

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 recent trend for the isolation 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, its reported to have lots of medicinal potential against microbes and some forms of cancers with significantly low or no side effects. The plant Daffodil is one of the commonest source of this alkaloid and is traditionally used 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.

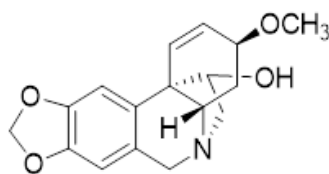
Key words: Haemanthamine, Daffodil, Alkaloids, NMR, Mass Spectroscopy

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'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°C. The plant grows excellently in a well-drained acidic soil. The flower of daffodil consists of a 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 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.

Comment [SJ1]: Details of control, all chemicals and solvent are required. Purity, %, manufacturer/supplier. Environmental conditions, name of instruments, column details.

Comment [SJ2]: % yield calculation, amount of yield missing.

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 further a pure isolate.

Comment [S13]: Temperature, pressure (if reduced pressure applicable)

Results and Discussion

¹HNMR 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 (CDCl₃).

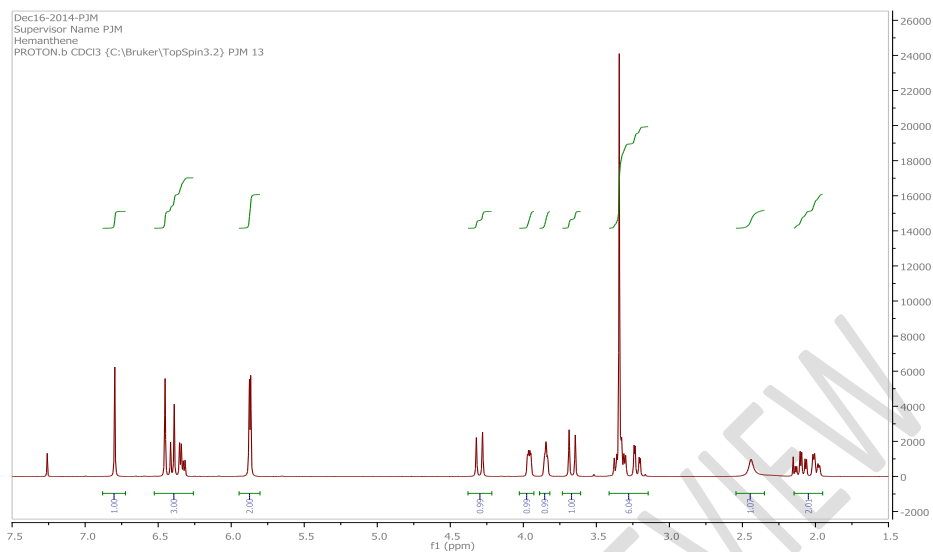


Figure 1: NMR Spectra of the Standard Haemanthamine

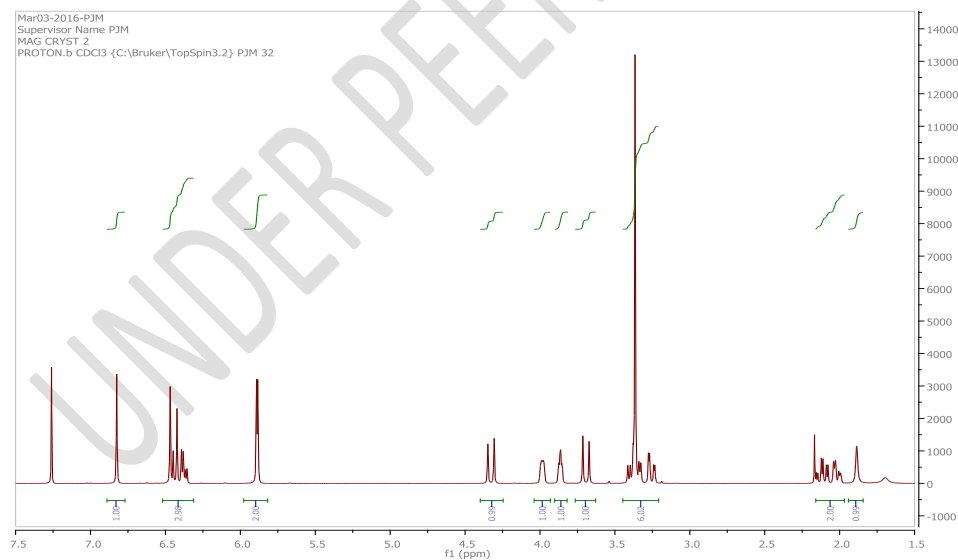


Figure 2: HNMR of Haemanthamine of Isolated Alkaloids

Comparison of a Commercial Haemanthamine with Isolated Haemanthamine

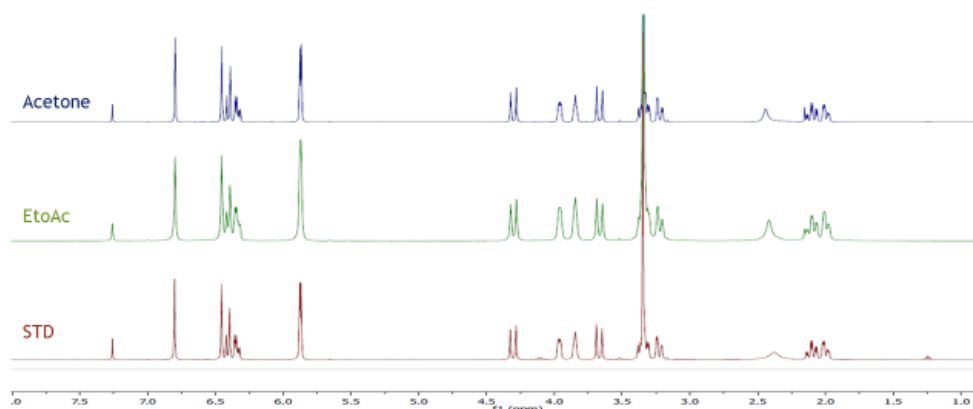


Figure 3: Comparison of a Commercial Haemanthamine with Recrystallized Using Different Solvents (Acetone and Ethyl-acetate)

The agreement between the NMR spectra of the isolated haemanthamine with that reported earlier by Bessa et al., (2017) and Bastida et al., (1989) are clearly presented in Table 1. The only difference is the position of the hydroxyl proton, which could be attributable to the presence of trace of moisture in NMR solvent. The spectra data of the isolated haemanthamine presented in figure 3 show the various positions, proton number and the splitting pattern of the different protons present in the alkaloids positions of other functional groups in the alkaloids in relation to the standard.

Furthermore, the vital signals for the characterisation of haemanthamine as obtained from the ¹H NMR spectrum are assigned 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 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)	Protons	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 data obtained from the compound isolated was further confirmed using mass spectrometry (Figure 4), which revealed that the isolated compound has a molecular formula of $\text{C}_{17}\text{H}_{18}\text{NO}_4$ 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 $\text{C}_2\text{H}_6\text{N}$ and the loss of CHO . $301 [\text{M}-\text{H}]^+$, $270 [\text{M}-\text{CH}_3\text{OH}]^+$, $269 [\text{M}-\text{CH}_3\text{O}]^+$, $259 [\text{M}-\text{CH}_2-\text{CHOH}]^+$, $240 [\text{M}-\text{CH}_3\text{OH}-\text{CHO}]^+$, $225 [\text{M}-\text{CH}_2\text{NCH}_3-\text{CH}_3\text{O}]^+$, $211 [\text{M}-\text{CH}_2\text{CHOH}-\text{OCH}_2\text{OH}]^+$, $118 [\text{M}-\text{CH}_3\text{OH}-\text{CHO}-\text{CH}_2=\text{NH}-\text{CH}_2\text{O}]^+$. These

differences could be isotopic, thus, the results are within the acceptable range as corroborated by the work of Bessa et al., (2017).

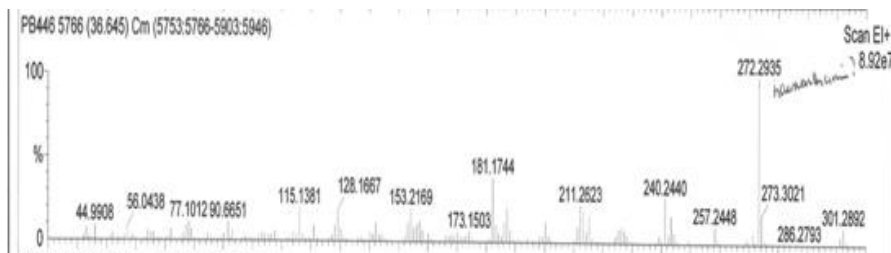


Figure 4. Mass Spectrum Showing the Fragmentation pattern of Haemanthamine

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 daffod bulb extract and the results were in agreement with previous literatures.

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