Antimicrobial and Isolation of Stigmasterol from Ethyl Acetate Extract of Musa Acuminate Calla Flowers

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Abstract. Banana blossom is traditionally used in northern Nigeria to treat a variety of ailments, including microbiological infections; 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) present in banana flowers. Based on the antimicrobial activity results, the highest inhibitory concentration for ethyl acetate extracts of the Banana blossom flower is 12.5 mg/ml, as indicated below.

Compounds are isolated using column chromatography. Isolation was performed using an ethyl acetate extract of the banana blossom flower. The fractions from the column are subjected to TLC testing to determine the separation of the component combination using a thin stationary phase supported by an inert backing, resulting in BF16 fractions. The goal of TLC is to produce well-defined, well-separated spots. Each component has a retention factor (RF), which joins fractions with equal RF values. We extracted stigmasterol from *Musa acuminata calla* ethyl acetate extracts and analysed it using NMR spectroscopy and FT-IR.

Keywords: Musa acuminata calla; antimicrobial activities; stigmasterol; extraction.

INTRODUCTION

Bananas (including plantains) are one of the world's most significant and oldest food crops, with evidence of cultivation dating back to 4,000 BC in New Guinea, and are thought to have originated in Southeast Asia's hot, tropical regions [1]. Malaysia is believed to be the origin of *Musa acuminata*, which moved to India and Burma, where *Musa balbisiana* is the native species. Malaysia and Indonesia are regarded as the diversity hotspots. Human mobility was most likely the sole cause of dispersal from Asia. There are thousands of domesticated Musa cultivars, and their genetic diversity is high, indicating various origins from diverse wild crosses between two main ancestral species [2]. *Musa acuminata* is the most common among the Musa species [1]. Dwarf Cavendish is the most extensively distributed banana.

*Traditional uses***.** Many conventional banana applications have been extensively documented. For example, the leaf and stem are used to treat diarrhoea; the stem is helpful for asthenia and wounds; and the leaf is useful for inflammation, headache, and rheumatism [3]. Previous studies reported that *Musa Paradisiaca* had antimicrobial and healing activities. Nevertheless, only a few studies have reported on the efficacy of this plant against nematodes – various parts of *Musa Paradisiaca* have been used for medicinal purposes [4]. It has traditionally been used for antidepressant, antibacterial, antihypertensive, antiulcerogenic, urolithiasis, laxatives, antihelmintic, analgesic, antifungal, constipation, wound healing,

fevers, burns, diarrhoea, inflammation, pains and antivenom for snake bites. Flowers are used in dysentery and menorrhagia. Stem juice of fruited plants treats diarrhoea, dysentery, cholera, otalgia, and haemoptysis and flowers in dysentery, diabetes and menorrhagia. The root is used for antihelmintic, blood disorders and venereal diseases [3].

*Banana blossom***.** The plant stem, upon maturing, gives rise to a bunch of rolled leaves and then protrudes as a dark purple bud that is elongated and oval-shaped, known as inflorescence or flower or banana blossom. Blossom has two main parts: Bract is an outer sheath-like substance that ranges in hue from reddish to purple. Florets are the inner little tubular-toothed whitish flowers positioned along the floral stacks. Every time a banana blossom is generated, a banana stem is produced beside it. After removing the fruits from the stem, the flowers and other leftovers of the banana plant are typically discarded [5].

*Florets or flowers***.** The flowers of the banana plant are one of the widely consumed vegetables in several cuisines in Asian countries. The male flowers are usually creamy white, while the stigma is orange or rich golden. The basal flowers have female hands that vary in quantity from 1 to 10 and are placed in 2 rows per bract, while male flowers are found in the upper hands and have around 20 florets arranged in 2 rows per bract. The compound tepal is about 2.5 cm long, white, yellowish, or somewhat purple, with a white or yellow tip and lobes, and is made up of translucent free tepals that are roughly half the length of the compound tepal. It has pale green, yellowish green, or purplish ovaries that are either glabrous or have a few fine hairs near the base. Male buds in the advanced blooming stage are ovoid to turbinate. The stamens and perianth are the same length, and their anthers usually turn pink before dehiscence [5]. South Asians and Southeast Asians use banana flowers raw or steamed with dips (as vegetables) in curries, soups, fried foods, etc. The flavour of the flowers resembles artichokes [6].

Authors [7] the 100 g of banana flower offers a lot of nutritious benefits like 51 kcal, 1.6 g of Protein, 0.6 g of Fat, 9.9 g of Carbohydrates, 5.7 g of Fiber, 56 mg of Calcium, 73.3 mg of Phosphorous, 56.4 mg of Iron, 13 mg of Copper, 553.3 mg of Potassium, 48.7 mg of Magnesium and 1.07 mg of Vitamin E [6].

Common flower uses include allergies, infections, bronchitis, dysentery, joint pain, and improved blood circulation [8]. The flowers are also used to manage diabetes and anaemia, help nursing mothers, boost mood and reduce anxiety, and help reduce free radical activity and menstrual bleeding [6]. The major bioactive principles in the flowers' methanol extract were glycosides, tannins, saponins, phenols, steroids and flavonoids.

Plant collection. Banana Blossom was collected from Song, a local government area in Adamawa State. The plant part was authenticated by the Department of Biology (Botany), School of Physical Science, Modibbo Adama University Yola, Nigeria.

*Sample Preparation***.** The Banana Blossom was thoroughly washed with tap water to remove dust and impurities, while some impurities were carefully picked and rinsed with tap water again. The plants are 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 [9].

Column Chromatography. Extracts of banana flowers 6 g of distilled hexane, ethyl acetate, and methanol extracts were individually adsorbed onto Celite by dissolving the extract in minute volumes of solvent and thoroughly mixing with 10 g of Celite, following which the adsorbed extract was crushed into powdery form and dried entirely. A little piece of cotton wool was inserted at the base of the column and gently tapped with a rubber applicator. A silica gel slurry was created by combining 50 g (230-400 mesh ASTM) with n-hexane and stirring with a glass rod. Allow it to cool for about fifteen minutes before swiftly transferring to the column.

More of the solvent was added to rinse the slurry down the column and tapped with a rubber applicator to make the bed compact and remove any air bubbles. A beaker was placed under the column, and then the tap was allowed to run until the solvent got close to the top level of the bed. The sample was then loaded into the column

carefully while the tap was closed. To start elution, solvent mixtures were added. 20 cm^3 vials were used to collect fractions from the column [9].

Thin Layer Chromatography (TLC). Thin layer chromatographic analysis was performed on the hexane, ethyl acetate, and methanol plant extracts to determine which solvent system would better separate the components. This will enable the use of pre-coated TLC plates. A small sample solution was spotted on TLC pre-coated (MERCK) plates and developed with several ratios of organic solvents (hexane, ethyl acetate, methanol, and chloroform), considering their polarity. The solvent system that can separate components with high resolution was considered. The plates were illuminated with visible and UV light (366 and 254 nm, respectively). The plates were sprayed with 10% sulphuric acid in methanol and heated at 100 °C for 1–5 minutes. The fractions were taken from the column that TLC monitored. The fractions with the same retention factor Rf values $\left(\frac{\text{distance move by solvent}}{\text{distance move by sample}}\right)$ was pooled and concentrated, and the spots and observations were recorded [9].

Spectroscopic Measurement. The final products of the fractions BF 16 were analysed spectroscopically using a JEOL-LA-400 MHz FT-NMR spectrophotometer at SIPBS, University of Strathclyde, Glasgow, UK. Fourier-transform infrared (F-TIR) measurements were performed using a Shimadzu FTIR-8400 S Spectrophotometer.

*The antimicrobial screening***.** The antimicrobial activities of the BF16 compound were determined using some pathogenic microbes. The microbes were obtained from the Department of Medical Microbiology ABU teaching hospital Zaria.

The 0.001 mg of the compound was weighed and dissolved in 10 ml of DMSO to obtain a concentration of 100 µg/ml. This was the initial concentration of the compound used to determine its antimicrobial activities. The diffusion method was used to screen the compound. Mueller Hinton agar and Sabouraud dextrose agar were the media used to grow the microbes. The media were prepared according to the manufacturer's instructions, sterilised at 121 °C for 15 mins, poured into the sterile Petri dishes, and allowed to cool and solidify.

The sterilised media were seeded with 0.1 ml of the standard inoculum of the test microbe, and the inoculum was spread evenly over the surface of the media with a sterile swab. A standard cork borer of 6 mm in diameter was used to cut a well at the centre of each inoculated media. 0.1 ml of solution of the 100 µg/ml concentration compound was then introduced into the well on the inoculated media. Incubation was made at 37 °C for 24 hr for the bacteria and at 30 °C for 1-7 days for the fungi, after which the plates of the media were observed for the zone of inhibition of growth. The zone was measured with a transparent ruler and recorded in millimetres [10].

Minimum Inhibition Concentration. The minimum inhibition concentration of the compound was determined using the broth dilution method. Mueller Hinton broth and Sabouraud dextrose broth were prepared, 10 ml were dispensed into test tubes, sterilised at 121 °C for 15 mins, and the broth was allowed to cool. MC-farland's turbidity standard scale number 0.5 was prepared to give a solution. Normal saline was prepared, 10 ml was dispensed into a sterile test tube, and the microbe was inoculated and incubated at 37 °C for 6 hrs. Dilution of the microbe was done in the normal saline until the turbidity marched that of the MC-Farland's scale by visual comparison at this point, the test microbe has a concentration of about $1.5X10^8$c/ml.

Two-fold serial dilution of the compound was done in the sterile broth to obtain 100, 50, 25, 12.5 and 6.25 µg/m concentrations. The initial concentration was obtained by dissolving 0.001 mg of the compound in 10 ml of the sterile broth. Having obtained the different concentrations of the compound in the sterile broth, 0.1 ml of the test microbe in the normal saline was then inoculated into the other concentrations; incubation was made at 37 °C for 24 hrs for the bacteria and at 30 °C for the fungi, after which the test tubes of the broth were observed for turbidity (growth) the lowest concentration of the compound in the sterile broth which shows no turbidity was recorded as the minimum inhibition concentration [10].

Minimum bactericidal /fungicidal concentration (MBC/MFC). MBC/MFC were carried out to determine whether the test microbes were killed or only their growth was inhibited. Mueller Hinton agar and Sabouraud dextrose agar were prepared and sterilised at 121 °C for 15 mins, poured into sterile Petri dishes, and cooled solidly. The contents of the MIC in the serial dilutions were then subcultured onto the prepared media;

incubation was made at 37 °C for 24 hrs for the bacteria and at 30 °C for the fungi, after which the plates of the press were observed for colony growth, MBC/MFC were the plates with the lowest concentration of the compound without colony growth [10].

RESULTS AND DISCUSSION

Yield of extract banana blossom. The air-dried powdered Banana blossom flowers (1 kg) were extracted by excellent maceration method to yield Ethyl acetate 9.2 g dark green (529 nm) Colorimeter was used to identify the colour wavelength above. The solvent was removed by evaporating the extracts in a rotary evaporator under vacuum. To get rid of any solvent.

Fractions BF15 with a retention factor of 0.55 were sent for analysis with the confirmation of thin layer chromatography using a solvent system of hexane and ethyl acetate (1:2) 20 ml displayed apparent single spots that were also confirmed by antimicrobial tests (zone of inhibition/sensitivity test, MIC, and MBFC).

Table 1 – Zone of inhibition/sensitivity test of the compound and the control against the test microorganism

Test Organism	BF16	Ciprofloxacin	Fluconazole	Fulcin
MRSA	22 (S)	34 (S)	0 (R)	0 (R)
VRE	0 (R)	0 (R)	0 (R)	0 (R)
Staphylococcus aureus	27 (S)	30 (S)	0 (R)	0 (R)
Escherichia coli	0 (R)	40 (S)	0 (R)	0 (R)
Klebsiella pneumonia	0 (R)	0 (R)	0 (R)	0 (R)
Proteus mirabilis	28 (S)	32 (S)	0 (R)	0 (R)
Pseudomonas aeruginosa	26 (S)	0 (R)	0 (R)	0 (R)
Candida albicans	28 (S)	0 (R)	34 (S)	0 (R)
Candida krusei	22 (S)	0 (R)	32 (S)	0 (R)
Aspergillus fumigatus	0 (R)	0 (R)	0 (R)	32 (S)
Aspergillus flavus	0 (R)	0 (R)	0 (R)	30 (S)
Microsporum canis	25 (S)	0 (R)	0 (R)	29 (S)

Notes: S – Sensitive=Resistance

Table 1 deals with pathogens to determine whether they are sensitive to fractions collected from the plant extract and their zone of inhibition. It was determined that the fractions BF 15 show the zone of inhibition (ZOI) sensitivity against *Methicillin-resistant staph aureus (*MRVA*)* 22 mm and 24 mm, respectively. In comparison, the drug control (Ciprofloxacin) is 34 mm. *Staphylococcus aureus* is 27 mm and 22 mm, the drug control (Ciprofloxacin) is 30 mm. *Candida albicans is* 28 mm and 26 mm, respectively, while the drug control (Fluconazole) is 34 mm. *Candida krusei* is 22 mm and 24 mm, respectively, while the drug control (Fluconazole) is 32 mm. *Microsporum canis* is 25 mm and 20 mm, respectively, while the drug control (Fulcin) is 29 mm.

BF16 revealed *Escherichia coli* 27 mm while 40 mm drug control (Ciprofloxacin) showed *Proteus mirabilis* BF15 28 mm while 34 mm drug control (Ciprofloxacin), *Aspergillus flavus*. *Vancomycin-resistant enterococci* (VRE*) Klebsiella pneumonia* and *Aspergillus fumigatus* were resistant to the fractions. *Pseudomonas aeruginosa* of BF15 show sensitivity of 26 mm and 23 mm, respectively.

The fractions' minimum inhibition concentration (MIC) ranged from 6.25 to 100 µg/ml. The *Escherichia coli,* and *Candida albicans* have the highest minimum inhibition concentration of 12.5 µg/ml in BF 25 and *Staphylococcus aureus*, *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Candida albicans* 12.5 µg/ml in BF15.

The lowest minimum inhibition concentration of *Methicillin resistant staph aureus* (MRVA), *Staphylococcus aureus, Pseudomonas aeruginosa, Candida krusei, Aspergillus flavus, and Microsporum canis* is 25 µg/ml in BF15. While *Vancomycin resistant enterococci* (VRE), *Klebsiella pneumonia*, *Proteus mirabilis* and *Aspergillus fumigatus* in BF15 were resistant to the fraction Table 2.

Table 2 – Minimum inhibition concentration of the compound against the test microorganism, μg/ml

Test Organism	100	50	25	12.5	6.25
MRSA	-	-	0*	+	++
VRE					
Staphylococcus aureus	-	-	0*	+	++
Escherichia coli	-	-	-	0*	+
Klebsiella pneumonia					
Proteus mirabilis					
Pseudomonas aeruginosa	-	-	0*	+	++
Candida albicans	-	-	-	0*	+
Candida krusei	-	-	0*	+	++
Aspergillus fumigatus					
Aspergillus flavus	-	-	0*	+	++
Microsporum canis	-	-	0*	+	++

Notes: "-" No turbidity (no growth); "0*" MIC, "+" Turbid (light growth); "++" light turbidity

The fraction's minimum bactericidal/fungicidal concentration ranged from 6.25 to 100 μg/ml. The *Methicillin-resistant staph aureus* (MRVA*)*, *Escherichia coli, Candida albicans* and *Candida krusei* show 50 μg/ml in BF15.

The lowest minimum bactericidal/fungicidal concentration of the *Staphylococcus aureus*, *Pseudomonas aeruginosa, Aspergillus flavus* and *Microsporum canis* is 100 μg/ml in BF15. At the same time, Vancomycin-resistant enterococci (VRE), Klebsiella pneumonia, Proteus mirabilis and Aspergillus fumigatus in BF 16 and Vancomycin-resistant enterococci (VRE) were resistant to the fraction Table 3.

Table 3 – Minimum bactericidal/fungicidal concentration of the compound against the test microorganism, μg/ml

Test Organism	100	50	25	12.5	6.25
MRSA	-	0*	+	++	+++
VRE					
Staphylococcus aureus	0*	+	++	+++	+++
Escherichia coli	-	0*	+	++	+++
Klebsiella pneumonia					
Proteus mirabilis					
Pseudomonas aeruginosa	0*	+	++	+++	+++
Candida albicans	-	0*	+	++	+++
Candida krusei	-	0*	+	++	+++
Aspergillus fumigatus					
Aspergillus flavus	0*	+	++	+++	+++
Microsporum canis	0*	+	++	+++	+++

Notes: "-" No colony; "0*" MBC/MFC; "+" Scanty colony growth; "++" Moderate colony growth; "+++" Heavy colony growth.

Characterisation of BF16 as stigmasterol. From the positive tests for steroids given by the compound, it is assumed to be a compound containing a steroidal nucleus. The compound has Rf value of 0.54 (EtAc/Hex: 2/3). Regarding IR spectroscopic analysis, the observed absorption bands are 3456.17, which is characteristic of O-H stretching. Absorption at 973.80 is due to cyclic olefinic –HC= CH- structure and 2871.31 assigned to C-H structure. 1468 is a bending frequency for cyclic CH_2 and 1373.37 for $–CH_3$. The absorption frequency at 728.22 is aromatic. These absorption frequencies resemble the absorption frequencies observed for stigmasterol. The proton NMR showed the proton of H-3 appeared as a multiplet at δ 3.53 ppm and revealed the existence of signals for Olefinic proton at δ5.08 (m), 5.20 (m), and 5.37 (m) - angular methyl proton at 1.03 and 0.72.

Table 4 – Experimental and literature data for stigmasterol ^{1}H NMR

[11]	[12]	^{1}H NMR Experimental data	Type of carbon absorption on FT-IR
3.53 (m, 1 H)	3.53 (m 1 H)	3.53 (m 1 H)	CH
5.38 (s, 1 H)	5.38 (s, 1 H)	5.37 (s, 1 H)	C=CH
1.29 (d, 3 H)	1.29 (d, 3 H)	1.28 (d, 3H)	CH_3
0.74 (d, 3 H)	0.74 (d, 3 H)	0.72 (d, 3H)	CH_3
1.20 (d, 3 H)	1.20 (d, 3 H)	1.21 (d, 3H)	CH_3
5.07 (m, 1 H)	5.07 (m, 1 H)	5.08 (m, 1 H)	C=C
5.20 (m, 1 H)	5.20 (m, 1 H)	5.20 (m, 1 H)	C=C
0.84 (d, 3 H)	0.84 (d, 3 H)	0.83 (d, 3 H)	CH_3
0.97 (d, 3 H)	0.97 (d, 3 H)	0.98 (d, 3 H)	CH_3
1.04 (t, 3 H)	1.40 (t, 3 H)	1.03 (t, 3 H)	CH_3

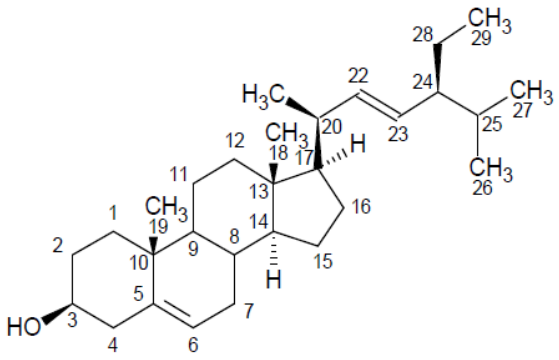

Figure – Stigmaterol

CONCLUSIONS

Musa acuminate calla flower fraction was tested against seven bacteria and five fungi for its antimicrobial activities. The sample fraction was compared with the positive control. The ethyl acetate fraction of plant isolate has the highest minimum inhibitory concentration, 12.5 mg/ml. The sterols were isolated from the commercial extract obtained from the *Musa acuminate calla* flower. The structure of the isolated new compound was identified as stigmasterol based on FT-IR, spectroscopic and by comparing their physical properties reported in the literature.

The study of *Musa acuminate calla* has yielded three known compounds. It is recommended that more work should be carried out on the plant to isolate more compounds. Other assays such as antimalarial, anthelmintic, anti-inflammatory and cytotoxicity tests may be carried out on the extracts.

REFERENCES

1. Ghosh, S. (2022). *Tropical fruit crop: Theory to Practical*. New Delhi: Jaya.
2. Elaveniya, E., & Jayamuthunagai, J. (2014). Functional, Physicochemical and Anti-oxidant properties of Dehydrated Banana Blossom Powder and its Incorporation in Biscuits. *International Journal of Chemtech Research*, *6*(9), 4446-4454.
3. Marie-Magdeleine, C., Udino, L., Philibert, L., Bocage, B., & Archimede, H. (2014). In vitro effects of Musa x paradisiaca extracts on four developmental stages of Haemonchus contortus. *Research in Veterinary Science, 96*(1), 127–132. doi: 10.1016/j.rvsc.2013.12.004
4. Newman, D. J., & Cragg, G. M. (2020). Natural Products as Sources of New Drugs over the Nearly Four Decades from 01/1981 to 09/2019. *Journal of Natural Products, 83*(3), 770–803. doi: 10.1021/acs.jnatprod.9b01285
5. Mathew, N. S., & Negi, P. S. (2017). Traditional uses, phytochemistry and pharmacology of wild banana (Musa acuminata Colla): A review. *Journal of Ethnopharmacology, 196*, 124–140. doi: 10.1016/j.jep.2016.12.009
6. Netmeds.com. (2020). *7 Brilliant Health Benefits of Banana Flowers*. Retrieved from https://www.youtube.com/watch?v=1NqHaLiiLGc&ab_channel=Netmeds.com
7. Shwetha, A., Humeera, T., & Preethi, B. (2019). *Review on: Production of Natural Banana Blossom Concentrate*. Retrieved from https://www.researchgate.net/profile/Humeera-Tazeen/publication/333641058_REVIEW_ON_PRODUCTION_OF_NATURAL_BANANA_BLOSSOM_CONCENTRATE/links/5cf8a75c4585153c3db7408a/REVIEW-ON-PRODUCTION-OF-NATURAL-BANANA-BLOSSOM-CONCENTRATE.pdf
8. Liyanage, R., Gunasegaram, S., Visvanathan, R., Jayathilake, C., Weththasinghe, P., Jayawardana, B. C., & Vidanarachchi, J. K. (2016). Banana Blossom (Musa acuminate Colla) Incorporated Experimental Diets Modulate Serum Cholesterol and Serum Glucose Level in Wistar Rats Fed with Cholesterol. *Cholesterol, 2016*, 1–6. doi: 10.1155/2016/9747412
9. Cyprian, T. A., Sewuese, S., & Akacha, L. U. (2019). Preliminary Phytochemical Screening and Thin Layer Chromatography Analysis of Stem Bark Extracts of African Mistletoe Parasitic on Vitellaria paradoxa, Piliostigma thonningii and Combretum fragrans. *Asian Journal of Applied Chemistry Research*, 1–6. doi: 10.9734/ajacr/2019/v3i230087

10. Dikko, Y., Khan, M., Tor-Anyiin, T., Anyam, J., & Linus, U. (2016). In vitro Antimicrobial Activity of Fruit Pulp Extracts of Azanza garckeana (F. Hoffm.) Exell & Hillc. and Isolation of One of its Active Principles, Betulinic Acid. *British Journal of Pharmaceutical Research, 14*(1), 1–10. doi: 10.9734/bjpr/2016/30152

11. Okoro, I., Tor-Anyiin, T., Igoli, J., Noundou, X., & Krause, R. (2018). Isolation and Characterisation of Stigmasterol and β–Sitosterol from Anthocleista djalonensis A. Chev. *Asian Journal of Chemical Sciences, 3*(4), 1–5. doi: 10.9734/ajocs/2017/37147

12. Pierre, L. L., & Moses, M. N. (2015). Isolation and Characterisation of Stigmasterol and B - Sitosterolfrom Odontonema Strictum (Acanthaceae). *Journal of Innovations in Pharmaceuticals and Biological Science, 2*(1), 88-95.