Original Article

Antidiarrheal, Antioxidant and Antimicrobial Activities of the *Musa sapientum* Seed

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Abstract

Musa sapientum (*M.sapientum*) commonly known as 'banana' is widely used in Bangladeshi folk medicine for the treatment of various ailments including diarrhea. Hence, the present study was designed to investigate antidiarrheal, antioxidant and antibacterial potential of the methanolic extract of *M.sapientum* seed (MMSS). The extract was studied for antidiarrheal property using castor oil and magnesium sulfate induced diarrheal model and charcoal induced gastrointestinal motility test in mice. Total phenolic and flavonoids content, total antioxidant activity, scavenging of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, as well as nitric oxide (NO) and assessment of reducing power were used to evaluate antioxidant potential of MMSS. In addition, disc diffusion methods were used for antibacterial assay using various diarrheal induced bacterial strains. At the doses of 100 and 200 *mg/kg* body weight, the extract reduced the frequency and severity of diarrhea in test animals throughout the study period. At the same doses, the extracts significantly ($p<0.001$) delayed the intestinal transit of charcoal meal in test animals as compared to the control. In DPPH and NO scavenging method, MMSS showed good antioxidant potentiality in a dose dependent manner with the IC_{50} value of 12.32±0.33 *µg/ml* and 18.96±1.01 *µg/ml*, respectively with a significant ($p<0.001$) good reducing power. The extract also displayed strong antibacterial effect against when tested against *Escherichia coli, Shigella dysenteriae,* and *Pseudomonas aeruginosa.* Altogether, these results suggest that the MMSS could be used as a potential antidiarrheal agent along with its antioxidant and antibacterial potentiality.

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Key words: Antibacterial agents, Diarrhea, Free radicals, *Musa sapientum*

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Introduction

Diarrhea is an alteration in the normal bowel movement, characterized by increased frequency of bowel sound and movement, wet stool, and abdominal pain [(1)]. Clinically it is used to describe increased liquidity of stool, usually associated with increased stool weight and frequency [(2)]. Regardless of the understanding causes, treatment and prevention of diarrheal diseases, an estimated 4.6 *million* people, with 2.5 *million* children, die from diarrhea every year, particularly in developing countries [(3)]. Diarrhea, may be acute or

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chronic. Acute diarrhea being the most common, is usually caused by an infectious agent, even though drugs, poisons or acute inflammatory reactions can contribute a lot [4]. Now a days, *rotavirus* is the major causative agent for infectious diarrhea, particularly in young children, however, other viral (*adenovirus, enterovirus and norovirus*), bacterial (*Escherichia coli, Salmonella sp., Shigella sp., Camphylobacter* and *Vibrio cholerae*) and parasitic (*Cryptosporidium* and *Giardia*) agents are important pathogens [5].

Oral Rehydration Therapy (ORT) has been identified as a key factor in the decline of child mortality rate due to diarrhea, although it does not reduce the volume or duration of diarrhea [6]. Likely, antibiotics and gut motility suppressing agents bid the other treatment option, wherein reverse dehydration, shorten the length of illness and reduce the period of time when an individual is infected [7]. Treatment with pharmacological agents that are pathogen specific or that suppress severe symptoms would be of benefit to patients suffering from prolonged diarrhea [8].

Despite the availability of a vast spectrum of approaches for diarrheal management, a vast majority of the people of Bangladesh have been known to treat diarrhea with a variety of medicinal plants one of which being *M.sapientum* [9]. *M.sapientum* L. (family Musaceae) grows in humid lowland to upland tropical areas whose vernacular name is banana in Bengali. Agarwal et al [10] has studied the wound healing activity of plantain banana extracts. Plants parts like fruits, leaves, peels, root, and stalks from banana plants have been utilized orally or topically as a medicine for treating diarrhea and dysentery. It is also used in inflammation, pains and snakebite [11] as well as it has antilithic [12], antiulcerogenic [13], hypoglycemic [14], hypolipidemic and antioxidant actions [15]. Houghton and Skari [16] have also reported the antivenom action of the stem juice from banana plant.

Literature reviews indicated that no studies combining the antidiarrheal, antioxidants as well as antibacterial of the seeds of M.sapientum have so far been undertaken. Taking this in view and as a part of our ongoing research [17,18] on Bangladeshi medicinal plants, the present study aimed to evaluate the antidiarrheal activity of seeds extracts of *M.sapientum* along with their antioxidant and antibacterial activities.

Materials and Methods

Plant materials

The fruits of *M.sapientum* were collected from the local market in Mirpur, Dhaka, Bangladesh in the month of April, 2008 and identified by experts in Bangladesh National Herbarium, Mirpur, Dhaka where the Voucher specimen no: 38765 has been retained for future reference.

Preparation of plant extract

The seeds of *M.sapientum* were manually separated from the whole fruits, dried in hot air woven, pulverized into coarse powder using mechanical grinder, seiving through sieve #40, and stored in an air tight container. The dried powder material (500 *g*) was refluxed with methanol for three *hr*. The total filtrate was concentrated to dryness, in vacuum at 40 ℃ to render the methanolic extract (80 *g*).

Chemicals

Ammonium molybdate, Folin-chiocaltu phenol reagent, sodium nitroprusside, were purchased from E. Merck (Germany). 1,1-diphenyl- 2-picryl-hydrazyl (DPPH), sodium nitroprusside, ascorbic acid, quercetin, and potassium ferric cyanide were purchased from Sigma Chemical Co. Ltd, (St. Louis, MO, USA). All other chemicals and reagents were of analytical grade.

In vivo antidiarrheal activity

Castor oil-induced diarrhea: The experiment was performed according to the method described by Shoba & Thomas [19]. Briefly, mice fasted for 24 *hr* were randomly allocated to four groups of five animals each. The animals were all screened initially by giving 0.5 *ml* of

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castor oil. Only those showing diarrhea were selected for the final experiment. Group I received 1% carboxy-methyl cellulose (CMC) (10 *ml/kg*, *p.o*), groups III and IV received *p.o* the drug extract (100 and 200 *mg/kg*), respectively. Group II was given antidiarrheal drug loperamide (3 *mg/kg*, *p.o*) in suspension. After 60 *min*, each animal was given 0.5 *ml* of castor oil, each animal was placed in an individual cage, the floor of which was lined with blotting paper which was changed every hour, observed for 4 *hr* and the characteristic diarrheal droppings were recorded.

Magnesium sulfate-induced diarrhea

Diarrhea was induced by oral administration of magnesium sulfate at the dose of 2 *g/kg* to the animals 30 *min* after pre-treatment with vehicle (1% Tween 80 in water, 10 *ml/kg*, *p.o*) to the control group, loperamide (3 *mg/kg*) to the positive control group, and the methanol extract at the doses of 100 and 200 *mg/kg* to the test groups [20].

Effect on gastrointestinal motility

Animals were divided into four groups of five mice each and each animal was given *p.o* 1 *ml* of charcoal meal (5% activated charcoal suspended in 1% CMC) 60 *min* after an oral dose of drugs or vehicle. Group I was administered 1% CMC (10 *ml/kg*) and animals in groups III and IV received extract at the dose of 100 *mg/kg* and 200 *mg/kg* body weight, respectively. Group II received atropine sulfate (0.1 *mg/kg*), which decreased gastrointestinal tract motility and was used as the standard drug. After 30 *min*, animals were killed by light ether anaesthesia and the intestine was removed without stretching and placed lengthwise on moist filter paper. The intestinal transit was calculated as a percentage of the distance travelled by the charcoal meal compared to the length of the small intestine [21].

Acute toxicity study

Animals were divided into groups of five mice each. The test was performed using increasing doses of test extract, given *p.o*, in a 10 *ml/kg* volume to different groups serving as test groups [22]. Another group of mice was administered saline (10 *ml/kg*, *p.o*) as negative control. The mice were allowed food *ad libitum* during the 24 *hr* test and kept under regular observation for mortality.

In vitro antioxidant activity

Determination of total antioxidant capacity: The antioxidant activity of the extract was evaluated by the phosphomolybdenum method according to the procedure of Prieto et al [23]. The assay is based on the reduction of Mo (VI)–Mo(V) by the extract and subsequent formation of a green phosphate/ Mo(V) complex at acid pH. Extract (0.3 *ml*) was combined with 3 *ml* of reagent solution (0.6 *M* sulfuric acid, 28 *mM* sodium phosphate and 4 *mM* ammonium molybdate). The tubes containing the reaction solution were incubated at 95 ℃ for 90 *min*. Then the absorbance of the solution was measured at 695 *nm* using a spectrophotometer (Shimadzu, UV-150-02) against blank after cooling to room temperature. Methanol (0.3 *ml*) was used as the blank experiment. The antioxidant activity is expressed as the number of equivalents of ascorbic acid using the following formula:

$C = (c \times V)/m$

where: C-total antioxidant activity, *mg/g* plant extract, in Ascorbic acid; c-the concentration of ascorbic acid established from the calibration curve, *mg/ml*; V-the volume of extract, *ml*; m-the weight of pure plant extract, *g*.

Free radical scavenging activity measured by 1,1-diphenyl-2-picryl-hydrazyl (DPPH)

The free radical scavenging activity of extract, based on the scavenging activity of the stable 1,1-diphenyl-2- picrylhydrazyl (DPPH) free radical, was determined by the method described by Braca et al [24]. Plant extract (0.1*ml*) was added to 3 *ml* of a 0.004% MeOH solution of DPPH. Absorbance at 517 *nm* was determined after 30 *min*, and the percentage inhibition activity was calculated from $[(A_0 – A_1)/A_0] \times 100$, where A_0 is the absorbance of the control, and A_1 is the absorbance of the extract/ standard. IC_{50} value was calculated from the equation of line obtained by plotting

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a graph of concentration (*µg/ml*) versus % inhibition.

Nitric oxide radical scavenging assay

The procedure is based on the method, where sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions that can be estimated using Greiss reagent. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitrite ions. For the experiment, sodium nitroprusside (10 *mM*) in phosphate buffered solution (pH=7.4) was mixed with different concentrations of extract dissolved in 10% DMSO and incubated at room temperature for 150 *min*. The same reaction mixture without the extract but the equivalent amount of the solvent used served as the control. After incubation, 0.5 *ml* of Griess reagent (1% sulfanilamide, 2% H_3PO_4 and 0.1% N-(1- naphthyl) ethylenediamine dihydrochloride was added. The absorbance was measured at 546 *nm* and the percentage inhibition activity was calculated from $[(A_0–A_1)/A_0] \times 100$, where A_0 is the absorbance of the control, and A_1 is the absorbance of the extract/ standard [25]. IC_{50} value was calculated from the equation of line obtained by plotting a graph of concentration (*µg/ml*) versus % inhibition.

Reducing power activity

The reducing power of *M.sapientum* seed extract was determined according to the method previously described [26]. Extract at different concentrations in 1 *ml* of 10% DMSO were mixed with 2.5 *ml* of phosphate buffer (0.2 *M*, pH=6.6) and 2.5 *ml* potassium ferricyanide [K3Fe (CN) 6] (1%), and then the mixture was incubated at 50 ℃ for 30 *min*. Afterwards, 2.5 *ml* of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 *rpm* for 10 *min*. Finally, 2.5 *ml* of upper layer solution was mixed with 2.5 *ml* distilled water and 0.5 *ml* FeCl3 (0.1%), and the absorbance was measured at 700 *nm*. Increased absorbance of the reaction mixture indicated increased reducing power.

The amount of phenolic compounds and flavonoids

The total phenolic content of extract was determined using Folin-Ciocalteu reagent [27]. *M.sapientum* seed extracts (100 *µl*) were mixed with the Folin-Ciocalteu reagent (500 *µl*) and 20% sodium carbonate (1.5 *ml*). The mixture was shaken thoroughly and made up to 10 *ml* with distilled water. The mixture was allowed to stand for 2 *hr*. Then the absorbance at 765 *nm* was determined with a Shimadzu UV-160A spectrophotometer (Kyoto, Japan). These data were used to estimate the phenolic contents using a standard curve obtained from various concentration of gallic acid.

The flavonoids content was determined by aluminium chloride colorimetric method [28]. Quercetin was used to make the calibration curve. The different concentration of extract (0.5 *ml*) were separately mixed with 95% ethanol (1.5 *ml*), 10% aluminum chloride (0.1 *ml*), 1 *M* potassium acetate (0.1 *ml*) and distilled water (2.8 *ml*). After incubation at room temperature for 30 *min*, the absorbance of the reaction mixture was measured at 415 *nm*. The amount of 10% aluminum chloride was substituted by the same amount of distilled water in blank. All the determinations were carried out in duplicates. These data were used to estimate the flavonoid contents using a standard curve obtained from various concentration of quercetin.

Antimicrobial activity

Sterile 6.0 *mm* diameter blank discs (BBL, Cocksville, USA) were impregnated with test substances at the dose of 500 *µg/disc*. This disc, along with standard discs (Ciprofloxacin, Oxoid Ltd, UK) and control discs were placed in petri dishes containing a suitable agar medium seeded with the test organisms using sterile transfer loop and kept at 4 ℃ to facilitate maximum diffusion. The plates then kept in an incubator (37 ℃) to allow the

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growth of the bacteria. The antibacterial activities of the test agents were determined by measuring the diameter of the zone of inhibition in terms of *millimeter*. Antimicrobial activity was tested against *Staphylococcus aureus*, *Escherichia coli, Pseudomonus aeruginosa, Salmonella typhi, Shigella boydii, Shigella flexneri* and *Shigella dysenteriae* were obtained from International Centre for Diarrheal Disease Research, Bangladesh (ICDDR, B) [29].

Statistical analysis

All values were expressed as the mean ± standard error of the mean (SEM) of three replicate experiments and were analyzed using the GraphPad program (GraphPad, San Diego, CA, USA). The analysis was performed using student's *t*-test. P<0.001 was considered to be statistically significant.

Results

Effect on castor oil-induced diarrhea

In the castor oil induced diarrheal mice, the methanolic extract of the seeds of *M.sapientum,* at the dose of 100 and 200 *mg/kg*, significantly (p<0.001) lessened the total number of faeces as well as delayed the onset of diarrhea in a dose dependent manner (Table 1).

Effect on magnesium sulfate-induced diarrhea

M.sapientum seed extract exhibited significant antidiarrheal activity against magnesium sulfate-induced diarrhea (Table 2). The extract at both dose levels significantly (p<0.001) reduced the extent of diarrhea and also notably delayed the onset of diarrhea in a dose dependent manner.

Effect on gastrointestinal motility

With the gastrointestinal transit experiment, the methanolic extract, at the dose of 100 and 200 *mg/kg*, retarded (p<0.001) the intestinal transit of charcoal meal in mice when compared to the control (Table 3).

Acute toxicity studies

Methanolic seed extract of *M.sapientum* (500-5000 *mg/kg*, body weight) given *p.o* did not cause any death in the different dose groups. The LD_{50} value for oral administration of the plant extract was found to be greater than 5000 *mg/kg*.

Total phenolic and flavonoid contents

The total phenols and flavonoids content was found to be 15.94±0.12 *mg/g* plant extract (in GAE) and 29.98±0.32 *mg/g* plant extract (in quercetin equivalent), respectively, in crude extract of *M.sapientum* seeds (Table 4).

Total antioxidant capacity

Percentage yield of methanol extract of *M. sapientum* seeds and its total antioxidant capacity are given in table 4. Total antioxidant capacity of the extract is expressed as the number of equivalents of ascorbic acid and was found to be 197.24±0.69 *mg/g* equivalent of ascorbic acid.

DPPH radical scavenging activity

The percentage (%) scavenging of DPPH radical was found to be concentration dependent *i.e.* concentration of the extract between 5-80 *µg/ml* greatly increasing the inhibition activity (Figure 1). Crude extract of *M.sapientum* seed (IC_{50} value 12.32±0.33 *µg/ml*) showed similar activity than the standard ascorbic acid (IC_{50} value 12.30±0.15 *µg/ml*).

Nitric oxide (NO) scavenging activity

The percentage inhibition of nitric oxide production was illustrated in figure 2. It is observed that scavenging of nitric oxide by

Table 1. Effect of *M.sapientum* seed extract on castor oil-induced diarrhea in mice

	Dose (*mg/kg*)	Onset of diarrhea (*min*)	Animals with diarrhea	No. of faeces in 4 *hr*	% Inhibition of defecation
Group I	Vehicle	24.45±2.19	5/5	22.7±0.98	
Group II	10	180±0.13**	1/5	2.8±0.28**	87.38
Group III	100	50.67±2.73**	3/5	11.6±1.05**	47.75
Group IV	200	61.23±3.03**	2/5	7.8±0.79**	64.86

Values are mean±SEM, (n = 5); p<0.001, student's *t*-test compared to control. Group I animals received vehicle (1% Tween 80 in water), Group II received Loperamide 10 *mg/kg* body weight, Group III and Group IV were treated with 100 and 200 *mg/kg* body weight (*p.o.*) of the MMSS

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Table 2. Effect of *M.sapientum* seed extract on magnesium sulfate-induced diarrhea in mice

	Dose (*mg/kg*)	Onset of diarrhea (*min*)	Animals with diarrhea	No. of faeces in 4 *hr*	% Inhibition of defecation
Group I	Vehicle	41.23 ± 1.11	5/5	19.0 ± 0.61	
Group II	10	188 ± 0.10**	0/5	3.2 ± 0.18**	83.15
Group III	100	56.69 ± 2.03	2/5	13.2 ± 1.25	30.53
Group IV	200	78.22 ± 2.19**	1/5	9.4 ± 0.99**	50.53

Values are mean±SEM, (n = 5); p<0.001, student's *t*-test compared to control. Group I animals received vehicle (1% Tween 80 in water), Group II received Loperamide 10 *mg/kg* body weight, Group III and Group IV were treated with 100 and 200 *mg/kg* body weight (*p.o.*) of the MMSS

Table 3. Effect of *M.sapientum* seed extract on charcoal meal stimulated gastrointestinal transit in mice

	Dose (*p.o*)	Mean intestinal length (*cm*)	Mean distance traveled by charcoal (*cm*)	% GI transit
Group I	0.4 *ml/mouse*	68.4±0.98	50.4±1.18	73.86±3.57
Group II	0.1 *mg/kg*	63.2±1.61	20±1.04**	31.62±1.32**
Group III	100 *mg/kg*	63.8±2.41	35±0.91**	55.05±1.39**
Group IV	200 *mg/kg*	66.2±2.60	24.8±1.61**	37.82±3.04**

Values are presented as mean±SEM, (n=5); p<0.001, student's t test compared to control. Group I animals received vehicle (1% Tween 80 in water), Group II received atropine sulfate 0.1 *mg/kg* body weight, Group III and Group IV were treated with 100 and 200 *mg/kg* body weight (*p.o.*) of the MMSS

Table 4. Yield, total amount of plant phenolic compounds, flavonoids and total antioxidant capacity of methanolic extract of *M.sapientum* seeds

Sample	Yield (%)	Total phenols *mg/g* plant extract (in GAE)[a]	Total flavonoids *mg/g* plant extract (in QA)[b]	Total antioxidant capacity *mg/g* extract (in ASC)[c]
MMSS	30.0%	15.94±0.12	29.98±0.32	197.24±0.69

[a] Gallic acid equivalents (GAE), (*mg/g* of each extract) for the total phenolic content
[b] Quercetin equivalents (QA), (*mg/g* of each extract) for the total flavonoid content
[c] Ascorbic acid equivalents (ASC), (*mg/g* of each extract) for the total antioxidant capacity
The GAE, QA and ASC values are expressed as means±SEM of triplicate experiments

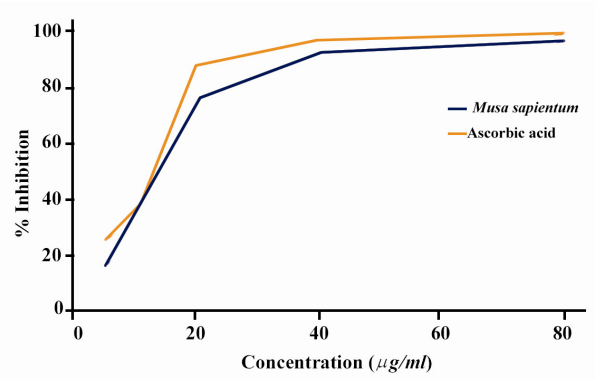

Figure 1. Free radical scavenging activity of different concentrations of crude extract of *M.sapientum* seed and ascorbic acid by DPPH radicals

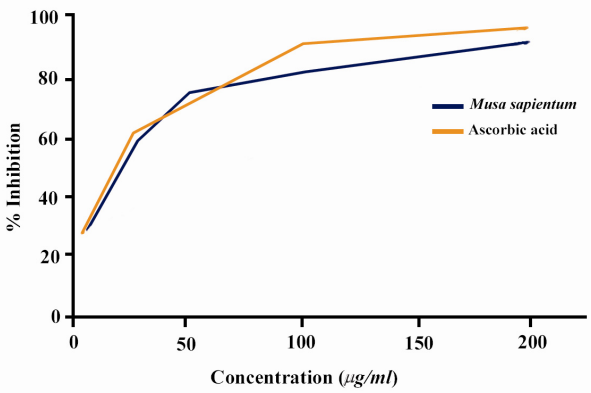

Figure 2. Percentage inhibition of nitric oxide radical by different concentrations of crude extract of *M.sapientum* seed and ascorbic acid

the extract is also concentration dependent and statistically significant (p<0.001). The IC_{50} value of the extract of *M.sapientum* seed was 18.96±1.01 *μg/ml*, while ascorbic acid showed the value of 8.22±0.22 *μg/ml*.

Reducing power ability

For the measurement of the reductive ability, we investigated the Fe^{3+} to Fe^{2+} transformation in the presence of crude extract of *M.sapientum*. Like the antioxidant activity,

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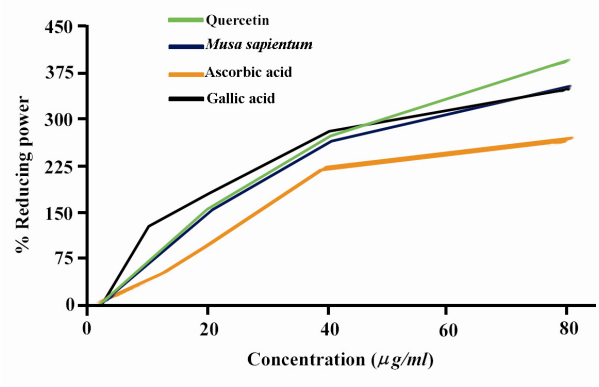

Figure 3. Reducing power of MeOH extract of *M.sapientum* and quercetin, ascorbic acid and gallic acid by spectrophotometric detection of Fe^{3+} to Fe^{2+} transformation

the reducing power of *M.sapientum* seed extract increased with increasing concentration of the sample and effect was statistically significant ($p<0.001$). Figure 3 shows the reductive capabilities of the *M.sapientum* compared with quercetin, gallic acid and ascorbic acid.

Antibacterial activity

Table 5 expressed the antibacterial activity (zone of inhibitions) of the seed extract of the *M. sapientum*. The extract showed significant activity against the entire tested bacterial flora except *Shigella flexneri* and *Shigella boydii*. The highest zone of inhibition was found against *Escherichia coli* (zone of inhibition 18.59±0.22 *mm*), followed by *Shigella dysenteriae* (zone of inhibition 16.92±0.62 *mm*) and the moderate activity was shown against *Pseudomonas aeruginosa* (zone of inhibition 12.21±0.14 *mm*). The weakest activity was shown against *Staphylococcus aureus.*

Table 5. Antibacterial activity of methanolic extract of *M. sapientium* seeds

Bacterial strain	Diameter of zone of inhibition (*mm*)	
	Ciprofloxacin	MMSS
Staphylococcus aureus	28.03±0.12	8.89±0.14
Pseudomonas aeruginosa	29.13±0.21	12.21±0.14
Salmonella typhi	25.41±0.11	10.39±0.12
Shigella flexneri	27.34±0.12	NA
Shigella dysenteriae	28.01±0.11	16.92±0.62
Shigella boydii	29.39±0.14	NA
Escherichia coli	30.23±0.18	18.59±0.22

Assay was performed in triplicate and results are the mean of three values±Standard Deviation. NA- Zone of inhibition < 5 *mm* consider as no activity

Discussion

Several mechanisms have been previously proposed to explain the diarrheal effect of castor oil including inhibition of intestinal Na^+, K^+-ATPase activity to reduce normal fluid absorption [30], activation of adenylate cyclase or mucosal cAMP mediated active secretion [31], stimulation of prostaglandin formation [32], platelet activating factor and recently nitric oxide has been claimed to contribute to the diarrheal effect of castor oil [33]. However, it is well evident that castor oil produces diarrhea due to its most active component recinoleic acid which causes irritation and inflammation of the intestinal mucosa, leading to release of prostaglandins, which results in stimulation of secretion [34]. Since the methanol extract of the seeds of *M. sapientum* successfully inhibited the castor oil-induced diarrhea, the extract might have exerted its antidiarrheal action via antisecretory mechanism which was also evident from the reduction of total number of wet faeces (not shown separately) in the test groups in the experiment. Again, flavonoids present in the plant extract [13] are reported to inhibit release of autacoids and prostaglandins, thereby inhibit motility and secretion induced by castor oil [35].

On the other hand, magnesium sulfate has been reported to induce diarrhea by increasing the volume of intestinal content through prevention of reabsorption of water. It has also been reported that it promotes the liberation of cholecystokinin from the duodenal mucosa, which increases the secretion and motility of small intestine and thereby prevents the reabsorption of sodium chloride and water [36]. The methanol extract was found to improve the diarrheal condition in this model. The extract may increase the absorption of water and electrolyte from the gastrointestinal tract, since it delayed the gastrointestinal transit in mice as compared to the control. The delay in the gastrointestinal transit prompted by the extract might have contributed, at least to

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some extent, to their antidiarrheal activity by allowing a greater time for absorption.

To determine the efficacy of natural antioxidants either as pure compounds or as plant extract, a great number of *in vitro* methods have been developed in which antioxidant compounds act by several mechanisms. The phosphomolybdenum method was based on the reduction of Mo(VI) to Mo(V) by the compounds having antioxidant property and is successfully used to quantify vitamin E in seeds [23].

DPPH is a stable free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule [37] and is usually used as a substrate to evaluate the antioxidant activity of a compound [28]. Based on the data obtained from this study, DPPH radical scavenging activity of *M.sapientum* seed extract (IC_{50} 12.32±0.16 *µg/ml*) was similar than the standard (IC_{50} 12.30±0.11 *µg/ml*). It was revealed that *M.sapientum* seed extract did show the proton donating ability and could serve as free radical inhibitor or scavenger. In fact, the radical scavenging capability of phenolic compounds are due to their hydrogen donating ability/number of hydroxyl groups present, which in turn is closely related both to the chemical structure and spatial conformation, that can modify the reactivity of the molecules [38].

A direct correlation between antioxidant capacity and reducing power of certain plant extracts has been reported. The reducing properties are generally associated with the presence of reductones, which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom [39]. Moreover, it has been reported that the phenol and polyphenolic compound (flavonoids)-constituents of the plant-possess antioxidant properties mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers. In addition, they have a metal chelation potential [40].

In the present study this possibility is supported by the estimation of total polyphenols and flavonoids [41], which was found to be present in high concentration in the *Musa sp.* extracts. Our study also supported the previous study [42] on producing the antioxidant effect of the hydroalcoholic extract of *M. sapientum* against various *in vitro* model.

Flavonoids and sugars obtained from selected traditional medicinal plants in Bangladesh were reported by Rahman and Wilcock having antidiarrheal properties [43]. Longanga Otshudi et al screened a number of medicinal plants and showed that antidiarrheal activity of those plants were due to tannins alkaloids, saponins, flavonoids, sterols, triterpenes and reducing sugars contained in them [44]. The flavonoids presence of these types of compounds, such as kaemferol, myricetin, apigenin, and leucocyanidin in *M.sapientum* is likely to contribute to its grastrointestinal effects [41]. Moreover, nitric acid mechanism has also been shown to be involved in castor oil induced diarrhea [33]. In our study plant extract showed remarkable NO scavenging capacity may partially contribute the antidiarrheal activity.

The MMSS was shown to exhibit good antibacterial activity when tested against *Escherichia coli, Shigella dysenteriae,* and *Pseudomonas aeruginosa* and also supported to the previous study [45]. Phytoconstituents such as saponin, phenolic compounds, flavonoids and glycosides have been reported to inhibit bacterial growth and to be protective to plants against bacterial and fungal infections [46]. In the present study this possibility is supported by the estimation of total polyphenols and flavonoids [41], which was found to be present in high concentration and was found to be 15.94±0.12 *mg* equivalent of gallic acid/*g* plant extract and 29.98 *mg* equivalent of quercetin/*g* plant extract, respectively. Moreover, Mokbel et al,[47] isolated various antibacterial compound viz. ß-sitosterol, malic acid, succinic acid, palmatic acid, 12-hydroxystrearic acid, glycoside, the d-malic and 12-hydroxystrearic acid. So the antibacterial activity showed by the extract may be due to the presence of those compounds. Also some

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plants show antidiarrheal properties by their antimicrobial activities [48].

Conclusion

The MMSS showed antidiarrheal as well as antioxidant activities in a number of models of diarrheic condition in the test animals and *in vitro* antioxidant assay method, respectively. The obtained results thus give the experimental basis to understand the use of *M.sapientum* in traditional medicine, as an antidiarrheal agent. However, further bioassay guided phytochemical and pharmacological studies are required to identify the active principles and exact mechanism(s) of action.

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