In Vitro Bioactivity and Phytochemical Screening of *Musa Acuminata* Flower

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Summary

The current study was performed to evaluate the bioactivity and to screen the phytochemicals that are present in *Musa acuminata* flower. *M. acuminata* flower extract showed good antimicrobial activity against the tested microorganisms with zone of inhibition ranging from 12 mm to 22 mm and the MIC values ranging from 1.562 mg/ml to 12.5 mg/ml. Brine shrimp toxicity test performed on *Artemia salina* proves that banana flower extract is not toxic and the LC_{50} value obtained was 9.97 mg/ml above the cut-off point of toxicity level 1.0 mg/ml. *M. acuminata* flower antioxidant study reveals that the flower is a good antioxidant source as butylated hydroxytoluene (BHT) with a LC_{50} value of 7.63 mg/ml. Phytochemical screening confirmed the presence of active compounds like glycosides, tannins, saponnins, phenols, steroids and flavonoids in the *M. acuminata* flower methanolic extract.

Key words: *Musa acuminata*, antimicrobial, toxicity, antioxidant, phytochemical screening

Introduction

Today with the globalization of the world, opportunistic diseases have also developed to a serious state where the survival rate of patients is decreasing drastically. The prime agent that leads to these diseases is microorganisms. Almost all the microorganisms are becoming resistant to the current antimicrobial agents used today. Newer prototype, antimicrobial agents are necessary to overcome the current problem. Across the globe, the plant kingdom has become the choice of researchers to develop alternative antimicrobial agents from the chemical compounds that are present in the plant which can resist the growth of microorganism in the human body. Since ancient times, many types of vegetables, medicinal plants and spices were used to treat many different types of diseases.

Banana blossom or commonly known as banana heart belongs to the family of Musaceae. It is produced on the banana stem of a banana tree. Generally each banana stem has a single banana flower but in some cases there are more than one. Banana plant is known as the largest herbaceous flowering plant in the world. All the parts of a banana plant are beneficial to mankind in the medical aspects and ornamental uses. Banana blossom that is considered a vegetable is cooked in a variety of dishes in Asian countries like curry, deep fried, cutlet and more. Besides cooked, banana flower is also used to treat some diseases. In China, this flower is used to treat heart pain, asthma and endocrine problem like diabetes. Consumption of banana flower also helps to treat diarrhea and stomach cramps. For women, eating this flower helps to reduce painful menses and menopausal bleeding. Banana blossom that carries many nutrients and vitamins is also used for infantile malnutrition and weak body[1].

Materials and Methods

Plant and Extract Preparation

Matured *Musa acuminata* flower was harvested from a banana tree farm in Kulim, Kedah. The outer sap was removed and the flowers were collected. Banana flowers were washed and dried in an oven at 50ºC for 24 hours and blended using an electronic blender to obtain powdered sample in. 100g of powdered flowers was soaked in 300ml of methanol and left at room temperature for 3 days. The extract was dried using a rotary evaporator and maintained in room temperature for further test.

Microorganisms

Stock cultures of *Escherichia coli, Proteus mirabilis, Staphylococcus aureus, Micrococcus sp, Salmonella sp, Bacillus subtilis, Bacillus thuringensis, Candida albicans* and *Aspergillus niger* were obtained from the School of Biological Sciences, USM and used throughout this project. All bacteria were maintained on Nutrient Agar (NA) at 37 ºC and fungi on Potato Dextrose Agar (PDA) at 28 ºC.

Antimicrobial Screening

Disc diffusion method was used for the antimicrobial susceptibility testing. Inoculum suspensions of the bacteria and fungi were spread on the surface media. The plates were divided into three portions for negative control, positive control and extract treatment. Sterile 6mm discs were placed on the plates and 20µL of (100mg/ml extract, 30 µg/ml \ Micanazole nitrate or 30 µg/ml Chloramphenicol and 100% of methanol) were applied on each disc. All the NA plates were incubated at 37ºC for 24 hour and PDA plates at 28ºC for 48 hours. The zone of inhibition was measured for all the plates.

Determination of Minimum inhibitory concentration (MIC)

Minimum inhibitory concentration (MIC) was determined using the broth dilution method. The crude *M. acuminata* flower extract was dissolved in methanol to obtain the initial concentration as 100mg/ml. Two-fold dilution was performed from the initial test concentration and each tube was inoculated with 500 µL of suspension containing 10^8 CFU/ml of bacteria and 10^4 spore/ ml of fungi. The test tube for bacteria was incubated at 37 ºC for 24 hours and at 28 ºC for 48 hours for fungi. MIC was determined as the lowest concentration of the extract inhibiting the visual growth of the test cultures in the tubes based on turbidity [2].

Brine Shrimp lethality assay

Brine shrimp eggs *Artemia salina* was purchased from Cheaw Thean Yeang Aquarium Sdn. Bhd in Penang. The eggs were hatched in artificial seawater prepared by dissolving 38g of sea salt in 1L of distilled water and incubated at room temperature (25-29 ºC) for 48h with light source. After the incubation period, the nauplii were used for the brine shrimp assay. The extract were dissolved in dimethylsulphoxide (DMSO) and diluted with artificial seawater. The extract concentration in the first tube was 100mg/ml and two- fold dilutions were performed to obtain a final concentration of 0.195mg/ml. One hundred microliters of nauplii suspension containing 10 larvae were added into each tube and incubated for 24 hours with light source. Potassium dichromate was used as a positive control. The number of dead nauplii was calculated under a microscope and the percentage of mortality was determined using the equation:

% Mortality= (no. of dead nauplii / initial no. of live nauplii) x 100%

Lethality concentration (LC_{50}) for the assay was calculated from triplicates experiments[3].

DPPH radical scavenging activity

Quantitative measurement of radical scavenging properties was performed using universal bottles. The reaction mixture contained 50 µL of test sample (80% Methanol as blank) and 5ml of 0.004% of 2, 2-diphenyl-1- picrylhydrazyl radical (DPPH) scavenging in methanol. Commercial antioxidant butylated hydroxytoluene (BHT, Sigma) was used as standard in this study. The bottles were incubated for 30 min and the optical density (OD) was measured at 517 nm. DPPH radical's concentration was calculated using the following equation:

DPPH scavenging effect (%) = $A_0 - A_1 / A_0$ x 100%

Where A_0 was the absorbance for the control and A_1 was the absorbance for sample (Sangetha et al., 2008).

Phytochemical Screening

Phytochemical screening of the crude methanol extract of *M. acuminata* was carried out using standard phytochemical procedure [4, 5, 6.]

Test for Carbohydrate

2ml of Molish's reagent and 2ml of concentrated sulphuric acid (H_2SO_4) was added to 2ml boiling methanolic extract. A reddish ring indicates the presence of carbohydrate.

Test for reducing sugar

2ml of methanolic extract was added to boiling Fehling's solution for 5minutes. A brick red precipitate indicates the presence of reducing sugar.

Test for tannins

To 2ml of methanolic extract 1ml of ferric chloride ($FeCl_3$) was added and blue-black or greenish –black precipitate indicates presence of tannins.

Test for Saponins

To 2ml of methanolic extract 5ml of distilled water was added and the solution shaken vigorously for 30s, stable persistent frothing indicates saponin.

Test for flavonoids

Magnesium ribbon and few drops of concentrated HCL were added to 2ml of methanolic extract, pink or red colour indicates the presence of flavonoids.

Test for alkaloids

10ml of ammoniacal chloroform solution was added to 2ml of methanolic extract. The extract was than treated with 10 drops of 10% sulphuric acid and tested with Meyer's reagent. Formation of white precipitate indicates the presence of alkaloids.

Test for phenols

To 2ml of methanol extract 0.5ml of Folin-cicocalteau reagent and 2ml of 20% of Na_2CO_3 was added and presence of bluish colour indicates the presence of phenols.

Test for anthraquinones

To 2ml of methanolic extract 2ml of 10% NH_4OH was added. A bright pink colour indicates the presence of anthraquinones.

Test for steroids

To 2ml of methanolic extract, 2ml of chloroform, acetic acid and 1ml of concentrated H_2SO_4 was added. A blue-green indicates the presence of steroids.

Results and Discussion

Extraction of the flower of *Musa acuminata* by absolute methanol yielded a crude extract in paste form of 20.3%. The *in vitro* antimicrobial susceptibility of *M. acuminata* flower against Gram positive, Gram negative, yeast and molds were qualitatively and quantitatively assessed by the presence of inhibition zones and MIC. Out of nine tested microorganisms, eight showed potent inhibitory effect on the growth of the cultures with different range of inhibition zone (Table 1). The zone of inhibition for negative control (methanol) was zero. However, larger clear zones were observed on the plates with 30µg/ml of Chloramphenicol and 30µg/ml of Micanazole nitrate (antibiotics) when compared to the tested methanol crude extract. *M. acuminata* flower extract was more sensitive to *S. aureus* with inhibition zone of 22mm whereas the range on inhibition for

Table 1. Antimicrobial activity (zone of inhibition[a]) and (Minimum inhibitory concentration[b]) of *Musa acuminata*

Mircoorganisms	Zone of Inhibition (mm)[c]			MIC
	Extract	C	M	(mg/ml)
Escherichia coli	12	24	ND	12.5
Micrococcus sp	14	26	ND	6.25
Salmonella sp	13	21	ND	12.5
Proteus mirabilis	19	23	ND	3.13
Staphylococcus aureus	22	24	ND	1.56
Bacillus subtilis	17	23	ND	3.13
Bacillus thuringiensis	-	25	ND	-
Candida albicans	15	ND	23	6.25
Aspergillus niger	14	ND	21	6.25

[a] Disk diffusion technique. [b] Broth dilution method. [c] The values (average of triplicate) are diameter of zone of inhibition at 100mg/ml crude extract, 30 µg/ml Chloramphenicol and 30 µg/ml Micanozole nitrate

other microorganisms was between 12- 19mm. In our study, *Bacillus thuringiensis* did not show any resistance against banana flower extract. The MIC value ranged from 1.562mg/ml to 12.5mg/ml for the cultures tested. The extract had the highest antimicrobial activity against *S. aureus* with the lowest MIC value of 1.562mg/ml. The antimicrobial activity observed from the disc diffusion and broth dilution method could be due to the active compounds that are present in the *M. acuminata* flower extract. Negative results do not indicate that the flower extract is inactive nor the absence of active compounds. Thus, the reason may be due to insufficient quantities of active

compounds present in crude extract with the dose applied[6.] Another reason for less antimicrobial activity observed in the study could be due to poor absorptions of the active compounds by the microorganisms cell that occurs due to medium precipitation that happens upon diluting the methanol extract with the aqueous medium [7.]

Although banana flower is consumed as a vegetable or used for medical purposes in humans it is necessary to know the toxicity level to avoid overdoses or poisoning. Safety should be the major criteria in selecting medicinal plants for therapeutic uses because many plants used as medicine are poisonous [1.] Mortality of *Artemia salina* tested with different extract concentration in the toxicity assay was determined based on the linear graph of mean percentage mortality versus the logarithm of concentration plotted. The concentration at which 50% of the nauplii could be dead (LC_{50}) was determined from the graph. LC_{50} value of *M. acuminata* flower extract was 9.97mg/ml (Figure 1a) and LC_{50} value of positive control was 0.27mg/ml (Figure 1a and 1b). This indicates that *M. acuminata* flower extract is not toxic compared to potassium dichromate because the LC_{50} value was greater than 1.0 mg/ml, the cut-off point for detecting the toxicity level and can be consumed at any doses [8.]

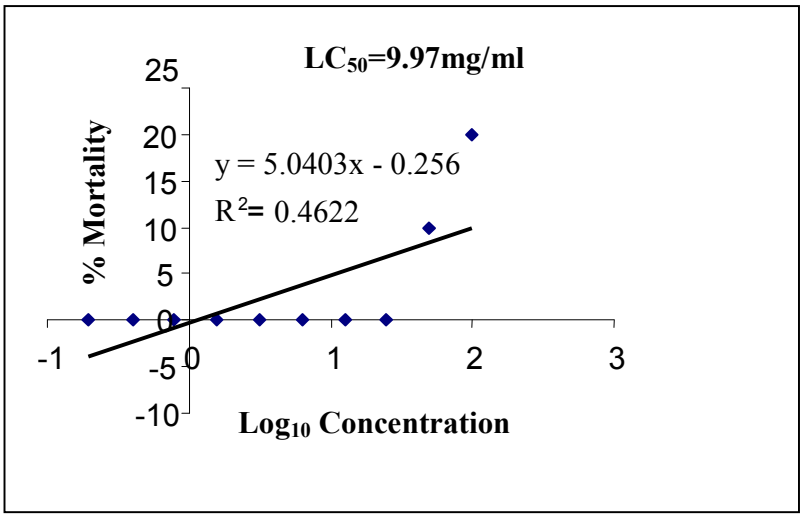

Figure 1 (a). Toxicity effect of the *Musa acuminata* flower extract using brine shrimp lethality assay after 24 hours

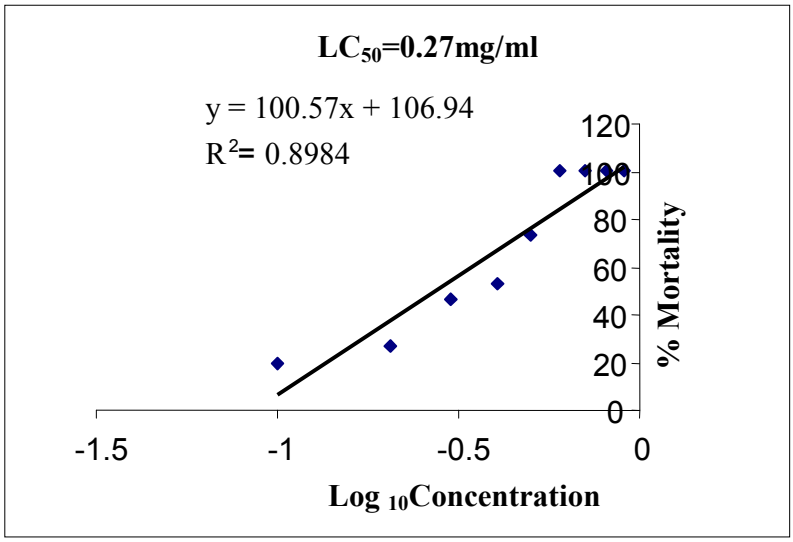

Figure 1(b). Toxicity effect of potassium dichromate using brine shrimp lethality assay after 24 hours

Antioxidant activity basically refers to the action of a molecule that is capable of slowing or preventing the oxidation of other molecules. Antioxidants that scavenge free radicals play an important role for medical reasons. There are a few synthetic antioxidants that are available in the market like buthylhyroxyanisole (BHA) and butylhydroxtoulene (BHT) but alternative antioxidants are necessary due to the side effects of the commercial antioxidants [9.] Thus, there is currently great attention placed on the search for alternative antioxidants from natural sources. In this study, the antioxidant activity of *M. acuminata* flower extract was investigated using the DPPH radical scavenging assay. Antioxidant activity was determined using DPPH radical scavenging assay because it gives reliable information concerning the antioxidant ability of the tested compounds [10.] The percentage of scavenging effect is basically determined by the colour change of DPPH solution from purple to yellow or colourless. DPPH radical scavenging can be expressed in terms of IC_{50}, the concentration required to achieve 50% scavenging of DPPH radical under experiment condition [11.] BHT had the highest scavenging activity compared to *Musa acuminata* flower extract (Figure 2) The IC_{50} value exhibited by banana flower was 7.63mg/ml slightly higher than BHT which was 6.12mg/ml. From the figure below it is clearly observed that *M. acuminata* flower is an equally good antioxidant as the BHT standard used. In previous article it has been reported that banana plant can protect itself from oxidative stress by producing large amounts of antioxidant, thus it is considered as a good source of natural antioxidant [12.]

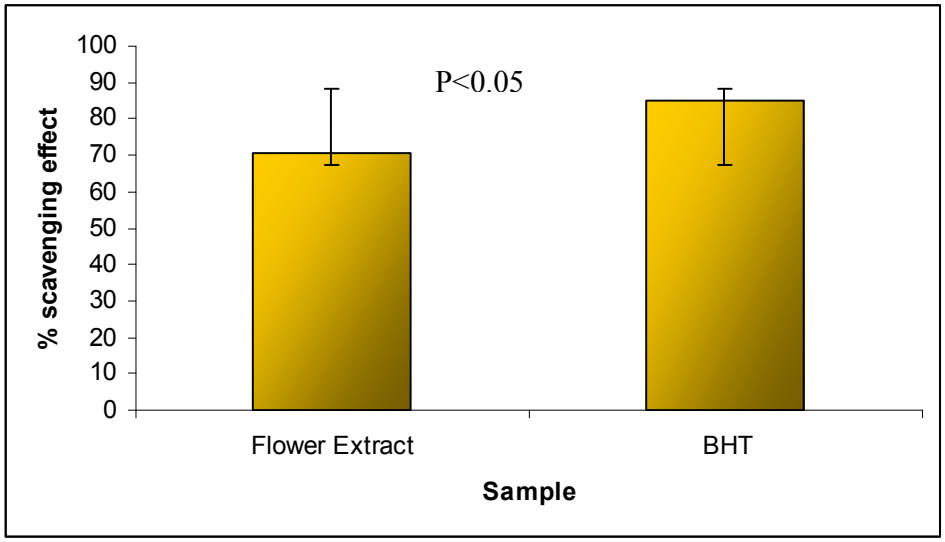

Figure 2. Scavenging effect (%) of crude extract of *Musa acuminate* flower and known antioxidant BHT, at 1.0mg/ml

In the past decades, secondary metabolites that are present in the Angiosperms have contributed a lot in the production of new drugs to cure different types of diseases. These active compounds include saponins, tannins, flavanoids, alkaloids, terpenoids and many others. Different isolation methods are available to identify active compounds present in crude extract like Thin Layer Chromatography (TLC), Gas Chromatography (GC), High Performance Liquid Chromatography (HPLC) and more. But the easiest and simplest method is phytochemical screening. The phytochemical screening method employed in this study is qualitative. The phytochemical analysis of *Musa acuminata* flower extract reveals that glycosides, tannins, saponins, steroids, phenols and flavonoids were present in the methanolic crude extract (Table 2). Glycosides detected in phytochemical screening are used in the treatment of congestive heart failure and cardiac arrhythmias. It helps to protect the heart from coronary heart disease [5]. Tannins are believed to have some general antimicrobial and antioxidant activities where

Table 2. Phytochemical analysis of *Musa acuminata* flower extract

Chemical compounds	Results
Carbohydrates	-
Glycosides	+
Tannins	+
Saponin	+
Steroids	+
Phenols	+
Alkaloids	-
Antraquinones	-
Flavanoids	+

Key = "+" active compound present, "-" active compound absent

at low concentrations it can inhibit bacterial growth and act as an antifungal agent at higher concentrations. Tannins are known as polymeric phenolic substances because of its capability of precipitating gelatin from solution which is known as astringency[9]. Saponins present in this flower are commonly used medically for treating epilepsy, excessive salivation, chlorosis and migraines[5]. Phenols are also believed to show therapeutic effect against some diseases and classified as active antimicrobial compounds. Recently, it has been reported that flavonoids possess many pharmacological properties like antifungal, antioxidant, antiallergenic, anti-inflammatory, antithrombic, anticarcenogenic and hepatoprotective that narrows researcher interest to work on this secondary metabolites. Since ancient times, flavonoids have been used as anti-inflammatory and for cosmetic purposes in the Chinese traditions. The inconsistent in the results obtained in the antimicrobial activities could be due to the absence of other active compounds like anthraquinones and alkaloids. Antraquinones is believed to possess antiparasitic and antimicrobial properties. This compound is used to treat herpes simplex and skin diseases in the folkloric community[9].

Conclusion

Our results allow us to sum up that *Musa acuminata* flower is a good natural source of antioxidant. Thus, the antioxidant property of this flower may be a good substitute for synthetic ones. Banana blossom do exhibit some significant antimicrobial activity against few tested microorganism culture like *Staphylococcus aureus*, *Proteus mirabilis*, *Bacillus subtilis*, *Aspergillus niger*, *Candida albicans*, *Micrococcus sp* and *Salmonella sp* so it can act as antimicrobial agents to some selected microorganism. All this properties probably explains the use of banana flower by the indigenous people to treat and heal number of infections and diseases.

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