Wall ex Benth. leaf extracts, methanol extract (ABME-L), and water extract (ABWE-L) are the main focus of this study's assessment. The goal of this study is to offer a thorough understanding of their therapeutic potential by investigating their total phenolic content, glucose utilisation in L6 myoblasts, DPP-4 inhibition, and ROS scavenging capabilities. The findings not only highlight the pharmacological relevance of these extracts but also pave the way for their potential application in developing natural remedies for metabolic and oxidative stress-related disorders.

EXPERIMENTAL

Drugs, Chemicals, reagents and kits

Every chemical and reagent used in this study was of analytical grade. Water, petroleum ether, methanol, acetone, and chloroform were employed as extraction solvents. We purchased streptomycin, penicillin, gallic acid, and silymarin from Himedia Laboratories in Mumbai, India. All other biochemical kits and the Folin-Ciocalteu reagent were acquired from R&D Systems in India and Sigma Aldrich in Mumbai, India, respectively. Penicillin, streptomycin, foetal bovine serum (FBS), and Dulbecco's Modified Eagle Medium (DMEM) were all acquired from Himedia (India) and used in L6 myoblast cell culture tests. Merck (Germany) supplied hydrogen peroxide ($H2O_2$) to cause oxidative stress. A well-known DPP-4 inhibitor, diprotin A, was acquired from Sigma-Aldrich

(USA). While DPP-4 inhibition was assessed using a commercially available DPP-4 assay kit (Bayer), glucose levels were measured using the glucose oxidase-peroxidase (GOD-POD) assay kit. Using 2',7'-dichlorodihydrofluorescein diacetate (DCF-DA), which is also sourced from Sigma-Aldrich, the reactive oxygen species (ROS) were measured. All of the other chemicals and reagents were of excellent quality, analytical grade, and were only bought from reputable, pre-screened vendors. The reagents were stored appropriately to ensure their stability and efficacy, and each experiment was conducted in compliance with the manufacturer's instructions.

Gathering the plant material

For this investigation, the leaves of the therapeutic herb Ajuga bracteosa Wall ex Benth. were gathered from the Majhitar area of Sikkim. The plant was identified and confirmed by Dr. Gaurav Pant, a botanist from the Department of Botany. To facilitate future reference and validation, a herbarium specimen of the plant, labelled with the accession number BKSPH/19292/AB/298, has been meticulously prepared and deposited in the pharmacognosy laboratory. This preserved specimen will serve as an important reference point for ongoing and future research related to this plant.

The Extract Preparation Process

To maintain their phytochemical qualities, the plant's leaves were dried in the shade for a few days. The dry leaves were then ground into a coarse powder using a mechanical grinder. A total of 500 grams of the powdered plant material underwent successive extraction using a Soxhlet apparatus. The extraction technique required the use of two solvents: methanol and water. Each extraction cycle lasted for several hours to ensure maximum extraction of the plant's bioactive compounds. The extracts obtained from each solvent were then concentrated using a rotary vacuum evaporator (M. M. Traders, New

Delhi, India) at a controlled temperature of less than 45°C to prevent the degradation of heat-sensitive components. The two extracts, labelled as ABME-L(methanol), and ABWE-L (water), were stored at 4°C to maintain their stability until further use. Subsequently, both the extracts were evaluated for their total phenolic content. To measure the effectiveness of the extraction procedure, the percentage yield of each extract was calculated using a standard formula.

% Yield= Extract Amount Received(gm)/ Dry powder drug amount taken initially (gm) ×100

Initial Screening for Phytochemicals

Both the extracts underwent a preliminary phytochemical screening to find different bioactive substances, including proteins, carbohydrates, alkaloids, glycosides, flavonoids, phytosterols, phenols, and saponins. Standardized tests were used to ensure reliable identification of these constituents, providing a foundational understanding of the plant's chemical profile and offering insights into the potential therapeutic applications of the extracts.

Assay for Total Phenolic Compounds

The total phenolic content of the extracts was determined using the Folin-Ciocalteu method(19). The necessary materials included the Folin-Ciocalteu reagent, gallic acid as the standard, sodium carbonate solution, distilled water, the extracts labeled ABME-L and ABWE-L, a UV-Visible spectrophotometer, and standard laboratory glassware. The Folin-Ciocalteu reagent was diluted 1:10 with distilled water, and 7.5 grammes of Na_2CO_3 were dissolved in 100 millilitres of distilled water to create a 7.5% (w/v) sodium carbonate solution. Gallic acid was used to create a stock solution of 1 mg/mL, which was then further diluted to create working standards ranging from 10 to 100 µg/mL. After

adding 0.5 mL of the diluted extract or standard gallic acid solution to a test tube, 2.5 mL of the diluted Folin-Ciocalteu reagent was added. After letting the mixture remain at room temperature for five minutes, two millilitres of the sodium carbonate solution were added.After fully mixing the solution, it was allowed to sit at room temperature for half an hour in the dark. Using a UV-visible spectrophotometer, the absorbance of the resulting blue hue at 765 nm was measured. A calibration curve of absorbance vs concentration for the gallic acid standards was produced in order to determine the total phenolic content. The total phenolic content of the extracts was determined by interpolating their absorbance values on the standard calibration curve. Milligrammes of gallic acid equivalents (GAE) per gramme of extract (mg GAE/g extract) was then used to express the results.To prevent interference, all glassware was kept clean and clear of impurities, and for accuracy, every measurement was done three times. To preserve their reactivity, the sodium carbonate solution and Folin-Ciocalteu reagent were maintained properly.

Antidiabetic activity

Investigation on the Utilisation of Glucose by L6 Myoblasts

The utilisation of glucose by L6 myoblast cells was determined using the methods described by van de Venter et al. (2008) earlier (20). The cells were cultivated in Dulbecco's Modified Eagle Medium (DMEM), which was supplemented with 10% foetal bovine serum and 1% penicillin-streptomycin, to maintain optimal growth conditions. After being sown in 24-well plates, the cells were given time to attain about 80% confluence. After that, the cells were exposed to varying concentrations of the test substances for a full day. Following the incubation period, the cells were washed with phosphate-buffered saline (PBS) to eliminate any leftover materials from the solution. To

finish the glucose uptake experiment, a glucose solution was then given to each well, and the cells were incubated for two more hours. The amount of glucose in the media was measured using a glucose oxidase-peroxidase test kit, which involved the enzymatic conversion of glucose to gluconic acid and hydrogen peroxide, followed by a colorimetric reaction that was observed using a microplate reader at 540 nm. By comparing the glucose concentration in the fluid before and after the incubation period, the amount of glucose used by the cells was determined. This experimental approach provided valuable insights into the cellular mechanisms of glucose utilization, which is critical for understanding the potential antidiabetic effects of the test compounds. The data obtained from this assay were analyzed statistically to determine the significance of the observed effects and to identify the most effective concentrations of the test compounds (21).

Assay for DPP-4 Inhibition

The technique suggested by Al-Masri et al. (2009) was adapted and used to perform the DPP-4 inhibition experiment(22). This assay involved preparing a series of test compound solutions at different concentrations to evaluate their inhibitory effects on the DPP-4 enzyme. The enzyme activity was measured by monitoring the release of a chromogenic product resulting from the cleavage of a specific DPP-4 substrate. The following formula was used to determine the percentage inhibition of the DPP-4 enzyme by each test compound or control: % inhibition = (Control Absorbance – Test Absorbance) / Control Absorbance × 100. Control samples contained the DPP-4 enzyme and substrate without any test compound, providing a baseline absorbance value for comparison. Each test compound was incubated with the DPP-4 enzyme at 37°C for a specified period, allowing the reaction to proceed. Following incubation, a stop solution

was added to halt the reaction, and the absorbance was measured using a spectrophotometer at a specific wavelength, often about 405 nm.. This technique made it possible to evaluate the test drugs' inhibitory effects on the DPP-4 enzyme precisely, yielding important information about their possible therapeutic usefulness.In order to assess the effectiveness of the inhibitors, the IC50 values—the concentration of the test substance required to inhibit 50% of the enzyme activity—were computed from the findings. To guarantee the validity and importance of the results, statistical analysis was performed on the data gathered from this experiment.

Neuroprotective activity

Cell culture and treatment

To investigate the neuroprotective properties of the extracts, SK-N-SH cells, a human neuroblastoma cell line sourced from the American Type Culture Collection (ATCC), were grown in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% foetal bovine serum, 1% penicillin-streptomycin, and 1% non-essential amino acids. The cells were maintained at 37°C in a humidified atmosphere with 5% CO2. After the cells had achieved roughly 80% confluence, they were exposed to varying doses of the test compounds, extracts, for a duration of 24 hours. Only the vehicle was applied to the control cells. Following the removal of the media at the end of the incubation period, the cells were washed twice with phosphate-buffered saline (PBS) to eliminate any leftover materials. The neuroprotective effectiveness of the treated cells was then assessed using several assays (23, 24).

Level of intracellular reactive oxygen species

Intracellular reactive oxygen species (ROS) levels were measured in order to quantify oxidative stress, a significant cause of dementia. ROS levels were assessed using the

DCFH-DA (2',7'-dichlorofluorescin diacetate) test, which involves subjecting the cells to DCFH-DA for 30 minutes at 37°C. The extremely fluorescent chemical DCF (2',7'-dichlorofluorescein) is created when ROS further oxidises the non-fluorescent DCFH-DA molecule after cellular esterases have deacetylated it inside the cell.The fluorescence intensity, which is proportional to the quantity of ROS present, was measured using a fluorescent microplate reader at an excitation wavelength of 485 nm and an emission wavelength of 535 nm (24, 25).

Statistical analysis

The data was statistically analysed using GraphPad Prism Software Version 8 to guarantee accuracy and reliability. Descriptive statistics such as the mean and standard deviation were calculated for each experimental group. One-way analysis of variance (ANOVA) was used to compare the means of the test substances and control groups across different concentrations. Post hoc tests such as Tukey's test was employed to identify specific group differences. To get IC50 values, nonlinear regression analysis was also employed. Each result was given the proper confidence range, and a significance criterion of $p < 0.05$ was set.

RESULTS AND DISCUSSION

Initial Screening for Phytochemicals

Several bioactive chemicals were found in ABWE-L (aqueous extract) and ABME-L (methanolic extract) during initial phytochemical screening. Both extracts tested positive for phenols, carbohydrates, flavonoids, terpenoids, phytosterols, proteins, and tannins, indicating their rich phytochemical profile. However, alkaloids, glycosides, and saponins were absent in ABWE-L but present in ABME-L. This suggests that methanol as a solvent is more effective in extracting these specific compounds. The results highlight the

potential of these extracts for further exploration of their pharmacological activities based on their diverse phytochemical composition.

Tablet 1. The initial findings of the extracts' phytochemical screening

Phytochemical Type	**ABWE-L**	**ABME-L**
Phenols	+	+
Alkaloids	-	+
Glycosides	-	+
Carbohydrates	+	+
Flavonoids	+	+
Terpenoids	+	+
Phytosterols	+	+
Saponins	-	+
Proteins	+	+
Tannins	+	+

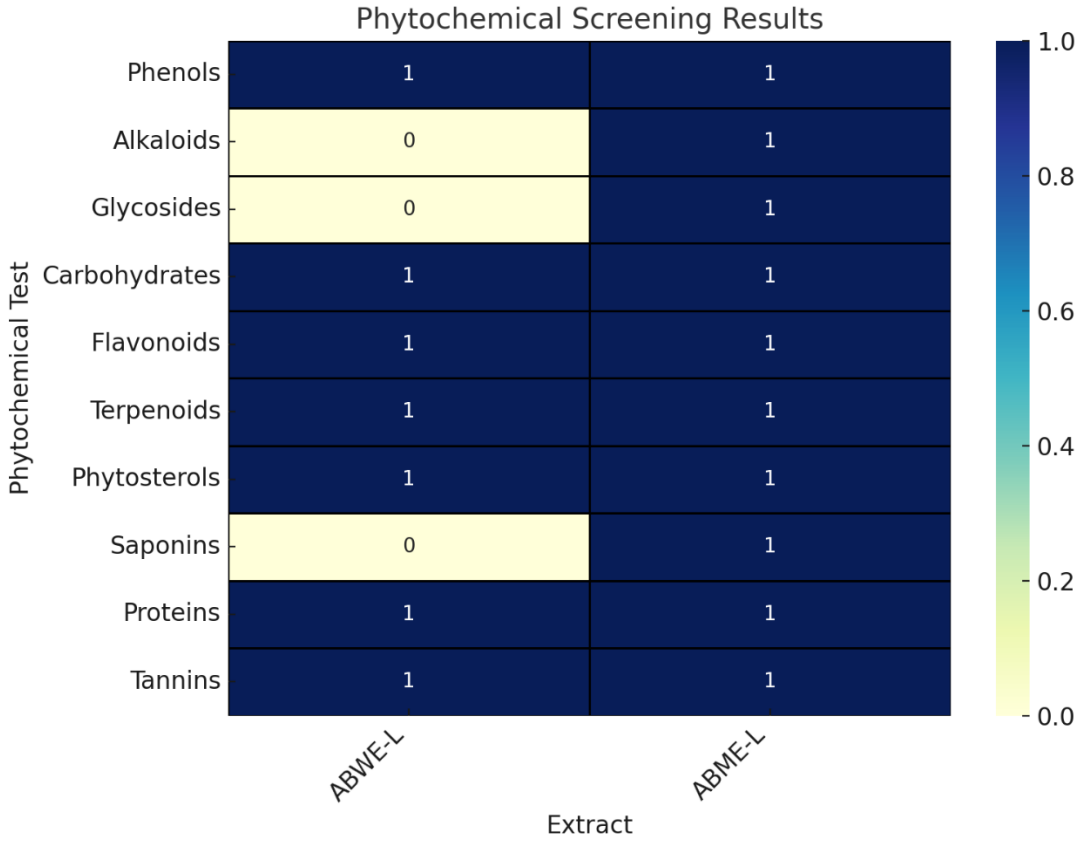

Figure 1. A heatmap showing phytochemical screening results for various extracts.

Assay for Total Phenolic Compounds

The extracts' linear regression equations and total phenolic content (TPC), which is expressed in gallic acid equivalents (GAE) per gramme, highlight significant findings. ABME-L exhibited a higher TPC of 379.74 mg GAE/g compared to ABWE-L, which had 288.95 mg GAE/g, indicating methanol's superior extraction efficiency for phenolic compounds. Both extracts demonstrated excellent linearity in their calibration curves, as reflected by high R^2 values (ABWE-L: 0.9891, ABME-L: 0.9874). The regression equations suggest a consistent relationship between absorbance and phenolic concentration, underscoring the reliability of the method used for phenolic quantification.

This data underscores the potential of ABME-L for antioxidant-related applications due to its richer phenolic content.

Table 2. The linear regression analysis equations and the total phenolic content of each extract

Extracts	**ABWE-L**	**ABME-L**
Regression Equation	y = 0.0073x + 0.0833 R^2 = 0.9891	y = 0.0082x + 0.0693 R^2 = 0.9874
Total Phenolic content in GAE per gram of extract	288.95	379.74

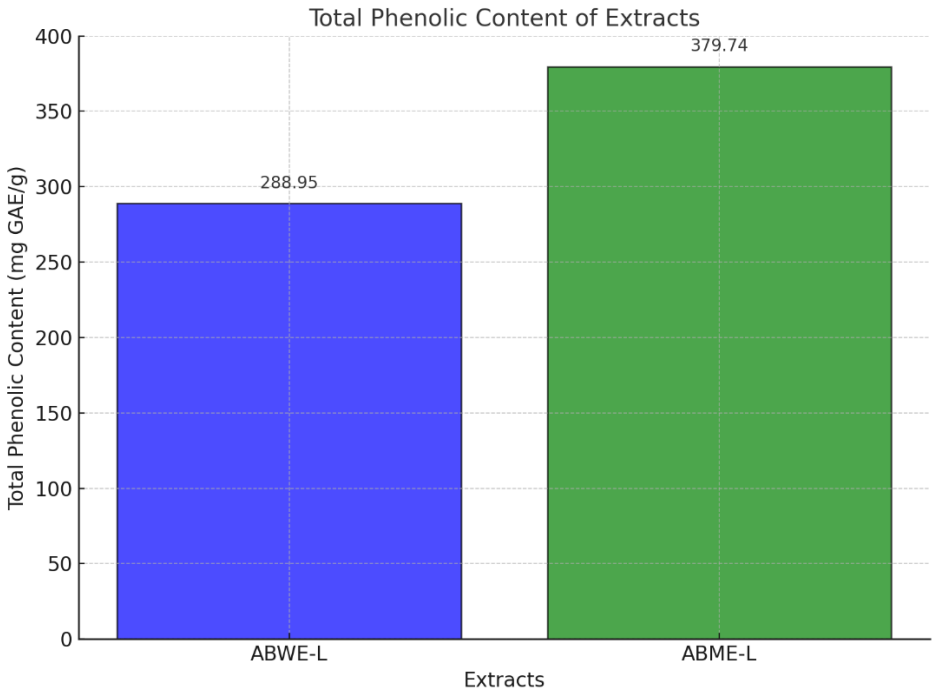

Figure 2. The GAE per gram for all extracts' total phenolic content.

Antidiabetic activity

Utilisation of Glucose by L6 Myoblasts

The effects of ABME-L were emphasised in the glucose utilisation research in L6 myoblasts when compared to insulin and a control group. The baseline glucose utilisation for the control group was 99.99 ± 2.45.Insulin's effectiveness in improving glucose metabolism was confirmed by the considerable increase in glucose absorption to 143.87 ± 3.16 at a concentration of 6 µg/mL.ABME-L at 15 µg/mL resulted in a moderate increase in glucose utilization to 113.32 ± 2.45, indicating its potential insulin-mimetic activity. ABME-L further improved glucose utilisation to 123.87 ± 2.56 at a higher dosage of 25 µg/mL, indicating a dose-dependent effect. These results imply that ABME-L might be a viable choice for improving the absorption of glucose, which necessitates more investigation into its mechanisms and applications.

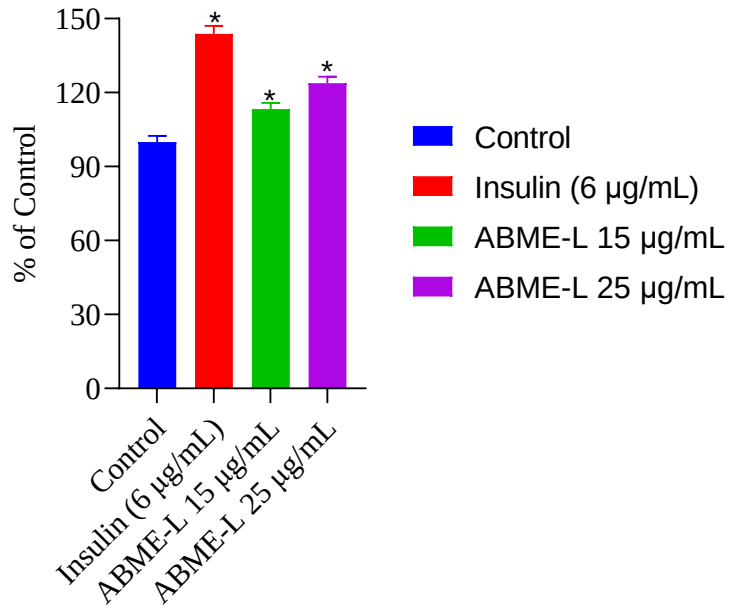

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Figure 2. ABME-L's effect on the absorption of glucose by L6 myoblasts. For 48 hours, the cells were treated with varying doses of the plant extract or a constant quantity.

The glucose utilization study in L6 myoblasts for the aqueous extract (ABWE-L) revealed its potential to enhance glucose uptake compared to the control and insulin

groups. The control group exhibited a baseline glucose utilization of 99.99 ± 2.45, while insulin at 6 µg/mL significantly increased glucose utilization to 143.87 ± 3.16, serving as the positive control. ABWE-L at 15 µg/mL moderately improved glucose uptake to 110.22 ± 2.12, indicating a slight insulin-mimetic activity. At a higher concentration of 25 µg/mL, ABWE-L further enhanced glucose utilization to 120.98 ± 2.49, demonstrating a dose-dependent effect. These findings suggest ABWE-L as a promising agent for improving glucose metabolism, warranting further exploration to elucidate its mechanisms and therapeutic potential.

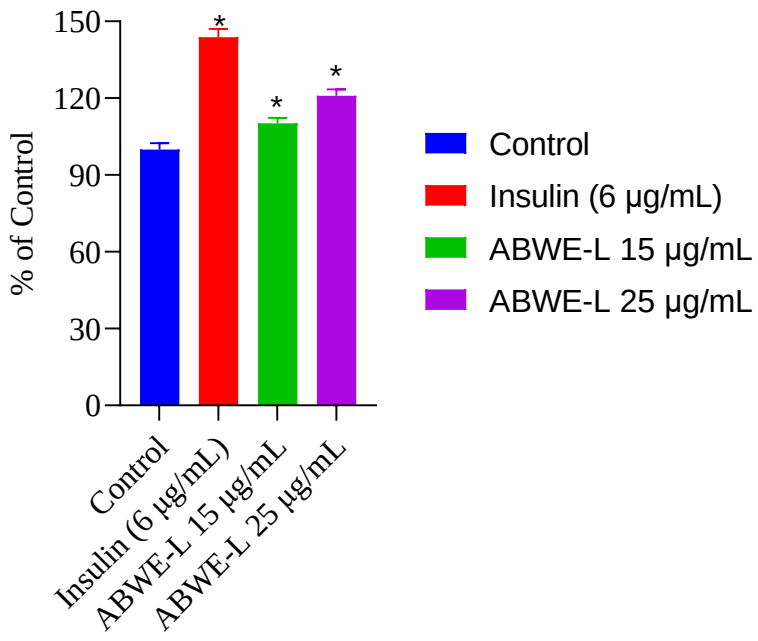

Figure 3. ABWE-L's effect on the absorption of glucose by L6 myoblasts. For 48 hours, the cells were treated with varying doses of the plant extract or a constant quantity.

Inhibition Assay of DPP-4

The DPP-4 inhibition assay evaluated the effectiveness of ABME-L (methanolic extract) at different concentrations compared to Diprotin A as a standard inhibitor and a control. The control group exhibited no inhibitory activity. Diprotin A (65 µg/mL) showed a

strong inhibitory effect, with an inhibition rate of 88.65 ± 1.57, validating its role as a potent DPP-4 inhibitor. ABME-L at 65 µg/mL demonstrated a lower inhibition rate of 18.87 ± 1.16, while increasing the concentration to 125 µg/mL significantly improved the inhibitory activity to 41.89 ± 1.14. According to these findings, ABME-L may have use in antidiabetic applications since it shows dose-dependent DPP-4 inhibitory action. To identify and describe the active ingredients causing this behaviour, more research is advised.

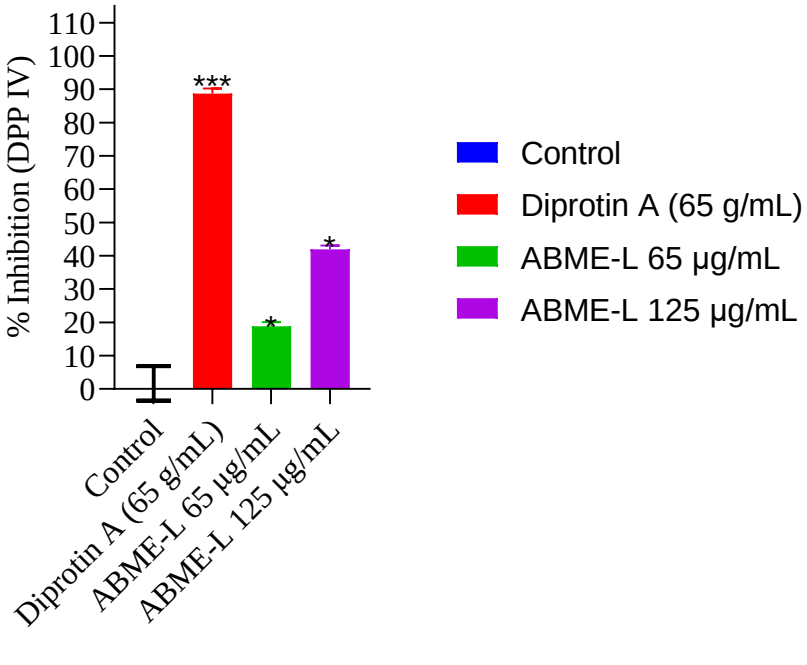

Figure 4. The impact of ABME-L on the percentage of DPP-4 activity inhibition.

In comparison to Diprotin A and a control, the DPP-4 inhibition experiment for ABWE-L (aqueous extract) showed its inhibitory potential. The control group exhibited no inhibition activity. Diprotin A at 65 µg/mL showed a high inhibitory effect of 88.65 ± 1.57, confirming its efficacy as a DPP-4 inhibitor. ABWE-L at 65 µg/mL displayed moderate inhibition with a rate of 16.33 ± 1.13, while increasing the concentration to 125

µg/mL improved the inhibition to 37.97 ± 1.13. These findings indicate a dose-dependent inhibitory effect of ABWE-L on DPP-4 activity, suggesting its potential role in managing diabetes. Further research is warranted to identify the active components responsible for this activity and optimize its therapeutic efficacy.

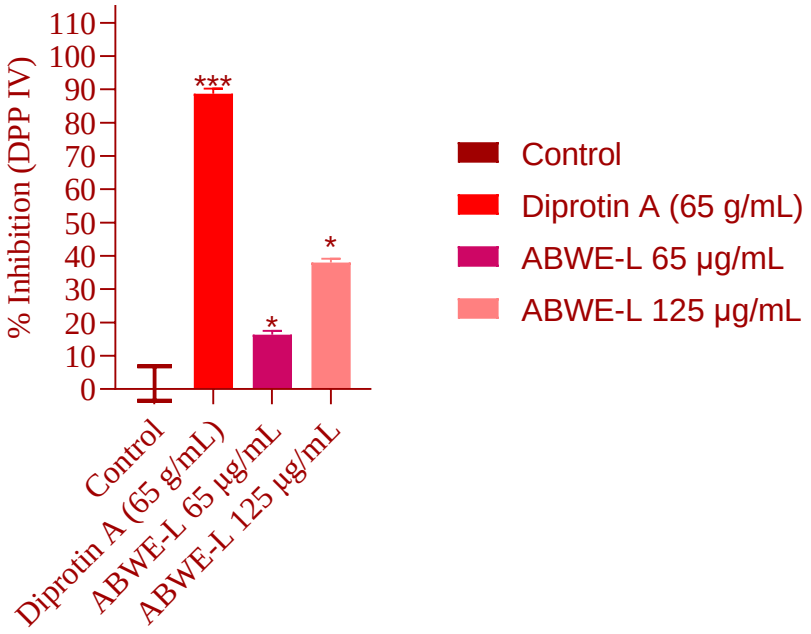

Figure 5. The impact of ABWE-L on the percentage of DPP-4 activity inhibition

Neuroprotective activity:

Intracellular reactive oxygen species levels are lowered by the extracts

The impact of ABME-L on intracellular reactive oxygen species (ROS) levels was evaluated across varying concentrations, with H_2O_2 serving as an oxidative stress inducer. The control group exhibited baseline ROS levels normalized to 100%. H_2O_2 (500 µM) significantly elevated ROS levels to 270 ± 5.45, confirming oxidative stress induction. ROS levels were shown to decrease in a dose-dependent manner by ABME-L, with 5 µg/mL and 10 µg/mL producing 269 ± 5.19 and 229 ± 5.38, respectively. Higher concentrations of 20, 40, and 80 µg/mL further reduced ROS levels to 211 ± 4.41, 151 ±

4.04, and 147 ± 4.11, respectively. These results indicate that ABME-L effectively mitigates oxidative stress, potentially due to its antioxidant properties, with greater efficacy observed at higher concentrations. This suggests its potential for therapeutic applications in oxidative stress-related conditions.

Table 3. ABME-L's effect on the amount of intracellular reactive oxygen species.

Control	**Hydrogen Peroxide (H_2O_2)**	**ABME-L (µg/ml)**				
	500 µm	5	10	20	40	80
100	270±5.45	269±5.19	229±5.38	211±4.41	151±4.04	147±4.11

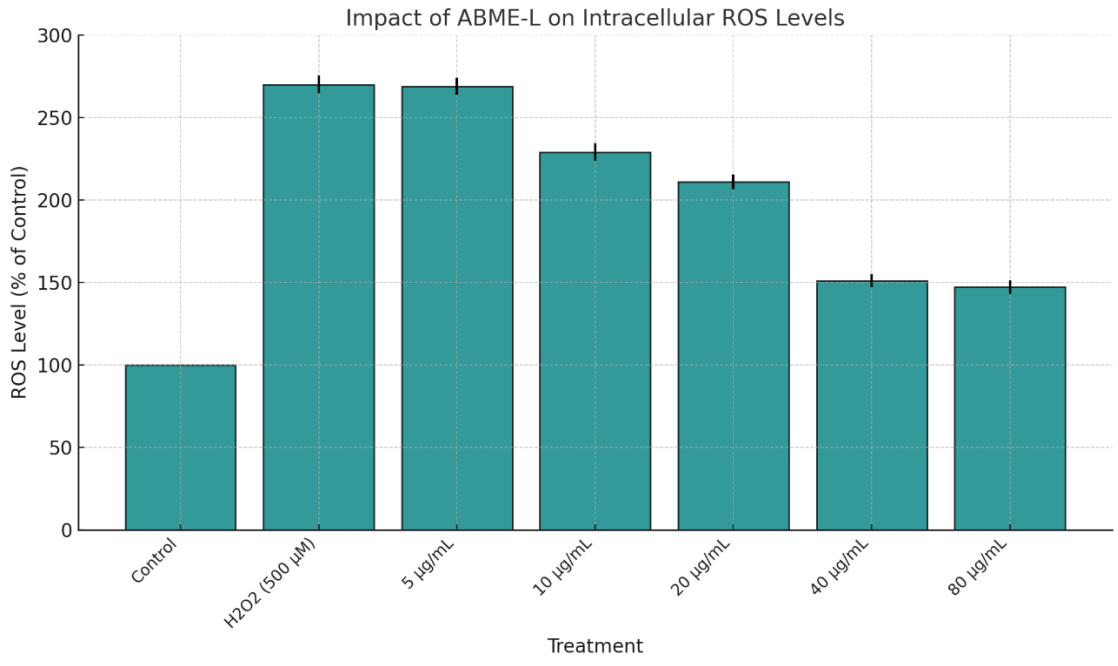

Figure 6.ABME-L lowers intracellular reactive oxygen species levels.

The impact of ABWE-L on intracellular reactive oxygen species (ROS) levels was assessed across varying concentrations in the presence of H_2O_2-induced oxidative stress. The control group showed a baseline ROS level of 100%, while H_2O_2 (500 µM) significantly elevated ROS levels to 272 ± 8.62, confirming its ability to induce oxidative stress. Treatment with ABWE-L at 5 µg/mL resulted in a negligible reduction, with ROS levels at 271 ± 5.86. At higher concentrations, ABWE-L exhibited a dose-dependent decrease in ROS levels, with values of 231 ± 6.01 at 10 µg/mL, 213 ± 5.19 at 20 µg/mL, 152 ± 4.21 at 40 µg/mL, and 148 ± 4.11 at 80 µg/mL. These results indicate that ABWE-L possesses antioxidant properties capable of mitigating oxidative stress, with higher concentrations being more effective. This dose-dependent response suggests its potential utility in managing oxidative stress-related disorders.

Table 4. Impact of ABWE-L in intracellular reactive oxygen species level.

Control	**Hydrogen Peroxide (H_2O_2)**	**ABWE-L (µg/ml)**				
	500 µm	5	10	20	40	80
100	272±8.62	271±5.86	231±6.01	213±5.19	152±4.21	148±4.11

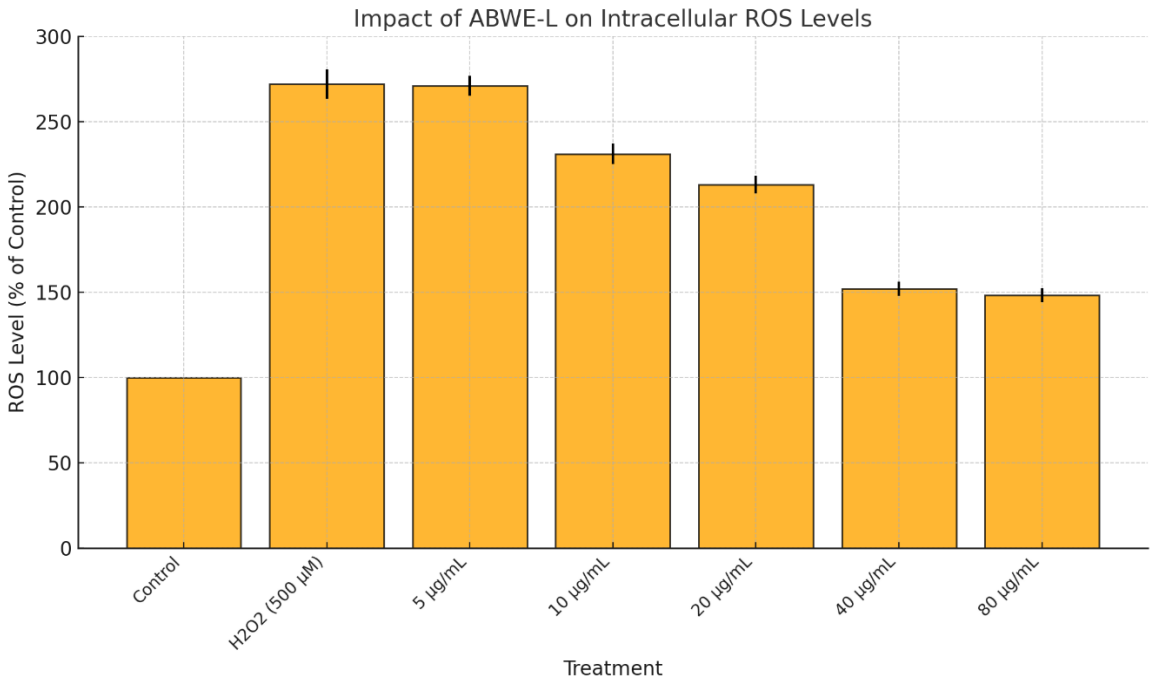

Figure 7.ABWE-L lowers the quantity of intracellular reactive oxygen species.

CONCLUSIONS

The comprehensive evaluation of *Ajuga bracteosa Wall ex Benth.* Leaf extracts, ABME-L and ABWE-L revealed their diverse phytochemical composition and significant pharmacological activities. ABME-L demonstrated superior antioxidant and antidiabetic potential, as evidenced by its higher phenolic content, enhanced glucose uptake, and stronger DPP-4 inhibitory activity compared to ABWE-L. Both extracts effectively reduced intracellular ROS levels, with ABME-L exhibiting a more pronounced dose-dependent response, highlighting its neuroprotective capabilities. The results underscore the therapeutic potential of these extracts, particularly ABME-L, in oxidative stress-related conditions and metabolic disorders. In order to prepare the way for their use in the creation of innovative treatments, it is advised that future research isolate active ingredients and look into their modes of action.

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