

Original Research Article

Isolation and Characterisation of Haemanthamine Alkaloid from Daffodil Extract using NMR and Mass Spectroscopic Techniques

ABSTRACTS

The recent surge in drug resistance and failure is belief to be a factor in the trend for the isolation of natural products from plants and other sources. This is largely due to the efficacy and safety of their components. Haemanthamine is an alkaloid of *Amaryllidaceae* family, the compound was reported to have lots of medicinal effects against microbes and some forms of cancers with significantly low or no side effects. The plant Daffodil is one of the commonest reservoir of this alkaloid and used traditionally in the treatment of urinary disease, headache, fever, swelling growth, joint ailments, skin diseases, bruises, sprains, respiratory problems, gastrointestinal disorders among others. These medicinal potential of the plant stimulated our interest to isolate and characterized the Haemanthamine alkaloids from the plant using NMR and Mass Spectroscopic Techniques. The results obtained are in good agreement with the previously reported literatures which indicate that Haemanthamine was successfully isolated.

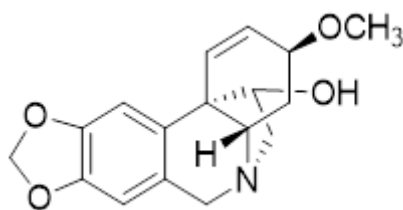
Key words: Haemanthamine, Daffodil, Alkaloids, NMR, Mass Spectroscopy

Introduction

The recent inclinations toward natural products are due to their perceived potency, affordability, accessibility and ability to elicit biological actions with less toxicity (Naiho et al., 2015). Available data revealed that plants are bio-reservoirs of variety of natural products capable of treating different ailments. Such bioactive principles inherent in plants are flavonoids, phenols, phenolic glycosides, alkaloid and cyanogenic glycoside among others.

Accordingly, the World Health Organization (WHO) estimates that 80% of the populations in Asian and Africa depend solely on herbal medicine and about 25% of world's pharmaceutical products are sourced from phytochemicals (Golam-Rasul, 2018). These drugs are either directly extracted from plants or plants are used as precursors for their synthesis (Veeresham, 2012). For instance, Fulata et al., (2019) and Usman et al (2020) attributed the medicinal potentials of *Laggera aurita* Linn and *Ficus polita* to flavonoids, terpenoids, alkaloids and likewise many other recent scholars. Thus, it is worthy of note that medicinal plants have and will continue to play a crucial role in the discovery and treatment of many tropical and genetic disorders in both developed and developing countries.

Daffodil is a species in the family *Amaryllidaceae*, it is commonly called *Narcissus* in English. It is 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°C. The plant grows excellently in a well-drained acidic soil. The flower of daffodil consists of petals 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 an 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. Thus, this study aimed at the isolation and characterisation of important alkaloids (haemanthamine) from the plant using NMR and mass spectroscopic technique.



Scheme 1: Structure of haemanthamine

Material and Methods

Chemicals and Sample Collection

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

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 portion of chloroform (ca. 200 mL) was used to wash the filter pad, following which the filtrate was separated and the aqueous phase was further extracted with chloroform (2 x 100 mL). The chloroform layers were dried in (MgSO₄), filtered and evaporated under reduced pressure and the yield obtained was analysed. 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 (Breiterová *et al.*, 2018). The yield was subjected to column chromatography for further purification and isolation as described below.

Chromatography

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. Thus, the column fractions are pooled on the basis of their appearance and R_f values as follows; Fractions 1-3 (A), 4-5 (B), 6-8 (C), 10-55 (D) respectively. It was evident that fraction C was mainly haemanthamine, hence, re-crystallized to give a pure isolate.

Results and Discussion

^1H NMR Analysis of Haemanthamine: The recrystallized haemanthamine from each fraction was compared with a standard and the work of Bastida *et al* (1989). The NMR of the standard sample is shown in figure 1. Whilst our sample is shown below in Figure 2. The correlation between these spectra (Figure 3) is very significant, the only minor differences are observed in the position of the hydroxyl proton which tends to be variable depending on the concentration of the sample and the presence of varying amounts of trace moisture and acid in the NMR solvent (CDCl_3).

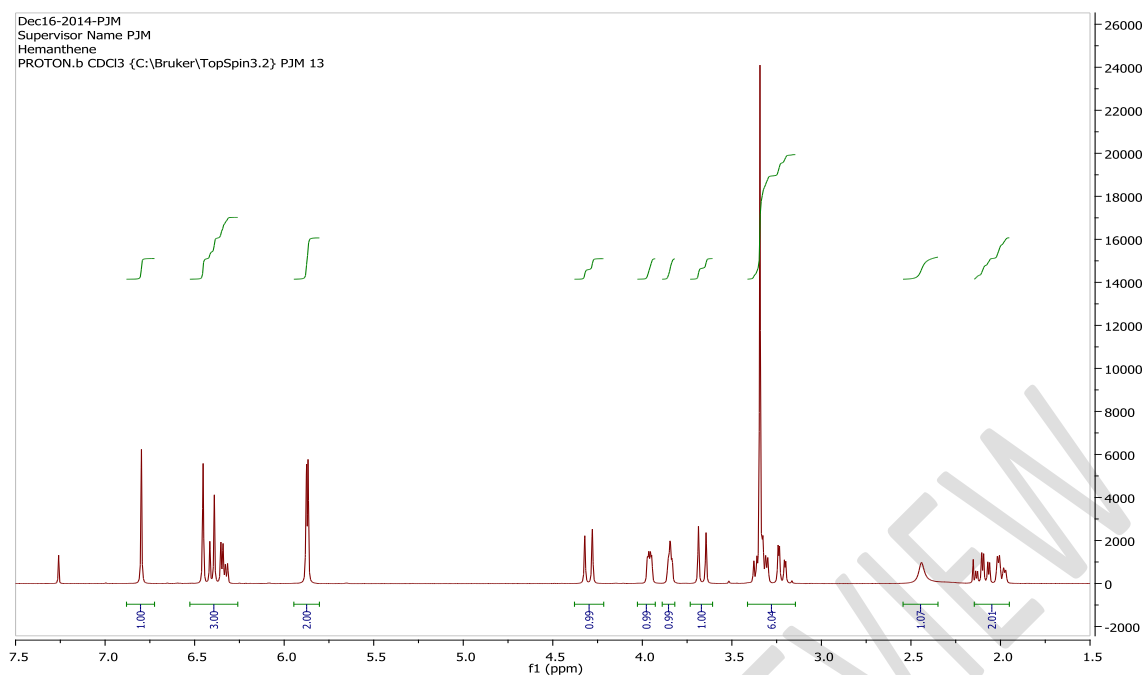


Figure 1: NMR Spectra of the Standard Haemanthamine

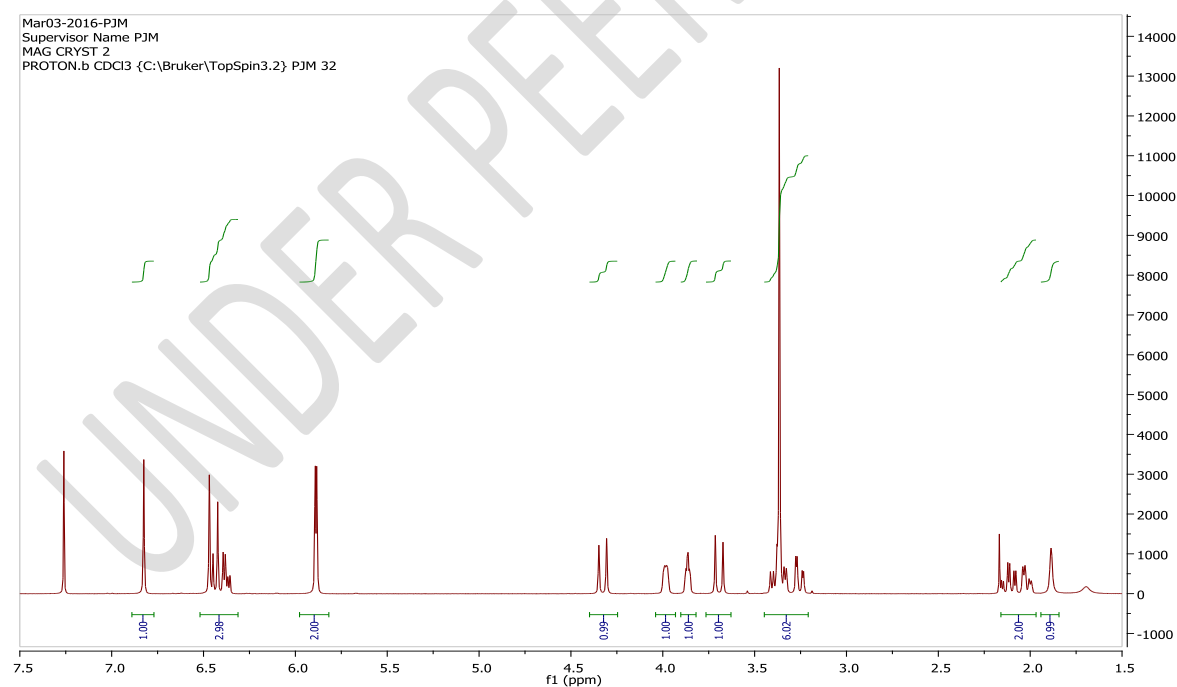


Figure 2: HNMR of Haemanthamine of Isolated Alkaloids

Comparison of a Commercial Haemanthamine with Isolated Haemanthamine

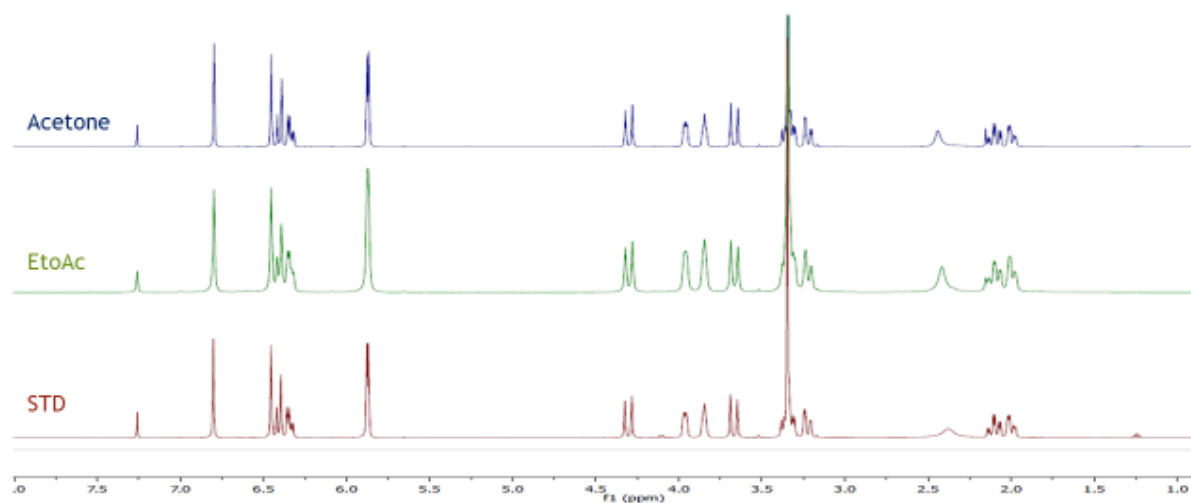


Figure 3: Comparison of a Commercial Haemanthamine with Recrystallized Using Different Solvents (Acetone and Ethyl-acetate)

The NMR data of the isolated compound is in line with the spectral data of haemanthamine earlier isolated and characterised by Bessa et al., (2017) and Bastida et al., (1989). The spectral data of the isolated compound shows significant similarity in the number of proton and splitting pattern with the standard as well as the literatures values of Bessa et al., (2017) and Bastida et al., (1989). The only notable difference in the spectra is the position of hydroxyl proton which could be due to solvent effect as presented in Table1 and figure 3 respectively.

Furthermore, the vital signals for the characterisation of haemanthamine as obtained from the ¹H NMR spectrum are as follows: a singlet at δ 3.37 corresponding to methoxy group and a doublet at δ 5.9 corresponds to the methylenedioxy group; singlets at δ 6.47 and 6.83 for aromatic protons H-7 and H-10 respectively. A singlet at δ 6.44 for H-1 and a double doublet at δ 6.38 for H-2, two doublets at δ 3.70 and 4.34 for C-6 protons. The NMR spectral data is in agreement with the work of Bessa et al., (2017) and Bastida et al (1987). Thus, the isolated alkaloid is confirmed to be haemanthamine.

Table 1: The ^1H NMR of the Isolated Haemanthamine, (400 MHz, CDCl_3)

Carbon No	δ (ppm)	Multiplicity	J (Hz)	Protons
1	6.44	d	10.10	1
2	6.38	dd	4.63, 10.08, 14.79	1
3	3.86	td	1.56, 4.51, 5.82, 8.64, 10.03	1
4	2.12, 2.03	td, dd	4.27, 13.68, 17.87, 26.97, 31.16 4.47, 13.90, 17.56	2
5	3.36	dd	6.93, 12.46, 18.57	1
6	3.70, 4.34	d, d	17.85, 15.85	2
7	/	/		0
8	6.83	s		1
9	/	/		0
10	/	/		0
11	6.47	s		1
12	/	/		0
13	3.27, 3.40	dd, dd	6.67, 14.11, 20.84 3.02, 14.01, 17.04	2
14	3.99 (OH not there)	q (may be tetra doublet)	2.44, 6.65, 8.06	1 (+1OH)
15	/	/		0
16	5.89	d (with shoulders)	2.72	2
17	3.37	s		3

Table 2: The $^{13}\text{C}\{^1\text{H}\}$ NMR of the isolated Haemanthamine, (101 MHz, CDCl_3)

Carbon No.	δ (ppm)	No. of Proton	Lit. δ (ppm)
1	132.37	1	131.8

2	127.21	1	127.3
3	72.61	1	72.7
4	28.33	2	28.2
5	62.64	1	62.6
6	61.54	2	61.3
7	126.95	0	126.7
8	103.10	1	103.3
9	146.16	0	146.1
10	146.43	0	146.4
11	107.06	1	106.8
12	135.19	0	135.3
13	63.56	2	63.5
14	80.20	1 (+1OH)	80.1
15	50.08	0	50.0
16	100.82	2	100.8
17	56.42	3	56.5

Mass spectrometry: The structure of the isolated haemanthamine was further confirmed using mass spectrometry (Figure 4), which revealed that the isolated compound has a molecular formula of $C_{17}H_{18}NO_4$ which directly corresponds to haemanthamine. Its mass spectrum displays a parent peak at m/z 301 and shows fragments at 301, 272, 257, 225, 181, 115, 77 which are all typical of haemanthamine as presented in figure 4. The result slightly differs from the literature values for the fragmentation of haemanthamine in the following pattern; loss of CH_3OH , loss of C_2H_6N and the loss of CHO . 301 $[M-H]^+$, 270 $[M-CH_3OH]^+$, 269 $[M-CH_3O]^+$, 259 $[M-CH_2-CHOH]^+$, 240 $[M-CH_3OH-CHO]^+$, 225 $[M-CH_2NCH_3-CH_3O]^+$, 211 $[M-CH_2CHOH-OCH_2OH]^+$, 118 $[M-CH_3OH-CHO-CH_2=NH-CH_2O]^+$. These differences could be isotopic, thus, the results are within the acceptable range as corroborated by the work of Bessa et al., (2017).

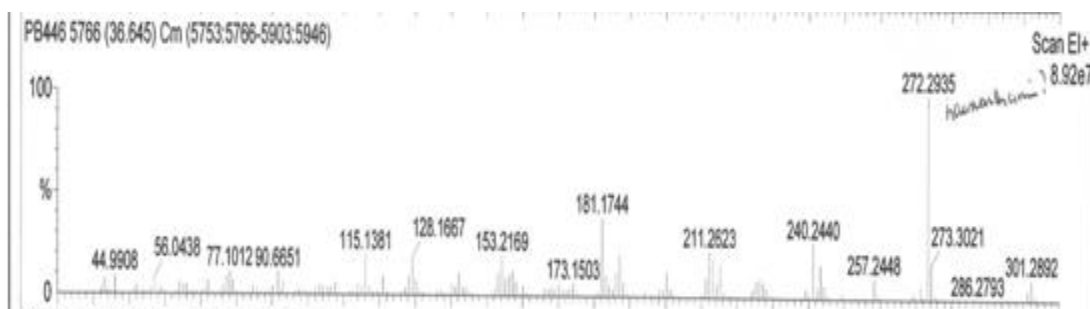


Figure 4. Mass Spectrum Showing the Fragmentation pattern of Haemanthamine

Moreover, the pharmacological relevance of haemanthamine was established by other researchers using different pharmacological models. For instance, Haemanthamine was reported to be a potent inducer of apoptosis in tumour cells at micromolar concentration (McNulty *et al.*, 2014). Other biological properties of this alkaloid include protein synthesis inhibition, anti-parasitic, antiretroviral, anti-inflammatory and anti-malaria effects and cytotoxicity agent against various cancer cells (Toriizuka *et al.*, 2008); (Rezanka and Dembitsky, 2001). Thus, the isolated alkaloid is indeed valuable pharmaceutical asset capable of treating different diseases.

Conclusion

In conclusion, the adopted methods and techniques proved to be vital in the isolation and characterisation of haemanthamine alkaloids in daffodil bulb extract and the results were in agreement with previous literatures.

REFERENCES

- Bastida J. F. Viladomat, J. Manuel, L. Guillermo, R. Carles, C. and Mario, R. (1989). Narcissus Alkaloids, VIII. Mesembrenone: An Unexpected Alkaloid From *Narcissus Pallidulus*, *J. Nat. Prod.* **52**(3): 478–480
- Bessa, C. D. P. B., De-AndradeRenata, J. P. S. de Oliveira, E., Domingos H., S. Wanderson, R. Bastida, J., Warley S. B. (2017). Identification of Alkaloids from *Hippeastrum aulicum* (Ker Gawl.) Herb. (Amaryllidaceae) Using CGC-MS and Ambient Ionization Mass Spectrometry (PS-MS and LS-MS) *J. Braz. Chem. Soc.* **28** (5): 819-830
- Breiterová, K., Miroslav L. , Eliška K. , Markéta, T. , Daniela H., Lubomír, O. and Lucie C. (2018). Daffodils as Potential Crops of Biologically-active Compounds: Assessment of 40 Ornamental Taxa for their Alkaloid Profile and Cholinesterases Inhibition Activity . *Natural Product Communications* **13**(4): 419 - 422
- Chandrakant, K., Arun, G., Satyajyoti, K. and Shefali, K. (2012). Drug discovery from plant sources: An integrated approach, *Ayu.* **33**(1): 10–19.
- Evidente, A. Anna, A. Amina, H. A. Soad, M. T. Hala, M. H. Eman, S. And Andrea, M. (2004). Amarbellisine, a lycorine-type alkaloid from *Amaryllis belladonna* L. growing in Egypt *Phytochemistry* **65**(14): 2113-2118
- Fulata, A. M. Usman, H., Arastus, W., Sule, I., Zadvá, A. I., Mohammed B., Umar, H. A. and Ngulde, S. I. (2018). Studies on the Phytochemical Constituents, Analgesic and Anti-Inflammatory Properties of the n-Butanol Column Fractions of the Aerial Parts of *Lagdera aurita* Linn in Rats. *Nigerian journal of Pharmaceutical and Biomedical Research* **3**(3): 217-224.
- Gilman E. F. (1999). *Narcissus* spp. Daffodil, *Narcissus*1 Environmental Horticulture Department, UF/IFAS Extension, Gainesville, FL 32611.
- Golam-Rasul, M. (2018). Extraction, Isolation and Characterization of Natural Products from Medicinal Plants. *International Journal of Basic Sciences and Applied Computing (IJBSAC)* ISSN: 2394-367X, **2** (6): 1-6
- McNulty, J., Jerald, J. N., Carles, C., Jaume, B., Siyaram, P., Jenny, G., Carly, G. (2007). Selective apoptosis-inducing activity of crinum-type Amaryllidaceae alkaloids *Phytochemistry*; **68**(7):1068-74.
- Naiho, A. O. Okonkwo, B. C. and Okoukwu, C. (2015). Anti-Sickling and Membrane Stabilizing Effects of Carica papaya Leaf Extract *British Journal of Medicine & Medical Research* **6**(5): 484-492,
- Nair. J. J. Anke, W. Susanna, L. B. and Johannesvan, S. (2017). Antibacterial constituents of the plant family Amaryllidaceae, *Bioorganic & Medicinal Chemistry Letters* Volume **27**(22): 4943-4951

Nehir Unver Somer, Gulen Irem Kaya , Christa Werner ,Rob Verpoorte (2003). Galanthindole: A New Indole Alkaloid from *Galanthus plicatus* ssp. *byzantinus* Article in *Planta Medica* **68**:869-871

Rezanka T. and Dembitsky, V. M. (2001). *Tetrahedron*, , **57**, 8743–8749.

Toriizuka, Y. Eri, K. Noriyuki, K. Mariko, K. Aki, I. Kazuhiko, O., Haruki, Y., Satoshi, Ō. Hiromitsu, T. (2008). New lycorine-type alkaloid from *Lycoris traubii* and evaluation of antitrypanosomal and antimalarial activities of lycorine derivatives *Bioorganic & Medicinal Chemistry* **16** (24): 10182-10189

Usman, H., Abubakar, U K., Olajide O Ibisagba, Fulata, A. M., Ibrahim, A. A. (2018). Phytoconstituents Evaluation and Anti-microbial Efficacy of the Crude Flavonoids and Saponins Root Bark Extracts of *Terminalia aviccinoides* and *Ficus polita*. *Journal of Herbmedicine Pharmacology*.; **7**(2): 106-111.

Veeresham, C. (2012). Natural products derived from plants as a source of drugs *J Adv Pharm Technol Res.* **3**(4): 200–201.