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# Biologically active Amaryllidaceae alkaloids in *Narcissus pseudonarcissus* cv. Carlton and Andrew's Choice dormant bulbs

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Since the ancient times, Amaryllidaceae genus *Narcissus* has been reported for the production of biologically important alkaloids, many of which are used as therapeutic tools for human diseases. Among them, galanthamine (Gal) has received a considerable attention for its acetylcholinesterase inhibitory activity for the treatment of early to mid-stage Alzheimer's diseases. In this study, gas chromatography-mass spectrophotometry was used to determine the galanthamine content along with the detection of other biologically important alkaloids in dormant bulb and basal plate of two cultivated varieties of *Narcissus pseudonarcissus*, Carlton and Andrew's Choice. The amount of galanthamine was higher in Carlton bulb (860 µg Gal/g) and basal plate (1254 µg Gal/g) than Andrew's Choice bulb (674 µg Gal/g) and basal plate (1051 µg Gal/g). Non-dormant Carlton bulb was used as control, which represented the galanthamine content of 1117 µg Gal/g. Besides galanthamine, other biologically important alkaloids were detected in Carlton such as lycoramine, lycorine, lycorenine, crinamine, and pancracine; while tazettine, oxoassoanine, lycorine, and *o*-methyl-macronine were detected in Andrew's Choice. Findings of this study suggest that both *Narcissus* cultivars could be the important sources for the isolation of naturally occurring bioactive alkaloids for their commercial availability and could be potentially used in pharmaceuticals.

**Keywords:** Amaryllidaceae alkaloids, Galanthamine, Gas chromatography, *Narcissus pseudonarcissus*, Pharmaceuticals.

**IPC code; Int. cl. (2021.01)-**A61K 36/00, A61K 125/00

## Introduction

Due to climate change, urbanization, deforestation, several emerging biotic and abiotic stresses, and other related factors are extremely challenging to the potential natural resources containing bioactive compounds[1]. Plants are the natural reservoir of various bioactive compounds, the chemical synthesis of those are very expensive[2]. Hence, it is mandatory to identify the potential natural sources as the alternatives of chemical sources. The genus *Narcissus* belongs to the monocotyledon family Amaryllidaceae[3], originated from the Mediterranean area, the centre of diversity was in the Iberian Peninsula and predominantly distributed in North Africa and South-Western Europe, with some populations in the Balkans, Italy and France[4]. *Narcissus* comprises about 100 wild species, commonly cultivated and hybridized with over 27,000 registered cultivars in the International Daffodil Register including Carlton and Andrew's Choice[5]. *Narcissus* are spring-flowering plants comprising an underground bulb, life cycle includes a summer dormancy period for bulbs immediately after the senescence of leaves and stems[6]. Bulb dormancy over the summer allows conserving moisture and reserves carbohydrate and protective secondary metabolites[7-8].

Besides the economic value as ornamentals, the *Narcissus* plants possess notable application in traditional and western medicines as it contains more than hundreds of biologically active secondary metabolites called Amaryllidaceae alkaloids[3,9]. Among them, galanthamine and lycorine are the two best studied alkaloids and only galanthamine is produced on a commercial scale for the pharmaceutical industry[6,10]. Besides galanthamine type, the abundant alkaloids found in the genus *Narcissus* are lycorine and homolycorine type, narciclasine and montanine type, haemanthamine type and tazettine type[6,11-12].

Galanthamine is used as an acetylcholinesterase (AChE) inhibitor by increasing the concentration of acetylcholine at sites of neurotransmission and widely used in the treatment of early to mid-stage

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Alzheimer's disease[13]. Recently, galanthamine, homolycorine, haemanthamine, and lycoramine were reported for human serum butyrylcholinesterase (HuBuChE), human erythrocytic acetylcholinesterase (HuAChE) and prolyl oligopeptidase (POP) inhibitory activities[9]. Lycorine is a powerful inhibitor of ascorbic acid biosynthesis, cell growth, division, and organogenesis, which has been demonstrated to have antitumor and antiviral activities[14]. Another biologically active *Narcissus* alkaloid, haemanthamine has anticancer properties, which inhibits proliferation and activates apoptosis in the cancer cells, also possesses antitumor and antiviral properties[15]. Narciclasine type alkaloids retain highly promising anti-cancer properties have also been reported in several *Narcissus* species[6,11]. Montanine-type alkaloids are predominantly present in low concentrations in plants, however, display promising biological activity in *in-vitro* cytotoxic activity against different cancerous cell lines[16].

Complex chemical structures of alkaloids and limited knowledge on their biosynthesis often challenges the nature of production and accumulation of them in plants. The Amaryllidaceae alkaloids biosynthesis starts with the activation of amino acids, tyrosine and L-phenylalanine, which is predominantly based on the core skeletons obtained from norbelladine, methylnorbelladine, and the phenol coupling steps following a series of complex biochemical pathways, and still far from completes to understand[17]. The alkaloid biosynthesis shares a common biochemical pathway with a key intermediate, norbelladine, originates from the condensation of tyrosine and phenylalanine, which is subsequently *O*-methylated to form *O*-methylnorbelladine. *O*-methylnorbelladine undergoes cyclization results in a regioselective phenol-phenol oxidative C-C coupling forms the *ortho-para'* leading to lycorine and homolycorine type; *para-para'*to haemanthamine, tazettine, narciclasine and montanine type or *para-ortho'* producing the galanthamine type alkaloids[6,17-18].

Gas chromatography-mass spectophotometry (GC-MS) is a rapid and robust method that requires a small amount of plant materials. GC-MS has been widely used for galanthamine qantification in both leaves and bulb of more than 100 ornamental varieties of *Narcissus*[19-20] along with the identification of other important alkaloids[20-21]. The data obtained by GC-MS is highly reproducible and data analysis is relatively simple because of the standard ionization settings and reproducibility of spectra[22-23]. However, metabolomic analysis using GC-MS relies on authentic standards for specific metabolite identification and isolation[24].

*Narcissus* cultivar Carlton is the natural and commercial source of galanthamine due to its easy cultivation process, availability and large bulb size (4 to 5 cm in diameter). Moreover, it is characterized by high galanthamine content along with other important Amaryllidaceae alkaloids such as lycorine, homolycorine, haemanthamine and narciclasine[12,25-29]. Andrew's Choice however a new cultivar of *Narcissus* developed from the cross between *N. jonquilla* and *N. cinel* presumed as low galanthamine producing cultivar used in a transcriptome analysis compared with Carlton[30], is also commercially available, cheap, easily cultivable with large bulb size of 4 to 6 cm in diameter[31]. Although many cultivated varieties of *Narcissus* have been studied for their alkaloid content along with galanthamine[14], Andrew's Choice has been hardly reported[30]. Therefore, a comparative study would provide knowledge on diverse alkaloids accumulation leading through different phenol coupling pathway branches in Carlton and Andrew's Choice[23,32]. Furthermore, the observations on alkaloid profile of Andrew's Choice would provide a suitable natural source along with Carlton for the isolation of galanthamine as well as other biologically active Amaryllidaceae alkaloids.

## Materials and Methods

### Plant materials

*Narcissus pseudonarcissus* bulbs from the variety 'Carlton' and 'Andrew's Choice' used were supplied by New Generation Daffodil Ltd, UK. The experiment was conducted from March 2015 to December 2016, in the Institute of Integrative Biology, University of Liverpool, UK. Dormant bulbs harvested in June/July after senescence of leaves and stem, basal plate tissues of dormant bulbs and bulbs harvested in April/May after flowering (non-dormant bulb) were cut into small pieces and stored at -80 °C. Dormant bulb and basal tissue samples were collected from three different bulbs of both cultivars. Non-dormant Carlton bulb tissues were used as control. Bulb and basal plate plant tissues are represented in Fig. 1.

### Sample preparation and extraction

About 100 mg of frozen bulb and basal tissues were taken in a 2 mL microfuge tube with 500 µL methanol (HPLC grade, Fisher Scientific, UK) and homogenised for 2 × 60 s. After which, an additional 500 µL methanol was added and the samples were

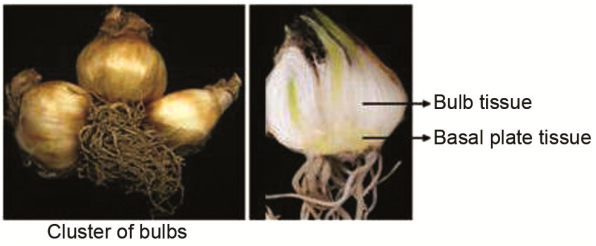


Fig. 1 — Plant materials: Bulb and basal plate tissues of daffodils (Carlton and Andrew's Choice).

vortexed for 30 s and then incubated for 5 hours at room temperature assisted by an ultrasonic bath (Grant Instruments Ltd. England) for 15 minutes, every 30 minutes. After incubation, the samples were centrifuged at 12,000 rpm for 1 minute and 500 µL supernatant was taken carefully without disturbing the pellet to 2 mL glass vials (Thermo Scientific, Germany). The freshly extracted samples (65 µL) were transferred to the GC-MS vial (Chromacol Ltd. USA) with 2 µL (5 µg/µL) codeine standard solution. Each alkaloid extract was analysed in triplicate.

**GC-MS analysis**

Methanol extracts of all samples (bulb and basal tissues of Carlton and Andrew's Choice obtained from three different bulbs) were analysed using GC-MS to quantify the amount of galanthamine. Standard solutions were prepared using galanthamine and codeine as external and internal standard respectively; following the method described by Ferdausi *et al.*[29]. Samples (42) were run in two batches of 21 samples each. Standards were run three times with each batch of samples, i.e. at the beginning, in the middle after 10 samples, and at the end of 21 samples, in the Centre of Proteome Research, University of Liverpool using GC-MS. The GC-MS mass spectra were recorded on an automated Micromass GCT instrument operating in EI mode of 70 eV (Waters Ltd. UK). The separation was carried on a non-polar general purpose chromatography column, BPX5 (30 m × 0.25 mm × 0.25 µm) (SGE Analytical Science). The GC-MS chromatograms of Carlton dormant bulb and basal plate showed a slight shift in retention time to high values due to a change of column to DB5M5 (30 m × 0.25 mm × 0.25 µm) (J & W Scientific, Agilent Science 2000). The temperature program was initially 70 °C for 2 minutes, then an increase of 10 °C/min up to 320 °C and finally held for 8 minutes. The flow rate of the carrier gas (helium) was 0.7 mL/min. The injection of 1 µL of extract was introduced in spitless injection mode and the injection temperature was 250 °C.

**Identification of alkaloids**

The NIST mass spectral library database software that was accessed via the Waters Mass Lynx was used to identify and quantify the amount of galanthamine in samples under analysis. An authentic galanthamine standard was used for quantification purposes[29]. Furthermore, GC-MS chromatograms for all samples under analysis were downloaded from the NIST mass spectral library database which contains the mass spectra, compound structures, and retention index information of each sample based on which the individual alkaloids were identified by comparing their individual MS along with reported spectra in the literature.

**Calculations and data interpretation**

The calibration curves were prepared by plotting the galanthamine peak areas (TIC) versus the standard of galanthamine molarities (0.0002671, 0.0001336, and 0.00002671 M), to generate equations to calculate the amount of galanthamine using Microsoft Excel 2010. The equations generated from the calibration curves of Gal standards, which were used as the external standard, were used to calculate the amount of galanthamine from all sample extracts. As the calibration curve analysis was based on external standards (Gal) therefore, calculations were more accurate and reproducible than internal standard (Codeine) equations. Standards were analysed in triplicate along with bulb and basal plate samples and showed high linearity over the range of galanthamine molarities with correlation coefficient ($R^2$) of 0.914 to 0.954. A representative calibration curve showing a typical linear regression equation for standards is shown in Fig. 2, where $x$ represents galanthamine molarities and $y$ is the total Gal peak area (TIC). The galanthamine molarities were then converted to galanthamine content in Gal % FW (fresh weight) following the equation below:

$$\text{Gal \% FW} = \frac{287.354 \times \text{Gal molarities} \times 100}{100\ (\text{sample})}$$

where, Gal molecular weight is 287.354 g/mol, Gal molarities is obtained from calibration curve analysis, and the weight of fresh sample used for the analysis is 100 mg.

The amount in Gal % FW was then converted into µg Gal/g (FW). The amount of galanthamine was

quantified on fresh weight (FW) basis because about 60% galanthamine might be lost during the drying process and drying process incurs additional costs in industry (personal communication with Agroceutical Products Ltd. Powys, UK, 2020).

## Results and Discussion

The calibration curve (Fig. 2) model was used for the determination of galanthamine content in all the tissues under study. A similar calibration was performed plotting the ratio of galanthamine peak areas standardized with internal standard codeine and the regression line was constructed and tested on slope and intercept. The calibration model was suitable to quantify the galanthamine content in *Narcissus* samples ranging from 0.03 to 1.59% of dry weight[26]. The amount of galanthamine was quantified in leaves and bulbs of 97 ornamental species of *Narcissus* by a calibration curve method using galanthamine standards (1-100 µg/mL) standardised with codeine[19].

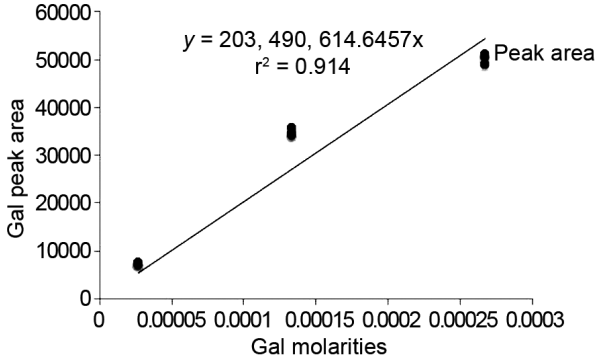


Fig. 2 — Representative calibration curve of galanthamine standards showing galanthamine molarities versus Gal peak area.

### Alkaloids in *N. pseudonarcissus* cv. Carlton

The amount of galanthamine was quantified using GC-MS where the non-dormant Carlton bulb was used as control and showed that the galanthamine content varied from 1018 to 1195 µg Gal/g FW. The dormant Carlton bulb tissue exhibited 813 to 887 µg Gal/g FW which was lower than the amount observed in control. However, basal plate tissue of Carlton dormant bulb represented a higher amount of galanthamine content ranging from 1220 to 1320 µg Gal/g FW than both dormant and non-dormant Carlton bulb tissues (Table 1).

Several studies have reported the amount of galanthamine in different ornamental varieties of *Narcissus* bulbs[13,19,26,28,33]; however, it was not mentioned whether the source of bulb samples included basal plate or not. In addition, the amount of galanthamine represented a wide range of 196 to 1358 µg Gal/g based on dry weight (DW) in different ornamental cultivars of *Narcissus* bulb tissues[19]. *N. pseudonarcissus* cv. Carlton is an eminent commercial source of galanthamine and several metabolomic approaches such as gas chromatography, liquid chromatography, nuclear magnetic resonance (NMR) were used for the determination of galanthamine in Carlton[19,21,27,33]. GC-MS based determination showed 1000 to 1300 µg/g DW galanthamine content in Carlton bulb[34], while 151-164 µg/g DW[26] and 891 µg/g DW galanthamine were also reported in Carlton bulb using GS-MS. On the

Table 1 — Amount of galanthamine in *N. pseudonarcissus* cv. Carlton and Andrew's Choice (values expressed as µg Gal/g FW±SD, n = 3 sample extracts)

| *N. pseudonarcissus* samples | Bulbs | µg Gal/g FW±SD | Mean±SD |
|---|---|---|---|
| Non-dormant bulb (Carlton) | Bulb 1 | 1018±80.16 | 1117±58.32 |
| | Bulb 2 | 1137±69.39 | |
| | Bulb 3 | 1195±25.41 | |
| Dormant bulb tissue (Carlton) | Bulb 1 | 880±86.95 | 860±75.72 |
| | Bulb 2 | 887±75.55 | |
| | Bulb 3 | 813±66.66 | |
| Dormant basal plate tissue (Carlton) | Bulb 1 | 1220±58.94 | 1254±50.69 |
| | Bulb 2 | 1320±53.02 | |
| | Bulb 3 | 1223±40.13 | |
| Dormant bulb tissue (Andrew's Choice) | Bulb 1 | 630±75.32 | 647±73.45 |
| | Bulb 2 | 577±82.58 | |
| | Bulb 3 | 733±62.45 | |
| Dormant basal plate tissue (Andrew's Choice) | Bulb 1 | 1179±80.32 | 1051±61.01 |
| | Bulb 2 | 942±52.91 | |
| | Bulb 3 | 1031±50.01 | |

SD = Standard deviation; FW = Fresh weight

other hand, NMR based metabolomic studies showed a galanthamine level in bulb tissues ranging from 2000 to 3000 µg/g DW in different plant growth stages from shoot initiation, flowering, to shoot senescence in Carton[27-28]. A previous study also reported the amount of galanthamine in *Narcissus* Carlton basal plate tissue ranging from 830 to 1250 µg/g FW using GC-MS[29]. Therefore, the accumulation of galanthamine varies widely in *Narcissus* pseudonarcissus cv. Carlton based on the use of fresh or dry sample for quantification, tissue type, plant growth stages, bulb harvesting time, quantification method etc.

Besides galanthamine, other notable Amaryllidaceae alkaloids, their derivatives and related compounds were identified from the GC-MS chromatograms of Carlton non-dormant bulb, Carlton and Andrew's Choice dormant bulb and basal plate using GC-MS data system library search using database software, the NIST mass spectral library. Lycoramine (galanthamine type), crinamine (haemanthamine type), lycorine and lycorenine (homolycorine type) were identified as the top matched compounds in the database from the GC-MS chromatogram of Carlton non-dormant bulb extracts along with galanthamine (Gal) and internal standard codeine (Cod) (Fig. 3). The top matched mass spectra obtained from Carlton dormant bulb extract were lycorenine (homolycorine type) and crinamine (haemanthamine type) (Fig. 4). Whereas Carlton dormant basal plate chromatogram showed one extra matched compound, pancracine (narciclasine and montanine type) along with lycorenine and crinamine as identified in bulb extract (Fig. 5).

In all Carlton samples, the highest peaks or abundance values were observed for galanthamine than any other identified alkaloids, from which it could be predicted that the highest value could relate to the high accumulation of galanthamine in Carlton bulb or basal plate than the other identified alkaloids (Fig. 3-5). Therefore, it can be assumed that the corresponding peaks could provide knowledge on the proportion or relative amount of the identified alkaloids. However, it would be worth to perform further quantification of the identified alkaloids using authenticated standards to observe their actual amount in Carlton bulb or basal plate.

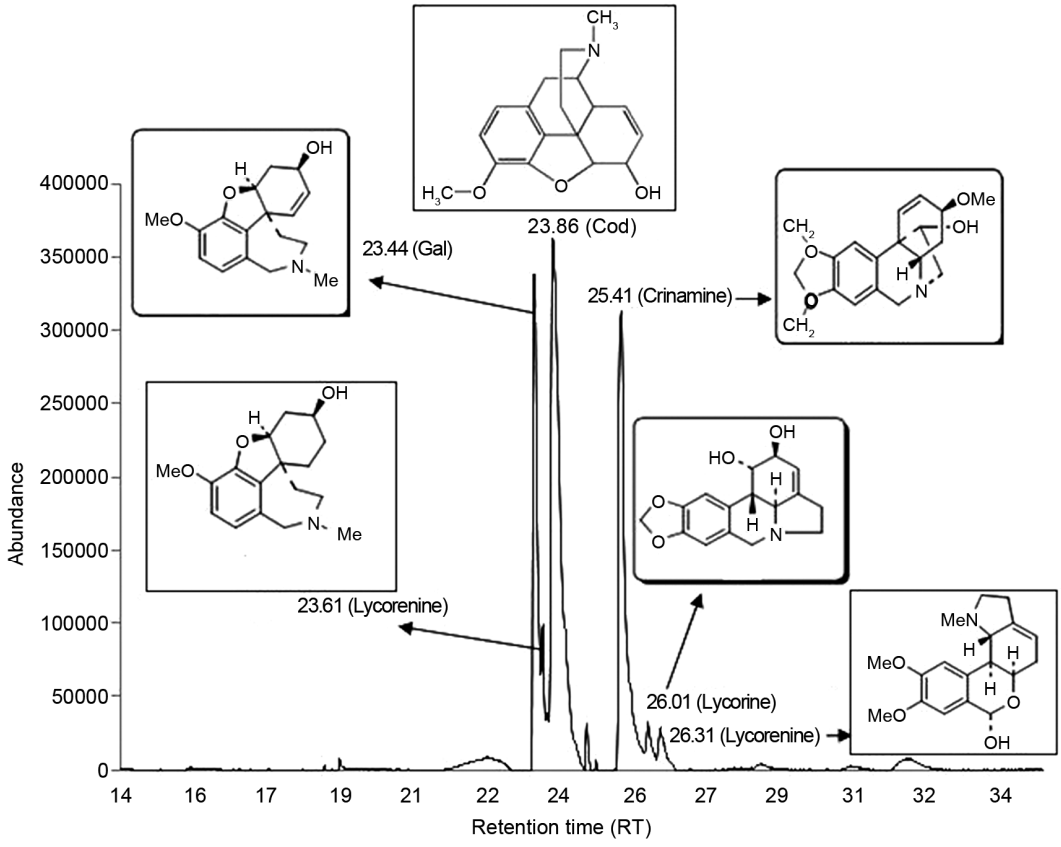


Fig. 3 — GC-MS chromatogram of alkaloid extract of Carlton non-dormant bulb tissues (control) showing all detected alkaloids with their representative chemical structures using column: BPX5; Gal = Galanthamine, Cod = Codeine.

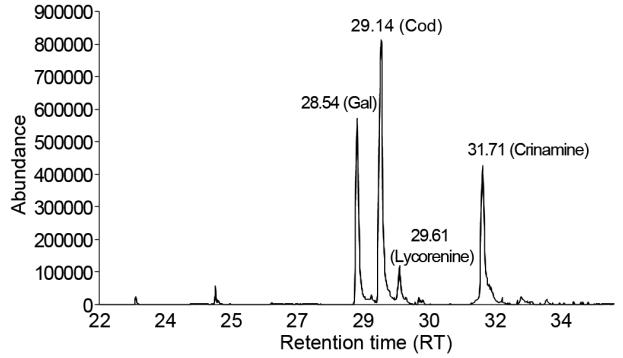


Fig. 4 — GC-MS chromatogram of alkaloid extract of Carlton dormant bulb showing all detected alkaloids using column: DB5M5; Gal = Galanthamine, Cod = Codeine.

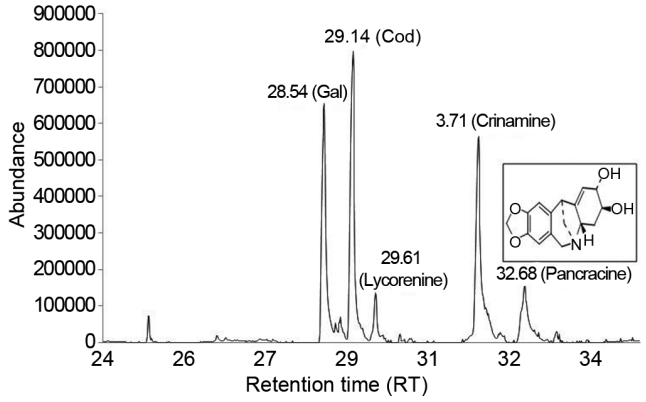


Fig. 5 — GC-MS chromatogram of alkaloid extract of Carlton dormant basal plate showing all detected alkaloids using column: DB5M5; Gal = Galanthamine, Cod = Codeine.

The alkaloids were identified by comparing the RT (Retention time), molecular fragmentation, and mass spectra with the information of reference data and previously reported data (Fig. 6 a-h). When such samples were not available, tentative structures were proposed based on the mass spectral fragmentation. Shawky *et al.*, also used similar method of alkaloid identification in Narcissus[33]. Galanthamine type (lycoramine) and homolycorine type (lycorine and lycorenine) alkaloids have been previously identified through GC-MS in *N. pseudonarcissus* cv. Carlton bulb extracts[26-27]. Lycoramine (MW 289) and lycorine (MW 287) were detected in Carlton bulb tissues at 21.87 RT and 23.10 RT; respectively using GC-MS spectra analysis in NIST database[26], which was detected at a slightly higher RT of 23.61 and 26.01 for lycoramine and lycorine in the present study (Fig. 3). The lower retention time observed could be due to the use of different column (DB-5 MS column) for sample separation by Berkov *et al.*[26]. They also reported the similar GC-MS fragmentation pattern of Lycorine as observed in this study at MW 227, 250, 266 (Fig. 6b). The GC-MS ion fragmentation patterns for lycoramine and lycorenine in *Narcissus* Carlton bulb[27] also represented the similar fragmentation patterns (Fig. 6a,c) observed in this study. The fragmentation pattern of suggested alkaloid lycorenine (MW 300) with the prominent fragments of homolycorine (MW 108 and 109) was detected (Fig. 6c). Hanks and Bastida *et al.*, have previously reported similar mass fragments of homolycorine[7,11]. Crinamine (MW 301), detected in Carlton dormant bulb and basal plate was observed as the most abundant alkaloids along with lycorine and lycorenine in *Narcissus* bulb[35] which showed anti-tumour and moderate antimalarial activity[9,11,36]. A similar GC-MS fragmentation pattern as observed for crinamine in this study (Fig. 6d) was also reported for a similar compound, crinamine acetate (MW 343) isolated from an Amaryllidaceae species *Crinum jagus* L.[37]. Another biologically important Amaryllidaceae alkaloid, pancracine (MW 278), reported for its anticancer and antibacterial activity which was detected only in Carlton dormant basal plate (Fig. 6e) was previously isolated from *Narcissus* cv. Professor Einstein[11,14]. Pancracine was also detected in several *Narcissus* species; however, it was absent in the alkaloid profile of *N. pseudonarcissus*[13].

**Alkaloids in *N. pseudonarcissus* cv. Andrew's Choice**

The quantification of galanthamine was also performed using GC-MS in Andrew's Choice dormant bulb and basal plate tissues. Andrew's Choice dormant bulb extract showed 577 to 733 µg Gal/g FW with a mean value of 647 µg Gal/g FW; which was the lowest amount among the all studied samples (Table 1). However, the galanthamine content was higher in Andrew's Choice dormant basal plate ranging from 942 to 1179 µg Gal/g FW than Carlton dormant bulb (860 µg Gal/g FW) but lower than the galanthamine content observed in Carlton non-dormant bulb (1117 µg Gal/g FW) and Carlton dormant basal plate (1254 µg Gal/g FW) (Table 1). Several previous research showed a wide range of galanthamine content in different *Narcissus* cultivars which varied from 100 to 4000 µg/g DW[13,14,19], however, limited research has been executed on *Narcissus* Andrew's Choice, a recently developed cultivar through hybridization[30]. The reported galanthamine content in Andrew's Choice bulb showed a range of 100 to 600 µg/g FW[30], which is in accordance with our findings of bulb galanthamine content. Nevertheless, Andrew's Choice basal plate tissue was

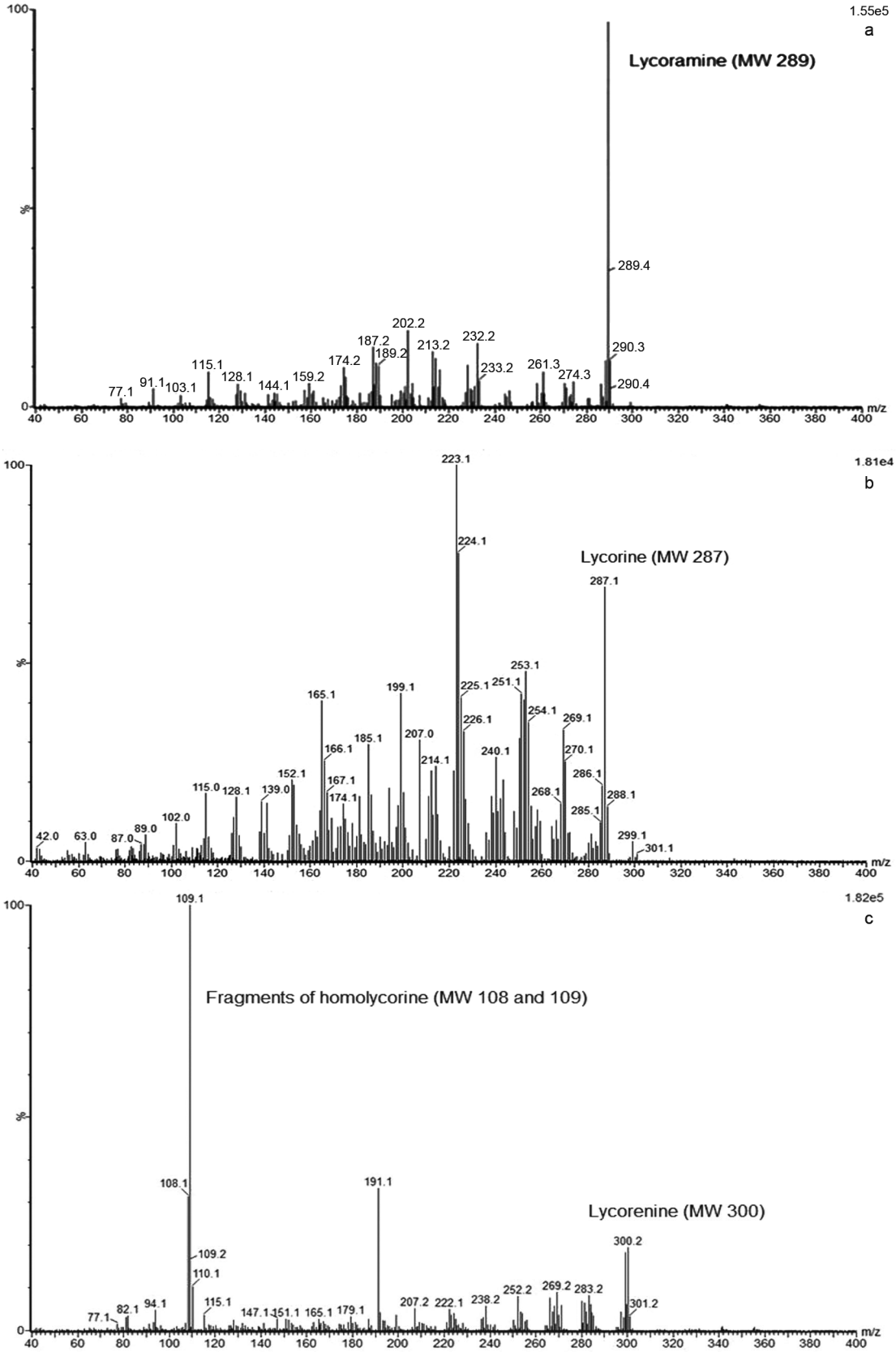
1.55e5
a
Lycoramine (MW 289)
289.4
202.2
187.2
213.2
232.2
189.2
290.3
174.2
233.2
261.3
115.1
128.1
159.2
274.3
144.1
290.4
77.1
91.1
103.1
m/z
1.81e4
b
Lycorine (MW 287)
223.1
224.1
287.1
253.1
251.1
225.1
165.1
199.1
254.1
269.1
226.1
185.1
207.0
166.1
270.1
240.1
214.1
152.1
286.1
167.1
115.0
128.1
139.0
174.1
268.1
288.1
102.0
285.1
89.0
42.0
63.0
87.0
299.1
301.1
1.82e5
c
109.1
Fragments of homolycorine (MW 108 and 109)
108.1
191.1
Lycorenine (MW 300)
300.2
109.2
110.1
269.2
252.2
283.2
115.1
238.2
301.2
94.1
82.1
77.1
147.1
151.1
165.1
179.1
207.2
222.1

Fig. 6 *Contd*.

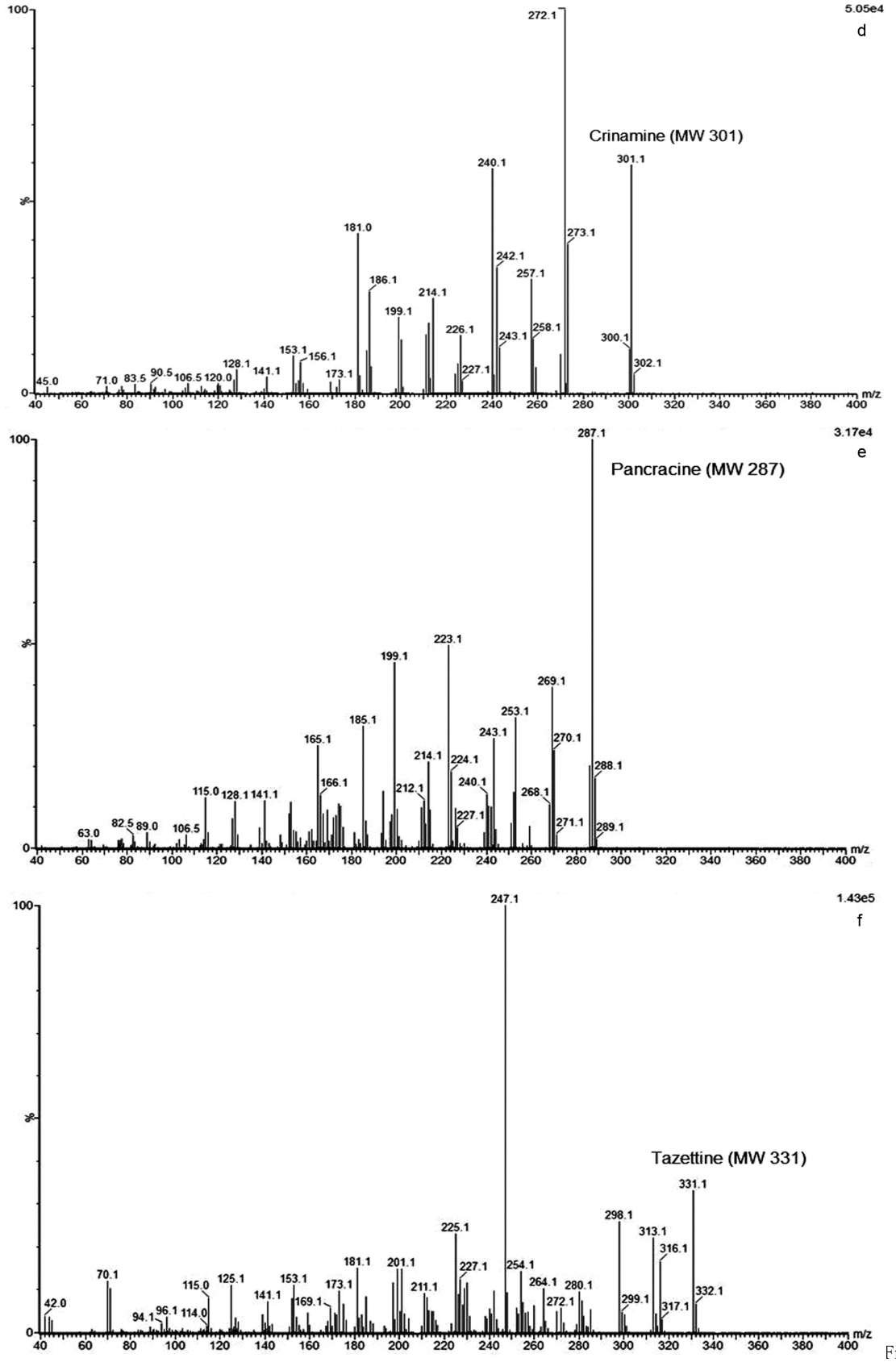
5.05e4
d
Crinamine (MW 301)
272.1
301.1
240.1
273.1
181.0
242.1
257.1
186.1
214.1
199.1
226.1
243.1
258.1
153.1
156.1
128.1
141.1
173.1
227.1
300.1
302.1
45.0
71.0
83.5
90.5
106.5
120.0
3.17e4
e
Pancracine (MW 287)
287.1
223.1
199.1
269.1
253.1
185.1
165.1
243.1
270.1
214.1
224.1
288.1
166.1
240.1
212.1
268.1
115.0
128.1
141.1
227.1
271.1
289.1
63.0
82.5
89.0
106.5
1.43e5
f
Tazettine (MW 331)
247.1
331.1
298.1
225.1
313.1
316.1
181.1
201.1
227.1
254.1
70.1
125.1
153.1
264.1
280.1
115.0
173.1
211.1
332.1
141.1
272.1
42.0
169.1
299.1
94.1
96.1
114.0
317.1
%
m/z

Fig. 6 (*Contd*.)

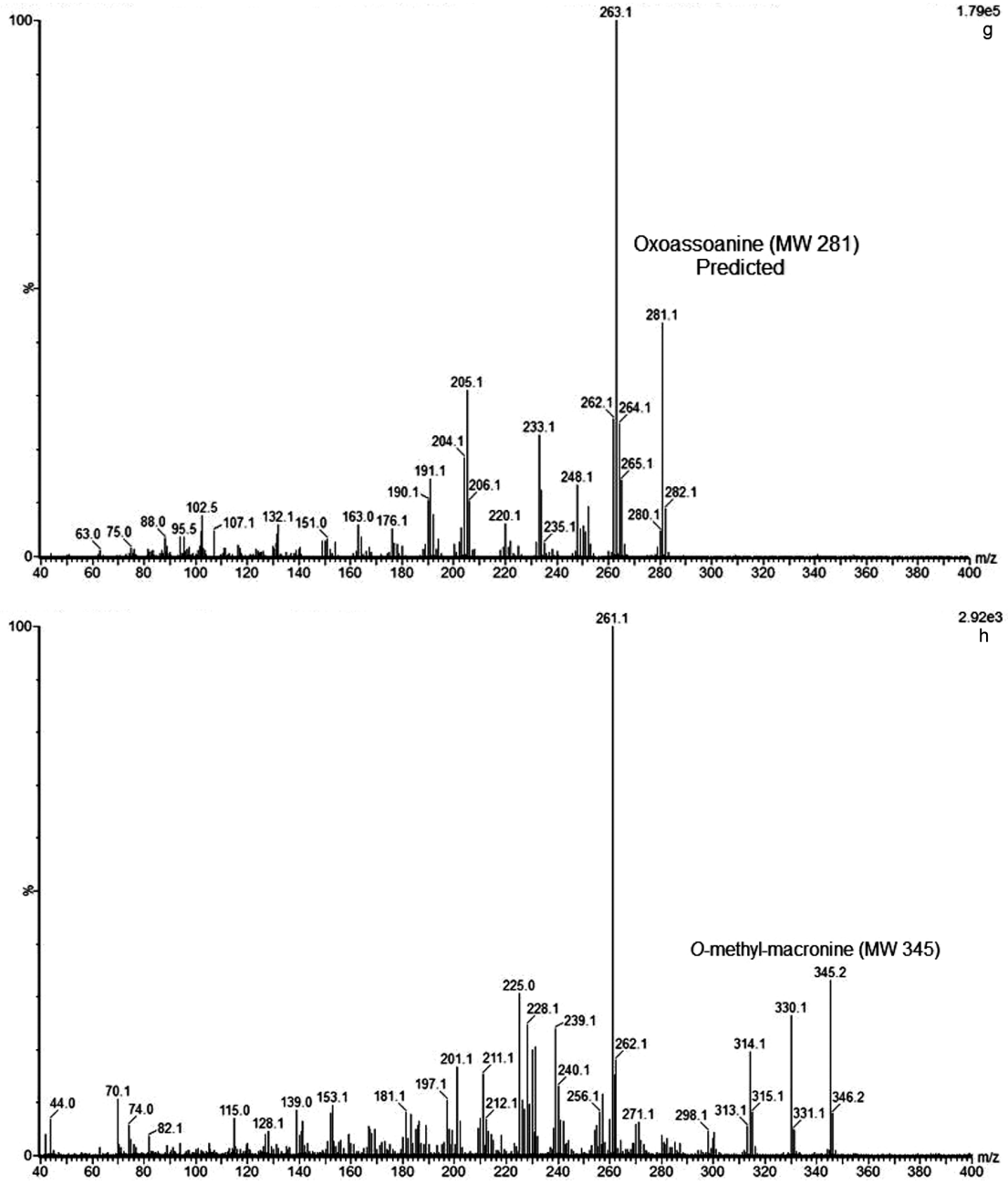


Fig. 6 — GC-MS mass fragmentation patterns of suggested alkaloids found in Carlton and Andrew's Choice bulb and basal plate tissues based on their corresponding molecular weights, a) Lycoramine (MW 289), b) Lycorine (MW 287), c) Lycorenine (MW 300), d) Crinamine (MW 301), e) Pancracine (MW 287), f) Tazettine (331), g) Oxoassoanine (MW 281) and, h) *O*-methyl-macronine (MW 345).

not used previously for galanthamine isolation, which showed relatively higher galanthamine content (1051 µg/g FW) in this study and the amount is in accordance with the galanthamine content of many other ornamental species of *Narcissus*[13,19]. Hence, besides Carlton and other *Narcissus* ornamental cultivars, Andrew's Choice also could be used as a potential source of galanthamine isolation.

The GC-MS fragmentation patterns showed the presence of slightly different compounds in Andrew's Choice than Carlton chromatograms such as tazettine, oxoassoanine, and *O*-methyl-macronine (Fig. 6f-h). The alkaloids identified in Andrew's Choice dormant bulb were tazettine, oxoassoanine, and lycorine (Fig. 7). The Andrew's Choice dormant basal plate showed the occurrence of same compounds such as

oxoassoanine, tazettine and lycorine as identified in the bulb extract with an additional suggested compound, *o*-methyl-macronine (MW 345) (Fig. 8).

Tazettine was previously isolated and detected based on mass spectra and retention time in a large collection of *Narcissus* cultivars using GC-MS[14]. Although the biological activity of tazettine is not noticeable, it can be chemically transformed into plant labile precursor pretazettine, which displays cytotoxicity against fibroblastic cell lines, inhibits cell growth and therapeutically effective against several types of leukaemia[14]. The GC-MS mass spectra of the related compound, pretazettine having same molecular weight (331) and similar fragmentations like tazettine (Fig. 6f) was reported in Carlton bulb[20]. The compound assigned in 24.29 RT (Fig. 7) did not match with any compound in NIST mass spectral library database search. Yet oxoassoanine, a lycorine type alkaloid with same molecular weight (281) and similar fragmentation pattern (Fig. 6g) observed in this study was previously

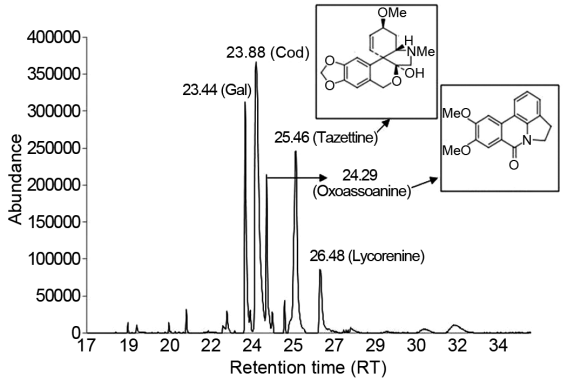


Fig. 7 — GC-MS chromatogram of alkaloid extract of Andrew's Choice bulb showing all detected alkaloids using column: BPX5; Gal = Galanthamine, Cod = Codeine.

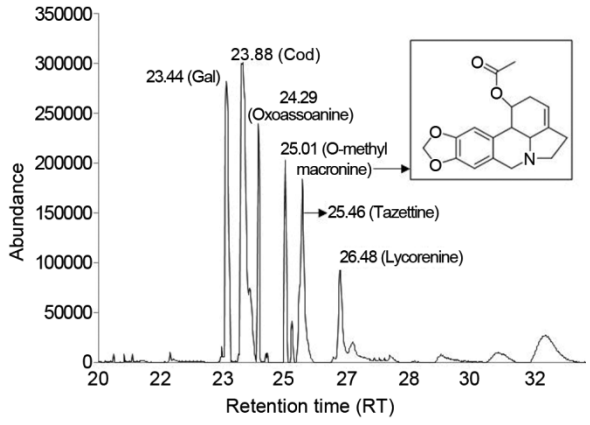


Fig. 8 — GC-MS chromatogram of alkaloid extract of Andrew's Choice basal plate tissues showing all detected alkaloids using column: BPX5; Gal = Galanthamine, Cod = Codeine.

detected and isolated from *N. assoanus* and was detected in several ornamental varieties of *Narcissus*[38,39]. Hence, the compound at 24.29 RT with 281 MW was predicted as oxoassoanine. The compound *O*-methyl-macronine (MW 345), a tazettine type alkaloid, detected in Andrew's Choice basal plate was previously detected in Amaryllidaceae species *Crinum jagus*[37]and showed the same fragmentation pattern (Fig. 6h) as observed in this study. *O*-methyl-macronine having the same molecular weight has also been isolated from *N. confusus* bulbs and leaves[20] and a similar molecule, macronine was reported as a tazettine type alkaloid in a well reported Amaryllidaceae species, *Leucojum aestivum*[12].

The alternative ways of oxidative phenol coupling i. e. *ortho-para'*, *para-para'* and *para-ortho'* of *o*-methylnorbelladine produce a vast range of alkaloids in Amaryllidaceae that differs between species, cultivars and even between tissues[36]. In the present study, lycorine (oxoassoanine) and homolycorine type (lycorenine) alkaloids; haemanthamine (crinamine), tazettine (*o*-methyl-macronine) and narciclasine and montanine type (pancracine) alkaloids; and galanthamine type (lycoramine) alkaloids were derived through *ortho-para'*, *para-para'*; and *para-ortho'* phenol oxidative coupling, respectively, which suggests that all the biosynthetic routes might be active within the studied tissues (Fig. 9).

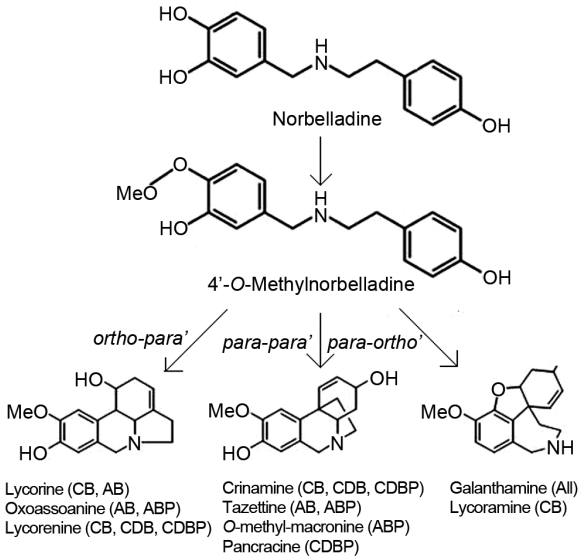


Fig. 9 — Proposed Amaryllidaceae alkaloid biosynthesis pathway relating the alkaloids detected in *N. pseudonarcissus* cv. Carlton bulb (CB), dormant bulb (CDB) and dormant basal plate (CDBP); and Andrew's Choice dormant bulb (AB) and basal plate (ABP); All = CB, CDB, CDBP, AB and ABP (Postulated from Takos and Rook, 2013 and Ferdausi, 2017).

The present study explained a suitable method for the quantification of considerable amount of galanthamine in different bulb tissue types such as bulb (including bulb scales) and basal plate (the bottom, condensed part of bulb)[29] in two *Narcissus* cultivar's bulbs harvested under dormant condition. The higher amount of galanthamine was quantified in basal plate which could relate to the most condensed storage portion of bulb or due to the presence of meristematic tissue for root initiation near the basal plate making basal plate more metabolically active[29]. Although the quantification of other detected alkaloids was not possible due to the lack of authentic standards, and the provided data do not express a real quantification, the abundance of alkaloids form the GC-MS spectra could be compared with the galanthamine spectra, which would provide the knowledge on the relative amount of the detected alkaloids[33].

## Conclusion

The results showed clear differences in galanthamine content in two different commercially available *N. pseudonarcissus* cv. Carlton and Andrew's Choice in different tissue types i.e. bulb and basal plate. This confirmed along with Carlton and other reported Amaryllidaceae species Andrew's Choice could be a promising natural source of galanthamine isolation. Further, several other Amaryllidaceae alkaloids were detected based on mass fragmentation patterns, which were also differentially found in cultivars and tissues. Yet confirmation of their identity and quantification is required using authentic standards. Future research on the confirmation of the biologically active alkaloids detected in the present study would provide new materials as a source of these alkaloids, which could potentially contribute to the pharmaceutical industry.

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## Conflict of interest

The authors declare that there are no conflicts of interest.

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