Overview of Importance and Isolation of Β-Sitosterol From Ethyl Acetate Extracts of *Musa Acuminata Calla* (Banana Blossom)

Linus Usman Akacha [1], **Kevin Shadrach Ndyakalah** [2]

[1] *Adamawa State College of Education, Hong*
P. M. B. 2237, Adamawa State, Nigeria

[2] *Adamawa State College of Agriculture Science and Technology*
P. M. B. 2088, Ganye, Adamawa State, Nigeria

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Corresponding Author:
Linus Usman Akacha
linusakacha@yahoo.com

Abstract. In northern Nigeria, the banana blossom has traditionally been used to treat a variety of ailments, including microbiological infections; it reduces menstrual bleeding, aids in the treatment of diabetes, anaemia, and ulcers, reduces anxiety, aids in weight loss, and produces an undetermined amount of filtrate decoction to facilitate conception. As a result, the study intends to isolate secondary metabolites to identify the bioactive compound(s) found in banana blossoms. The ethyl acetate extract was utilised in column chromatography to isolate the compound. Fractions were collected from the column and subjected to thin-layer chromatography to confirm their purity for component separation using a thin stationary phase supported by an inert backing, yielding BF58 fractions. Researchers characterised the fraction BF 58 using NMR spectroscopy. β-Sitosterol was isolated from Musa acuminata calla.

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

INTRODUCTION

The banana blossom, sometimes called banana heart or banana flower, is a large, teardrop-shaped purple flower that grows at the end of a bunch of bananas on the banana plant (Musa spp.). While the banana fruit is widely recognised and consumed worldwide, the blossom often goes overlooked despite its rich culinary potential [1].

The banana blossom is composed of tightly packed layers of deep purple petals. As the banana fruit develops, the blossom emerges from the end of the banana cluster, gradually unfurling its layers. If left unpicked, it eventually transforms into bananas. However, when harvested at the right stage, usually before the bananas begin to form, the blossom is a culinary delicacy enjoyed in many cultures, particularly in Southeast Asia [1].

Preparing banana blossom for consumption can be labour-intensive due to its numerous tough outer layers and a central core filled with sticky sap. One must carefully peel away the outer layers to reveal the tender, edible inner portions. Once cleaned, the blossom can be sliced, shredded, or chopped and used in various dishes [1]

One popular way to prepare banana blossoms is in salads. The thinly sliced or shredded blossom adds a unique texture and flavour to salads, often paired with onions, tomatoes, herbs, and sometimes seafood. The blossom's mild, slightly bitter taste complements the freshness of the salad ingredients, making for a refreshing and nutritious dish [2].

Banana blossom contains low calories and fat but is rich in fibre, vitamins, and minerals. It contains antioxidants, potassium, calcium, iron, and vitamin E, among other elements, making it an excellent supplement to any diet. In addition to its culinary usage, banana blossom has therapeutic qualities. In some traditional medicine practices, extracts from the blossom are used to treat various ailments, including menstrual disorders, ulcers, and respiratory problems. While more research is needed to fully understand its medicinal potential, banana blossom continues to be valued for its health benefits in many cultures [3].

It also indicates that banana blooms of various types have little protein [4]. Banana roots are used for culinary purposes in Malaysia, Indonesia, India and Sri Lanka. It is also quoted by [4]. The flowers can cure diarrhoea, ulcers, and other ailments. Banana blossoms can also be utilised as an alternative to diabetes treatment since they are enzyme inhibitors obtained from plant extract. According to prior research, scientists have reported that blooms have antioxidative and antibacterial effects [5].

Biological activities of banana blossom. The data on various biological activities of several edible flowers have shown potential health-promoting benefits for humans. The banana blossom has also demonstrated biological activities that might symbolise its potential health-enhancing properties. Many research outcomes have indicated a positive effect of banana blossom's antioxidant and anti-diabetic properties [6]. 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. The banana blossom also depicted properties like anti-carcinogenic, anti-microbial, cardio-protective activities, and anti-inflammatory due to specific bioactive components present in the inflorescence, discussed in the next section [7]. The nutritional content of banana blossoms plays a different role when tackling health issues and infections.

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 [4]. 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. 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. Food components with antioxidant properties may prevent cardiovascular diseases by inhibiting the oxidative damage to cholesterol [4].

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 Banana Blossom was 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 is air-dried at ambient temperatures to a constant weight over four weeks. It was powdered in a wooden mortar and then blended.

Sample Extraction. The extraction was done using 1 kg of powdered sample, poured into a glass container, and macerated successively with 1200 ml of each 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 [8].

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 [8].

Thin Layer Chromatography (TLC). The plant's hexane, ethyl acetate, and methanol extracts were subjected to thin-layer chromatographic analysis to find the solvent system and better separate the components; this will be achieved using pre-coated TLC plates. Researchers spotted a micro quantity of the sample solution on TLC pre-coated (MERCK) plates. Considering their polarity, they developed it with various ratios of organic solvents (hexane, ethyl acetate, methanol, chloroform). Researchers considered the solvent system, which separates the components with high resolution, and visualised the plates under visible and UV light (366 and 254 nm). Researchers 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 ($\frac{\text{distance moved by the solvent}}{\text{distance moved by sample}}$) was pooled and concentrated, and researchers recorded the spots and observations. [8]

Spectroscopic Measurement. Spectroscopic analysis of the final products of the fractions was carried out. Characterisation using NMR-spectrophotometer at SIPBS, University of Strathclyde, Glasgow, UK. on a JEOL-LA-400MHz FT-NMR spectrophotometer. Fourier-transform infrared (F-TIR) was carried out using Shimadzu FTIR-8400S Spectrophotometer and mass spectroscopy.

RESULTS AND DISCUSSION

Characterisation of BF 58 as β-Sitosterol. The compound was obtained as colourless needles with melting points 134-137 °C and on the TLC plate gave the Rf of 0.78 using the solvent system mixture of ethyl acetate/diethyl ether (50:50). The ^{1}H-NMR spectrum revealed one proton multiplet at δH-3.51, the multiplicity and position indicate three protons of the steroid nucleus. The typical six protons of the steroidal skeleton were evident of a doublet at δH- 5.36 integrated with one proton. The spectral also showed doublets at δH-0.83 and δH-0.87, which could be attributed to methyl groups at Carbon 26 and 27, and other methyl groups could be assigned to carbon 21 and 29 at δH-0.93 and δH-0.85. At the same time, The chemical shift values were similar to those of the stigmasterol, except that two Olefinic protons were absent while methylene signals of δH-1.05 and δH-1.19 were present at H-22 and H-23, respectively. The spectral properties obtained for β-Sitosterol are consistent with the literature [9–13].

Table 1 – Experimental and Literature data for β-Sitosterol ^{1}H-NMR

Position	Experimental data ^{1}H(δ ppm)	[11] ^{1}H(δ ppm)	[13] ^{1}H(δ ppm)	[9] ^{1}H(δ pm)
	1.09	1.13		1.08
2	1.84	1.56		1.85
3	3.51	3.58	3.51	3.50
4	2.29	2.34		2.28
5				
6	5.36	5.40	5.34	5.35
7	1.54	1.50		1.54
8	2.03	2.03		2.0
9	0.98	0.98		0.96
10				
11	1.55	1.55		1.54
12	1.16	1.21		1.16
13				
14	1.05	1.04		1.04
15	1.61	1.63		1.61
16	1.86	1.89		1.86
17	1.14	1.16		1.13
18	0.70	0.74	0.67	0.68
19	1.01	1.06	1.0	1.01
20	1.35	1.40		1.35
21	0.93	0.93	0.92	0.93
22	1.05	1.07		1.04
23	1.19	1.21		1.19
24	0.95	0.97		0.95
25	1.71	1.71		1.71
26	0.83	0.88	0.83	0.82
27	0.87	0.87	0.79	0.83
28	1.28	1.31		1.27
29	0.85	0.89	0.85	0.85
30	OH(at C3 position)			

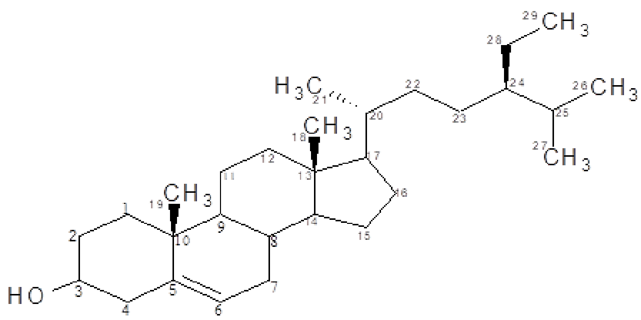

Figure 1 – β-Sitosterol structure

Overview Beta-sitosterol importance. Beta-sitosterol is a sterol found in almost all plants. It is one of the main subcomponents of a group of plant sterols known as phytosterols that are very similar in composition to cholesterol. These plant sterols are the active ingredients in popular margarine spreads (Take Control, Benecol) used to lower cholesterol. Beta-sitosterol is found in rice bran, wheat germ, peanuts, corn oils, soybeans and banana blossom. High levels are also found in botanicals such as saw palmetto, rye grass pollen, pygeum, and stinging nettles, which are beneficial for Prostate enlargement or benign prostatic hyperplasia (BPH). Unlike cholesterol, beta-sitosterol cannot be converted to testosterone. It also inhibits aromatase and 5-alpha-reductase. Beta-sitosterol is likely one of the reasons eating vegetables is good for health. Encourage adequate consumption of these plants in the diet [14].

Beta-sitosterol is a plant sterol (cholesterol is the principal animal sterol). Sources include rice bran, wheat germ, corn oil, soybeans, peanuts and their products, Serenoa repens, avocados, pumpkin seed, Pygeum africanum, and cashew fruit.

Beta-sitosterol improves BPH. On average, urinary flow increases by 4.5 mL/s; residual urinary volumes decrease by 33.5 mL. The International Prostate Symptom Score (IPSS) shows statistically significant improvement [15].

CONCLUSIONS

From ethyl acetate, exacts of Musa acuminata calla β-sitosterol were isolated by column chromatography and characterised by NMR spectroscopy 1D NMR. Based on the study's findings, the study recommends isolating more bioactive compounds from the plant.

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