Chagas disease, caused by the protozoan parasite Trypanosoma cruzi, is an anthropozoonosis affecting over six million people worldwide. It is endemic in Latin America, where it is the parasitic disease with the greatest socioeconomic impact, causing over 800,000 disability-adjusted life years and ~7500 deaths per year (Kratz, 2019; WHO, 2021). In the last decades, the disease has spread beyond its natural geographical boundaries due to migratory movements and is now a global health issue (WHO, 2021).
The clinical course of Chagas disease usually comprises two phases. The initial acute phase is generally asymptomatic and thus frequently goes undiagnosed and untreated. This is followed by a chronic phase that can be clinically silent for life. Nevertheless, around 30–40% of chronically infected individuals will develop digestive and/or cardiac symptomatology, cardiac tissue alterations being the most frequent and leading cause of morbidity and mortality.

There is no vaccine available for Chagas disease and current specific chemotherapy for *T. cruzi* infections relies only on benznidazole (BNZ) and nifurtimox (NFX). Both drugs, in use since the early 1970s, have several drawbacks, including variable efficacy in the chronic phase and frequent toxic side-effects that often lead to treatment interruption (Kratz, 2019). Therefore, new drugs with improved efficacy and safety profiles are urgently needed.

In this regard, natural products constitute a source of highly explored chemical entities with privileged structures and bioactivity, which could serve as templates for the synthesis of new drugs. In fact, 61% of new chemical entities approved as anti-inflammatory drugs over the period 1981–2019 were of natural origin (Newman and Cragg, 2020).

Almost 400 species belonging to more than 100 plant families have been studied for a possible Chagas disease treatment (Santos et al., 2020; Varela et al., 2018). Several compounds with anti-trypansomocyte activity have been extracted from plants, including alkaloids, terpenoids, flavonoids, and quinonoids (Salem and Werbovetz, 2006). Alkaloids are of particular interest in biomedicine and drug discovery research due to their structural diversity and specific biological potential (Daley and Cordell, 2021). The Amaryllidaceae is a plant family that contains an exclusive, large and still expanding alkaloids group, which is characterized by unique skeleton arrangements and a broad spectrum of biological activities (Berkov et al., 2020).

Overall, Amaryllidaceae alkaloids are represented by four major types (lycorine, crinine, haemanthamine and galanthamine (GAL)), three minor types (tazettine, homolycorine and montanine), as well as less-conspicuous congeners such as cherylline, trisphaeridine and ismine (Berkov et al., 2020).

Amaryllidaceae are distributed in tropical and subtropical regions, coinciding with areas conventionally associated with a rich culture in folk medicine. For instance, they have been used for their therapeutic activity in cancer, fever, swelling, and some parasitic diseases (Berkov et al., 2020). Indeed, around 15 species of Amaryllidaceae have been indicated for their use against parasitic infections (Nair and van Staden, 2020). Among them, extracts or alkaloids isolated from the genera *Crinum*, *Hippeastrum*, *Rhodophiala*, *Stenomesson* and *Zephyranthes* have been previously reported for their anti-parasitic activity (Martinez-Peinado et al., 2021a, 2020b; Nair and van Staden, 2019). In Argentina, the Amaryllidaceae family includes around 61 species belonging to ten genera (*Chilanthus*, *Crinum*, *Habranthus*, *Haylockia*, *Hieronymiella*, *Hippeastrum*, *Phycella*, *Rhodophiala*, *Stenomesson* and *Zephyranthes*).

*Habranthus brachyandrus* (Baker) Sealy (synonym *Hippeastrum brachyandrus* Baker & Beknoped, *Zephyranthes brachyandrus* (Baker) Backer, *Zephyranthes porphyrospila* E. Holmb.) (WFO, 2021), an endemic species from South America (Arroyo-Leuenberger, 1996) has been previously studied for its chemical composition. Among the reported alkaloids there were lycorine, lycocine, habranthine haemanthamine, haemanthidine, (-)-bulbispermine, galanthine, 10-O-demethylgalanthine and pancratistatin (Boit and Düpke, 1959; Jitsuno, 2009; Wildman and Brown, 1968). In this work, the alkaloid extract obtained from the bulbs of *H. brachyandrus* and four isolated alkaloids were evaluated for their activity against *T. cruzi*.

Material and methods

**Plant material**

*H. brachyandrus* was cultivated under greenhouse conditions, kindly provided for research in May 2017 and authenticated by German Roitman, MSc, (Facultad de Turismo y Urbanismo, Universidad Nacional de San Luis, Av. del Libertador San Martín 721 (D5881DFN) Villa de Merlo, San Luis, Argentina). A sample was deposited in the herbarium of Universidad de Buenos Aires under the code: IBT-UNSJ-Arg.19.

**Alkaloid extraction and isolation**

Dry powdered bulb material (200 g) was macerated in H$_2$SO$_4$ 2% for 4 h in an ultrasonic bath (3 × 1000 ml). Subsequently, samples were centrifuged at 5000 × g (10 min), and the supernatant was transferred to another flask where it was defatted with di-ethyl-ether (3 × 500 ml). The aqueous solution was led to pH 11 – 12 with 10% NaOH and the alkaloids were extracted with dichloromethane (3 × 500 ml). The organic phase was dried with anhydrous sodium sulfate and then evaporated to obtain the basic alkaloid extract, named AE-Hbr (194 mg).

AE-Hbr (120 mg) was roughly separated by SiO$_2$ (100 g) flash column chromatography (CC) using an n-hexane/EtOAc/MeOH gradient to give three fractions (A – C): 24 mg A (n-hexane), 58.5 mg B (EtOAc), 32.25 mg C (MeOH). CC on Sephadex LH-20 (30 cm length, 2.5 cm i.d.) of fraction A gave 9 subfractions (6.75 mg A1, 11.55 mg A2, and 2.25 mg A3). Subfraction A2 was then subjected to preparative TLC using n-hexane/EtOAc/MeOH 4.5:5:0.5 as the mobile phase in NH$_3$ atmosphere to give 3.9 mg of ismine (1). Likewise, fraction B was permeated through a Sephadex LH-20 column using MeOH as the eluent to give four subfractions (9.6 mg B1, 18.1 mg B2, 23.25 mg B3, and 6.52 mg B4). Crystallization of subfraction B3 afforded tazettine (9) (14.2 mg). Subfraction B2 was subjected to preparative TLC using EtOAc/MeOH 9:5:0.5 as the mobile phase in NH$_3$ atmosphere to give 10.3 mg of hippeastidine (8). Finally, a silica gel CC using gradient elution from EtOAc/MeOH (8:2 – 1:9) was applied to fraction C to give four subfractions (2.5 mg C1, 3.1 mg C2, 20.4 mg C3, and 3.6 mg C4). Subfraction C3 was subjected to preparative TLC using EtOAc/MeOH 9:1 as the mobile phase in NH$_3$ atmosphere to give 2.8 mg of 3-epimacronine (11). To monitor the isolation process, column fractions were subjected to analytical TLC. Then column fractions were analyzed, applying UV light, iodine vapors resublimed and/or Dragendorff’s reagent, and similar ones were combined. Their structures were confirmed by NMR spectroscopy, the results being in agreement with literature data (Bastida et al., 2006). Based on the $^1$H and $^{13}$C NMR data, the purities of the isolated alkaloids ismine (1), tazettine (9), hippeastidine (8), and 3-epimacronine (11) were 96%, 94%, 96%, and 95%, respectively (Figs. S3–6).

**GC-MS analysis**

The alkaloids were identified by comparing their GC-MS spectra and Kovats retention index (RI) values against authentic Amaryllidaceae alkaloids previously isolated and identified. Spectral data were processed with AMDIS 2.64 software. Alkaloids were identified by comparing their fragmentation pattern and RI with those of the Amaryllidaceae alkaloids of our laboratory library, in which the isolated compounds were identified by NMR and other spectroscopic techniques (UV, CD, MS), as well as the NIST database and literature data. RI values were calibrated with an n-hydrocarbon calibration mixture (C9-C36), and compounds were semi-quantified according to Torras-Claveria et al. (2014) and expressed as µg GAL/mg AE-Hbr as well as µg GAL/100 mg of bulb dry weight.

**Host cells and *T. cruzi* parasite cultures**

Vero (green monkey kidney epithelial cells), LLC-MK2 (Rhesus monkey kidney epithelial cells) and HepG2 (human liver epithelial cells) were cultivated as previously described (Martinez-Peinado et al., 2020b). *T. cruzi* Tulahuen-β-galactosidase parasites (Discrete Typing Unit VI) were kept in culture by infection of LLC-MK2 cells as described (Martinez-Peinado et al., 2020b).
**Phytomedicine 101 (2022) 154126**

**T. cruzi growth inhibition assay**

The anti-*T. cruzi* assay was performed as previously described (Martinez-Peinado et al., 2020b). The assay test plates were prepared with starting concentrations of 754 μg/ml of extract and 500 μM of alkaloid, which were diluted following a dose-response pattern. The percentage of DMSO was kept below 0.5% in all the wells. Vero cells and purified trypomastigotes were diluted at a concentration of 1 × 10⁶ per ml and mixed at a volume/volume (1:1). Then, 100 μl of the solution was added per well (50,000 Vero cells and trypomastigotes), the multiplicity of infection being 1 (MOI) (Martinez-Peinado et al., 2020b). Each run contained the reference drug BNZ as well as positive and negative controls, as described (Martinez-Peinado et al., 2020b). Plates were incubated for 4 days at 37 °C. Assay read out was performed by adding 50 μl per well of a PBS solution containing 0.25% NP40 and 500 μM chlorophenol red-β-D-galactoside (CPRG) substrate, incubating for 4 days at 37 °C and recording the absorbance at 590 nm, as described (Buckner et al., 1996). All experiments were performed at least in triplicate.

**Anti-amastigote specific activity**

An anti-amastigote assay was performed as previously reported (Martinez-Peinado et al., 2021b). Briefly, 5 × 10⁴ Vero cells were seeded in a T-175 flask and cultured for 24 h. Then, cells were washed and infected with 1 × 10⁷ trypomastigotes (MOI ~ 1). After 18 h, infected cell monolayers were washed with PBS and detached from the flask. Cells were counted and diluted to a concentration of 5 × 10⁵ cells per ml, before adding 100 μl per well to test plates already containing the extract or alkaloids as previously described. We included BNZ, positive and negative controls, as described (Martinez-Peinado et al., 2021b).

**Cell toxicity assays**

Vero and HepG2 cell toxicity assays were performed as described (Martinez-Peinado et al., 2020b). Briefly, Vero and HepG2 cells were respectively diluted at a concentration of 5 × 10⁵ and 3.2 × 10⁵ cells per ml, before adding 100 μl per well. Each run contained its own negative and positive controls as described (Martinez-Peinado et al., 2020b). Plates were incubated at 37 °C for 4 days in the case of Vero cells, and 2 days for HepG2 cells. Then, 50 μl of a PBS solution containing 10% AlamarBlue was added to each well and the plates were incubated for another 6 h at 37 °C before reading the fluorescence intensity (excitation: 530 nm, emission: 590 nm) as described (Martinez-Peinado et al., 2020b). All experiments were performed at least in triplicate.

**Statistical analysis**

The absorbance and fluorescence values were normalized to the controls as described (Martinez-Peinado et al., 2020b). IC₅₀ and TC₅₀ values were determined with GraphPad Prism 7 software (version 7.00, 2016) using a non-linear regression analysis model ((Inhibitor) versus normalized response – Variable slope) (Martinez-Peinado et al., 2020b). Values provided are means and standard deviation (mean ± SD) of at least three independent experiments.

**Results**

**Alkaloid profile analysis**

To elucidate the alkaloid profile, AE-Hbr was analyzed by GC/MS, comparing the fragmentation patterns of the compounds in the extract against those of the alkaloids belonging to the GC-MS library (Fig. S1). Twelve alkaloids were identified from AE-Hbr by GC-MS analysis, involving nine Amaryllidaceae skeleton types (Berkov et al., 2020). The GC-MS results are presented in Table 1, which shows the alkaloid composition of the extract, as well as the retention index and semi-quantitation expressed as μg GAL/mg AE-Hbr and μg GAL/100 mg of dry weight (DW).

| Alkaloid                | RI¹ | μg GAL/mg AE-Hbr | μg GAL/100 mg DW |
|------------------------|-----|------------------|-----------------|
| Crinine-type           |     |                  |                 |
| Hippeastidine (8)      | 2650.9 | 54.77              | 18.13           |
| Galanthamine-type      |     |                  |                 |
| Lycoramine (3)         | 2457.4 | 0.91               | 0.30            |
| Galanthidine-type      |     |                  |                 |
| Galanthidine (5)       | 2535.2 | 2.68               | 0.89            |
| Haemanthamine-type     |     |                  |                 |
| 8-Demethylamartidine (6) | 2540.6 | 1.15               | 0.38            |
| Homolycorine-type      |     |                  |                 |
| 8-Demethylhomolycorine (10) | 2781.3 | 1.01               | 0.33            |
| Nerinine (4)           |     |                  |                 |
| Ismine-type            |     |                  |                 |
| Ismine (1)             | 2306.0 | 3.99               | 1.32            |
| Narcilasine-type       |     |                  |                 |
| Triphaeridine (2)      | 2328.8 | 1.91               | 0.63            |
| Pretazettine-type      |     |                  |                 |
| Deoxytazettine (7)     | 2570.9 | 0.98               | 0.33            |
| Tazettine (9)          | 2689.2 | 42.77              | 14.16           |
| 3-epimacronine (11)    | 2840.8 | 3.49               | 1.16            |
| Miscellaneous-type     |     |                  |                 |
| Tazettamide (12)       | 2961.2 | 0.91               | 0.30            |

¹ RI: Kovats retention index.

Based on the semi-quantitation analysis, the alkaloids found in highest abundance were hippeastidine (also named aulicine), tazettine and, to a lesser extent, ismine and 3-epimacronine (see μg GAL/mg AE-Hbr in Table 1). Alkaloid structures are shown in Fig. 1 (MS data of the alkaloids are provided in Table S1).

**Anti-*T. cruzi* activity of AE-Hbr**

AE-Hbr was tested against *T. cruzi* Tulahuen-β-galactosidase parasites, using Vero cells as hosts (Martinez-Peinado et al., 2020b). AE-Hbr showed an IC₅₀ value of 0.56 ± 0.03 μg/ml, which is similar to that of BNZ (IC₅₀ = 0.4 ± 0.01 μg/ml) (Table 2, Fig. 2A).

AE-Hbr was then evaluated in a Vero cell toxicity assay to discern whether the activity observed was specific against the parasite. Compared to BNZ, the extract displayed higher toxicity against Vero cells, showing a TC₅₀ value of 14.24 ± 4.53 μg/ml (Table 2, Fig. 2B). As previously described, a selectivity index (SI, or TC₅₀ to IC₅₀ ratio) > 10 was considered for progression of the extract (Martinez-Peinado et al., 2020b). AE-Hbr committed to this threshold with a SI value of 25.42, so it was tested in a HepG2 cell toxicity assay, a model used to predict acute liver toxicity. Digi toxin was included in every round and an average TC₅₀ value of 0.19 ± 0.06 μM [i.e., 0.15 ± 0.04 μg/ml] was obtained. As in the Vero cell toxicity assays, AE-Hbr was more toxic than BNZ against HepG2 cells, with a TC₅₀ value of 16.20 ± 3.56 μg/ml (Table 2, Fig. 2C).

Amastigotes, the intracellular replicative forms infecting mammals in the parasite life cycle, are likely the main target for any prospective drug to treat chronic *T. cruzi* infections. Thus, AE-Hbr was evaluated by means of a biological assay specifically targeting amastigotes and found to have a potent IC₅₀ value of 0.66 ± 0.02 μg/ml and a SI window > 10 (SI = 21.57), indicating specific anti-amastigote activity (Table 2, Fig. 2D).

AE-Hbr exhibited highly specific anti-parasitic activity and low toxicity in both mammalian cell lines. In addition, we assessed the anti-parasitic effects of the alkaloids isolated from AE-Hbr: hippeastidine (8), tazettine (9), ismine (1) and 3-epimacronine (11).

Ismine exhibited an IC₅₀ value of 31.13 ± 1.10 μM (Table 2), which was less potent than that of BNZ (Fig. 3A). After progressive testing in the Vero and HepG2 cell toxicity assays, it showed TC₅₀ values ≥ 300...
Fig. 1. Isolated alkaloids: Ismine (1), Hippeastidine (8), Tazettine (9), and 3-Epimacronine (11). Alkaloids identified by means of GC-MS: Trisphaeridine (2), Lycoramine (3), Nerinine (4), Galanthindole (5), 8-O-demethylmaritidine (6), Deoxytazettine (7), 8-O-demethylhomolycorine (10).

Table 2

| Extract / Compounds | Anti-T. cruzi | Toxicity | HepG2 | Anti-amastigote |
|---------------------|---------------|----------|-------|----------------|
|                      | IC₅₀ [μM]     | TC₅₀ [μM] | SI    | IC₅₀ [μM]      |
| HBr-AE               | 0.56 ± 0.03 μg/ml | 14.24 ± 4.53 μg/ml | 25.42 | 16.20 ± 3.56 μg/ml | 0.66 ± 0.02 μg/ml | 21.57 |
| Ismine (1)           | 3.13 ± 1.10 μM | > 300 μM | > 10 | > 300 μM | 191.7 ± 3.75 μM | 1.56 |
| Hippeastidine (8)    | > 150 μM      | –        | –    | –        | –            | –     |
| 3-Epimacronine (11)  | > 400 μM      | –        | –    | –        | –            | –     |
| BNZ [d]              | 1.55 ± 0.05 μM | 269.4 ± 14.39 μM | 174.05 | 193.8 ± 28.92 μM | 2.02 ± 0.09 μM | 131.36 |

a Half-maximal inhibitory concentration,.
b half-maximal toxic concentration,.
c selectivity index,.
d positive control. (-) Not assayed.
µM, with low toxicity against both cell lines and specificity against the parasite. In addition, ismine was less toxic than BNZ against both cell lines (Table 2, Fig. 3B and C). However, it was found to be inactive against the amastigotes (Table 2, Fig. 3D).

Hippeastidine and 3-epimacronine, tested for the first time against T. cruzi in this study, were both found to be inactive (IC$_{50}$ values > 150 µM) (Table 2, Fig. S2). Tazettine, which has been evaluated previously, was also inactive against T. cruzi (Martinez-Peinado et al., 2020b).

**Discussion**

The chemical profile of AE-Hbr showed the presence of twelve alkaloids, four of which, ismine (1), hippeastidine (8), tazettine (9) and 3-epimacronine (11), were isolated and characterized by means of NMR data. In other phytochemical studies of H. brachyandrus, using samples obtained in the Netherlands, Boit and Döpke (1959) and Wildman and Brown (1968) found a different alkaloid profile. Likewise, Jitsuno et al. (2009) reported a different alkaloidal composition of a H. brachyandrus bulb alkaloidal extract obtained from a sample purchased from a garden.
center in Heiwaen, Japan. Causes of alkaloid diversity can be genetic or environmental, or an interaction between both factors, as well as the geographical origin of the species (Berkov et al., 2011). Finally, a mistake in taxonomic characterization is possible.

In this study, AE-Hbr was found to specifically inhibit the parasite growth. It also showed low toxicity against HepG2 cells and Vero cells, and was active against amastigote forms, with an IC50 value of 0.66 ± 0.02 µg/ml and an SI value of 21.57 (Table 2). The low toxicity observed against both mammalian cell lines is of interest and could be related to the absence of lycorine- and crinine-type alkaloids, which are reported to have cytotoxic properties (Nair et al., 2012; Nair and van Staden, 2014).

To determine their individual contributions to the biological activity of the extract, the alkaloids hippeastidine (B), ismine (1), and 3-epimacronine (11) were isolated and tested against the parasite. Ismine had specific activity against the T. cruzi mammalian forms with IC50 values equal to 31.13 ± 1.10 µM. Notably, it showed very low toxicity against Vero and HepG2 cells, which contributed to a good selectivity index.

Gasca et al. (2020) conducted in silico studies with Amaryllidaceae alkaloids, showing that ismine is highly hydrophobic, and therefore able to penetrate biological membranes such as the human intestinal and blood-brain barrier, and a substrate of the human P-glycoprotein. These pharmacokinetic properties could allow effective distribution of the compound if administrated orally, which is an important requirement for new antichagas drugs.

Information on the mechanisms of action involved in the anti-protozoal effects of Amaryllidaceae alkaloids is scant in the literature. Amaryllidaceae alkaloids have shown potent antioxidant activities due to the presence of enol or phenol groups able to stabilize reactive oxygen species and reactive nitrogen species (Cortes et al., 2018). Since the 1980s, it has been known that several synthetic antioxidants inhibit the respiration and growth of T. cruzi in culture (Aldunate et al., 1986). Some aperiphine alkaloids with antioxidant properties have also shown anti-T. cruzi activity (Barbosa et al., 2021; Morello et al., 1994). For instance, Morello et al. (1994) found that boldine and other aperiphine alkaloids were able to inhibit T. cruzi epimastigote growth. The most active compounds also inhibited cell respiration, suggesting that these alkaloids may act by blocking mitochondrial electron transport. Moreover, their anti-T. cruzi activity appears to be correlated with their antioxidative properties (Morello et al., 1994). Recently, another aperiphine alkaloid, dicentrine-β-N-oxide, was described to have specific activity against T. cruzi trypomastigotes (IC50 = 18.2 µM, SI = 11) and the mechanism of parasite death was mitochondrial depolarization (Barbosa et al., 2021). Aperiphine and Amaryllidaceae alkaloids, which belong to the isoquinoline class, are characterized by a benzene ring fused to a pyridine ring, and share antioxidant properties. Taking these previous works into consideration, it could be hypothesized that ismine is acting through a similar mechanism of action. In addition, genes encoding enzymes involved in mitochondrial oxidative phosphorylation are upregulated in amastigotes compared to trypomastigotes, suggesting that the respiratory capacity differs between these two parasite life stages (Li et al., 2016). Thus, this mechanism of action would also account for the lack of activity of ismine against amastigote forms. However, other mechanisms of action reported for other Amaryllidaceae alkaloids in cancer cell lines, such as apoptosis induction and/or interference with DNA, RNA, or protein synthesis, should not be discarded (Nair and van Staden, 2019). The identification of the molecular mode of action of ismine as potential targets of ismine is a major challenge that might be explored with in silico or chemical genomics approaches.

In any case, ismine was non-active when tested specifically against T. cruzi amastigotes and thus it would only be partially responsible for the anti-parasitic effects observed in AE-Hbr. Its lack of activity against amastigotes suggests that the anti-parasitic properties of the extract are caused by other less abundant alkaloid/s, or by a synergic action among them. Hippeastidine and 3-epimacronine were found to be inactive. Regarding the alkaloids with a lower content (< 1 µg GAL/mg AE), only 8-O-demethylmaritidine and 8-O-demethylhomolycorine have been tested against T. cruzi and both proved inactive (de Andrade et al., 2012; Kaya et al., 2011). Thus, the composition of AE-Hbr deserves further study, focusing on the anti-T. cruzi activity of the remaining alkaloids. Lycoramine is a GAL-type alkaloid that structurally differs from GAL in the absence of a double bond between C4 and C4a. GAL has been found to be inactive against T. cruzi (Osorio et al., 2010). Thus, hypothesizing that this small structural difference would not have a modifying effect, lycoramine is unlikely to contribute to the AE-Hbr anti-T. cruzi activity. Similarly, we would expect nerine to be a poor antiprotozoal agent, based on previous work by de Andrade et al. (2012), in which three homolycorine-type alkaloids (homolycorine, 8-O-demethylhomolycorine and 6-O-methyllycorenine) were inactive against the T. cruzi strain Tulahuen C2C4. Deoxytazettine would also be expected to lack anti-T. cruzi activity, as tazettine and 3-epimacronine are reported to be inactive (Martinez-Peinado et al., 2020a). Therefore, the anti-T. cruzi effects of AE-Hbr may be due to the presence of triphenyaeridine, galanthidone and/or tazettamine. Structure–antiprotozoal activity relationships have been poorly studied in Amaryllidaceae alkaloids. However, some results suggest that the presence of a methylenedioxy group, a feature common to the aforementioned alkaloids, and a tertiary non-methylated nitrogen, found in triphenyaeridine, induces a higher anti-parasitic activity (Osorio et al., 2008). In any case, the alkaloids found in AE-Hbr in low amounts (< 1 µg GAL/mg AE) warrant testing against T. cruzi to elucidate their potential contribution to the extract activity. To the best of our knowledge, this is the first time AE-Hbr and its isolated alkaloids have been evaluated for their anti-T. cruzi properties.

Conclusions

H. brachyandrus specifically inhibited T. cruzi growth, showed low toxicity to HepG2 cells and was active against amastigote forms. The chemical profile of AE-Hbr showed the presence of twelve alkaloids and four of them were isolated. Among them, ismine was shown to be partially responsible for the anti-parasitic effects of AE-Hbr. The results obtained in the present work encourage us to continue exploring the alkaloids that are also present in AE-Hbr in different amounts, including those that are present in minor proportions.

Funding

We thank the support of the Generalitat of Catalonia Universities and Research Department, Spain (AGAUR; 2017SGR00924), and the funding from the Carlos III Health Institute (ISCIIC), RICET Network for Cooperative Research in Tropical Diseases (ISCIIC; RD16/0027/0004), and FEDER. N.-M.-P., N.C.-S. and J.G.’s work was supported by the ISCIIC project PI18/01,054. M.-J.P.’s research was supported by the Generalitat of Catalonia Department of Health (PERIS 2016–2010 SLT008/18/00,132). We acknowledge support from the Spanish Ministry of Science, Innovation, and Universities through the “Centro de Excelencia Severo Ochoa 2019–2023” Program (CEX2018–000,806-S), and from the Generalitat of Catalonia through the “CERCA Program”. This work was partially supported by ANPCyT (PICT-2020-SERIEA-03,883), CONICET and CICITCA-UNISJ (Argentina), and BIFRENES RED 416RTOS11 CYTED España.

Declaration of Competing Interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.
Nieves Martinez-Peinado: Conceptualization, Writing – original draft. Javier E. Ortiz: Conceptualization, Writing – original draft. Nuria Cortes-Serra: Conceptualization, Writing – original draft. Joaquim Gascon: Funding acquisition. Alejandro Tapia: German Roitman: Jaume Bastida: Conceptualization, Writing – original draft. Gabriela E. Feresin: Conceptualization, Writing – original draft. Julio Alonso-Padilla: Conceptualization, Writing – original draft.

Acknowledgments

J.O. holds a fellowship from CONICET. G.E.F. is researcher from CONICET. CIBERINFEC is co-funded with FEDER funds.

Supplementary materials

Supplementary material associated with this article can be found in the online version, at doi:10.1016/j.phymed.2022.154126

References

Aldunate, J., Ferreira, J., Letelier, M.E., Repetto, Y., Morello, A., 1986. 1-Butyl-4-hydroxyxiloxane, a novel respiratory chain inhibitor. Effects on Trypanosoma cruzi epimastigotes. FEBS Lett. 195, 295–297. https://doi.org/10.1016/0014-5793(86)80186-6.

Arroyo-Leuenberger, S.C., Zaloa, F.O., Morrone, O., 1996. Amaryllidaceae. Catálogo de las plantas vasculares de la República Argentina I. Missouri Botanical Garden Press, St Louis, MO, USA, pp. 90–100.

Barbosa, H., Costa-Silva, T.A., Alves Conserva, G.A., Araujo, A.J., Lordello, A., Antar, G., de Andrade, J.P., Pigni, N.B., Torras-Claveria, L., Berkov, S., Codina, C., Viladomat, F., Daley, S., Cordell, G.A., 2021. Alkaloids in contemporary drug discovery to meet global disease needs. Molecules 26, 3800. https://doi.org/10.3390/molecules26133800.

Buckner, F.S., Verlinde, C.L., La Flamme, A.C., Van Voorhis, W.C., 1996. Efficient spectral studies. J. Pharm. Biomed. Anal. 70, 137–142. https://doi.org/10.1016/j.jpba.2013.05.009.

Boit, H.G., Dumas, M., Lefebvre, A., Caten, M., 1992. Trypanocidal effect of boldine and related alkaloids upon several strains of Trypanosoma cruzi. Comp. Biochem. Physiol. C Pharmacol. Toxicol. Endocrinol. 107, 367–371. https://doi.org/10.1016/S1099-4831(94)90063-9.

Nair, J.J., Bastida, J., Viladomat, F., van Staden, J., 2012. Cytotoxic agents of the crinane series of Amaryllidaceae alkaloids. Nat. Prod. Commun. 7, 1677–1688.

Nair, J.J., van Staden, J., 2014. Cytotoxicity studies of lycorine alkaloids from Amaryllidaceae plants: a potential natural resource for the treatment of Chagas disease. Parasites Vectors 7, 337. https://doi.org/10.1186/s13071-014-0178-y.

Martinez-Peinado, N., Cortes-Serra, N., Torras-Claveria, L., Pinazo, M.J., Gascon, J., Bastida, J., Alonso-Padilla, J., 2021a. Amaryllidaceae plants: a potential natural resource for the treatment of Chagas disease. Parasites Vectors 14, 337. https://doi.org/10.1186/s13071-021-04839-7.

Martinez-Peinado, N., Cortes-Serra, N., Torres-Claveria, L., Pinazo, M.J., Gascon, J., Battista, J., Alonso-Padilla, J., 2020b. Amaryllidaceae alkaloids with anti-Trypanosoma cruzi activity. Parasites Vectors 13, 299. https://doi.org/10.1186/s13071-020-04171-6.

Nair, J.J., Bastida, J., Viladomat, F., van Staden, J., 2012. Cytotoxic agents of the crinane series of Amaryllidaceae alkaloids. Nat. Prod. Commun. 7, 1677–1688.

Nair, J.J., van Staden, J., 2014. Cytotoxicity studies of lycorine alkaloids from Amaryllidaceae plants. Bioorg. Med. Chem. Lett. 24, 1266–1272. https://doi.org/10.1016/j.bmclet.2014.12.038.

Salem, M., Werbovetz, K., 2006. Natural products from plants as drug candidates and lead compounds against leishmaniasis and trypanosomiasis. Curr. Med. Chem. 13, 787–803. https://doi.org/10.2174/092986706778063003.

Bastida, J., van Staden, J., 2014. Cytotoxicity studies of lycorine alkaloids from Amaryllidaceae plants. Bioorg. Med. Chem. Lett. 24, 1266–1272. https://doi.org/10.1016/j.bmclet.2014.12.038.

Daley, S., Cordell, G.A., 2021. Alkaloids in contemporary drug discovery to meet global disease needs. Molecules 26, 3800. https://doi.org/10.3390/molecules26133800.

Nair, J.J., van Staden, J., 2019. Antiprotozoal alkaloid principles of the plant family Amaryllidaceae. Bioorg. Med. Chem. Lett. 29, 1266–1272. https://doi.org/10.1016/j.bmcl.2019.126642.

Newman, D.J., Cragg, G.M., 2020. Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. J. Nat. Prod. 83, 770–803. https://doi.org/10.1021/acs.jnatprod.9b00285.

Osorio, E.D., Berkov, S., Brun, R., Codina, V., Viladomat, F., Cabezas, F., Bastida, J., 2010. In vitro anti-Trypanosoma cruzi activity of alkaloids from Pholisma tuberosum (Amaryllidaceae). Phytochemistry 71, 161–163. https://doi.org/10.1016/j.phytochem.2010.06.004.

Osorio, E.D., Robledo, S.M., Bastida, J., Cordell, G.A., 2008. Alkaloids with antipROTOZOAL activity. In: The Alkaloids, 66. Elsevier Inc. Press, Amsterdam, Netherlands, pp. 113–190. https://doi.org/10.1016/S0076-6341(08)00202-2.

Salem, M., Werbovetz, K., 2006. Natural products from plants as drug candidates and lead compounds against leishmaniasis and trypanosomiasis. Curr. Med. Chem. 13, 2571–2598. https://doi.org/10.2174/092986706780676201611.

Santos, M.S., de Araújo, R.V., Giongola, J., El-Souad, O., Ferreira, E.L., 2020. Searching for drugs for Chagas disease, leishmaniasis and schistosomiasis: a review. Int. J. Antimicrob. Agents 55, 105906. https://doi.org/10.1016/j.ijantimicag.2020.105906.

Torras-Claveria, L., Berkov, S., Codina, V., Viladomat, F., Bastida, J., 2012. Biactive alkaloid extracts from Narcissus tazetta: mass spectrometric studies. J. Pharm. Biomed. Anal. 70, 13–25. https://doi.org/10.1016/j.jpba.2012.05.009.

Gasca, C.A., Moreira, N.C.S., de Almeida, F.C., Dutra Gomes, J.V., Castillo, W.O., Fagg, C.W., Magalhães, P.O., Fonseca-Brazzo, Y.M., Sakamoto-Hojo, E., de Medeiros, V.K., de Souza Borges, W., Silvério, D., 2020. Acetylicholinoesterase inhibitory activity, anti-inflammatory, and neuroprotective potential of Hipppeastrum psittacinum (Ker Gawl.) herb (Amaryllidaceae). Food Chem. Toxicol. 145, 111703. https://doi.org/10.1016/j.fct.2020.111703.

Jitsuno, M., Yokosuka, A., Sakagami, H., Mimaki, Y., 2009. Chemical constituents of the bulbs of Habranthus brachyandrus and their cytoxic activities. Chem. Pharm. Bull. 57(10), 1153–1157. https://doi.org/10.1246/cpb.57.1153.