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Antidiarrheal Potentiality of Methanolic Extract of Different Parts of *Musa sapientum* Fruits

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Abstract: To investigate the antidiarrheal activities of the methanolic extract of peel (MSPL) and pulp (MSPP) of *Musa sapientum* fruits, the extract was studied for antidiarrheal property using castor oil and magnesium sulphate induced diarrheal model and charcoal induced gastrointestinal motility as well as PGE_2-induced enterolooping test in mice. In addition, activities against some causative diarrheal pathogenic bacteria were also determined. At the doses of 100 and 200 mg/kg body weight, both extracts significantly reduced the frequency and severity of diarrhea in test animals throughout the study period. Both extracts also showed a significant (p<0.001; p<0.05) reduction in the gastrointestinal motility in charcoal meal test as well as PGE_2-induced intrafluid accumulation. MSPL extract also displayed strong antibacterial effect against *Staphylococcus aureus, Salmonella typhi, Shigella dysenteriae and Escherichia coli.* Altogether, these results suggest that the *Musa sapientum* fruit extracts could be used as a potential antidiarrheal agent.

Key words: Diarrhea · Flavonoid · Antibacterial · *Musa Sapientum*

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]. 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 [2]. Diarrhea, may be acute or chronic. With 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 [3]. Nowadays, 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 [4]. 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 [5]. 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 [6]. Treatment with pharmacological agents that are pathogen specific or that suppress severe symptoms would be of benefit to patients suffering from prolonged diarrhea [7].

Medicinal herbs constitute an indispensable component of the traditional medicine practiced worldwide due to their economical viability, accessibility and ancestral experience. 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 *Musa sapientum* [8]. *Musa Sapientum* L. (family Musaceae) grows in humid lowland to upland tropical areas whose vernacular name is banana in Bengali has studied the wound healing activity of *Plantain banana* extracts [9]. Plants parts like fruits, leaves, peels, root and stalks from banana plants have been utilized orally or topically as a medicine for treating diarrhoea and dysentery. It is also used in inflammation, pains and snakebite [10] as well as it has antilithic [11], antiulcerogenic [12], hypoglycemic [13], hypolipidemic and antioxidant actions [14] Houghton and Skari [15] have

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also reported the antivenom action of the stem juice from banana plant. As a part of our ongoing research [16] on Bangladeshi medicinal plants, the present study aimed to evaluate the antidiarrheal activity of peel and pulp extracts of *Musa sapientum* fruits.

MATERIAL AND METHODS

Plant Materials: The fruits of *Musa sapientum* were collected from the local market in Mirpur, Dhaka, Bangladesh in the month of April, 2010 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: Around 250 gm of powdered materials of both peel and pulp were taken in two different clean, flat bottomed glass containers and were immersed in 500 ml of 95% methanol. The containers with their contents were sealed and kept for a period of 7 days associated with occasional shaking and stirring. The two mixtures then underwent a coarse filtration by a piece of clean, white cotton material and were filtered through Whatman filter paper (Bibby 200, Sterilin Ltd., UK). The filtrates (methanolic extract) obtained were evaporated using rotary evaporator. The extracts were transferred to two different closed containers for further use and fortification.

Chemicals: Folin-chiocaltu phenol reagent, were purchased from E. Merck (Germany). Galic acid and quercetin, were purchased from Sigma Chemical Co. Ltd, (St. Louis, MO, USA). All other chemicals and reagents were of analytical grade.

The Amount of Phenolic Compounds and Flavonoids: The total phenolic content of extract was determined using Folin–Ciocalteu reagent [17]. 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 hrs. 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 [18]. The different concentration of extracts (0.5 ml) were separately mixed with 95% ethanol (1.5 ml), 10% aluminum chloride (0.1 ml), 1M 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.

Acute Toxicity Study: Animals were divided into groups of five mice each. The test was performed using increasing doses of both test extracts, given orally, in a 10 ml/kg volume to different groups serving as test groups [19]. 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 hrs test and kept under regular observation for mortality.

Castor Oil-Induced Diarrhea: The experiment was performed according to the method described by Shoba & Thomas [20]. Briefly, mice fasted for 24 hrs were randomly allocated to six groups of five animals each. The animals were all screened initially by giving 0.5 ml of castor oil. Only those showing diarrhea were selected for the final experiment. Group I received 1% tween 80 (10 ml/kg, *p.o.*), groups III-VI received orally MSPL and MSPP extracts (100 and 200 mg/kg), respectively. Group II was given 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 hrs and the characteristic diarrhoeal droppings were recorded.

Magnesium Sulphate-Induced Diarrhea: Diarrhoea was induced by oral administration of magnesium sulphate 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 (MSPL and MSPP) at the doses of 100 and 200 mg/kg to the test groups [21].

Effect on Gastrointestinal Motility: Animals were divided into six groups of five mice each and each animal was given orally 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-VI received extract of both MSPL and MSPP at the dose of 100 mg/kg and 200 mg/kg body weight, respectively. Group II received atropine sulfate (0.1 mg/kg,) 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 [22].

PGE_2-Induced Enteropooling: The method of Robert *et al.* [23] was applied. Overnight fasted mice were divided into 7 groups of 5 animals each. Group I was given 2% gum acacia and kept as a control. Groups III-VI received 100 and 200 mg/kg p.o. of MSPL and MSPP extracts, respectively. Group II served as a vehicle control and received 2% gum acacia plus PGE_2 (0.5 ml of 100µg/kg, i.p.). Group VII received loperamide and kept as a positive control. Immediately afterwards, diarrhea was induced by 0.5 ml of 100µg/kg, i.p., dose of PGE_2 (Sigma Aldrich, USA). After 30 minutes, the animals were sacrificed, small intestine was removed and intestinal contents were collected and measured in a syringe. The percentage inhibition in intestinal fluid was determined by comparing the values with vehicle control.

Antimicrobial Activity: Sterile 6.0 mm diameter blank discs (BBL, Cocksville, USA) were impregnated with test substances of both MSPL and MSPP 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°C to facilitate maximum diffusion. The plates then kept in an incubator (37°C) to allow the 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 Diarrhoeal Disease Research, Bangladesh (ICDDR,B) [24].

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 by using student's t test. $p<0.001$ and $p<0.05$ were considered to be statistically significant.

RESULTS

Total Phenolic and Flavonoid Contents: The total extractable phenolic contents of the peel and pulp of *M. sapientum* were 84.23 ± 0.81 and 21.95 ± 0.12 mg/g plant extract (in GAE), respectively. As shown in Table 1, the polyphenol contents of MSPL extract was approximately 4 fold higher than the MSPP extract. In case of flavonoid, MSPL also displayed the highest flavonoid content (21.34 ± 0.39 mg/g plant extract in QA).

Acute Toxicity Studies: Methanolic extract of both peel and pulp of *Musa sapientum* (500 – 5000 mg/kg, body weight) given orally did not cause any death in the different dose groups. The LD_{50} values for oral administration of the plant extracts were found to be greater than 5000 mg/kg in both cases.

Effect on Castor Oil-Induced Diarrhea: The extracts significantly reduced the number of diarrheal episodes in a dose dependent manner when compared with the untreated controls. At 200 mg/kg doses, MSPL showed 55.30% and MSPP 30.97% reduction in the number of fecal episodes, whereas loperamide offered 83.18% protection. (Table 2).

Effect on Magnesium Sulphate-Induced Diarrhea: Both extracts (MSPL and MSPP) exhibited significant antidiarrheal activity against magnesium sulphate-induced diarrhea (Table 3). The extracts at both dose levels significantly ($p< 0.001$, $p< 0.05$) 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 treated groups showed significant difference compared with control ($p< 0.001$, $p< 0.05$). The intestinal transit of charcoal meal was 75.00% in the control group, but at 200 mg/kg b.wt. dose 37.82% in MSPL and 59.26% in MSPP (Table 4).

PGE_2 – Induced Enteropooling: The plant extracts reduced the intestinal fluid accumulation induced by PGE_2 in a dose dependent manner (Figure 1). At 200 mg/kg b.wt. dose, MSPL showed a greater reduction (25.60%) than MSPP (16.04%) compared with the vehicle control.

Table 1: Yield, total amount of plant phenolic compounds and flavonoids of methanolic extract of *Musa sapientum* peel and pulp.

Sample	Yield (%)	Total phenols mg/g plant extract (in GAE)[a]	Total flavonoids mg/g plant extract (in QA)[b]
MSPL	15.0%	84.23 ± 0.81	21.34 ± 0.39
MSPP	12.1%	21.95 ± 0.12	8.23 ± 0.19[a]

Gallic acid equivalents (GAE, mg/g of each extract) for the total phenolic content. [b]Quercetin equivalents (mg/g of each extract) for the total flavonoid content. The GAE and QA are expressed as means ± SEM of triplicate experiments.

Table 2: Effect of *M. sapientum* peel and pulp extracts on castor oil-induced diarrhea in mice.

Treatment	Onset of diarrhea (min)	Animals with diarrhea	No. of faeces in 4 h	% inhibition of defaecation
Group I	25.45 ± 1.19	5/5	22.6 ± 0.68	
Group II	160 ± 0.13**	1/5	3.8 ± 0.58**	83.18
Group III	38.67 ± 2.73	4/5	20.2 ± 1.05	10.62
Group IV	51.23 ± 3.03*	3/5	15.6 ± 0.29*	30.97
Group V	48.67 ± 2.73**	3/5	15.2 ± 1.05**	32.74
Group VI	64.23 ± 3.03**	2/5	10.1 ± 0.29**	55.30

Values are presented as mean ± SEM, (n=5); **,* p<0.001; <0.05, respectively, compared to control by student's *t*-test. Group I animals received vehicle (1% Tween 80 in water), Group II received Loperamide 10 mg/kg body weight, Group III, IV, V and VI were treated with 100 and 200 mg/kg body weight (p.o.) of the MSPP and MSPL respectively

Table 3: Effect of *M. sapientum* peel and pulp extract on magnesium sulphate-induced diarrhea in mice.

Treatment	Onset of diarrhea (min)	Animals with diarrhea	No. of faeces in 4 h	% inhibition of defaecation
Group I	32.05 ± 1.09	5/5	20.2 ± 1.68	
Group II	160 ± 0.10**	1/5	4.8 ± 0.51**	76.23
Group III	38.67 ± 1.73	4/5	15.4 ± 1.05	23.76
Group IV	45.23 ± 1.03*	4/5	11.2 ± 0.49*	44.55
Group V	41.67 ± 2.03**	3/5	10.4 ± 1.15**	48.51
Group VI	59.23 ± 2.03**	2/5	5.4 ± 0.39**	73.27

Values are presented as mean ± SEM, (n=5); **,* p<0.001; <0.05, respectively, compared to control by student's *t*-test. Group I animals received vehicle (1% Tween 80 in water), Group II received Loperamide 10 mg/kg body weight, Group III, IV, V and VI were treated with 100 and 200 mg/kg body weight (p.o.) of the MSPP and MSPL respectively

Table 4: Effect of *M. sapientum* peel and pulp extract on charcoal meal stimulated gastrointestinal transit in mice.

Treatment	Dose (p.o.)	Mean intestinal length (cm)	Mean distance traveled by charcoal (cm)	% GI transit
1% Tween 80 in water	0.4 mL/mouse	69.6 ± 0.91	52.0 ± 1.08	75.00 ± 1.57
Atropine	0.1 mg/kg	64.0 ± 1.19	19.4 ± 0.79**	30.27 ± 0.80**
MSPP	100 mg/kg	68.8 ± 1.11	50.6 ± 1.06	73.73 ± 2.32
	200 mg/kg	69.2 ± 1.51	40.8 ± 2.02*	59.26 ± 3.82*
MSPL	100 mg/kg	67.2 ± 1.23	30.2 ± 0.83**	45.23 ± 2.30**
	200 mg/kg	66.2 ± 1.60	24.8 ± 1.01**	37.82 ± 1.04**

Values are presented as mean ± SEM, (n=5); **,* p<0.001; <0.05, respectively, compared to control by student's *t*-test.

Table 5: Antibacterial activity of the methanolic extracts of *M. sapientium* peel and Pulp.

	Diameter of zone of inhibition (mm)		
Bacterial strain	Ciprofloxacin	MSPL	MSPP
Staphylococcus aureus	28.03 ± 0.12	11.89 ± 0.14	NA
Pseudomonas aeruginosa	29.13 ± 0.21	NA	NA
Salmonella typhi	25.41 ± 0.11	8.09 ± 0.12	NA
Shigella flexneri	27.34 ± 0.12	NA	NA
Shigella dysenteriae	28.01 ± 0.11	9.02 ± 0.62	10.02 ± 0.02
Shigella boydii	29.39 ± 0.14	NA	NA
Escherichia coli	30.23 ± 0.18	10.59 ± 0.22	7.09 ± 0.12

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.

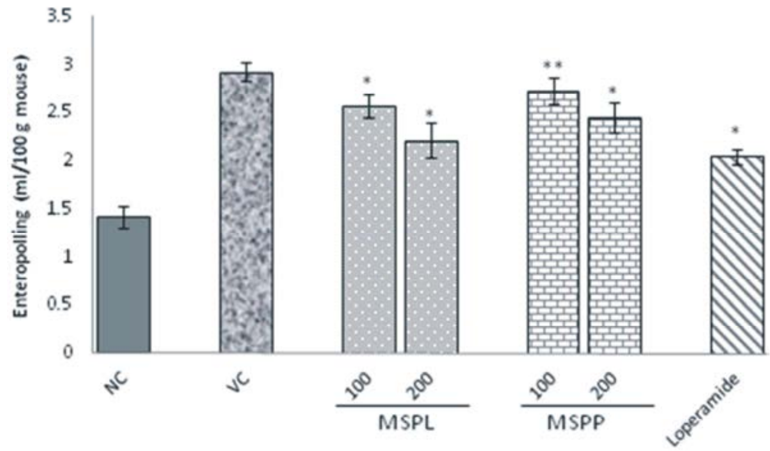

Fig. 1: Effect of the methanolic extract of peel and pulp of *M. sapientum* fruits on PGE_2-induced enteropooling in mice. Values are presented as mean ± SEM, (n=5); **,* p<0.001; <0.05, respectively, compared to vehicle control by student's *t*-test. NC: Normal Control; VC: Vehicle control.

Antibacterial Activity: Table 5 expressed the antibacterial activity (zone of inhibitions) of the MSPL and MSPP extracts. The MSPL extract showed significant to moderate activity against *Staphylococcus aureus, Salmonella typhi, Shigella dysenteriae and Escherichia coli,* whereas MSPP showed activity against *Shigella dysenteriae and Escherichia coli* only. Both extracts have not shown any activity against *Pseudomonas aeruginosa, Shigella flexneri* and *Shigella boydii*. The highest zone of inhibition was found against *Escherichia coli* (zone of inhibition 11.89 ± 0.22 mm), followed by *Staphylococcus aureus* (zone of inhibition 10.59 ± 0.14 mm) for MSPL.

DISCUSSION

Plants or plant derived preparations are used abundantly by mass population against diarrheal disorders without any scientific explanation. Imbalance between absorptive and secretary mechanisms in the GIT accompanied by intestinal hurry results in frequent loose stools or diarrhea [25]. Use of medicinal plants against diarrhea have been validated by several studies i.e. antispasmodic effects, delay intestinal transit, suppress gut motility, stimulate water adsorption, or reduce the intraluminal fluid accumulation [26,27]. Those experimental procedures were therefore employed to judge the antidiarrheal efficacy of *Musa sapientum* peel and pulp in the current study.

In the present investigation, MSPL and MSPP at large dose (200 mg/kg, b.wt.) exhibited significant antidiarrheal effects in one or the other experimental models. With respect to the castor oil induced diarrhea model, the results revealed that MSPL showed lightly better protection from diarrhea in the animals as compared with MSPP and so was the case in PGE_2 induced enteropooling. It is likely that the extracts bring out the aforementioned action either through their pro-absorptive property that promotes faster fluid absorption in the intestine or through an anti-secretory mechanism. Our first speculation gains support from the fact that castor oil, which was used as a diarrhea inducing agent in the experimental protocol. Several mechanisms have been previously proposed to explain the diarrhoeal effect of castor oil including inhibition of intestinal Na^+, K^+-ATPase activity to reduce normal fluid absorption [28], activation of adenylate cyclase or mucosal cAMP mediated active secretion [29], stimulation of prostaglandin formation [30], platelet activating factor and recently nitric oxide has been claimed to contribute to the diarrhoeal effect of castor oil [31] (Mascolo *et al*., 1996). 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 [32]. The prostaglandins of the E series are considered to be good diarrheogenic agents in experimental animals as well as in human beings [33]. The inhibitors of prostaglandins biosynthesis are therefore considered to delay the castor oil induced diarrhea [34].

On the other hand, magnesium sulphate 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 [35]. Both MSPL and MSPP extracts were found to improve the diarrheal condition in this model. The extracts 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 some extent, to their antidiarrheal activity by allowing a greater time for absorption.

In the small intestinal transit test, both extracts suppressed the propulsion of charcoal marker in a dose dependent manner. This finding suggests that the extracts act on all parts of the intestine. A decrease in the motility of gut muscles increases the stay of substances in the intestine [36]. This allows better water absorption. It is therefore presumed that the reduction in the intestinal propulsive movement in the charcoal meal model may be due to antispasmodic properties of the extracts. Salah *et al*. [37] has reported that flavonoids inhibit the intestinal motility in experimental induced diarrhea in rats.

Flavonoids and sugars obtained from selected traditional medicinal plants in Bangladesh were reported by Rahman and Wilcock [38] having antidiarrheal properties. Longanga *et al*. [39] 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. The flavonoids presence of these types of compounds, such as kaemferol, myricetin, apigenin and leucocyanidin in *Musa sapientum* is likely to contribute to its grastrointestinal effects [40]. Also some plants show antidiarrheal properties by their antimicrobial activities [41]. MSPL was shown to exhibit good antibacterial activity when tested against *Escherichia coli, Shigella dysenteriae* and *Staphylococcus aureus* and also supported to the previous study [42]. 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. In the present study this possibility is supported by the estimation of total polyphenols and flavonoids [43], which was found to be present in high concentration and was found to be 84.23±0.81 *mg* equivalent of gallic acid/*g* plant extract and 21.34 ±0.39 *mg* equivalent of quercetin/*g* plant extract, respectively. Moreover, Mokbel *et al*. [44] 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.

In conclusion, the results obtained in the present study suggest that *M. sapientum* peel and pulp extracts have beneficial effect in controlling the diarrhea in experimental animals. The antidiarrheal property of *M. sapientum* is mediated through inhibition of hypersecretion, gastrointestinal motility and increase of gastric transit time. The *Musa sapientum* could be used in the treatment of diarrhea.

REFERENCES

1. Guerrant, R.L., T. Van Gilder, T.S. Steiner, M.N. Theilman, L. Slutsker and R.V. Tauxe, 2001. Practice guidelines for the management of infectious diarrhea. Clinical Infectious Diseases, 32(3): 331-335.
2. Kosek, M., C. Bern and R.L. Guerrant, 2003. The global burden of diarrheal disease, as estimated from studies published between 1992 and 2000. Bulletin of the World Health Organization, 81: 197-204.
3. Thapar, N. and I.R. Sanderson, 2004. Diarrhea in children: an interface between developing and developed countries. Lancet, 363: 641-653.
4. Allen, S.J., B. Okoko, E. Martinez, G. Gregorio and L.F. Dans, 2004. Probiotics for treating infectious diarrhea. The Cochrane Database of Systemic Reviews, 2: 48-50.
5. Subbotina, M.D., V.N. Timchenko, M.M. Vorobyov, Y.S. Konunova, Y.S. Aleksandrovih and S. Shushunov, 2003. Effect of oral administration of tormentil root extract (*Potentilla tormentilla*) on rotavirus diarrhea in children: a randomized, double blind, controlled trial. The Pediatric infectious Disease Journal, 22(8): 706-711.
6. Palombo, E.A., 2006. Phytochemicals from traditional medicinal plants used in the treatment of diarrhea: Modes of action and effects on intestinal function. Phytotherapy Research, 20(9): 717-724.
7. Takahashi, K., M. Matsuda, K. Ohashi, K. Taniguchi, O. Nakagomi, Y. Abe, S. Mori, N. Sato, K. Okutani and S. Shigeta, 2001. Analysis of antirotavirus activity of extract from stevia rebaudiana. Antiviral Research, 49(1): 15-24.

8. Rahmatullah, M., M.A.H. Mollik, A.K. Paul, R. Jahan, M.A. Khatun, S. Seraj, A.R. Chowdhury, A.B.M.A Bashar, S.M.R. Wahab and M.T. Rahman, 2010. A Comparative Analysis of Medicinal Plants used to treat Gastrointestinal Disorders in two sub-districts of Greater Khulna Division, Bangladesh. Advances in Natural and Applied Sciences, 4(1): 22-28.
9. Agarwal, P.K., A. Singh, K, Gaurav, S. Goel, H.D. Khanna and R.K. Goel, 2009. Evaluation of Wound healing activity of extracts of plantain Banana (*Musa sapientum* Var. Paradisiaca) in rats. Indian Journal of Experimental Biology, 47: 32-40.
10. Coe, F. and G.J. Anderson, 1999. Ethnobotany of the Sumu (Ulwa) of southeastern Nicaragua and comparisons with Miskitu plant lore. Economic Botany, 53: 363-383.
11. Prasad, K.V., K. Bharathi and K.K. Srinivasan, 1993. Evaluation of *Musa Paradisiaca* Linn. cultivar "Puttubale" stem juice for antilithiatic activity in albino rats. Indian Journal of Physiology and Pharmacology, 37: 337-341.
12. Lewis, D.A., W.N. Fields and G.P. Shaw, 1999. A natural flavonoid present in unripe plantain banana pulp (*Musa sapientum* L. var. paradisiaca) protects the gastric mucosa from aspirin-induced erosions. Journal of Ethnopharmacology, 65: 283-288.
13. Ojewole, J.A. and C.O. Adewunmi, 2003. Hypoglycemic effect of methanolic extract of *Musa sapientum* (Musaceae) green fruits in normal and diabetic mice. Methods and Findings in Experimental and Clinical Pharmacology, 25: 453-456.
14. Krishnan, K. and N.R. Vijayalakshmi, 2005. Alterations in lipids & lipid peroxidation in rats fed with flavonoid rich fraction of banana (*Musa sapientum*) from high background radiation area. Indian Journal of Medial Research, 122: 540-546.
15. Houghton, P.J. and K. Skari, 1992. The effect of Indian plants used against snakebite on blood clotting. Journal of Pharmacy and Pharmacology, 44: 1054-1060.
16. Alam, M.B., N.S. Chowdhury, M.E.H. Mazumder and M.E. Haque, 2011. Antimicrobial and toxicity study of different fractions of *Dillenia indica* Linn. International Journal of Pharmaceutical Sciences and Research, 2(4): 860-866.
17. Yu, L., S. Haley, J. Perret, M. Harris, J. Wilson and M. Qian, 2002. Free radical scavenging properties of wheat extracts. Journal of Agricultural Food and Chemistry, 50(6): 1619-1624.
18. Chang, C.C., M.H. Yang, H.M. Wen and J.C. Chern, 2002. Estimation of Total Flavonoid Content in Propolis by Two Complementary Colorimetric Methods. Journal of Food and Drug Analysis, 10(3): 178-182.
19. Sanmugapriya, E. and S. Venkataraman, 2006. Toxicological investigations on Strychnos potatorum seeds in experimental models. Journal of Health Sciences, 52(4): 339-343.
20. Shoba, F.G. and M. Thomas, 2001. Study of antidiarrheal activity of four medicinal plants in castor oil induced diarrhea. Journal of Ethnopharmacology, 76(1): 73-76.
21. Doherty, S.S., 1981. Inhibition of arachidonic acid release, mechanism by which glucocorticoids inhibit endotoxin-induced diarrhea. British Journal of Pharmacology, 73(2): 549-554.
22. Abdullahi, A.L, M.O. Agho, S. Amos, K.S. Gamaniel and C. Watanabe, 2001. Antidiarrheal activity of the aqueous extract of *Terminalia avicemmoides* roots. Phytotherapy Research, 15(5): 431-434.
23. Robert, A., J.E. Nezamis, C. Lancaster, A.J. Hanchar and M.S. Klepper, 1976. Enteropooling assay: A test for diarrhea produced by prostaglandins. Prostaglandins, 11(5): 809-828.
24. Bauer, A.W., W.M.M. Kirby, J.C. Sherris and M. Truck, 1966. Antibiotic susceptibility testing by a standardized single disk method. American Journal of Clinical Pathology, 45(4): 493-496.
25. Yegnanarayan, R. and M.D.D.S. Shrotri, 1982. Comparison of antidiarrheal activity of some drugs in experimental diarrhea. Indian Journal of Pharmacology, 14: 293-299.
26. Atta, A.H. and S.M. Mouneir, 2005. Evaluation of some medicinal plant extracts for antidiarrheal activity. Phytotherapy Research, 19(6): 481-485.
27. Almeida, C.E., M.G. Karnikowshi, R. Foleto and B. Baldisserotto, 1995. Analysis of antidiarrhoeic effect of plants used in popular medicine. Revista de Saude Publica, 29: 428-433.
28. Nell, G. and W. Rummel, 1984. Action mechanism of secretagogue drugs. In: Csaky TZ (Ed.). Pharmacology of Intestinal Permeation, 2nd ed. Berlin; Springer, pp: 451.
29. Capasso, F., N. Mascolo, A.A. Izzo and T.S. Gaginella, 1994. Dissociation of castor oil induced diarrhea and intestinal mucosal injury in rat: effect of NG-nitro-L-arginine methyl ester. British Journal of Pharmacology, 113(4): 1127-1130.

30. Galvez, A., M.E. Zarzuelo, M.D. Crespo, M. Lorente, A. Ocete and J. Jimenez, 1993. Antidiarrhoeic activity of *Euphorbia hirta* extract and isolation of active flavonoid constituents. Planta Medica, 59(4): 333-336.
31. Mascolo, N., A.A. Izzo, T.S. Gaginella and F. Capasso, 1996. Relationship between nitric oxide and platelet activating factor in castor oil induced mucosal injury in the rat duodenum. Naunyn Schmiedeberg's Archive of Pharmacology, 353: 680-684.
32. Gaginella, T.S., J.J. Stewart, W.A. Olsen and P. Bass, 1975. Action of recinoleic acid and structurally related fatty acid on the gastrointestinal tract. II. Effect on water and electrolyte absorption *in vitro*. The Journal of Pharmacology and Experimental Therapeutics, 195(2): 355-356.
33. Jaffe, B.M., 1979. Prostaglandins and serotonin: Nonpeptide diarrhoegenic hormones. World Journal of Surgery, 3: 565-578.
34. Pierce, N.F., C.C. Carpenter, H.L. Elliot and W.B. Greenough, 1971. Effects of prostaglandins, theophylline and cholera exotoxin upon transmucosal water and electrolyte movement in canine jejunum. Gastroenterology, 60(1): 22-32.
35. Zavala, M.A., S. Perez, C. Perez, R. Vargas and R.M. Perez, 1998. Antidiarrheal activity of Waltheria Americana, *Commelina coelestis* and *Alternanthera repens*. J Ethnopharmacology, 61(1): 41-47.
36. Tangpu, V. and A.K. Yadav, 2004. Antidiarrheal activity of *Rhus javanica* ripen fruit extract in albino mice. Fitoterapia, 75(1): 39-44.
37. Salah, A.M., J. Gathumbi and W. Vierling, 2002. Inhibition of intestinal motility by methanol extracts of *Hibiscus sabdariffa* L. (Malvaceae) in rats. Phytotherapy Research, 16(3): 283-285.
38. Rahman, M.A. and C.C. Wilcock, 1991. A report on flavonoid investigation in some Bangladesh Asclepiads. Bangladesh Journal of Botany, 20(2): 175-178.
39. Longanga, O.A., A. Vercruysse and A. Foriers, 2000. Contribution to the ethnobotanical, phytochemical and pharmacological studies of traditionally used medicinal plants in the treatment of dysentery and diarrhea in Lomela area, Democratic Republic of Congo (DRC). Journal of Ethnopharmacology, 71: 411-423.
40. Kongkachuichai, R., R. Charoensiri and P. Sungpuag, 2010. Carotenoid, flavonoid profiles and dietary fiber contents of fruits commonly consumed in Thailand. International Journal of Food Sciences and Nutrition, 61(5): 536-548.
41. Ilyas, M., A.K. Haruna and N. Ilyas, 1995. Plant constituents with antidiarrheal properties. Bulletin of the Science Association of Nigeria., 10: 5-12.
42. Ferdinand, F.J., U. Esther, A. Tayo and A. Omotoyin, 2009. Evaluation of the antimicrobial properties of unripe banana (*Musa sapientum* L.), lemon grass (*Cymbopogon citratus* S.) and turmeric (*Curcuma longa* L.) on pathogens. African Journal of Biotechnology, 8(7): 1176-1182.
43. Kongkachuichai, R., R. Charoensiri and P. Sungpuag, 2010. Carotenoid, flavonoid profiles and dietary fiber contents of fruits commonly consumed in Thailand. International Journal of Food Sciences and Nutrition, 61(5): 536-548.
44. Mokbel, M.S. and F. Hashinaga, 2005. Antibacterial and antioxidant activities of banana (Musa, AAA cv. Cavendish) fruits peel. American Journal of Biochemistry and Biotechnology, 1(3): 126-132.