British Journal of Pharmaceutical Research
10(1): 1-9, 2016, Article no.BJPR.22593
ISSN: 2231-2919, NLM ID: 101631759

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Phytochemical and Antimicrobial Analysis of Banana Pseudo Stem (*Musa acuminata*)

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Authors' contributions

This research was carried out through joint collaboration of all authors. Their responsibilities spanned through the design of the study, literature survey, experimental, writing protocols, preparation of manuscript, financing and proof reading of approved manuscript.

Article Information

DOI: 10.9734/BJPR/2016/22593
Editor(s):
(1) Sami Nazzal, College of Pharmacy, University of Louisiana at Monroe, USA.
(2) Faiyaz Shakeel, King Saud University, Riyadh, Saudi Arabia.
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Reviewers:
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(2) Anonymous, Universidad de Los Andes, Merida, Venezuela.
(3) Charu Gupta, Amity University, UP, India
Complete Peer review History: http://sciencedomain.org/review-history/12871

Original Research Article

Received 13th October 2015
Accepted 19th December 2015
Published 1st January 2016

ABSTRACT

Preliminary phytochemical screening of extracts of banana pseudo stem and antimicrobial activities revealed the presence of phenolic compounds, flavonoids, terpenoids, alkaloids, protein, tannins, saponins, cardiac glycosides. The phytochemical constituents were: Alkaloid (8.16%), Flavonoids (4.02%), Saponin (3.5%), Phenols (5.5743 mg/kg, Tannin (9.13%), Oxalate (0.162%), Heamaglutinin (1.8814 mg/kg), Phytate (1.2967 mg/kg), and Cardiac Glycoside (1.6%). Some of the extracts inhibited the growth of pathogens while some did not. Water extract notably had a high Zone Inhibition Diameter of 21 mm to *Streptococcus aureus* and 17 mm to *Pseudomonas aerugenosa*. The results obtained indicated that the pseudo stem of banana plant have pharmaceutical values and can be utilized in treatment of some ailments.

Keywords: Phytochemical constituents; antifungal; antimicrobial activity and banana pseudo stem.

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1. INTRODUCTION

Banana is the common name for herbaceous plants of the genus Musa. Binomial nomenclature of banana plant is *Musa acuminata*. Locally known as '*unere*', bananas come in a variety of sizes and colours when ripe, including yellow, purple, and red. The banana plant is the largest herbaceous flowering plant. Bananas and plantains constitute the fourth most important global food commodity (after rice, wheat and maize) grown in more than 100 countries over a harvested area of approximately 10 million hectares, with an annual production of 88 million tonnes [1]. The all year round fruiting habit of bananas puts the crop in a superior position in bridging the 'hunger gap' between crop harvests. It therefore contributes significantly to food and income security of people engaged in its production and trade, particularly in developing countries. In Africa they provide more than 25% of the carbohydrate requirements for over 70 million people [2]. Eastern and Southern Africa produces over 20 million tonnes of bananas which accounts for 25.58% of the total World output [3]. The Great Lakes region covering parts of Uganda, Rwanda, Burundi, Tanzania, Kenya and DRC is the largest producer and consumer of bananas in Africa [4] where per capita consumption has been estimated at more than 250 kg; the highest in the world [5].

2. MATERIALS AND METHODS

2.1 Sample Collection and Preparation

Matured banana pseudo stem was obtained on 30th July, 2015 during the rainy season from a humus farmland in Amawbia, Awka, South L.G.A of Anambra State. The banana pseudo stem was washed under running tap to remove debris; it was sliced and then air dried. After drying, the sample was ground into powder and stored in an airtight container for future use.

2.2 Preliminary Phytochemical Screening

Phytochemcial screening (quantitative and qualitative) was carried out on water, 50% ethanol, 90% ethanol, 50% methanol and 90% methanol extract of banana pseudo stem using Harbone [6] method for plant analysis. 150 ml of each solvent was used to extract the banana pseudo stem separately and the presence of the phytochemicals: alkaloids, saponins, flavonoids, phenol, steroids, glycosides and tannins were tested. The phytochemicals present were estimated quantitatively.

2.2.1 Qualitative analysis

10 g of the ground sample was weighed into three (3) different labelled conical flasks. 100 ml of the three (3) different solvents (distilled water, 50% methanol and 90% methanol) were poured into the three different conical flasks to extract the phytochemicals. After 24 hrs, the mixtures were filtered into conical flasks using whatmann filter paper (No.1). The filtrates were concentrated by placing the flasks into water bath at 100℃. The resulting filtrate were cooled to room temperature, Qualitative test was then carried out on the cool solution (extract) using [6,7] to ascertain the presence of the different phytochemicals in banana pseudo stem.

2.2.1.1 Test for alkaloids

1.0 ml of the above extract was pipetted into a test tube followed by 5.0 ml of 2% HCl and heated on a waterbath (Memmert) for 10 minutes. The solution was filtered using Whatmann No 1 filter paper and used for Wagner's reagent test.

For the test, 1.0 ml of filtrate was pippetted into a test tube followed by 1.0 ml of Wagner's reagent. A reddish brown precipitate indicated the presence of alkaloids.

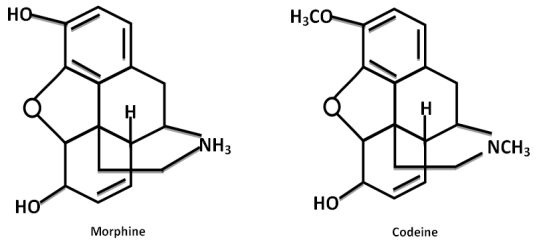

Fig. 1. Basic structures of some pharmacologically important plant derived alkaloids

2.2.1.2 Test for flavonoids

1.0 ml of filtrate was pippetted into a test tube followed by 1.0 ml 10% ferric chloride. Greenish brown or black colour/precipitate is an indication

of presence of phenolic nucleus an indicator of the presence of flavonoids.

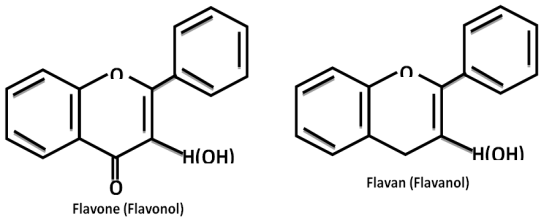

Fig. 2. Basic structures of some pharmacologically important plant derived flavonoids

2.2.1.3 Test for tannins

2.2.1.3.1 Acid test

3.0 ml of the extract was added to 2.0 ml of 1% HCl. The observation of a red colour or precipitate indicated the presence of phlobotannins.

2.2.1.3.2 Lead acetate test

To 2 ml of the extract was added 3 drops of 5% lead acetate solution to the extract. A dark blue to black precipitate indicated presence of phlobotannins.

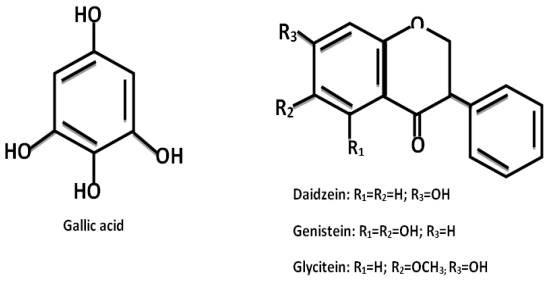

Fig. 3. Basic structures of some pharmacologically important plant derived tannins

2.2.1.4 Test for proteins

To a little portion of the filtrate in a test tube, 2 drops of Million's reagent were added. A white precipitate indicated the presence of protein.

2.2.1.5 Test for saponins

2 g of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. 10 ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion.

2.2.1.6 Test for cardiac glycosides

1 ml of the extract was added to 10 cm^3 of 50% H_2SO_4 and was heated in boiling H_2O for 5 minutes. 10 cm^3 of Fehling's solution (5 cm^3 of each solution A and B) was added and boiled. A brick red precipitate indicating presence of glycosides was observed.

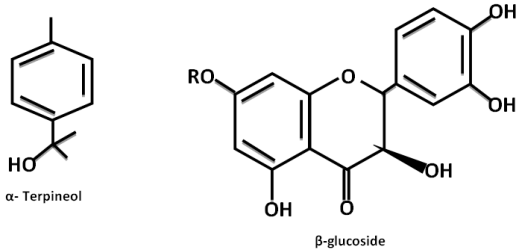

Fig. 4. Basic structures of some pharmacologically important plant derived glycosides

2.2.1.7 Test for steroids

To 0.5 ml of the chloroform, 1 ml concentrated H_2SO_4 was added to form a lower layer. Then the colour at the interface was observed and recorded.

2.2.1.8 Test for terpenoids

5 ml of each extract was mixed in 2 ml of chloroform (Numex, India), and concentrated H_2SO_4 (3 ml) was carefully added to form a layer. A reddish brown coloration of the inner face was formed to show positive results for the presence of terpenoids.

2.2.2 Quantitative analysis

Quantitative analysis were done using various procedures as follows: [8] (phytate), [9] (Tannin), [10] (Saponin), [11] (Flavonoids), [8,10] (Alkaloids and Oxalate).

2.2.2.1 Oxalate determination by titration method

This determination involved 3 major steps digestion, oxalate precipitation and permanganate titration.

2.2.2.1.1 Digestion

2 g of sample was suspended in 190 ml of distilled water in a 250 ml volumetric flask followed by the addition of 10 ml (6 M) HCl. The suspension was digested at 100℃ for 1 hour, Cooled, and then made up to 250 ml mark before filtration.

2.2.2.1.2 Oxalate precipitation

Duplicate portions of 125 ml of the filtrate were measured into beakers and four drops of methyl red indicator added. This was followed by the addition of NH_4OH solution (drop wise) until the test solution changed from salmon pink colour to a faint yellow colour (pH 4-4.5). Each portion was then heated to 90℃, cooled and filtered to remove precipitate containing ferrous ion. The filtrate is again heated to 90℃ and 10 ml of 5% $CaCl_2$ solution was added while being stirred constantly. After heating, it was cooled and left overnight at 25℃. The solution was then centrifuged at 2500 rpm for 5 minutes. The supernatant is decanted and the precipitate completely dissolved in 10 ml of 20% (v/v) H_2SO_4 solution.

2.2.2.1.3 Permanganate titration

At this point, the total filtrate resulting from the digestion was made up to 300 ml. Aliquots of 125 ml of the filtrate was heated until near boiling and then titrated against 0.05 M standardized $KMnO_4$ solution to a faint pink colour which persists for 30 seconds. The calcium oxalate content was calculated using the formula

$$\frac{T \times (Vme)(Df)}{(ME) \times Mf} \text{ (mg/100 g)}$$

Where T is the titre of KMnO4 (ml), Vme is the volume-mass equivalent (i.e. 1 ml of 0.05 M $KMnO_4$ solution is equivalent to 0.00225 g anhydrous oxalic acid). Df is the dilution factor Vt/A (2.4) where Vt is the total volume of titrate (300 ml) and A is the aliquot used (125 ml), ME is the molar equivalent of $KMnO_4$ in oxalate ($KMnO_4$ redox reaction) and Mf is the mass of sample used [6].

2.2.2.2 Alkaloid determination

5 g of the sample was weighed into a 250 ml beaker and 200 ml of 20% acetic acid in ethanol was added and covered and allowed to stand for 4 hours at 25℃. This was filtered with filter pape r No. 42 and the filtrate was concentrated using a water bath (Memmert) to one quarter of the original volume. Concentrated NH_4OH was added drop wise to the extract until the precipitate was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute NH_4OH (1% NH_3 solution), then filtered with pre-weighed filter paper. The residue on the filter paper (alkaloid) was dried in the oven (precision electrothermal model BNP 9052 England) at 80℃. The alkaloid content was calculated and expressed as a percentage of the weight of the sample analysed [7,10].

Calculation: %weight of alkaloid = (Weight of filter paper with residue – weight of filter paper / Weight of sample analysed) ×100

2.2.2.3 Flavonoids determination

10 g of the plant sample was extracted repeatedly with 100ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatmann filter paper No. 42 (125mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight [11].

Calculation: % flavonoids = {(weight of crucible + residue) – (weight of crucible) / Weight of sample analysed}× 100

2.2.2.4 Determination of saponin

5 g of the sample was put into 20% acetic acid in ethanol and allowed to stand in a water bath at 50℃ for 24 hours. This was filtered and the extract was concentrated using a water bath to one-quarter of the original volume. Concentrated NH_4OH was added drop wise to the extract until the precipitate was complete. The whole solution was allowed to settle and the precipitate was collected by filtration and weighed. The saponin content was weighed and calculated in percentage [10].

Calculation: % saponin content = {(Weight of filter paper + residue) – (weight of filter paper) / Weight of sample analysed ×100}

2.2.2.5 Cardiac glycosides determination

Wang and Filled method was used. To 1 ml of extract was added 1 ml of 2% solution of 3,5-DNS (Dinitro Salicylic acid) in methanol and 1 ml

of 5% aqueous NaOH. It was boiled for 2 minutes (until brick-red precipitate was observed) and the boiled sample was filtered. The weight of the filter paper was weighed before filtration. The filter paper with the absorbed residue was dried in an oven at 50℃ till the dryness and weight of the filter paper with residue was noted. The cardiac glycoside was calculated in %.

Calculation: % cardiac glycoside= {(Weight of filter paper + residue) – (weight of filter paper) / Weight of sample analysed × 100}

2.2.2.6 Tannin determination by titration

The Follins Dennis titration method as described by Pearson [9] was used. To 20 g of the crushed sample in a conical flask was added 100 ml of petroleum ether and covered for 24 hours. The sample was then filtered and allowed to stand for 15 minutes allowing petroleum ether to evaporate. It was then re-extracted by soaking in 100 ml of 10% acetic acid in ethanol for 4 hours. The sample was then filtered and the filtrate collected.

25 ml of NH_4OH were added to the filtrate to precipitate the alkaloids. The alkaloids were heated with electric hot plate to remove some of the NH_4OH still in the solution. The remaining volume was measured to be 33 ml. 5 ml of this was taken and 20 ml of ethanol was added to it. It was titrated with 0.1 M NaOH using phenolphthalein as indicator until a pink endpoint is reached. Tannin content was then calculated in % ($C_1V_1=C_2V_2$) molarity.

Calculation:

Data

C_1= concentration of tannic acid
C_2= concentration of base
V_1= volume of tannic acid
V_2= volume of base

Therefore $C_1 = \frac{C_2V_2}{V_1}$

$$\% \text{ of tannic acid content} = \frac{C_1 \times 100}{\text{Weight of sample analysed}}$$

2.2.2.7 Phytate determination

Phytate contents were determined using the Young and Greaves method. 0.2 g of the sample was weighed into different 250 ml conical flasks. Each sample was soaked in 100 ml of 2% concentrated HCl for 3 hours, the sample was then filtered. 50 ml of each filtrate was laced in 250 ml beaker and 100 ml distilled water added to each sample. 10 ml of 0.3% ammonium thiocynate solution was added as indicator and titrated with standard iron (III) chloride solution which contained 0.00195 g iron per 1 ml.

$$\text{Phytic acid} = \frac{\text{Titre value} \times 0.00195 \times 1.19 \times 100}{\text{Weight of sample}}$$

2.2.2.8 Phenol determination

The quantity of phenols is determined using the Spectrophotometer method. The plant sample was boiled with 50 ml of $(CH_3CH_2)_2O$ for 15 minutes. 5 ml of the boiled sample was then pipetted into 50 ml flask and 10 ml of distilled water added. After the addition of distilled water, 2 ml of NH_4OH solution and 5 ml of concentrated $CH_3(CH_2)_3CH_2OH$ was added to the mixture. The samples was made up to the mark and left for 30 minutes to react for colour development and measured at 505 nm wavelength using a spectrophotometer (Apel PD-3000UV spectrophotometer).

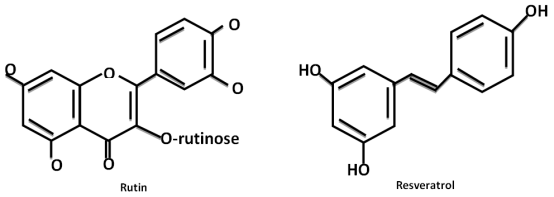

Fig. 5. Basic structures of some pharmacologically important plant derived phenolics

2.2.2.9 Heamaglutinin determination

To 2 g of the sample was added 20 ml of 0.9% NaCl and suspension shaken vigorously for 1 minute. The supernatant were left to stand for 1 hour, the samples were then centrifuged at 2000 rpm for 10 minutes and the suspension filtered. The supernatants in each were collected and used as crude agglutination extract. Absorbance was read at 420 nm.

2.3 Antimicrobial Susceptibility Test

The sensitivity of the various banana pseudo stem extracts against selected test organisms (*Streptococcus sp., Staphylococcus aureus, Escherichia coli*, *Pseudomonas aeruginosa, Aspergillus niger & Candida albican*) was carried out using the agar well diffusion method.

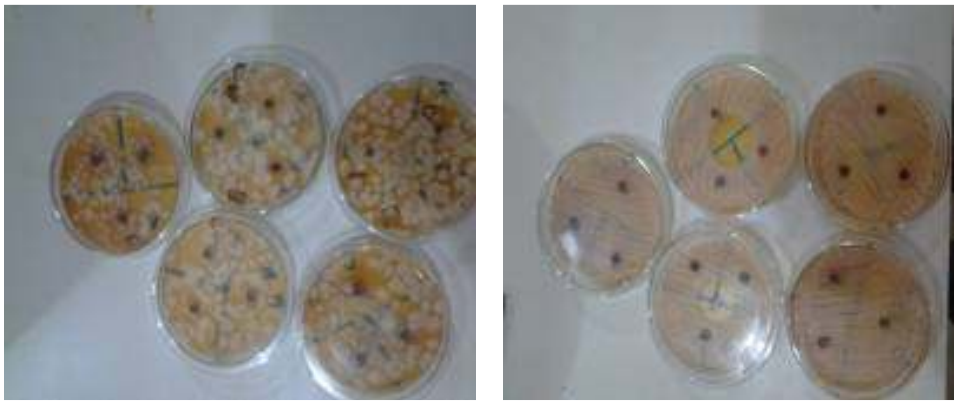

Fig. 6. Zone of inhibition for antifungal organisms

Fig. 7. Zone of inhibition for antibacterial organisms

3. RESULTS AND DISCUSSION

3.1 Phytochemical Screening Result

From Table 1 generally, water extract fared better with respect to amount of phytochemicals found in it except for Cardiac glycoside where it was notably absent. The presence of virtually all the phytochemicals in the water extract connotes that water could be used as the main solvent for the extraction of the metabolites for pharmaceutical purposes. The lapses observed by the absence of cardiac glycoside in the water extract should not be a problem as cardiac glycosides which serve as flavoring agents in pharmaceuticals [12] could be replaced by other flavorings.

Worthy of note also is the efficiency difference between methanol and ethanol. 50% methanol and 50% ethanol showed better efficiency than 90% methanol and 90% ethanol extract as all the phytochemicals were extracted by it unlike 90% methanol and 90% ethanol where alkaloids, tannins and saponins were conspicuously absent in the extract.

Also of interest is the extraction trend associated with the various extracting solvents wherein both 50% and 90% methanol and ethanol had the same Phytochemical constituents. This goes to show that there is the possibility of obtaining the same Phytochemical constituents in different alcohols of the same concentration.

The results in Table *2* show that the banana pseudo stem contains phytochemicals in relative abundance with percentage comparative measure following the trend Tannin>Alkaloids>Flavonoids>Saponins>Cardiac glycosides while weight by weight comparison shows that Phenol> Haemaglutinin>Phytate>Oxalate.

Phytochemicals are known to occur in various parts of plants with diverse functions which include provision of strength to plants, attraction of insects for pollination and feeding, defence against predators, provision of colour, while

Table 1. Result of qualitative analysis of various solvent extracts of banana pseudo stem

S/N	Phytochemical	50% Methanol	90% Methanol	Water extract	50% Ethanol	90% Ethanol
1	Alkaloid	+	-	+	+	-
2	Cardiac glycoside	+	++	-	+	++
3	Flavonoid	++	+	+++	++	+
4	Phenol	+	+	+	+	-
5	Protein	++	++	++	+	+
6	Saponin	+	-	+	+	-
7	Steroid	++	+	+	++	+
8	Tannin	+	-	+	-	-
9	Terpenoid	++	+	+	+	+

Key: - Absent
+ Present in low concentration
+ + Present in moderate concentration
+ + +Present in high concentration

some are simply waste products [13]. When ingested by animals these secondary metabolites exhibit varied biochemical and pharmacological actions [14]. The presence of tannins in diets for livestock have been reported to have anti-nutritional and toxic effects including reduced fed intake, growth, feed efficiency and net metabolizable energy [15].

Table 2. Result of quantitative analysis of various fractions of banana pseudo stem

S/N	Phytochemical	Quantity
1	Alkaloids	8.16%
2	Cardiac glycosides	1.6%
3	Flavonoid	4.02%
4	Heamaglutinin	1.8814 mg/kg
5	Oxalate	0.1620 mg/Kg
6	Phenol	5.5743 mg/kg
7	Phytate	1.2976 mg/kg
8	Saponin	3.5%
9	Tannin	9.13%

Tannins in plants have been shown to confer antidiarrhoeic and anti-haemorrhagic properties on plants [16]. This is consistent with the traditional use of the pseudo stem of *Musa acuminata* for the treatment of diarrhoea, fresh wounds, cuts and insect bites as they have shown to contain these phytochemicals. Saponins have been reported to have antifungal properties [17] as well as serve as an expectorant and emulsifying agent [18]. Alkaloids, flavonoids and tannins have been known to show medicinal activity as well as exhibiting physiological activity [19]. Flavonoids are known to have antioxidant effects and have been shown to inhibit the initiation, promotion and progression of tumours [20]. The presence of these phytochemicals in the banana pseudo stem confers medicinal properties on the plant and this explains the use of this plant for treatment of different ailments. The findings of this study are consistent with reports of the presence of these phytochemicals in various parts of the banana plant as documented by [21].

3.2 Antimicrobial Test Results

The results of the antibacterial activity of the aqueous and methanolic extracts of banana pseudo stem against the test organisms are shown in Table 3. The zone of inhibition of the growth of the isolates was found to be a function of the relative antibacterial potency of the extracts. Thus zones of inhibition decreased as the concentration of the extracts decreased. Higher growth inhibition was obtained with the water extract compared with methanol extracts. In Table 3 the antibacterial activity of the aqueous extract of banana pseudo stem revealed the highest zone of inhibition to be 21 mm against *Streptococcus sp.* compared to 14 mm of methanolic extract. The bacteria *Staphylococcus aureus and Escherichia coli* were not inhibited with the water extract and thus showed no zone of inhibition.

Microorganisms vary widely in their degree of susceptibility to anti-microbial agents. A high MIC value indicates low activity and vice versa. In this study the gram- negative organisms had the lowest MICs and MBCs. This suggests their higher susceptibility to the extract of the banana pseudo stem. On the basis of the result obtained in this investigation it can be concluded that water extract of *Musa acuminata* pseudo stem had significant *in vitro* broad spectrum antimicrobial activity although do not really compete with the standard drug. Nevertheless,

Table 3. Result of the antibacterial activity of various fractions of banana pseudo stem extract

	Banana extracts IZD (mm)			
Extract	***Pseudomonas aerugenosa***	***Escherichia Coli***	***Staphylococcus aureus***	***Streptococcus spp.***
Water	*17*	*0*	*0*	*21*
50% Methanol	0	0	16	14
90% Methanol	0	0	0	13
50% Ethanol	0	0	0	18
90% Ethanol	0	18	0	13
Ampicillin	-	-	27	28
Ciprofloxacin	54	26	-	-

the extracts from the plant can be used to control infections caused by *Pseudomonas aerugenosa & Streptococcus sp.* for water extract and *Escherichia coli and Staphylococcus aureus* for 50% methanol.

Table 4. Result of the antifungal activity of various fractions of banana pseudo stem extract

	Banana extracts/IZD (mm)	
Extract	***Candida albicans***	***Aspergilus niger***
Water	0	0
50% Methanol	0	0
90% Methanol	0	7
50% Ethanol	0	0
90% Ethanol	0	2
Ketoconazole 50 µg/mL	30	0

According to Ibeh et al. [22], an inhibitory zone diameter of 11-15 mm shows intermediate effect while 26 mm and above indicated that the organism was susceptible to the compound. Therefore with the extracts having an inhibitory zone of 0-7 mm, we could infer that these extracts do not have any antifungal effect on the test organisms.

4. CONCLUSION

The aqueous, ethanol and methanolic extracts of banana pseudo stem have been found to contain significant amount of phytochemicals. Water, ethanol and methanol (50%) extracts were effective against some of antibacterial organisms. All the extracts however did not show any activity against fungal test organisms.

5. RECOMMENDATION

Further work could be done to isolate specific secondary metabolites present in this plant and structurally elucidate them.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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