Isolation of Stigmasterol and B-Sitosterol From Ethyl Acetate Extracts of *Musa Acuminata Colla* Bract

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Abstract. The research focuses on uncovering the bioactive chemicals or compounds in the banana bloom by isolating secondary metabolites. The researchers used column chromatography to isolate the compounds from the ethyl acetate extract. The BFB 57 fractions were obtained as a white needle-like crystal compound after being recovered from the column and put via thin-layer chromatography to verify their purity for component separation. 1D nuclear magnetic resonance (1D NMR) and 2D nuclear magnetic resonance (2D NMR) spectroscopy were used to characterize the fraction. By carefully analysing the NMR spectral data and comparing the measured NMR spectral data with those found in the literature, the structures of the separated compounds were determined to reveal stigmasterol and β-sitosterol.

Keywords: Banana blossom; β-Sitosterol; stigmasterol; NMR spectroscopy.

INTRODUCTION

Musa acuminata can reach six meters in height despite being a perennial herbaceous plant with a stiff fibrous pseudostem composed of overlapping leaf bases [1]. The leaves are large and spirally clustered around the stem. The inflorescence, which can be large, horizontal, or deflexed, grows from the top of the pseudostem. The inflorescence bracts range in colour from crimson to dark purple and contain both male and female flowers [2].

The consumption of banana blossom is a culinary tradition in many cultures, particularly in Southeast Asia, where it is prized for its unique flavour, texture, and nutritional benefits. Here's a look at how banana blossom is consumed [3].

One of the most popular ways to enjoy banana blossom is in salads. The blossom is often thinly sliced or shredded and added to salads along with other vegetables, herbs, and sometimes proteins like shrimp or chicken. The mild flavour and crunchy texture of banana blossom add a delightful contrast to the fresh ingredients in the salad. Cooks can incorporate banana blossom into cooked dishes such as curries and stir-fries. In these dishes, it is typically chopped and added along with other vegetables, meats, or seafood [4]. The blossom absorbs the flavours of the other ingredients while contributing its unique taste and texture to the dish. Banana blossom is used in soups and stews to enhance flavour and nutritional value. It can be added to broths, other vegetables, and protein sources to create healthy meals [5].

Fritters and Fritter-like Preparations: In some regions, banana blossoms are coated in batter and deep-fried to make fritters or patties. These crispy snacks are served as appetisers or street food, often with dipping sauces or chutneys [6]. In certain cultures, banana blossom is pickled or preserved to extend its shelf life and create flavorful condiments. These pickled preparations can be enjoyed as accompaniments to meals or added to sandwiches and wraps for extra flavour. Smoothies and Juices of the Blossoms Recently, there has been growing interest in using banana blossoms as an ingredient in smoothies and juices [6]. Blending the blossom with fruits, vegetables, and other nutritious ingredients creates refreshing and healthful beverages packed with vitamins, minerals, and antioxidants [7].

When preparing banana blossom for consumption, it's essential to properly clean and trim away any tough outer layers or fibrous parts. People should consume fresh banana blossoms quickly, as they spoil soon after being harvested. With its versatility and nutritional benefits, banana blossom offers a delicious and nutritious addition to various dishes and culinary creations [8].

Banana blossom is a versatile and nutritious ingredient enjoyed in traditional cuisines for centuries [9]. With its mild flavour, unique texture, and numerous health benefits, banana blossom adds depth and complexity to various dishes, ranging from salads and curries to soups and stir-fries. Banana blossom, which is packed with vitamins, minerals, antioxidants, and dietary fibre, offers a host of nutritional advantages, contributing to overall health and well-being [10]. While its consumption has long been a culinary tradition in regions like Southeast Asia, the global interest in diverse and nutritious foods has led to increased recognition of banana blossom in modern cuisine [11].

METHODS

Plant collection. Researchers obtained banana flowers from the Song Local Government Area of Adamawa State. The Department of Biology (Botany), Faculty of Life Sciences, Modibbo Adama University Yola, Nigeria, validated the plant.

Sample Preparation. The researchers thoroughly washed the banana blossom with tap water to eliminate dust and contaminants, carefully picked out inevitable impurities, and then rinsed it with tap water again. Over four weeks, the plant is air-dried at ambient temperatures until it reaches a constant weight. It was powdered in a wooden mortar and then blended.

Sample Extraction. The extraction was performed using one kilogram of powdered material, which was placed into a glass container and macerated with 1200 ml each of distilled hexane, ethyl acetate, and methanol. The researchers carried out each extraction cycle for three days with periodic shaking, and then they filtered the mixture and evaporated the filtrate at room temperature to obtain crude extracts [12].

Column Chromatography. The researchers independently adsorbed 6 g of banana blossom extracts from distilled hexane, ethyl acetate, and methanol onto celite by dissolving them in small volumes of solvent and thoroughly mixing them with 10 g of celite. The adsorbed extract was crushed into powdery form after drying entirely. The researchers placed a small amount of cotton wool at the bottom of the column and gently tapped it with a rubber applicator. They made a silica gel slurry by mixing 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, then tapped it with a rubber applicator to compact the bed and remove any air bubbles. The researchers placed a beaker beneath the column and left the tap running until the solvent reached the top level of the bed. The sample was then gently placed into the column with the tap closed. The researchers introduced solvent combinations to begin elution and collected fractions from the column into 20 cm^3 vials [13].

Thin Layer Chromatography (TLC). The researchers performed thin-layer chromatographic analysis on the plant's hexane, ethyl acetate, and methanol extracts to determine the best solvent for separating the constituents. As a result, using pre-coated TLC plates was possible. Using different ratios of organic solvents (hexane, ethyl acetate, methanol, and chloroform) while considering their polarity, a microquantity of the sample solution was spotted on TLC pre-coated (MERCK) plates. The researchers considered the solvent system, which achieved a high resolution in component separation. They sprayed the plates with 10% sulfuric acid in methanol and heated them at 100 °C 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 [12].

Spectroscopic Measurement. The end products of the fractions were subjected to spectroscopic examination. NMR spectrophotometer characterisation at SIPBS, University of Strathclyde, Glasgow, UK, on a JEOL-LA-400MHz FT-NMR spectrophotometer. Fourier-transform infrared (FTIR) was carried out using Shimadzu FTIR-8400S Spectrophotometer and mass spectroscopy.

RESULTS AND DISCUSSION

The below results were isolated from the spectra of BFB 57.

Compound 1 was isolated as crystals. The mass spectral data of the compound gave a molecular formula C29H48O, which was supported by the ^{13}C-NMR spectral data. ^{1}H-NMR spectra of Compound 1 showed the presence of two methyl singlets at δ 0.71 and 1.03; three methyl doublets that appeared at δ 0.80, 0.82, and 0.91; and a methyl triplet at δ 0.83. Compound 1 also showed protons at δ 4.98, 5.14, and 5.31, suggesting three protons corresponding to the olefinic bond – sterol skeleton [14, 15]. The proton corresponding to the H-3 of a sterol appeared as a triplet of doublet of doublets at δ 3.51. The researchers assigned the ^{1}H and ^{13}C-NMR data for all the protons and carbons based on COSY, HMQC, and HMBC correlations and provided the data in Table 1.

The above spectral data supported the presence of a sterol skeleton with a hydroxyl group at the C-3 position with two double bonds at C-5/C-6 and C-20/C-21 with six methyl groups supported by the key COSY and HMBC correlations. Thus, the structure of compound one was assigned as the known compound stigmasterol. The physical and spectral data are consistent with the reported literature data [16–18] of stigmasterol.

Compound 2 was also isolated as crystals, and its mass spectral data suggested the molecular formula to be $C_{29}H_{50}O$. The ^{1}H-NMR spectra of compound 2 showed the presence of six methyl signals that appeared as two methyl singlets at δ 0.68 and 1.01, three methyl doublets that appeared at δ 0.81, 0.83 and 0.93, and a methyl triplet at δ 0.84 in Table 2; same as compound 1.

Table 1 – ^{1}H and ^{13}C-NMR Spectrum Data for Stigmasterol (compound 1)

	Experimental data		[14]		[15]	
	^{1}H-NMR	^{13}C-NMR	^{1}H-NMR	^{13}C-NMR	^{1}H-NMR	^{13}C-NMR
1	1.85 (m)	37.28	1.85 (m)	37.39	1.08 (o), 1.85 (o)	37.24
2	1.89 (m)	31.93	1.95 (m)	32.02	1.52 (o), 1.84 (o)	31.64
3	3.52 (m)	71.83	3.52 (m)	71.93	3.52 (m)	71.78
4	2.29 (dd, *J*=1.44; 1.06) and 2.31, t)	42.33	2.24 (dd, *J*=1.44; 1.06) and 2.38, t)	42.42	2.24 (m), 2.28 (m)	42.20
5	-	140.78	-	140.87		140.74
6	5.36 (t)	121.73	5.36 (t)	121.84	5.35 (brd)	121.68
7	1.89 (m)	31.69	1.99 (m)	31.78	1.50 (o), 1.96 (o)	31.90
8	2.02(m)	31.89	2.00(m)	32.05	1.49 (o)	31.88
9	0.95 (m)	50.19	0.94 (m)	50.26	0.93 (o)	50.12
10	-	36.53	-	36.64		36.49
11	1.05 (m,)	21.10	1.02 (m,)	21.22	1.45 (o), 1.50 (o)	21.02
12	1.17 (m)	39.80	1.16 (m)	39.82	1.17 (o), 1.99 (o)	39.67
13	-	42.34	-	42.35		42.31
14	1.03 (m)	56.79	1.00 (m)	56.99	0.98 (o)	56.75
15	1.06 (m) and 1.56 (m)	24.38	1.06 (m) and 1.58 (m)	24.45	1.15 (o), 1.57 (o)	24.35
16	1.59 (m) and 1.28 (m)	28.26	1.66 (m) and 1.25 (m)	29.07	1.24 (o), 1.73 (o)	28.90
17	1.13 (m)	56.08	1.12 (m)	56.08	1.15 (o)	56.04
18	0.86 (s)	12.00	0.85 (s)	12.13	0.65 (s)	11.80
19	1.05 (s)	19.41	1,01 (s)	19.54	1.05 (s)	19.10
20	1.16 (m)	40.49	1.16 (m)	40.65	2.04 (o)	40.48
21	1.03 (d, *J*=7.2 Hz, 3H)	21.23	1.03 (d, *J*=7.2 Hz, 3H)	21.23	1.02 (d)	21.51
22	5.00 (dd, *J* =1.73 Hz and 1.72 Hz)	138.32	5.00 (dd, *J* =1.73 Hz and 1.72 Hz)	138.46	5.15 (brd)	138.30
23	5.15 (dd, j=1.75 Hz and 1.73 Hz)	129.30	5.15 (dd, j=1.75 Hz and 1.73 Hz)	129.39	5.05 (brd)	129.26
24	1.55 (m)	51.25	1,55 (m)	51.38	1.53 (o)	51.22
25	1.46 (m)	33.97	1.45 (m)	32.03	1.37(o)	36.13
26	0.99 (d, ,*J* =13 Hz)	21.23	1.02 (d, ,*J* =13 Hz)	21.21	0.83(d)	19.80
27	0.84 (br s)	19.83	0.84 (br s)	19.97	0.80 (d)	18.91
28	1.17 (m)	25.41	1.16 (m)	25.56	1.16 (o), 1.43 (o)	25.39
29	0.81 (t)	12.25	0.81 (t)	12.41	0.65 (t)	11.97

Notes: s = singlet, d = doublet, t = triplet, m = multiplet, O = overlap

Table 2 – ^{1}H and ^{13}C-NMR Spectrum Data for β-Sitosterol (compound 2)

	Experimental data		[14]		[15]	
	^{1}H-NMR	^{13}C-NMR	^{1}H-NMR	^{13}C-NMR	^{1}H-NMR	^{13}C-NMR
1	1.85 (m), 1.09	37.28	1.85 (m)	37.39	1.08 (o), 1.85(o)	37.243
2	1.89 (m), 1.53	31.93	1.95 (m)	32.05	1.52 (o), 1.84 (o)	31.864
3	3.52 (m)	71.83	3.52(m)	71.93	3.52 (m)	71.775
4	2.29 (dd, *J*=1.44; 1.06) and 2.31, t)	42.33	2.24 (dd, *J*=1.44; 1.06) and 2.38,1H)	42.42	2.23 (m), 2.28 (m)	42.285
5	-	140.78	-	140.87		140.743
6	5.36 (t)	121.73	5.36 (t)	121.85	5.35 (brd)	121.691
7	1.89 (m),	31.69	1.99 (m)	31.78	1.95, 1.98(o)	31.897
8	2.02(m)	31.89	2.00 (m)	32.05	1.47(o)	32.401
9	0.95 (m)	50.19	0.94 (m)	50.28	0.93(o)	50.143
10	-	36.53	-	36.64		36.498
11	1.07 (m,), 1.49	21.10	1.02 (m)	21.22	1.46 (o), 1.49(o)	21.05
12	1.17 (m), 1.19	39.80	1.16 (m)	39.91	1.16 (o), 1.19(o)	39.761
13	-	42.34	-	42.46		42.305
14	1.03 (m)	56.79	1.00 (m)	56.90	1.06(o)	56.851
15	1.06 (m) and 1.56 (m)	24.38	1.06 (m) and 1.58 (m)	24.51	1.16 (o), 1.43(o)	25.500
16	1.20 (m), 1.88	28.26	1.09 (m)	28.39	1.26 (o), 1.85(o)	28.233
17	1.13 (m)	56.08	1.12 (m)	56.18	1.11(o)	56.096
18	0.86 (s)	12.00	0.85 (s)	12.00	0.68 (s)	12.20
19	1.05 (s)	19.41	0.82 (s)	19.18	1.01 (s)	19.41
20	1.35 (m)	36.16	1,35 (m)	36.30	1.38 (o)	35.865
21	0.92 (d, *J*= 7.2 Hz, 3H)	18.79	0.92 (d, *J*=5.12 Hz, 3H)	18.92	0.92 (d)	18.60
22	1,33 (m), 1.07	33.97	1,33 (m)	34.07	1.02, 1.34 (o)	33.930
23	1.16 (m), 1.18	26.11	1.16 (m)	26.20	1.16, 1.18 (o)	26.062
24	0.94 (m)	45.87	0.94 (m)	45.96	0.92 (o)	45.818
25	1.58 (m)	29.19	1.66 (m)	29.27	1.67 (o)	29.137
26	0.87 (d, *J* =13 Hz)	21.23	0.83(d, *J*=11 Hz)	21.36	0.86 (d)	21.00
27	0.84 (br s)	19.83	0.84 (br s)	19.13	0.90 (d)	18.60
28	1.24 (m), 1.27	23.09	1,25 (m)	23.20	1.23, 1.28 (o)	22.90
29	0.81 (t)	12.06	0.85 (t)	12.19	0.86 (t)	12.01

Notes: s = singlet, d = doublet, t = triplet, m = multiplet, O = overlap

The ^{1}H-NMR spectra of compound two also showed one olefinic proton at δ 5.36 instead of three in compound 1. The absence of protons corresponds to the double bond between C-20/C-21 in compound two and the appearance of mass spectral data showing a double bond at C-5/C-6 in its structure. The ^{1}H-NMR spectra of compound 2 showed a proton corresponding to the proton connected to the C-3 hydroxy group, which appeared as a triplet of doublet of doublets at δ 3.53. The ^{13}C-NMR and COSY, HMQC, and HMBC showed twenty-nine carbon signals, including six methyls, eleven methylenes, ten methanes, and three quaternary carbons.

Thus, the structure of compound two was assigned as β-sitosterol, which was consistent with the reported literature data [19, 20] and was further supported by the key COSY and HMBC correlations.

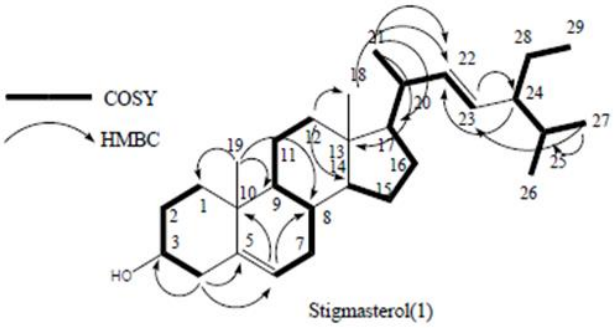

Figure 1 – COSY and HMBC Correlations of Compound 1

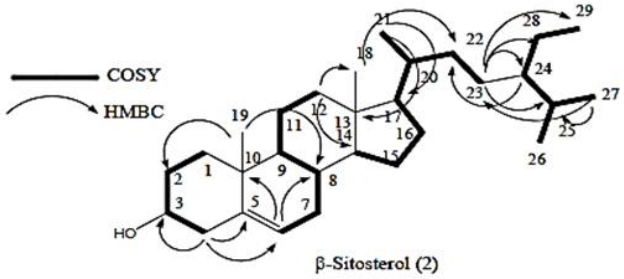

Figure 2 – COSY and HMBC Correlations of Compound 2

^{13}C-NMR Spectrum data shows there were 50 signals overall. The signals at 140.87 (C5), 121.84 (C6), and 140.87 (C5), 121.85 (C6) were carbon double bonds for stigmasterol and β-sitosterol, respectively. The signal at 71.93 was one carbon oxymetin C-sp3 for C3. The presence of carbon double bonds was shown in signals at 8.46 (C22) and 129.39 (C23) for stigmasterol (1). Stigmasterol and β-sitosterol are two types of steroids with similar molecular formulas that differ only at C-22 and C-23. Based on NMR data, including NMR-2D and supported by literature data, compound (1) is a mixture of stigmasterol and β-sitosterol. Stigmasterol and β-sitosterol are two plant sterols that are difficult to separate.

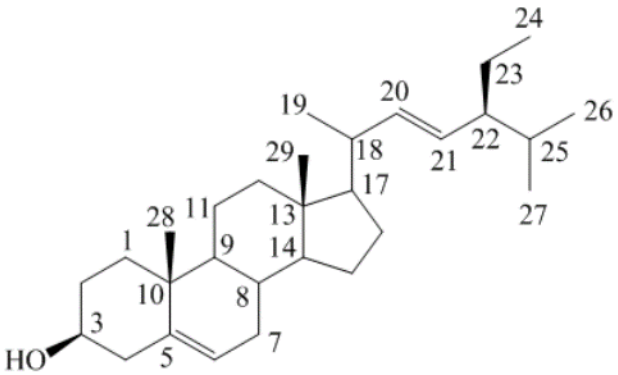

Figure 3 – Structure of Stigmasterol (1)

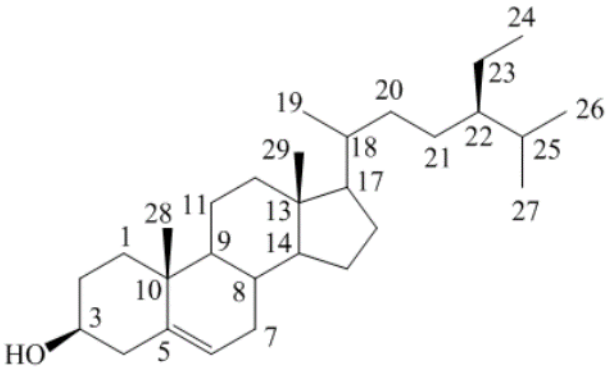

Figure 4 – Structure of β-Sitosterol (2)

CONCLUSIONS

Two sterols were isolated from the bract Musa acuminata colla extract. The researchers identified the structures of the isolated compounds as stigmasterol (compound 1) and β-sitosterol (compound 2) based on spectroscopic data and by comparing their physical properties with those reported in the literature.

The two isolated compounds' complete ^{1}H and ^{13}C-NMR spectral assignments were based on COSY, HSQC, and HMBC data.

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