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# IDENTIFICATION OF THE ALKALOIDS OF *STENOMESSON AURANTIACUM* (KUNTH) HERB., AN AMARYLLIDACEAE SPECIES FROM THE ECUADORIAN ANDES

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## Abstract

The plant family Amaryllidaceae presents a group of isoquinoline type alkaloids that have been subject of active research for almost 200 years and many of these compounds have been studied for diverse biomedical activities. This study has been focused on the analysis of *Stenomesson aurantiacum* from the Ecuadorian Andes, being one of the first studies of an Amaryllidaceae from Ecuador, where 33 Amaryllidaceae species have been described. The plant *S. aurantiacum* was collected in March 2013 from Cuicococha (Imbabura). The purified alkaloid extracts, of the different organs, were analyzed by gas chromatography coupled to mass spectrometry (GC-MS). In total, 22 different Amaryllidaceae alkaloids were identified, with haemanthamine as the most abundant, found in concentrations above 15 % of the Total Ion Current (TIC) in all the organs, but higher in leaves. The next major alkaloids were tazettine in leaves, stem and flowers; and lycorine in bulb. This is an important finding, as previous, *in vitro* studies have shown that haemanthamine, lycorine and tazetine have antineoplastic and antiparasitic properties. We consider *S. aurantiacum* extracts could be useful in medical studies as these compounds may be effective treating tropical diseases such as Chagas or Malaria, as well as world-wide diseases such as cancer.

Key words: alkaloids, Amaryllidaceae, antineoplastic, antiparasitic, *Stenomesson aurantiacum*, Gas Cromatography-Mass Spectroscopy (GC-MS), haemanthamine, lycorine, tazettine,

## Introduction

The alkaloids of the Amaryllidaceae family have been studied over the world because of their pharmacological potential. Some *in vitro* studies have found that these compounds have antiviral, antibacterial, and antifungal activities [1-2]. Other Amaryllidaceae alkaloids, such as lycorine, augustine and crinamine have antimalarial activity [3]. These compounds also show *in vitro* antitumor activity [4-6]. Galanthamine type alkaloids have acetylcholine esterase inhibitory activity; making galanthamine the second most used medicine in the treatment of Alzheimer [7-8].

There are 33 species of Amaryllidaceae in Ecuador, 12 of which are endemic for the country [9]. Of these taxa, the *Stenomesson* genus is interesting because of its ornamental and ethnobotanical properties. *Stenomesson aurantiacum* is a species spread between southern Colombia and northern Peru at altitudes between 2,700 and 4,000 meters. In Perú *S. aurantiacum* lives in allopatry with similar species as *S. pearcei* and *S. flavum* [10].

Although this plant is widely known, there are no studies of the phytochemicals or the biological activity of the species. The research on *S. aurantiacum* is mostly in the form of botanical surveys [10]. Thus, the aim of this present study is to identify alkaloids present in the Ecuadorian Andes *S. aurantiacum* species.

## Methods

### *Plant material*

*S. aurantiacum* was collected during flowering stage around the Andes' Cuicocha Lake (Imbabura, Ecuador), at 3,000 meters above sea level. The different organs of the plant (bulb, stem, leaves and flowers) were cut into pieces of about 2 cm and dried for 48 hs with vacuum at 60 °C. The resulting dry material was weighed and recorded. The final dry weigh of the samples were of 1.246 g of bulbs, 2.329 g of stems, 1.389 g of leaves and 0.543 g of flowers.

### *Extraction Procedure*

The dried samples were left in methanol for 48 hours. Alternatively, for a proper maceration, ultrasonic baths of 2 hs per day were made, with solvent replacement every 24 hs. After the maceration time, the methanolic extract was filtered and the solvent was evaporated using a rotary evaporator under reduced pressure at 50 °C. The dried crude extract was then redissolved with $H_2SO_4$ (2% v/v) to remove the neutral material (waxes, chlorophylls, mucilages, etc.) with ethyl ether (4 times). The acidic aqueous phase was then subjected to basification with $NH_4OH$ (25% v/v) to pH ~ 10 for alkaloid extraction with ethyl acetate. The extract was evaporated under reduced pressure at 45 °C.

### *Analysis by Gas Chromatography coupled to Mass Spectrometry (GC-MS).*

Five mg of purified extract were dissolved in 300 µl of methanol. The equipment consisted in a gas chromatograph Agilent 6890 coupled to an EI-MS detector (5975) operating at 70eV at 230 °C in the ion source. The chromatograph had a SAPIENS-X5-MS column (30m x 0,25mm x 0,25µm). The temperature program used was: 55 - 100 ºC (60 °C/min), 2 minutes at 100 ° C, 100 - 180 °C (15 °C/ min), 1 minute at 180 °C and 180 °C to 300 °C (5 °C/min). The injector temperature was 280 °C and the flow of helium used was 0.8 ml/min. Codeine was used as an internal standard alkaloid in all the samples analyzed.

AMDIS 2.71(NIST) software was used to analyze the spectral data obtained. The identification of compounds was carried out by comparison with reference compounds, considering fragmentation patterns and retention indices (RI). A standard hydrocarbon mixture was used to perform the retention indices (RI) calibration.

## Results

The analysis of the alkaloid extract of *S. aurantiacum* showed the presence of 22 known Amaryllidaceae alkaloids together with 5 unidentified compounds that presented MS fragmentation patterns also typical of this group of structures.

Twelve alkaloids were identified in the analysis of the bulb with haemanthamine as the most abundant (17.5% of the total ion current (TIC)), followed by lycorine with 9,5% and galanthamine, vittatine, 8-O-demethylmaritidine, tazettine, 1-*O*-acethyllycorine with percentages between 2-10%. Additionally, four compounds, with MS similar to alkaloid fragmentation patterns, were not possible to identify (Figure 1 and Table 1). Fourteen alkaloids were found in *S. aurantiacum* leaves. Haemanthamine was the most abundant (33.44%), followed by tazettine (16.00%), and galanthindole, galanthamine, pancratine C, hippeastrine with percentages ranging from 2 to 10. Moreover, one compound was not possible to identify (Figure 1 and Table 1).

The stem of *S. aurantiacum* showed 13 Amaryllidaceae alkaloids. As in bulbs and leaves, haemathamine was the most abundant with 30.00%, followed by tazzetine (16.00%), and galanthindol, pancratinine C and hippeastrine with percentages varying from 2 to 5 (Figure 1 and Table 1). Finally, twelve alkaloids were detected in the flowers. Haemanthamine was the most abundant (28.54%), followed by tazettine (14,56%), and epigalanthamine, narwedine, galanthindol with percentages between 2-10 (Figure 1 and Table 1).

## Discussion

This study showed a great diversity of alkaloids in *S. auranticum*. Haemanthamine was the most abundant, with concentrations above 15% in all parts of the plant. Tazettine was the second major alkaloid in leaves, stems and flowers. Our results show that the alkaloids profile in leaves, stems and flowers is quite similar, but varies in the bulbs. Our findings are that there is greater diversity of alkaloids in the bulbs. Also, we found that the bulb have a lower concentration of haemathamine than we found in leaves, stems and flowers. Finally, the second major alkaloid in the bulbs was lycorine (as opposed to the tazettine we found in the rest of the parts of the plant).

The presence of the most abundant alkaloids (haemanthamine, tazettine and lycorine) has a great biomedical importance considering previous studies about these alkaloids. *In vitro* studies with haemanthamine have proved antineoplastic and antiparasitic activities against *Plasmodium falciparum*, *Trypanosoma brucei rhodesiense* and *Trypanosoma cruzi* [11-12]. Lycorine has antineoplastic, antiviral, antifungal, antiparasitic activities and it is also an inhibitor of acetilcolinesterase [13]. Tazetine has antimalarial and antineoplastic activities [14-15]. The properties of this alkaloid are of interest as it may have activity against Malaria and Chagas, two neglected tropical diseases to which there are no cure. There is also a need for further studies of the anti-tumoral activity of these compounds.

In addition, the chemical changes found in the different parts of the plant and increasing levels of heamanthamine and other minor alkaloid types in aerial parts, especially in leaves, may be related to defence against pests or diseases [16]. The fact that bulbs have lower alkaloid contents is similar to studies in other Amaryllidaceae species as Narcissus *assoanus* Duf. and *Sternbergia lutea* Ker-Gawl. Ex Schult.f. [17]. A study on the species *Phaedranassa dubia* from Colombia, Amaryllidaceae also present in the Andes of Ecuador, identified the alkaloids: fedramina,pseudolicorina,sanguinine,galanthamine,epinorgalantamina,hemantamina, urgenremina and zefbetaina. *S. aurantiacum* presents greater number of alkaloids and they have only two alkaloids in common (galanthamine and haemanthamine) [18].

It is also interesting to highlight that our analysis has shown the presence of several unindentified components with mass spectra characteristics of Amaryllidaceae alkaloids. Three of these compounds showed base peaks at m/z 286, m/z 174 and m/z 319, with fragmentation patterns similar to lycorine, pancratinine C and hippeastidine, respectively. Moreover, there was a base peak at m/z 372. In the analysis of leaves, the mass spectra of the compound with base peak at m/z 238 reminds to that of ismine. For future studies, it is necessary to confirm these structures by Nuclear Magnetic Resonance (NMR).

We also suggest the use of chemical data, especially alkaloids, of Ecuadorian Amaryllidaceae as a tool of chemotaxonomic markers to a proper identification and to promote the conservation of their genetic biodiversity. Moreover, we consider of high interest to start studies to determine differences in alkaloids content in different vegetative stages of *S. aurantiacum,* because in other Amaryllidaceae these kind of differences had been found [17].

Further research needs to be focused on the identification of the possible new alkaloids and the study of biological activity of extracts and isolated compounds from *S. aurantiacum*, with the purpose of develop a new phytophamaceutical product.

## Acknowledgments

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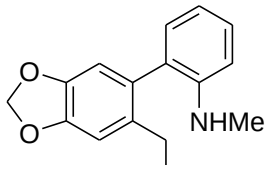
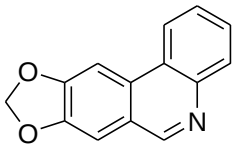
## References

1. Lewis JR. Amaryllidaceae, sceletium, imidazole, oxazole, thiazole, peptide and miscellaneous alkaloids. Nat Prod Rep. 2002; 19(2):223-58
2. Bastida J, Lavilla R & Viladomat F. Chemical and Biological Aspects of *Narcissus* Alkaloids. In: Geoffrey A. Cordell, editor. The Alkaloids: Chemistry and Biology. Academic Press; 2006. Vol. 63. pag 87–179
3. Sener B, Orhan I, Satayavivad J. Antimalarial activity screening of some alkaloids and the plant extracts from Amaryllidaceae. Phytother Res. 2003; 17(10):1220-3.
4. Furusawa E, Suzuki N et al. Anticancer activity of *Narcissus* extracts in mice. Proc Soc Exp Biol Med. 1973; 143(1):33-8.

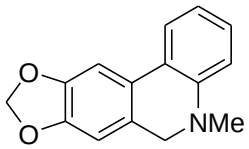
5. Furusawa E, Furusawa S, Marimoto S et al.Therapeutic activity of *Narcissus* alkaloid on Rauscher leukemia and comparison with standard drugs. Proc Soc Exp Biol Med. 1971. 136(4):1168-73.
6. Ceriotti, G. Narciclasine: an antimitotic substance from *Narcissus* bulbs. Nature. 1967; 213: 595- 596.
7. G. Wilcock and D. Wilkinson. In: K. Iqbal, B. Winblad, T. Nishimura, M. Takeda, and H. M. Wisniewski, editors. Alzheimer's Disease: Biology, Diagnosis and Therapeutics. West Sussex: Wiley. 1997 p. 661.
8. Sramek JJ, Frackiewicz EJ, Cutler NR. Review of the acetylcholinesterase inhibitor galanthamine. Expert Opin Investig Drugs. 2000; 9(10):2393-402.
9. Jorgensen PM & León-Yánez. Catalogue of the vascular plants of Ecuador. Monogr. Syst. Bot. Missouri Bot. Gard. 1999. Vol. 75:1-1181.
10. Weber O, Wilkin P. *Stenomesson Pearcei*. Curtis's Botanical Magazine. 2007; 24: 114-120.
11. Likhitwitayawuid K. et al. Cytotoxic and antimalarial alkaloids from the bulbs of *Crinum amabile*. Journal of Natural Products. 1993; 56: 1331–1338.
12. Sener B, Orhan I & Satayavivad J. Antimalarial activity screening of some alkaloids and the plant extracts from Amaryllidaceae. Phytotherapy Research. 2003; 17: 1220-1223
13. Herrera MR et al. Crinane and Lycorane Type Alkaloids from *Zephyranthes citrina*. Planta medica. 2001; 67: 191-193.
14. Furusawa E, Irie H, Combs D et al. Therapeutic Activity of Pretazettine on Rauscher Leukemia: Comparison with the Related Amaryllidaceae Alkaloids. Chemotherapy. 1980; 26: 36-45
15. Furusawa E, Furusawa S, Lee JYB et al. Therapeutic Activity of Pretazettine, a *Narcissus* Alkaloid on Rauscher Leukemia: Comparison with Tazettine and Streptonigrin. Exp Biol Med (Maywood). 1976; 152: 186-191
16. Ríos S, Berkov S, Martínez-Francés V et al. Biogeographical Patterns and Phenological Changes in *Lapiedra martinezii* Lag. Related to Its Alkaloid Diversity. Chem. Biodiv. 2013; 10:1220-1237.
17. Elgorashi E, Drewes S, Van Staden J. Organ-to-organ and seasonal variation in alkaloids from *Crinum macowanii*. Fitoterapia. 2002; 73: 490-495.
18. Osorio E, Brun R, Viladomat F et al. Alkaloids from *Phaedranassa dubia* (Amaryllidaceae). In vitro antiprotozoal activity. Planta Med 2006; 72: 32 studies in other Amaryllidaceae species as Narcissus



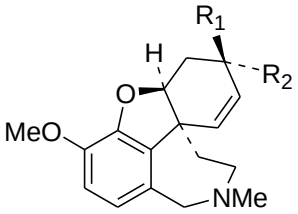
**1** ismine



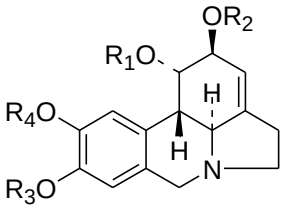
**2** trisphaeridine



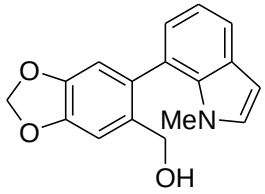
**3** 5,6-dihydrobicolorine



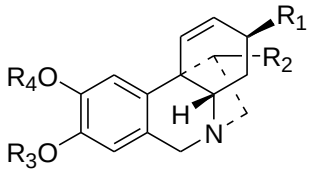
**4** epigalanthamine: $R_1$=H, $R_2$=OH
**5** galanthamine: $R_1$=OH, $R_2$=H
**6** narwedine: $R_1$+$R_2$=O



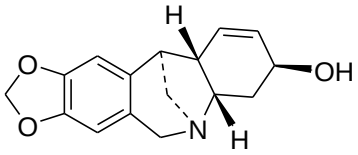
**7** pluviine: $R_1$=H, $R_2$=H, $R_3$=Me, $R_4$=Me
**17** galanthine: $R_1$=H, $R_2$=Me, $R_3$=Me, $R_4$=Me
**18** 1-*O*-acetyllycorine: $R_1$=Ac, $R_2$=H, $R_3$+$R_4$=$CH_2$
**20** lycorine: $R_1$=H, $R_2$=H, $R_3$+$R_4$=$CH_2$



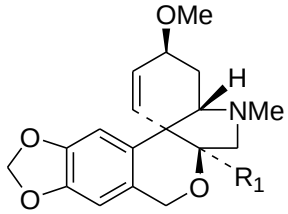
**9** galanthindole



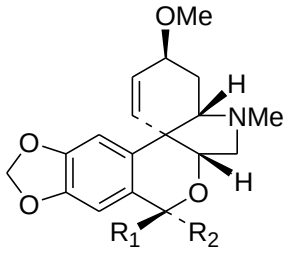
**8** vittatine: $R_1$=OH, $R_2$=H, $R_3$+$R_4$=$CH_2$
**10** 8-*O*-demethylmaritidine: $R_1$=OH, $R_2$=H, $R_3$=H, $R_4$=Me
**15** haemanthamine: $R_1$=OMe, $R_2$=OH, $R_3$+$R_4$=$CH_2$



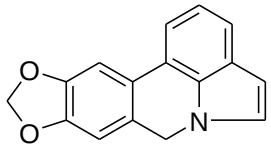
**11** pancratinine C



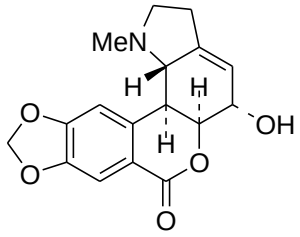
**12** didehydroungvedine: $R_1$=OMe
**16** tazettine: $R_1$=OH



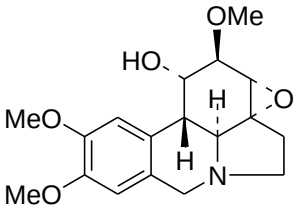
**13** 6-*O*-methylpretazettine: $R_1$=OMe, $R_2$=H
**21** epimacronine: $R_1$+$R_2$=O



**14** 11,12-dehydroanhydrolycorine



**22** hippeastrine



**19** incartine

**Fig. 1.** Alkaloids identified in *S. aurantiacum*

**Table 1**. GC-MS data for *S. aurantiacum* alkaloids.

| Compound | RI | *m/z* [M$^+$] | *m/z* Base peak | % TIC Bulb | % TIC Leaves | % TIC Stem | % TIC Flowers |
|---|---|---|---|---|---|---|---|
| Ismine (**1**) | 2352.6 | 257 | 238 | tr | tr | tr | tr |
| Trisphaeridine (**2**) | 2345.0 | 223 | 223 | | tr | tr | |
| 5,6-Dihydrobicolorine (**3**) | 2387.6 | 239 | 238 | | tr | | |
| Epigalanthamine (**4**) | 2451.4 | 287 | 286 | | | tr | 3.18 |
| Galanthamine (**5**) | 2452.6 | 287 | 286 | 4.65 | 2.87 | | |
| Unknown (m/z 286) | 2461.4 | (?) | 286 | tr | | | |
| Narwedine (**6**) | 2483.0 | 285 | 284 | | tr | | 2.85 |
| Pluviine (**7**) | 2489.0 | 287 | 242 | tr | | | |
| Unknown (m/z 238) | 2514.1 | 299 | 238 | | tr | | |
| Vittatine (**8**) | 2536.0 | 271 | 271 | 6.20 | | | |
| Galanthindole (9) | 2542.0 | 281 | 281 | tr | 5.19 | 4.13 | 2.74 |
| 8-*O*-demethylmaritidine (**10**) | 2555.6 | 273 | 273 | 3.18 | | | |
| Unknown (m/z 174) | 2594.0 | 301 | 174 | 3.73 | | | |
| Pancratinine C (**11**) | 2646.3 | 287 | 176 | tr | 2.54 | 3.42 | tr |
| Didehydroungvedina (**12**) | 2651.8 | 345 | 261 | | tr | | |
| 6-*O*-Methylpretazettine (**13**) | 2652.0 | 345 | 245 | | | tr | 1.14 |
| Unknown (m/z 319) | 2658.3 | (?) | 319 | tr | | | |
| 11,12-Dehydroanhidrolycorine (**14**) | 2663.9 | 249 | 248 | | | tr | |
| Haemanthamine (**15**) | 2691.0 | 301 | 272 | 17.53 | 33.44 | 30.55 | 28.54 |
| Tazettine (**16**) | 2699.5 | 331 | 247 | 4.07 | 16.07 | 16.20 | 14.56 |
| Galanthine (**17**) | 2740.0 | 317 | 242 | tr | | | |
| 1-*O*-acetyllycorine (**18**) | 2762.1 | 329 | 226 | 3.38 | | | |
| Incartine (**19**) | 2787.6 | 333 | 258 | | tr | tr | tr |
| Lycorine (**20**) | 2801.4 | 287 | 226 | 9.54 | | tr | tr |
| Epi-macronine (**21**) | 2849.9 | 329 | 245 | | tr | tr | tr |
| Unknown (*m/z 372*) | 2879.5 | 373 | 372 | tr | | | |
| Hippeastrine (**22**) | 2918.7 | 315 | 125 | | 2.63 | 2.45 | tr |

*RI; Retention Index [M$^+$]; Molecular Ion TIC; Total Ion Current (?); molecular ion with no detectable abundance tr; traces (<2% of TIC)*