Cycloeucalenone Isolation and Characterisation from *Musa Acuminata Colla*

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Abstract. The isolation and characterisation of bioactive compound(s) from *Musa acuminata colla* (banana blossom) As a result, the research aimed to isolate secondary metabolites. Sample Extraction was carried out using the maceration method to obtain ethyl acetate extracts. The Ethyl acetate extract of Banana blossom flower and bract was used for isolation. Compounds isolation using Column Chromatography, the 260 fractions from the column are subjected to TLC testing to see the separation of the component combination by applying a thin stationary phase supported by an inert backing that yields 30 fractions. The purpose of TLC is to obtain well-defined, well-separated spots. Each component has a retention factor (RF), which combines fractions with the same RF value. The four fractions obtained from TLC activities were analysed using NMR spectroscopy (FT-IR, 1D NMR, 2D NMR and Mass spec), which resulted in the isolation of triterpenes compounds named Cycloeucalenone.

Keywords: *Musa acuminata Colla*; 1D NMR; 2D NMR; mass spectrometry; FT-IR.

INTRODUCTION

The data on various biological activities of several edible flowers have shown potential health-promoting benefits for humans [1]. The banana blossom has also demonstrated biological activities that might symbolise its potential health-enhancing properties [2]. Many research outcomes have indicated a positive effect of banana blossom's antioxidant and anti-diabetic properties [3]. These properties are available for human consumption upon incorporation into foods. The latter part of this paper discusses the ethnic delicacies prepared using banana inflorescence in various parts of India [4]. The banana blossom also depicted properties like anti-carcinogenic, antimicrobial, cardio-protective activities, and anti-inflammatory due to specific bioactive components present in the inflorescence, discussed in the next section [4]. The nutritional content of banana blossoms plays a role in tackling different health issues and infections [5].

The banana blossom (Musa acuminata Colla), a byproduct of banana farming, is commonly consumed as a vegetable in several Asian nations, including Sri Lanka, Malaysia, Indonesia, and the Philippines. In Sri Lanka, it is enjoyed as a curry, boiling or deep-fried salad with rice and wheat bread [6]. Banana blossoms have tremendous nutritional value and are a rich source of dietary fibre and some biologically active compounds like vitamin C, tannin, myoinositol phosphates, and alpha-tocopherol [7]. High levels of dietary fibre intake are associated with significantly lower prevalence rates for coronary heart disease, stroke, and peripheral vascular disease. However, the average fibre intake for children and adults is alarmingly less than that of the recommended level [8]. Food components with antioxidant properties may prevent cardiovascular diseases by inhibiting the oxidative damage to cholesterol [9].

METHODS

Sample Extraction. The extraction was carried out using 1 kg of the powdered sample, poured into a reagent bottle and macerated successively with 1200 mL each of distilled hexane, ethyl acetate and methanol. Each extraction cycle was carried out for three days with occasional shaking, after which it was filtered, and the filtrate evaporated at room temperature to obtain crude extracts according to [10, 11]

Sample collection. Researchers collected 67 banana blossoms from Dumne (Gollabwazange) in the Song Local Government Area of Adamawa State. The Department of Plant Science, Faculty of Life Science, Modibbo Adama University Yola, Nigeria, authenticated the plant part. The Banana Blossoms were thoroughly washed with tap water to remove dust and impurities, while some impurities were carefully picked and thereafter rinsed with tap water again. The plant sample was air-dried at ambient temperatures to a constant weight over four weeks. It was pulverised using a wooden mortar and then blended [11].

Isolation of compounds. The hexane extract (6.8 g) was adsorbed unto Celite and subjected to VLC using 30 g of silica gel (GF 254 MERCK). The column was eluted 20 times with hexane (40 ml), then with increasing amounts of ethyl acetate in hexane until 100% ethyl acetate. TLC examined the fractions, combining similar fractions based on their TLC profiles. Combined fractions BF 02 (10–14) showed better separation. The column fractions were monitored by Thin Layer Chromatography (TLC); from these, sub-fractions BF 25 were confirmed to be pure by TLC with ethyl acetate/diethyl ether (50:50) as they gave single spots. These fractions were allowed to dry and labelled BB 25 and were analysed using NMR spectroscopy.

Structure Elucidation of Isolated Compounds by Spectroscopic Analyses. The phytochemical screening of Banana blossom gave positive tests for steroid, alkaloid and triterpenoids. Below are the results obtained after the analysis.

RESULTS AND DISCUSSION

Table 1 – Experimental and literature data for Cycloeucalenone ^{13}C-NMR

Position	[12] (Appendix S5)	[13]	Experimental data
C-1	32.80	32.80	32.78
C-2	41.00	40. 80	40.96
C-3	213.50	212.20	213.8
C-4	50.00	49.00	49.98
C-5	46.10	45.90	46.05
C-6	25.90	25.80	25.90
C-7	25.20	25.10	25.20
C-8	47.10	46.90	47.08
C-9	25.00	24.90	24.95
C-10	29.30	29.30	29.28
C-11	27.20	26.90	27.20
C-12	32.90	32.80	32.85
C-13	45.40	45.20	45.37
C-14	48.80	48.70	48.77
C-15	35.40	35.30	35.42
C-16	28.10	28.00	28.11
C-17	52.20	52.10	53.83
C-18	18.00	17.90	17.92
C-19	27.00	27.10	26.95
C-20	36.10	36.00	36.10
C-21	18.30	18.30	18.33
C-22	34.90	35.00	34.57
C-23	31.30	31.30	31.30
C-24	156.90	156.10	156.78
C-25	33.80	33.70	33.91
C-26	22.00	21.80	22.01
C-27	21.90	21.80	21.87
C-28	19.20	19.10	19.17
C-29	10.80	10.70	10.75
C-30	106.00	105.60	104.42

Table 2 – Experimental and literature data for Cycloeucalenone ^{1}H-NMR

Position	[12] (Appendix S5)		[13]		Experimental data	
C-1	1.59	1.90	1.61	1.89	1.65	1.91
C-2	2.45 (2H)		2.45 (2H)		2.24 (2H}	
C-3						
C-4	2.26		2.26		2.23	
C-5	1.60		1.60		1.65	
C-6	0.77	1.73	0.76	1.72	0.78	1.72
C-7	1.14	1.38	1.12	1.38	0.97	1.39
C-8	1.67		1.66		1.67	
C-9						
C-10						
C-11	1.28	2.06	1.27	2.06	1.35	2.00
C-12	1.69 (2H)		1.68 (2H)		1.69 (2H}	
C-13						
C-14						
C-15	1.34 (2H)		1.32 (2H)		1.36 (2H}	
C-16	1.34	1.97	1.32	1.93	1.36	1.93
C-17	1.65		1.61		1.66	
C-18	1.03 (3H)		1.02 (3H)		0.96 (3H}	
C-19	0.42	0.64	0.42	0.64	0.42	0.66
C-20	1.44		1.32		1.40	
C-21	0.93 (3H)		0.89 (3H)		0.93 (3H}	
C-22	1.19	1.60	0.95	1.36	0.95	1.67
C-23	1.92	2.15	1.19	1.48	1.92	2.19
C-24			2.13			

Position	[12] (Appendix S5)		[13]		Experimental data	
C-25	2.26				2.22	
C-26	1.05 (3H)		1.66 (3H)		0.96 (3H}	
C-27	1.06 (2H)		1.03 (2H)		0.95 (2H}	
C-28	4.69 (3H)	4.74	4.69 (3H)		4.47 (3H}	4.52
C-29	1.01 (3H)		1.01 (3H)		0.92 (3H}	
C-30	0.94 (3H)		0.92 (3H)		0.94 (3H}	

The compound was obtained as a white crystalline solid. It gave a characteristic of sodium salt that resembles the molecular ion $[M^+H]^+$ at m/z 425.3778 in its high-resolution liquid chromatography-mass spectrometry (HR-LCMS) corresponding to the molecular formula $C_{30}H_{48}O$ (calc. 425.3783) that known as cycloeucalenone. The compound's Fourier-transform infrared spectroscopy (FTIR) showed a signal at 1750 showing the presence of the carbonyl C=O group. ^{13}C show the presence of the carbonyl group at 213 peaks. The ^1H-NMR spectrum displayed a cluster of downfield signals suggesting the presence of a triterpenoid's methyl, methylene and methine groups. It showed two singlets at δH 4.47 and 4.52 ppm, characteristic of a terminal olefinic double bond with geminal protons. Two highly shielded signals at 0.42 (1H, J = 4.0 Hz) and δ 0.66 ppm (1H, J = 3.8 Hz) corresponded to a cyclopropane ring [12] in the compound; hence the compound must be a cyclobutane type triterpene. The ^{13}C- NMR revealed 30 carbon signals, including a double bond between C-24 (δC 156.9) and C-30 (δC 106.3) and a carbonyl group at C-3 (δC 213.4). The compound was identified as cycloeucalenone based on its 2D NMR correlation as follows: correlations in its COSY spectrum identified geminal and vicinal protons, hence confirming H-2 and H-3, H-22 and H-23, H-4 and H-28, and the various methyl doublets. The HSQC confirmed the proton-bearing carbons and thus also identified the olefinic proton-bearing carbon at 106.3 (C-30) and the cyclopropane carbon at 26.9 ppm (C-19). The long-range ^2J and ^3J correlations in its HMBC spectrum further confirmed the structure as correlations from the methyl at H-28 to the carbonyl carbon at 213.4 (C-3) confirmed the position of the C=O as well as C-4 and C-5. Long-range correlations ^3J from the cyclopropane protons indicated C-1, C-5 and C-11; thus, the cyclopropane is attached to C-9 and C-10. Correlations from H-30 olefinic protons identified C-23 and C-25, while the geminal methyl groups at C-18 and 29 also confirmed C-26 and C-27. These correlations showed that a long chain with an isopropyl ending and a double bond adjacent to the isopropyl group was present in the compound; this is a standard chain in tetracyclic triterpenes with a methyl group at C-21 and a cyclopentane ring D. Therefore, the compound was identified as cycloeucalenone, and its NMR data agreed with the reports of [12, 13]. The complete chemical shift assessments and comparison with literature reports are in Tables 1 and 2.

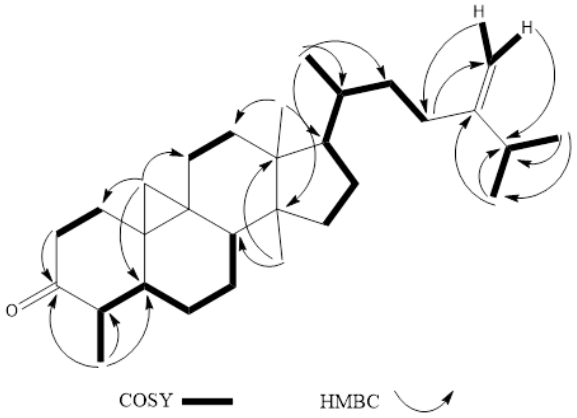

Figure 1 – Long-range (HMBC) and COSY correlations for the compound

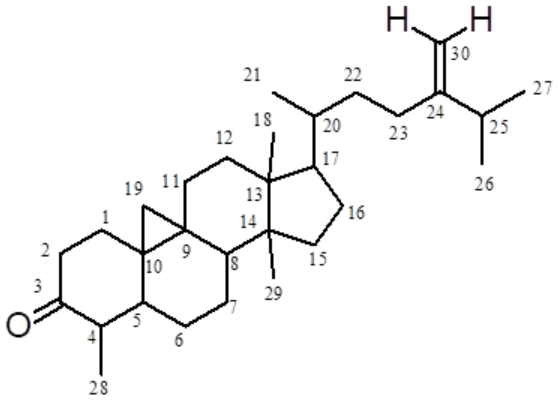

Figure 2 – Cycloeucalenone

CONCLUSIONS

Cycloeucalenone was isolated from ethyl acetate extracts of *Musa acuminata calla* as a member of the secondary metabolite.

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