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Isolation and Characterisation of Some Amaryllidaceae Alkaloids from Daffodils Extract Using GCMS Techniques

Ogbu Magnus Onyemaechi
Senior Lecturer, Department of Chemistry Education,
Federal College of Education (Technical) Omoku, Nigeria
Dr. Baba Fugu Mohammed
Senior Lecturer, Department of Pure and Applied Chemistry,
University of Maiduguri, Nigeria
Nnam-Obi Chukudi Victor
Lecturer, Department of Chemistry Education,
Federal College of Education (Technical) Omoku, Nigeria
Ndukwu Didacus Emeka
Principal Lecturer, Department of Chemistry Education,
Federal College of Education (Technical) Omoku, Nigeria
Ali Muhammad Fulata
Lecturer, Department of Remedial Sciences,
Ramat Polytechnic Maiduguri, Nigeria

Abstracts:
Haemanthamine an amaryllidaceae alkaloid has growth inhibitory activities against tumour cells such as Rauscher viral leukaemia. It has also been reported to possessed many interesting biological properties including protein synthesis inhibition, anti-parasitic, anti-retroviral activity and cytotoxicity agent against various cancer cells, anti-inflammatory activities and analgesic property. Daffodil is one of the specie in the family Amaryllidaceae, it is commonly called Narcissus in English. In recent years, a lot of technological advances have been deployed in the quest for the discovery of natural products. Most of which majorly favours constituents present in high concentrations and negating those in low concentrations. Thus, this research employed GC-MS techniques being among the most reliable and efficient analytical tools for the evaluation and characterisation of haemanthamine and other minor alkaloids. The results revealed that a total of 12 alkaloids tentatively isolated and characterised by GC-MS.

Keywords: *Amaryllidaceae, Daffodils, alkaloids, GC-ms, chromatography*

1. Introduction

Amaryllidaceae alkaloids are isoquinoline alkaloids exclusive to *Amaryllidaceae* plant family (Nehir, 2007). They exhibit a range of pharmacological activities to mention but few; antibacterial, anti-malarial, anti-tumour, antiviral, acetylcholinesterase inhibitory and cytotoxic activities (Cahlíková *et al.,* 2019). It was estimated that about 12,000 different alkaloids are distributed in the plant kingdom and a significant number have been identified and pharmacologically proven to be active (Takos and Rook, 2013). Some of the alkaloids successfully isolated and characterised from the family include among others; homolycorine, crinine, tazettine, lycorine, galanthamine, narciclassine, haemanthamine (Saheed and Jamiu, 2019). Consequently, the biological relevance of these alkaloids continues get greater attention, hence, the trend in the discovery, isolation, as well as the quantification of alkaloids from different plant families (Nehir, 2007).

Daffodil is a species in the family *Amaryllidaceae*, it is commonly called *Narcissus* in English. It's simply described as a central trumpet, disc-shaped corona surrounded by a ring of six floral leaves united into a tube at the forward edge of the 3- ocular ovary (Gilman, 1999). It is cultivated from bulbs in the fall before the first frost, usually when ground temperature reaches 15-18^{o}C. The plant grows excellently in a well-drained acidic soil. The flower of daffodil consists of petal and a cup-like corona. The stem is usually 16 inches in length and light weight (Gilman, 1999). Traditionally, it's used in the treatment of urinary disease, headache, fever, swelling growth, joint ailments, skin diseases, bruises, sprains, respiratory problems, gastrointestinal disorders and as internal purifier (Nair *et al.,* 2017). Despite its wide medicinal application and numerous traditional functions only a few of its constituents have been isolated, identified, elucidated and studied. In recent years, a lot of technological advances have recently deployed in the quest of the discovery of natural

products. Classical extraction techniques have been developed and structural elucidation methods but yet only a few successes have been recorded for most of the high concentration constituents negating those in low concentrations. Therefore, GC-MS being among the most reliable and efficient analytical tools was used for the evaluation and analysis of haemanthamine and other minor alkaloids.

2. Material and Methods

2.1. Chemicals and Sample Collection

All chemicals and reagents used in this investigation are certified analytical grade. While the isolation and characterisation investigation were carried out with a quantifiable amount of commercially produced daffodil extract and the procedure is briefly described below.

2.2. Isolation of Haemanthamine

About 50 g of the dark crude extract was weighed into a beaker and 500 ml of water added, an aqueous solution of NaOH (2 M) was added until pH = 11 (UI paper) whereupon a black semi-solid precipitate forms. Chloroform (100 mL) was added to the solution together with celite© filter aid (100 g) and after mixing for 5-10 mins, the mixture was filtered through a pad of celite© on a Buchner funnel as reported by Bastida *et al* (1989). Further small portions of chloroform (ca. 200 mL) were used to wash the filter pad, following which the filtrate was separated and the aqueous phase extracted with further chloroform (2 x 100 mL). The combined chloroform layers were dried in ($MgSO_4$), filtered and evaporated under reduced pressure and the yield obtained was analysed by GCMS. To the residue 280 ml Acetone was added and the mixture heated to reflux until the sample dissolved. On slow cooling to room temperature and then cooling in ice (with stirring) an off-white precipitate formed which was removed by filtration. The yield obtained and the mother liquor were recovered and analysed by GCMS. This process was repeated on a scale of 20 g and 200 g respectively.

2.3. Thin layer Chromatography and GC-MS Analysis

The combined mother liquor residues were dissolved in chloroform and silica gel (100 g) was added and the mixture evaporated to dryness. This was then loaded onto a pre-flushed (chloroform) column of silica gel (100 g) in chloroform and flushed sequentially with 0-4 % MeOH in chloroform (300 mL) increasing the polarity by 0.5 % in each flush collecting 50 mL fractions (boiling tubes). The fractions were analysed by TLC (1% MeOH in chloroform) in order to identify wide range of compounds and was visualized using reactive spray reagents and the figure of the plate TLC. The spots with appearance and R*f* values are pooled and analysed using GC-MS techniques.

3. Results and Discussion

3.1. GC-MS Analysis Result

The result of the GC-MS analysis was based on the computer evaluation of mass spectra of samples through NIST based AMIDS v 2.69 automated mass spectra deconvolution and identification software as well as direct comparison of peaks and retention time with those in literature. The general fragmentation pattern of each type of alkaloid was used in the identification of a particular type of alkaloid. Analysis of the crude extract from the plant by GC-MS (Figure 1) indicated the presence of a variety of alkaloids which are listed in Table 1 along with their retention time (RT) and percentage composition.

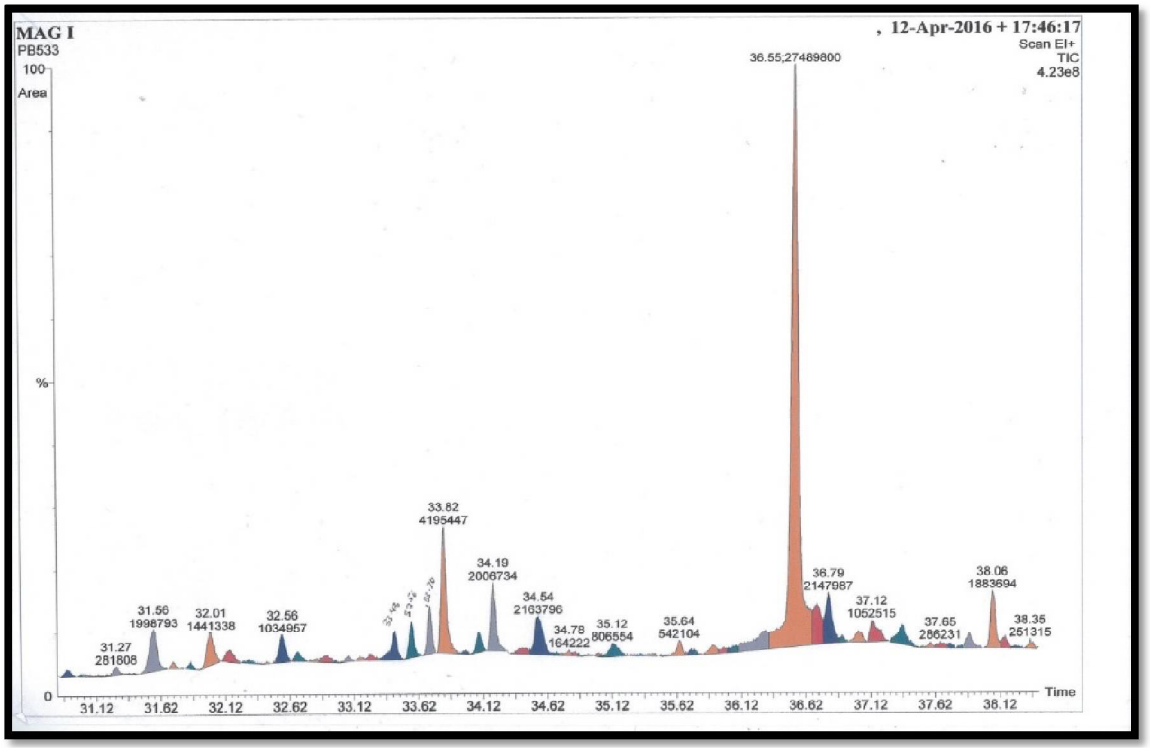

Figure 1: GC Spectra of the Isolated Compounds in the Sample

Peak	RT	Peak area	%	Compound
1	32.01	1441338	3.1	Unknown
2	33.43	1188909	2.6	Unknown
3	33.56	1147754	2.5	Galanthamine
4	33.7	1273447	2.8	Unknown
5	33.82	4195447	9.1	Lycoramine
6	34.19	2006734	4.7	Epigalanthamine
7	34.54	2163796	4.7	Unknown
8	36.55	27489800	59.8	Haemanthamine
9	36.79	2147987	4.7	Unknown
10	37.12	1052515	2.3	Galanthine
11	38.06	1883694	4.0	Nerinine

Table 1: Shows the GC Analysis of Isolated Compounds from the Sample

The result of the recrystallized the mother liquors of these fractions by GCMS indicated a significant separation with the following percentage proportions of compounds as shown in Table 2.

Sample (g)	1	2	3	4	5	6	7	8	9	10	11	12
Original (69.73)	4.7	2.5		59.8		4	2.1	9.1				
Fraction A (26.70)	8.5	6.5	15.5	0.7			7.5	6.9	20.0		2.9	
Fraction B (17.87)	4.4	10.3	5.6	7.5	6.7		6.6	34.6	2.8	4.6	5.1	
Fraction C (18.78)		27.2	1.5	46.9				8.0	0.7	6.0	0.5	7.5
Fraction D (1.43)		84.7		2.7				2.8		9.7		

Table 2: Shows the % Proportion of Compounds in the Recrystallized Mother Liquors
Key: 1 = Epigalanthamine, 2 = Galanthamine, 3 = Pluvine, 4 = Haemanthamine, 5 = Tazettine, 6 = Nerinine, 7 = Galanthine, 8 = Lycoramine, 9 = Homolycorine, 10 = Norpluvine, 11 = Galanthaminone, 12= Hippeatrine

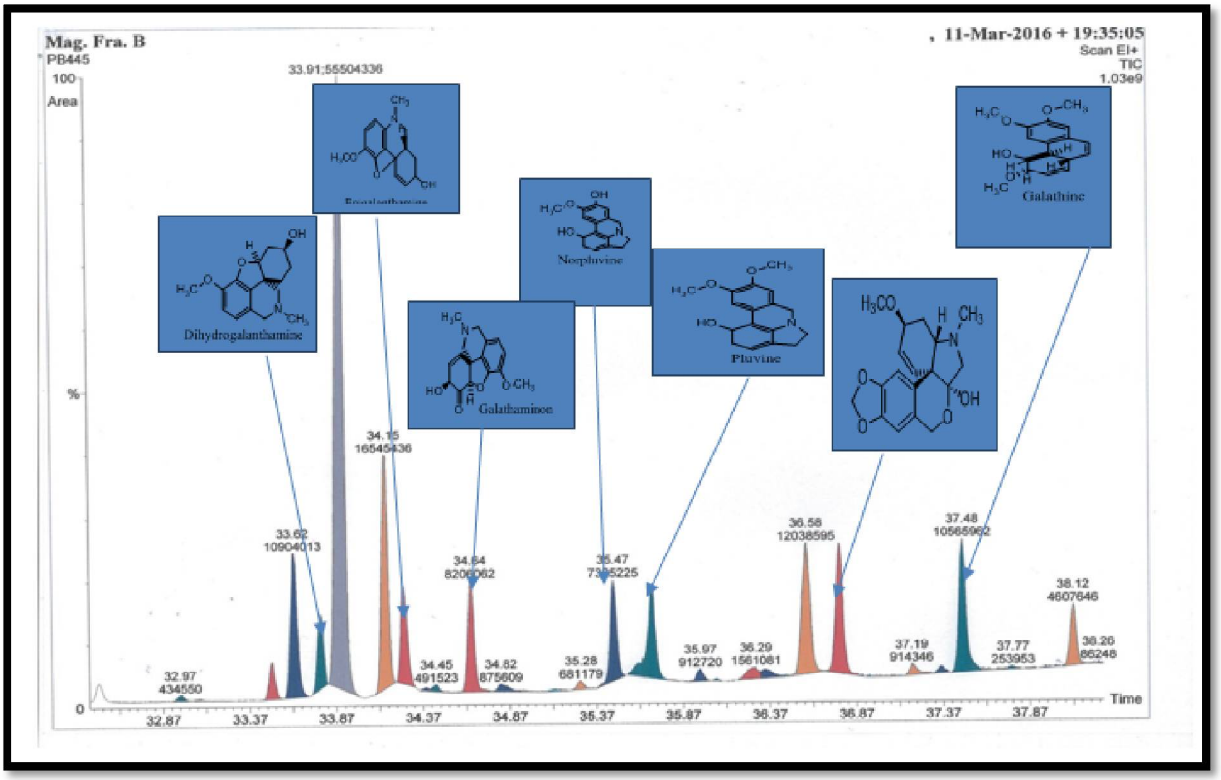

Figure 2: The GC Spectra Showing the Minor Alkaloids in Daffodils Extract

4. Discussion

Table 1 is a presentation of compounds isolated from the extract. Haemanthamine accounts for an approximately 60 % of the bulk alkaloids. The next major alkaloid is lycoramine (also known as dihydrogalanthamine) in approximately 10%. Several other alkaloids are present in in an amount ranging from 2-5% respectively. Other minor peaks in the GC spectrum correlate to unknown alkaloids and also to known silicone impurities which are common in GC technique and arise from the instrument and materials used in sample preparation. The GC-MS analysis of underivatized sample of the main extract in this study shows the presence of five skeletal type alkaloids namely: the galanthamine type (galanthamine, norpluvine), homolycorine type (homolycorine, hippeastine, and masonine); haemanthamine type (haemanthamine); tazettine type (tazettine). A total of 17 amaryllidaceae alkaloids were identified and the alkaloids were in line to those identified by Khalifa *et al.,* (2018) and Kreh et al (1994).

Furthermore, a new alkaloid was observed, thats masonine and which classically belongs to homolycorine type alkaloid. Similarly, Goti et al (2000) and Lubbe et al (2013) reported galanthamine as the most abundant alkaloid in the bulb of *N. pseudonarcissus* followed by haemanthamine and then lycoramine. This result is contrary to their findings because of the fact that galanthamine was carefully isolated from the sample prior to its used in the present study, while all other alkaloids are in agreement with previous literatures.

Moreover, GC-MS was effectively used in the analysis of N. jonquilla and the results revealed about 5% of N-dimethyl galanthamine and 7% dimethyl lycoramine alkaloids (Gotti et al 2006). Same technique was deployed in the phytochemical differentiation of *Amaryllidaceae* alkaloids in N. *Broussonetii,* revealing the presence of lycorine, tazettine, homolycorine and less 8-O-demethylhomolycorine and galanthindole (Andrade *et al.,* 2012). Thus, the selection of GCMS for the quantification of some alkaloid was scientifically backed by earlier researchers.

5. Conclusion

In conclusion, a total of 12 alkaloids were isolated as *Amaryllidaceae* alkaloids by GC-MS and haemanthamine was discovered to the most abundant with a concentration (up to 60%).

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