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Identification of Amaryllidaceae alkaloids in bulbs and in vitro cultures in various species of Narcissus using GC-MS and LC-MS

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INTRODUCTION

Various species of *Narcissus*, bulbous flowering plants belonging to the Amaryllidaceae family, are known to synthesize Amaryllidaceae alkaloids (galanthamine, lycorine and tazettine) from the same precursor: 4'-O-methylnorbelladine. There is a growing interest for these plants due to the medicinal use of one of the major alkaloids in this genus. *Narcissus* bulbs are considered one of the most important natural sources of galanthamine (selective, reversible and competitive acetylcholinesterase inhibitor) other than synthetic solution. Amaryllidaceae alkaloids possess some interesting biological activities such as anti-malarial, antiviral, antitumor, antifungal and insect antifeedant activity [1]. The aim of work is the evaluation of Amaryllidaceae alkaloids diversity in various varieties of *N. tazetta* (*N. tazetta* var. *Shastpar* (G1), *N. tazetta* var. *Meskin* (G2), *N. tazetta* var. *Panjehgorbei* (G3) and *N. tazetta* var. *Shahla* (G4)) and *Narcissus papyraceus* (G5), growing wild populations in Iran using GCMS and LCMS and in *in vitro* culture.

MATERIALS AND METHODS

Plant materials: : the *in vivo* bulbs of the four varieties of *Narcissus tazetta* and *N. papyraceus* were harvested 2 months after full flowering stage from Behbahan, Ahvaz province in Iran in April 2016. The *in vitro* cultures are initiated using thin slices of leaves (about 3–5 mm) isolated from *N. tazetta* var. *Meskin* (G2) bulbs. Initial explants were cultivated on the Murashige and Skoog medium supplemented with various auxins, 2,4-dichlorophenoxyacetic acid (2,4-D), picloram or naphthalene acetic acid (NAA) at different concentrations (25 μM and 50 μM) and the cytokinin 6-benzylaminopurine (BAP, 0.5 μM) for induction of callogenesis and organogenesis. Calli and bulblets of *N. tazetta* var. *Meskin* (G2) were subcultured in medium containing precursor 4'-O-methylnorbelladine at various concentrations (0.05 and 0.15 g/L) and incubated for two periods of time (10 and 20 days) [1].

Preparation of sample (Figure 1): lyophilized start material was extracted, overnight, with acidified methanol (HCOOH) with addition of internal standard hyoscyamine.

GCMS method: Column DB5MS (30*0,25 mm), mobile phase :He at 1mL/mn, injector : 280°C

LCMS method: Column Mixed mode HILIC (150*2,1 mm), mobile phase A : acetonitrile, mobile phase B : water 10mM HCOONH₄, Flow : 200 μL/mn

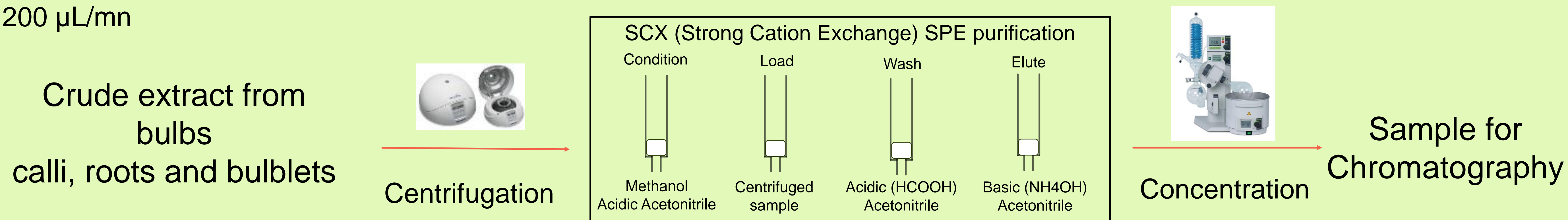


Figure 1: Preparation of analytical sample

RESULTS:

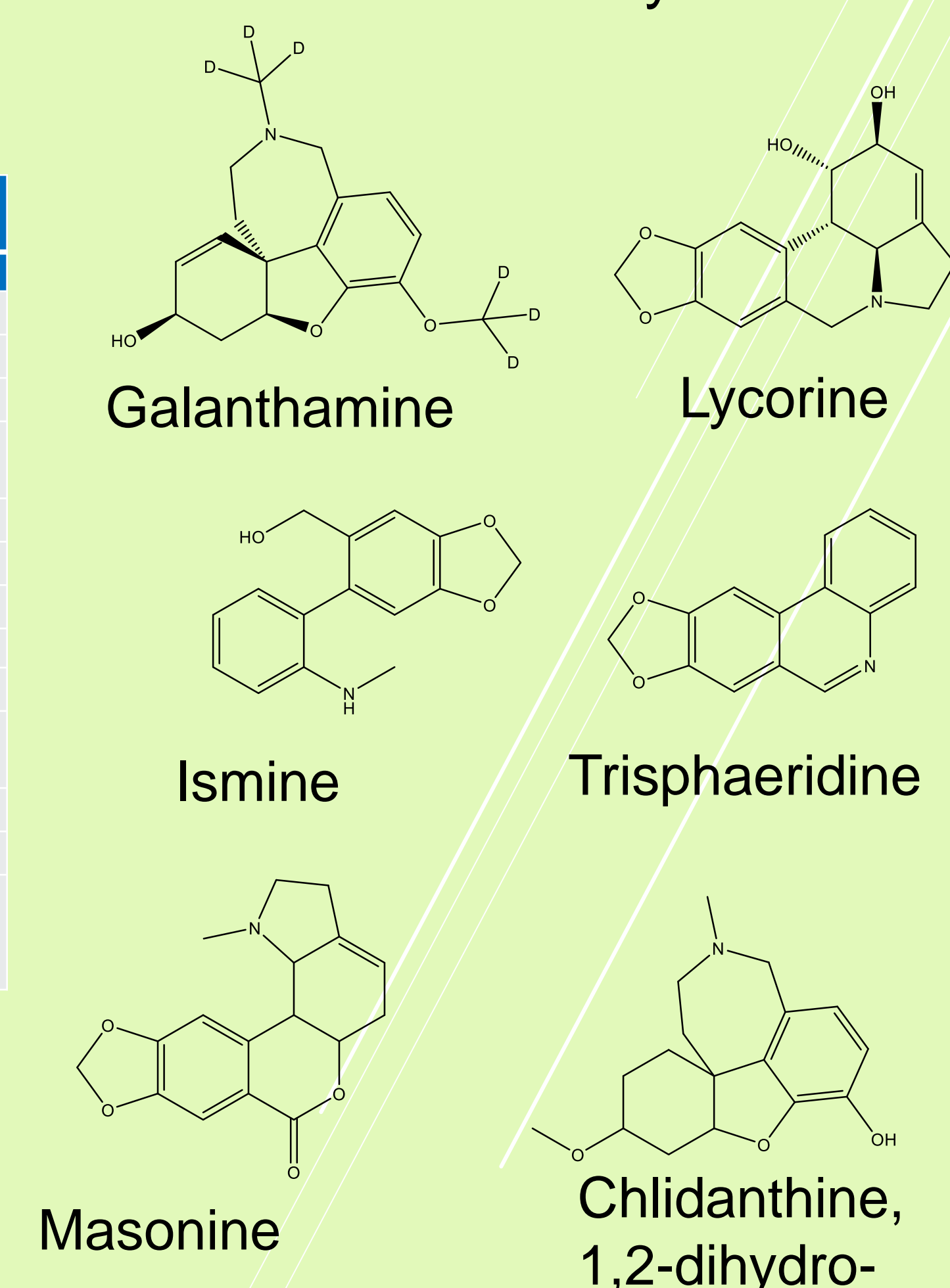
For the *in vivo* bulbs: Thirty (30) alkaloids were identified among them nine alkaloids were observed with the both methods of analysis. The variety *Meskin* of *N. tazetta* (G2), showed the highest diversity of alkaloids and the highest content in galanthamine (red square in tables 1 and 2). By GCMS, six compounds were found in all *Narcissus* varieties bulbs and showed MS fragmentation patterns characteristic of the Amaryllidaceae alkaloids.

Table 1: LCMS analysis

Alkaloids	Retention time (min)	Formula	theoretical (m/z)	Genotypes of <i>Narcissus</i>				
				G1	G2	G3	G4	G5
7-Methoxyoxoasoanine (Cabezas et al. 2003)	2.1	C ₁₈ H ₁₇ NO ₄	312.1230	-	-	-	+	+
Bulbocapnine	2.1	C ₁₉ H ₁₉ NO ₄	326.1387	-	-	-	-	+
Oxoasoanine	2.2	C ₁₇ H ₁₅ NO ₃	282.1125	-	+	-	-	-
5,6-dihydrobicolorine (Ortiz et al. 2012)	2.2	C ₁₅ H ₁₃ NO ₂	240.1019	-	+	-	+	+
Trisphaeridine	2.7	C ₁₄ H ₉ NO ₂	224.0706	+	+	+	+	+
asoanine	3.1	C ₁₇ H ₁₇ NO ₂	268.1332	-	-	-	-	+
N-methylphenylalanine	3.1	C ₁₀ H ₁₃ NO ₂	180.1019	-	-	+	+	+
Albomaculine	3.4	C ₁₉ H ₂₃ NO ₅	346.1649	+	+	+	+	+
Aknadicine	4.1	C ₁₉ H ₂₃ NO ₅	346.1649	-	+	-	-	-
Narcissidine	4.4	C ₁₈ H ₂₃ NO ₅	334.1649	-	+	+	-	-
Ismine	4.6	C ₁₅ H ₁₅ NO ₃	258.1125	+	-	+	+	+
Acetylcaranine	4.6	C ₁₈ H ₁₉ NO ₄	314.1387	+	+	+	+	+
Crinamidine	4.6	C ₁₇ H ₁₉ NO ₅	318.1336	-	-	+	+	+
3-Epimacronine	5.1	C ₁₈ H ₁₉ NO ₅	330.1336	-	+	+	+	+
Cavinine	5.1	C ₁₈ H ₂₁ NO ₆	348.1442	-	+	+	-	-
pretazettine	6.8	C ₁₈ H ₂₁ NO ₅	332.1492	-	-	-	-	+
Haemanthamine	7.8	C ₁₇ H ₁₉ NO ₄	302.1387	-	+	-	-	-
Hippeastrine	8.5	C ₁₇ H ₁₇ NO ₅	316.1179	+	+	+	+	+
Haemanthidine	8.5	C ₁₇ H ₁₉ NO ₅	318.1336	+	-	+	+	+
Lycorenine	9.6	C ₁₈ H ₂₃ NO ₄	318.1700	-	+	-	-	-
Lycorine	10.8	C ₁₈ H ₁₇ NO ₄	288.1230	+	+	+	+	+
Apoaemanthamine	11.0	C ₁₆ H ₁₅ NO ₃	270.1125	-	-	-	-	+
Erythratine	11.1	C ₁₈ H ₂₁ NO ₄	316.1543	+	-	-	+	-
Crinamine	12.4	C ₁₇ H ₁₉ NO ₄	302.1387	+	+	+	+	+
Pseudolycorine	13.4	C ₁₆ H ₁₉ NO ₄	290.1387	+	-	+	+	+

Table 2: GCMS analysis

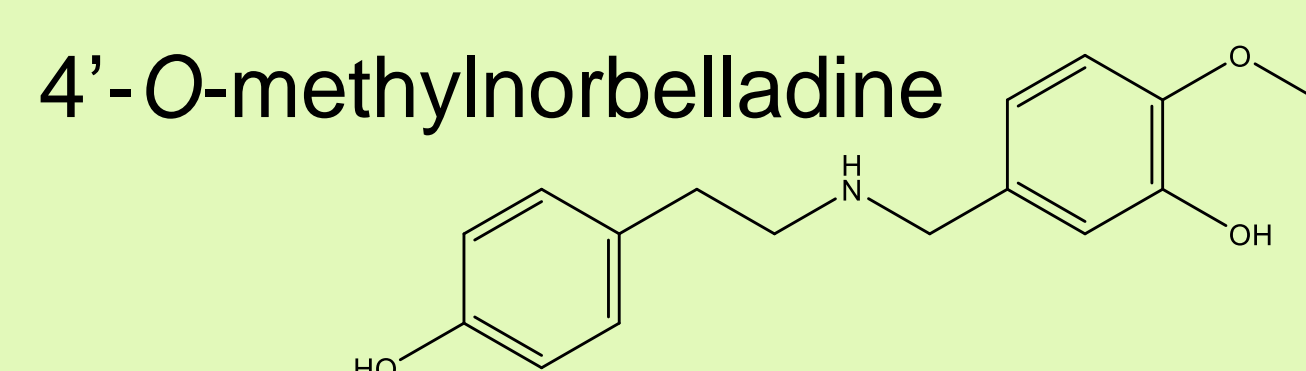
Alkaloids	formula	Molecular mass	Retention time (min)	Fragmentation m/z (Relative intensity, %)	Genotypes of <i>Narcissus</i>				
					G1	G2	G3	G4	G5
Ismine	C ₁₅ H ₁₅ NO ₃	257	8.1	238/100; 257/39; 239/19; 196/8; 168/8	+	+	+	+	+
Trisphaeridine	C ₁₄ H ₉ NO ₂	223	8.3	223/100; 222/36; 138/31; 164/21; 111/18	+	+	+	+	+
Galanthamine	C ₁₇ H ₂₁ NO ₃	287	9.7	286/100; 287/84; 174/37; 216/34; 244/29	+	+	+	+	+
Alpha-dihydrocaranine	C ₁₆ H ₁₉ NO ₃	273	10.8	272/100; 273/37; 162/9; 77/8; 58/8	-	-	-	-	+
Tazettine	C ₁₈ H ₂₁ NO ₅	331	13.2	247/100; 70/41; 71/39; 331/21; 298/18	+	+	+	+	-
Lycorenine	C ₁₈ H ₂₃ NO ₄	317	13.8	243/100; 242/88; 244/18; 317/18; 268/14	-	+	-	-	-
Lycorine	C ₁₆ H ₁₇ NO ₄	287	14.6	226/100; 227/84; 287/38; 268/28; 286/21	+	+	+	+	+
Masonine	C ₁₇ H ₁₇ NO ₄	299	15.3	109/100; 108/21; 110/7; 82/6; 81/4	+	+	+	+	+
3-Epimacronine	C ₁₈ H ₁₉ NO ₅	329	15.4	245/100; 201/54; 109/43; 70/36; 329/26	+	-	-	-	-
Chlidanthine, 1,2-dihydro-	C ₁₇ H ₂₃ NO ₃	289	15.7	228/100; 229/83; 289/41; 270/24; 288/21	+	+	+	+	+
Assoanine	C ₁₇ H ₁₇ NO ₂	267	15.9	266/100; 267/52; 104/9; 268/9; 89/9; 77/8	-	+	-	-	+
Hippeastrine	C ₁₇ H ₁₇ NO ₅	315	16.3	125/100; 96/42; 126/11; 229/11; 124/10	+	-	+	+	-
4,5-Ethylene-8,9-dimethoxy-6-phenanthridone	C ₁₇ H ₁₅ NO ₃	281	17.5	280/100; 281/80; 264/14; 282/14; 140/13	-	+	-	-	-



The identification of the alkaloids was performed by comparing the measured data with literature data

For the *in vitro* cultures: the medium containing NAA (50 μM) is appropriate both for the organogenesis induction and for leading to the highest diversity of alkaloid profile in this variety *Meskin*..

Precursor 4'-O-methylnorbelladine (MN) of Amaryllidaceae alkaloids feeding was found to significantly improve the accumulation of both galanthamine (82 μg/g DW) and lycorine (1800 μg/g DW) in bulblets of *N. tazetta* var. *Meskin* (G2).



CONCLUSION

- It is the first time that these various varieties of *Narcissus tazetta* were analysed by GC-MS and LC-MS for their alkaloid diversity
- *N. tazetta* var. *Meskin* (G2) is a potential source of Amaryllidaceae alkaloids of interest.

REFERENCES:

[1] Tarakemeh, A., Azizi, M., Rowshan, V., Salehi, H., Spina, R., Dupire, F., Arouie, H., & Laurain-Mattar, D, *Journal of Pharmaceutical and Biomedical Analysis*, Elsevier, 2019, 172, pp.230-237 and all references cited in the article.