MONOTERPENES FROM Placamium Cartilagineum AS INHIBITORS OF THE ALPHA-GLUCOSIDASE ENZYME

VICTOR SANHUEZA[1], DAFNE DÍAZ-HERNANDEZ[1], BORJA MARTINEZ-ALBARDONEDO[1], ANDRÉS F. OLEA[2], MITCHELL BACHO[3], VICTOR FAJARDO[1], AURELIO SAN-MARTÍN[1].*

[1]*Facultad de Ciencias, Departamento de Ciencias y Recursos Naturales, Universidad de Magallanes, Punta Arenas Chile.*
[2]*Grupo QBAB, Instituto de Ciencias Químicas Aplicadas, Facultad de Ingeniería, Universidad Autónoma de Chile, Santiago Chile.*
[3]*Laboratorio de Síntesis Orgánica y Organometálica, Facultad de Ciencias Exactas, Departamento de Ciencias Químicas, Universidad Andrés Bello, Santiago, Chile.*

ABSTRACT

Seaweed is an inexhaustible source of chemical compounds of varied biological activity, characterized by the synthesis of various secondary metabolites that have antioxidant, anti-inflammatory, anticancer and antidiabetic activity. This study focused on the species *Placamium cartilagineum* in search of a potential inhibitor of the enzyme α-D-glucosidase associated with type 2 diabetes mellitus. In recent years, bioautographic assays in TLC have become an effective tool to identify the presence of a possible enzyme inhibitor in a short time. The extract of *P. cartilagineum* was evaluated against the enzyme α-D-glucosidase and 4 compounds were isolated that showed important inhibitory activity of the enzyme by TLC. Pure compounds were identified by employing IR, EIMS, NMR and compared with authentic samples.

The CH_2Cl_2 extract of *P. cartilagineum* shows bioactive compounds, which were identified as: Mertensene; Violacene; 1S,2S,4R,5R)-1,2,4-trichloro-5-((E)-2-chlorovinil)-1,5-dimethyl cyclohexane and (1R,2S,4S,5S)-1,2,4-trichloro-5-((E)-2-chlorovinyl)-1,5-dimethylcyclohexane.

Keywords: *Placamium, Diabetes, Mertensene Inhibitions, α-D-glucosidase.*

INTRODUCTION

Bioautography is a straightforward and rapid technique that combines the benefits of thin-layer chromatography and the detection of biological activity in plant species, allowing for the direct visualization of active fractions. Assessing the efficacy of this technique can simplify the isolation of hypoglycemic substances present in complex mixtures, such as marine algae. An example is marine algae of the Plocamiaceae family (order Gigartinales), which are distributed in both hemispheres and are widely researched today [1]. Each of the eight different *Placamium* species contains either monocyclic or acyclic halogenated monoterpenes [2]. The greatest variety of halogenated monoterpenes has been isolated from *P. cartilagineum*, a cosmopolitan species found along the Pacific coasts of North America and Australia, the Mediterranean, the Isle of Wight, and the inhospitable Janus Island of Antarctica [3].

Placamium cartilagineum L. (Dixon) is a marine alga from the Rhodophyceae division with a wide geographical distribution. Over four halogenated monoterpenes have been detected in samples collected from the central coasts of Chile. Mertensene and Violacene are the most common monoterpenes [4]. The relative concentration of these compounds is variable, suggesting that this variation is due to the geographical area where the algae are found [5].

Marine algae are inexhaustible sources of chemical compounds with diverse biological activities. They synthesize various secondary metabolites that exhibit antioxidant, anti-inflammatory, anticancer, and antidiabetic activities [6]. Two bromophenols with activity against α-D-glucosidase have been isolated from the red alga *Grateloupila elliptica* [7], and bromophenols with the same activity have been isolated from the alga *Symphyocladia latiuscula* [8].

Diabetes refers to a group of metabolic diseases characterized by hyperglycemia due to defects in insulin secretion, insulin action, or both. Chronic hyperglycemia in diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels [9].

Various techniques are tested in the search for α-D-glucosidase inhibitors. Among these, bioautographic assays on TLC combine chromatographic separation with in situ determination of biological activity, allowing for the rapid localization of active compounds in complex organism extracts [10], providing a simple "yes/no" response [11]. These detection methods are highly sensitive, simple, inexpensive, time saving, and do not require sophisticated equipment. The most analyzed biological activities with this method include antibacterial, antifungal, enzymatic, antitumoral, and antiprotozoal activities, among others [11].

This research enables the standardization of a bioautographic method [12] and the analysis of various metabolites isolated from algae for identifying α-D-glucosidase inhibitors, an enzyme associated with the development of diabetes.

An appropriate method for detecting α-D-glucosidase inhibition is presented in this work, applied to pure compounds obtained from marine algae. The test is based on the cleavage of 2-Naphthyl-α-D-glucopyranoside to form 2-naphthol, which subsequently reacts with Fast Blue B salt to produce a purple diazonium dye. This reaction is analogous to the TLC assay used for detecting cholinesterase inhibitors [13].

MATERIALS AND METHODS

Materials

The enzyme α-D-glucosidase from yeast, TLC Silica gel 60 F 254, Dibasic Potassium Phosphate, Pluronic F127, Monobasic Potassium Phosphate, and 4-Nitrophenyl-α-D-glucopyranoside, absolute EtOH, were acquired from SIGMA Aldrich. Acarbose was obtained from Cayman Chemical Company. 2-Naphthyl-α-D-glucopyranoside was procured from Gbiosciences, while Fast Blue B Salt was sourced from Glentham.

The ^{1}H NMR and ^{13}C NMR spectra were obtained using the Nanalisys 100Pro equipment, operating at 104 MHz for ^{1}H and 26 MHz for ^{13}C, with $CDCl_3$ as the internal standard.

Collection

Ten grams (dry) of *P. cartilagineum* (Dixon) were collected from the intertidal zones of Totoralillo, Isla Negra, and Algarrobo. Subsequently, 430 grams of dry algae were collected from Totoralillo and dried using a cold air flow. The samples were authenticated using bibliographic references [14]. A voucher specimen is deposited in the Herbarium of the University of Magallanes, Chile.

Extraction

Three samples, one from each locality, were extracted, and a preliminary screening in dichloromethane was conducted to determine which extract had the best inhibitory power in the TLC test against the enzyme. It was decided to carry out the work with samples from Totoralillo.

Purification

Mixtures of hexane and ethyl acetate of increasing polarity were used as the mobile phase. Through successive chromatographic columns, the following compounds were isolated and identified by spectroscopic methods and comparison with authentic samples: Mertensene, 110 mg (**1**); Violacene, 107 mg (**2**); (1S,2S,4R,5R)-1,2,4-trichloro-5-((E)-2-chlorovinyl)-1,5-dimethylcyclohexane, 76 mg (**3**); and (1R,2S,4S,5S)-1,2,4-trichloro-5-((E)-2-chlorovinyl)-1,5-dimethylcyclohexane, 61 mg (**4**).

*Corresponding author email: aurelio.sanmartin@umag.cl

Mertensene ^{1}H NMR (104 MHz, Chloroform-d) δ 6.10 (d, J = 13.2 Hz, 1H), 5.84 (d, J = 13.5 Hz, 1H), 4.17 (dd, J = 12.2, 5.0 Hz, 1H), 3.95 (dd, J = 12.3, 4.8 Hz, 1H), 2.72 (dd, J = 9.2, 5.0 Hz, 1H), 2.59 (d, J = 5.0 Hz, 1H), 2.49 (d, J = 9.3 Hz, 1H),

2.72 (dd, J = 9.2, 5.0 Hz, 1H), 2.59 (d, J = 5.0 Hz, 1H), 2.49 (d, J = 9.3 Hz, 1H), 2.35 (d, J = 9.1 Hz, 1H), 1.78 (s, 3H), 1.28 (s, 3H).[1]

^{3}C NMR (26 MHz, Chloroform-d) δ 140.80, 119.28, 70.72, 67.23, 55.30, 52.84, 43.56, 40.75, 26.20, 20.20.

Violacene ^{1}H NMR (104 MHz, Chloroform-d) δ 6.56 (d, J = 13.6 Hz, 1H), 6.07 (d, J = 13.6 Hz, 1H), 4.34 (dd, J = 11.3, 4.8 Hz, 1H), 3.97 (d, J = 10.6 Hz, 1H), 3.70 (dd, J = 11.7, 4.9 Hz, 1H), 3.54 (d, J = 10.7 Hz, 1H), 2.86 – 2.57 (m, 2H), 2.50 (dd, J = 5.6, 3.8 Hz, 1H), 2.29 (d, J = 6.7 Hz, 1H), 1.29 (s, 3H).

^{13}C NMR (26 MHz, Chloroform-d) δ 135.55, 119.38, 71.29, 64.37, 59.30, 49.04, 42.12, 39.03, 38.52, 27.60.

Compound 3 ^{1}H NMR (104 MHz, Chloroform-*d*) δ 5.98 (s, 2H), 4.38 (dd, *J* = 11.2, 3.9 Hz, 1H), 4.09 (s, 1H), 2.77 (d, *J* = 14.1 Hz, 1H), 2.37 (d, *J* = 4.1 Hz, 1H), 2.17 (s, 2H), 1.79 – 1.63 (m, 3H), 1.49 (s, 3H).

Compound 4 ^{1}H NMR (104 MHz, Chloroform-*d*) δ 6.19 (d, 2H), 4.17 (dd, *J* = 12.3, 4.6 Hz, 1H), 3.74 (dd, *J* = 12.4, 4.3 Hz, 1H), 2.74 – 1.99 (m, 4H), 1.67 (s, 3H), 1.27 (s, 3H).

Bioautography of α-D-glucosidase

a. Preparation of Working Solutions for TLC

The method of Simões [13] was used with modifications. The enzyme α-D-glucosidase was used at a concentration of 0.15 mg/mL, dissolved in phosphate buffer at pH 7.4 at a concentration of 100 mM. The 2-Naphthyl-α-D-glucopyranoside was dissolved in 50% EtOH at a concentration of 2 mg/mL. The Fast Blue B chromophore was prepared in Milli-Q water at a concentration of 2 mg/mL. A positive control of Acarbose was prepared at a concentration of 10 mg/mL in phosphate buffer at pH 7.4. The buffer solution was prepared with KH_2PO_4 (127 mg) and $K_2HPO_4x3H_2O$ (926 mg), dissolved in 50 mL of Milli-Q water.

b. Bioautographic Method in TLC

Pure samples of the isolated compounds were prepared at a concentration of 10 mg/mL in CH_2Cl_2. The positive control Acarbose was dissolved in phosphate buffer, and 1 µL per spot was applied to the TLC. Once the TLC plate was dried, it was sprayed with the α-D-glucosidase enzyme solution. The Fast Blue B solution and the 2-Naphthyl-α-D-glucopyranoside substrate solution were prepared just before sample application. After the incubation period, the substrate/chromophore mixture (1:1) was sprayed. Within 15 minutes, the TLC plate displayed coloration and inhibition spots, indicating the activity of the compounds as inhibitors.

c. Standardization of Bioautographic Method in TLC

To standardize this method, tests with Acarbose [15] were conducted at concentrations of 50, 20, 10, 5, 2, and 1 mg/mL, determining that the minimum inhibitory concentration is 10 mg/mL. To visualize the optimal inhibition signal of an extract, an Acarbose-extract ratio of 1/10 [13] was used. Similarly, bioautography in TLC (8/2 Hexane/Acetate) was performed on the extract of *P. cartilagineum* collected in Totoralillo and on the isolated compounds to determine their behavior against α-D-glucosidase (Figure 1).

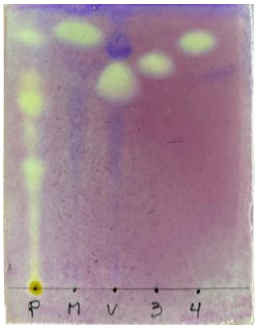

Figure 1: White zones of inhibition; P: Totoralillo plocamium, M: Mertensene, V: Violacene, Compound 3, Compound 4.

d.-Pluronic F-127

Pluronic F-127 (Aldrich) was used without further purification. Aqueous solutions of Pluronic F-127 (0.001M in a molar chain unit basis) were prepared by direct dissolution in distilled and deionized water [16, 17]. To dissolve active compounds into this micellar solution, the emulsion method was used. Briefly, a compound is dissolved in 5 mL of CH_2Cl_2 and then added to 10 mL of Pluronic solution under constant agitation. The temperature of the resultant emulsion is raised to 40 °C to slowly eliminate the organic solvent. After all CH_2Cl_2 has been removed a transparent solution with a known concentration of organic compound is obtained.

e.- Cálculo de IC_{50}

For the calculation of IC_{50}, 7 µL of α-D-glucosidase enzyme at a concentration of 0.15 mg/mL was used, along with 7 µL of 4-nitrophenyl-α-glucopyranoside substrate at a concentration of 1.5 mg/mL. The sample was tested at concentrations of 0.028, 0.054, 0.082, and 0.100 mg/mL, with respective volumes of 14, 27, 41, and 50 µL for each concentration. To stop the reaction, 7 µL of Na_2CO_3 at a concentration of 20 mg/mL was used, completing 250 µL of solution in a microplate, which was read on a UV spectrophotometer at 405 nm. All measurements were done in triplicate. The calculation used the method of Sindhu [18], where the control consisted of enzyme-substrate-buffer-salt and the extract comprised sample-enzyme-substrate-salt (Figure 2).

$$Inhibition\ (\%) = \frac{Abs_{405}(control) - Abs_{405}(extract)}{Abs_{405}(control)} \times 100$$

Figure 2: % Inhibition Formula

IC_{50} values were determined from graphs of inhibition percentage versus inhibitor concentration and were calculated using linear regression analysis of the mean inhibition values obtained. Acarbose was used as the reference inhibitor for the α-D-glucosidase enzyme. All tests were performed in triplicate.

f. Molecular Docking of Isolated Monoterpenes

The molecular docking study of the isolated monoterpenes and Acarbose was conducted using Autodock Vina (version 1.5.7) [19]. These studies provide the binding modes of the compounds with α-D-glucosidase. The protein structure was obtained from the Protein Data Bank with the code PDB 5NN8. Water molecules were removed from the protein structure, and missing polar hydrogens were added using the Autodock tool package [20]. For the docking calculations, a grid was prepared with dimensions -14.245, -33.657, and 94.927 Å in the x, y, and z orientations. The selection of these coordinates and points through space was related to the amino acids present in the active site [21]. The Autogrid tool was used to establish the space where the compounds were docked, consisting of16 points through the x, y, and z coordinates. Additionally, the grid space was selected with a resolution of 0.375 Å, and the exhaustiveness value was set to 20.

The 3D coordinates of the isolated compounds and the control were generated and minimized using the MMF94 force field in the Avogadro package [22]. After optimization, the ligands were used for docking calculations. The visualization and interactions between the ligands and the protein's amino acids were observed using Discover Studio 2024 (BIOVIA, Dassault Systèmes, Discovery Studio Visualizer, v24.1.0.23298, San Diego: Dassault Systèmes, 2023).

g. Validation of Molecular Docking

The docking procedure was validated by removing Acarbose from the α-D-glucosidase and performing a new molecular docking at the active site using AutoDock Vina. The co-crystallized complex was opened in Discover Studio 2024, and the heteroatoms of the inhibitor were removed from the α-D-glucosidase. The enzyme was then saved in PDB format. The previously used protocol and grid parameters were kept unchanged. This step was undertaken to verify that the inhibitor docks precisely in the active site, showing minimal deviation from the original co-crystallized complex. The re-docked complex was then superimposed with the reference complex, and the root-mean-square deviation (RMSD) was calculated using DockRMSD [23].

h. ADME Properties of Isolated Monoterpenes

Lipinski's Rule of Five consists of guidelines used to predict the oral bioavailability of a compound based on its chemical properties. The rule suggests that a compound is more likely to be orally active if it meets the following criteria: molecular weight ≤ 500 Da, octanol-water partition coefficient (LogP) ≤ 5, hydrogen bond donors (HBD) ≤ 5, and hydrogen bond acceptors (HBA) ≤ 10 [24]. ADME properties are essential for determining the pharmacological profile, safety, and therapeutic potential of compounds as drugs. The analysis of Absorption, Distribution, Metabolism, and Excretion (ADME) was conducted on the isolated monoterpenes from *P. cartilagineum*using the online server Molinspiration (Molinspiration Cheminformatics free web services, https://www.molinspiration.com, Slovensky Grob, Slovakia).

RESULTS AND DISCUSSION

Structural determination of active secondary metabolites

The obtained compounds were identified through 1H NMR and comparison with authentic samples [4] (Figure 3). The identified compounds are as follows: Compound 1, Mertensene; Compound 2, Violacene; Compound 3 [25],[3]; and Compound 4.

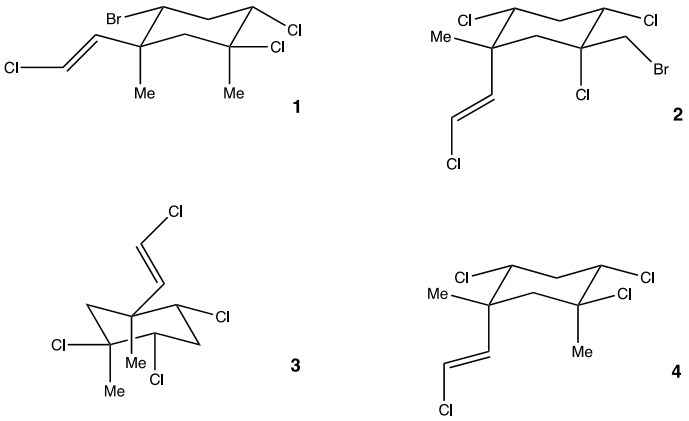

Figure 3: Halogenated monoterpenes isolated from Plocamium *cartilagineum*

Quantification of inhibitory activity of Acarbose, extract, and calculation of IC_{50}.

Bioautography

Due to the low water solubility of the extract and isolated compounds, the inhibition of the α-D-glucosidase enzyme was measured by preparing micellar solutions of Pluronic F-127 containing different concentrations of CH_2Cl_2 extract from *P. cartilagineum* collected in Totoralillo, Mertensene, Violacene, and Compounds 3 and 4. Acarbose was used as the reference. The obtained IC_{50} values are shown in Table 1.

Table 1: IC_{50} of Acarbose and *P.cartilagineum* compounds; s/d no data.

COMPOUND	IC_{50} mg/mL Pluronic	IC_{50} mg/mL Buffer
Acarbose	0,100±0,042	0,138±0,073
Plocamium	0,410±0,070	s/d
Mertensene	0,110±0,052	s/d
Violacene	0,314±0,069	s/d
Compound 3	0,317±0,081	s/d
Compound 4	0,202±0,096	s/d

For the *P. cartilagineum* extract, an IC_{50} value of 0.410 mg/mL was obtained, whereas for Acarbose, the value is four times lower at 0.100 mg/mL. Additionally, the isolated components from the extract, namely Mertensene, Violacene, and Compounds 3 and 4, exhibited intermediate IC_{50} values (see Table 1).

To validate the experimental conditions, the IC_{50} for Acarbose in phosphate buffer was determined, yielding a value of 0.138 mg/mL (Table 1), indicating that Acarbose is more efficient in Pluronic when acting on the enzyme. It was not possible to obtain IC_{50} values in Buffer for the pure compounds due to their low polarity, which made it impossible to dissolve these halogenated monoterpenes; these are indicated as n/d (no data) in Table 1.

The IC_{50} value obtained for the *P. cartilagineum* extract suggests that it could be a potential inhibitor of the α-D-glucosidase enzyme, and its activity likely results from the large number of compounds present in the extract.

Molecular Docking

A molecular docking study was conducted to explore the potential binding orientations of the isolated compounds with α-D-glucosidase, and the results are illustrated in Figure 4. The binding affinities between α-D-glucosidase with Acarbose, Compound 1, and Compound 2 were -7.3, -4.6, and -4.8 Kcal/mol, respectively (Table 2). In the active site, hydrogen bonds and hydrophobic interactions were identified for Acarbose, while π-alkyl interactions were observed for Compounds 1 and 2.

For Compound 1, hydrophobic interactions occur between the methyl groups and the residues TRP376 and TRP481 at distances of 4.89 and 4.88 Å, respectively. Additionally, interactions between chlorine and PHE649 were observed at a distance of 4.28 Å. In Compound 2, similar interactions were noted, with the methyl groups interacting with TRP376 and TRP481 at distances of 4.80 and 5.10 Å, respectively, and the chlorine group interacting with PHE649 at 4.27 Å.

These observed interactions could partially explain the inhibition measurements obtained for the α-glucosidase inhibition by Compounds 1 and 2. Generally, the distance between the ligand and the enzyme residues plays a key role in determining the interaction energy, with shorter distances typically resulting in stronger interactions and, consequently, greater inhibition [24]. In this context, Compound 1 forms interactions at shorter distances than Compound 2, which might explain the better biological response observed for these compounds.

Table 2: Molecular docking results of the isolated compounds

Molecule	Binding energy (Kcal/mol)
Acarbose	- 7.3
1	- 4.6
2	- 4.8

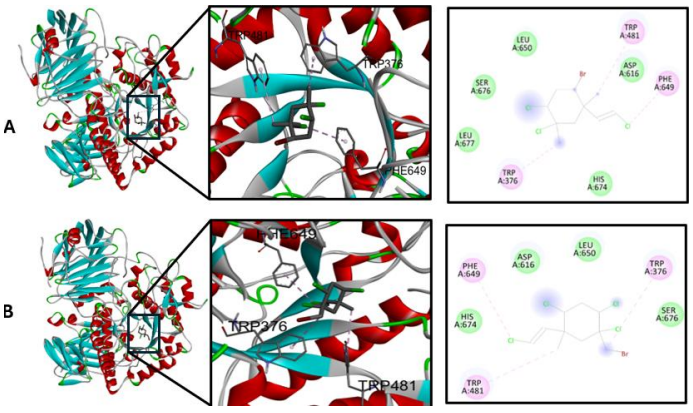

Figure 4: 3D and 2D binding modes for compound 1 (A) and 2 (B) in α -D-glucosidase.

Validation of Molecular Docking

The re-docking procedure was conducted to evaluate the docking process and its efficiency. The same methodology used in the initial docking was applied during the re-docking process. Acarbose, as an inhibitor, bound to the active site

with a binding energy of -7.3 Kcal/mol. Ten hydrogen bond interactions were observed with the residues ASP282, ALA284, ASP518, ARG600, ASP616, TRP618, and HIS674, at distances ranging from 2.3 to 3.2 Å. The re-docked complex was superimposed with the co-crystallized α-D-glucosidase/Acarbose complex, where the DockRMSD interface delivered an RMSD of 2.543 Å, indicating that the docked poses have a good fit according to the employed methodology [21]. Figure 5 shows the pose for Acarbose in the ligand/protein complex (grey) and its re-docking (red).

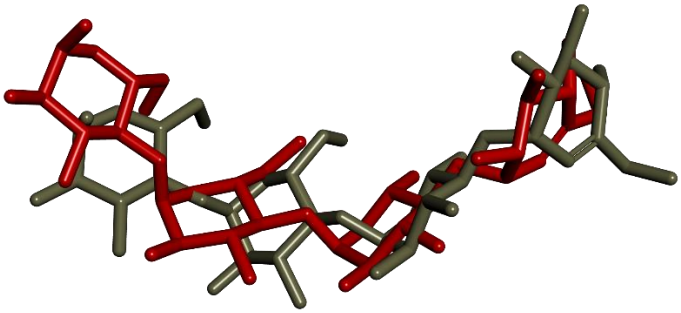

Figure 5: Superposition of the re-docking of Acarbose (red) with the co-crystallized inhibitor in the active site (RMSD 2.543 Å).

ADME Properties of Isolated Compounds

The approach used by Molinspiration is highly reliable, based on a combination of corrections and fragment contributions, and it can handle a wide range of organic molecules. According to Lipinski's Rule of Five, the LogP value of a potential pharmacological compound should be less than 5. The isolated metabolites 1 and 2 exhibited lipophilicity in the range of 4.4 to 4.7. The molecular weight of a compound is related to its in vivo administration. All the isolated compounds have a molecular weight within the acceptable range of 320-360 g/mol. The compounds showed HBA values below 10 and HBD values below 5, which also comply with Lipinski's rule limits. The metabolites exhibited a topological polar surface area (TPSA) of 0 Å², indicating that the compounds are hydrophobic. This attribute could impact their oral absorption and distribution within the body. This property justifies the use of micelles, such as Pluronic F-127, to improve the aqueous solubility of the isolated terpenes.

Table 3: Predicted molecular properties for compounds 1 and 2

Property	Compound 1	Compound 2
Octanol-water partition coefficient (LogP)	4.47	4.70
Polar surface area (TPSA)	0.00	0.00
Number of non-hydrogen atoms	14	15
Molecular weight (g/mol)	320.49	354.93
Number of hydrogen bond acceptors (O and N atoms)	0	0
Number of hydrogen bond donors (OH and NH groups)	0	0
Number of violations of Lipinski's rule	0	0
Number of rotational bonds	1	2
Molecular volume (Å³)	221.09	234.87

CONCLUSION

Both the *P. cartilagineum* extract and the isolated halogenated monoterpenes exhibit significant inhibitory power against the α-D-glucosidase enzyme in TLC chromatography. Regarding the IC50, Mertensene stands out with a value very close to that of Acarbose. Although the three remaining compounds are less efficient in inhibiting the enzyme, these halogenated monoterpenes could serve as a foundation for synthesis work in the search for new enzymatic inhibitors. The ADME prediction results showed that all compounds comply with Lipinski's Rule of Five, suggesting they possess potential drug-like characteristics.

Given that the isolated monoterpenes lack a polar surface area, it became necessary to use Pluronic F-127, which acts as an effective agent to solubilize low-polarity compounds such as the metabolites isolated from *P. cartilagineum*. This improvement facilitated the necessary assays for determining α-D-glucosidase inhibition. Simultaneously, molecular modeling revealed the main interactions between the halogenated monoterpenes and the active site of the enzyme. These interactions helped elucidate the inhibition mechanism of these halogenated compounds against α-D-glucosidase. This finding paves the way for generating derivatives that contain polar functional groups, which could enhance solubility and provide stronger interactions with the α-D-glucosidase active site.

AUTHOR CONTRIBUTIONS

Conceptualization, V.S, A.S-M and V.F; methodology V.S D.D-H and B.M-A; software, M.B; validation, A.S-M,V.F and A.O; formal analysis, V.S and D.D-H ; investigation, V.S; resources, V.S,A.S-M and M.B.; data curation, V.S.; writing—original draft preparation, V.S; writing— review and editing, V.S, A.S-M and V.F; visualization, V.S; supervision, A.S-M, V.F and A.O.

FUNDING

This research work received no external funding.

ACKNOWLEDGMENTS

The authors greatly thank the ANID Project, Strengthening of Doctoral Programs, call 2022 number (86220036), and the FONDEQUIP project, medium scientific and technological equipment competition RMN 100MHz, 2021 number (EQM210006), V.S deeply thanks the scholarship of the doctoral program of the University of Magallanes

REFERENCES

1. J. Cremades, R. Barreiro, I. Maneiro, and G. W. Saunders, *Eur. J. Phycol.* 46, 125 (2011).
2. V. Gressler, M. Lechtenberg, F. Petereit, H. Luftmann, A. Hensel, M. T. Fujii, P. Colepicolo, and E. Pinto, *Soc. Bras. Química (SBQ)* 31, 2002 (2006).
3. D. Dias and S. Urban, *Phytochem. Anal.* 19, 453 (2008).
4. A. San-Martin, R. Negrete, and J. Rovirosa, *Phytochemistry* 30, 2165 (1991).
5. J. Rovirosa, J. Moena, and A. San-Martín, *Biochem. Syst. Ecol.* 16, 593 (1988).
6. V. Quitral R., C. Morales G., M. Sepúlveda L., and M. Schwartz M., *Rev. Chil. Nutr.* 39, 196 (2012).
7. K. Y. Kim, K. A. Nam, H. Kurihara, and S. M. Kim, *Phytochemistry* 69, 2820 (2008).
8. H. Kurihara, T. Mitani, and J. Kawabata, *Fish. Sci.* 65, 300 (1999).
9. A. American Diabetes, *Diabetes Care* 33, S62 (2010).
10. T. W. Zhihong Cheng, *Comb. Chem. High Throughput Screen.* 16 (2013).
11. I. M. Choma and E. M. Grzelak, *J. Chromatogr. A* 1218, 2684 (2011).
12. A. Marston, J. Kissling, and K. Hostettmann, *Phytochem. Anal.* 13, 51 (2002).
13. C. A. Simões-pires, B. Hmicha, A. Marston, and K. Hostettmann, *Phytochem. Anal.* 20, 511 (2009).
14. M. E. Ramirez, M. R. García-Huidobro M., and N. Orellana A., *Boletín Mus. Nac. Hist. Nat.* 57, 9 (2008).
15. J. J. DiNicolantonio, J. Bhutani, and J. H. O'Keefe, *Open Hear.* 2, 1 (2015).
16. A. F. Olea, H. Carrasco, L. Espinoza, and B. Acevedo, *J. Chil. Chem. Soc.* 59, 2451 (2014).
17. L. Taborga, K. Díaz, A. F. Olea, P. Reyes-Bravo, M. E. Flores, H. Peña-Cortés, and L. Espinoza, *J. Agric. Food Chem.* 63, 6890 (2015).
18. Sindhu.Nair, V. Kavrekar, and A. Mishra, *Pelagia Res. Libr.* 3, 128 (2013).
19. A. J. Olson, *J Comput Chem* 31, 455 (2009).
20. E. W. Bell and Y. Zhang, *J. Cheminform.* 11, 1 (2019).
21. A. Castro-Alvarez, A. M. Costa, and J. Vilarrasa, *Molecules* 22 (2017).
22. M. D. Hanwell, D. E. Curtis, D. C. Lonie, T. Vandermeerschd, E. Zurek, and G. R. Hutchison, *J. Cheminform.* 4 (2012).
23. C. A. Lipinski, F. Lombardo, B. W. Dominy, and P. J. Feeney, *Adv. Drug Deliv. Rev.* 64, 4 (2012).
24. A. Allouche, *J. Comput. Chem.* 32, 174 (2012).
25. M. Assef, 829 (1989).