Alkaloid Profiling, Anti-Enzymatic and Antiproliferative Activity of the Endemic Chilean Amaryllidaceae Phycella cyrtanthoides

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Abstract: This research aims to identify the alkaloid profile and to evaluate the enzyme inhibitory potential and antiproliferative effects of the Amaryllidaceae plant Phycella cyrtanthoides. The alkaloid extracts from bulbs and leaves were analyzed using ultrahigh performance liquid chromatography orbitrap mass spectrometry (UHPLC-Orbitrap-MS) analysis. A total of 70 alkaloids were detected in the P. cyrtanthoides extracts. The enzyme inhibition potential against cholinesterases (AChE: acetylcholinesterase, and BChE: butyrylcholinesterase) and tyrosinase were studied. Bulbs displayed the best IC50 values against AChE (4.29 ± 0.03 µg/mL) and BChE (18.32 ± 0.03 µg/mL). These results were consistent with docking experiments with selected major compounds in the active sites of enzymes, while no activity was observed against tyrosinase enzyme. Antiproliferative effects were investigated against human cervical (HeLa), lung (A549, SW1573), colon (WiDr), and breast (HBL-100, T-47D) tumor cell lines. Bulbs and leaves were active in all cell lines (GI50 < 2.5 µg/mL). These findings suggest that the endemic Chilean plant P. cyrtanthoides contains diverse types of bioactive alkaloids with antiproliferative activities and inhibitory effects with potential therapeutic applications for neurodegenerative diseases.

Keywords: Amaryllidaceae alkaloids; Phycella; cholinesterase; tyrosinase; antiproliferative; UHPLC-MS.

1. Introduction

Plants belonging to the Amaryllidaceae family are known for the biosynthesis of pharmacologically active alkaloids [1]. Traditionally, plants extracts of this family have been used as folk medicine for cancer in Ancient Greece, Asia, Africa, and Polynesia for a variety of ailments [2,3]. Galanthamine, an acetylcholinesterase (AChE) inhibitor, is well known for being the most important Amaryllidaceae alkaloid (AA) extracted and the first commercial natural product for the treatment of Alzheimer’s disease (AD). The inhibition of AChE enzyme restored the levels of acetylcholine (ACh) in the postsynaptic neuronal membrane, improving the decline of cognitive function. Similarly, butyrylcholinesterase (BChE) enzyme also has an important function in cholinergic transmission and their levels are increased in AD [4]. Thus, several research groups have focused on finding new sources of bioactive alkaloids from Amaryllidaceae plants with cholinesterase inhibitory potential. On the other hand, these plants showed strong antiproliferative activity. Amaryllidaceae alkaloids, such as lycorine, haemantamine, pancratistatine, and montanine, have been
extensively screened for their antiproliferative effects [3,5–8]. Considering the structural variety and pharmacological properties of AAs, further studies aiming to identify and characterize active compounds would contribute to optimize their therapeutic applications. Different analytical methods have been used for the analysis of AAs, including thin layer chromatography (TLC) [9], capillary electrophoresis (CE) [10], and capillary-electrophoresis-MS (CE-MS) [11]. However, GC-MS and HPLC-MS have been widely and successfully employed in the analysis of AAs from plant sources [12–15]. Recently, ultra-high-performance liquid chromatography quadrupole time-of-flight mass spectrometry (UHPLC-QTOF-MS) has been used for the analysis of crude extracts in different Lycoris species [16].

The Amaryllidaceae family comprises 85 genera and approximately 1100 species, which are widely distributed in tropical, subtropical, and warm regions around the world [17]. In Chile, approximately 9 genera and 45 species have been described [18]. Additionally, some species belonging to the Traubia, Placea and Phycella genera have been cataloged as endemic [19,20].

Phycella genus is distributed in central Chile (from the Coquimbo to Biobio region), where the following five species have been identified: *P. australis*, *P. scarlatina*, *P. herbetiana* (also present in Argentina), *P. brevituba*, and *P. cyrtanthoides* [21]. *P. cyrtanthoides* (local name: Añañuca de Fuego) is an endemic plant mainly found in the Metropolitana (Santiago) region and characterized by having a paraperigonium with fimbriae and six red flowers for umbel (Figure 1). To the best of our knowledge, no reports have been published that describe the chemistry and pharmacological properties of *P. cyrtanthoides* plants.

![Figure 1. Phycella cyrtanthoides (Amaryllidaceae) plants.](image-url)

In previous reports, the bulbs of *P. australis* were analyzed by GC-MS and showed a high content of haemantamine-type alkaloids. Pharmacological studies of the alkaloid fractions on human neuroblastoma cells suggested remarkable neuroprotective properties [22]. This study is one of the few regarding the chemical and pharmacological properties of the Chilean *Phycella*. The alkaloid profile and the AChE inhibitory potential from bulbs of the Argentinian *P. herbetiana* have also been investigated [23].

Considering the potential of AAs, we decided to explore the chemistry and pharmacological properties of *P. cyrtanthoides* for the first time using UHPLC-Orbitrap-MS. We screened the biological properties of the extracts based on enzymatic and cell models. The main objective of the present study is to investigate the alkaloid profile from the endemic *Phycella cyrtanthoides* (Amaryllidaceae) from bulbs and leaves organs. The alkaloid-rich
extracts were subjected to cholinesterase and tyrosinase inhibitory evaluation. Selected major compounds were studied by molecular docking to investigate intermolecular interactions with cholinesterase and tyrosinase enzymes. Antiproliferative activities were also evaluated using a panel of six solid tumor cells lines.

2. Results and Discussion

2.1. Alkaloid Profiling of Phycella Cyrtanthoides Extracts

In the present work, the alkaloid profiling of bulbs and leaves from the methanolic extracts of Phycella cyrtanthoides was investigated. This is the first report concerning the alkaloid profiling of P. cyrtanthoides. In total, 70 alkaloids were detected by UHPLC-PDA-Orbitrap-Mass Spectrometry, among them 47 were tentatively identified and 23 could not be identified using the techniques described herein (see Table 1, and Figure 2). One alkaloid was identified by spiking experiments with available standards (lycorine hydrochloride) using positive mode of detection. The generation of molecular formulas was performed using high resolution accurate mass analysis (HRAM) and matching with the isotopic pattern. Finally, analyses were confirmed using MS/MS data, fragmentation pattern, database of MassBank of North America (MoNA), and the published literature.

Lycorin and homolycorine-type alkaloids were the most common alkaloids identified on the extracts. In addition, crinine, haemanthamine, tazettine and belladine-type alkaloids were also detected. The distribution of the alkaloids in P. cyrtanthoides varied according to the organs. However, a total of nine alkaloids (lycorine, pseudolycorine, hippeastrine, candimine, haemanthamine, vittatine, 7-methoxy-O-methyllycorine, 5-methyl-2-epihippamine and 1-O-acetyl(buphanamine) were found both in bulbs and leaves (Figure 3).

The representative interpretations among the identified alkaloids are discussed below.

Peak 1 with a [M+H]+ ion at m/z = 362.13348 was identified as 2-hydroxyalbomaculine, previously isolated from the aerial parts of Zephyranthes candida [24]. Peak 2 was identified as tazettine (C16H22O3N+, 332.15756), which was reported previously from bulbs of Chilean Phycella australis [22] and the Argentinian Phycella herbetiana [23]. Peak 3 was identified as powelline (C17H20O4N+, 302.14523) based on the formation of [M+H-H2O]+ ion at m/z = 284.13364, suggesting the presence of hydroxyl group at C-3 [25]. Peak 4 with a [M+H]+ ion at m/z = 288.12869 and diagnostic fragments formed after the retro Diels–Alder rearrangements (RDAr) at m/z = 239.05540, m/z = 147.04440, and m/z = 119.04981 was identified as lycorine (C16H18O4N+) [23]; peak 5 with a m/z = 290.14432 was identified as pseudolycorine (C16H20O4N+) according to the fragmentation data [26] and previously reported from P. australis [22] and P. herbetiana [23].

Peak 6 with a [M+H]+ ion at m/z = 316.12579 showed diagnostic fragments detected at m/z = 298.11389 (loss of H2O), m/z = 280.10153 (loss of 2H2O) and m/z = 191.03445 (formed by RDAr) in agreements with hippeastrine (C17H15O2N+). Peak 7 and peak 8 produced the same fragmentation ions and were identified as hippeastrine isomers [27]. Peak 9 was identified as pluviine (C17H22O3N+, 288.12869), peak 10 as dihydrolycorine (C16H20O2N+, 290.14432) and also detected on Phycella australis [22], and peak 11 as 3-O-methyl-epimacowine (C16H18O4N+, 288.12872), previously identified from the bulbs of the Brazilian Hippeastrum calyptratum [28]. Peak 12 with a [M+H]+ ion at m/z = 316.12589 was identified as 11-O-methylcrinamine [29], peak 13 as 3-hydroxydihydrocaranine (C16H20O4N+, 290.14420), and peaks 14 and 15 were proposed as epi-zephyranthine isomers (C16H20O4N+, 290.14420). Peak 16 and peak 17 were identified as hippeastrine isomers, while peak 18 with a [M+H]+ ion at m/z = 346.13831 was identified as candimine, previously reported from Hippeastrum morelianum bulbs [30]. Peak 19 with a [M+H]+ ion at m/z = 274.14804 was identified as 8-O-demethylmaritidine [31] and peak 20 with fragments at m/z = 272.13251, m/z = 180.10258 and m/z = 167.11855 was identified as haemanthamine (C17H20O4N+, 302.14548). Haemanthamine was also identified on Phycella australis [22].
| Peak | UV Max | Tentative Identification Name (AA-Type) | Elemental Composition [M+H]⁺ | Rt | Theoretical Mass (m/z) | Measured Mass (m/z) | Accuracy (ppm) | MSn Ions (ppm) | Organs |
|------|--------|----------------------------------------|-----------------------------|----|------------------------|---------------------|----------------|----------------|--------|
| 1    | 236–281| 2-Hydroxyalbomaculine (homolycorine-type) C₁₉H₂₄O₆N⁺ | 0.86 | 362.15981 | 362.13348 | −72.719 | 274.14774, 251.09735, 214.06267, 199.07582, 165.07063, 121.06544, 107.04959 | L |
| 2    | 231–248-271–307 | Tazettine isomer (tazettine-type) C₁₈H₂₅O₆N⁺ | 1.37 | 332.14925 | 332.15756 | 25.020 | 316.12589, 274.14835, 247.12236, 228.14095, 181.06543, 152.06271, 115.05478, 107.04965 | L |
| 3    | 233–247-306 | Powelline (crinine-type) C₁₇H₂₀O₄N⁺ | 2.06 | 302.13868 | 302.14523 | 21.663 | 284.13364, 274.14798, 251.09767, 228.06765, 181.06557, 165.07068, 127.05473, 115.05476 | L |
| 4    | 236–286 | Lycorine * (lycorine-type) C₁₆H₁₈O₄N⁺ | 3.07 | 288.12303 | 288.12869 | 19.628 | 274.14774, 251.09735, 214.06267, 199.07582, 165.07063, 121.06544, 107.04959 | B; L |
| 5    | 235–283 | Pseudolycorine (lycorine-type) C₁₆H₂₀O₄N⁺ | 3.11 | 290.13923 | 290.14432 | 19.423 | 274.14774, 251.09735, 214.06267, 199.07582, 165.07063, 121.06544, 107.04959 | B; L |
| 6    | 233–278 | Hippeastrine isomers (homolycorine-type) C₁₇H₁₈O₅N⁺ | 3.25 | 316.11850 | 316.12579 | 25.689 | 291.14764, 274.14828, 191.03468, 166.12335, 147.04440, 119.04981, 103.05488 | B; L |
| 7    | 231–276 | Hippeastrine isomers (homolycorine-type) C₁₇H₁₈O₅N⁺ | 4.54 | 316.11850 | 316.12579 | 24.803 | 291.14764, 274.14828, 191.03468, 166.12335, 147.04440, 119.04981, 103.05488 | B; L |
| 8    | 232–278 | Hippeastrine isomers (homolycorine-type) C₁₇H₁₈O₅N⁺ | 4.59 | 316.11850 | 316.12616 | 25.973 | 272.13245, 257.15308, 220.12132, 161.06518 | B; L |
| 9    | 233–289 | Pluviine (lycorine-type) C₁₇H₂₂O₃N⁺ | 5.35 | 288.15942 | 288.12869 | 19.628 | 274.14774, 251.09735, 214.06267, 199.07582, 165.07063, 121.06544, 107.04959 | B; L |
| 10   | 232–289 | Dihydrolycorine (lycorine-type) C₁₆H₂₀O₅N⁺ | 6.59 | 290.13923 | 290.14432 | 19.423 | 274.14774, 251.09735, 214.06267, 199.07582, 165.07063, 121.06544, 107.04959 | B; L |
| 11   | 233–290 | 3-O-methyl-eipimacowine (crinine-type) C₁₆H₂₀O₅N⁺ | 6.73 | 288.12358 | 288.12872 | 19.732 | 291.14764, 274.14828, 191.03468, 166.12335, 147.04440, 119.04981, 103.05488 | B; L |
| 12   | 231–277-310 | 11-O-methylecrinamine (crinine-type) C₁₈H₂₂O₄N⁺ | 6.84 | 316.15433 | 316.12589 | −89.979 | 274.14807, 182.11839, 121.06535 | L |
| 13   | 231–277 | 3-Hydroxydihydrocaranine (lycorine-type) C₁₆H₂₀O₄N⁺ | 7.74 | 290.13868 | 290.14420 | 19.940 | 268.67581, 239.88618, 167.34416, 137.10796, 111.88570, 107.48717 | B |

Table 1. Ultrahigh performance liquid chromatography orbitrap mass spectrometry (UHPLC-Orbitrap-MS) identification of Phycella cyrtanthoides.
| Peak | UV Max | Tentative Identification Name (AA-Type) | Elemental Composition \([M+H]^+\) | Rt | Theoretical Mass \((m/z)\) | Measured Mass \((m/z)\) | Accuracy (ppm) | MSn Ions (ppm) | Organs |
|------|--------|----------------------------------------|-------------------------------|----|---------------------------|---------------------------|----------------|----------------|---------|
| 14   | 232–278| *epi*-Zephyranthine isomers (lycorine-type) | C_{16}H_{20}O_{4}N^+ | 8.34 | 290.1386 | 290.14413 | 18.768 | 272.1322, 262.1116, 244.0999, 214.0874, 181.0653, 169.0654, 147.0445, 120.0813, 118.06568, 272.1324, 268.74606, 181.17052, 170.64424, 148.16725, 129.39676, 118.06547 | L |
| 15   | 232–278| *epi*-Zephyranthine isomers (lycorine-type) | C_{16}H_{20}O_{4}N^+ | 8.81 | 290.1386 | 290.14435 | 19.526 | 298.11267, 290.14447, 272.13245, 268.74606, 181.06534, 169.06546, 147.04451, 120.08131, 118.06568 | B |
| 16   | 232–275| Hippeastrine isomers (homolycorine-type) | C_{17}H_{19}O_{5}N^+ | 9.67 | 316.1185 | 316.12579 | 24.80 | 316.12576, 288.12836, 274.14810, 228.14084, 155.15477, 138.05533, 121.06532 | L |
| 17   | 232–275| Hippeastrine isomers (homolycorine-type) | C_{17}H_{19}O_{5}N^+ | 10.09 | 316.1185 | 316.12610 | −22.476 | 316.12576, 288.12836, 274.14810, 228.14084, 155.15477, 138.05533, 121.06532 | B |
| 18   | 235–282-314| Candidine (homolycorine-type) | C_{18}H_{20}O_{6}N^+ | 10.75 | 346.12906 | 346.13831 | 28.301 | 316.12576, 288.12836, 274.14810, 228.14084, 155.15477, 138.05533, 121.06532 | B; L |
| 19   | 234–385| 8-O-demethylmaritidine (haemanthamine-type) | C_{16}H_{20}O_{3}N^+ | 11.39 | 274.14377 | 274.14804 | 15.576 | 267.06967, 223.07715, 191.03459, 177.01894, 149.02383, 121.06531, 107.04959 | L |
| 20   | 232–273| Haemanthamine (haemanthamine-type) | C_{17}H_{20}O_{3}N^+ | 11.78 | 302.13923 | 302.14548 | 22.490 | 289.13168, 272.13251, 228.14088, 183.57773, 180.10258, 167.11855, 161.0791, 144.08138 | B; L |
| 21   | 235–282| 5-Methyl-epimethylpseudolycorine (lycorine-type) | C_{18}H_{22}O_{4}N^+ | 11.89 | 318.17053 | 318.17819 | 25.788 | 287.12823, 162.06857, 147.04459, 125.98681, 115.05488, 103.05471 | B |
| 22   | 241–325| 2α-Methoxy-6-O-ethyloduline (homolycorine-type) | C_{20}H_{26}O_{5}N^+ | 12.16 | 360.18110 | 360.19092 | 28.792 | 330.14169, 274.14807, 270.11676, 228.14082, 153.10274, 151.07596, 121.06521 | L |
| 23   | 240–291| Vittatine (haemanthamine-type) | C_{16}H_{18}O_{3}N^+ | 12.43 | 272.12867 | 272.13257 | 16.242 | 269.1075, 247.12251, 199.21875, 180.10249, 167.99812, 153.13918, 121.06526, 115.05033 | B; L |
| 24   | 233–288| 10-Norpluviine (lycorine-type) | C_{16}H_{20}O_{3}N^+ | 12.63 | 274.14432 | 274.14813 | 15.904 | 274.14813, 256.13666, 228.14076, 175.03946, 147.04443, 121.06533, 118.06563, 102.03407 | L |
## Table 1. Cont.

| Peak | UV Max   | Tentative Identification Name (AA-Type) | Elemental Composition [M+H]^+ | Rt     | Theoretical Mass (m/z) | Measured Mass (m/z) | Accuracy (ppm) | MSn Ions (ppm) | Organs |
|------|----------|----------------------------------------|-------------------------------|--------|------------------------|--------------------|---------------|----------------|--------|
| 25   | 236–287  | Kirkine (lycorine-type)                | C_{16}H_{20}O_{3}N^+          | 12.73  | 274.14432              | 274.14822          | 16.232        | 270.11679, 256.13672, 231.15173, 228.07106, 197.16562, 175.03972, 120.08125, 118.06599 | B |
| 26   | 232–272  | Albomaculine (homolycorine-type)       | C_{19}H_{24}O_{5}N^+          | 12.87  | 346.16490              | 346.17456          | 27.994        | 320.15652, 304.16083, 274.14795, 193.05043, 180.10242, 178.06317, 152.06268, 103.05467 | L |
| 27   | 232–256-309 | 3-Epimacronine (tazettine-type)   | C_{18}H_{20}O_{5}N^+          | 13.05  | 330.13415              | 330.14191          | 25.173        | 326.09451, 316.12592, 247.12227, 231.15181, 202.13469, 167.15497, 144.08133, 111.09221 | B |
| 28   | 234–252  | 10-O-methylpseudolycorine (lycorine-type) | C_{17}H_{22}O_{4}N^+         | 13.12  | 304.15488              | 304.16098          | 21.848        | 304.16074, 276.12741, 258.11655, 193.05020, 178.06328, 165.07066, 147.04482, 125.08409, 118.06564 | L |
| 29   | 232–271  | Aknadicine                             | C_{19}H_{24}O_{5}N^+          | 13.29  | 346.16545              | 346.17526          | 29.929        | 316.12640, 304.16125, 193.05049, 178.06313, 125.08411, 121.06541, 110.06061 | L |
| 30   | 248–271  | 3-O-Acetylarcissidine (lycorine-type)  | C_{20}H_{26}O_{5}N^+          | 13.43  | 376.17601              | 376.18796          | 33.217        | 316.12589, 304.16135, 258.11682, 193.05042, 165.07048, 153.07048, 147.04456, 125.08416, 118.06568 | L |
| 31   | 238–284  | 10-O-Dimethylgalanthine (lycorine-type) | C_{17}H_{22}O_{4}N^+         | 13.48  | 304.15488              | 304.16119          | 22.539        | 274.14847, 266.08557, 258.11697, 191.15506, 167.15486, 125.98666, 118.06574 | B |
| 32   | 248–271  | 7-Methoxy-O-methyllycorine (homolycorine-type) | C_{20}H_{26}O_{5}N^+       | 13.75  | 362.19675              | 362.20648          | 29.405        | 346.13885, 330.14188, 247.12267, 221.16689, 191.15508, 167.01363 | B; L |
| 33   | 238–271  | Unknown alkaloid                       | C_{24}H_{26}O_{4}N^+         | 13.97  | 392.18618              | 392.18298          | –6.769        | 376.18741, 344.15909, 304.16098, 252.10530, 212.14474, 180.10254 | L |
| 34   | 249–278  | Unknown alkaloid                       | C_{20}H_{24}O_{4}N^+         | 14.05  | 342.17053              | 342.17990          | 28.977        | 337.19955, 316.12595, 259.18481, 247.12242, 194.11845, 144.08136 | B |
| 35   | 241–287  | Unknown alkaloid                       | C_{19}H_{22}O_{5}N^+         | 14.24  | 344.16183              | 344.15912          | 28.681        | 337.19943, 316.16238, 282.11783, 227.08345, 191.14377, 110.02042 | L |
| Peak | UV Max | Tentative Identification Name (AA-Type) | Elemental Composition [M+H]^+ | Rt | Theoretical Mass (m/z) | Measured Mass (m/z) | Accuracy (ppm) | MSn Ions (ppm) | Organs |
|------|--------|----------------------------------------|-------------------------------|----|------------------------|---------------------|----------------|----------------|---------|
| 36   | 248–284| Homolycorine (homolycorine-type)        | C_{18}H_{22}O_{4}N^+          | 14.29 | 316.15488              | 316.16257            | −22.206      | 312.16726, 284.18124, 272.13272, 251.15782, 201.13953, 181.17062, 125.98682, 110.02058 | B       |
| 37   | 249–284| Unknown alkaloid                        | C_{14}H_{30}O_{6}N^+          | 14.42 | 356.19151              | 356.19595            | 12.471       | 226.28473, 201.05020, 143.05002, 115.05481, 108.08139 | B; L    |
| 38   | 249–282| Unknown alkaloid                        | C_{18}H_{34}O_{7}N^+          | 14.60 | 376.23298              | 376.22391            | −24.105      | 356.19583, 322.15192, 240.15221, 181.17058, 167.01340, 125.98679 | B        |
| 39   | 242    | Unknown alkaloid                        | C_{18}H_{34}O_{8}N^+          | 14.68 | 392.22789              | 392.21906            | −22.522      | 374.17166, 346.17444, 290.15924, 197.11798, 150.09190, 121.06519 | B; L    |
| 40   | 243    | Unknown alkaloid                        | C_{24}H_{24}O_{3}N^+          | 14.85 | 406.16490              | 406.16412            | −7.395       | 392.21921, 346.10187, 290.15942, 274.14810, 211.17101, 197.11813, 179.10728 | L        |
| 41   | 253–282| 5-methyl-2-ephippamine isomers (lycorine-type) | C_{18}H_{22}O_{4}N^+          | 14.99 | 316.15433              | 316.12613            | 25.878       | 284.18137, 272.13257, 254.16881, 247.12253, 197.08179, 144.08141, 125.98682, 111.09224 | B; L    |
| 42   | 252–276| Unknown alkaloid                        | C_{19}H_{30}O_{6}N^+          | 15.37 | 368.20676              | 368.19748            | 32.171       | 316.12601, 249.14214, 228.14104, 209.20259, 167.01352, 110.02060 | B        |
| 43   | 252–278| Unknown alkaloid                        | C_{28}H_{33}O_{7}N^+          | 15.53 | 463.23532              | 463.24121            | 12.705       | 449.26157, 431.21402, 346.13846, 249.14217, 225.12744, 163.07600 | B        |
| 44   | 251–301| 3-O-methynarcissidine (lycorine-type)    | C_{19}H_{26}O_{5}N^+          | 15.85 | 348.18055              | 348.19052            | 28.636       | 346.17471, 274.14804, 197.11795, 171.14987, 138.09195, 121.06521 | L        |
| 45   | 250–280| Unknown alkaloid                        | C_{26}H_{35}O_{3}N^+          | 15.96 | 406.23767              | 406.23715            | −1.281       | 346.13843, 316.12604, 247.12244, 203.11884, 144.08141, 121.06521 | B        |
| 46   | 252–297| 2-O-acetyl-4-O-methyllicorine (lycorine-type) | C_{19}H_{25}O_{4}N^+          | 16.33 | 360.14471              | 360.15427            | 28.061       | 314.14539, 267.12448, 247.12241, 211.07701, 171.14987, 121.06534, 103.05473 | L        |
| 47   | 253–283| Jonquailine (tazettine-type)             | C_{19}H_{24}O_{3}N^+          | 16.45 | 346.16490              | 346.13846            | −76.384      | 321.20441, 314.14566, 288.05582, 247.12248, 171.14989, 102.03439 | B        |
| 48   | 261–305| Unknown alkaloid                        | C_{19}H_{25}O_{4}N^+          | 16.63 | 360.14416              | 360.15417            | 27.783       | 344.12250, 326.14676, 316.12579, 304.16077, 247.12231, 180.10242, 164.10765 | L        |
| 49   | 252–282| Unknown alkaloid                        | C_{27}H_{33}O_{3}N^+          | 16.76 | 419.24550              | 419.24805            | 6.093        | 398.21057, 346.13849, 316.12604, 268.13763, 210.12157, 121.06532 | B        |
| Peak | UV Max | Tentative Identification Name (AA-Type)                                                                 | Elemental Composition [M+H] + | Rt   | Theoretical Mass (m/z) | Measured Mass (m/z) | Accuracy (ppm) | MSn Ions (ppm) | Organs |
|------|--------|--------------------------------------------------------------------------------------------------------|-----------------------------|------|-----------------------|---------------------|-----------------|----------------|--------|
| 50   | 250–275| Bulbocapnine (isoquinoline alkaloid)                                                                  | **C_{19}H_{20}O_{4}N^{+}**  | 16.85| 326.13923             | 326.14673           | 24.668          | 282.08136, 274.14810, 240.1322, 225.15038, 207.13930, 138.09189, 121.06530 | L       |
| 51   | 253–278| Unknown alkaloid                                                                                        | **C_{21}H_{32}O_{4}N^{+}**  | 17.18| 362.23258             | 362.22394           | −23.866         | 360.21735, 316.12598, 247.12244, 167.01346, 102.03439 | B       |
| 52   | 254–297| Unknown alkaloid                                                                                        | **C_{18}H_{26}O_{7}N^{+}**  | 17.39| 368.17038             | 368.17639           | 16.328          | 346.17471, 316.12595, 304.16098, 274.14807, 164.10770, 102.03436 | L       |
| 53   | 253–302| Nerinine (homolycorine-type)                                                                             | **C_{19}H_{25}O_{5}N^{+}**  | 18.34| 347.17272             | 347.17801           | 59.168          | 334.12265, 316.12579, 274.14801, 256.06381, 167.01337, 110.02050, 102.03431 | L       |
| 54   | 242–277| Unknown alkaloid                                                                                        | **C_{24}H_{16}O_{3}N^{+}**  | 18.82| 366.11247             | 366.10648           | 28.525          | 304.16092, 278.08636, 270.15344, 252.10562, 247.12234, 226.18208, 191.03456, 102.03433 | L       |
| 55   | 285–310| 1-O-acetylcaranine (lycorine-type)                                                                      | **C_{18}H_{20}O_{4}N^{+}**  | 19.24| 314.13866             | 314.10910           | −94.186         | 336.02965, 314.10925, 278.08649, 191.03464, 102.03435 | L       |
| 56   | 266–309-356| Unknown alkaloid                                                                                      | **C_{23}H_{20}O_{3}N^{+}**  | 19.41| 358.14377             | 358.13867           | −14.240         | 250.14955, 228.14110, 186.09221, 167.01358, 102.03442 | L       |
| 57   | 251–282| Unknown alkaloid                                                                                        | **C_{18}H_{20}O_{3}N^{+}**  | 20.32| 251.15159             | 251.15793           | 25.223          | 399.18460, 388.15140, 376.18735, 316.12582, 247.12230, 167.01335, 122.54755 | L       |
| 58   | 253–327| Carltonine A (belladine-type)                                                                            | **C_{27}H_{33}O_{3}N_{2}^{+}** | 20.44| 433.24857             | 433.26575           | 39.654          | 449.26141, 429.29208, 346.13840, 247.12242, 144.08139 | B; L    |
| 59   | 251–278| Unknown alkaloid                                                                                        | **C_{17}H_{18}O_{3}N^{+}**  | 20.79| 472.26936             | 472.28061           | 23.811          | 330.10541, 316.12585, 274.14807, 225.55421, 187.12723, 167.01343 | B; L    |
| 60   | 245–284| Unknown alkaloid                                                                                        | **C_{24}H_{25}O_{4}N^{+}**  | 21.06| 388.15433             | 388.15140           | −7.561          | 267.19000, 247.12218, 235.17180, 184.10028, 150.09189, 121.06524 | B; L    |
| 61   | 251–285| Unknown alkaloid                                                                                        | **C_{15}H_{25}O_{3}N^{+}**  | 21.27| 269.19909             | 269.20554           | 25.983          | 352.34933, 316.12595, 269.20566, 228.14091, 221.15578, 144.08133 | B       |
| 62   | 252–281| Unknown alkaloid                                                                                        | **C_{20}H_{33}O_{6}N^{+}**  | 21.38| 383.23024             | 383.23532           | 13.258          | 277.19623, 269.20547, 240.25401, 239.25075, 211.08781, 180.10248, 102.03432 | L       |

Table 1. Cont.
| Peak | UV Max  | Tentative Identification Name (AA-Type) | Elemental Composition [M+H]⁺ | Rt  | Theoretical Mass (m/z) | Measured Mass (m/z) | Accuracy (ppm) | MSn Ions (ppm) | Organs |
|------|---------|----------------------------------------|-----------------------------|-----|------------------------|-------------------|---------------|----------------|--------|
| 64   | 253–282 | Unknown alkaloid                       | C₁₄H₂₁O₃N⁺                  | 21.89 | 251.15159              | 251.15799         | 25.462        | 247.12265, 212.14493, 186.12865, 167.01361, 144.08147, 102.03445 | B      |
| 65   | 255–302 | 1-O-acetylbuphanamine (crinine-type)   | C₁₉H₂₅O₅N⁺                  | 22.23 | 344.14925              | 344.15903         | –14.851       | 294.11795, 274.14795, 197.11797, 181.12305, 174.12837, 138.09187 | B; L   |
| 66   | 257     | 11-Acetylambelline (crinine-type)      | C₂₀H₂₄O₆N⁺                  | 22.33 | 374.16036              | 374.17181         | 32.060        | 358.13858, 324.13116, 304.16089, 197.11806, 174.12848, 151.11229, 121.06525 | B; L   |
| 67   | 255–306 | Maritidine (haemanthamine type)        | C₁₇H₂₂O₃N⁺                  | 22.78 | 288.15942              | 288.12863         | 27.502        | 244.13684, 216.14043, 191.03470, 167.01357, 122.54771, 102.03446 | B; L   |
| 68   | 257–296 | 9-Norpluviine (lycorine-type)          | C₁₆H₂₆O₃N⁺                  | 23.05 | 274.14432              | 274.14798         | 15.357        | 228.27116, 191.03436, 174.12823, 147.18108, 121.06519, 102.03426 | L      |
| 69   | 258     | 5-Methylipseudolycorine (lycorine-type) | C₁₇H₂₅O₄N⁺                  | 24.22 | 304.15433              | 304.16098         | 21.848        | 258.28345, 242.28685, 228.27103, 174.12837, 151.11229, 102.03432 | L      |
| 70   | 257–292 | Unknown alkaloid                       | C₂₄H₂₆O₃N⁺                  | 24.37 | 376.19072              | 376.18744         | –8.720        | 352.34924, 274.14795, 258.28336, 174.12845, 166.11258, 146.09694, 132.08127 | L      |

Abbreviations: L = leaves; B = bulbs; RT = retention time; * = identified using authentic compounds.
Figure 2. UHPLC chromatogram of *Phycella cyrtanthoides* bulbs (black) and leaves (red) in positive mode.

Figure 3. Lycorine (a), pseudolycorine (b), hippeastrine (c), candimine (d), haemanthamine (e), vittatine (f), 7-methoxy-O-methyllycorenine (g), 5-methyl-2-epihippamine (h) and 1-O-acetylbuphanamine (i).

Peak 21 was identified as 5-methyl-epimethylpseudolycorine [16], peak 22 as 2α-methoxy-6-O-ethyloduline previously isolated from *Lycoris radiata* [32], peak 23 with diagnostic fragments at *m/z* = 180.10249 and *m/z* = 167.01340 was proposed as vittatine (C_{16}H_{18}O_{3}N^+, 272.13257) previously detected from *P. australis* [22] and *P. herbetiana* [23], peak 24 as 10-norpluviine (C_{16}H_{20}O_{3}N^+, 274.14432) [16], peak 25 as kirkine (C_{16}H_{20}O_{3}N^+, 274.14822) [16], and peak 26 as albomaculine (C_{19}H_{24}O_{5}N^+, 346.17456) [33].

Peak 27 with a [M+H]^+ ion at *m/z* = 330.14191 was identified as 3-epimacronine also found on *P. australis* [22], peak 28 as 10-O-methylpseudolycorine, peak 29 as the
isoquinoline alkaloids aknadicine detected on bulbs of *Narcissus tazetta* [34] and peak 30 as 3-O-acetylhaemanthamine [35]. Peak 31 was identified as 10-O-dimethylgalanthine [16], peak 32 as 7-methoxy-O-methyllycorenine previously isolated from Brazilian *Hippeastrum aulicum* [28], peak 36 was identified as homolycorine [36], and peak 41 was identified as 5-methyl-2-epihippamine, peak 46 as 2-O-acetyl-4-O-methyllicorine [16], peak 47 as jonquailine [38], and peak 50 as the classical isoquinoline bulbocapnine. These alkaloids have been isolated previously from *Galanthus nivalis* [39] and detected recently from bulbs of *Narcissus tazetta* [34]. Peak 53 was identified as nerinine detected in some Chilean *Rhodophiala* species [12], peak 55 was identified as 1-O-acetylcaranine [40], and peak 58 was identified as the belladine-type alkaloid carltonine A isolated from the bulbs of *Narcissus pseudonarcissus* cv. Carlton [41]. Peak 63 with a [M+H]⁺ ion at m/z = 300.12955 was identified as 11-oxo-haemanthamine [28], peak 65 as 1-O-acetylbuphanamine isolated from the bulbs of *Boophone disticha* [42], and peak 66 as 11-acetylambelline [43]. Peak 67 with a [M+H]⁺ ion at m/z = 288.12863 was identified as maritidine, and was detected previously from *P. australis* bulbs [22], peaks 68 and 69 were proposed as 9-norpluviine [16], and 5-methylipseudolycorine [16].

2.2. Enzyme Inhibition Studies

*Phycella cyrtanthoides* bulbs and leaves alkaloid extracts were evaluated *in vitro* for acetylcholinesterase, butyrylcholinesterase and tyrosinase inhibitory effects (Table 2, expressed as IC₅₀ values). Bulbs and leaves were active against AChE and BChE. Bulbs showed the highest inhibitory effects compared to leaves with IC₅₀ values for AChE of 4.29 ± 0.04 and for BChE of 18.32 ± 0.03 µg/mL. *P. cyrtanthoides* bulbs were more active than Chilean *P. australis* bulbs (IC₅₀ = 80.12 ± 1.03 µg/mL) [22], while the Argentinian *P. herbetiana* bulbs showed strong inhibitory activity against AChE (IC₅₀ = 1.2 ± 0.12 µg/mL) [23]. No results regarding BChE inhibitory activity have been reported for other *Phycella* species. Conversely, no activity was detected against tyrosinase enzyme on the bulbs and leaves of the alkaloid extract of *P. cyrtanthoides*. These results are in agreement with previous studies using isolated and alkaloid fractions [44], since the presence of phenolic groups has positive effects on the inhibitory activity due to the chelating properties of metals such as copper [45].

Table 2. Enzymatic inhibitory activity of *Phycella cyrtanthoides* alkaloid extracts.

| Assay                      | AChE Inhibition IC₅₀ (µg/mL) | BChE Inhibition IC₅₀ (µg/mL) | Tyrosinase Inhibition IC₅₀ (µg/mL) |
|----------------------------|-------------------------------|-----------------------------|---------------------------------|
| *P. cyrtanthoides* bulbs   | 4.29 ± 0.04                   | 18.32 ± 0.03                | ND                              |
| *P. cyrtanthoides* leaves  | 8.66 ± 0.03                   | 37.70 ± 0.02                | ND                              |
| Galanthamine               | 0.55 ± 0.03                   | 3.82 ± 0.02                 |                                  |
| Kojic acid                 | -                             | -                           | 0.76 ± 0.05                     |

All values are expressed as means ± SD (n = 3). Abbreviations: AChE, acetylcholinesterase; BChE, butyrylcholinesterase; ND, not detected (>250 µg/mL).

2.3. Docking Studies

All compounds subjected to docking assays in the *Torpedo californica* acetylcholinesterase (TaAChE) catalytic site and human butyrylcholinesterase (hBChE) catalytic site turned out to be the major alkaloids selected from *Phycella cyrtanthoides* bulbs and leaves extracts according to the UHPLC chromatogram (Figure 2). Docking experiments were performed to determine the pharmacological behavior of these main alkaloids, and therefore, their contributions to the cholinesterase inhibitory activities. The best docking binding energies expressed in kcal/mol of each selected compound are shown in Table 3.
Table 3. Binding energies obtained from docking experiments of selected major alkaloids in *Phycella cyrtanthoides* bulbs and leaves extracts, as well as the known inhibitor galanthamine over acetylcholinesterase (TcAChE) and butyrylcholinesterase (hBChE).

| Compound                        | Binding Energy (kcal/mol) | Acetylcholinesterase | Butyrylcholinesterase |
|---------------------------------|--------------------------|----------------------|-----------------------|
| 3-hydroxydihydrocaranine (13)   | −8.67                    | −8.06                |
| Kirkine (25)                    | −8.42                    | −8.33                |
| 10-O-dimethylgalanthine (31)    | −8.27                    | −7.1                 |
| 2-α-methoxy-6-O-ethyloduline (22)| −9.38                    | −8.21                |
| 10-norpluviine (24)             | −8.56                    | −7.48                |
| 3-O-acetylnarcissidine (30)     | −8.88                    | −7.68                |
| Galanthamine                    | −11.81                   | −9.5                 |

2.3.1. Acetylcholinesterase (TcAChE) Docking Results

The binding energies shown in Table 3 indicate that 2-α-methoxy-6-O-ethyloduline is the main compound responsible for acetylcholinesterase inhibition, even though all derivatives displayed good binding energies over the enzyme, such as 3-O-acetylnarcissidine and 3-hydroxydihydrocaranine, which showed energy descriptors of −8.88 kcal/mol and −8.67 kcal/mol, respectively, turning them into good candidates as acetylcholinesterase inhibitors. These results are consistent with the experimental data that showed both bulbs and leaves extracts demonstrated good half-maximal inhibitory concentration (IC₅₀ values were within the same order of magnitude in µg/mL), as depicted in Table 2.

The docking assays indicated that all the main alkaloids that are reported in the present study establish hydrogen bond interactions with acetylcholinesterase. In addition to the hydrogen bond interactions, some alkaloids also establish π–π interactions, T-shaped interactions, and salt bridges. For instance, 3-hydroxydihydrocaranine performs the following interactions with acetylcholinesterase: one π–π interaction between the benzene ring of Phe330 and the 1,3-benzodioxole moiety, and two hydrogen bond interactions (one of which occurs between the Glu199 carboxylate group (–COOH) and one of its secondary alcohols (–OH), while the other hydrogen bond interaction occurs between another secondary –OH and the amino acid Ser200 of the acetylcholinesterase catalytic site), as depicted in Figure 4A. Kirkine, as well as 3-hydroxydihydrocaranine, show one π–π interaction with Trp84, and two hydrogen bond interactions with Ser122 and Glu199, respectively (Figure 4B). The compound 10-norpluviine is arranged in a similar manner compared to Kirkine, both having their phenyl moieties of the 2-methoxyphenol frameworks overlapped between them in the enzyme’s catalytic site, leaving the tertiary amino groups of both compounds in opposite directions. This way, 10-norpluviine cannot perform a π–π interaction with Trp84, but instead still shows a good binding energy since it exhibits two hydrogen bond interactions with Tyr130 and through the carbonyl group (C=O) of Trp84. Additionally, 10-norpluviine exhibits a salt bridge with Asp72, which probably contributes to its binding stabilization into the enzyme catalytic site (Figure 4E). 3-O-acetylnarcissidine displays an analogous pose into the acetylcholinesterase catalytic site with Kirkine and 10-norpluviine, but the 1,2-dimethoxybenzene core of 3-O-acetylnarcissidine is superimposed with the cycloaliphatic rings of the former compounds, performing two hydrogen bond interactions with Gly118 and Asp72, as well as a π–π interaction with Phe330 (Figure 4F).

10-O-dimethylgalanthine showed a good binding energy of −9.38 kcal/mol. Inside the acetylcholinesterase catalytic site, this derivative performs an important salt bridge interaction between the amino group and Asp72 amino acid, but also carries out a hydrogen bond interaction with Ser122, as well as π–π and T-shaped interactions through its aromatic 2-methoxyphenol moiety and the residues Phe330 and Tyr334, respectively. Therefore, these data confirm 10-O-dimethylgalanthine as a good candidate for acetylcholinesterase binding and inhibition (Figure 4C).
2-α-methoxy-6-O-ethyloduline, which demonstrated to possess the best binding energy of all derivatives, presents a slightly different binding mode in the acetylcholinesterase catalytic site compared to 3-hydroxydihydrocaranine. Notwithstanding, both compounds share the same direction of their 1,3-benzodioxole cores and their tertiary amino groups. However, the difference in the binding orientation of the 2-α-methoxy-6-O-ethyloduline allows it to execute two hydrogen bond interactions with Glu199 and Tyr121, as well as a T-shaped interaction with Phe330, suggesting that these features are presumably responsible for the better binding energy profile (Figure 4D).

2.3.2. Butyrylcholinesterase (hBuChE) Docking Results

The binding energies from the docking assays of the major selected alkaloids from the Phycella cyrtanthoides extracts over butyrylcholinesterase also showed good binding energy profiles (Table 3). The half-maximal inhibitory concentration values (IC_{50}) for P. cyrtanthoides bulbs and P. cyrtanthoides leaves extracts were 18.32 ± 0.03 µg/mL and 37.70 ± 0.02 µg/mL, respectively, indicating that they effectively inhibit the human butyrylcholinesterase. In this manner, the docking experiments confirm the inhibitory potential mentioned above. Kirkine was the alkaloid that exhibited the best binding energy (−8.33 kcal/mol). Kirkine displays three hydrogen bond interactions, two of which are performed by the hydroxyl group (−OH) of its 2-methoxyphenol moiety, where the hydrogen atom of the −OH interacts with the carboxylate group of Glu197, whereas the oxygen atom of the same −OH interacts with Ser198. The third hydrogen bond interaction occurs between the secondary alcohol (−OH) present in one of the cycloaliphatic rings of Kirkine and the amino acid His438 (Figure 5B). Furthermore, the same 2-methoxyphenol aromatic ring of Kirkine is in charge to perform other two T-shaped interactions with the residues Trp82 and His438, whereupon this alkaloid derivative achieves good stability within the butyrylcholinesterase catalytic site (Figure 5B). In the same way, 10-norpluviine is arranged in a similarly mode into the enzyme pocket; in fact, the same hydrogen bond interactions with Glu197, Ser198 and His438, as well as the T-shaped interaction with His438 could be perceived (Figure 5E). Nonetheless, this derivative does not perform the...
T-shaped interaction seen in Kirkine with Trp82, which could explain the lower energy shown for 10-norpluviine (Table 3). 10-O-dimethylgalanthine is positioned in an opposite manner relative to Kirkine and 10-norpluviine; therefore, the interactions performed by 10-O-dimethylgalanthine are executed with different residues of the catalytic cavity, showing two hydrogen bond interactions with Ala328 and His438, one π-cation interaction between the amino group and Trp82, as well as a salt bridge with the latter amino group and Glu197 (Figure 5C).

3-hydroxydihydrocaranine carries out three hydrogen bond interactions with the amino acids Asp72, Trp82 and Tyr128. Moreover, 3-hydroxydihydrocaranine shares a relatively common binding pose with 3-O-acetylnarcissidine into the butryrylcholinesterase catalytic site. Nonetheless, the fact that these two derivatives show similar, but not identical, orientations results in a common hydrogen bond interaction with the amino acid Asp70. A hydrogen bond interaction through one of the oxygen atoms of the 1,3-benzodioxole framework in the case of 3-hydroxydihydrocaranine, and the same hydrogen bond interaction through one of the oxygen atoms of the 1,2-dimethoxybenzene core of 3-O-acetylnarcissidine. Likewise, 3-O-acetylnarcissidine also carries out another hydrogen bond interaction with Ser198 and the only methoxy group (–OCH₃) of its structure, which is present in one of its cycloaliphatic rings (Figure 5A,F).

The 2-α-methoxy-6-O-ethyloduline established pose into the butryrylcholinesterase catalytic site is quite different relative to the other major alkaloids studied; however, this derivative is still stabilized through two hydrogen bond interactions with Gly116 and Ser198, as well as two T-shaped interactions performed by the 1,3-benzodioxole moiety and the amino acids Trp82 and His438 (Figure 5D).

Figure 5. Predicted binding mode and predicted intermolecular interactions of major alkaloids in leaves and bulbs Phycella cyrtanthoides extracts and the residues of the human butyrylcholinesterase (hBuChE) catalytic site; (A) 3-hydroxydihydrocaranine in the catalytic site; (B) Kirkine in the catalytic site; (C) 10-O-dimethylgalanthine in the catalytic site; (D) 2-α-methoxy-6-O-ethyloduline in the catalytic site; (E) 10-norpluviine in the catalytic site; (F) 3-O-acetylnarcissidine in the catalytic site. Yellow dotted lines indicate hydrogen bond interactions; cyan dotted lines represent π–π interactions; magenta dotted lines represent T-shaped interactions; blue dotted lines indicate π-cation interactions; and red dotted lines indicate salt bridge interactions.

2.4. Antiproliferative Effects

The antiproliferative effects of bulbs and leaves of P. cyrtanthoides alkaloid extracts were tested in the following six tumor cell lines: A549 (lung), HBL-100 (breast), HeLa (cervix), SW1573 (lung), T-47D (breast) and WiDr (colon). To the best of our knowledge, no previous studies regarding the antiproliferative potential have been conducted on Phycella genera. The alkaloid extracts showed activity against all tumor cell lines in this study. Both
extracts display a GI$_{50}$ (50% growth inhibition) <2.5 µg/mL against all cell lines. These results indicate that the potency of the compounds present in the extract is comparable to standard anticancer drugs. For instance, cisplatin under the same six cell lines displayed GI$_{50}$ values in the range 0.54–6.9 µg/mL (Table S1, Supplementary Material). Additionally, several alkaloids contained in the extracts have been previously identified to have anticancer activity. Lycorine showed significant antiproliferative effects against A2780 and MV4-11 cells [46]. Previous studies have demonstrated that lycorine and haemanthamine were able to inhibit cell proliferation using a panel of 16 tumor cell lines [5]. In a previous report, some alkaloids, such as norpluviine, caranine, dihydrolycorine, pseudolycorine, and lycorine, were also investigated against A549, OE21, Hs683, U373, SKMEL, and B16F10 cancer cells lines [47]. In addition, from Hippeastrum solandriflorum, several isolated alkaloids, including narcissidine, 11-hydroxyvittatine, narciclasine, among others, were evaluated against HCT-116 (colon adenocarcinoma), HL-60 (leukemia), OVCAR-8 (ovarian carcinoma) and SF-295 (glioblastoma) cancer cell lines [48]. On the other hand, the homolycorine-type alkaloid hippeastrine inhibited the proliferation of Hep G2 and HT-29 cells [27]. In addition, albusmaculine have been evaluated against breast (Hs578T, MDA-MB-231, MCF7), colon (HCT-15), melanoma (SK-MEL-28) and lung (A549) cells lines [33]. Lycorine and homolycorine-type alkaloids were the most common alkaloids identified in P. cyrthanthoides, which could be strongly associated as the main compounds responsible for the antiproliferative effects.

3. Materials and Methods

3.1. Chemicals

Ultra-pure water (<5 µg/L TOC) was obtained from a water system of purification (Milli-Q Merck Millipore, Chile). Methanol (HPLC grade) and formic acid (puriss. p.a. for mass spectrometry) from J. T. Baker (Phillipsburg, NJ, USA). Acetonitrile (HPLC grade) was from Merck (Santiago, Chile). 2,2-diphenyl-1-picrylhydrazyl (DPPH), gallic acid, DMSO, NaCl, MgCl$_2$, acetyl-thiocholine iodide (ATCI), butyryl-thiocholine chloride (BTCl), 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB), sulforhodamine B (SRB), galanthamine, acetylcholinesterase (AChE), butyrylcholinesterase (BChE), and tyrosinase were purchased from Sigma-Aldrich Chem. Co. (St Louis, MO, USA). Lycorine hydrochloride was purchased from Sigma-Aldrich Chem. Co. (St Louis, MO, USA). Fetal calf serum (FCS) was purchased from Gibco (Grand Island, NY, USA). Trichloroacetic acid (TCA), glutamine, and gentamicin were purchased from Merck (Darmstadt, Germany).

3.2. Plant Material

Phyllea cyrthanthoides was collected during the flowering state in the locality of Cachagua, Region de Valparaiso, Chile, in November of 2019 (22°34′26.7″ S, 68°01′24.4″ W). A voucher herbarium specimen (voucher number PC-52019) was deposited in the Laboratory of Natural Products of the Universidad Austral de Chile (Chile). The sample was authenticated by the botanist Jorge Macaya, University of Chile, Santiago, Chile. The entire plant was cleaned and separated into the different organs (Figure 6), dried, and stored without light, and then ground using an electric processor (Ursus Trotter, UT-PETRUS320) to prepare the extracts.

3.3. Extraction

Bulbs and leaves (100 g) were extracted three times with 100 mL MeOH using an ultrasonic water bath (UC-60A Biobase, Guanzhou, China) with a procedure similar to that usually reported to Amaryllidaceae alkaloids [49] with some modifications. Briefly, extracts were combined, filtered, and concentrated under reduced pressure. The raw extracts were acidified to pH 2 with H$_2$SO$_4$ (2% v/v) and extracted with Et$_2$O (3 × 30 mL). The aqueous solutions were basified with 25% NH$_3$·H$_2$O, up to pH 10. The alkaloids were extracted with EtOAc (3 × 50 mL). The organic layer was evaporated under reduced pressure to obtain the alkaloid extracts.
interactions with cholinesterase and tyrosinase enzymes. Antiproliferative activities were also evaluated using a panel of six solid tumor cell lines.

2.3. Extraction

Bulbs and leaves (100 g) were extracted three times with 100 mL MeOH using an ultrasonic water bath (UC-60A Biobase, Guanzhou, China) with a procedure similar to the man's method as previously reported [51,52]. A sample solution (50 µL, 2 mg/mL) was mixed with 120 µL of 5,5-dithio-bis (2-nitrobenzoic) acid (DTNB) 0.3 mM, and AChE (0.26 U/mL, acetylcholinesterase from Electric eel), or BChE (0.26 U/mL, butyrylcholinesterase from horse serum) solution (25 µL) in Tris-HCl buffer 50 mM (pH = 8.0) in a 96-well mi-
croplate and incubated for 20 min at 37 °C. The reaction was initiated by the addition of 25 µL of acetylthiocholine iodide (ATCI) 1.5 mM or butyrylthiocholine chloride (BTCl) 1.5 mM. A blank was prepared to all reaction reagents without enzymes solution. The absorbances were recorded at three times at 405 nm during 30 min at 37 °C using microplate reader (Synergy HTX Multi-Mode). Galanthamine hydrobromide was used as a positive control. The cholinesterase inhibitory activity was expressed as IC₅₀ (µg/mL, concentration range 0.5 to 50 µg/mL). All data were recorded in triplicate.

3.7. Determination of Tyrosinase Inhibition

Tyrosinase inhibitory activity was evaluated by utilizing the dopachrome method as previously reported [45]. P. cyrtanthoides extract solution (20 µL, 2 mg/mL) was mixed with Mushroom tyrosinase solution (100 unit/mL, 40 µL) and phosphate buffer 0.067 M (30 µL, pH = 6.8) in a 96-well microplate and incubated for 15 min at 30 °C. The reaction was initiated with the addition of 40 µL L-DOPA 2.5 mM and the mixture was incubated for 15 min at 25 °C. A blank was prepared to all reaction reagents without enzyme. The sample and blank absorbances were recorded at 492 nm using a microplate reader (Synergy TM HT Multi-Mode). Kojic acid was used as a positive control. The tyrosinase inhibitory activity was expressed as IC₅₀ (µg/mL, concentration range 31.25 to 250 µg/mL). All data were recorded in triplicate.

3.8. Docking Assays

Docking simulations were carried out for selected major alkaloids, shown in Figure S1 (Supplementary Material), obtained from Phycella cyrtanthoides leaves or bulbs extracts. The geometries and partial charges of each alkaloid were fully optimized using the DFT/B3LYP method with standard basis set 6-311G+/dp [53,54] in Gaussian 09W software. Crystallographic enzyme structures of Torpedo Californica acetylcholinesterase (TcAChE; PDBID: 1DX6 code) [55], and human butyrylcholinesterase (hBuChE; PDBID: 4BDS code) [56] were downloaded from the Protein Data Bank RCSB PDB [57] (for full description, see Supplementary Material).

3.9. Antiproliferative Activity

Antiproliferative activity was evaluated using human solid tumor cell lines. Cells were inoculated onto 96-well plates using 100 µL per well at densities of 2500 (A549, HBL-100, and HeLa) and 5000 (SW1573, T-47D, and WiDr) cells per well. Extract solutions dispersed in water were dissolved in DMSO at 400 times the final maximum test concentration (250 µg/mL). Control cells were exposed to an equivalent concentration of DMSO (0.25% v/v, negative control). The extracts were tested in triplicate at concentrations ranging from 250 to 2.5 µg/mL. Treatment with compounds started on day 1 after plating. Incubation time with compounds was 48 h, after which cells were precipitated with ice-cold trichloroacetic acid (TCA) (50% w/v, 25 µL) during 60 min at 4 °C. Then, the sulforhodamine B (SRB) assay was performed. The optical density (OD) was measured at 530 nm using BioTek PowerWave XS microplate reader. The results were expressed as GI₅₀ values (µg /mL, calculated according to NCI formulas).

3.10. Statistical Analysis

The results obtained from these experiments were repeated five times and expressed as mean ± standard error of mean. Statistical analysis of the data was performed using analysis of variance (two-way ANOVA) where applicable followed by post hoc Bonferroni test. In addition, the determination of the sensitivity (EC₅₀ or IC₅₀) was performed using nonlinear regression (sigmoidal) via origin Pro 9.0 software package (Origin lab Corporation, Northampton, MA, USA). Statistical significance was set at p < 0.05.
4. Conclusions

In summary, seventy alkaloids were detected in bulbs and leaves from the endemic Amaryllidaceae plant *Phycella cyrtanthoides* using UHPLC-DAD-Orbitrap-MS mass spectrometry analysis. Lycorine and haemanthamine type were the major alkaloids identified. The alkaloids extract showed activity against AChE and BChE, but no activity was observed against tyrosinase enzymes. Bulbs’ extracts proved to be the most active against both cholinesterase enzymes. The docking results indicated that hydrogen bond and T-shaped interactions are responsible for better binding over AChE and BChE enzymes. The assessment of the antiproliferative activity indicates that bulbs and leaves exert activity against several human tumor cell lines. The results reported herein are promising and indicate that alkaloid compounds that are present in *Phycella cyrtanthoides* extracts should be further studied for their antiproliferative activities as well as their potential therapeutic applications against neurodegenerative diseases. However, the isolation of major alkaloids as well as in *in vivo* studies are needed to further evaluate the pharmacological properties of this plant.

**Supplementary Materials:** The following are available online at https://www.mdpi.com/article/10.3390/metabo12020188/s1, Figure S1: Alkaloids subjected to docking assays in the corresponding catalytic sites of *Torpedo californica* acetylcholinesterase (TcAChE) and human butyrylcholinesterase (hBuChE), Table S1. Antiproliferative activity (GI$_{50}$, in µg/mL) of *P. cyrtanthoides*, against human solid tumor cell lines.

**Author Contributions:** M.J.S. and C.F.-G. conceived and designed the experiments and wrote the paper; C.F.-G. performed the enzymatic experiments; M.J.S. and C.F.-G. analyzed the data of HPLC/MS. J.R.-P. performed the docking studies and wrote the results. A.P. and J.M.P. performed the antiproliferative experiments. All authors have read and agreed to the published version of the manuscript.

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**Data Availability Statement:** Data is contained within the article or Supplementary Material. The raw UHPLC MS data or other additional data presented in this study are available on request from the corresponding author. The raw data are not publicly available due to privacy.

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