

Research Article

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

Achyut Adhikari ¹, Binita Adhikari,¹ Dipesh Shrestha ², Ram Jeevan Tharu,¹
Nisha Gyawali,¹ and Hem Raj Paudel³

¹Central Department of Chemistry, Tribhuvan University, Kathmandu 44618, Nepal

²Department of Chemistry, University of North Texas, 1155 Union Circle, Denton, TX 76203–5017, USA

³National Herbarium and Plant Laboratories, Godawari, Lalitpur, Nepal

Correspondence should be addressed to Achyut Adhikari; achyutraj05@gmail.com

Received 18 October 2022; Revised 19 March 2023; Accepted 20 March 2023; Published 1 April 2023

Academic Editor: Beatriz P. P. Oliveira

Copyright © 2023 Achyut Adhikari et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

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 *Erythralpalum 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 $59.35 \pm 5.47 \mu\text{g/mL}$, followed by its dichloromethane (DCM) and ethyl acetate (EA) fractions with an IC_{50} of $66.45 \pm 2.46 \mu\text{g/mL}$ and $80.46 \pm 2.69 \mu\text{g/mL}$, respectively, as compared to the standard quercetin's IC_{50} value of $6.29 \pm 1.02 \mu\text{g/mL}$. 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 $17.92 \pm 0.88 \mu\text{g/mL}$ and $44.51 \pm 0.12 \mu\text{g/mL}$, 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].

Erythralpalum scandens, a wildy 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 *Erythralpalum 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-maltotriose (CNP3), 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 *Erythralum 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 fractionated 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 μ L of plant sample, 100 μ L of FC reagent, and 80 μ L of Na₂CO₃ solution were mixed and left in the dark for 15 minutes. Finally, the absorbance at 765 nm was measured using a microplate reader (Epoch™ 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 μ L of plant sample, 110 μ L of distilled water, 60 μ L of ethanol, 5 μ L of AlCl₃, and 5 μ L 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 μ L of plant sample and 100 μ L 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 μ L of sample extract (in 30% DMSO) was mixed with 20 μ L of α -glucosidase (final concentration of 0.5 U/mL) and 120 μ L of buffer solution and incubated at 37°C for 15 minutes. The reaction was then terminated by adding 40 μ L of *p*-NPG and incubating it for another 15 minutes. Finally, using a microplate reader, the absorbance was measured at 405 nm (Epoch™ 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 μ L from different concentrations were mixed with 80 μ L 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 μ L 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 *Erythralum scandens* showed an inhibitory activity $\geq 50\%$ against DPPH free radical at a concentration of 500 μ g/mL. Hence, the samples are further diluted to obtain the IC₅₀ value. The inhibition percentage of the crude extract and its fraction at 500 μ g/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 *Erythralum scandens* against DPPH free radical, α -glucosidase, and α -amylase at 500 $\mu\text{g/mL}$.

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 *Erythralum scandens*.

Plant (fractions)	Antioxidant ($\mu\text{g/mL}$)	α -amylase ($\mu\text{g/mL}$)	α -glucosidase ($\mu\text{g/mL}$)
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 $59.35 \pm 5.47 \mu\text{g/mL}$ compared to the standard quercetin $6.29 \pm 1.02 \mu\text{g/mL}$. Similarly, the IC_{50} values for EA and DCM fractions were reported to be $80.46 \pm 2.69 \mu\text{g/mL}$ and $66.45 \pm 2.46 \mu\text{g/mL}$, 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 $\geq 50\%$ against α -glucosidase and α -amylase at a concentration of 500 $\mu\text{g/mL}$. The inhibition percentage of the crude extract and its fractions at 500 $\mu\text{g/mL}$ 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 $17.92 \pm 0.88 \mu\text{g/mL}$ and $44.51 \pm 0.12 \mu\text{g/mL}$, respectively. Similarly, the crude extract revealed an IC_{50} value of $35.45 \pm 0.69 \mu\text{g/mL}$ against α -glucosidase and $45.24 \pm 0.89 \mu\text{g/mL}$ against α -amylase. The DCM fraction of the bark of the plant showed an IC_{50} value of $38.02 \pm 7.25 \mu\text{g/mL}$ and $100.83 \pm 0.08 \mu\text{g/mL}$ 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 *Erythralum 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 $0.38 \pm 0.03 \text{ mg GAE/g DW}$ and $0.068 \pm 0.01 \text{ mg CE/g DW}$, respectively [19]. In the present study, the TPC and TFC of this plant were reported as $179.43 \pm 3.40 \text{ mg GAE/g}$, and the TFC was reported as $10.63 \pm 0.34 \text{ mg QE/g}$, 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 $2.28 \pm 0.01 \text{ mg/mL}$ and $2.97 \pm 0.03 \text{ mg/mL}$, respectively [19]. The antioxidant activity of *E. scandens* in the previously carried research work was found to be $2.72 \pm 0.00 \mu\text{g AAE/g DW}$. Our study disclosed the most potent antioxidant activity in the crude extract with an IC_{50} value of $59.35 \pm 5.47 \mu\text{g/mL}$ among its fractions. In the present study, crude extract of the plant revealed an IC_{50} value of $35.45 \pm 0.69 \mu\text{g/mL}$ against α -glucosidase and $45.24 \pm 0.89 \mu\text{g/mL}$ against α -amylase. The EA fraction revealed the highest inhibition activity against α -glucosidase and α -amylase, with the IC_{50} value of $17.92 \pm 0.88 \mu\text{g/mL}$ and $44.51 \pm 0.12 \mu\text{g/mL}$, respectively. The DCM fraction revealed an IC_{50} value of $38.02 \pm 7.25 \mu\text{g/mL}$ and $100.83 \pm 0.08 \mu\text{g/mL}$ 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.

Acknowledgments

The authors would like to acknowledge HERP Tribhuvan University, Nepal, for a startup research grant to A. Adhikari.

References

- [1] D. Shrestha, T. Pokhrel, K. Dhakal, A. Pandey, P. Sharma, and S. Sapkota, " α -Glucosidase and α -amylase inhibition study and in silico analysis of *Mimosa pudica* L. of Nepalese origin," *Current Bioactive Compounds*, vol. 18, pp. 2–8, 2022.
- [2] A. B. Olokoba, O. A. Obateru, and L. B. Olokoba, "Type 2 diabetes mellitus: a review of current trends," *Oman Medical Journal*, vol. 27, no. 4, pp. 269–273, 2012.
- [3] C. Lankatillake, T. Huynh, and D. A. Dias, "Understanding glycaemic control and current approaches for screening antidiabetic natural products from evidence-based medicinal plants," *Plant Methods*, vol. 15, no. 1, p. 105, 2019.
- [4] A. I. Martinez-Gonzalez, ÁG. Díaz-Sánchez, L. A. Rosa, C. L. Vargas-Requena, I. Bustos-Jaimes, and E. Alvarez-Parrilla, "Polyphenolic compounds and digestive enzymes: in vitro non-covalent interactions," *Molecules*, vol. 22, no. 4, p. 669, 2017.
- [5] D. Shrestha, P. Sharma, A. Adhikari, A. K. Mandal, and A. Verma, "A review on Nepalese medicinal plants used traditionally as α -amylase and α -glucosidase inhibitors against diabetes mellitus," *Current Traditional Medicine*, vol. 7, no. 5, p. 1, 2021.
- [6] T. Pokhrel, D. Shrestha, K. Dhakal, P. M. Yadav, and A. Adhikari, "Comparative analysis of the antioxidant and antidiabetic potential of *Nelumbo nucifera* Gaertn. and *Nymphaea lotus* L. Var. *pubes1*," *Journal of Chemistry*, vol. 2022, Article ID 4258124, 5 pages, 2022.
- [7] Y. M. Kim, M. H. Wang, and H. I. Rhee, "A novel α -glucosidase inhibitor from pine bark," *Carbohydrate Research*, vol. 339, no. 3, pp. 715–717, 2004.
- [8] M. Toeller, " α -Glucosidase inhibitors in diabetes: efficacy in NIDDM subjects," *European Journal of Clinical Investigation*, vol. 24, no. S3, pp. 31–35, 2010.
- [9] N. Saito, H. Sakai, S. Suzuki, H. Sekihara, and Y. Yajima, "Effect of an α -glucosidase inhibitor (voglibose), in combination with sulphonylureas, on glycaemic control in type 2 diabetes patients," *Journal of International Medical Research*, vol. 26, no. 5, pp. 219–232, 1998.
- [10] K. Dhakal, D. Shrestha, T. Pokhrel et al., "Antioxidant, antibacterial, antidiabetic potential, and in silico analysis of *Rhus chinensis* from western Nepal," *Current Topics in Medicinal Chemistry*, vol. 22, no. 26, pp. 2145–2151, 2022.
- [11] Z. X. Zhu, J. H. Wang, Y. C. Cai, K. K. Zhao, M. J. Moore, and H. F. Wang, "Complete plastome sequence of *Erythralum scandens* (Erythralaceae), an edible and medicinally important liana in China," *Mitochondrial DNA Part B*, vol. 3, no. 1, pp. 139–140, 2018.
- [12] D. T. Vu and T. A. Nguyen, "The neglected and underutilized species in the Northern mountainous provinces of Vietnam," *Genetic Resources and Crop Evolution*, vol. 64, no. 6, pp. 1115–1124, 2017.
- [13] S. Sutha, A. Maruthupandian, V. R. Mohan, and T. Athiparamsami, "Anti-inflammatory activity of leaf of *Erythralum scandens* bl., bjd against carrageenan induced paw edema," *Int J PharmTech Res CODEN*, vol. 3, no. 1, pp. 24–26, 2011.
- [14] S. Kupina, C. Fields, M. C. Roman, and S. L. Brunelle, "Determination of total phenolic content using the Folin-C assay: single-laboratory validation, first action 2017.13," *Journal of AOAC International*, vol. 101, no. 5, pp. 1466–1472, 2018.
- [15] C.-C. Chang, M.-H. Yang, H.-M. Wen, and J.-C. Chern, "Estimation of total flavonoid content in propolis by two complementary colorimetric methods," *Journal of Food and Drug Analysis*, vol. 10, no. 3, 2020.
- [16] L. L. Mensor, F. S. Menezes, G. G. Leitão et al., "Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method," *Phytotherapy Research*, vol. 15, no. 2, pp. 127–130, 2001.
- [17] M. Telagari and K. Hullatti, "In-vitro α -amylase and α -glucosidase inhibitory activity of *Adiantum caudatum* Linn. and *Celosia argentea* Linn. extracts and fractions," *Indian Journal of Pharmacology*, vol. 47, no. 4, pp. 425–429, 2015.
- [18] M. R. Senger, L. D. C. A. Gomes, S. B. Ferreira, C. R. Kaiser, V. F. Ferreira, and F. P. J. Silva, "Kinetics studies on the inhibition mechanism of pancreatic α -amylase by glyco-conjugated 1H-1,2,3-triazoles: a new class of inhibitors with hypoglycemic activity," *ChemBioChem*, vol. 13, no. 11, pp. 1584–1593, 2012.
- [19] P. Dedvisitsakul and K. Watla-iad, "Antioxidant activity and antidiabetic activities of Northern Thai indigenous edible plant extracts and their phytochemical constituents," *Heliyon*, vol. 8, no. 9, Article ID e10740, 2022.