Isolation of Cyloeucalenol from Ethyl Acetate Extracts of *Musa Acuminata Colla* Bract

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Abstract. The investigation seeks to separate secondary metabolites to identify the bioactive compound(s) found in banana blossoms. The researchers divided the ethyl acetate extract into compounds using column chromatography. The fractions retrieved from the column were subjected to thin-layer chromatography to ensure their purity for component separation, yielding BF57 fractions as a white needle-like crystal substance. The proportion was determined using 1D Nuclear Magnetic Resonance (1D NMR) and 2D Nuclear Magnetic Resonance (2D NMR) spectroscopy. The extracted chemical structures were identified as cycloeucalenol after thoroughly interpreting NMR spectral data and comparison to the literature.

Keywords: Banana blossom; Cycloeucalenol; NMR spectroscopy.

INTRODUCTION

The scientific landscape has transformed the Banana flower (Musa Acuminata Colla) into various therapeutic effects. It also has a particular flavour and has established itself in the culinary world. It is primarily consumed in South East Asian nations such as India [1]. Thai and Vietnamese cuisines play a vital role in Ayurvedic cookery. People can eat it fresh or cooked; they think its petals taste like artichoke leaves. As with artichokes, the heart and fleshy part of the bracts are both edible [2]. The aromatic profile is less intense and more delicate in blossom form than in the fruits. The flower is starchy and slightly bitter, with more vegetal flavours. In India, people primarily use banana blossoms in salads, curries, or soups. In South Indian cuisine, they mainly use it in curries, soups, fritters, stir-fried, or fried dishes [3]. In Thailand, it is typically served raw on the side with pad Thai, whereas in Indonesia, it is combined with pork and spicy sambal, fried in a piece of bamboo, and offered at festivals. People can also use it in stews, like kari-kari, a popular beef stew in the Philippines. In Laos, they use it with galangal, a rhizome similar to ginger [4].

Over 32 million banana bunches are produced annually in Sri Lanka (Department of Census and Statistics, 1998, Agricultural Statistics, Sri Lanka). Bananas are considered a popular ingredient in their cuisine and are delicious in curries, fritters, and salads [5].

Keeps mood elevated and reduces anxiety. Banana blossoms can boost moods in every person, especially kids with mood swings who are naturally anxious. They can also act as a remedy for mentally imbalanced kids who suffer from bouts of anxiety as they reduce feelings of anxiety. The reducing property of banana blossoms can be attributed to the fact that they contain magnesium, which acts as an antidepressant without side effects [6].

Helpful in infection treatment. Banana blossom extract is beneficial for treating the infection naturally. During research on the antimicrobial activity of banana blossom extract, it was suggested that certain bioactive compounds extracted from banana blossoms exhibited antibacterial activity against Bacillus [7]. The research further mentioned that the bioactive compound malic acid

found in blossom exhibited a more potent antibacterial activity against Bacillus subtilis, Bacillus cereus, and Escherichia coli. The flower extract is also helpful in healing wounds, especially in children and preventing the malarial parasite, Plasmodium falciparum, from growing and developing in the body. Besides fighting against infections, the juice of banana blossom is very helpful in healing wounds and burning faster [8].

Helpful in Diabetes. Regularly consuming banana blossoms for about a month reduces the blood sugar level and raises the body's haemoglobin level as it is rich in fibre and iron, which assists in producing red blood cells [9].

Musa acuminata Colla bracts. Musa acuminate collar bracts are generally purple or crimson (ranging from bright red to dark violet), although they can sometimes be yellow at the extreme tip and outer surfaces. They are convolute and overlap at the extreme tip. Bracts are glaucous, with a faint longitudinal rib and an inner surface that is lighter, light crimson, or yellowish. The bract is usually lanceolate or narrowly oval, tapers sharply towards the end, and has an acute apex. The bract shoulders are often high (ratio 0.28), and the curlings of the bracts appear to be reflex and roll back after opening [10].

Bracts lift from the first hand in 3 to 10 days, which will shed soon and give rise to fully grown fruits in a cluster. Only one bract lifts at a time, and it revolves around fading. The bracts are generally used as feed for cattle but can also be used to treat hypertension when taken in decoction for 3times a day [11]. The bracts contained certain bioactive constituents like alkaloids and cyclo glycosides in petroleum ether extract and flavonoids in ethyl acetate extract [11].

Methanol extract showed various constituents like alkaloids, glycosides, saponins, terpenoids, tannins, flavonoids, phenols, steroids and coumarins. In contrast, aqueous extracts have coumarins and phenols, but chloroform extracts do not show bioactive principles [12].

METHODS

Plant collection. Researchers collected banana blossoms from the Song local government area in Adamawa State. The Department of Biology (Botany), Faculty of Life Sciences, Modibbo Adama University Yola in Nigeria validated the plant.

Sample Preparation. Researchers thoroughly cleaned the banana blossom with tap water to remove dust and pollutants, then carefully removed any remaining impurities before rinsing it with tap water again. Over four weeks, the plant is air-dried at room temperature until it reaches a consistent weight. They pounded it into powder in a wooden mortar.

Sample Extraction. The extraction was carried out with 1 kilogram of powdered material placed in a glass container and macerated with 1200 mL 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 [13].

Column Chromatography. Extracts from the banana bloom 6 g of ethyl acetate extracts were adsorbed onto Celite separately by dissolving them in tiny amounts of solvent and thoroughly mixing them with 10 g of Celite. Researchers crushed the adsorbed extract into a powdery form after it dried utterly. A small amount of cotton wool was applied to the bottom of the column and gently tapped with a rubber applicator. Researchers created a silica gel slurry by combining 50 g (230-400 mesh ASTM) with n-hexane and stirring it with a glass rod. Allow it to cool for about fifteen minutes before swiftly transferring to the column. The researchers added more solvent to rinse the slurry down the column and then tapped it with a rubber applicator to compact the bed and remove any air bubbles. The researcher placed a beaker beneath the column and turned on the tap until the solvent reached the top of the bed. The sample was then carefully inserted into the column with the tap closed. Researchers introduced solvent combinations to start elution. Fractions of the column were collected into 20 cm^3 vials [14].

Thin Layer Chromatography (TLC). Researchers conducted thin-layer chromatographic analysis on the plant's hexane, ethyl acetate, and methanol extracts to find the optimal solvent system for separating the components. As a result, it became practical to use pre-coated TLC plates. A microquantity of the sample solution was spotted on TLC pre-coated (MERCK) plates using various ratios of organic solvents (hexane, ethyl acetate, methanol, and chloroform), considering their polarity. Researchers considered the solvent system that achieves a high resolution in component separation. They sprayed the plates with 10% sulfuric acid in methanol and heated them at 100 ºC for 1–5 minutes. Appropriate

fractions are collected from the column, which TLC monitored. The fractions with the same retention factor Rf values ($\frac{\text{distance moved by solvent}}{\text{distance move by sample}}$) was pooled and concentrated, and the spots and observations were recorded [14],

RESULTS AND DISCUSSION

The tables below are the extracted results from the spectrum (supplementary material).

The fraction BFB 25 was dissolved in deuterated chloroform for NMR analysis (1H, 13C and 2D experiments). The signals obtained from the 1H and 13C NMR spectra data revealed that the structure of the isolated compound was cycloeucalenol; the NMR data obtained was compared with that of the literature data on cycloeucalenol.

However, this class of compound includes cycloalkanes, including cycloeucalenol. The 1H NMR spectra of cycloeucalenol were observed in the double bond position on the side chain. The methyl protons, Me-28 and Me-21, appeared as broad singlets (0.92 and 0.86); Me-26 appeared as a multiplet (δH 1.05), while Me-29 was observed as a singlet (δH 1.01). A sextet was observed at δH 2.26 (J = 7.0 Hz), while an olefinic proton, which appeared as a doublet, was observed at δH 1.00 (J = 6.6 Hz). The 1H NMR data compares with [15]. The 13C NMR spectra of cycloeucalenol from C-1 to C-21, with the only difference observed in the side chain from C-22, because of the difference in position of the double bond C-25 is an olefinic quaternary carbon at δC 156.9. At the same time, C-27 is an exomethylene carbon at δC 106.1. The complete chemical shift assessments and comparison with literature reports are in Tables 1 and 2.

Table 1 – 13Carbon Spectrum Data of BFB 25 as Cycloeucalenol

	Experimental data	[15]
C-1	31.4	30.8
C-2	34.0	34.8
C-3	76.8	76.6
C-4	44.7	44.6
C-5	43.7	43.3
C-6	25.1	24.7
C-7	28.2	28.1
C-8	46.2	46.9
C-9	23.2	23.5
C-10	29.4	29.5
C-11	25.3	25.2
C-12	35.5	35.3
C-13	45.4	45.3
C-14	48.9	48.9
C-15	32.9	32.9
C-16	27.1	27.0
C-17	52.2	52.2
C-18	18.0	17.8
C-19	27.2	27.2
C-20	36.1	36.1
C-21	18.4	18.3
C-22	35.1	35.0
C-23	31.4	31.3
C-24	156.9	156.9
C-25	33.9	33.8
C-26	22.1	22.0
C-27	21.9	21.9
C-28	106.1	105.9
C-29	14.3	14.4
C-30	19.3	19.1

Table 2 – Proton Spectrum Data of BFB 25 as Cycloeucalenol

	Experimental data	[15]
C-1	1.30, 1.57	1.30, 1.57
C-2	1.44, 2.02	1.44, 2.01
C-3	3.98	3.24
C-4	1.20	1.20
C-5	1.24	1.23
C-6	0.63, 1.70	0.61, 1.70
C-7	1.33, 1.95	1.32, 1.95
C-8	1.63	1.60
C-9		
C-10		
C-11	1.09, 1.33	1.09, 1.33
C-12	1.65 (2H)	1.65 (2H)
C-13		
C-14		
C-15	1.32 (2H)	1.32 (2H)
C-16	1.23, 2.01	1.23, 2.01
C-17	1.61	1.62
C-18	0.99 (3H)	0.99 (3H)
C-19	0.17, 0.41	0.17, 0.41
C-20	1.42	1.42
C-21	0.92 (3H)	0.92 (3H)
C-22	1.16, 1.59	1.17, 1.60
C-23	1.92, 2.15	1.91, 2.15
C-24		
C-25	2.26	2.26
C-26	1.05 (3H)	1.05 (3H)
C-27	1.06 (3H)	1.06 (3H)
C-28	4.69, 4.74	4.69, 4.74
C-29	1.01 (3H)	1.01 (3H)
C-30	0.92 (3H)	0.92 (3H)

The compound was identified as cycloeucalenol 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 106.1 (C-30) and the cyclopropane carbon at 27.2 ppm (C-19).

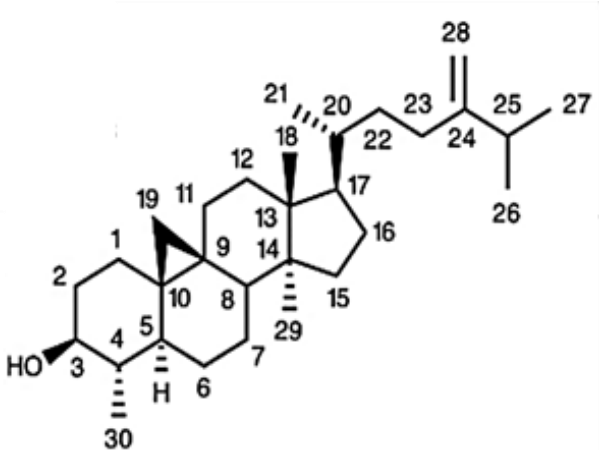

Figure 1 – Positions of the carbon atom of cycloeucalenol

The long-range 2J and 3J correlations in its HMBC spectrum further confirmed the structure as correlations 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 C-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 cycloeucalenol, and its NMR data agreed with the reports of [15; 16].

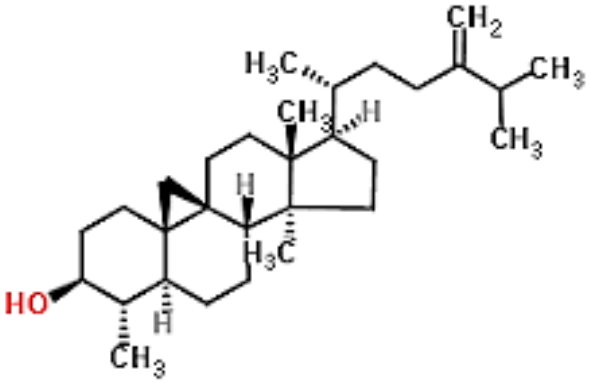

Figure 2 – Cycloeucalenol structure

CONCLUSIONS

The ethyl acetate extract of banana blossom was subjected to column and thin layer chromatography, yielding fraction BFB 25. It was analysed using NMR spectroscopy, which provided a triterpene-like molecule known as cycloeucalenol.

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