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Phytochemical and Biological Investigation of *Narcissus pseudonarcissus* Cultivated in Egypt

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Abstract

A phytochemical investigation of the alkaloidal content of the bulbs of *Narcissus pseudonarcissus* cultivated in Egypt resulted in the isolation of the alkaloid; 9-*O*-demethyl-7-*O*-methyllycorenine **(5)** in addition to the eight known alkaloids; homolycorine (**1**), 7-*O*-methyloduline (**2**), 7-*O*-methyllycorenine (**3**), hippeastrine **(4)**, 9-*O*-demethylhomolycorine **(6)**, galanthamine **(7)**, haemanthamine **(8)** and lycorine. Structural determination of the isolated alkaloids was established by different spectral analyses (UV, MS, NMR and 2D-NMR). The isolation of 9-*O*-demethyl-7-*O*-methyllycorenine **(5)** is reported here for the first time from family Amaryllidaceae.

The chemotaxonomic significance of the isolated alkaloids was also studied; six of the isolated alkaloids belong to the homolycorine series which is a distinctive feature for the section *Pseudonarcissi*. The alkaloids of the homolycorine series are absent from some tribes of the Amaryllidaceae, such as the *Amaryllideae* or *Hemantheae*. Moreover, all the *Narcissus* alkaloids of the homolycorine series display a B/C ring junction with a *cis* stereochemistry Furthermore; the antimicrobial activity of some isolated alkaloids has been studied. It is noteworthy that this is the first phytochemical and biological investigation to be carried out on the Amaryllidaceae plant *N. pseudonarcissus* grown in Egypt.

Keywords: Amaryllidaceae, Narcissus pseudonarcissus, homolycorine series, 9-*O*-demethyl-7-*O*-methyllycorenine**.**

1. Introduction

Genus Narcissus L. belongs to the Narcisseae, one of the 15 tribes of family Amaryllidaceae. The most common species of this genus are: *N. pseudonarcissus, N. tazzeta, N. poeticus, N. bulbocodium and N. confus* (Bastida, J., Lavilla, R., Viladomat, 2006). Plants of this genus have been used throughout history in traditional medicine to treat a variety of medicinal problems (Bastida, J., Lavilla, R., Viladomat, 2006)

. Arabian, North African, and Chinese medical Ages continued using Narcissus oil in cancer treatment (Bastida et al., 1997). Bulbs of *N. tazetta* are used in Turkey as a home remedy for the treatment of abscesses because of their antiphlogistic and analgesic properties (Çakici et al., 1997). In some regions of Spain, infusion of *N. pseudonarcissus* flowers is used for treatment of cough and cold, as well as for their emetic and purgative properties (Pigni et al., 2012).

As a part of our interest in the investigation of some Egyptian Amaryllidaceae plants (Evidente et al., 1999; Shawky, 2016), we carried out the present study on the title plant. This paper describes the isolation, structural elucidation and biological study of nine alkaloids; lycorine, homolycorine (1), 7-*O*-methyloduline (2), 7-*O*-methyllycorenine (3), hippeastrine (4), 9-*O*-demethylhomolycorine (6), galanthamine (7), haemanthamine (8) and the alkaloid 9-*O*-demethyl-7-*O*-methyllycorenine (5). The structures of the isolated alkaloids were determined using different spectral methods. The isolation of 9-*O*-demethyl-7-*O*-methyllycorenine (5) is reported here for the first time from family Amaryllidaceae and this is the second report for its isolation from a natural source (Wang et al., 2007). This is also the first phytochemical and biological investigation of the Amaryllidaceae plant *N. pseudonarcissus* grown in Egypt.

2. Materials and Methods

2.1 General:

Melting points were determined on a Stuart SMP heating stage microscope and are uncorrected. UV spectra were determined on Pye Unicam SP8-100 UV/VIS Spectrophotometer. 1D- and 2D-NMR (COSY, HSQC and HMBC) spectra were recorded at JEOL JNM A-500 (1H: 500 MHz, 13C: 125 MHz) for alkaloids 1-8 and JEOL JNM A-400 (1H: 400 MHz, 13C: 100 MHz) for alkaloid 5. EI MS and FAB-MS were taken at JEOL JMS GC mate. Analytical and preparative TLC were performed on silica gel (Merck, Kieselgel, 60 F254, 0.25 and 0.50 mm, respectively). Spots were visualized by UV radiation, anisaldehyde/H2SO4 and Dragendorff's spray reagents. Authentic reference alkaloids were stock samples at the Department of Pharmacognosy, Faculty of Pharmacy, Alexandria University, Alexandria, Egypt.

2.2 Plant material:

practitioners of the Middle *Narcissus pseudonarcissus* was collected in November, 2015 during flowering stage cultivated in Alexandria, Egypt. The plant was kindly identified by Professor Alam El-Din Noah (Head of Ornamental Plants Department, Faculty of Agriculture, Alexandria University, Alexandria, Egypt). A voucher sample is deposited in the Department of Pharmacognosy, Faculty of Pharmacy, Alexandria University, Alexandria, Egypt.

2.3 Extraction and isolation:

Freshly chopped bulbs of *N. pseudonarcissus* in the flowering stage (4 kg) were exhaustively extracted with EtOH by maceration. The combined extracts were concentrated under reduced pressure then defatted with pet. ether, acidified with 5% tartaric acid to pH 2 and then washed with Et_2O. The acidic aqueous phase was rendered alkaline with NH_4OH solution to pH 10, and then extracted successively with $CHCl_3$, EtOAc and n-BuOH. The $CHCl_3$ ¬extracts were concentrated to a small volume, at this stage a white residue (0.1 g) was precipitated, filtered out and identified as lycorine by comparison with a reference sample. The filtrate was evaporated under reduced pressure to give a residue (2.5 g), which was fractionated over a silica gel column (130 g, 4cm in diameter). Elution was started by chloroform, increasing the polarity with methanol. Fractions (100 ml. each) were collected and monitored by TLC using the solvent systems (chloroform: methanol, 9:1 and 8:2). Chromatographic separation resulted in the isolation of nine alkaloids.

2.4 Antibacterial and antifungal activities:

Antibacterial and antifungal screenings of some of the isolated alkaloids were carried out using the agar diffusion technique (Prabuseenivasan et al., 2006) against a Gram-positive bacterium Staphylococcus aureus, two Gram-negative bacteria, Shigella flexneri and Pseudomonas aeruginosa, and the fungus Candida albicans. The used organisms are local isolates provided from the Department of Microbiology, Faculty of Pharmacy, Alexandria University, Alexandria, Egypt. One ml of 24 hours broth culture of each of the tested organisms was separately inoculated into 100 ml of sterile molten nutrient agar maintained at 45°C. Each of the inoculated media was mixed well and poured into sterile 10 cm diameter Petri-dishes. After setting, ten cups, each 8 mm in diameter, were cut in the agar medium (Oxoid). Accurately weighed 2 mg of each tested alkaloid

were dissolved in 1 ml DMF and the solution was inserted in the cups then incubated at 37°C for 24 hours.

9-*O*-demethyl-7-*O*-methyllycorenine (5): Amorphous powder, [α]25D +130; (c 0.08, $CHCl_3$); UV λmax, nm (abs.) MeOH: 285.5 (0.17), 237 (0.34), 209 (1.29); FAB-MS (% rel. int.) m/z: 318 $[M+H]^+$ (56.5), 286 $[M-OCH_3]^+$ (27.0), 149 $[C_9H_9O_2]^+$ (90.0), 109 $[C_7H_{11}N]^+$ (77.6); 1H- and 13C- NMR Table (1).

Table 1: ^{1}H- and ^{13}C-NMR spectral data of alkaloid (5):

#	δ H, ppm (500 MHz, CD_3OD)	δ H, ppm (400 MHz, $CDCl_3$)	nOe's	δ C, ppm
2*α*	3.09 (1H, dd, *J*=9.2, 9.2 Hz)	3.14 (1H, ddd, *J*=9.8,6.3,3.4 Hz)		57.7 (t)
2*β*	2.18 (1H, m)	2.22 (1H, ddd, *J* =9.4,9.4, 9.4Hz)		
3	2.42 (2H, m)	2.47 (2H, m)		28.7 (t)
3a				140.7 (s)
4	5.42 (1H, m)	5.49 (1H, br-s)		117.7 (d)
5*α*	2.54 (1H, m)	2.63 (1H, m)		32.6 (t)
5*β*	2.18 (1H, m)	2.35 (1H,br-d, *J*=16.4 Hz)		
5a	4.14 (1H, dd, *J*=5.8, 1.8 Hz)	4.28 (1H, dd, *J*=5.9, 1.8 Hz)		68.1 (d)
7	5.34 (1H, s)	5.50 (1H, s)		99.7 (d)
7a				130.0 (s)
8	6.64 (1H, s)	6.86 (1H, s)		113.6 (d)
9				148.8 (s)
10				147.1 (s)
11	6.84 (1H, s)	6.86 (1H, s)	H-11c (7.5%) N-CH_3 (2.3%) C10-*O*CH_3(10.1%)	115.4 (d)
11a				127.5 (s)
11b	2.33 (1H, dd, *J*=9.8, 1.8 Hz)	2.40 (1H, dd, *J*=9.3, 1.8 Hz)		44.5 (d)
11c	2.74 (1H, m)	2.74 (1H, d, *J*=9.3 Hz)		69.0 (d)
C7-*O*CH_3	3.39 (3H, s)	3.55 (3H, s)		56.5 (q)
C10-*O*CH_3	3.77 (3H, s)	3.88 (3H, s)	H-11 (10.1%)	55.6 (q)
N-CH_3	2.02 (3H,s)	2.06 (3H,s)		44.1 (q)

Homolycorine (1): Colourless crystals, m.p: 173-175 ºC, [α]25D +95; (c 0.1, $CHCl_3$); UV λmax, nm (abs.) MeOH: 303.5 (0.58), 267.5 (0.94), 227 (2.66); ^{1}H NMR, δH 7.56 (1H, s, H-8), 7.00 (1H, s, H-11), 5.51 (1H, m, H-4), 4.81 (1H, m, H-5a), 3.95 (3H, s, C_9-*O*CH_3), 3.94 (3H, s, C_{10}-*O*CH_3), 3.15 (1H, ddd, J=10.1,6.5,3.6 Hz, H-2α), 2.74 (1H, br.d, J=10.6 Hz, H-11c), 2.66 (1H, dd, J=9.7, 2.0 Hz, H-11b), 2.62 (2H, m, H-5), 2.51 (2H, m. H-3), 2.24 (1H, ddd, J =9.3,9.3, 9.3Hz, H-2β), 2.00 (3H, s, NCH_3); 13C- NMR, δC: 165.9 (C, C-7),153.0 (C, C-10), 148.8 (C, C-9), 140.7 (C, C-3a), 137.6 (C, C-11a), 116.8 (C, C-7a), 115.3 (CH, C-4), 111.8 (CH, C-8), 110.7 (CH, C-11), 77.6 (CH, C-5a), 66.5 (CH, C-11c), 56.5 (CH_2, C-2), 56.4 (CH3, C9-*O*CH_3), 56.1 (CH_3, C10-*O*CH_3), 44.0 (CH, C-11b), 43.6 (CH_3, NCH_3), 31.2 (CH_2, C-5), 27.9 (CH_2, C-3); EI-MS (% rel. int.) m/z: 315 [M]+ (3), 109 $[C_7H_{11}N]$ (100).

7-*O*-methyloduline (2): Colourless crystals, m.p: 163-165 ºC, [α]25D +148; (c 0.1, CHCl3); UV λmax, nm (abs.) MeOH: 287.5 (0.38), 235 (0.69), 214 (1.19); ^{1}H NMR δH: 6.82 (1H, s, H-11), 6.77 (1H, s, H-8), 5.96 (1H, d, J=1.5 Hz, H-12α), 5.91 (1H, d, J =1.5 Hz, H-12β,), 5.49 (1H, overlapped, H-7), 5.49 (1H, overlapped, H-4), 4.26 (1H, br.s, H-5a), 3.53 (3H, s, C7-OCH3) 3.12 (1H, ddd, J=9.9,6.7,3.5 Hz, H-2α), 2.73 (1H, br.d, J=9.4 Hz, H-11c), 2.61 (1H, m, H-5α), 2.46 (2H, m, H-3), 2.39 (1H, dd, J=9.4, 1.8 Hz, H-11b), 2.34 (1H, m, H-5 β), 2.24 (1H, ddd, J =9.2,9.2, 9.2Hz, H-2β), 2.06 (3H, s, NCH_3); EI-MS (% rel. int.) m/z: 315 [M]+ (4), 284 $[M-OCH_3]$+ (11.5), 176 $[C_{10}H_8O_3]^+$ (30.0), 109 $[C_7H_{11}N]^+$ (100).

7-*O*-methyllycorenine (3): Colourless crystals, m.p: 124-126 ºC, [α]25D +164; (c 0.2, $CHCl_3$); UV λmax, nm (abs.) MeOH: 282 (0.57), 233 (1.73), 214.5 (2.70); ^{1}H NMR δH: 6.88 (1H, s, H-11), 6.80 (1H, s, H-8), 5.54 (1H, s, H-7), 5.50 (1H, m, H-4), 4.30 (1H, br.d, J=5.8 Hz, H-5a), 3.90 (3H, s, C_9-OCH_3), 3.88 (3H, s, C10-OCH_3), 3.55 (3H, s, C7-OCH_3), 3.14 (1H, ddd, J=9.9,6.2,3.7 Hz, H-2α), 2.74 (1H, br-d, J=9.2 Hz, H-11c), 2.64

(1H, m, H-5α), 2.48 (2H, m, H-3), 2.42 (1H, dd, J=9.4, 1.7 Hz, H-11b), 2.36 (1H, m, H-5β), 2.21 (1H, ddd, J =9.3,9.3, 9.3 Hz, H-2β), 2.06 (3H, s, NCH3); 13C- NMR, δC: 148.3 (C, C-9), 148.3 (C, C-10), 140.8 (C, C-3a), 130.6 (C, C-7a), 125.7 (C, C-11a), 115.7 (CH, C-4), 112.5 (CH, C-11), 109.8 (CH, C-8), 98.4 (CH, C-7), 67.5 (CH, C-11c), 66.9 (CH, 5a), 56.9 (CH_2, C-2), 56.1(CH_3, C7-OCH_3), 55.8 (CH3, C9-OCH_3), 55.4 (CH_3, C10-OCH_3), 44.3 (CH, C-11b), 44.2 (CH_3, NCH_3), 31.6 (CH_2, C-5), 28.1 (CH_2, C-3); EI-MS (% rel. int.) m/z: 331 $[M]^+$ (5), 300 [M-OCH_3]+ (20.9), 191 $[C_{11}H_{11}O_3]^+$ (34.6), 109 $[C_7H_{11}N]^+$ (100).

Hippeastrine (4): colourless crystals, m.p: 215°C, [α]25D +152; (c 0.1, $CHCl_3$); UV λmax, nm (abs.) MeOH: 308 (2.57), 268 (2.73), 236 (3.4); 1H NMR δH: 7.46 (1H, s, H-8), 6.92 (1H, s, H-11), 6.05 (1H, d, J=1.2 Hz, H-12α), 6.06 (1H, d, J =1.2 Hz, H-12β), 5.65-5.66 (1H, m, H-4), 4.59 (1H, br s, H-5a), 4.39-4.01 (1H, m, H-5), 3.14-3.15 (1H, m, H-2α), 2.84 (1H, dd, J=9.4, 2.1 Hz, H-11b), 2.63 (1H, d, J=9.4 Hz, H-11c), 2.52-2.54 (2H, m, H-3), 2.24 (1H, q, J =9.4 Hz, H-2β), 2.04 (3H, s, NCH3); ^{13}C- NMR, δC: 164.6 (C, C-7), 151.8 (C, C-9), 148.0 (C, C-10), 145.0 (C, C-3a), 139.0 (C, C-7a), 118.5 (C, C-11a), 118.0 (CH, C-4), 109.9 (CH, C-8), 108.7 (CH, C-11), 102.1 (CH_2, C-12), 82.0 (CH, C-5a), 67.0 (CH, C-5), 67.0 (CH, C-11c), 56.1 (CH_2, C-2), 43.5 (CH_3, NCH_3), 40.0 (CH, C-11b), 27.9 (CH_2, C-3); EI-MS (% rel. int.) m/z: 316 $[M+H]^+$ (4), 297 $[M-H_2O]^+$ (10), 190 [M-$C_7H_{11}NO]^+$ (28), 126 $[C_7H_{12}NO]^+$ (84), 125 $[C_7H_{11}NO]^+$ (100).

9-*O*-demethylhomolycorine (6): Colourless crystals, m.p: 138-140 ºC, [α]25D +71.6; (c 0.2, $CHCl_3$); UV λ_{max}, nm (abs.) MeOH: 307 (0.32), 266.5 (0.49), 228 (1.49); ^{1}H NMR, δH 7.59 (1H, s, H-8), 6.99 (1H, s, H-11), 5.50 (1H, m, H-4), 4.78 (1H, m, H-5a), 3.95 (3H, s, C10-OCH_3), 3.15 (1H, ddd, J=10.1,6.5,3.6 Hz, H-2α), 2.74 (1H, br.d, J=10.0 Hz, H-11c), 2.66 (1H, dd, J=9.6, 2.1 Hz, H-11b), 2.60 (2H, m, H-5), 2.51 (2H, m. H-3), 2.26 (1H, ddd, J =9.4,9.4, 9.4 Hz, H-2β), 2.06 (3H,s, NCH_3); ^{13}C- NMR, δC: 165.6 (C, C-7),150.9 (C, C-10), 145.7 (C-, C-9), 140.6 (C, C-3a), 136.6 (C, C-11a), 117.6 (C, C-7a), 116.0 (CH, C-4), 115.4 (CH, C-8), 110.3 (CH, C-11), 77.5 (CH, C-5a), 66.5 (CH, C-11c), 56.5 (CH_3, C10-OCH_3), 56.3 (CH_2, C-2), 43.9 (CH_3, NCH_3), 43.7 (CH, C-11b), 31.2 (CH_2, C-5), 27.9 (CH_2, C-3); EI-MS (% rel. int.) m/z: 301 [M]+ (4), 109 $[C_7H_{11}N]$ (100).

Galanthamine (7): colourless prisms, m.p: 124-126°C, [α]25D -95; (c 0.9, $CHCl_3$); UV λ_{max}, nm (abs.) MeOH: 217 (1.65), 285 (0.27); 1H NMR δH: 6.67 (1H, d, J=8.2 Hz, H-8), 6.63 (1H, d, J=8.2 Hz, H-7), 6.05 (1H, dd, J=10.4,1.4 Hz, H-4a), 6.00 (1H, ddd, J =10.3, 4.9, 1.4 Hz, H-4), 4.62 (1H, br.s, H-1), 4.15 (1H, br.dd, J=4.3, 4.3 Hz, H-3), 4.10 (1H, d, J=15.2 Hz, H-6β), 3.84 (3H, s, OCH_3), 3.69 (1H, d, J=15.2 Hz, H-6α), 3.28 (1H, dd, J=13.5, 13.5 Hz H-12β), 3.06 (1H, br.d, J=14.5 Hz H-12α), 2.69 (1H, ddt, J = 15.8, 3.5, 1.8, 1.8 Hz, H-2β), 2.41 (3H, s, NCH_3), 2.09 (1H, ddd, J= 13.4, 13.4, 3.0 Hz. H-11α), 2.01 (1H, ddd, J= 13.2, 5.0, 2.5 Hz, H-2α), 1.58 (1H, br.d, J= 13.9 Hz, H-11 β); ^{13}C- NMR, δC: 145.8 (C, C-9), 144.1 (C, C-10), 133.0 (C, C-10a), 129.0 (C, C-6a), 127.6 (CH, C-4a), 126.8 (CH, C-4), 122.1 (CH, C-7), 111.1 (CH, C-8), 88.7 (CH, C-1), 62.0 (CH, C-3), 60.5 (CH_2, C-6), 55.9 (CH_3, OCH_3), 53.7 (CH_2, C-12), 48.2 (C, C-10b), 41.9 (CH3, NCH_3), 33.7 (CH_2, C-11), 29.9 (CH_2, C-2); EI-MS (% rel. int.) m/z: 287 [M]+ (90.3), 286 (100), 269 (34.9), 216 (53.6), 174 (58.2).

Haemanthamine (8): colorless prisms, m.p: 196-198 °C; [α]25D +12° (c 0.6, MeOH); UV λmax, nm (abs.) MeOH: 218 (1.46), 242 (0.76) and 295 (1.24); 1H-NMR δH: 6.81 (1H, s, H-10), 6.47 (1H, s, H-7), 6.41 (1H, d, J= 10.1 Hz, H-1), 6.34 (1H, dd, J= 10.1, 4.9 Hz, H-2), 5.89 (2H, s, H-13) 4.31 (1H, d, J =16.8 Hz, H-6α), 3.97 (1H, dd, J=6.8, 3.1 Hz, H-11), 3.86 (1H, m, H-3), 3.68 (1H, d, J=16.8 Hz, H-6β), 3.36 (3H, s, OCH_3), 3.35 (1H, m, H-12α), 3.32 (1H, dd, J=13.6, 4.8 Hz, H-4a), 3.23 (1H, dd, J =13.9, 3.1 Hz, H-12β), 2.11 (1H, ddd, J=4.2, 13.5, 13.5 Hz, H-4α), 2.00 (1H, dd, J=13.7, 4.9 Hz, H-4β); ^{13}C- NMR δC: 146.3 (C, C-8), 146.0 (C, C-9), 135.3 (C, C-10a), 131.7 (CH, C-2), 127.4 (CH, C-1), 126.7 (C, C-6a), 106.8 (CH, C-7), 103.2 (CH, C-10), 100.7 (CH_2, C-13), 80.0 (CH, C-11), 72.7 (CH, C-3), 63.5 (CH_2, C-12), 62.6 (CH, C-4a), 61.5 (CH_2, C-6), 56.5 (CH_3, OCH_3), 50.0 (C, C-10b), 28.1 (CH_2, C-4); EI-MS (% rel. int.) m/z: $[M]^+$ 301 (100), 269 (59.8), 257 (49.0), 240 (38.2), 227(95.2), 225 (80.2), 181 (82.0).

3. Results and Discussion

3.1. Structural elucidation of alkaloids

FAB-MS of alkaloid (Figure 1) (5) showed a parent peak at m/z 318 $[M+H]^+$ and a fragment ion at m/z 109 originating from a retro-Diels-Alder cleavage of ring C, characteristic for Amaryllidaceae alkaloids of homolycorine series (Ibuka et al., 1966)

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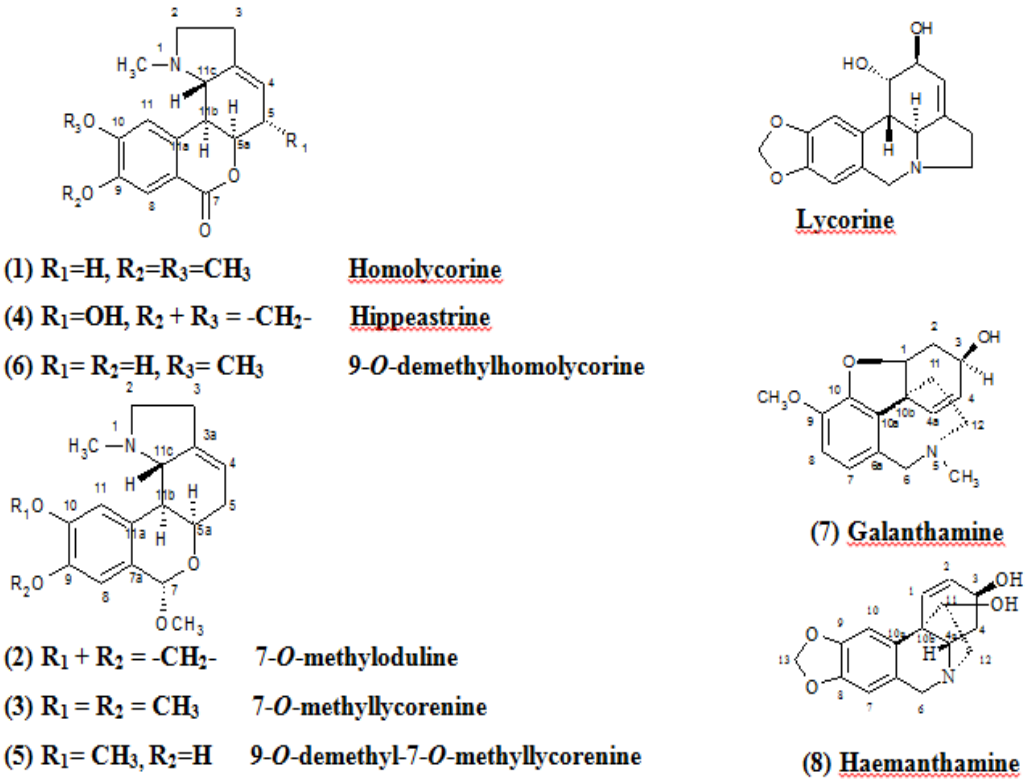

Figure-1: Structures of the isolated alkaloids

^{1}H-NMR spectrum (500MHz) showed the presence of two singlets at δ 6.64 and 6.84 assigned to the two aromatic protons H-8 and H-11, respectively. The assignment of the two signals belonging to the aromatic ring was carried out by nOe's experiments where spatial proximity is observed between H-11 and both the singlet appearing at δ 2.02 (N-methyl group) and the broad doublet observed at δ 2.74 (H-11c). Singlet observed at δ 5.34 was assigned for the methine proton (H-7), of the hemiacetal moiety, signal appeared at δ 5.42 was assigned to the olefinic proton (H-4). The two doublets of double doublets at δ 3.09 and 2.18 were assigned for α- and β-protons at C-2 position, the first being more deshielded due to its cis-relation with the nitrogen lone pair . ^{1}H-NMR spectrum also exhibited the presence of two intense signals at δ 3.39 and 3.77 corresponding to two methoxyl groups, the former assigned to C-7-OCH_3, while the assignment of the other methoxyl substituent to C-10 was supported by nOe's connecting the methoxyl group and H-11 (10.1%). The large coupling constant (J=9.8 Hz) observed between H-11b (δ 2.33, dd) and H-11c (δ 2.74, m) constituted a strong evidence for their trans-diaxial relationship which, in return, together with the small magnitude of coupling constant between H-11b and H-5a (J=1.8 Hz) clearly indicated the cis-junction of B and C rings (Jeffs et al., 1971). This relative stereochemistry was consistent with the inspection of a 3D-model of alkaloid (5) (Figure 2).

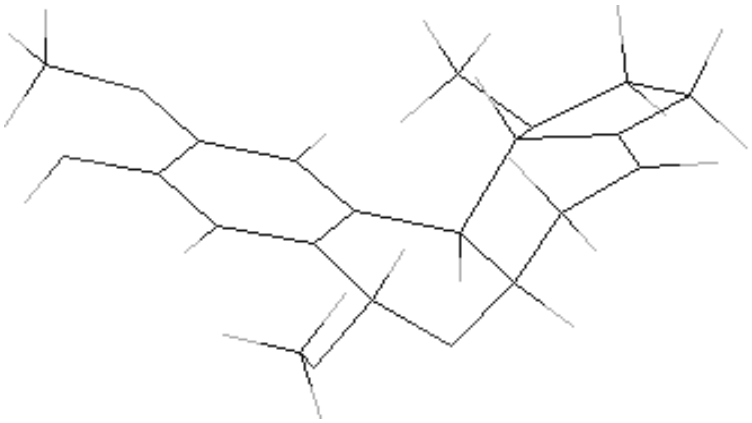

Figure-2: 3D-model of alkaloid (5)

^{13}C-NMR spectrum of alkaloid (**5**) was consistent with a structure of the homolycorine series lacking the carbonyl group (Almanza et al., 1996). The most characteristic signals were: three methyl groups at δ 56.5, 55.6 and 44.1 for two methoxyl groups and N-methyl group, respectively; three methylene carbons at δ 57.7, 32.6 and δ 28.7 for C-2, C-5 and C-3, respectively; three methine carbons at δ 117.7, 115.4 and 113.6 assignable to olefinic and aromatic carbons C-4, C-11 and C-8, respectively; one methine carbon at δ 99.7 assignable to C-7; this carbon responded to the change from hydroxylation to methoxylation by a down-field shift of about 8 ppm (Kreh et al., 1995); three methine carbons at δ 69.0, 68.1 and 44.5 and for the C-11c, C-5a and C-11b, respectively; and five singlets at lower field for quaternary carbons C-3a, C-7a, C-9, C-10 and C-11a, respectively.

In view of the afore-mentioned discussion, alkaloid (**5**) was identified as 9-*O*-demethyl-7-*O*-methyllycorenine, first time to be isolated from family Amaryllidaceae.

In addition to alkaloid (**5**) , eight alkaloids were isolated and identified using their physical properties and different spectral analyses as well as comparison with reference samples into; lycorine, homolycorine (**1**) (Bastida et al., 1987), 7-*O*-methyloduline (**2**) (Kreh et al., 1995), 7-*O*-methyllycorenine (**3**) (Labraña et al., 1999), galanthamine (**7**) (Feng et al., 2011), hippeastrine (**4**) (Almanza et al., 1996), 9-*O*-demethylhomolycorine (**6**) (Jeffs et al., 1985), haemanthamine (**8**) (Crouch et al., 2005) (200, 10, 3, 13, 11, 10, 14, 9 and 20 mg, respectively). It is note-worthy that six of the identified alkaloids (**1**, **2**, **3**, **5**, **6** and **7**) belong to the homolycorine series.

3.2 Chemotaxonomic significance of some of the isolated alkaloids

In general, a series of related alkaloids is found in each plant of the genus Narcissus which differ in the position of their substitutents. The alkaloids lycorine, galanthine, and pluviine (lycorine type) and homolycorine and lycorenine (homolycorine type) are particularly frequent in this genus, lycorine being the most abundant. The presence of these alkaloids is very significant in the sections Narcissi (mainly lycorine type) and Pseudonarcissi (mainly homolycorine type) (Bastida, J., Lavilla, R., Viladomat, 2006). The alkaloids of the homolycorine series are absent from some tribes of the Amaryllidaceae, such as the Amaryllideae or Hemantheae. For that reason, the presence of these alkaloids is a distinctive feature of the Narcisseae tribe (Bastida, J., Lavilla, R., Viladomat, 2006). Moreover, all the Narcissus alkaloids of the homolycorine series display a B/C ring junction with a cis stereochemistry (Bastida et al., 1988).

3.3 Antibacterial and antifungal activities:

The results of antibacterial and antifungal screening (Table 2) showed that the tested alkaloids have moderate antibacterial activity against the Gram-positive *Staphylococcus aureus* and the Gram-negative *Pseudomonas aeruginosa* and good antifungal activity against *Candida albicans*.

Table 2- Results of the antibacterial and antifungal screening of some isolates of *N. pseudonarcissus* bulbs

	Inhibition zone (IZ) in mm[a]			
	Bacteria			**Fungi**
	Gram-positive	**Gram-negative**		
	Staphylococcus aureus	***Shigella flexneri***	***Pseudomonas aeruginosa***	***Candida albicans***
Homolycorine	26	15	28	28
O-methyllycorenine	25	16	25	29
Galanthamine	22	19	26	27
9-*O*-demethylhomolycorine	30	15	28	25
9-*O*-demethyl-*O*-methyllycorenine	24	16	27	23
Haemanthamine	32	15	28	26
Clotrimazole (2 mg/ml)	0	0	0	33
Erythromycin (15 ug/disc)	33	0	0	0
Ciprofloxacin (5 ug/disc)	0	33	35	0
Sulfamethoxazole (25 ug/disc)	35	32	32	0

[a] **Values expressed are averages of three replicates**.

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