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Alkaloid profile in *Pyrolirion albicans* Herb. (Amaryllidaceae), a Peruvian endemic species

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ABSTRACT

The Amaryllidaceae family is widely distributed in different regions of the neotropics and temperate areas of the world. Species of the subfamily Amaryllidoideae are unique in producing the alkaloid galanthamine, which inhibits the action of acetylcholinesterase and is used for the treatment of Alzheimer's disease. In Perú, 15 genera and 68 species belonging to the Amaryllidoideae have been reported in different types of forest, ranging from wet montane to dry, as well as the sandy biomes of the Pacific coastal region, with the greatest diversity in the south. In the tribe Eustephieae, the Andean genus *Pyrolirion* Herb has eight species, six of which are endemic to Peru.

In this work, the leaves and bulbs of *Pyrolirion albicans* were analyzed for their alkaloid content for the first time, using gas chromatography (GC) coupled to mass spectrometry (MS). The alkaloids determined in the leaves were galanthamine, chlidanthine, tazettine and lycorine and those in the bulbs were galanthamine, *N*-demethylgalanthamine, vittatine/crinine, montanine, pancracine, sternbergine, lycorine and hippeastrine. Owing to their important bioactive properties, the high quantity of montanine and galanthamine determined in the bulbs is of particular interest.

1. Introduction

The Amaryllidoideae subfamily of the Amaryllidaceae belongs to the order Asparagales (APG IV, 2016), and consists of 14 tribes and about 70 genera (Chase et al., 2009; Meerow & Snijman, 1998; Meerow et al., 1999, 2006). The genus *Pyrolirion* Herb. belongs to the tribe Eustephieae, which is represented in Peru by four genera, *Eustephia* Cav., *Chlidanthus* Herb., *Hieronymiella* Pax and *Pyrolirion* Herb. (Meerow et al, 2000; León et al., 2006). Only the genus *Eustephia* is endemic and is represented by two species, *Eustephia darwinii* Vargas and *E. hugoei* Vargas. (Huaylla et al., in prep.).

According to World Checklist of Selected Plant Families (WCSP) (2014), *Pyrolirion* Herb. is restricted to the New World and consists of eight species *P. albicans* Herb., *P. arvense* (F. Dietr.) Erhardt, Götz & Seybold, *P. boliviensis* (Baker) Sealy, *P. cutleri* (Cárdenas) Ravenna, *P. flavum* Herb., *P. huantae* Ravenna, *P. tarahuasicum* Ravenna and *P. tubiflorum* (L'Hér.) M. Roem. The majority of these species are Andean, seven are found in Perú, two in Bolivia and one in Chile. *P. albicans* is unique because it is endemic to sandy biomes between 50 and 300 m in the coastal region of southern Perú, where it flowers during the season of mist and drizzle. According to the IUCN categories, its conservation status is vulnerable, because of its restricted geographical range and the degree of threat faced by the coastal biomes as a consequence of climate change (Huaylla & Llalla, in prep.).

The importance of the plants in the Amaryllidoideae subfamily lies in their alkaloid content, and more than 600 alkaloids with a wide range of biological activity have been extracted to date (Bastida et al., 2006, 2011; Berkov et al., 2020; Kulhánková et al., 2013; Cavallaro et al., 2014; Ortiz at al., 2016). Antitumoral activity has been reported in Amaryllidaceae plants, and several Amaryllidaceae alkaloids have demonstrated antitumoral properties (McNulty et al., 2009; Torras-Claveria et al., 2017). In particular, narciclasine and pancratistatine have attracted attention for their potent cell-line-specific anticancer activity and minimal effect on normal cells (Nair et al., 2012). Antiparasitic properties have also been reported for some Amaryllidaceae alkaloids, such as augustine, haemanthamine and haemanthidine, which are active against *Plasmodium falciparum* (Osorio et al., 2008). Other parasites inhibited by Amaryllidaceae alkaloids are *Trypanosoma cruzi* and *T. brucei-rhodesiense* (Osorio et al., 2010; Torras-Claveria et al., 2017).

Additionally, galanthamine is an Amaryllidaceae alkaloid approved by the FDA in 2001 for the treatment of mild to moderate states of Alzheimer's disease. It maintains acetylcholine levels in the brain (which are low in Alzheimer patients) by inhibiting acetylcholinesterase and by binding nicotinic receptors (Torras-Claveria et al., 2017).

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To the best of our knowledge, this is the first study of the alkaloid composition of a *Pyrolirion* species. According to chemosystematics, galanthamine and lycorine are characteristic of the tribe Eustephieae (Huaylla et al., in prep.). Within this tribe, the genus *Pirolyrion* is distinguished from *Hieronymiella* (Ortiz et al., 2018) and *Chlinanthus* (Cahlíková et al., 2011) by its content of montanine, *N*-demethylgalanthamine, pancracine, sternbergine, hippeastrine and 2α-hydroxyhomolycorine. The genus *Eustephia* has not been studied yet.

2. Materials and methods

2.1. Plant material

Pyrolirion albicans Herb. was collected in September 2018 on the sandy biomes south of Ilo, about 10 km from the toll station towards Moguegua, and the specimen was deposited in the Herbarium Moqueguensis of the Universidad Nacional de Moquegua (*H. Huaylla*

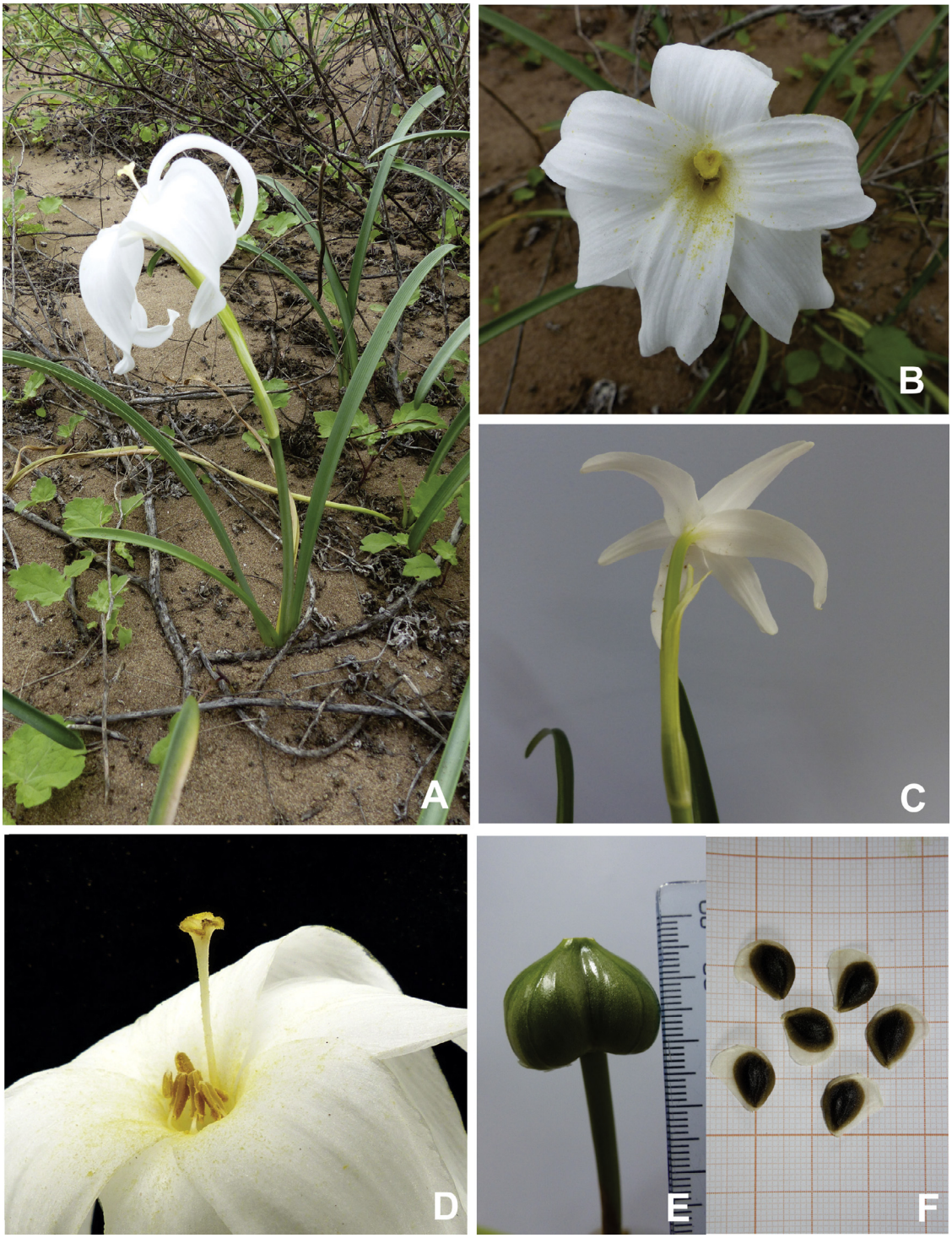

Fig. 1. Photograph of *Pyrolirion albicans* **A.** Habitat. **B.** Flower seen from above, inner and outer tepals. **C.** Lateral view of the flower. **D.** View of stamens and style branches. **E**–**F.** Fruit and seeds.

& R. Calderón 3884). The plant flowers abundantly and ephemerally during the season of drizzle from September to November. It grows in sandy ground on the sides of biomes between 50 and 300 meters in the coastal region of southern Peru. Associated species include *Tiquilia elongata* (Rusby) A.T. Richardson and *Nolana spathula* Ruiz & Pav.

2.2. Description of *P.* albicans *herb*

Bulbous perennial herb 25-38 cm high, flowering ephemerally; roots filiform, white, numerous; bulbs globose, 2.8 – 4.5 × 3.4 – 4 cm, covered in dark brown papery tunics, the neck ca. 13.4 - 18 cm long, clothed in brown linear segments and tunics. Leaves linear 2-4, 14 – 35 × 1.1 – 1.4 cm, erect, shiny-green, weakly bent apically, midrib prominent, lateral veins parallel, apex acute, the blade ribbed. Inflorescence 1-flowered, scape flattened, 15 – 17 × 0.6 – 1 cm, basally greenish-yellow, in the middle part shiny-green towards the insertion of the bracts. Bracts 2, 6.8 – 5.2 × 0.8 – 1.2 cm, fused at the base, 4.5 – 3.1 cm long, white-hyaline in color, apex acute. Flower actinomorphic, perianth funnel-shaped, fused at the base to form a floral tube, aromatic, 4.1 – 6.6 × 1.4 – 1.6 cm; sepals lanceolate, 4.5 – 7.5 × 1.8 cm, apex apiculate, the apiculum 1.5 mm long, furnished with scattered hairs; tepals lanceolate with undulate margin, 4.5 – 6.2 × 1.1 – 1.4 cm. Stamens 6, shorter than the style, filaments fused to the floral tube, 5 – 6.6 cm long, free for 2 mm. Anthers oblong, 0.3 – 0.4 cm long, pollen yellow. Style filiform, yellow-green, apex white, 8.2 – 7.2 × 0.1 cm. Stigma flattened, 3-lobed, yellow. c. 5 mm long. Ovary inferior ovoid, 1.5 × 0.3 cm. Fruit a trilocular green, dehiscent capsule, 2.5 – 1.7 × 1.8 – 2.4 cm. Seeds 1 × 0.7 – 0.35 cm, flattened, oblong or rounded, black with a white marginal wing (Fig. 1).

2.3. Alkaloid extraction and purification

The alkaloid purification was performed using the protocol reported by Torras-Claveria et al. (2013). Bulbs and leaves of *P. albicans* were dried and milled separately. Fifty mg of powder were macerated in MeOH, with codeine as the internal standard. After acidification with 2% H_2SO_4, non-polar compounds were eliminated with $CHCl_3$. After basification with NH_4OH, alkaloids were extracted with $CHCl_3$ (3 × 500 μL). After evaporation of the solvent, the alkaloid mixture was redissolved in 100 μL of $CHCl_3$ and directly injected to the GC-MS system.

2.4. GC-MS analysis

The GC-MS system consisted of a Hewlett Packard 6890 coupled with MSD 5975 (Hewlett Packard, Palo Alto, CA, USA). The injection volume was 1 μL and the operating mode EI at 70 eV. The gas column was

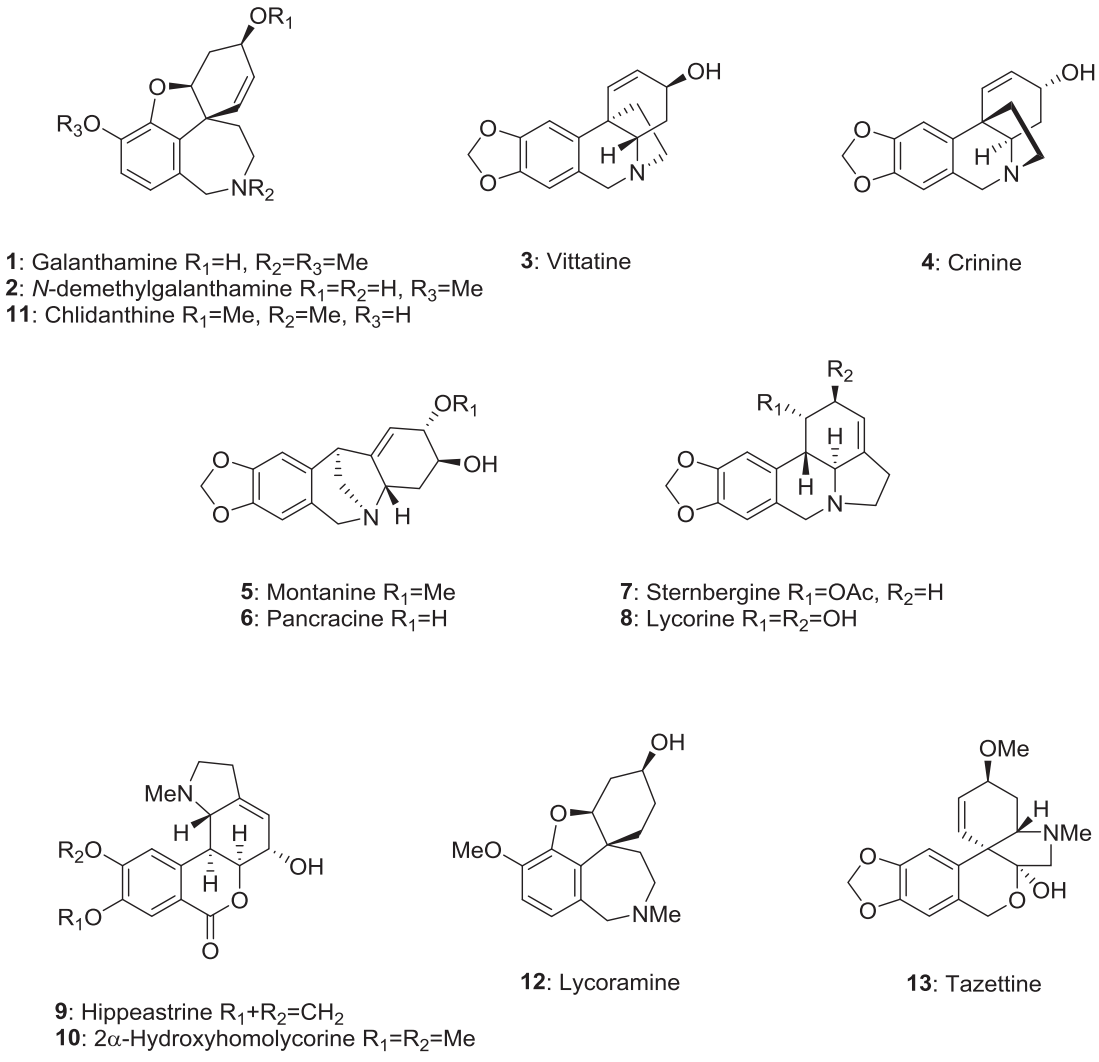

Fig. 2. Alkaloids identified in extracts of *Pyrolirion albicans*: galanthamine, *N*-demethylgalantha-mine, chlidanthine, vittatine, crinine, montanine, pancracine, sternbergine, lycorine, hippeastrine, 2α-hydroxyhomolycorine, lycoramine and tazettine

Table 1
Alkaloids identified by GC-MS in bulbs and leaves, with the corresponding retention time (RT), retention index (RI), the quantity expressed as (μg galanthamine / 100 mg dry weight) and the *m*/*z* fragments of each alkaloid.

Alkaloids	RT	RI	μg gal / 100 mg DW	*m*/*z* fragments
Bulbs				
Galanthamine (**1**)	22,7154	2418,2	10,407	174 (30), 216 (35), 244 (30), 286 (100), 287 (80)
N-Demethylgalanthamine (**2**)	22,9971	2436,6	9,849	160 (50), 202 (40), 230 (40), 272 (100), 273 (90)
Vittatine (**3**) / Crinine (**4**)	23,9485	2498,7	10,382	115 (25), 128 (25), 187 (60), 199 (70), 271 (100)
Montanine (**5**)	26,2267	2647,3	26,787	115 (10), 185 (20), 199 (10), 223 (20), 257 (40), 270 (80), 301 (100)
Pancracine (**6**)	27,2239	2712,3	10,693	115 (25), 185 (30), 199 (25), 223 (25), 243 (25), 270 (25), 287 (100)
Sternbergine (**7**)	27,3242	2718,9	9,861	228 (100), 270 (40), 287 (25), 331 (40)
Lycorine (**8**)	27,9599	2760,3	10,066	147 (15), 226 (100), 250 (25), 268 (30), 268 (30), 287 (40)
Hippeastrine (**9**)	29,8510	2883,7	12,262	96 (40), 125 (100), 315 (< 1)
2α-Hydroxyhomolycorine (**10**)	30,8636	2949,7	14,202	97 (40), 125 (100), 149 (10), 178 (10), 331 (< 1)
Leaves				
Galanthamine (**1**)	22,7139	2418,1	10,016	174 (30), 216 (35), 244 (30), 286 (100), 287 (80)
Chlidanthine (**11**)	22,8282	2425,6	10,275	202 (50), 225 (10), 256 (75), 287 (100)
Lycoramine (**12**)	23,0406	2439,4	traces	111 (55), 125 (35), 153 (35), 288 (100), 299 (50)
Tazettine (**13**)	26,4997	2665,1	10,628	153 (15), 181 (15), 201 (25), 230 (20), 247 (100), 298 (30), 316 (30), 331 (35)
Lycorine (**8**)	27,9511	2759,8	10,030	147 (15), 226 (100), 250 (25), 268 (30), 268 (30), 287 (40)

an HP-5-MS (30 m x 0.25 mm, film thickness 0.25 μm). The temperature gradient was the following: 100-180°C at 15°/min, followed by 180-300°C at 5°C/min, 10 min hold at 300°C, and 2 min at 100°C. The injector temperature was 250°C and the flow-rate of carrier gas (helium) was 1mL/min. The analysis was performed in splitless mode.

2.5. GC-MS identification and quantification of alkaloids

Spectral data were processed with AMDIS 2.64 software. Alkaloids were identified by comparing the fragmentation pattern and Kovats retention index (RI) with those of our library of Amaryllidaceae alkaloids isolated in our laboratory and identified with NMR and other spectroscopic techniques (UV, CD, MS), as well as with the NIST database and literature data. RI values were calibrated with an *n*-hydrocarbon calibration mixture (C_9-C_{36}) (Torras-Claveria et al., 2010).

The quantification was performed manually with AMDIS software, based on the area of the peaks, which depends not only on the quantity of the compound but also on the intensity of mass spectral fragmentation. A calibration curve of galanthamine using codeine as the internal standard (y = 38,636 x + 9,8269; y = μg galanthamine; x = galanthamine area /codeine area) was applied for the quantification, with galanthamine ranges of 5-900 μg, and 3 injections for each galanthamine level. Although not an absolute quantification, it is considered suitable for comparing the quantity of specific alkaloids between samples and with other analyses and Amaryllidaceae plants already quantified with this method (Torras-Claveria et al., 2013).

3. Results and discussion

This is the first phytochemical study of a *Pyrolirion* species and constitutes a new contribution to the field of Amaryllidaceae alkaloids. The bulbs and leaves were found to contain the main structural types of this group of alkaloids (Berkov et al., 2020).

The presence of montanine (the predominant alkaloid in the bulbs) and its derivative pancracine is notable. Montanine has recently attracted attention due to its valuable pharmacological activities. It has demonstrated anti-inflammatory and immunomodulatory (Reis et al., 2019), as well as antioxidant and antimicrobial (Castilhos et al., 2007) properties. It has also shown significant activity as an anxiolytic, antidepressant and anticonvulsant (da Silva et al., 2006) and as an anti-rheumatic (Farinon et al., 2017). Furthermore, montanine has also demonstrated acetylcho-linesterase inhibition activity (Rhee et al., 2001). Owing to its importance, new tools to improve montanine production, such as *in vitro* culture and regeneration, have been developed in *Rhodophiala bifida* (Reis et al., 2019). Notably, montanine has been patented for the treatment and prevention of rheumatoid arthritis and other inflammatory and fibrosing diseases such as ulcerative colitis, sepsis, osteoporosis, Castleman disease, and psoriatic arthritis (Oliveira et al., 2014).

The presence of galanthamine in both leaves and bulbs is also noteworthy, as well as some of its structural derivatives (chlidanthine, *N*-demethylgalanthamine and lycoramine) (Fig. 2). The identified alkaloids and their concentrations expressed as μg of galanthamine by 100 mg of dry weight are shown in Table 1.

It should be mentioned that GC-MS analysis does not distinguish between alkaloids of the enantiomeric series haemanthamine and crinine, and so we have listed both structures.

4. Conclusions

This is the first report of the phytochemical composition of a *Pyrolirium* species. Notably, high levels of montanine, an alkaloid with multiple important biological activities, and galanthamine, commercialized as a drug for the treatment of Alzheimer's disease, were found in the bulbs. Therefore, *P. albicum* is potentially an interesting candidate for culturing to obtain these valuable natural products on a large scale for the treatment of high-impact illnesses.

Declaration of Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.sajb.2020.03.016.

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