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Medicinal activities of the leaves of *Musa sapientum* var. *sylvestris* in vitroRepon Kumer Sahaa^{1*}, Srijan Acharyaa¹, Syed Sohiful Haque Shovon, Priyanka Royb²¹Department of Pharmacy, East West University, Dhaka, Bangladesh²Dhaka Medical College, Dhaka, Bangladesh

PEER REVIEW

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Comments

This paper added interesting new medicinal values such as antioxidant, antibacterial, hemagglutination inhibition activity and hydrogen peroxide induced hemolysis inhibitory effect to the methanolic extract of the leaves of plantain banana, *M. sapientum* var. *sylvestris* collected in Bangladesh. Therefore, *M. sapientum* var. *sylvestris* may be considered as an useful medicinal plant.

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ABSTRACT

Objective: This study is to investigate the medicinal value of methanolic extract of the leaves of *Musa sapientum* var. *sylvestris* in Bangladesh. **Methods:** Several biochemical assays, thin layer chromatography and ultra-violet spectroscopy were used to detect the presence of various types of compounds in this extract. Antioxidant effects were measured by DPPH scavenging assay, total reducing assay and hydrogen peroxide scavenging assay. Receptor binding activities and hydrogen peroxide induced hemolysis assay were performed by hemagglutination assay and hemolysis assay using erythrocytes. Disk diffusion assay was performed to show the antibacterial effect of the extract. **Results:** Methanolic extract of the leaves showed antioxidant and antibacterial activity *in vitro*. The extract showed hemagglutination inhibition activities and hydrogen peroxide induced hemolysis inhibition activity of human red blood cells. **Conclusion:** *Musa sapientum* var. *sylvestris* can be an useful medicinal plant.

KEYWORDS

Musa sapientum var. *sylvestris*, Antioxidant activity, Hemolysis activity, Flavanoids, Antimicrobial activity, Bangladesh

1. Introduction

Musa sapientum var. *sylvestris* (*M. sapientum* var. *sylvestris*) is a valuable medicinal plant which belongs to the family Musaceae. The plant widely grows in Bangladesh. Banana is generally consumed as a dessert or cooked as vegetable or made into various confections. In addition to these uses of banana as a highly nourishing

delicacy, banana fruits as well as various other parts of banana tree find diverse uses in medicine, fibre making, religious rituals etc. The leaves are used by the tribals of Western Ghats in India for bandaging cuts, blisters and ulcers[1]. There are various types of species in *Musa* genus and their pharmacological studies have been studied. Plantain banana (*Musa sapientum* var. *paradisiaca*) (*M. sapientum* var. *paradisiaca*) has been shown to possess

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ulcer healing activity in rats. They concluded that wound healing which could be due to its antioxidant effect and on various wound healing biochemical parameters present in plantain banana[2]. Methanolic extract of *M. sapientum* var. *paradisiaca* showed antiulcer and mucosal defensive factors in normal and non-insulin dependent diabetes mellitus rats. They concluded that ulcer protective effect of the extract could be due to its predominant effect on mucosal glycoprotein, cell proliferation, free radicals and antioxidant systems[3]. Studies with plantain banana (*M. sapientum* var. *paradisiaca*) have indicated its ulcer protective and healing activities through its predominant effect on various mucosal defensive factors and they concluded that its antioxidant activity may be involved in its ulcerprotective activity[4]. Previous study reported that dried unripe plantain banana powder contain flavanoid leucocyanidin and demonstrated a significant protective effect against aspirin-induced erosions[5]. Besides, soluble and insoluble components of dietary fibre participate in the hypocholesterolaemic effect of banana pulp present in banana fruit (*Musa sapientum* L. var. *cavendishii*)[6].

Here we examined the antioxidant, antibacterial, hemagglutination inhibition activity and hydrogen peroxide induced hemolysis inhibitory effect of the methanolic extract of the leaves of *M. sapientum* var. *sylvestris* collected local areas from Bangladesh.

2. Materials and methods

2.1. Plant collection and identification

The fresh leaves of the plant were collected from the surrounding of Dhaka, Bangladesh during January, 2010 and identified by the taxonomist of the Bangladesh National Herbarium, Mirpur, Dhaka as *M. sapientum* var. *sylvestris*. A voucher specimen of the plant has been deposited (Accession No.: DACB 36560) in the herbarium for further reference.

2.2. Extraction of the plant material

Sun-dried and powdered plant material (750 g $\times$ 2) was extracted with methanol by cold extraction process. The extracts were concentrated with a rotary evaporator (IKA, Germany) at low temperature (40–50 °C) and reduced pressure. A total of 5 g of the extracts were stored at 4 °C until used.

2.3. Phytochemical screening

The freshly prepared crude extracts were qualitatively tested for the presence of alkaloids (Hager's test), flavonoids (Modified Ammonia test), steroids (Salkowski test), terpenoids (Modified Salkowski test), reducing sugars (Fehling's test), saponins (Frothing test), tannins (FeCl₃ test),

cardiac glycosides (Killer–Killani's test) and anthraquinones (Chloroform layer test).

2.4. TLC analysis

The extracts were analyzed by performing TLC to determine the composition of each extract. TLC was done under polar basic solvent consisted of ethyl acetate, ethanol, and water (8.0:1.2:0.8). After completion of TLC, the plates were exposed to UV light for compound detection and identification. For charring the plates were exposed to 10% sulphuric acid solution, dried and then heated to 80–90°C for charring purpose. This helped in the fixation of spot and allowed it to be prominently visible. For detection of flavanoids the plates were dipped into 0.04% 2,2-Diphenyl-1-picrylhydrazyl (DPPH) solution and dried while keeping in a dark place. For detection of polyphenols the plates were washed with Folin–ciocalteu reagent and dried.

2.5. Chemical analysis of the extract

UV spectroscopy of the extract was performed within 200 nm to 400 nm using a Lambda UV spectrometer (Shimadzu, Japan)[7].

2.6. Determination of total phenolic content

The total phenolic content of extracts was determined using Folin–Ciocalteu method using gallic acid as standard[8]. The extracts were oxidized with 10% Folin–Ciocalteu reagent (Merck, Germany), and were neutralized with 700 mmol/L sodium carbonate solution. The absorbance of the resulting blue color was measured at 765 nm after 60 min. The total phenolic contents were determined using a standard curve prepared with gallic acid. The estimation of the phenolic compounds was carried out in triplicate. The results were mean $\pm$ standard deviations and expressed as milligram of gallic acid equivalent per gram of extract.

2.7. Total flavonoid assay

The total flavonoid compounds in each extract were determined as previously described by Jothy *et al*[8]. An aliquot (1.5 mL) of methanolic extract was added to 6 mL of deionized water and then 0.45 mL 5% (w/v) NaNO₂ and incubated for 6 min. Total of 0.45 mL 10% (w/v) AlCl₃ and 6 mL 4% (w/v) NaOH was added and the total volume was made up to 15 mL with distilled water. The absorbance was measured at 510 nm by using visible spectrophotometer. The results were expressed as milligram rutin equivalents per gram. The experiments were performed in three times.

2.8. Total proanthocyanidins test

About 0.5 mL of *M. sapientum* var. *sylvestris* extract

(1 mg/mL) was taken with 3 mL of vanillin–methanol solution (4%) and added 1.5 mL of HCl. The solution was mixed homogenously. After 15 min incubation in room temperature absorbance was measure at 500 nm. Total proanthocyanidins content were expressed as catechin equivalent (mg/g) using the following equation $y=0.5825x$; $R=0.9277$, where, y was the absorbance and x was the concentration[9].

2.9. DPPH radical scavenging activity

The free radical scavenging activity of the extract were measured by decrease in the absorbance of methanolic solution of DPPH[10]. The DPPH radical scavenging method was used for the determination of the antioxidant capacity of the extracts. Different concentrations of the plant extract (2, 4, 6, 8 and 10 µg/mL, in methanol) were added at an equal volume (10 mL) to methanol solution of DPPH (400 µg/mL). Different concentrations of L-Ascorbic acid (2–10 mg/mL) were used as the standard antioxidant. After 30 min at room temperature, the absorbance values were measured at 517 nm on a spectrophotometer and converted into the percentage antioxidant activity using the following equation: DPPH antiradical scavenging capacity (%)=(Absorbance of sample–Absorbance of blank)×100/Absorbance of blank. Methanol plus different concentration of plant extract solution was used as a blank, while DPPH solution plus methanol was used as a control. IC₅₀ values denote the concentration of the sample required to scavenge 50% of DPPH radicals.

2.10. Hydrogen peroxide scavenging assay

A solution of hydrogen peroxide (40 mmol/L) is prepared in phosphate buffer (50 mmol/L, pH 7.4). The concentration of hydrogen peroxide is determined by absorption at 230 nm using a spectrophotometer. Extract (0–20 µg/mL) in phosphate buffer is added to hydrogen peroxide and absorbance at 230 nm is determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. L-ascorbic acid was used for comparison. The percentage of hydrogen peroxide scavenging is calculated as follows: % Scavenged (H₂O₂)=(A₀–A₁/A₀)×100 Where; A₀ is the absorbance of control and A₁ is the absorbance of test. Ascorbic acid was used as a positive control.

2.11. Total reducing assay

The reducing power of the extracts of *M. sapientum* var. *sylvestris* was measured using the potassium ferricyanide reduction method. Various amount of extracts (0–200 mg) and L-ascorbic acid (0–1000 mg) were taken in different test tubes as previously described by Oyaizu et al[11]. Then 2.5 mL of distilled water and 2.5 mL of potassium ferricyanide [K₃Fe₃(CN)₆] solution were added in all test tubes and mixed well. After incubation at 50 °C for 20 min, 2.5 mL of

trichloro acetic acid (10% w/v) was added in all test tubes and centrifuged at 3000 rpm for 10 min. Afterwards, upper layer of solution(5 mL) was mixed with 5 mL distilled water. Then 1 mL of FeCl₃ was added each test tube. Then from each test tube we collected 1 mL of solution and mixed it with 9 mL of distilled water. Then the solution was incubated at 35 °C for 10 min. The formation of perls prussian color was measured at 700 nm in a spectrometer. Increased absorbance of the reaction mixture indicates increasing reducing power. L-ascorbic acid was used as a standard. The analysis was performed in twice.

2.12. Antibacterial assay

In order to screening the antibacterial assay, five different bacterial strains were used to carry out this assay. These are *V. mimicus*, *S. typhi*, *S. dysentery*, *S. aureus* and *B. serus*. Nutrient agar was used as the culture media. Stocks of these bacterial solutions were revived in nutrient agar by incubating at 37 °C for 24 h. A single disk diffusion method was used to assess the presence of antimicrobial activities of the methanolic extract of night jasmine leaves. Whatman's filter paper was punched, and 6 mm disks were collected in a beaker. The beaker was covered with foil paper and autoclaved. Then 20 µL of different concentration extracts (0–10 mg/mL) were loaded and pipetted per disk. The revived test organisms were plated onto nutrient agar plates. The disks were then placed equidistant on all plates for all extracts. Standard disc (Himedia, India) of Azithromycin (30 µg/disc) and blank discs (impregnated with solvents followed by evaporation) were used as positive and negative control, respectively. After incubation at 37 °C for 24 h, the antimicrobial activity of the test agents was determined by measuring the diameter of zone of inhibition expressed in millimeter.

In order to investigate the dose–response curve of the exacts of *M. sapientum* var. *sylvestris* on bacterial (*A. niger*) growth a serial diluted solution of the extract (0–63 mg/mL) were prepared and incubated with same concentration of bacterial solution (1×10⁵ CFU/mL) as previously described method. After incubation bacterial colonies were counted.

2.13. Hemagglutination inhibition assay

Hemagglutination activity of the crude extract and fractions was tested against human erythrocyte blood groups ABO as previously described by Saha et al[12]. Stock solution of the test samples was prepared at concentration of 5 mg/mL and each solution was serially diluted. Fresh blood was collected from healthy persons, centrifuged and the erythrocytes were separated. There was 4% erythrocyte suspension prepared in phosphate buffer (pH 7.4) of all blood groups. And 1 mL of the test sample dilution was taken with 1 mL of 4% erythrocyte and incubated at 4°C. After incubation, the results were noted. Smooth button formation

in bottom indicated negative activity, while a rough granular deposition at bottom showed positive activity. The intensity of hemagglutination was determined from the extent of deposition.

2.14. Antihemolytic assay

Inhibition of H_2O_2 induced red blood cell hemolysis of methanolic extract was examined by the *in vitro* method described previously by Tavazzi *et al*^[13]. The erythrocytes from human blood were separated by centrifugation and washed with saline or isotonic sodium phosphate buffer (pH 7.4) until the supernatant was colorless. The erythrocytes were then diluted with saline or phosphate buffer to give a 4% suspension. Varying amounts of sample (0–1 mg/mL) with saline or buffer were added to 2 mL of the suspension of erythrocytes and the volume was made up to 3.5 mL with saline or buffer. This mixture was preincubated for 120 min and then 0.5 mL H_2O_2 solutions of appropriate concentration in saline or buffer were added. The concentration of H_2O_2 in the reaction mixture was adjusted so as to bring 90% hemolysis of blood cells after 120 min incubation. Incubation was concluded after these time intervals by centrifugation during 5 min at $\times 1000\ g$ and the extent of hemolysis was determined by measurement of the absorbance at 540 nm corresponding to hemoglobin liberation. Antihemolytic activity was expressed as the inhibition percentage and was calculated using the following formula: Antihemolytic activity (%) = $(\text{Control}_{540\text{ nm}} - \text{Sample}_{540\text{ nm}}) \times 100 / \text{Control}_{540\text{ nm}}$, where, $\text{Sample}_{540\text{ nm}}$ was the absorbance of the sample and $\text{Control}_{540\text{ nm}}$ was the absorbance of the control.

3. Results

3.1. Phytochemical screening

Preliminary phytochemical screening showed (Table 1) the presence or absence of alkaloids, flavonoids, steroids, terpenoids, reducing sugars, saponins, tannins, cardiac glycosides, anthraquinones in varying amount in the *M. sapientum var. sylvestris* extracts.

Table 1

Phytochemical compositions of *Nyctanthes arbortristis* extracts.

Phytoconstituents	Name of the test	Result
Alkaloids	Hager's test	+++
Anthraquinones	Chloroform layer test	–
Cardiac glycosides	Killer–Killani's test	+
Flavonoids	Ammonia test (modified)	++
Reducing sugars	Fehling's test	–
Saponins	Frothing test	+++
Steroids	Salkowski test	++
Tannins	$FeCl_3$ test	+
Terpenoids	Salkowski test (modified)	–

+++; highly present; ++; moderately present; +; slightly present; –; absent.

Polar basic solvent consisted of ethyl acetate, ethanol, and water (8.0:1.2:0.8). The separation performed by the polar basic solvent is shown here in Figure 1. TLC plates were seen under UV light and found some compounds were separated at the bottom of the plates. Charring with H_2SO_4 in high temperature the separated compounds transformed into black color. Staining the plate with FC–reagent the color of the separated compounds changed into bluish and after staining the plate with DPPH solution the color of the separated compounds changed into yellow color. Such a result indicated the presence of flavanoids in the separated fractions of the extract in the polar mobile phase. The separation in polar solvent system has been shifted from the bottom to the top the stationary TLC plates.

Figure 1. Separation of methanolic extract of *M. sapientum var. sylvestris* using ethyl acetate, ethanol, and water (8.0:1.2:0.8) solvent system.

A: Normal view; B: UV view; C: Charring view; D: FC reagent staining view; E: DPPH staining view.

UV spectroscopic analysis of the methanolic extracts of *M. sapientum var. sylvestris*'s leaves showed the maximum absorbance values at 670 and 410 nm (Figure 2). Such an absorbance values in UV spectroscopic analysis indicates the presence of chlorophyll (670 nm) and other molecule (410 nm).

Figure 2. Scanning of methanolic extract of *M. sapientum var. sylvestris* in UV–spectroscopy.

3.2. Total phenolic and proanthocyanidines content

Quantitative analysis of polyphenols and flavanoids were performed as described previously. In case of polyphenols quantification a standard curve was used where the equation is $y=1.4456X-0.0186$, $R^2=0.999[8]$. From the standard curve, the total phenolic compounds as gallic acid equivalent of the extract was 0.092 ± 0.02 mg/100 mg sample. In case of flavanoid quantification a standard curve was used[5], where the equation is $y=0.0071X+0.1139$, $R^2=0.9927$. From the standard curve the amount of catechin present in the extract is 28.75 ± 1.85 mg of catechin equivalent per gram of sample. Total proanthocyanidins was 0.922 mg/1 g catechin.

3.3. DPPH radical scavenging activity

From the analyses of Figure 3, we can conclude that the scavenging effect of extracts of *M. sapientum* var. *sylvestris* increase as the concentration increases. Therefore, extracts of *M. sapientum* var. *sylvestris* showed stronger antioxidant activity than that of vitamin C. The 50% inhibitory effect of the extract was calculated from the curve and it was 0.039 mg/mL.

Figure 3. DPPH scavenging activity of the methanolic extracts of *M. sapientum* var. *sylvestris*.

3.4. Hydrogen peroxide scavenging activity

Hydrogen peroxide scavenging activity of the extract was measured in compared with ascorbic acid as shown in Figure 4. Their rate of hydrogen peroxide scavenging activity was measured comparing with ascorbic acid. It was found that methanolic extract showed stronger scavenging activity than that of ascorbic acid.

Figure 4. H_2O_2 scavenging activity of extracts of *M. sapientum* var. *sylvestris*.

3.5. Total reducing assay

Total reducing assay of the extract was investigated in compared with ascorbic acid as shown in Figure 5. Their 50% scavenging activity was measured and found that it has stronger scavenging activity than that of ascorbic acid.

Figure 5. Total reducing activity of MSS extract.

3.6. Hydrogen peroxide induced hemolytic inhibition activity

Hemolysis caused by hydrogen peroxide was inhibited by the extract at various concentration as shown in Figure 6. A volume of 300 μ L of H_2O_2 was used for complete lysis of red blood cell.

Figure 6. Hemolytic inhibition activity of extracts of *M. sapientum* var. *sylvestris*.

3.7. Hemagglutination inhibition assay

Various concentrations of extracts (0–5 mg/mL) were taken to investigate hemagglutination inhibition activity on different types of human blood groups. Hemagglutination inhibition activities of the extracts of *M. sapientum* var. *sylvestris* were tested against four different types of human blood groups and the results are in Table 2.

3.8. Antimicrobial assay

Antimicrobial activities of the extracts of *M. sapientum* var. *sylvestris* were tested against five pathogenic organisms and the results are in Table 3. In the antibacterial screening, the extracts showed average zone of inhibition 6–22 mm at concentrations of 20–200 μ g/disc (Table 3). The dose–

response curve of the extracts of *M. sapientum* var. *sylvestris* on bacterial (*A. niger*) were shown in Figure 7.

Table 2

Hemagglutination inhibition assay of extracts of *M. sapientum* var. *sylvestris*.

Blood group	5 mg/mL	2.5 mg/mL	1.25 mg/mL	Buffer only
A+	+++	+	+	---
B+	+++	++	+	---
O+	++	++	+	---
AB+	+	+	—	---

+++; no hemagglutination; ++; moderately hemagglutination; +; slightly hemagglutination; —; hemagglutination.

Table 3

Antibacterial effect of MSS extract.

Name of microorganisms	Zone of inhibition (mm)			
	Negative control	MSS extract 200 µg/disc	MSS extract 20 µg/disc	Azithromycin 30 µg/disc
<i>V. mimicus</i>	—	17	7	22
<i>S. typhi</i>	—	16	7	21
<i>S. dysentery</i>	—	16	7	23
<i>S. aureus</i>	—	16	11	25
<i>B. cereus</i>	6	7	6	23

—: no zone of inhibition.

Figure 7. Dose–response curve of antibacterial activity of extracts of *M. sapientum* var. *sylvestris* against *A. niger*.

4. Discussion

4.1. Phytochemical screening

Extracts of *M. sapientum* var. *sylvestris* showed the presence of various types of phytochemical active compounds including alkaloids, flavanoids, steroids, glycosides and saponins. Besides, TLC analysis assured the presence of flavanoids in the extract. UV spectroscopy scanning showed the possibility of the presence of pure active compounds in the extract.

4.2. Total phenolic and proanthocyanidines content

In order to investigate the total phenolic content in extracts of *M. sapientum* var. *sylvestris* we performed total phenolic and flavanoid content of it. We found that extracts of *M. sapientum* var. *sylvestris* contained a significant amount of phenols, flavanoids, and proanthocyanidines.

4.3. DPPH radical scavenging activity

Structural analysis of the phenol derivatives and the importance of the –R groups located on the phenolic ring in the molecule's showed the ability to act as free radical scavenging as well as their influence in the electrochemical behavior^[14]. We found that extracts of *M. sapientum* var. *sylvestris* showed DPPH scavenging activity.

4.4. Hydrogen peroxide scavenging activity, total reducing assay, and hydrogen peroxide induced hemolytic inhibition activity

Crude banana peel (*M. paradisica*) extracts of unripe, ripe, and leaky ripe banana fruit showed hydrogen peroxide–induced hemolysis and their antioxidant capacity^[15]. Here, we found that extracts of *M. sapientum* var. *sylvestris* also showed the similar types of results. It showed hydrogen peroxide scavenging activity, reducing activity and hydrogen peroxide induced hemolytic inhibition activity.

4.5. Hemagglutination inhibition assay

Hemagglutination inhibition assay was performed to investigate the receptor binding affinity of *M. sapientum* var. *sylvestris* extract on human erythrocytes. We found that *M. sapientum* var. *sylvestris* has binding affinity to the receptors of erythrocytes and prevent agglutination. The results showed a possible benefits of extracts of *M. sapientum* var. *sylvestris* as an antiviral therapeutics.

4.6. Antimicrobial assay

Extracts of *M. sapientum* var. *sylvestris* showed broad spectrum antibacterial activities. It showed clear zone of inhibition in all types of strains tested. It also showed the dose–response relationship of antibacterial activities. Therefore, extracts of *M. sapientum* var. *sylvestris* may be useful as an antibacterial compound. Our present study on the methanolic extract of the leaves of *M. sapientum* var. *sylvestris* showed the potentiality of it as an antioxidant, receptor binding activity, hydrogen peroxide induced hemolysis activity and antibacterial activity. Therefore, *M. sapientum* var. *sylvestris* may be considered as a medicinal plant for future benefits.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

M. sapientum var. *sylvestris* is a valuable medicinal plant which belongs to the family Musaceae. The plant widely grows in Bangladesh. Authors investigated several medicinal values of methanolic extract of the leaves of this plant collected in local areas from Bangladesh.

Research frontiers

The authors examined several medicinal activities such as antioxidant, antibacterial, hemagglutination inhibition activity and hydrogen peroxide induced hemolysis inhibitory effect of the methanolic extract of the leaves of *M. sapientum* var. *sylvestris* collected local areas from Bangladesh.

Related reports

Pushpangadan *et al.* (1989) reported that the leaves of plantain banana (*M. sapientum* var. *pareadisiaca*) can be used by the tribals of Western Ghats in India for bandaging cuts, blisters and ulcers. Agarwai *et al.* (2009) reported that plantain banana (*M. sapientum* var. *paradisiaca*) possess ulcer healing activity which could be due to its antioxidant effect.

Innovations and breakthroughs

This paper innovated several new medicinal value to the extract of the leaves of plantain banana, *M. sapientum* var. *sylvestris* collected in Bangladesh.

Applications

Banana leaves may be used as a local source of antibacterial and antifungal agents.

Peer review

This paper added interesting new medicinal values such as antioxidant, antibacterial, hemagglutination inhibition activity and hydrogen peroxide induced hemolysis inhibitory effect to the methanolic extract of the leaves of plantain banana, *Musa sapientum* var. *sylvestris* collected in Bangladesh. Therefore, *M. sapientum* var. *sylvestris* may be considered as an useful medicinal plant.

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