Trop J Nat Prod Res, January 2025; 9(1): 209 - 212 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

In Vitro Inhibitory Activity of *Lygodium Japonicum* Leaves on *A*-Amylase, *A*-Glucosidase, and Dipeptidyl Peptidase-IV

Nguyen H. My[1], Nguyen L. K. Loan[1], Do H. Tan[1], Le G. H. Long[1], Tran L. P. Linh[2], Nguyen K. Oanh[2], Nguyen T. Anh[2], Nguyen H. Phuc[1], Bui T. Phong[1*]

[1]*Department of General Medicine, Faculty of Medicine, Hong Bang International University (HIU), 215 Dien Bien Phu, Ward 15, Binh Thanh District, Ho Chi Minh City 70000, Vietnam.*
[2]*Department of Pharmacy, Faculty of Pharmacy, Hong Bang International University (HIU), 215 Dien Bien Phu, Ward 15, Binh Thanh District, Ho Chi Minh City 70000, Vietnam.*

ARTICLE INFO

Article history:
Received 19 May 2024
Revised 11 June 2024
Accepted 23 December 2024
Published online 01 February 2025

Copyright: © 2024 Nguyen *et al*. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

ABSTRACT

Diabetes mellites is a chronic disease of glucose metabolism affecting millions of people worldwide and is projected to be over 783 million by 2045. Key enzymes (*α*-amylase, *α*-glucosidase, and dipeptidyl-peptidase IV) have been reported to play a major role in glucose metabolism. Plant extracts have been used in traditional medicine to treat diabetes with encouraging results. *α*-amylase catalyses carbohydrate hydrolysis in the pancreas. In contrast, *α*-glucosidase catalyses the process of cutting the 1,4-α-glucosidic linkage of substrates to release *α*-D-glucose. DPP-IV decreases insulin secretion by decomposing peptides like glucagon-1 and gastric inhibitory polypeptides. This study investigates the inhibitory effects of *Lygodium japonicum* leaf extracts on *α*-amylase, *α*-glucosidase, and dipeptidyl-peptidase IV *in vitro*. *Lygodium japonium* leaves were extracted with 96% ethanol and concentrated to obtain a total extract and then fractionated with solvents in increasing order of polarity (n-hexane, chloroform, and n-butanol) to obtain different solvent extracts with the mother liquor (aqueous extract). *α*-amylase, *α*-glucosidase, and DPP-IV inhibitory activity of the extracts were investigated using established methods. Results of the study showed that the chloroform extract (CF) exhibited the highest inhibitory effect on *α*-glucosidase activity, with an IC_{50} value of 102.05 µg/mL. The butanol extract (BU) showed the highest *α*-amylase and DPP-IV inhibitory activity, with IC_{50} values of 227.35 µg/mL and 141.98 µg/mL, respectively. It was concluded that *Lygodium japonicum* extracts can inhibit *α*-amylase, α-glucosidase, and DPP-IV enzymes and could be a source of leads for the development of antidiabetic medicines.

Keywords: Diabetes, *Lygodium japonicum*, *α*-glucosidase, *α*-amylase, dipeptidyl-peptidase IV.

Introduction

Diabetes mellitus is a chronic disease characterized by high blood sugar levels and is classified into type 1 diabetes (insufficient insulin production) and type 2 diabetes (insulin resistance).[1] Currently, there are millions of people living with diabetes worldwide, and it is one of the leading causes of death in Vietnam.[2,3] Diabetes mellitus often has characteristic symptoms such as increased thirst, frequent urination, increased hunger, and unexplained weight loss.[4] If not well controlled, diabetes mellitus can lead to life-threatening complications such as ketoacidosis or nonketotic hyperosmolar syndrome.[5]
Glucose is provided by carbohydrates in food. After entering the body, carbohydrate is hydrolyzed into glucose by *α*-amylase in the pancreas and *α*-glucosidase in the small intestine before penetrating the blood.

*Corresponding author. E mail: phongbui0407@gmail.com
Tel: (+84)934188966

Citation: My NH, Loan NLK , Tan DH, Long LGH, Linh TLP, Oanh NK, Anh NT, Phuc NH, Phong BT. *In Vitro* Inhibitory Activity of *Lygodium Japonicum* Leaves on *A*-Amylase, *A*-Glucosidase, and Dipeptidyl Peptidase-IV. Trop J Nat Prod Res. 2025; 9(1): 209 - 212 https://doi.org/10.26538/tjnpr/v9i1.31

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

α-amylase is a catalyst for carbohydrate hydrolysis,[6] *α*-amylase is a polypeptide chain consisting of 496 amino acids that hydrolyses *α*-1,4 glycosidic linkages of *α*-linked polysaccharides into maltose and glucose.[7] *α*-glucosidase is one of the most critical enzymes in carbohydrate digestion.[8] It catalyses the process of cutting the 1,4-*α*-glucosidic linkage of the substrate to release *α*-D-glucose.[7,9] *α*-glucosidase enzymes are divided into three groups based on substrate specificity: Group I: Selective for heterogeneous substrates such as sucrose and aryl *α*-glucoside. Groups II and III: Selective for homogeneous substrates such as maltose, although enzymes in group II are highly selective for long-chain substrates.[8] The regulation of blood glucose concentration is closely related to two hormones from the pancreas: insulin and glucagon. Insulin is a polypeptide hormone produced by *β* cells in the Islets of Langerhans, while glucagon is produced by *α* cells. Insulin activates the absorption and storage of glucose in the liver, muscle, and fat tissue through the tyrosine kinase receptor path, thus reducing blood glucose. In contrast, when insulin is deficient or ineffective, the blood glucose levels in the blood increase, causing hyperglycemia; if this condition persists, it often leads to diabetes, which can lead to CNS complications, eyes, kidneys, and more severe chronic ketoacidosis.[9] Another enzyme that plays a role in glucose and insulin metabolism is dipeptidyl-peptidase IV (DPP-IV). DPP-IV decomposes peptides like glucagon 1 (GLP-1) and gastric inhibitory polypeptide (GIP), causing decreased insulin secretion; on the other hand, DPP-IV also degenerates GLP-1 to adjust glucose after meals, causing hyperglycemia.[10] One of the goals of diabetes mellitus treatment is to inhibit carbohydrate hydrolyzing enzymes (*α*-glucosidase and *α*-amylase), which helps slow glucose absorption,

© 2024 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License

 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

resulting in plasma glucose concentration decreases and inhibition of postprandial hyperglycemia.[8] DPP-IV inhibition is also advantageous in treating diabetes mellitus. DPP-IV inhibition helps increase GLP-1 and GIP levels, thereby helping to increase insulin secretion and reduce blood glucose.[8] Many common medications used to treat diabetes mellitus, including acarbose, miglitol, and voglibose, which effectively inhibit *α*-amylase and *α*-glucosidase, are expensive and are associated with many side effects.[11] Recent studies show that phytochemical compounds can inhibit *α*-glucosidase and *α*-amylase effectively.[12] Natural compounds in herbal medicine can act synergistically in the human body and provide optimum therapeutic properties without unwanted side effects.[13] Therefore, the use of herbal medicine can effectively treat diabetes mellitus and reduce the risks and complications caused by the disease. *Lygodium japonicum*, also known as Japanese climbing fern, is a member of the Lygodiaceae family. *Lygodium japonicum* is commonly found along forests or in shrubs in many regions of Vietnam. It is a climbing plant with pale yellow or brownish-yellow spores arranged in circular patterns on the sporangia. *Lygodium japonicum* holds enormous amounts of secondary metabolites such as tilianin, kaempferol-7-O-alpha-L-rhamnopyranoside, kaempferol, p-coumaric acid, hexadecanoic acid 2, 3-dihydroxy-propyl ester, daucosterol, beta-sitosterol, và 1-hentriacontanol.[14] The spores or the entire plant are often used as a traditional remedy due to their content of phenolic compounds, flavonoids, and polysaccharides.[13,15] *Lygodium japonicum* is also used to treat pain, inflammation, fever, and helminth diarrhoea.[16] Polysaccharides from *Lygodium japonicum* also exhibit vigorous antimicrobial and antioxidant activity.[13] *Lygodium japonicum* is noted to be used as a diuretic in China and for the treatment of colds, inflammations, and kidney diseases. In India, it is used to treat snake bites, diabetes, and ulcers.[17] In folk medicine, some herbal species in the same genus, *Lygodium*, are used in treating diabetes and high blood pressure; however, the potential to treat diabetes mellitus of *Lygodium japonicum* has not yet been confirmed through experimentation. Therefore, the objective of this research is to investigate the inhibitory potential of *Lygodium japonicum* extract on *α*-amylase and *α*-glucosidase enzymes, as well as DPP-IV and to elucidate its therapeutic capabilities for diabetes management through an *in vitro* experimental model.

Materials and Methods

Plant collection, identification and preparation.

Lygodium japonicum leaf samples were collected in October 2023 in uncultivated land in the Can Duoc district, Long An province, Vietnam. The sample was identified by a plant morphologist at the Faculty of Pharmacy Laboratory, Hong Bang International University (HIU), Vietnam, and assigned a code 23.10.005.

The fresh leaves were washed, cut into pieces, air-dried, ground and stored in an airtight container for further investigation.

Chemicals and other materials

The solvents include n-hexane, chloroform, n-butanol from Fisher, *α*-glucosidase from *Saccharomyces cerevisiae* yeast (≥ 10 U/mg), *α*-amylase from pig pancreas (≥ 5 U/mg), hippuryl-histidyl-leucine (HHL), captopril, p-Nitrophenyl α-D-glucopyranoside (pNPG), acarbose, dipeptidyl-peptidase IV (DPP-IV), Gly-Pro p-nitroanilide hydrochloride (GP-pNA), diprotin A were purchased from Sigma (USA) and UV-VIS spectrophotometer. Other chemicals used in this study were of analytical grades.

Plant material extraction

The dried powdered material was extracted with a 96% ethanol solvent ratio of 1:25 (w/v) by maceration and filtered with Whatman No. 1 filter paper. The extract was concentrated to obtain a crude (total extract (TP). An amount of the TP was dissolved in distilled water and successively extracted with solvents in order of polarity: n-hexane, chloroform, n-butanol, and water to obtain extracts of n-hexane (HE), chloroform (CF), n-butanol (BU) and the remaining aqueous solution (WA extract). The extracts were concentrated to dryness and stored in a refrigerator at 4°C until further biological activity investigation.

α-amylase inhibitory assay

Alpha-amylase inhibitory activity of the extracts was conducted using the method reported by Li *et al.*[18] Briefly, 200 µL of the extract was mixed with 40 µL of *α*-amylase enzyme (5 U/mL) and 0.36 mL of 6 mM sodium phosphate buffer at pH 6.9. The mixture was then incubated at 37°C for 20 minutes. After incubation, 300 µL of 1% starch was added to the mixture, and incubation was continued at 37°C for 20 minutes. Next, 0.2 mL of 1% DNSA reagent was added to the mixture, boiled for 5 minutes, and then brought to room temperature to cool. After that, 10 mL of distilled water was added and mixed well. The absorbance spectrum was measured at wavelength 540 nm to investigate the inhibition ability of the *α*-amylase enzyme. The positive control drug was Acarbose. *α*-amylase enzyme inhibitory activity was calculated using the formula:

$[(B-A)/B]x100\%$.

Where: A is the absorbance of the blank solution, and B is the absorbance of the sample solution.[18]

α-glucosidase inhibitory assay

The method previously reported by Nguyen *et al.* was adopted for this assay.[19] Briefly, 100 µL of extract was added to a mixture of 100 µL of enzyme and 2,200 µL of sodium phosphate buffer (0.01M; pH 7). After well mixed, the mixture was incubated at 37°C for 5 minutes. Then, 100 µL of pNPG substrate with a concentration of 3 mM was added to the mixture, mixed, and incubated for 30 minutes at 37°C. The reaction was stopped by adding 1,500 µL of Na_2CO_3 solution (0.1M) to the mixture. The pNPG generates a yellow product, p-nitrophenol, detected by measuring the optical absorbance at 405 nm using a spectrophotometer. The higher the *α*-glucosidase inhibitory activity of the sample, the lower the product produced. Acarbose was used as a positive control for this experiment. The *α*-glucosidase inhibitory activity was calculated from the formula:

$$Inhibitory\ activity\ = \frac{(B-A)}{B} x\ 100\%$$

Where A is the absorbance of the test sample, and B is the absorbance of the control sample.

The correlation equation between inhibition rate and substrate concentration y= a.lnx+b was built when measuring a series of 5 different concentrations of the samples, and the IC_{50} value was deduced from this equation.[19]

DPP-IV inhibitory assay

The extract was mixed well with the same volume of Gly - Pro - p - nitroaniline substrate (12 mM) (25 µL and incubated for 10 minutes at 37 °C. Then, 50 µL of DPP-IV (0.02 U/mL) was added. The mixture was incubated for 30 minutes at the same temperature. The reaction was completed by adding 100 µL of 1M sodium acetate buffer at pH 4.0. The DPP-IV inhibitory potential of the extract was measured at the absorbance of the reference spectrum at 405nm. DPP-IV inhibitory activity was calculated using the formula:

DPP-IV inhibitory activity = ((B-A)/B)x100%

Where A is the absorbance of the second sample, and B is the absorbance of the control sample. The inhibition ratio and substrate concentration y= a.lnx+b were constructed by measuring a series of 5 different concentrations of the sample, and the IC_{50} value was derived from this equation.[20]

Statistical analysis

Data was analysed using the Excel 2019 (Microsoft, Washington, USA) software package and expressed as mean ± SD. The correlation relationship was considered statistically significant when the correlation coefficient $R^2 > 0.95$.

© 2024 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Results and Discussion

Results of the investigation of the *α*-amylase inhibitory activity of *Lygodium japonicum* leaf extract are presented in Figure 1. The result shows that the BU extract had the highest *α*-amylase inhibitory effect with an IC_{50} value of 227.35 µg/mL. In contrast, the WA extract produced a weak *α*-amylase inhibitory activity with an IC_{50} value of 853.44 µg/mL. Compared to the positive control Acarbose, which has an IC_{50} value of 572.38 µg/ml, both BU and CF extracts of *Lygodium japonicum* leaves exhibit stronger inhibitions. BU extract was 2.5 times higher than the positive control. Research by Subba *et al.* (2016) showed that the ethanol extract of *Lygodium japonicum* contains chemical components such as Polyphenols, Terpenoids, and Glycosides.[21] Another report analysing the composition of species of the genus Lygodium also shows components such as Alkaloids, Flavonoids, Saponins, and Tannins.[22] Polyphenol compounds can be ingredients that effectively inhibit the *α*-amylase enzyme. As mentioned above, in the chemical constituents of Lygodium japonicum14-16, flavonoids were reported to inhibit *α*-amylase in over 80 studies with six different assay methods. The most used method in these six methods is using DNSA and measuring the absorbance spectrum at wavelength 540, which is the same method we used in this study.[23] Besides that, kaempferol, which was also found in *Lygodium japonicum,*[17] was also clarified that there is a possibility of inhibiting *α*-amylase through the non-competitive mechanism and kaempferol has fewer side effects than acarbose.[23] Thus, it is highly likely that the *α*-amylase inhibitory ability of *Lygodium japonicum* is due to flavonoids and kaempferol.

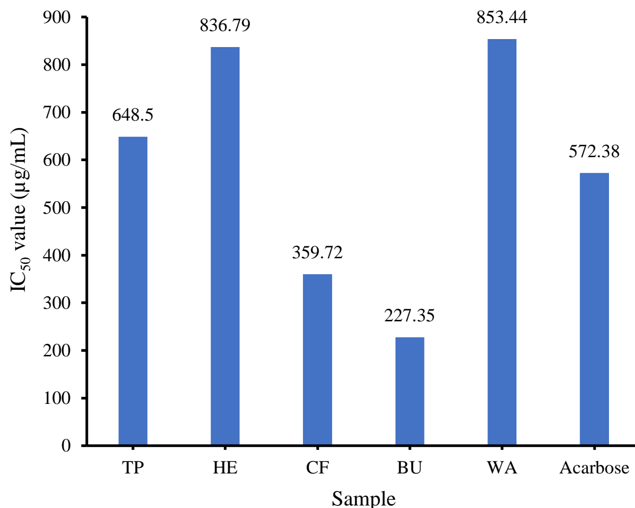

Figure 1: IC_{50} value (µg/mL) of *α*-amylase inhibitory activity of total and fractions extract of *Lygodium japonicum.*
Note: IC_{50} = Half-maximal inhibitory concentration; TP = total ethanol extract; HE = n-hexane extract; CF = chloroform extract; BU = n-butanol extract; WA = water.

As presented in Figure 2, the IC_{50} value of 102.05 µg/mL of CF extract revealed the highest inhibitory activity against α-glucosidase. The weakest inhibition was from WA extract with an IC_{50} value of 979.34 µg/ml. Compared with the Acarbose value, the *α*-glucosidase enzyme inhibitory activity of BU and CF extracts was 1.8 times higher than Acarbose. The chemical composition of *Lygodium japonicum* contains many components that can inhibit the *α*-glucosidase enzyme, such as Flavonoids, Alkaloids, Polyphenols, Tannins, and Terpenoids. In addition, a study has discovered and isolated the component 1,4-naphthoquinone from *L. japonicum* roots.[24] Anthraquinone derivatives can activate gamma receptors by peroxisomes and inhibit the *α*-glucosidase enzyme.[25] Besides, some species of the genus Lygodium, such as *Lygodium microphyllum*, show potential for treating diabetes. *Lygodium microphyllum* can prevent hyperglycemia in diabetic mice caused by alloxan.[26] As a result, *Lygodium japonicum* had the potential to treat diabetes mellitus by inhibiting the enzyme *α*-glucosidase.

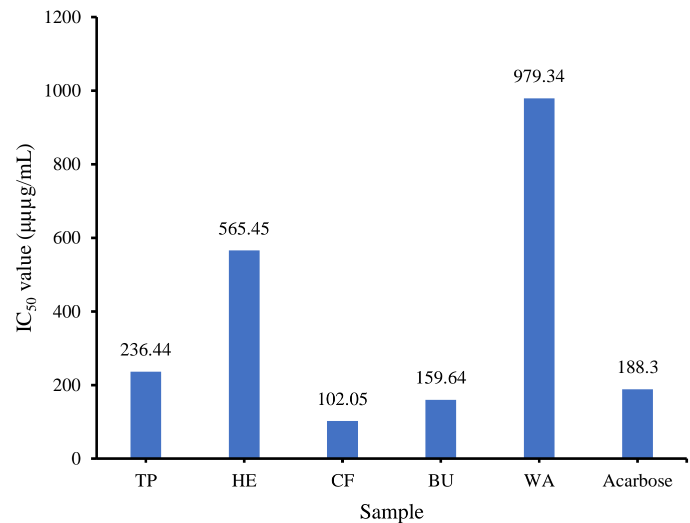

Figure 2: IC_{50} value (µg/mL) of *α*-glucosidase inhibitory activity of total and fractions extract of *Lygodium japonicum*
Note: IC_{50} = Half-maximal inhibitory concentration; TP = total ethanol extract; HE = n-hexane extract; CF = chloroform extract; BU = n-butanol extract; WA = water

Similarly, figure 3 shows that BU extract produced a vigorous DPP-IV inhibitory activity with an IC_{50} value of 141.98 µg/mL. Again, the WA extract showed the weakest DPP-IV inhibitory activity. The results from this study illustrate that five extracts from *Lygodium japonicum* leaves showed inhibitory effects on DPP-IV. Still, they did not inhibit DPP-IV as strongly as the positive control Diprotin A, which had an IC_{50} value of 1.55 µg/mL. Despite having the highest inhibitory activity in this study, BU extract was still 91.6 times lower than the positive control. However, in a study by Purnomo *et al.* (2015) comparing the antidiabetic potential of two leaf extracts, *Urena lobata* and *Lygodium japonicum,* through DPP-IV inhibition, the results showed *Lygodium japonicum* extract exhibited more potent inhibitory effects.[27]

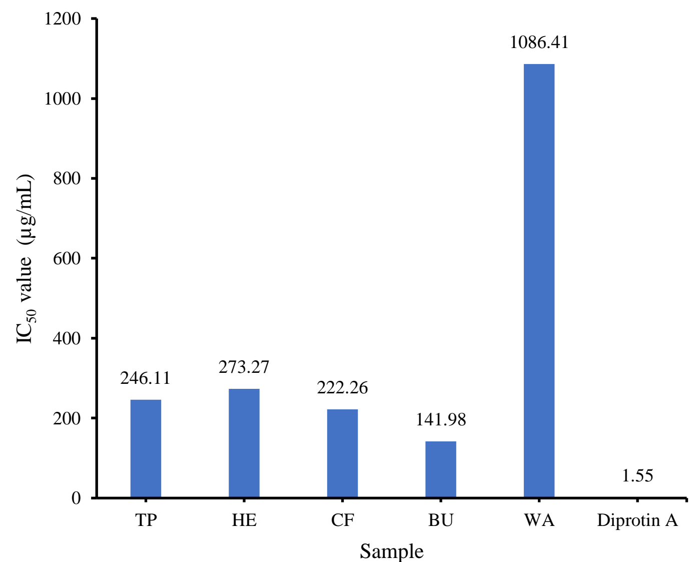

Figure 3: IC_{50} value (µg/mL) of DPP-IV inhibitory activity of total and fractions extract of *Lygodium japonicum.*

© 2024 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Note: IC_{50} = Half-maximal inhibitory concentration; TP = total ethanol extract; HE = n-hexane extract; CF = chloroform extract; BU = n-butanol extract; WA = water

Conclusion

Concentrated extracts from *Lygodium japonicum* leaves can inhibit *α*-amylase, *α*-glucosidase, and DPP-IV enzymes. BU extract demonstrated the highest potential against *α*-amylase and DPP-IV, with IC_{50} values of 227.35 µg/mL and 141.98 µg/mL, respectively. The CF extract exhibited an IC_{50} of 102.05 µg/mL, indicating the highest inhibitory activity of *α*-glucosidase. However, further research on isolating active compounds and conducting *in vivo* experiments is necessary to develop pharmaceuticals for treating and managing diabetes.

Conflict of Interest

The authors declare no conflict of interest.

Author's Declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgments

This work is funded partially by Hong Bang International University under grant code SVTC17.05.

References

1. Chaudhury A, Duvoor C, Reddy Dendi VS, Kraleti S, Chada A, Ravilla R, Marco A, Shekhawat NS, Montales MT, Kuriakose K, Sasapu A, Beebe A, Patil N, Musham CK, Lohani GP, Mirza W. Clinical Review of Antidiabetic Drugs: Implications for Type 2 Diabetes Mellitus Management. Front Endocrinol (Lausanne). 2017;8:6.
2. IDF Diabetes Atlas [Internet]. [cited 2024]. Available from: https://diabetesatlas.org/atlas/seventh-edition/
3. Abbafati C, Abbas KM, Abbasi M, Abbasifard M, Abbasi-Kangevari M, Abbastabar H, et al. Global burden of 369 diseases and injuries in 204 countries and territories, 1990-2019: a systematic analysis for the Global Burden of Disease Study 2019. Lancet. 2020;396(10258):1204–1222.
4. Diagnosis and Classification of Diabetes Mellitus. Diabetes Care. 2013;36(Supplement_1):S62–S69.
5. Papoutsis K, Zhang J, Bowyer MC, Brunton N, Gibney ER, Lyng J. Fruit, vegetables, and mushrooms for the preparation of extracts with *α*-amylase and *α*-glucosidase inhibition properties: A review. Food Chem. 2021;338:128119.
6. Rahman MS, Hossain KS, Das S, Kundu S, Adegoke EO, Rahman MA, Hannan MA, Uddin MJ, Pang MG. Role of Insulin in Health and Disease: An Update. Int J Mol Sci. 2021;22(12):6403. doi: 10.3390/ijms22126403
7. Ćorković I, Gašo-Sokač D, Pichler A, Šimunović J, Kopjar M. Dietary Polyphenols as Natural Inhibitors of α-Amylase and *α*-Glucosidase. Life. 2022;12(11):1692. doi: 10.3390/life12111692.
8. Kumar S, Narwal S, Kumar V, Prakash O. *α*-glucosidase inhibitors from plants: A natural approach to treat diabetes. Pharmacogn Rev. 2011;5(9):19–29.
9. Makrilakis, K. The role of DPP-4 inhibitors in the treatment algorithm of type 2 diabetes mellitus: when to select, what to expect. Int. J. Env Res. Pub. , 2019;*16*(15):2720.
10. Association AD. Standards of Medical Care in Diabetes—2022 Abridged for Primary Care Providers. Clinical Diabetes. 2022;40(1):10–38.
11. Cheng AYY, Fantus IG. Oral antihyperglycemic therapy for type 2 diabetes mellitus. CMAJ. 2005;172(2):213–226.
12. Doupis J, Veves A. DPP4 inhibitors: a new approach in diabetes treatment. Adv Ther. 2008;25(7):627–643.
13. Li XL, Zhou AG, Han Y. Anti-oxidation and anti-microorganism activities of purification polysaccharide from *Lygodium japonicum* in vitro. Carbohydr Polym. 2006;66(1):34–42.
14. Zhang LH, Yin ZQ, Ye WC, Zhao SX, Wang L, Hu F. Studies on the chemical constituents in herb of *Lygodium japonicum*. 2005;30(19):1522-1524.
15. Ye W, Fan C, Zhang L, Zhiqi Yin, Zhao S. A new phenolic glycoside from the roots of *Lygodium japonicum*. Fitoterapia. 2007;78(7–8):600–601.
16. Alam MM, Emon NU, Alam S, Rudra S, Akhter N, Mamun MMR, Ganguly A. Assessment of pharmacological activities of *Lygodium microphyllum* Cav. Leaves in the management of pain, inflammation, pyrexia, diarrhea, and helminths: *In vivo*, *in vitro* and *in silico* approaches. Biomed Pharmacother. 2021;139:111644.
17. Teoh WY, Yong YS, Razali FN, Stephenie S, Dawood Shah M, Tan JK, Gnanaraj C, Mohd Esa N. LC-MS/MS and GC-MS Analysis for the Identification of Bioactive Metabolites Responsible for the Antioxidant and Antibacterial Activities of *Lygodium microphyllum* (Cav.) R. Br. Separations. 2023;10(3), 215; doi.10.3390/separations10030215
18. Li K, Yao F, Xue Q, Fan H, Yang L, Li X, Sun L, Liu Y. Inhibitory effects against α-glucosidase and α-amylase of the flavonoids-rich extract from *Scutellaria baicalensis* shoots and interpretation of structure-activity relationship of its eight flavonoids by a refined assign-score method. Chem Cent J. 2018;12(1):82. doi: 10.1186/s13065-018-0445-y.
19. Nguyen T, Nguyen T, Nguyen V, Nguyen T, Tran T, Do A, Kim S. Antidiabetic and antioxidant activities of red seaweed *Laurencia dendroidea*. Asian Pac J Trop Biomed. 2019;9(12):501–509.
20. Lacroix IME, Li-Chan ECY. Inhibition of dipeptidyl peptidase (DPP)-IV and α-glucosidase activities by pepsin-treated whey proteins. J Agric Food Chem. 2013;61(31):7500–7506.
21. Subba B, Sharma A, Budhathoki A. Assessment of phytochemical content, antioxidant and antibacterial activities of three medicinal plants of Nepal. J. Med. Plant Res. 2016;10(45):829–837.
22. Zuraini Z, Radiah A, Roziahanim M. Phytochemical Analysis And Antibacterial Activity Of *Lygodium microphyllum* Extract. EPSBS. 2019. DOI: 10.15405/epsbs.2020.03.03.91
23. Lam TP, Tran NVN, Pham LHD, Lai NVT, Dang BTN, Truong NLN, Nguyen-Vo SK, Hoang TL, Mai TT, Tran TD. Flavonoids as dual-target inhibitors against *α*-glucosidase and *α*-amylase: a systematic review of in vitro studies. Nat Prod Bioprospect. 2024;14(1):4.
24. Chen L, Zhang G, He J, Guan J, Pan C, Mi W, Wang Q. New naphthoquinone from the root of *Lygodium japonicum* (Thunb.) Sw. J Nat Med. 2010;64(1):114–116.
25. Mohammed A, Ibrahim MA, Tajuddeen N, Aliyu AB, Isah MB. Antidiabetic potential of anthraquinones: A review. Phytother. Res. 2020;34(3):486–504.
26. Abdull Majid DGSNB, Iqbal M. Antihyperglycemic and Antioxidative Effects of *Lygodium microphyllum* in Alloxan Inducecd Diabetic Rats. Int J Pharm Pharm Sci. 2018;10(12):75-9.
27. Purnomo Y, Soeatmadji DW, Sumitro SB, Widodo MA. Antidiabetic potential of Urena lobata leaf extract through inhibition of dipeptidyl peptidase IV activity. Asian Pac J Trop Biomed. 2015;5(8):645–649.

© 2024 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License