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Research Article

Antioxidant, α-Glucosidase, and α-Amylase Inhibition Activities of *Erythropalum scandens* Blume

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Diabetes is a major health problem worldwide, which is increasing day by day. Since ancient times, medicinal plants have been a key source of medicinal agents, and many of them have been authorized as strong medications or drug candidates. This study evaluated the antioxidant, α-glucosidase, and α-amylase inhibition activities of *Erythropalum scandens* Blume. The plant revealed the significant antioxidant and *in vitro* antidiabetic activity. The crude methanolic extract reported the highest antioxidant activity with an IC_{50} of 59.35 ± 5.47 μg/mL, followed by its dichloromethane (DCM) and ethyl acetate (EA) fractions with an IC_{50} of 66.45 ± 2.46 μg/mL and 80.46 ± 2.69 μg/mL, respectively, as compared to the standard quercetin's IC_{50} value of 6.29 ± 1.02 μg/mL. Among the crude extract and its fractions, the EA fraction disclosed the significant inhibiting activity against α-glucosidase and α-amylase with an IC_{50} value of 17.92 ± 0.88 μg/mL and 44.51 ± 0.12 μg/mL, respectively. This research work has scientifically validated the traditional use of this plant.

1. Introduction

Type 2 diabetes mellitus (T2DM) is the most prevalent type of diabetes, characterized by hyperglycemia, insulin resistance, and relative insulin deficiency [1, 2]. One of the best methods to manage postprandial hyperglycemia is enzyme (α-glucosidase and α-amylase) inhibition [3]. α-Glucosidase and α-amylase are the two common hydrolyzing enzymes responsible for the breakdown of carbohydrates [4–6]. Inhibiting these enzymes, therefore, lessens the rise in postprandial blood glucose levels and has been successfully used to prevent the progression of poor glucose tolerance to T2DM [7–10].

Erythropalum scandens, a wildly grown species belonging to the family Olacaceae, can be found at an altitude of 100–1000 m above sea level. The roots of the plants constitute some therapeutic constituents such as resins, phenolic, and triterpenes [11]. Leaves of this plant have a high nutritional value due to the presence of high water content, lipid, protein, vitamins (B1, B2, and C), calcium, iron, phosphorus, and zinc [12]. This plant also possesses anti-inflammatory properties since it is traditionally used to treat pain and inflammation. Boiled water extract of the tender shoots and leaf paste mixed with honey is used to get relief from rheumatic ailments and complications [13].

These findings encouraged us to do further research on this plant. In this work, we studied the total phenolic and flavonoid content, *in vitro* antioxidant, and α-glucosidase and α-amylase inhibition activities of *Erythropalum scandens* methanolic extract and its fractions.

2. Methods

2.1. Chemicals. The gallic acid, Folin–Ciocalteu's reagent, quercetin, 2,2-diphenyl-1-picrylhydrazyl (DPPH), dimethyl sulfoxide (DMSO), methanol, acarbose, α-glucosidase from

Saccharomyces cerevisiae, α-amylase from porcine pancreases, 2-chloro-4-nitrophenyl- α -D-maltotrioside (CNPG3), and *p*-nitrophenyl-α-D-glucopyranoside (*p*-NPG) were purchased from Sigma-Aldrich. All chemicals and reagents used in this study were of analytical grade.

2.2. Plant Collection and Preparation of Extract. The barks of *Erythropalum scandens* Blume were collected from Nepal's Tanahun district. The plant was authenticated by Mr. Hem Raj Paudel, Research Officer, National Herbarium and Plant Laboratories, Godavari Kathmandu, Nepal. Collected barks of *E. scandens* were dried and ground to powder. The methanolic extract of the plant was prepared by the cold percolation method in which the powdered form of the bark in a conical flask was mixed with methanol for 72 hours. After that, filtration was conducted using a muslin cloth, and the filtrate was concentrated in a vacuum rotary evaporator to obtain the crude extract. Obtained crude extract was successively fractioned with dichloromethane (DCM) and ethyl acetate (EA) to get DCM and EA fractions.

2.3. Total Phenolic Content (TPC). The Folin–Ciocalteu (FC) reagent was used to calculate the total phenolic content [14]. In brief, 20 μL of plant sample, 100 μL of FC reagent, and 80 μL of Na_2CO_3 solution were mixed and left in the dark for 15 minutes. Finally, the absorbance at 765 nm was measured using a microplate reader (EpochTM 2 Microplate Spectrophotometer, BioTek Instruments, USA). The standard curve was created using varying amounts of gallic acid.

2.4. Total Flavonoid Content (TFC). The total flavonoid content was determined using the aluminium trichloride method [15]. Briefly, 20 μL of plant sample, 110 μL of distilled water, 60 μL of ethanol, 5 μL of $AlCl_3$, and 5 μL of potassium acetate were mixed and left to react at room temperature for 30 minutes. Finally, absorbance at 415 nm was measured with a microplate reader. A calibration curve was constructed using various concentrations of quercetin.

2.5. Antioxidant Activity. DPPH free radical scavenging activity was used to determine the antioxidant activity of the plant's crude extract and its fractions [16]. In short, 100 μL of plant sample and 100 μL of DPPH (0.1 mM) were mixed and allowed to react in the dark at room temperature for 30 minutes. Finally, absorbance was measured at 517 nm in a spectrophotometer, and the inhibition percentage was calculated using the following formula. The plant sample was dissolved in 50% DMSO, so it was taken as the control. Quercetin was taken as an antioxidant standard.

$$\%\ \text{inhibition} = \left(\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}}\right) \times 100, \quad (1)$$

where A_{control} is the absorbance of the control and A_{sample} is the absorbance of the sample.

2.6. α-Glucosidase Inhibition Activity. The *p*-NPG was used as a substrate in the α-glucosidase inhibition assay, which was carried out using previously described procedures with minor changes [17]. Briefly, 20 μL of sample extract (in 30% DMSO) was mixed with 20 μL of α-glucosidase (final concentration of 0.5 U/mL) and 120 μL of buffer solution and incubated at 37°C for 15 minutes. The reaction was then terminated by adding 40 μL of *p*-NPG and incubating it for another 15 minutes. Finally, using a microplate reader, the absorbance was measured at 405 nm (EpochTM 2 Microplate Spectrophotometer, BioTek Instruments, USA). Plant samples were dissolved in 30% DMSO; hence, it was taken as the control. Acarbose was taken as a standard. The inhibition percentage was calculated using the following formula:

$$\%\ \text{inhibition} = \left(\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}}\right) \times 100, \quad (2)$$

where A_{control} is the absorbance of the control and A_{sample} is the absorbance of the sample.

2.7. α-Amylase Inhibition Activity. The α-amylase inhibition activity was evaluated in a 50 mM of phosphate buffer of pH 7.0 with 0.9% NaCl [18]. Plant samples of 20 μL from different concentrations were mixed with 80 μL α-amylase (at the final concentration of 1.5 U/mL) and incubated at 37°C for 15 min. The reaction was started by the addition of 100 μL substrate, CNPG3 (at 0.5 mM final concentration) prepared in the buffer. Finally, the absorbance was measured at 405 nm using a microplate reader. DMSO (30%) was taken as the negative control, and acarbose was taken as the standard. The inhibition percentage was calculated using the following formula:

$$\%\ \text{inhibition} = \left(\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}}\right) \times 100, \quad (3)$$

where A_{control} is the absorbance of the control and A_{sample} is the absorbance of the sample.

3. Results

3.1. Total Phenolic and Flavonoid Content. Total phenolic content (TPC) was calculated by plotting a graph of various concentrations of gallic acid against the absorbance. Similarly, the total flavonoid content (TFC) was estimated by plotting a graph of different concentrations of quercetin against the absorbance. The TPC was expressed as mg GAE/g, and TFC was expressed as mg QE/g. The TPC of the methanolic extract of *E. scandens* was found to be 179.43 ± 3.40 mg GAE/g, and the TFC was reported 10.63 ± 0.34 mg QE/g.

3.2. Antioxidant Activity. All fractions and crude extracts of *Erythropalum scandens* showed an inhibitory activity ≥50% against DPPH free radical at a concentration of 500 μg/mL. Hence, the samples are further diluted to obtain the IC_{50} value. The inhibition percentage of the crude extract and its fraction at 500 μg/mL against DPPH free radical is shown in Table 1. The crude extract revealed the highest inhibiting

Table 1: Inhibition percentage (%) of the crude extract, EA, and DCM fractions of *Erythropalum scandens* against DPPH free radical, α-glucosidase, and α-amylase at 500 μg/mL.

Plant (fractions)	Antioxidant (%)	α-glucosidase (%)	α-amylase (%)
Crude	82.85	83.46	87.52
DCM	90.64	70.25	77.25
Ethyl acetate	91.10	85.78	83.81

Table 2: IC_{50} values of the antioxidant, α-glucosidase, and α-amylase inhibition activity of *Erythropalum scandens*.

Plant (fractions)	Antioxidant (μg/mL)	α-amylase (μg/mL)	α-glucosidase (μg/mL)
Crude	59.35 ± 5.47	45.24 ± 0.89	35.45 ± 0.69
Dichloromethane	66.45 ± 2.46	100.83 ± 0.08	38.02 ± 7.25
Ethyl acetate (EA)	80.46 ± 2.69	44.51 ± 0.12	17.92 ± 0.88
Acarbose (standard)	—	6.02 ± 1.04	5.65 ± 0.3
Quercetin	6.29 ± 1.02	—	—

—: not tested.

activity against the DPPH free radical with an IC_{50} value of 59.35 ± 5.47 μg/mL compared to the standard quercetin 6.29 ± 1.02 μg/mL. Similarly, the IC_{50} values for EA and DCM fractions were reported to be 80.46 ± 2.69 μg/mL and 66.45 ± 2.46 μg/mL, respectively. The IC_{50} value of the antioxidant activity is depicted in Table 2.

3.3. α-Glucosidase and α-Amylase Inhibition Activity. The crude extract and all the plant fractions showed an inhibitory activity of ≥50% against α-glucosidase and α-amylase at a concentration of 500 μg/mL. The inhibition percentage of the crude extract and its fractions at 500 μg/mL against both the digestive enzymes is depicted in Table 1. Among the crude extract and fractions, the EA fraction revealed potent inhibiting activity against both the enzymes α-glucosidase and α-amylase with the IC_{50} value of 17.92 ± 0.88 μg/mL and 44.51 ± 0.12 μg/mL, respectively. Similarly, the crude extract revealed an IC_{50} value of 35.45 ± 0.69 μg/mL against α-glucosidase and 45.24 ± 0.89 μg/mL against α-amylase. The DCM fraction of the bark of the plant showed an IC_{50} value of 38.02 ± 7.25 μg/mL and 100.83 ± 0.08 μg/mL against α-glucosidase and α-amylase, respectively. The IC_{50} value of the crude extract and fractions against α-glucosidase and the α-amylase enzyme is presented in Table 2.

4. Discussion

The current study attempted to assess the inhibitory action of *Erythropalum scandens* on the digestive enzymes (α-glucosidase and α-amylase) and the free radical DPPH. The methanolic extract and its fractions of *E. scandens* showed promising antioxidant, α-glucosidase, and α-amylase inhibition activity. In the previous study, the TPC and TFC of *E. scandens* were found to be 0.38 ± 0.03 mg GAE/g DW and 0.068 ± 0.01 mg CE/g DW, respectively [19]. In the present study, the TPC and TFC of this plant were reported as 179.43 ± 3.40 mg GAE/g, and the TFC was reported as 10.63 ± 0.34 mg QE/g, respectively. Previously, the IC_{50} value for the α-amylase and α-glucosidase inhibition of the 95% ethanolic extract of this plant was found to be 2.28 ± 0.01 mg/mL and 2.97 ± 0.03 mg/mL, respectively [19]. The antioxidant activity of *E. scandens* in the previously carried research work was found to be 2.72 ± 0.00 μg AAE/g DW. Our study disclosed the most potent antioxidant activity in the crude extract with an IC_{50} value of 59.35 ± 5.47 μg/mL among its fractions. In the present study, crude extract of the plant revealed an IC_{50} value of 35.45 ± 0.69 μg/mL against α-glucosidase and 45.24 ± 0.89 μg/mL against α-amylase. The EA fraction revealed the highest inhibition activity against α-glucosidase and α-amylase, with the IC_{50} value of 17.92 ± 0.88 μg/mL and 44.51 ± 0.12 μg/mL, respectively. The DCM fraction revealed an IC_{50} value of 38.02 ± 7.25 μg/mL and 100.83 ± 0.08 μg/mL against α-glucosidase and α-amylase, respectively.

5. Conclusion

The present study revealed the total phenolic and flavonoid content, antioxidant, and α-glucosidase and α-amylase inhibition activities of the methanolic extract and its fractions of *E. scandens*. The plant possesses significant antioxidant as well as α-glucosidase and α-amylase inhibition activity. Furthermore, research should be carried out for the isolation and identification of the bioactive compounds that are responsible for these activities.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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