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In Vitro Anticancer Study of Bioactive Compound Isolated from Musa Extract (Musa Acuminata)

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Abstract

Banana tree being one of the most economically important tree worldwide is cut once the fruits are harvested. However other parts of the tree are used in medicine worldwide. In this study, the flower extract of *Musa acuminata*is extracted by maceration with methanol and the phenolic and aliphatic compounds present in the flower extract is identified using GC-MS analysis. Phytochemical screening of the banana extract showed the presence of alkaloids, phlobatanning, triterpanoids, flavanoids, lipids, steroids and terpenoids. Potent antibacterial activity is observed from the flower extract against the tested gram positive and negative bacteria. Anticancer activity of the flower extract is assessed on the cervical cancer cell line HeLa. MTT assay is used to evaluate the antiproliferative effects. This study concluded that the economically important *Musa acuminata*is widely used in anticancer studies worldwide.

Keywords: *Musaacuminata, Phenolic compounds, GC-MS analysis, Phytochemical screening, Antibacterial activity, MTT assay.*

Introduction

Recently, *Musa sp*, (Musaceae) also known as Banana has evolved to be one of the largest herbaceous flowering plant recognized by the world. *Musa sp*, being a most popular fruit are widely exported to a number of industrialized countries [1]. Tropical and sub-tropical regions of the world consume more banana in common. Having south western pacific as native, banana is recognized as a tropical fruit.[2] Almost every part of banana plant has got its own significant use that is useful to mankind in many aspects. Being highly polymorphous *Musa acuminata* is spindly plants that are grown in clumps. Musa species is widely used in medicinal and traditional food. The optimum temperature for the growth of *Musa acuminata* is 80˚F (26.67°C) and the optimum mean rainfall is 4 in (10 cm) per month.

Being under the kingdom Plantae and coming under the order Zingiberales it belongs to the family Musaceae having genus Musa has a wide range of sub species. It is a perennial monocotyledonous herb. Musa is an bisexual flower having each spathe encloses 2 rows of flowers, upper spathe enclosing male flowers, lower spathe enclosing female flowers, and few middle spathe with bisexual flowers. The flowers are tricarpellary, syncarpous, ovary inferior, placentation axile, style long, stigma capitated. [3] It takes approximately 28 days for *Musa acuminata* for anthesis, 2 to 3 weeks after plantation.

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It has high protective action and is widely used to prevent food spoilage. The blossoms of banana to a wide extent is considered as a vegetable and is cooked in various dishes [4]. It is helpful in muscle contraction and response of nerve cells. The banana also enables the stabilization of blood pressure. Being known for its eternal evergreen, Musa species, are widely used for ornamental purposes.

Musa species, highly known for its medicinal purposes is beneficial to mankind. Heart pain, asthma, endocrine problem like diabetes can be treated by the flowers of banana. Stomach cramps and diarrhoea can be treated by consuming banana leaves. Menses pain and bleeding due to menopause can be reduced by the uptake of banana leaves by women. Infantile malnutrition and weak body can be suppressed with the help of banana blossoms. Antioxidants are components that reduce the oxidative stress level by prevention of free radicals from damaging DNA, proteins and lipids. Being a weak primary antioxidant source, *Musa acuminata* has proven to be a powerful secondary antioxidant source[5]. Ascorbic acid, beta carotene, phenolic groups, dopamine are the antioxidant compounds found in *Musa acuminata*. Being a good source of bioactive phytochemicals *Musa acuminata* provide opportunities for functional food industry.

Methodology

Identification and Handling of Sample: Arid flowers of *Musa acuminata* were collected from Sathyamangalam forest. Using plastic bags, the flowers collected were transferred to lab. The powdered and sieved arid flowers are finely powered and is stored in the Non-toxic- polyethylene bag.

Plant Extract Preparation: 10 gm powder mass was extracted using 200 ml Methanol solvent. Dark maceration for 72 hours at 27˚C was considered for the extraction process and muslin cloth was used for filtration. The pasty layer of extract was formed after the filtrate was condensed at 45˚C which were used for further assays.

Phytochemical Screening:

Test for alkaloids: To 3 ml of the extract 1 ml of Mayer's reagent was added and shaken well, presence of Alkaloids was indicated by the white precipitate at the bottom.[12]

Test for phlobatannins: 10 ml of aqueous extract of flower was boiled with 1% HCl. Presence of phlobatannins was indicated by the thick red precipitate deposition in the bottom.[12]

Test for triterpenoids: Salkawasaki Test was used to indicate Triterpenoids in the extract in which 2 ml of extract was taken and 5 drops of concentrated sulphuric acid was added, shaken and allowed to stand. Presence of triterpenoids was indicated by the appearance of greenish blue colour.[12]

Test for flavonoids: Alkaline reagent was used to indicate the presence of flavanoids in the extract. To 1 ml of the extract few drops (3 drops) of 10%NaOH solution was added, flavonoids was indicated by intense yellow colour, which disappeared on addition of a few drops of dilute acid.[12]

Test for lipids: To 10 ml of the extract 0.5 N alcoholic potassium hydroxide was added along with a drop of phenolphthalein. The mixtures were heated on water bath for 1 hour. The presence of lipids was indicated by the formation of foam or soapy layer.[12]

Test for steroids: To 5 ml of aqueous extract 2 ml of chloroform and few drops of concentrated H_2SO_4 was added. The presence of steroids was indicated by the appearance of red colour in the upper layer while yellow with greenish fluorescence appears in the H_2SO_4 layer.[12]

Test for terpenoids: To 1 ml of the aqueous extract 1 ml of chloroform was added and mixed well and left for 5 minutes, 1ml concentrated H_2SO_4 was added after 5 minutes. The presence of terpenoids was indicated by the appearance of greyish layer.[12]

Antioxidant Assay: The antioxidant capacity of *Musa acuminata* was identified through 2,2-Diphenyl-1-picryhydrazyl (DPPH) assay. Equal volumes samples at different concentrations were with 0.1 mM of DPPH. Then the mixture was stored in dark place for 30 minutes. The colour change from violet to yellow indicated the presence of antioxidants. Quantification was calculated by absorbance. The absorbance was performed in triplicates. Ascorbic acid was used as the standard to compare with samples and IC50 (inhibition concentration) was calculated for both sample and standard. The percentage of inhibition was calculated using the following formula.

$$\% \text{ of inhibition} = [A_0\text{-}A_1/A_0] \times 100$$

Where A_0 is absorbance of control (i.e., DPPH solution without sample) and A_1 is absorbance of sample or standard (i.e., DPPH solution with sample/ standard)[12]

GC-MS: The sample was subjected to GC-MS analysis to quantify the number of molecules and its structures. The analysis was carried out using GCMS (Perkin Elmer model: Clarus 680) and also it is equipped with mass spectrometer (Clarus 600(EI) analysed using (TurboMassver5.4.2) software. Fused silica which is packed with Elite-5MS. At a constant flow rate about 1 ml/min, carrier gas such as helium was used to separate the components. The temperature of the injector was adjusted to 260˚C while performing the experiment. The extract sample of 1µL was injected was into the equipment the temperature of the oven were 60˚C(2mins); followed by 300˚C at the rate of 10˚Cmin^{-1}; and 300˚C for 6 mins. The conditions of the mass detector were: the temperature of transfer line was 240˚C: and ionization mode electron impact at 70 eV, the duration time of scan interval is 0.2 sec and scan interval is 0.1 sec. The fragments from 40 to 600 Da. The spectrum of components was corresponding to the database of the spectrum of established components gathered in the GC-MS NIST library.

Antibacterial Assay: Antibacterial activity was determined by agar well-diffusion method. Swabbing using sterile cotton swabs was done on Nutrient agar (NA) plates with 8 hour old-broth culture of different bacteria such as *Streptococcus agalactiae, Bacillus cereus, Staphylococcus aureus, Enterobacteraerogenes, Eschericia coli, Bacillus subtilis*. In each of these plates wells (10 mm diameter and about 2 cm a part) were made using sterile gel puncher. From the methanolic flower extract 1 mg/ml concentration of stock solution was prepared.30 µl of varying concentrations of flower solvent extracts were added by sterile micropipette into the wells and allowed to diffuse at room temperature for 2 hrs. Inoculums without plant extract were set up for control experiments. Incubation of the plates is done at 37˚C for 18-24 h for bacterial pathogen. The zone of inhibition was observed and the diameter of the inhibition zone (mm) was measured and the experiment was also calculated. Triplicates were maintained and the experiment was repeated thrice, for each replicates the readings were taken in three different and the average values were recorded.

Cytotoxic assay (MTT method): The sample was performed with an in vitro Cytotoxicity test method. The culture medium from the Hela cells was replaced with fresh medium. The triplicates of the test sample were added on the cells. Incubation at 37°C for 18 hr was done. After incubation MTT (1 mg/ml) were added in the wells and incubation was done for 4 h. DMSO were added in the wells after incubation and read at 570 nm using photometer. Cytotoxicity and cell viability were calculated by

Cytotoxicity = [(Control-Treated)/Control]×100

Cell Viability = (Treated/Control)×100

Result and Discussion

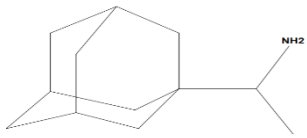
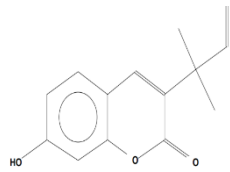
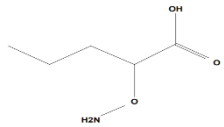
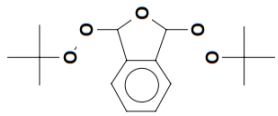
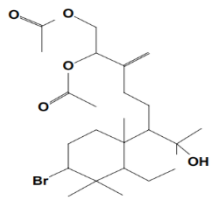
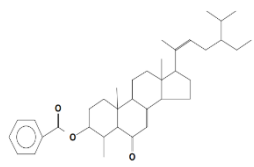
The chemical composition of the extract was studied using GC-MS analysis. The peak was identified as pentanoic acid, 2-(aminooxy)- an aromatic phenolic group of salicylic acid at a retention time of 26.508. The next compound was identified as 1,3-bis-t-butylperoxy-phthalan belonging to aromatic phenolic group of salicylic acid giving a peak at 27.663 retention time. The next peak was observed at 29.364 retention time which was identified as 1,2-pentanediol, 5-(6-bromodecahydro-2-hydroxy-2,5,5a,8a-tetramethyl-1-naphthalenyl)-3-methylene that belongs to the aromatic phenolic groups of salicylic acid. The final peak was observed at 29.544 retention time and the compound was identified as carpesterol dehydrate an aromatic phenolic group of salicylic acid compound.[6] The GCMS analysis result for major phytocomponents in*Musa acuminata* flower is given in Table 1. Chromatogram of compounds present in *Musa acuminata*is represented in Figure 1.

The crude extract of *Musa acuminata* was obtained from maceration with 80% methanol. Antibacterial assay of the flower extract against gram positive and negative bacteria were determined by the formation of zone of inhibition around the wells. The negative control (methanol) showed zero zone of inhibition. Larger zones of inhibition were observed on the plates with 20 µg/ml of Ampicillin (antibiotic) to the methanolic extract tested. The zone of inhibition of *S.aureus* shows greater diameter of 10mm over the flower extract while the zone of inhibition of other bacterial strains ranges from 5mm to 8mm. The antibacterial activity that is the formation of the zone of inhibition in the agar well diffusion method is due to the presence of active compounds in the flower extract.[7-12]Different Bacteria and its zone of inhibition is tabulated in Table 2.The phytochemical constituents of *Musa acuminata*flower extract is tabulated in Table 3.

The MTT assay resulted in the sample showed Slight to Severe Cytotoxic reactivity to Hela cells. The cell death was increased with increase in concentration on the sample. Another experimental study of anthocyanin extracted from *Musa acuminata* bract showed a strong anticancer activity against of MCF-7 cell lines(Breast cancer)[13-15]. Another experimental result tested against carcinoma of cervix (HeLa) showed increased effect on a dose-dependent manner against the extracts from rhizome of *Musa acuminata*.[16-17] The control gave no cytotoxic reactivity.The activity of sample at different concentrations against the cells are displayed in Figure 2, the cytotoxicity is represented in Figure 3 and the cytotoxic reactivity of *Musa acuminata* flower extract displayed in Table 4.

Table 1: GCMS analysis result for major phytocomponentsinMusaacuminata flower

(RT- Retention Time)

RT	Compound Name	Molecular Formula	Molecular Weight	Peak Area	Molecular Structure
25.933	1-adamantanemethylamine, alpha.-methyl-	$C_{12}H_{21}N$	179	3.555	
26.108	7-hydroxy-3-(1,1-dimethylprop-2-enyl)coumarin	$C_{14}H_{14}O_3$	230	4.025	
26.508	pentanoic acid, 2-(aminooxy)-	$C_5H_{11}O_3N$	133	10.832	
27.663	1,3-bis-t-butylperoxy-phthalan	$C_{16}H_{24}O_5$	296	5.798	
29.364	1,2-pentanediol, 5-(6-bromodecahydro-2-hydroxy-2,5,5a,8a-tetramethyl-1-naphthalenyl)-3-methylene	$C_{24}H_{39}O_5Br$	486	18.411	
29.544	Carpesterol Dehydrate	$C_{37}H_{52}O_3$	544	57.378	

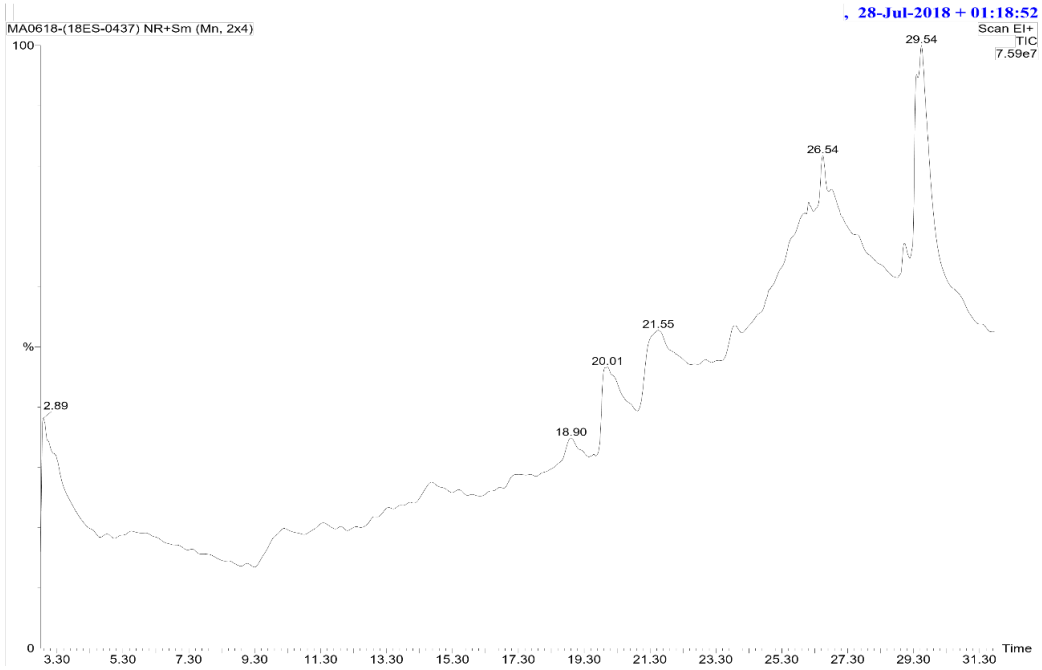


Figure 1: Chromatogram of compounds present in Musa acuminata

Table 2: Different Bacteria and its zone of inhibition

Microorganism	Zone of Inhibition in Extracts (mm)	Zone of Inhibition in Ampicillin (mm)
Streptococcus agalactiae	6	8
Bacillus cereus	5.4	7.1
Staphylococcus aureus	10	12
Enterobacteraerogenes	7	8.1
Eschericia coli	6.6	7.9
Bacillus subtilis	8	9

Table 3:The phytochemical constituents of Musa acuminataflower extract

Phytochemicalconstituents	Presence
Alkaloids	+
Phlobatannins	+
Triterpenoids	+
Flavonoids	+
Lipids	+
Steroids	+
Terpenoids	+

(+ present)

Table 4: Cytotoxic reactivity of Musa acuminata flower extract

Vol (µl)	Cytotoxicity (%)	Cell Viability (%)	Cytotoxic Reactivity
5	16.2	83.8	Slight
25	22.8	72.8	Mild
50	33.2	66.8	Mild
75	59.5	40.5	Moderate
100	71.9	28.1	Severe

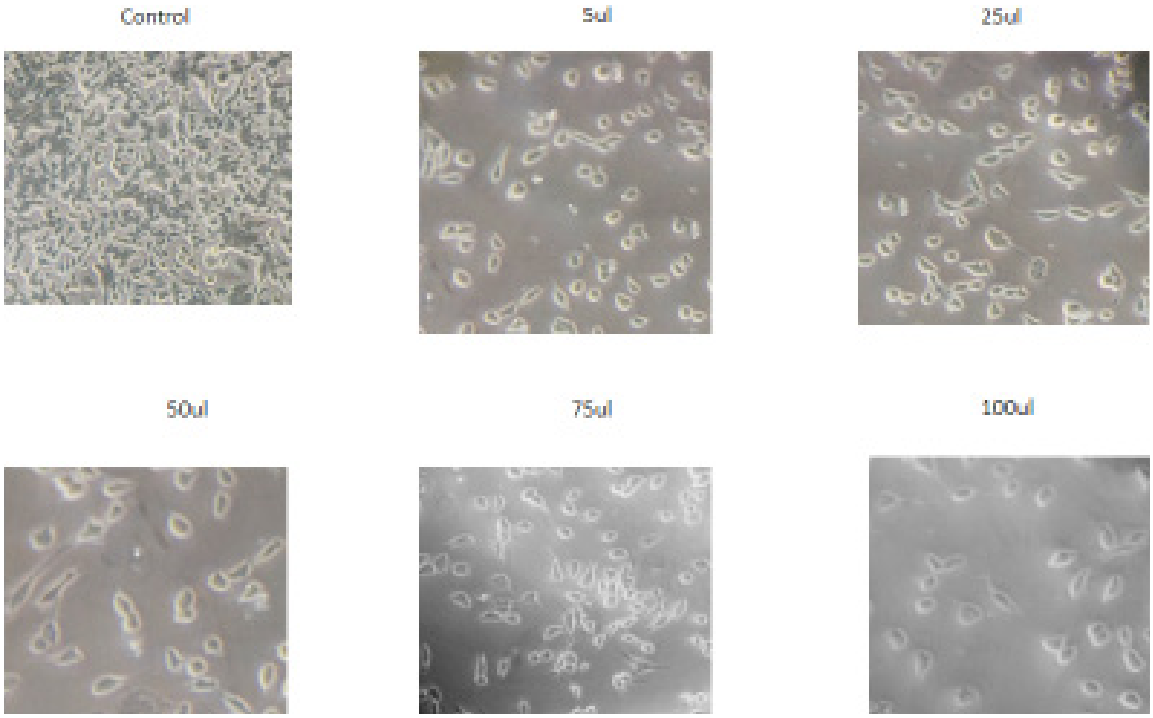


Figure 2: Hela cells reactions at different sample concentrations.

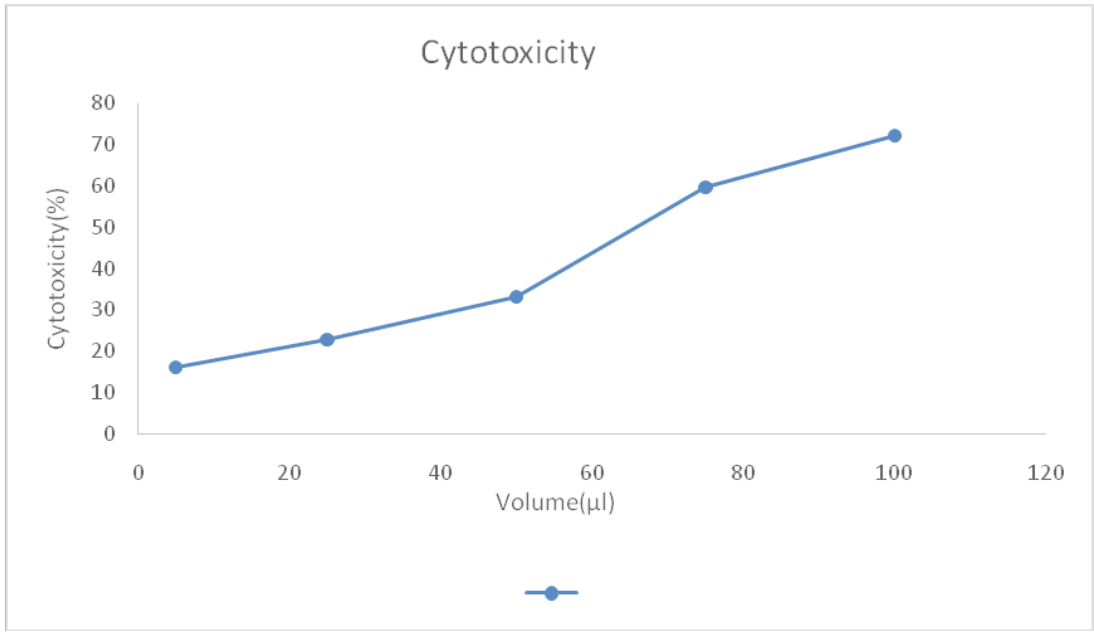


Figure 3: Cell death percentage of Hela cells against Musa acuminata

Conclusion

This study shows us the compounds present in *Musa acuminata*. *Musa acuminata* is rich in phenolic compounds as per this study. Phytochemical study of the extract revealed the presence of certain group of compounds. *Musa acuminata* acts as good antimicrobial agent since it shows a significant inhibition zone against some bacteria. Further Musa extracts acts as good antioxidant sources. Musa extracts are also involved in anticancer studies in medicinal field.

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Ethical Clearance: Ethical approval is taken from IEC. This work is carried out by following the strict guidelines of IEC.

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Conflict of Interest: Nil

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