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ORIGINAL ARTICLE

LIFE SCIENCE

GROWTH AND YIELD EVALUATION OF MUSTARD VARIETIES GROWN IN A MEDIUM HIGHLAND OF KHULNA REGION

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Abstract

The present research was conducted at the experimental field of Agrotechnology Discipline, Khulna University, Khulna during *Rabi* season 2016-17 to investigate the growth and yield performance of mustard varieties. The experiment was arranged in a randomized complete block design consisting of eight mustard varieties (*viz.* BARI Sarisha-8, BARI Sarisha-11, BARI Sarisha-13, BARI Sarisha-14, BARI Sarisha-15, BARI Sarisha-16, Rai and Tori-7) as treatment and replicated thrice. All the growth, yield attributes and yield were substantially influence among the mustard varieties except the phenological parameters. Results of the experiment showed that the highest plant height (131.33 cm), seed yield (1813.33 kg ha^{-1}) and stover yield (3876.67 kg ha^{-1}) were found in BARI Sarisha-16. BARI Sarisha-11 was found better in respect of maximum siliqua $plant^{-1}$, weight of seeds $plant^{-1}$, 1000-seed weight and harvest index. Besides this, BARI Sarisha-14 showed the maximum number of seeds $siliqua^{-1}$. Therefore, findings of this study suggested that BARI Sarisha-16 would be suitable for better productivity and recommended for cultivation in the medium highland of Khulna region of Bangladesh.

Keywords: Mustard, Varieties, Southwestern Bangladesh, Growth and Yield

Introduction

Mustard is one the most vital oil seed crop next to soybean throughout the world (FAO, 2014). Among the oil seed crops grown in Bangladesh, mustard is considered as the principal oil seed crop which belongs to the genus *Brassica* of the family Cruciferae. It is well adapted to all agro-climatic zones of the country and is grown in *Rabi* season (November-March). Mustard seeds have high energy content, having 28–32% oil with relatively high protein content (28–36%) by weight, although these values can vary slightly between varieties, growing regions and crop years.

Actually mustard is covering above 69.94% of the oil cropped area and producing 38.80% of the total oil seed production in Bangladesh. Total area coverage and production of mustard in Bangladesh is 2,94,737 ha and 1,94,000 tons, respectively and rank first among the oil seed crops grown (BBS, 2013). The per capita consumption of edible oil in Bangladesh is 10-12g/day. The internal production of edible oil only meet less than one-third of the annual requirement (Mondal and Wahab, 2001). The major reasons for low yield of mustard in Bangladesh are lack of high yielding variety, appropriate population density and inadequate knowledge of sowing time, sowing methods and proper management practices (Mamun *et al.*, 2014). There is a great scope of increasing yield of mustard by selecting appropriate high yielding varieties, soil topography, weather condition with improved management practices (Bhuiyan *et al.,* 2011).

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The area under mustard cultivation is declining in Bangladesh due to late harvesting of high yielding T. aman rice and increased cultivation of boro rice losing an area of 104 thousand hectare with a production 68 thousand tons of mustard and rapeseed in last ten years (Anon., 2006). In Khulna region of southwestern coastal Bangladesh Fallow-Fallow-T. aman is major cropping pattern and after harvest of T. aman most of the land is remains fallow due to excess soil wetness, lack of fresh irrigation water and later increase in soil salinity. Mustard is a short duration crop which can be introduced in the existing cropping pattern of this region to make a better use of the fallow land and increase the copping intensity. In southwestern region only in few areas farmers usually cultivate mustard varieties which are mainly local and low yield potential. Besides local varieties Bangladesh Agriculture Research Institute (BARI) developed a number of short duration improved mustard varieties. After harvest of T. aman there is a scope to cultivate short duration high yield mustard varieties using residual soil moisture. Therefore, the present study was carried out to evaluate the growth and yield performance of mustard varieties and screen out the suitable variety for the medium highland in Khulna region of southwestern Bangladesh.

Materials and methods

The study was carried out in the experimental field of Agrotechnology Discipline, Khulna University, Khulna during *Rabi* season (from November 2016 to February 2017). Eight released varieties of mustard namely BARI Sarisha-8, BARI Sarisha-11, BARI Sarisha-13, BARI Sarisha-14, BARI Sarisha-15 BARI Sharisha-16, Rai Sharisha and Tori-7 were used as treatments in this experiment. Seeds were collected from the Regional Agricultural Research Station (RARS), Jashore.

Land Preparation and fertilizer application

The land was prepared by ploughing and cross ploughing followed by laddering and fertilized uniformly with recommended fertilizer doses of Urea, TSP, MoP, Gypsum, Zinc Sulphate and Boric Acid at the rate of 200 kg, 150 kg, 100 kg, 150 kg, 5 kg and 10 kg ha^{-1}, respectively. One-half of the urea and full doses of others fertilizer were applied during final land preparation and properly incorporated into the soil. The remaining urea was top dressed at 30 days after emergence (DAE).

Experimental design and layout

The experiment was laid out in a randomized complete block design with three replications. The size of unit plot was 4.0 m x 2.5 m. The distance between two rows were 30 cm and plant to plant 5 cm in line sowing method with intra plot spacing 0.50 m and intra block spacing of 1.0 m.

Germination test

Germination test was performed at the laboratory before sowing the seeds in the field. Petridishes were used for laboratory test. Seeds were distributed randomly in eight petridishes. Each petridish contained 25 seeds. On an average, the germination was above 80%. Data on emergence were collected on percentage basis by using the following formula:

$$\text{Germination (\%)} = \frac{\textit{No of seed germinated}}{\textit{No of seed taken for germination}} \times 100$$

Seed sowing

Before sowing the seeds were treated with vitavax-200 @ 2.5 g/ kg seed. The seeds were placed continuously in the furrow at a depth of 3-4 cm from the soil surface after that covered the furrow and slightly pressed. Light irrigation was done immediately after sowing.

Intercultural operations

Intercultural operation such as thinning, weeding, irrigation and plant protection measure were taken as and when necessary.

Sampling and data collection

Five sample plants were selected randomly avoid the border plants and marked in each plot.

Data on the following parameters were collected, calculated and recorded:

- First seedlings emergence and duration of emergence
- First flowering and duration of flowering
- First siliqua formation and duration of siliqua formation
- Plant height (cm) at 30, 40 , 50, 60 DAS and at harvest
- Number of leaves plant^{-1} at 30, 40 , 50, 60 DAS and at harvest
- Number of siliqua plant^{-1}
- Number of seeds siliqua^{-1}
- Weight of 1000 seeds (g)
- Seed yield (kg ha^{-1})
- Stover yield (kg ha^{-1})
- Biological yield (kg ha^{-1})
- Harvest index (%)

Harvest and post-harvest operations

Harvesting was done at full maturity when 80% of the siliqua turned yellowish in color. Harvesting was done in the morning to avoid shattering. The harvest area (1 m^2) was maintained leaving the border rows from which the seed weight, stover weight and 1000 seed weight were collected after proper drying and then converted to t ha^{-1}. Biological yield and harvest index were later calculated.

Statistical analysis

The collected data were compiled and statistically analyzed following analysis of variance (one-way ANOVA) using the MSTAT-C computer package program. Means were compared by using the Duncan's New Multiple Range Test (DMRT) at 5% level of probability (Gomez and Gomez, 1984).

Results and discussion

Seedlings emergence and duration of emergence

Among the varieties there was no significant difference on first seedlings emergence and duration of seedling emergence (Fig. 1). However, visually the fastest emergence (3.3 days) was observed in BARI Sharisha-13 whereas the slowest was observed (4.7 days) in Tori-7. The duration of seedlings emergence was maximum (12.0 days) in BARI Sharisha-16 and minimum (11.3 days) was recorded from BARI Sharisha-8 and BARI Sharisa-13.

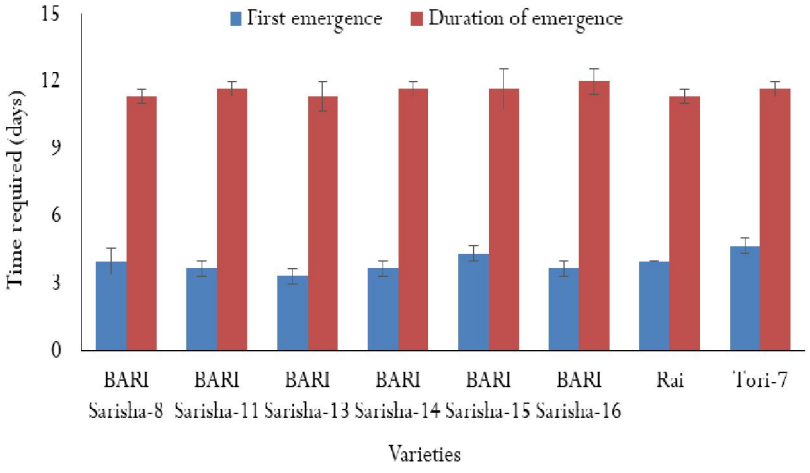

Figure 1. First seedling emergence and duration of emergence of different mustard varieties

First flowering and duration of flowering

On first flowering and duration of flowering there was no significant variation among the varieties (Fig. 2). But numerically the earliest flowering (21.7 days) was found in BARI Sharisha-11 while the late flowering was observed (27.0 days) in BARI Sharisha-13. The duration of flowering was observed maximum (8.3 days) in BARI Sharisha-13, BARI Sharisha-16, Rai and Tori-7 while minimum (7.7 days) was observed in BARI Sharisa-14.

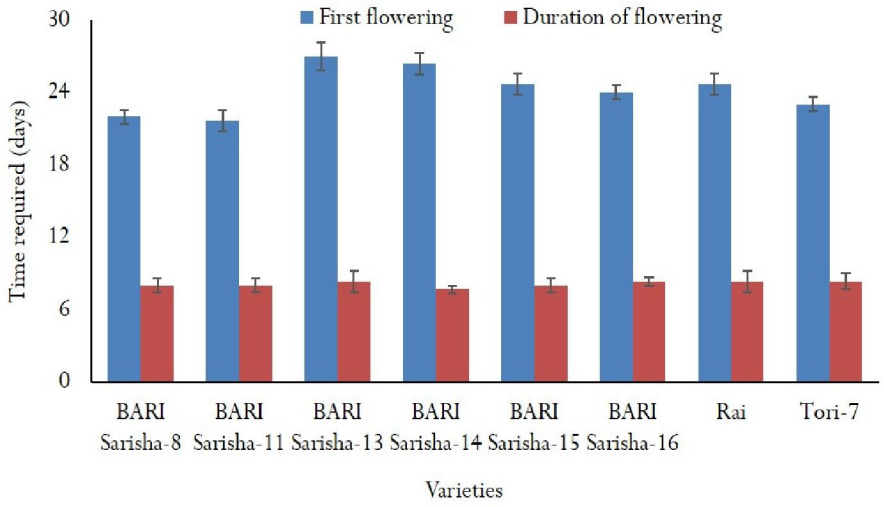

Figure 2. First flowering and duration of flowering of different mustard varieties

First siliqua formation and duration of siliqua formation

Analysis of variance showed that first siliqua formation was statistically significant but duration of siliqua formation was non-significant among the varieties (Fig. 3). The siliqua formation was earlier in BARI Sarisha-8 (26.3 days) which was statistically identical with Rai and BARI Sarisha-11 whereas siliqua formation was delayed in Tori-7 (39.3 days) which was similar to BARI Sarisha-14, BARI Sarisha-15 and BARI Sarisha-16. In BARI Sarisha-15 and Rai the duration of siliqua formation was highest whereas the lowest was observed (11.3 days) in BARI Sarisha-8, BARI Sarisha-11, BARI Sarisha-16 and Tori-7.

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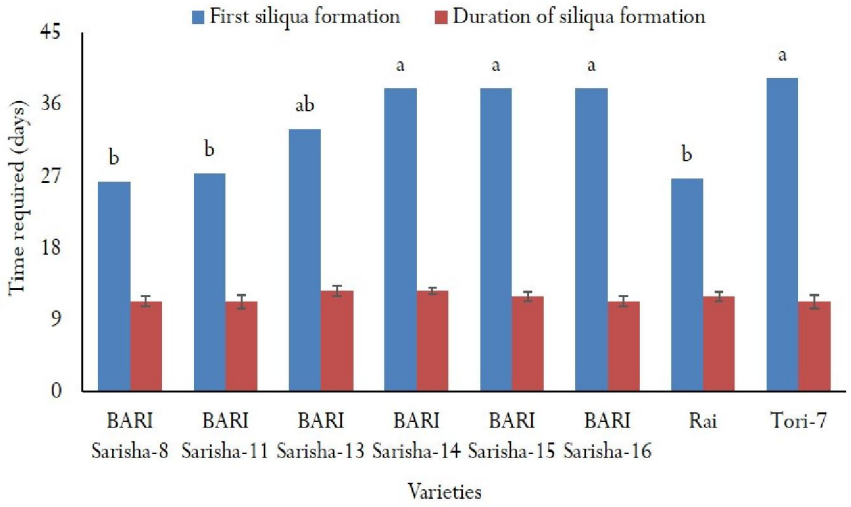

Figure 3. First siliqua formation and duration of siliqua formation of different mustard varieties

Plant height

Plant height was significantly influenced among the different varieties throughout the growing period (Table 1) and at harvest except at 50 DAS. At 30 and 40 DAS, BARI Sarisha-8 scored the tallest plant (18.60 cm & 38.33 cm) which was statistically identical with Tori-7 and Rai while the lowest was recorded from BARI Sarisha-11 (15.53 cm & 23.73 cm). At 50 DAS and at harvest, the tallest plant height was observed in BARI Sarisha-16 (118.67 cm & 131.33 cm) which was statistically identical with BARI Sarisha-11 but the lowest was found in BARI Sarisha-14 (46.00 cm & 49.40 cm) which was similar to BARI Sarisha-8. Variation of plant height was occurred due to the genetic makeup of the varieties. Akhter (2005) also found the variation of plant height among the different varieties. Similar variation of plant height among rapeseed/mustard varieties was also reported by many scientists (Ahmed et al., 2017; Roy, 2007; Zakaria and Jahan, 1997; Hossain et al., 1996). Yeasmin (2013) disagreed with this finding who reported that varietal effect was insignificant on plant height.

Table 1. Plant height (cm) and number of leaves plant-1 of different mustard varieties

Mustard varieties	Plant height (cm)			Leaf number		
	30 DAS	40 DAS	At harvest	30 DAS	40 DAS	At harvest
BARI Sarisha-8	18.60a	38.33a	54.53d	7.07	11.87a	19.00
BARI Sarisha-11	15.53b	23.73d	112.00b	6.47	8.93b	17.33
BARI Sarisha-13	16.70ab	23.80d	89.67c	6.73	10.00ab	18.33
BARI Sarisha-14	15.90ab	29.93bcd	49.40d	6.73	8.80b	17.67
BARI Sarisha-15	17.17ab	30.20bcd	74.53c	7.00	10.00ab	18.33
BARI Sarisha-16	17.00ab	27.53cd	131.33a	6.40	9.13ab	18.67
Rai	17.80ab	32.53abc	55.27d	6.80	10.33ab	19.33
Tori-7	18.47 a	35.67ab	55.90d	7.07	11.47ab	20.00
Level of significance	*	**	**	NS	**	NS
CV (%)	5.63	9.03	9.39	3.55	9.44	8.36

In a column figure having similar letter (s) do not differ significantly
CV = Co-efficient of variation, * = Significant at 5% level of level significance, **** =** Significant at 1% level of significance, DAS = Days after sowing, NS = Non- significant

Number of leaves plant-1

Leaf number was significantly varied among the varieties at 40 and 60 DAS (Table 1) but at 30 DAS, 50 DAS and at harvest there was no significant variation among the varieties. At 40 DAS, the maximum number of leaf was recorded from BARI Sarisha-8 (11.87) which was statistically similar to

all other varieties except BARI Sarisha-11 and BARI Sarisha-14 in which the number of leaf was minimum (8.93 and 8.80 respectively). At 60 DAS maximum leaf number was recorded at Tori-7 (18.93) which was statistically similar to all other varieties except BARI Sarisha-11 in which the number of leaves was minimum (15.47).

Number of siliqua plant^{-1}

Among the varieties number of siliqua plant^{-1} differed significantly (Fig. 4). Maximum number of siliqua plant^{-1} (130.8) was recorded in BARI Sarisha-11 which was identical to BARI Sarisha-16 while the minimum (22.8) was recorded from BARI Sarisha-14. Number of siliqua plant^{-1} is the result of genetic makeup of the crop and environmental conditions (Sana et al., 2003). The findings of Akhter (2005), Roy (2007) and Mamun et al. (2014) are in conformity with the results of this finding that the number of siliqua plant^{-1} of mustard was significantly affected by the varieties.

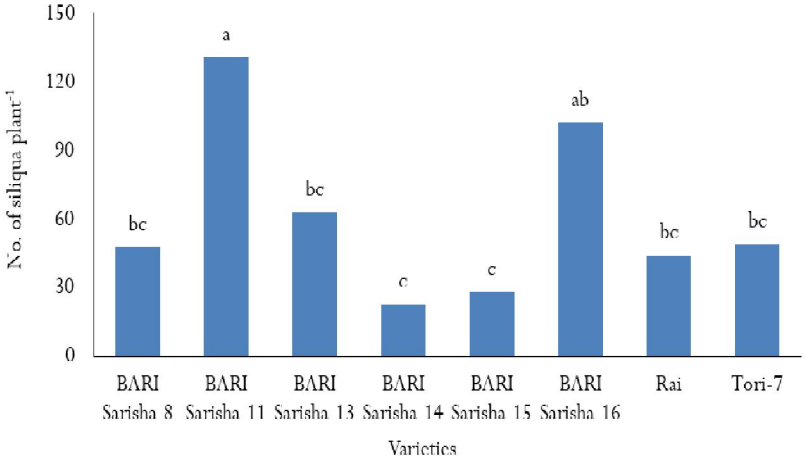

Figure 4. Number of siliqua plant^{-1} of different mustard varieties

Number of seeds siliqua^{-1}

Number of seeds siliqua^{-1} was significantly influenced due to the varietal difference (Table 2). The maximum number of seeds siliqua^{-1} (25.90) was resulted in BARI Sarisha-14 which was on parity with BARI Sarisha-13 whereas the minimum (11.43) was recorded from Tori-7. Variation in seeds siliqua^{-1} among the varieties was in conformity with Mamun et al. (2014), who found the highest seeds siliqua^{-1} in BARI Sarisha-13 and the lowest seeds siliqua^{-1} in BARI Sarisha-16 and this results are in agreement with the findings of Jahan and Zakaria (1997) and Gurjar and Chauhan (1997). But the results are in contradiction with Roy (2007) who found the highest seeds siliqua^{-1} in improved Tori-7 and the lowest number of seeds siliqua^{-1} in SAU Sarisha-1.

Weight of 1000 seeds

There was a significant variation among the varieties on weight of 1000 seeds (Table 2). Weight of 1000 seeds was higher (4.20 g) in BARI Sarisha-11 which was statistically at par with BARI Sarisha-13 and BARI Sarisha-8 while Tori-7 produced the lowest 1000 seed weight (1.68 g). The result of this finding was in conformity with that of Mamun et al. (2014). They also observed that BARI Sarisha-13 had the highest 1000 seed weight (4.00 g) whereas the lowest one (2.82 g) was found in SAU Sarisha-3. The 1000-seed weight is the stable part of yield and it varied from variety to variety which is in agreement with that of Mondal and Wahab (2001).

Seed yield

Analysis of variance revealed that seed yield among the varieties were differed significantly (Table 2). BARI Sarisha-16 resulted the higher seed yield (1813.33 kg ha^{-1}) while the lower (286.67 kg ha^{-1}) was obtained from Tori-7. Higher seed yield was attributed by the yield components. The results agreed

with Rahman (2002), BARI (2001), Mondal et al. (1995), Zaman et al. (1991) and Mendham et al. (1981) who reported that seed yield of rape and mustard varied with different varieties. Yeasmin (2013) also found significant varietal effect on seed yield. This findings are in conformity with the findings of Zaman et al. (1991), Chakrabarty et al. (1991) and Uddin et al. (1987) who reported that yields were different among the varieties. But the result was in contradiction with Roy (2007) and McNeilly (1987) who reported that seed yield of rapeseed and mustard was not significantly influenced by the variety.

Stover yield

Stover yield of different varieties of mustard were varied significantly (Table 2). BARI Sarisha-16 produced the highest stover yield (3876.67 kg ha^{-1}) whereas the lowest (633.33 kg ha^{-1}) was recorded from Tori-7.

Table 2. Yield and yield components of different mustard varieties

Treatments	Number of seeds $siliqua^{-1}$	1000-seed weight (g)	Seed yield (kg ha^{-1})	Stover yield (kg ha^{-1})	Biological yield (kg ha^{-1})	Harvest index (%)
BARI Sarisha-8	14.00c	3.62ab	416.67def	1086.67d	1503.33de	27.67c
BARI Sarisha-11	11.87c	4.20a	1403.33b	2713.33b	4116.67b	33.67a
BARI Sarisha-13	23.90ab	4.08ab	946.67c	1913.33c	2860.00c	33.00a
BARI Sarisha-14	25.90a	1.18de	313.33ef	893.33de	1206.67de	26.00c
BARI Sarisha-15	20.33b	2.38cd	496.67d	1273.33d	1770.00d	28.00c
BARI Sarisha-16	13.67c	3.59b	1813.33a	3876.67a	5716.67a	32.00ab
Rai	14.87c	2.61c	453.33de	1090.00d	1543.33de	29.33bc
Tori-7	11.43c	1.68e	286.67f	633.33e	1020.00e	28.33c
Level of significance	**	**	**	**	**	**
CV (%)	7.38	8.23	8.63	9.43	10.37	3.96

In a column figure having similar letter (s) do not differ significantly
CV = Co-efficient of variation, * = Significant at 5% level of level significance, **** =** Significant at 1% level of significance

Biological yield

Biological yield of different varieties of mustard varied significantly (Table 2). Maximum biological yield (5716.67 kg ha^{-1}) was obtained from BARI Sarisha-16 and the minimum (1020.00 kg ha^{-1}) was obtained from Tori-7. Mamun et al. (2014) found similar results on biological yield due to varieties. But this results are in contradiction with the findings of Yeasmin (2013) who found insignificant varietal effect on biological yield.

Harvest index

Harvest index was differed significantly among the tested varieties (Table 2). BARI Sarisha-11 contributed the highest harvest index (33.67%) which was statistically at par with BARI Sarisha-13 and BARI Sarisha-16 while the lowest (26.00%) was calculated from BARI Sarisha-14. Roy (2007) also found the similar result, lowest harvest index in was found in Tori-7. Similar results were also observed by Islam et al. (1994) that harvest index varied significantly among the varieties. Yeasmin (2013) found insignificant varietal effect on harvest index.

Conclusion

The result of this study revealed that the growth, yield and yield attributes of mustard varied substantially among the tested varieties used in this experiment yet no significant variation in phenological parameters. Considering the productivity, BARI Sharisha-16 is suitable and can be recommended for cultivation in the medium highland of Khulna region of southwestern Bangladesh.

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ORIGINAL ARTICLE

LIFE SCIENCE

MODULATORY ANTI-DIARRHEAL EFFECTS OF ASCORBIC ACID IN *SWISS* ALBINO MICE

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Abstract:
Ascorbic acid (AA) has been reported for the management of diarrhea. The anti-diarrheal potential and modulatory activities of AA on some commonly used anti-diarrheal drugs were investigated. For this purpose, the activities of AA on castor oil-induced diarrhea in *Swiss* mice were examined. As standard anti-diarrheal agents, we used prazosin, propranolol, loperamide, and nifedipine with or without AA. The results revealed that AA at 25 mg/kg (i.p.) and all other standard drugs exhibited significant ($p < 0.05$) diarrheal attenuating activities in mice. However, the impact was more pronounced in the loperamide and propranolol groups. AA administrated with prazosin and propranolol had a higher rate of latent periods and a lower rate of diarrheic secretion during the study period (4 h) than that of the other single or mixed groups. Furthermore, a molecular docking study illustrated that AA displayed good binding affinities with (α1) (–5.2 Kcal/mol), α2b (–5.4 Kcal/mol), α2c (-5.6 Kcal/mol), β1(–5.3 Kcal/mol) and β2(–6.4 Kcal/mol) adrenoceptors. Of note, AA exerted a significant anti-diarrheal effect and it was seen to modulate the anti-diarrheal effects of α- and β-adrenergic receptor blocking agents in *Swiss* mice.

Keywords: Ascorbic acid; Castor oil; Diarrhea; *Mus musculus*; Prazosin

Introduction

The global burden of morbidity and mortality associated with diarrhea remains a major concern all over the world (Guandalini and Vaziri, 2007). Many broad types of diarrhea which depend on severity, source or origin, epidemiological observations, causative organisms, duration of episode and fatality have been made. However, diarrhea has been generally and most acceptably classified into three categories depending on the number of days it lasts. It is termed acute if it lasts up to 14 days, persistent if it lasts between 14 and 29 days, and chronic if it lasts up to 30 days or more (DuPont et al., 2014). While the clinical manifestations of the disease vary in attendant severity and duration, the burden and economic toil it takes on affected individuals can not be overemphasized (Zimmermann et al., 2019). Some clinical manifestations of diarrhea include abdominal cramps, nausea and vomiting, which often make patients live a low-quality sedentary lifestyle (Cavalcanti et al., 2019).

While contaminated food and drinks are the most renowned cause of diarrhea in humans, the effect includes an imbalance in the body electrolytes which results in hyper-secretory feedback and induction of intestinal contraction. It should be noted that many therapeutics act by inhibiting gut motility as well as preventing electrolyte discharge (Shah et al., 2010). Commonly used anti-diarrheal

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agents such as loperamide, nifedipine, prazosin, and propranolol are without some mild or acute side effects such as abdominal discomfort, altered pulsation, nausea, dizziness, stomach disorder, constipation, drowsiness, and tiredness (Guandalini and Vaziri, 2007).

Ascorbic acid (AA), otherwise called vitamin C, is renowned for its antioxidant potential and other therapeutic applications in humans (Knight et al., 2016). In a study, pre-treatment of AA (150 mg/kg/day) has been seen to prevent gastrointestinal damage induced by radiation in mice (Yamamoto et al., 2010). However, a reduced concentration (5 mg/day) of AA has been suggested as a tolerable dose for Charcot-Marie-Tooth type 1A patients (Toth, 2009). An earlier report suggests that AA at one table (1000 mg) three times/day after the third week significantly lowers the number of diarrheic frequency in neonatal calves (Seifi et al., 1996). Due to the multi-therapeutic nature of the AA, with recognized relevance and application in the management of stress-related conditions linked to inflammation of the immune system, it has been used in combination therapy with other therapeutic agents (Sorice et al., 2014).

Opioid receptors have three pharmacological subtypes, μ, δ and ϰ (Connor and Christie, 1999; Dietis et al., 2011). The conventional opioid receptors are found in the vas deferens, knee joint, gastrointestinal, heart, and immune system, among many other places, across the central nervous system and, to a lesser degree, across the periphery (Stein et al., 2003). The opioid receptor is a class of receptor with many pharmacological importances, such as analgesia, anxiety, alleviating depression symptoms (Ide et al., 2010), diarrhea, irritable bowel syndrome (Maltz and Fidler, 2017), increasing gastrointestinal motility and some other biological effects.

In both health and sickness, the adrenergic system is critical for maintaining bodily homeostasis. Adrenergic receptor, belong to G-protein couple receptor family (Pierce et al., 2002; Philipp and Hein, 2004), has two major classes of receptor: α- adrenoceptor (aα1A, α1B, α1D, α2A, α2B,and α2C) and β-adrenoceptors (β_1-, β_2 , and β_3-) (Molinoff, 1984; Philip and Hein, 2004). They are found in the heart, brain, and adipose tissue and regulate several effects, including pain sensation (Diatchenko et al., 2006), vasodilation, decrease blood pressure (Rokosh and Simpson, 2002).), breakdown glycogen, diarrhea (Bricker et al., 2001) etc.

This study evaluates the anti-diarrheal potential of AA in a castor oil-induced diarrheal mouse model. Additionally, the interaction capacity of AA with the commonly used anti-diarrheal agents such as loperamide, prazosin, propranolol, and nifedipine has also been investigated.

Materials and Methods

Animal (in vivo) study

Reagents and Chemicals

Square Pharmaceuticals Ltd provided loperamide (LOP) and prazosin (PRA) while castor oil was acquired from a local market in Bangladesh. ACI Ltd. and Drug International Ltd. Bangladesh graciously contributed propranol (PRO) and nifedipine (NIF), respectively. Merck, India, produced ascorbic acid (AA) and tween 80.

Experimental mice

The animal resource section of Jahangir Nagar University (JU) in Dhaka provided the mature albino mice utilized in the experiments (weighing 22 - 30 g). The mice were housed in sterilized polypropylene cages with sterile rice husk as bedding under conventional climatic conditions (temperature: 25 ± 2 ºC, humidity: 50 ± 5%, and 12 hour light/dark cycles). All the housed mice with unrestricted access to pellets as their basal diet and water *ad libitum* were naturalised for approximately seven days before commencement of study. The mice, experimental and control groups were randomly assigned to have their food withdrawn 12 hours before the experimental hours. The test protocol was approved by the Department of Pharmacy, BSMRSTU, Gopalganj, Bangladesh (Approval number # 20150109004).

Groups and Treatments (Castor Oil-Induced Diarrhea in Mice)

The standard approach, which was based on the Awouters et al (1978) method with minor changes, was used. Briefly, 30 minutes following the sample (Group-II) and control (Group-I & Group-VI) treatments, each mouse was given 0.5 mL of castor oil. Similarly, AA (Group-II) was co-treated for 15 minutes with Group-III to Group-VI (Group-VII to Group-X) (Table 1). The animals (n = 5) were then observed for latency and total defecation for up to 4 hours in each group.

Table 1. Treatment given the overnight-fasted mice

Treatment groups	Dose
Gr-I: VEH (i.p.)	10 mL/kg
Gr-II: AA (i.p.)	25 mg/kg
Gr-III: LOP (p.o.)	3 mg/kg
Gr-IV: PRA (i.p.)	1 mg/kg
Gr-V: PRO (i.p.)	10 mg/kg
Gr-VI: NIF (i.p.)	2.5 mg/kg
Gr-VII: (Gr-II + Gr-III)	AA25+LOP3
Gr-VIII: (Gr-II + Gr-IV)	AA25+PRA1
Gr-IX: (Gr-II + Gr-V)	AA25+PRO10
Gr-X: (Gr-II + Gr-VI)	AA25+NIF2.5

VEH (Vehicle): 0.05% Tween 80 dissolved in 0.9% NaCl solution, i.p.: intra-peritoneal, p.o.: Per oral.

Statistical Analysis

This study's data were submitted for a one-way analysis of variance (ANOVA), and the findings were presented as the mean standard deviation (SD). GraphPadPrism® - GraphPad Software, Inc. (Version: 6.0) was used for statistical analysis, and the Newman-Keuls post hoc test was used; differences at $p < 0.05$ were judged significant at the 95% confidence interval.

Molecular docking study

For the molecular studies, therapeutically relevant proteins which include α1, α2b, α2c, β1 and β2 adrenoceptors were used for the ligand-protein interaction evaluation. Models of proteins were obtained from the protein data bank (PDB) as homology models of the proteins (Table 4) were built on Modeller 9.19. The docking grid of 40 × 40 × 40, 0.8 Å (enclosing the active binding sites) generated on the ProBiS server was used. The ligands, AA, were docked into the predicted binding pocket of selected proteins utilizing Auto dock Vina.

Results

Animal study

In comparison to the VEH group, AA and the standards (LOP, PRA, PRO, NIF) dramatically ($p< 0.05$) enhance latent durations in diarrheal mice. The latency duration was longer in the LOP (24.2 1.6 min) and PRO (21.8 2.4 min) groups than in the AA, PRA, and NIF groups. However, AA when co-administered with the standards resulted PRA group (26.8 3.3 min) led to the greatest increase in latent time, followed by AA + PRO, AA + LOP, and AA + NIF groups, respectively. AA co-treated with the LOP, significantly increased the latency period than the AA group, but remained unchanged compared to the LOP group. Furthermore, AA co-treated with NIF decreased the latency period compared to the individually treated groups, AA and NIF (Table 2).

Table 2. The latent duration of the treatment groups was determined in castor oil-induced diarrheal mice

Therapeutic groups	Dose (route of administration)	Latency (min)
Gr-I: VEH	10 ml/kg (i.p.)	9.2 ± 2.8
Gr-II: AA	25 mg/kg (i.p.)	18.2 ± 1.9*
Gr-III: LOP	3 mg/kg (p.o.)	24.2 ± 1.6$^{*\#}$
Gr-IV: PRA	1 mg/kg (i.p.)	16.8 ± 1.8*
Gr-V: PRO	10 mg/kg (i.p.)	21.8 ± 2.4^{*b}
Gr-VI: NIF	2.5 mg/kg (i.p.)	16.8 ± 3.9*
Gr-VII: (AA + LOP)	25 + 3 mg/kg	24.3 ± 2.8$^{*\#bcd}$
Gr-VIII: (AA + PRA)	25 + 1 mg/kg	26.8 ± 3.3$^{*\#bcd}$
Gr-IX: (AA + PRO)	25 + 10 mg/kg	25.2 ± 2.1$^{*\#bcd}$
Gr-X: (AA + NIF)	25 + 2.5 mg/kg	14.8 ± 1.8*

Values are mean ± SD (n = 5); anova one way followed by newman-keuls *post hoc* test; $p < 0.05$ when compared to the *Gr-I, $^{\#}$Gr-II, ^{a}Gr-III, ^{b}Gr-IV, ^{c}Gr-V, ^{d}Gr-VI in respective hour; VEH: 0.05% Tween-80 dissolved in 0.9% NaCl solution; AA: Ascorbic acid; LOP: Loperamide; PRA: Prazosin; PRO: Propranolol; NIF: Nifedipine

Similarly, findings in Table 3 show that AA at 25 mg/kg lowers the quantity of diarrheal discharges substantially ($p < 0.05$) when compared to the VEH group. On the 4th hour, AA showed the greatest decrease in diarrheal secretions (3.6 ± 2.8); the LOP and PRO groups showed the greatest attenuation in diarrheal secretions. In this case, the activities of AA piqued the PRA group's interest. In addition, in the AA + LOP, AA + PRA, and AA + PRO groups, AA co-treated with the standards substantially decreased diarrheal secretions. In comparison to the LOP group, AA co-administered with PRA and PRO was more successful in decreasing diarrheal secretions. AA co-treated with NIF resulted in augmented diarrheic secretions when compared to the NIF group animals (Table 3).

Table 3. Diarrheal secretions of mice in different treatment groups at 1st, 2nd, 3rd and 4th hours (stool number count)

Treatment groups	1st h	2nd h	3rd h	4th h
GR-I: VEH	15.2 ± 2.3	10.8 ± 2.6	9.8 ± 1.8	7.8 ± 2.8
GR-II: AA	10.0 ± 2.8*	6.8 ± 2.4*	6.4 ± 1.8*	3.6 ± 2.8*
GR-III: LOP	6.2 ± 2.6$^{*\#}$	4.8 ± 2.5$^{*\#}$	3.8 ± 1.3$^{*\#}$	1.8 ± 1.6$^{*\#}$
GR-IV: PRA	8.8 ± 2.4$^{*\#}$	6.6 ± 1.2*	4.2 ± 2.2$^{*\#}$	3.6 ± 2.8*
GR-V: PRO	7.4 ± 3.1$^{*\#}$	5.4 ± 2.2$^{*\#b}$	5.0 ± 1.0$^{*\#}$	4.2 ± 0.8*
GR-VI: NIF	8.2 ± 3.7$^{*\#}$	7.4 ± 1.7*	5.2 ± 2.8$^{*\#}$	2.2 ± 1.8$^{*\#abc}$
GR-VII: (AA + LOP)	6.0 ± 1.2$^{*\#bcd}$	3.0 ± 1.8$^{*\#abcd}$	2.2 ± 1.2$^{*\#bcd}$	1.6 ± 1.4$^{*\#bc}$
GR-VIII: (AA + PRA)	4.6 ± 1.1$^{*\#abcd}$	3.4 ± 1.4$^{*\#bcd}$	1.8 ± 0.8$^{*\#bcd}$	0.4 ± 1.3$^{*\#abcd}$
GR-IX: (AA + PRO)	4.8 ± 2.7$^{*\#c}$	3.4 ± 1.8$^{*\#abcd}$	2.6 ± 1.7$^{*\#bcd}$	1.6 ± 0.9$^{*\#bc}$
GR-X: (AA + NIF)	8.8 ± 1.6$^{*\#}$	7.8 ± 2.9*	5.6 ± 1.9*	3.2 ± 0.8^{*c}

Values are mean ± SD (n = 5); anova one way followed by newman-keuls *post hoc* test; $p < 0.05$ when compared to the *Gr-I, $^{\#}$Gr-II, ^{a}Gr-III, ^{b}Gr-IV, ^{c}Gr-V, ^{d}Gr-VI in respective hour; VEH: 0.05% Tween-80 dissolved in 0.9% NaCl solution; AA: Ascorbic acid; LOP: Loperamide; PRA:Prazosin; PRO: Propranolol; NIF: Nifedipine

In silico study

The results of the molecular docking experiment indicated that AA possessed good to moderate energies towards all the target proteins studied. AA showed the best interaction with (α1) (–5.2 Kcal/mol), α2b (–5.4 Kcal/mol), α2c (-5.6 Kcal/mol), β1 (-5.3 Kcal/mol) and β2 (-6.4 Kcal/mol) (Table 4). The observed ligand-protein interactions at the binding site together with interactions with some amino acid residues are as indicated. Hydrophobic interactions and hydrogen bonding are indicated by the red and green lines respectively. The 2D and 3D structures of non-bond interactions of AA with adrenoceptor subunits are shown in Figure 1.

Table 4. Molecular docking study of AA with alpha and beta adrenoceptors

Target protein	Binding affinity (Kcal/mol)	Interacting amino acids
α1	-5.2	SER90, PRO184, PHE106
α2b	-5.4	GLN68, ASP432
α2c	-5.6	ASN111, TYR427, SER108, CYS202, ASP131
β1	-5.3	ASN329, ASP200
β2	-6.4	SER207, ASN293, TYR316, ASN312, ASP113

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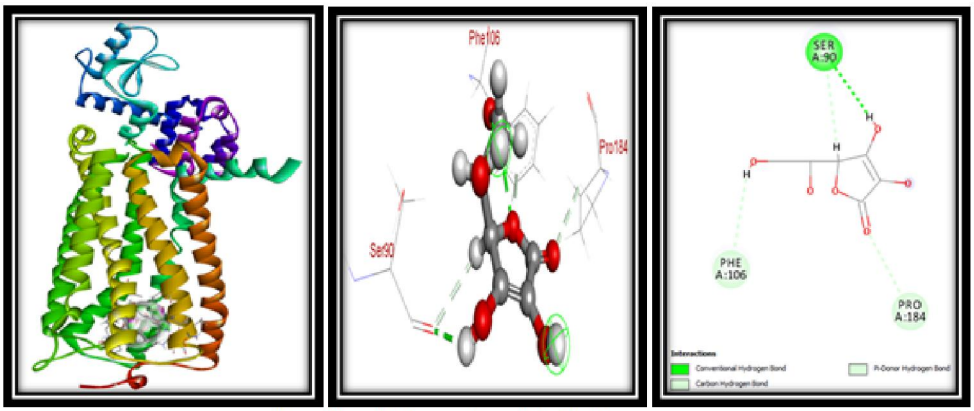
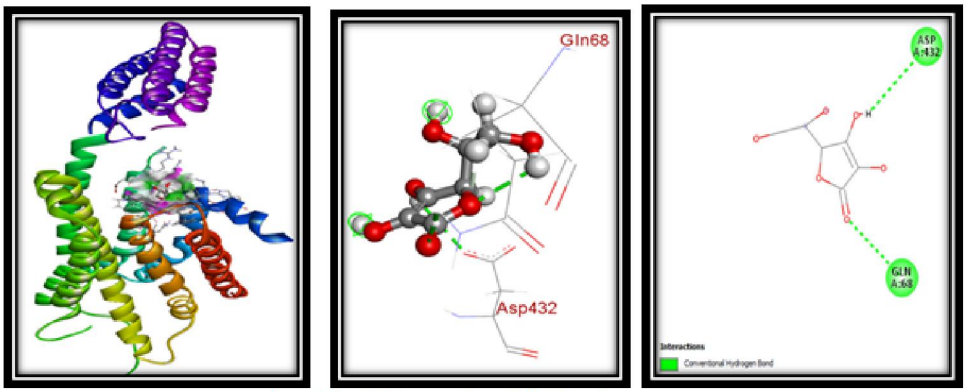
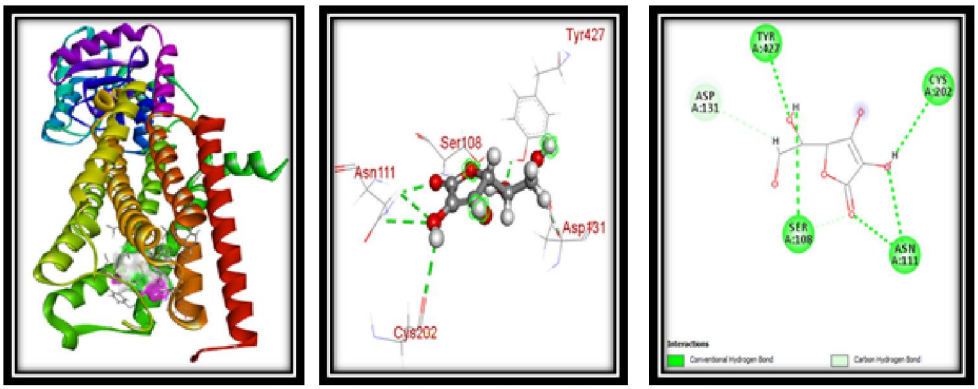


Ascorbic acid interaction with α1 adrenoceptor



Ascorbic acid interaction with α2b adrenoceptor



Ascorbic acid interaction with α2c adrenoceptor

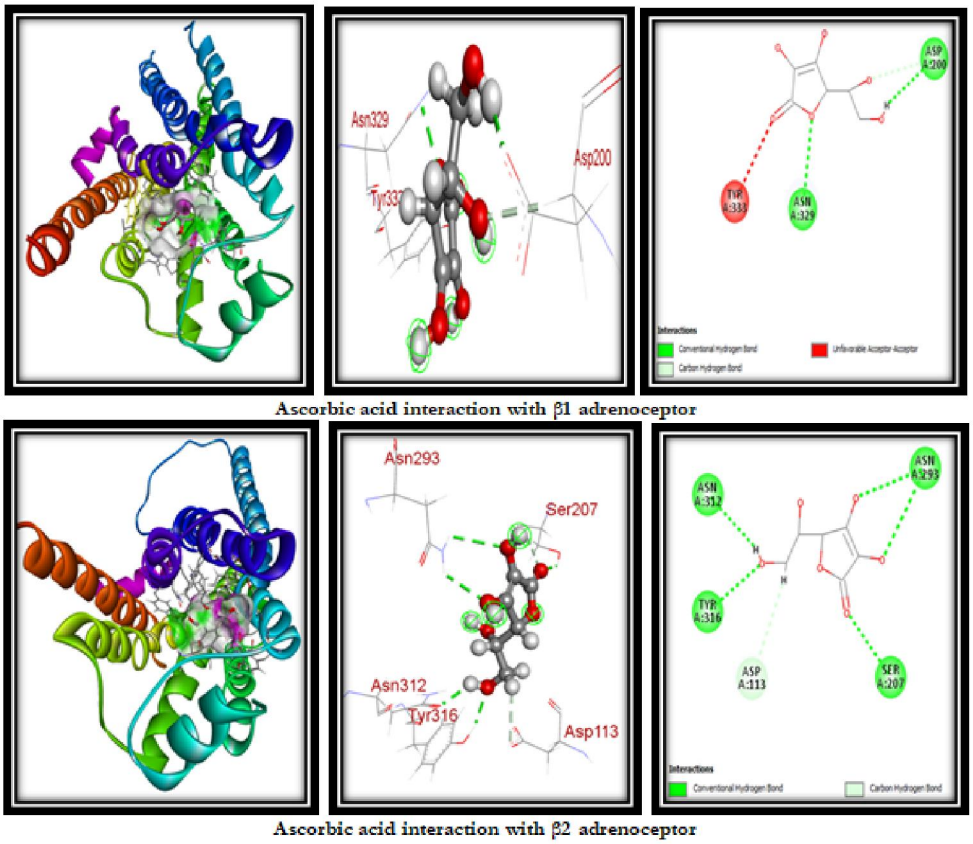


Figure 1. Ascorbic acid interactions with some selected adrenoceptors

Discussion

Diarrhea is one of several intestinal disorders (Chang et al., 1985). Various natural products and synthetic compounds have been reported to possess diarrheal activities. Ricinoleic acid, a primary compound from castor plant seeds and also in the sclerotium of ergot, has been reported to be responsible for the diarrheal effect in animals (Yoshio, 1999). Ricinoleic acid is known to trigger secretion of prostaglandins and platelet-activating factor (Mascolo et al. 1993), thereby inducing mucus release, smooth muscular contraction, and vasodilation in the gastrointestinal track, leading to a condition known as diarrhea (Bello et al. 2016). Other studies have also suggested that ricinoleic acid further stimulates the secretion of nitric oxide (NO) and likewise activates adenylyl cyclase, which causes an increase in cyclic adenosine monophosphate (cAMP) agglomeration. In the intestine, elevation of cAMP congregation reportedly decreases the absorption of sodium and potassium ions, induces peristaltic effect, distorts the membrane viability and reduces Na^+/K^+/ATPase pump activity. The entire process causes electrolyte assembly and H_2O in the gut lumen (Uchida et al. 2000; Rawat et al. 2017). AA is evident to ameliorate ischemia-reperfusion-influenced acute renal damage through a decrease in the renal nitric oxide level in rats (Koul et al. 2015).

Hemingway, (1991) revealed that AA suppressed diarrhea in neonatal calfs (Hemingway, 1991). Another study has demonstrated that AA (100 mg/kg, p.o.) along with *A. melegueta* (500 mg/kg) possess greater protection against diarrhea (Umukoro and Ashorobi, 2005). Several studies have shown that ascorbic acid reduces prostaglandin concentrations, possibly through inhibition of peroxidation of phospholipids (Stickel et al., 1997; Child et al., 1999). Considering the role of prostaglandin in the regulation of intestinal fluid secretion, these antioxidant nutrients may offer beneficial effects on castor oil–induced diarrhea, whereas prostaglandin induces gastrointestinal motility, which probably induced diarrhea in mice models (Umukoro and Ashorobi, 2005).

Candelario-Jalil et al. (2006) claimed that AA drastically suppresses prostaglandin synthesis (Fiebich et al., 2003; Candelario-Jalil et al., 2006). An excessive amount of prostaglandin may induce gastrointestinal motility and induce diarrhea (Robert et al., 1976; Riviere et al., 1991).

Furthermore, the opioid receptor has antidiarrheal properties with its subtypes including supraspinal μ and δ and peripheral μ, δ and k (Shook et al., 1989) and these are involved in GI motility (Maltz and Fidler, 2017). Other studies revealed that AA blocks GI motility in an experimental model in a dose dependent manner (Umukoro and Ashorobi, 2005). In our study, we found that after administration of AA to a mouse group, it reduced the stool count, possibly via interacting with opioid receptors and blocking GI movement, and increased gut transit time.

In this study, we saw that AA and the standards (LOP, PRA, PRO, NIF) drastically ($p < 0.05$) increased the latency in diarrheal mice when compared to the VEH group. In other words, AA when co-administered with the standards, induced a latent period and a reduction in diarrheic secretions, especially in the AA + PRA and AA + PRO groups.

The alpha-1 (α1*)* adrenergic receptor is a G protein-coupled receptor (GPCR) associated with the Gq heterotrimeric G-protein. Study suggests that adrenoceptor blockade is helpful in reducing the time it takes for food to pass through the intestines and alleviating diarrhea (Bricker et al., 2001). PRA is evident to exert its anti-diarrheal effect through blocking alpha-1 (α1*)* adrenergic receptors. The report suggests that AA has an inhibitory effect on this receptor (Wolfman et al. 1983). Moreover, in a recent clinical trial, AA together with the non-specific beta blocker, PRO, were found effective, when administered previously and continually, in the prophylaxis of fibro dysplasia ossificans progressive flare-ups (Palhares et al., 2019). This study establishes the combined potencies of AA and/or PRA/PRO in diarrheal-induced mice.

K, Na, and Ca ion channel blockers are often given medicine for a range of health issues, including angina pectoris, cardiac arrhythmias, and hypertension, among others. Some studies have also shown that using ascorbic acid with its complex in the therapeutic treatment of coronary heart disease to counteract the negative effects of voltage-gated channel blockers is effective (Ivanov et al. 2016). In this study, AA was seen to augment NIF (a calcium channel blocking agent) mediated decreasing diarrheic secretions, while decreasing the latency period in diarrheal mice.

Molecular docking studies suggested that AA exerts good to moderate binding energies with adrenoceptors, which reveals that AA can regulate adrenoceptor functions. It might induce an antidiarrheal effect. After in vivo study results, we can claim that AA has the ability to suppress diarrhea via regulating several mechanistic pathways like opioids, adrenoceptors, and calcium channels in experimental mice models.

Conclusion

In summary, findings from this investigation suggest that AA at 25 mg/kg displayed remarkable diarrheal-preventing potency in castor oil-induced diarrheal mice as it drastically increased the latency time while lowering diarrheic portions. When used with the conventional medicines PRA and PRO, AA had a higher anti-diarrheal effect. AA had no interference with the anti-diarrheal effect of LOP, but it slightly suppressed the NIF-mediated anti-diarrheal effects in experimental animals. Further studies are required to understand the molecular mechanism (s) behind the combined antidiarrheal effect of AA with PRA or PRO in animal models.

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Ethics approval and consent to participate

As appropriate, this project was approved by the authorizing Department of Pharmacy (Approval No. 20150109004), BSMRSTU, Gopalganj-8100, Bangladesh.

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SELECTION OF FUELWOOD TREE SPECIES USING FUEL VALUE INDEX IN JHENAIDAH DISTRICT

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Abstract

Four tree species *Acacia nilotica, Samanea saman, Leucaena leucocephala, Tamarindus indica* were collected from different wood vendors of Jhenidah district in Bangladesh to identify the potential fuelwood tree species through fuelwood properties. The calorific value and density of the wood considered as positive characteristics and high moisture and ash content as negative characteristics that helped develop a fuelwood value index (FVI). A 8×2×2 cm^3 of three wood blocks were collected from each tree species in order to determine the fuelwood properties. The highest calorific value and wood density were found in *Acacia nilotica* and *Tamarindus indica* respectively but the ash content was found to be lowest in *Leocoena leucocephala* (1.09%) and highest in *Tamarindus indica* (2.21%). Moreover, the lignin content was the highest in the *Acacia nilotica.* According to FVI, the better quality of fuelwood species in descending order are *Acacia nilotica> Leucaena leucocephala>Tamarindus indica >Samanea saman.* This study also revealed the significant negative correlation between FVI and ash content.

Keywords: Fuelwood, Ash content, Fuel value index, Wood properties

Introduction

According to Arnold and Jongma (1977) fuelwood is considered to be the main source of energy in rural and urban areas of many developing nations such as Africa, extending its use from household to industrial activities. Food and Agricultural Organization (FAO) (2011) reported that the fuel wood percentage of the total wood production for both developing and developed countries where it is 84.2% in case of developing and is 12.3% for developed countries.

Since 1950, fuelwood was regarded as a free commodity particularly in the rural areas of Bangladesh. Different sources like biomass, natural gas, electricity, coal, kerosene, diesel/gas oil and others met the energy requirement of the country. Conventional fuels, such as wood and agricultural residues like paddy husk, straw, jute stick, leaves, cowdung, bran, baggasse and charcoal are widely

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used particularly in the rural area for domestic consumption. But, one report depicts from Forestry Master Plan (FMP) (1993) which is only 13%, of the entire biomass energy consumption in the country comes from fuel wood. (FMP) (1993) also stated that overall, tree and bamboo (48%), agricultural residues (36%), dung (13%) and peat deposits (3%) fulfill the domestic energy demands. In recent times, natural forests as well as the village groves are being over exploited and as a result the fuelwood shortage is severe throughout the country. This has made the fuelwood a marketable product. According to Forestry Master Plan (FMP) (1993) in Bangladesh, fuelwood consumption is lower than any other countries in the world (0.43 m^3/ per capita). FMP estimation of fuelwood demand and supply from 1993 to 2013 portrays that fuelwood deficit is nearly one-third of the overall fuel demands of 2003-2013 which is disappointing. The shortage is likely to be massive in case of the country's failure to protect its forest covers and inadequacy to promote fuelwood plantation all over the country. Therefore, establishing energy plantation on degraded and unoccupied premises is necessary in order to avert this situation. Moreover, in this situation Jungerius (1985) said that indigenous knowledge should be taken under consideration for identifying the tree species because of their intimate knowledge of the local tree species and environment. Fuelwoods are selling according to the preference of the local people.

The choice of fuelwood is generally considered by the availability, the burning duration, the maximum temperature and the ash content (Núñez-Regueira *et al*., 2003). For this reason, people are using hardwoods to fulfill their basic needs of fuelwood that is, in turn responsible for the rapid deforestation of the country.

According to Kataki and Konwer (2001) the physical properties, such as moisture content, chemical composition and wood density are affecting the performance of fuelwood species. For example, increased moisture content in the wood decreases the amount of heat obtained, because more energy is used for evaporating water. Senelwa and Sims (1999) stated that for this reasons combustion efficiency becomes low. So, it is very urgent to evaporate the water present in wood to increase the heating value. Sometimes, moisture of the wood creates the negative effect on its calorific value (Junge, 1980). Structurally, cellulose, hemicelluloses and lignin are the main components of wood cell walls. Moreover, Nasser and Aref (2014) reported that species type and position of wood plays an important role to determine the chemical composition of the wood. According to Fengel and Wengener (2011) wood also consists of small amount of extractives that affect the characteristics of wood and wood products. After hand, Wang *et al*., (1982); Fuwape, (1990); Kataki and Konwar, (2002) stated that these extractives vary greatly in their quantity and chemical characteristics. So according to Wang *et al.,* (1982) to determine the suitability of a species to be considered as fuelwood; extractive, cellulose, hemicelluloses and lignin components are important criteria.

Earlier, fuelwood potentiality was determined either by calorific value or wood density which alone does not reveal the actual preferences of wood for combustion. Therefore, Fuel value index (FVI) was introduced for ranking fuelwood species (Deka *et al*., 2007). This index considers ash content, moisture content, wood density and calorific value of a wood collectively Goel and Behl, (1996); Bhatt and Todaria, (1990); Abbot *et al*., (1997). Recently, FVI value of six mangrove species in Bangladesh has been reported by Islam *et al*., (2019) and Miah and Islam (2020) demonstrated the local preferences of firewood for domestic use with the physical characteristics of firewood species using FVI but there have no data on FVI value with wood physicochemical properties in Bangladesh.

Research work done so far, focuses on the silvicultural aspects and environmental influences the four mentioned tree species in Bangladesh. But till date, no evidential information is found regarding fuel properties and chemical composition of the referred species. Thus, in this study, an attempt has been made to evaluate the fuelwood potentials of these four species for the first time in Bangladesh context and to investigate the relationship between the fuel value index, (FVI) with

physiochemical properties of wood, such as, wood density, mc, cellulose, hemicelluloses, lignin, extractives and ash content.

Materials and Methods

Sample collection: Four tree species were selected according to their local use as fuelwood from wood vendors in Jhenaidah district. The species were *Acacia nilotica, Samanea saman, Leucaena leucocephala* and *Tamarindus indica*. Randomly, three logs of each species were sampled among the four species from the wood vendors and 45 cm^3 long and 10-18 cm^3 in diameter were cut from the logs and put into a bag for further conversion in sawmill.

Sample preparation: The logs were converted into 8×2×2 cm^3 wood block for the determination of moisture content, wood density and also into some wood powder for the determination of ash content, calorific value, extractive, cellulose, hemicelluloses and lignin content of the selected wood species. Then the collected samples were transferred to the laboratory for various experiments.

Determination of Moisture content percentage (MC%): The green moisture content of a sample is the trees moisture content that was extracted from a log. The wood blocks taken from log were weighed and dried at 103°C for 24h according to the ASTM-D-4442-16 standard (ASTM) (2016). After that, the wood blocks were weighed again to obtain the dry weight. After that, the moisture content was determined by the following equation-

$$MC\,\% = \frac{\left(\text{Green Weight - Oven Dry Weight}\right)}{\text{Oven Dry Weight}} \times 100 \qquad (1)$$

Determination of wood density: According to Chave (2005) the density of a wood is a variable which tells how much carbon the plant uses for their constructing purpose. Wood density varies within the plants. The wood blocks taken from the log were weighed and volumes were estimated according to the ASTM D-143-94 standard (ASTM) (1997). The volume was determined by taking the measurement of the length, wide and height of the wood blocks with the help of slide calipers. After that the density was determined by the following equation-

$$\text{Wood Density } (g/cm^3) = \frac{\text{Oven dry weight}}{\text{Oven dry volume}} \qquad (2)$$

Determination of calorific value: The calorific value was determined on a dry weight basis. Around 1g of oven dry wood powder of per sample was tested by oxygen bomb calorimeter and was estimated using the Dulong's formula from the Laboratory of Mechanical Engineering Department of Khulna University of Engineering and technology (KUET), Bangladesh.

Determination of Extractives, Cellulose, Hemicellulose and Lignin content: Cellulose, hemicellulose and lignin in lingo-cellulosic contents of the biomass samples were calculated as stated in an experimental method reported elsewhere (Mitu *et al.*, 2019). In brief, solvent extraction carried out extractives determination experiments (100ml acetone for 1g of dried biomass sample) at 60°C. The biomass sample was dried at 110°C until a constant weight was obtained. After that, the solid residue was cooled in a drier until it reached room temperature and was weighted. Amount of extractives is the weight difference before and after of the extraction. To determine the hemicellulose content, 0.5g of dry biomass sample without the extractives was added in a 150 mL NaOH solution

(20 g/L) and was boiled with recycled distilled water for 3.5 hours. The residue then went through filtration and was washed in order to remove Na^+. After that, the remaining residue was respectively dried and weighted. The amount of hemicellulose is the difference of the weight before and after the treatment. Lignin determination is carried out by Klason method. In this method, 15 ml of H_2SO_4 (71%) was added to 0.5 g of extractive-less sample of dried biomass. The mixture was then heated and stirred for 2h. This mixture then was diluted to H_2SO_4 (4% concentration) and resulting remains were boiled with the recycled water for 4 hours. After that, the obtained residues were respectively filtered, washed and dried. Lignin is determined by the weight difference before and after this treatment. Lastly, cellulose was measured by calculating the weight difference from lignin, hemicellulose and extractives.

Determination of Ash content percentage: At first, in the laboratory, an appropriate number of small pots were taken, marked using a graphite marker and weighted. Then 1g oven dry weight was taken, ODW wood powder of per sample. After that, they were placed in the muffle furnace at 450°C for four hours according to ASTM standard Method Number E1755-01 (ASTM) (2007). Then, the pots were removed from the furnace and put them into a drier and weighted them. After that, the ash content percentage was determined by following formula-

$$\text{Ash content } \% = \frac{\text{Weight}_{\text{pot plus ash}} - \text{Weight}_{\text{only pot}}}{\text{ODW}_{\text{sample}}} \times 100 \quad (3)$$

Determination of Fuel Value Index, FVI: The fuelwood value index (FVI) was calculated by following method (Purohit and Nautival 1987) –

$$\text{FVI} = \frac{\text{Calorific value}(\text{MJ/kg}) \times \text{Wood Density}(\text{g/cm}^3)}{\text{Ash content}(\text{g/g}) \times \text{Moisture content}(\text{g/g})} \quad (4)$$

Results and discussions

Extractives, Cellulose, Hemicellulose and Lignin content of the species: In the present study, Table1 presents the extractive, cellulose, hemicelluloses and lignin content of the four species. It was demonstrated by Nasser *et al.,* (2014) that, statistically, all of the extractive, cellulose, hemicelluloses and lignin content shows significant differences from each other among the species. In present study, it can be clearly seen that, the selected four different species presented different values in their extractive, cellulose, hemicellulose and lignin content (Table 1). Here, higher extractives content of four tree species was in order of *Leucaena leucocephala* (5.05%) >*Samanea saman* (5.00%)>*Acacia nilotica* (3.34%)>*Tamarindus indica* (1.34%). Cellulose content of four tree species of present studied was in order of *Leucaena leucocephala* (48.45) >*Acacia nilotica* (47.27) > *Tamarindus indica* (41.42) >*Samanea saman* (41.6). Hemicellulose content of four tree species of present studied was in order of *Samanea saman* (29.0)>*Acacia nilotica* (24.9)> *Tamarindus indica* (20) >*Leucaenaleucocephala* (19.35). Lignin content of four tree species of present studied was in order of *Tamarindus indica* (37.25) > *Acacia nilotica* (28.1)>*Leucaena leucocephala* (27.15)>*Samanea saman* (24.5). Fengel and Wengener (2011) stated that, generally, the extractives, cellulose, hemicelluloses and lignin content range from 2- 6%, 45-50%, 15-35% and 23-30 in tree species respectively. But in present study, the extractive, cellulose, hemicelluloses and lignin content of the samples ranged from 1.34-5.05%, 41.6-48.45%, 19.35-29% and 24.5-37.25% respectively. It can be seen that, the *Tamarindus indica* had higher lignin content than normal range of wood species.

Table 1. Extractives, cellulose, hemicellulose and lignin content of the four species

Species	Wood Component %			
	Extractives(%)	Cellulose(%)	Hemicellulose(%)	Lignin(%)
Leucaena leucocephala	5.05	48.45	19.35	27.15
Acacia nilotica	3.34	47.27	24.90	28.10
Samania saman	5.00	41.60	29.00	24.50
Tamarindus indica	1.34	41.42	20.00	37.25

Ash content: For determining fuelwood quality, one of the negative factors is indicated by the highest ash percentage value (Meetei *et al.,* 2014). In general, Panshin and Zeeuw (1980) found that the ash content is usually between 0.1%- 0.5% for the tree species. However, Chow and Lucas (1988) mentioned that wood species in the tropical zone require more mineral components than others for their growth and this may be the logical explanation of the higher value obtained here. Devi *et al*., (2013) reported that the wood of *Leucaena leucocephala* burns steadily with little smoke, few sparks and produces less than 1% ash . Another study by Puri *et al*., (1994) reported the ash content of *Acacia nilotica* was 2.1%. Whereas, the ash content of *Tamarindus indica* and *Samanea saman* were found was 2.13% and according to Kumar (2003) 1.56% in heartwood.

Table 2. Fuelwood characteristics of four tree species in Bangladesh

Species	Moisture Content %	Density (g/cm^3)	Ash content %	Calorific value (kJ/g)	FVI
Leucaena leucocephala	57.45	0.65	1.09	19.17	1998.51
Acacia nilotica	48.88	0.75	1.12	19.18	2623.97
Samania saman	47.74	0.63	1.99	17.04	1124.35
Tamarindus indica	40.05	0.82	2.21	16.34	1513.62

In the present study, Table 2 shows that, of all the four selected tree species, exhibited lowest ash content in order to *Leucaena leucocephala* (1.09%)> *Acacia nilotica* (1.12%)>*Samanea saman* (1.99%)> *Tamarindus indica* (2.21%).Thus, higher amount of minerals are absorbed from the soil and are stored into the cell cavities and cell walls in these trees. That's why, they exhibits more ash content percentages than normal range of ash content percentages. But Kataki and Konwer (2001) mentioned that the bark of tree produce higher amount of ash forming materials and relatively lower heating content. So, heating values can be increased by removing the ash contents from the plant parts. Generally, ash content can be considered as an undesirable component and therefore, it is needed to be managed during direct wood combustion (Chow and Lucas, 1988). FAO (1985) revealed that, about 3% of ash content can be found in high quality lump charcoal. Usually, when a species produces a great amount of ash content, it is preferred less (Cardoso 2015). Meetei *et al*., (2014) found that, in the tree component of 5 distinctive oak tree species, ash percentage demonstrated considerable difference in the biomass components of wood and bark. Ash percentage of the 5 oak species was not only correlated negatively but was also significant with calorific values ($p<0.01$).

Moisture content: According to Abbot *et al.,*(1999) and Bhatt and Tomar (2002) the moisture in wood depends on the seasonal variation, state of the wood and many other factors. Available heat for combustion decreases if the moisture content is high. Kumar (2003) revealed that the moisture content of *Tamarindus indica* was 41.2% and *Samanea saman* was 57.25 in heartwood.

Table 2 showed that, among the four studies tree species, moisture content was highest in *Leucaena leucocephala* (57.45%) followed by *Acacia nilotica* (48.88%), *Samanea saman* (47.74%) and lowest

moisture content was observed in *Tamarindus indica* (40.05%). In this study, *Tamarindus indica* has shown high density wood with low MC%.

Wood Density: Another important factor for estimating the quality of a fuelwood is the density of tree species. Kataki and Konwer (2002) and Khoo *et al.,* (1982) said that, due to slow burning rates as well as the high content of energy in per unit of volume, denser species are likely to demonstrate better fuel quality. Moreover, wood density determines a positive factor in fuelwood quality as it has more heating durability. From a study of Meetei *et al.,* (2014), a consequential positive correlation ($p<0.05$) was found between wood density of the 5 oak species and calorific values of the wood samples. High wood density is likely to be subjected to higher lignifications, presence of the lignin and other denser fractions. Another possible explanation given by Kumar (2006) is that complex wood ultrastructure is capable of reiterating a strong linkage between the physical properties of the wood and the chemical substances. Therefore, Cunningham (2001) mentioned that, wood density is one of the most significant factors as it not only identifies the suitable wood for burning but it also gives the wood additional use value. In this study, the highest wood density in g/ cm^3 was found for *Tamarindus indica* (0.820)>*Acacia nilotica* (0.749)>*Leucaena leucocephala* (0.653)>*Samanea saman* (0.627). Lowest wood density was noticed in *Samania saman* (0.627).

Calorific value: Calorific values depend on many attributes such as the development type, growth or aging of the different species, seasonal variations and many other factors according to Klasnja *et al., (* 2010). Cardoso *et al*., (2015) reported that, these values coexist with the standard average values of species that maintains other related combustion qualities. The combustion heat of wood is depended mainly on the genetic characteristics of the species and chemical composition of the wood. The species containing a high amount of volatile matter, resin, wax and lignin in general has greater energy content stated by Jain and Singh (1999). From Kataki and Konwer (2001) and Demirbas and Demirbas (2009), it is assumed that calorific values are high when the extractives concentrations as well as lignin contents in wood are high. Along with this, Moya and Tenorio (2013) added that, higher dichloromethane extractives endorse higher calorific values in a tree species. In the present study, all the four selected tree species, exhibited highest calorific values in order of *Acacia nilotica* (19.179 kJ/g)>*Leucaena leucocephala* (19.165 kJ/g)>*Samanea saman* (17.036 kJ/g)> *Tamarindus indica* (16.338 kJ/g).

Ranking based on FVI: For screening desirable fuelwood species, Purohit and Nautiyal (1987) and Jain (1993) emphasized on FVI as it considers major fuel properties that described in Eq. 4. FVI of four tree species of present study was in order of *Acacia nilotica* (2623.97) > *Leucaena leucocephala* (1998.51) >*Tamarindus indica* (1513.62) >*Samanea saman* (1124.35). Among the four observed species, the highest FVI was noticed for *Acacia nilotica* (2623.97) due to higher calorific value in comparison with the other species. So, *Acacia nilotica* (2623.97) is likely to be considered as the best one in terms of being a fuelwood species followed by *Leucaena leucocephala* >*Tamarindus indica* >*Samanea saman* in Jhenaidah district.

Relationship between FVI and Physiochemical properties: Table 3 showed that, cellulose, hemicelluloses, lignin, extractives, wood density, moisture content has no significant effect on FVI. Only ash content showed a highly significant effect on FVI with a negative coefficient ($r= -0.78$). That means, if the value of ash content increases, the value of FVI will decrease. Here, two tailed test of significance is used. In most of the industrial activities, ash is regarded as an undesirable component. The increase of biomass utilization as a fuel may cause an ash disposal problems because it will accumulate in furnaces. This result is in agreement with Nasser *et al.,* (2014). Because, the result of Nasser *et al*., (2014) also showed a highly negative significant effect with heating value ($r= -0.93$).

In present study, species with high ash percentage such as, *Tamarindus indica, Samanea saman* yielded lower heat values than others. Bhatt and Todaria (1990) and Kataki and Konwer (2001) also reported the decreasing heat of combustion with increasing ash percentage in their study.

Table 3. Relation with FVI and other calculated properties

Properties	Pearson correlation coefficient	Significance status*
Cellulose	0.52745	0.17915
Hemicellulose	-0.17436	0.67964
Lignin	-0.02729	0.94886
Extractives	-0.0125	0.97656
Density	-0.06062	0.88661
Moisture content,%	0.16632	0.69386
Ash content,%	-0.78266	0.02166

*at 5% level of significance.

Conclusion

The findings from this study emphasize that, evaluating fuel wood should not solely be subjected to the calorific value, but other factors such as extractive, cellulose, hemicellulose, lignin, ash content and wood density must also be taken into account. Calorific values of the investigated species differ from each other. *Leucaena leucocephala* is the preferred species with regards to low ash content and high extractive and cellulose. In terms of calorific value *Acacia nilotica* seems to be the best option for fuelwood but *Leucaena leucocephala* also have higher calorific value and that is very near to *Acacia nilotica.* Although, the other two species *Tamarindus indica* and *Samanea saman* shown the lowest calorific value, ash content and extractive, but they have a high density and lignin content than other species. On the other hand the *Samanea saman* has exhibited the lowest density, cellulose and lignin content but they have relatively more extractive content than *Acacia nilotica and Tamarindus indica.* Considering the FVI value, in the present study, the preferred wood species are *Acacia nilotica* followed by *Leucaena. leucocephala, Tamarindus indica and Samanea saman.* This study also revealed the highly negative significant correlation between FVI and ash content.

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ORIGINAL ARTICLE

LIFE SCIENCE

PROXIMATE COMPOSITION AND HPLC-DAD ANALYSIS OF BIOACTIVE POLYPHENOLS IN LEAFY VEGETABLES CONSUMED IN THE DIET FOUND IN SOUTHERN PART OF BANGLADESH

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Abstract:
Four leafy vegetables, *Phyla nodiflora*, *Amaranthus spinosus*, *Amaranthus viridis* and *Chenopodium album* usually consumed by the Southern people of Bangladesh. In this study, we tested proximate composition and antioxidant potential of four leafy vegetables and further HPLC content of bioactive polyphenols in the most promising vegetable. The results revealed that they are rich in protein (12-17g/100g), carbohydrate (10- 20 g/100g) and fibre (29 - 44 g/100 g). The ash, glucose, sucrose and xylose contents were found within the ranges of 14-20 g/100 g, 35-77 mg/100 g, 32.79-68.72 mg/100 g and 3.79-7.71 mg/100 g, respectively. All the vegetables showed a lower content of lipid (0.73 – 1.47 g/100 g) and higher moisture content (81-85 g/100 g). The methanolic extracts of the vegetables were found to possess notable amount of total phenolic (11-60 mg GAE/g), total flavonoid (365-565 mg QE/g) and tannin content (28-49 mg TAE/g). All the samples showed significant DPPH free radical scavenging (IC_{50} 53-1097 µg/ml) and hydrogen peroxide scavenging activity (IC_{50} 41-96 µg/ml). Out of these four species, *C. album* was found to be the most promising leafy vegetable because of its high protein and fibre content, low lipid content and good antioxidant activities. HPLC-DAD analysis revealed the presence of 3,4-dihydroxy benzoic acid, catechol, vanillic acid, syringic acid, rutin hydrate, p-coumaric acid, trans-ferulic acid, rosmarinic acid and quercetin in *C. album*. The results of this study provide evidence for the importance of these leafy vegetables in improving the nutritional and health status of Southern rural people of Bangladesh.

Keywords: Bangladeshi vegetables, Antioxidant, Proximate Nutritional values; Polyphenols

Introduction

Leafy vegetables are long admired for their essential biochemical and nutritional importance as they contain good amounts of proteins, fats, carbohydrates, vitamins and minerals as well as provide us with dietary antioxidants (Ebert, 2014; Saikia & Deka, 2013). Dietary antioxidants from vegetables have been considered beneficial in preventing different chronic diseases and maintenance of good health (Oseni & Olawoye, 2015). According to numerous epidemiological evidences, consumption of leafy vegetable has protective effect against oxidative damage and reduces risk of cardiovascular disease, diabetes, inflammatory diseases, cancer, alzheimer's, parkinsonism and other chronic diseases

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(Dimitrios, 2006), attributed to different classes of bioactive constituents including micronutrients, polyphenols, flavonoids and sterols (Sree, Joshna, Lakshmi, & Kumar, 2013). The increase of dietary fresh vegetables intake can reduces the risk of mortality by 20% (Shetty, Magadum, & Managanvi, 2013). Native people tend to collect various indigenous leafy vegetables from the wild and their surroundings, and consume them hardly acknowledging their nutritional values and potential health benefits (Asyira, Sarbini, & Harah, 2017). Therefore, the awareness of consumption of fresh leafy vegetables needs to be promoted among common people to improve their nutritional and health benefits.

In Bangladesh, a huge portion of the total population suffers from malnutrition creating an alarming public health concern. A survey revealed that, the average household consumption of vegetable per person is about 166 g in Bangladesh, which is well below the minimum required amount (200 g) (Satter et al., 2016). The main concern in Bangladesh is the micronutrient deficiency that is far greater zinc extent than energy malnutrition which makes the general people vulnerable to a variety of health problems. The people of southern coastal region face more food scarcity than other areas of Bangladesh due to soil salinity and lack of cultivable land. They mainly rely on seasonal and native grown leafy vegetables for their daily dietary demand (Zihad et al., 2019). In this study, four coastal leafy vegetables, namely *Phyla nodiflora*, *Amaranthus spinosus*, *Amaranthus viridis* and *Chenopodium album* were analysed for their nutritional composition and antioxidant potential as well as further HPLC-DAD analysis of bioactive polyphenols in the most promising vegetable. These vegetables are commonly consumed particularly in the southern region of Bangladesh, especially in Khulna, Satkhira and Bagerhat district, not only as food but also as traditional medicine in different ailments. In spite of their local popularity very little scientific knowledge are found regarding their nutritional value, antioxidant potential and their bioactive polyphenols. Thus, the aim of this study is to provide a scientific basis for the dietary and ethnomedicinal utilization of these leafy vegetables.

Materials and Methods

Collection and preparation of plant samples

Four common leafy vegetables (*P. nodiflora*, *A. spinosus*, *A. viridis* & *C. album*) were collected from Southern part of Bangladesh including Patkelghata, Satkhira and Dumoria of Khulna division. All samples were authenticated by Professor Md. Asaduzzaman, Forestry and Wood Technology Discipline, Khulna University and a voucher number was recorded against each specimen. Plant samples were shed-dried to reduce their moisture content and dried samples were then ground and stored in an air-tight container until used for analysis. The dried powdered sample was extracted by maceration for 5 to 7 days using methanol. Methanol is an ideal solvent for extracting both polar and non-polar bioactive compounds, especially polyphenols. The solvent was filtered and evaporated by means of a rotary evaporator and freeze drying system to obtain crude extract. This crude extract was used of HPLC-DAD detection of polyphenolic compounds.

Proximate composition

The fat, fibre, ash, and protein contents of the vegetables were determined according to Association of Official Analytical Chemists (2000) on dry weight basis. We determined the protein contents of the vegetables using the Kjeldahl method where the conversion factor for converting nitrogen content to protein content was 6.25 (Kjeldahl, 1883). Fat content was determined by Soxhlet method (Soxhlet, 1879). We determined the fibre content by sequentially extracting with 0.225 N boiling Sulphuric acid (H_2SO_4) and 0.313 N sodium hydroxide (NaOH). To determine the ash content, the samples were left in a muffle furnace at 550°C for 6 h for ignition. The pH of chopped fresh sample was determined using basic digital pH meter (Konuk, Afyon, & Yagiz, 2006). Finally the percentages of moisture, ash, fibre, protein and fat contents were subtracted from 100 yielding the amount of carbohydrate in each sample (Zihad et al., 2019). Sugar contents (mainly hexose, pentose and glucose) were determined by Phenolic-Sulphuric method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956).

Determination of antioxidant properties

Total phenolic, flavonoid and tannin contents of the methanol extracts of the vegetables were determined in our study as measures of main antioxidant components. Folin–Ciocalteu's method was used to determine the total phenolic content where we prepared standard curve with gallic acid (Amorim et al., 2008; Hazra, Biswas, & Mandal, 2008). We followed the Aluminum trichloride

colorimetric method to determine total flavonoid content (TFC) of the samples where quercetin was used as the standard (Kariuki & Mwonjoria, 2013). Finally, total tannin content (TTC) was measured by Folin-Ciocalteu's method where tannic acid was used to construct the standard curve (Amorim et al., 2008).

We evaluated the ability of the vegetable samples to scavenge free radicals through the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging method (Sadhu, Okuyama, Fujimoto, & Ishibashi, 2003) and expressed the results as IC_{50} values. This value denotes the concentration of the sample needed to scavenge 50% of the free radicals present in the reaction mixture that we derived from the following equation: DPPH scavenged (%) = [1- (Abs_{sample} / $Abs_{control}$)] x 100. In addition, we assayed the hydrogen peroxide (H_2O_2) scavenging potential of the samples following the method described by Ruch, Cheng, and Klaunig (1989). Here the results are also expressed as IC_{50} values calculated using the following equation: H_2O_2 scavenged (%) = [(A_o-A_1)/A_o] x 100; where A_o is the absorbance of the control and A_1 is the absorbance in the presence of the sample of extract and standard.

HPLC-DAD analysis for estimation of polyphenols

The *C. album* showed most promising antioxidant and nutrition values and was further analyzed by HPLC to estimate different polyphenols. A rapid separation LC (Dionex UltiMate 3000, Thermo Fisher Scientific Inc., MA, USA) system equipped with an C_{18} (4.6 × 250 mm; 5 µm) column (Acclaim®, USA) was utilized to conduct HPLC analysis using Dionex Chromeleon software (Version 6.80 RS 10) as per Chuanphongpanich and Phanichphant 2006 method (Chuanphongpanich & Phanichphant, 2006). A gradient system containing of acetonitrile (A), acetic acid pH 3.0 (B) and methanol (C) were used as the solvents using the following elution: 0 to 9 min 5%A/95%B, 10 to 19 min 10%A/80%B/10%C and 20 to 30 min 20%A/60%B/20%C. The injection volume was 20 µl, constant flow rate at 1 ml/min and the temperature was at 30 °C. The peaks were detected by an UV detector at 280, 320, and 380 nm for 18, 24 and 30 min as well as a photodiode array detector was adjusted to acquisition all the peaks within the range of 200-700 nm. A total of sixteen bioactive polyphenols, namely 3,4-dihydroxy benzoic acid, catechol , catechin hydrate, (-) epicatechin, caffeic acid, p-coumaric acid, gallic acid, kaempferol, myricetin, quercetin, rutin hydrate, rosmarinic acid, syringic acid, trans-ferulic acid, trans-cinnamic acid and vanillic acid was prepared in a solution of methanol to prepare the standard calibration curve. The *C. album* extract solution was also prepared in methanol at a concentration of 5 mg/ml.

Statistical analysis

The results were presented as mean ± SD (Standard deviation) and each experiment was performed in triplicates. One way ANOVA followed by Bonferroni's post-hoc test was conducted for statistical analysis.

Results and Discussions

Proximate composition

The moisture contents of the tested leafy vegetables ranged between 81.06 and 85.49 g/100 g (Table 1). The water content of green leafy vegetables is of great importance when eaten raw as it helps the body to digest them and facilitating absorption of all the nutrients (Lussier, 2010). The studied leafy vegetables showed low amount of fat content ranging between 0.73-1.47 g/100 g of dry weight (DW), with *C. album* showed the lowest fat content among the four leafy vegetables (Table 1), hence making them ideal diet components for people suffering from obesity, cardiovascular complications and cancer. Dietary fibres has been reported to possesses an essential health promoting role to prevent the development and further advancement of different chronic diseases like diabetes, metabolic syndrome, cardiovascular diseases, inflammatory bowel syndrome and cancer (Soliman, 2019). In this study, we found the amount of dietary fibre present in the leafy vegetable samples in the range between 29.45 and 43.68 g/100 g DW (Table. 1), with *A. spinosus* showing the highest value (43.68%). This study shows that the leafy vegetables is capable of contributing 19-38 g/day of recommended dietary intake (RDA) of fibre if a person consumes the equivalent amount of 100 g dried leaves and, thus, can be regarded as significant sources of dietary fibre for nutrition and disease prevention (Akubugwo, Obasi, Chinyere, & Ugbogu, 2007). The ash contents of these vegetables

ranged from 14.04 to 19.48 g/100 g DW (Table. 1). *Phyla nodiflora* showed the highest ash content (19.48%) while the lowest ash content was found in *A. spinosus* (14.04 %). Ash content is the un-burnable salt reflecting the mineral content in a food (Zihad et al., 2019). Minerals are important dietary element that are playing as key contributors in several biochemical pathways including enzyme activation, oxygen transport and metabolism (Staszowska-Karkut & Materska, 2020). The high ash content in these leafy vegetables makes them excellent source of dietary minerals for people suffering from malnutrition. According to RDA, every person requires to consume 8 g/kg body weight of dietary protein (Lonnie et al., 2018).

Table 1. Proximate composition (g/100 g) dry weight of collected four green leafy vegetables

Nutritional value		Result			
		Phyla nodiflora	*Amaranthus spinosus*	*Amaranthus viridis*	*Chenopodium album*
Moisture %		83.35±0.32	80.90±0.56$^{£}$	81.06±0.35$^{£}$	85.49±0.41
pH		7.7±0.16	7.2±0.07*	6.87±0.13$^{*,\#}$	6.80±0.14$^{*,\#}$
Lipid %		1.09±0.61	1.27±0.7	1.47±0.5	0.73±0.61
Fiber %		37.78±0.49	43.68±0.23	29.45±0.05$^{*,\#}$	41.47±0.17$^{¥}$
Ash %		19.48±1.49	14.04±0.07*	19.05±0.06$^{\#}$	19.33±1.09$^{\#}$
Protein %		12.15±0.35	15.60±1.1*	16.89±0.55*	15.64±0.29*
Carbohydrate %		14.18±1.07$^{£}$	19.99±0.87$^{*,£}$	19.19±1.14$^{*,£}$	10.87±0.76
Sugar (mg/100g)	Glucose	35.52±0.69	76.10±0.76$^{*,£}$	75.73±1.98$^{*,£}$	39.03±0.33*
	Sucrose	32.78±0.61	68.72±0.45$^{*,£}$	68.37±1.17$^{*,£}$	35.75±1.52
	Xylose	3.79±0.07	7.71±0.19$^{*,£}$	7.59±0.19$^{*,£}$	4.07±0.03

***Data are expressed as mean ±SD of triplicate. $^{*}p < 0.05$ vs. *Phyla nodiflora*, $^{\#}p < 0.05$ vs. *Amaranthus spinosus*, $^{¥}p < 0.05$ vs. *Amaranthus viridis*, $^{£}p < 0.05$ vs. *Chenopodium album*. Data was analysed by one way ANOVA followed by Bonferroni's test.

In this case, consumption of plant protein contributes to the reduction of mortality risk than animal protein (Song et al., 2016). In our study, the leafy vegetables showed high amount of protein content ranged between 12.15-16.89 g/100 g DW (Table. 1), with *P. nodiflora* showing the lowest and *A. viridis* showing the highest amount of protein among the tested leafy vegetables. Furthermore, more than 12% of the calorific value provided by these leafy vegetables is attributed to the protein content, hence, making them good sources of protein (Pearson, 1976). The carbohydrate contents of these leafy vegetables ranged between 10.87-19.99 g/100 g DW (Table. 1). The previous studies of nutritional values (e.g. lipid, protein, carbohydrate, ash and fibre) showed different results from our study for *A. spinosus* (South African study) (Odhav, Beekrum, Akula, & Baijnath, 2007), *A. viridis* (Indian study) (Sharma, Gupta, & Rao, 2012), *C. album* (Indian study) (Poonia & Upadhayay, 2015). This might be due to difference of soil and environmental conditions, such as amount of photosynthetically active radiation, temperature, water availability and analysis technique (Saxena, Venkaiah, Anitha, Venu, & Raghunath, 2007). In this study, we found the pH of these leafy vegetables within the range 6.8 - 7.7 (Table. 1). It is reported that leafy vegetables contain constituents which are slightly acidic or slightly basic and they are mentioned in the list of slightly acidic or slightly basic foods. Thus, we can say that the selected edible leafy vegetables are as safe food since they don't contain extremely acidic or basic constituents. Diets containing foods that are rich in natural sugars are pivotal to the health of patient with chronic diseases. It is evident that these foods nourishes brain and nervous system, thus delivers benefits ranging from sleep and memory to depression and anxiety. Moreover, natural sugars are essential for glycosylation and help the body in controlling fat and cholesterol. Leafy vegetables contain different types and amount of sugar. The sugar content of the selected four leafy vegetables ranges as glucose 35.53-76.1 mg, sucrose 32.79-68.37 mg and xylose 3.79-7.71 mg per 100 g DW (Table. 1). *Phyla nodiflora* contain the lowest percentage and *A. spinosus* contain the highest percentage of glucose, *A. spinosus* contain the highest percentage (68.37 mg) and *P. nodiflora* contain the lowest percentage of sucrose (32.79 mg), *A. spinosus* also contain the highest percentage of xylose (7.71 mg) and *P. nodiflora* contain the lowest percentage of xylose (3.79 mg) among these four leafy vegetables. This is the first time study on the sugar contents of these species of leafy vegetables.

Antioxidant properties of leafy vegetables

Total phenolic, flavonoid and tannin content

The results of TPC, TFC and TTC of methanolic extracts of four leafy vegetables are presented in Table 2. Phenolics are non-nutritive secondary metabolites produced by plants. Though the role of these compounds in plant growth and metabolism is still unclear, they are proven to possess significant health benefit in human and provide protection against different chronic diseases linked to oxidative damage (Delgado, Haza, García, & Morales, 2009). The TPC of these leafy vegetables ranged between 11.39 -59.91 mg GAE/g. *A. spinosus* contain the lowest level and *P. nodiflora* contain the highest level of phenolic content. These levels are higher than those found in the commonly consumed plant based foods from India (0.0-1.2 mg GAE/g raw foodstuff) (Saxena et al., 2007). Among the diverse classes of natural polyphenols, flavonoids are the most widespread group with potent free radical scavenging activity. Generous intakes of dietary flavonoid are beneficial to human health as flavonoids lower the risk of a wide range of chronic diseases including cardiovascular diseases, stroke and some cancers (Saeed, Khan, & Shabbir, 2012). The total flavonoid contents of the sample vegetables varied considerably between 365 and 564 mg QE/g, and the order was found to be *P. nodiflora*> *A. viridis* > *C. album* > *A. spinosus* (Table 2). The levels of flavonoid content reported here are higher than the range (16.72-51.0 mg QE/g) of previous analysis (Ayoka, Ojo, Imafidon, Ademoye, & Oladele, 2016; Rjeibi, Saad, & Hfaiedh, 2016). Tannins are another unique class of water-soluble phenolic biomolecules, primarily with astringent property that are well-known for their diverse pharmacological activities with antioxidant potential being the most prominent among them (Vit et al., 2008). The total tannin content of these leafy vegetables ranged between 28.35-48.9 mg TAE/g in which *A. viridis* contain the lowest level and *P. nodiflora* contain the highest level of tannin content.

Table 2. Different antioxidant potential of four green leafy vegetables

Green leafy vegetables	TPC (mg GAE/g)	TFC (mg QE/g)	TTC (mg TAE/g)	DPPH free radical (IC_{50}) µg/ml	H_2O_2 (IC_{50}) µg/ml
Phyla nodiflora	59.91±0.04	564.6±0.05	48.90±0.01	1096.48±1.05	95.49±0.11
Amaranthus spinosus	11.39±0.09*	365.9±0.07*	29.90±0.01$^{*,£}$	53.58±0.39*	41.11±0.52$^{*,¥}$
Amaranthus viridis	22.16±0.07$^{*,\#}$	393.7±0.04*	28.35±0.01$^{*,£}$	151.35±0.23$^{*,\#}$	68.39±0.21*
Chenopodium album	16.84±0.09*	391.7±0.05*	41.11±0.04	141.25±0.47$^{*,\#}$	47.32±0.42$^{*¥}$

***Values expressed are means ± SD of triplicate. . $^{*}p < 0.05$ vs. *Phyla nodiflora*, $^{\#}p < 0.05$ vs. *Amaranthus spinosus*, $^{¥}p < 0.05$ vs. *Amaranthus viridis*, $^{£}p < 0.05$ vs. *Chenopodium album*. Data was analysed by one way ANOVA followed by Bonferroni's test.

DPPH free radical scavenging assay

Oxidative stress develops when the amount of free radicals generated from metabolic pathways that results in the emergence of a wide range of pathological conditions in human (McCord, 2000). DPPH free radical scavenging test is a simple, rapid and efficient technique to assay the ability of phytochemicals, foods and beverages to scavenge free radicals and impart anti-oxidant effect (Marinova & Batchvarov, 2011). Table 2 demonstrated the comparative data of DPPH radical scavenging activity expresses as the IC_{50} values of the investigated leafy vegetables. The lowest IC_{50} value and the highest activity were found in methanolic extract of *A. spinosus* followed by *C. album*, *A. viridis* and *P. nodiflora* as compared to ascorbic acid. We observed insignificant correlation between the total phenolic contents of the leafy vegetables and their DPPH free radicals scavenging activity. The total phenolic content generally resembles the antioxidant activity of crude extracts but exceptions have also been seen. Therefore it can be assumed that that there are unknown components present in these leafy vegetables other that polyphenols that contributed a significant part in the observed antioxidant activities, though, among the plant originated antioxidants, polyphenols reside at the top.

Determination of Hydrogen peroxide scavenging assay

Hydrogen peroxide present inside the cell is usually non-reactive and imparts no ill effect. But when they are converted to hydroxyl radical, they become is toxic and cause oxidative damage. As it happens, hydroxyl radicals are the major contributor to the free radical induced oxidative damage as it adversely affects most of the macromolecules present in living cells altering the habitual redox status (Halliwell, 1991). Through this assay we examined the ability of the selected leafy vegetables to

convert H_2O_2 into water by donating hydrogen and neutralize its toxic effects (Khan, Khan, Sahreen, & Ahmed, 2012). In our study, these four leafy vegetables showed concentration dependent inhibition of H_2O_2 and the results are expressed as IC_{50} values (Table 2). Hence, lower the IC_{50}, higher the antioxidant activity. The extract of *A. spinosus* showed highest and *P. nodiflora* showed the lowest antioxidant properties manifested by H_2O_2 scavenging capacity, thus, possess the ability to prevent the detrimental effects of hydroxyl radical in living system.

Estimation of polyphenols by HPLC

Polyphenols are one important class of plant constituents due to their ability to scavenge free radicals and prevent oxidative cell damage. As *C. album* showed the most promising antioxidant effects in our study, we performed HPLC analysis to trace polyphenolic compounds and confirmed the presence of 3,4-dihydroxy benzoic acid, catechol, vanillic acid, syringic acid, rutin hydrate, p-coumaric acid, trans-ferulic acid, rosmarinic acid and quercetin (Table 3). Figure 1 represents the HPLC chromatogram exhibited by methanolic extract of *C. album*.

Table 3: Polyphenolic compounds in the methanolic extract of *Chenopodium album* identified by HPLC analysis

Polyphenolic compounds	Contents (mg/100 g dry extract)
3,4-Dihydroxy benzoic acid	11.05
Catechol	65.65
Vanillic acid	59.73
Syringic acid	40.75
Rutin hydrate	36.19
p-Coumaric acid	45.95
trans-Ferulic acid	12.77
Rosmarinic acid	10.84
Quercetin	18.43

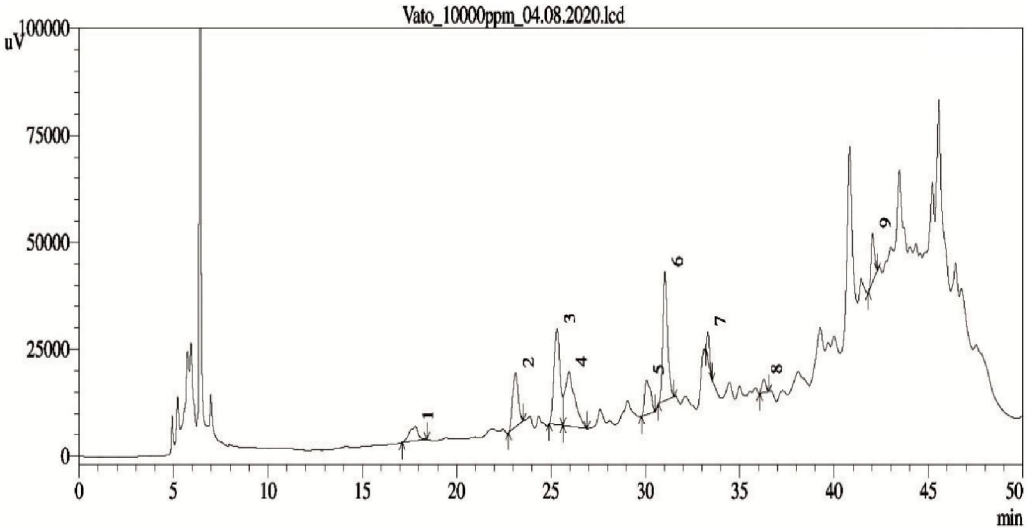

Figure 1. HPLC chromatogram of *Chenopodium album* extract. Peaks: 1. 3, 4-dihydroxy benzoic acid, 2.catechol, 3.vanillic acid, 4.syringic acid, 5.rutin hydrate, 6.p-coumaric acid, 7.trans-ferulic acid, 8.rosmarinic acid, 9.quercetin.

Out of the nine phenolic compounds (Figure 2) catechol was found in the highest concentration compared to others amounting 65.65 mg/100 g in *C. album* extract whereas vanillic acid was found 59.73 mg/100g (Table 3). The identified polyphenols have numerous pharmacological activities. It has been reported that catechol has significant antioxidant potential (Justino et al., 2006)

and protects melanin from photo and free radical induced damage (Seagle et al., 2005); vanillic acid prevents biomembrane damage caused by free radicals (Tai, Sawano, & Ito, 2012), improves cognitive impairment and attenuates $A\beta_{1-42}$-induced oxidative stress (Amin, Shah, & Kim, 2017); *p*-Coumaric acid also possess antioxidant potential (Luceri et al., 2007). These polyphenols have previously been identified in *C. album* . p-Coumaric acid, Vanillic acid, Ferulic acid, Syringic acid and 3,4-Dihydroxy benzoic acid in *C. album* extract was identified by HPLC-DAD and mass spectrometry in methanol leaf and flower extracts (Laghari, Memon, Nelofar, Khan, & Yasmin, 2011), quercetin were identified by flash chromatographic separation of acetone extract (Arora & Itankar, 2018), rutin and quercetin were identified by HPLC analysis from methanol extract (Chludil, Corbino, & Leicach, 2008). All polyphenolic constituents found from *C. album* extract are well established antioxidants and free radical scavengers. Thus, they can be held responsible for strong free radical scavenging ability and can be recommended as a food supplement to inhibit H_2O_2-induced cellular damage.

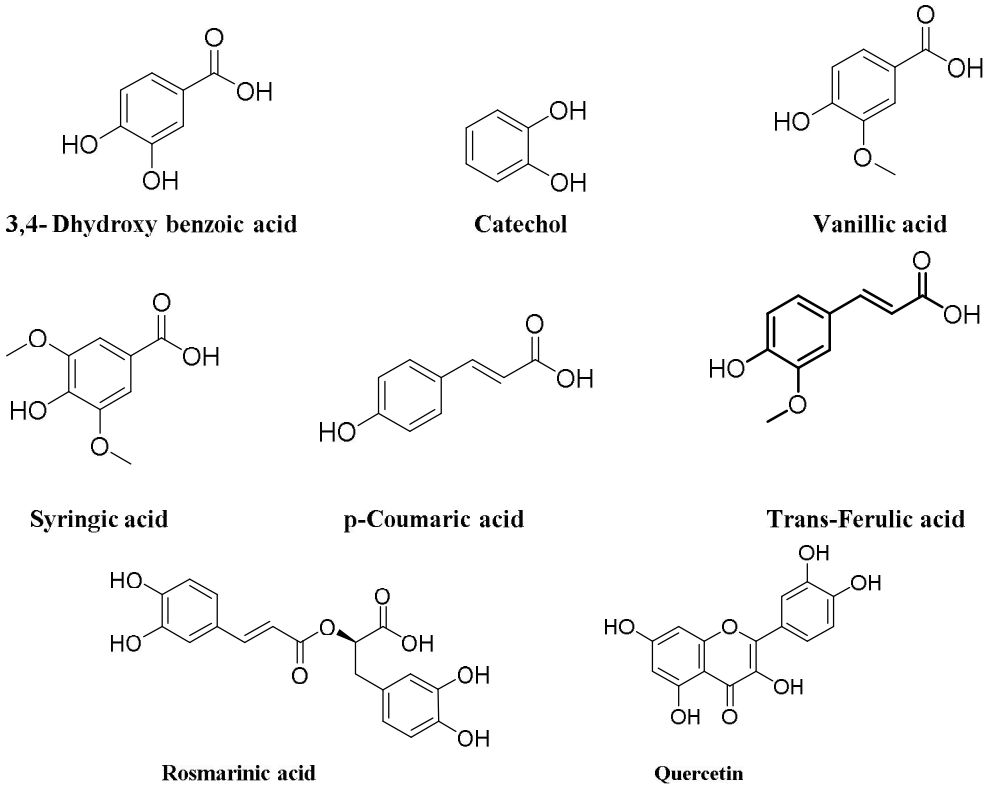

Figure 2. HPLC-DAD identified polyphenols from the methanolic extract of *Chenopodium album*.

Conclusion

The present observations revealed the nutritional and antioxidant potential of *P. nodiflora*, *A. spinosus*, *A. viridis* and *C. album*, and among these, *C. album* showed the most promising results. Significant amounts of a number of bioactive polyphenols were identified in *C. album*. The consumption of these green leafy vegetables may play a protective role against chronic diseases, especially those mediated by free radical induced oxidative stress, such as aging, cardiovascular diseases, and cancer. However, activity guided isolatioin of individual bioactive components responsible for the observed antioxidant activity, their *in vivo* effect and specific antioxidant mechanisms need to be revealed by further study. Furthermore, the great variability found in the antioxidant activity of the experimented leafy vegetables needs to be addressed by studying the influence of different factors like maturity stage and harvesting time.

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Conflict of interest

The authors declare no conflict of interest.

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ORIGINAL ARTICLE

SOCIAL SCIENCE

MUNICIPAL SOLID WASTE MANAGEMENT IN BANGLADESH: A STUDY OF X MUNICIPALITY

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Abstract: Proper solid waste disposal is very important for the nature itself and its' inhabitants. However, a study covering various aspects of solid waste management including management practices, prevailing problems and possible solutions are scarce in the literature. Accordingly, this study investigates a municipality of Bangladesh to understand the practices, problems and probable solutions of solid waste management. The dwellers of the surveyed municipality throw solid waste in various places like dustbin, drain, roadside and other crude dumping sites. Only about one-fourth of the respondents throw wastes to designated dustbins. More than half of the respondents don't have knowledge about proper disposal of solid wastes. The study findings indicate that the survey respondents throw waste throughout the whole day. More than 80 percent of the respondents are either dissatisfied or highly dissatisfied with the existing solid waste management services of the municipality. The data indicate that about two-third of the respondents are willing to pay for improving the waste disposal system. The respondents informed that they are affected by various diseases such as diarrhea, pneumonia, asthma, and bronchitis during the last one year which they perceive as the outcome of improper solid waste management. Therefore, this study suggests for awareness development, time management of waste disposal, establishment of more disposal and collection points, employment of more manpower and vehicles, introduction of the 'door to door' waste collection mechanism, segregation of industrial and clinical wastes for ensuring a better managed solid waste disposal system in the municipality.

Keywords: Solid Waste, Management, Municipality, Bangladesh

Introduction

Human being is surrounded by animal, plant, air, water, land, energy, and other objects, which are elements of environment. Environment is the sum of social, biological, physical and chemical factors, which compose the surround of human being (Ahmed, 1994). Public cleanliness and safe disposal of waste are essential to public health and environmental protection (Hamer, 2003). Solid waste creates

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environmental problems (Zurbrügg, 2002). Usually, solid waste refers to the waste generated from household kitchen, slaughterhouse, market, industry and so on. Urban solid waste management is considered to be one of the most immediate and serious environmental problems confronting urban governments in developing countries (Schertenleib and Meyer, 1992).

The economic development of a country, its' increased habitation, population density, changing food habit, social and cultural habits, and education grossly affects the physical composition of waste in the country which again has been changing over the years (Yousuf and Rahman, 2007). Only 0.2 kg/day out of the estimated 0.5 kg/day per capita solid wastes generated in the main cities of Bangladesh is carried out to the final disposal points and the rest is disposed-off locally due to the poor waste management system (Farzana and Kabir, 2004).

Solid Waste Management (SWM) is defined as the control, generation, storage, collection, transfer, transport, process and disposal of solid waste consistent with the best practices of public health, economic, financial, engineering, administrative, legal and environmental considerations (Bruvoll, 2001). Municipality refers to a town or district that has local government. Municipal solid waste management signifies the management of waste within a stable and rational financial expenditure of a municipality for a nourishing environment and healthy society (Kassim and Ali, 2006). Inadequate management of municipal solid waste is an obvious cause for degradation of the environment in most cities of the third world (Zohoori and Ghani, 2017). It is, therefore, a prime task to ensure proper disposal of municipal solid waste for protecting the environment (Memon, 2002).

According to McDougall et al. (2001), the concept of Integrated Waste Management (IWM) takes an overall approach and manages waste in an environmentally effective, economically affordable and socially acceptable way. It involves the use of a range of different treatment options at a local level and considers the entire solid waste stream. However, cities in developing countries are confronting a twin dilemma. On one hand, the urban population is growing rapidly, causing a huge increase in demand for waste management services, and on the other hand, the traditional public sector is responding poorly to the growing demand for such services (Ahmed and Ali, 2006).

The solid waste management in the urban areas of Bangladesh is mostly managed by the public sector, more specifically by the municipal/city corporation of the Bangladesh government. A study covering various aspects including management practices, prevailing problems and possible solutions are scarce in the literature. Hence, it is very important to investigate the management of municipal solid waste in the country with a holistic approach covering all related dimensions. Accordingly, this study attempts to fill in this gap. The main objective of this study is to examine the practices, problems and probable solutions of municipal solid waste management in Bangladesh. Accordingly, the corresponding research questions are: 1) What are the prevailing solid waste management practices in the municipality? 2) What are the problems faced by the municipality in managing solid wastes? and 3) What are the probable solutions of the problems?

Through analyzing the data, this study attempts to understand the prevailing solid waste management behavior of the city dwellers, problems faced by the municipality for managing wastes in response to the said behavior and probable solutions for better management of municipal solid wastes. Accordingly, the embedded research framework involves actions of two major parties: city dwellers and municipal authority. The actions of city dwellers impose constraints to the municipal authority in managing solid wastes. In addition, infrastructural and other facilities also hinder smooth functioning of solid waste management by the municipality. Hence, role of both the dwellers and municipal authority are important in handling the problem.

This section portrays the background of the study, while the next section explains the material and methods of the research. Section 3 reveals the study results and section 4 discusses study findings compared to available literature. Finally, section 5 concludes by highlighting recommendations.

Materials and Methods

This study purposively selects 'X'[1] municipality of Bangladesh to observe the practices, problems and probable solutions regarding solid waste management. It is declared as a municipality on 10 July, 1996. Currently there are nine wards in the municipality. Table 1 lists ward-wise population distribution and estimated solid waste generation scenario in the municipality. Among the nine wards of the municipality, this study randomly selects five wards (ward no. 1, 4, 5, 6 and 9) for collecting primary data. A total of 175 respondents of the municipality are surveyed in year 2012 randomly taking 35 from each of the five selected wards. Voter list of the area is used to randomly select samples in the selected wards. Personal, socioeconomic and waste management related information is collected through a questionnaire survey from the sample respondents. Accordingly, this study analyzes the data collected from the field to grasp a clear picture about solid waste management practices, constraints and probable solutions. A five-point likert scale is used to measure the satisfaction level.

Table 1: Generation of Solid Waste in the Municipality

Ward No.	Population	Area (acre)	Estimated waste generation		
			Kg/capita/day	**Kg/day**	**Percent**
1	18,250	747	0.27	4,928	31
2	9,500	38	0.29	2,755	17
3	12,200	181	0.15	1,830	12
4	9,020	277	0.15	1,353	9
5	10,400	225	0.20	2,080	13
6	7,500	454	0.08	600	4
7	11,600	328	0.07	811	5
8	10,850	348	0.07	760	5
9	14,583	718	0.04	583	4
Total	103,903	3,316	0.15	15,700	100

Source: XM (2012)

Results

The dwellers of the municipality throw solid waste in various places like dustbin, drain, roadside and other crude dumping sites. The sweepers of the municipality usually collect wastes from crude dumping sites and dump in secondary dumping places like dustbins, from where a municipality truck collects waste and dump in the final dumping ground. There are 16 dustbins in the area during the survey period. The final dumping ground is about four km far from the municipal area. The municipality has 12 vans, three handcarts and one truck for collecting and dumping wastes (XM, 2012). Assuming four trips in the morning and three trips in the afternoon and 1.5 ton per trip carrying capacity, the municipality has the capacity to carry about two-thirds of generated waste to the dumping ground per day.

According to the survey data, most of the surveyed respondents are literate. About half of them have six to ten years of formal schooling, and another one-fourth of them have more than ten years of formal schooling, while the rests have five or less than five years of schooling experience or can sign only. About one-third of them are engaged in business, while other important occupation sources include government sector, teaching, private sector and agriculture.

[1] The name of the municipality is not disclosed here to maintain secrecy and privacy.

Table 2 describes the waste disposal behavior of the respondents. This data portrays the answer of the first research question of this study. It seems that only about one-fourth of the respondents throw solid waste to designated dustbins, while the rests throw in roadside, open field, river, drain or ditch. Survey findings indicate that more than half (53 percent) of the respondents don't have knowledge about proper disposal of solid wastes.

Table 2: Waste Disposal Behavior

Waste disposal behavior	No. of Respondent	Percent
Throw in the dustbin	41	23
Throw in the roadside	62	36
Throw in open field	27	15
Throw in the river/drain/ditch	45	26
Total	175	100

The data indicate that the respondents don't throw waste in any specific time. For example, about 54 percent of the respondents throw waste in the afternoon, 22 percent in the morning, 14 percent at night and 10 percent at noon.

This study tries to understand the satisfaction level of the respondents about the ongoing waste disposal service of the municipality. The data signal that none of them are highly satisfied with the ongoing service facilities, while only 3 percent of the respondents are moderately satisfied and another 16 percent are somewhat satisfied. However, more than 80 percent of the respondents are either dissatisfied or highly dissatisfied with the ongoing solid waste management services of the municipality.

This study tries to estimate the willingness to pay of the respondents for improving the waste disposal system. Based on pilot survey findings, the survey puts four options (BDT[2] 20, 15, 10 and 0 per month) and asks the respondents to express their wiliness to pay. The survey data indicate that about one-third (31 percent) of the respondents are not willing to pay at all in this purpose. The findings indicate that about 10 percent, 22 percent and 37 percent of the respondents are willing to pay BDT 10, 15 and 20 per month respectively for improving the solid waste disposal system. The respondents are asked about the reasons behind their willingness to pay. About one-third (31 percent) of them want to pay for getting a better environment in the city. Around 26 percent and 16 percent respondents express the importance of hygiene issue and aesthetic views, respectively, behind their willingness to pay. About 60 percent of the respondents, who are not willing to pay, respond that they perceive waste disposal as the duty of the city government and hence they are not willing to pay for that. Only about 5 percent respondents are not willing to pay due to non-affordability, while 11 percent respondents think that waste is not a problem for them and 6 percent respondents are not willing to pay due to low volume of waste generation by themselves.

Wastes like broken bottles thrown here and there may become a breeding ground for mosquitoes and spread diseases. Improper disposal of wastes may lead to human injury. For example, when a person steps on the broken bottles or nails or other shaped objects, he/she can get injured. Wastes like human stool can cause diseases when poorly dumped, since the flies will carry the germ from the stool. This study attempts to understand the incidence of diseases among the respondents due to improper waste disposal. The respondents are asked to report disease history related with improper solid waste management in one year. About 42 percent of the respondents informed that they are affected by various diseases, such as diarrhea (21 percent), pneumonia (10 percent), asthma (6 percent), bronchitis (2 percent) and others (3 percent) in the last one year and they think that improper management of solid waste is the probable reason behind the said sufferings.

[2] BDT refers to the currency of Bangladesh. 1 US$ = 84.93 BDT (as on 10 January, 2021).

The surveyed municipality is lacking from a sewerage system. Therefore, people dispose household sewer to the surface drains or surface water bodies. Most of the outlets of the drains are blowing towards the rivers without any treatment measure. Most of the industrial units in the municipality are dumping their generated solid and liquid wastes in river, drain and in the municipal solid waste collection points. This study finds that the hospital and industrial wastes of the study area are not separated before dumping, which is a serious threat to human health and environment.

In response to the second research question of this study, it is found that lack of uniformity in terms of time of throwing solid wastes by the dwellers, throwing wastes here and there instead of throwing in designated places, lack of a sewerage system, mixing hospital and industrial wastes together are the major problems faced by the municipality in managing solid wastes.

In response to the third research question, this study recommends for capitalizing the willingness to pay of the respondents in addition to awareness creation, waste disposal time management, establishment of more disposal points and procuring more vehicles for improving the solid waste management services.

Discussion

This study identifies shortage in waste management handling capacity of the investigated municipality which is similar to the finding of Saifullah and Islam (2016) and Zahur (2007) who state that there is shortage of waste management capacity in the municipalities of Bangladesh.

This study discovers the lack of knowledge of most of the respondents regarding proper disposal of solid wastes. Such finding is similar to the finding of Banga (2011) who observe that about 60 percent of the households in Uganda don't have knowledge about segregation of solid waste.

This study observes heterogeneity in waste disposal time among the surveyed respondents. Such finding is similar to the finding of Sheheli (2007) and Sobhan et al. (2013) who find that people of Bangladesh dispose waste in different time periods.

The survey data signals that the satisfaction level of the respondents regarding ongoing waste management services of the municipality is disappointing. Such finding is similar to the finding of Sarker et al. (2012) who state that more than 60 percent people are not satisfied at all on present solid waste management system in a municipality of Bangladesh.

This study recognizes that most of the surveyed city dwellers are willing to pay additional money for getting improved waste management services. Such finding is similar to the finding of Barmon et al. (2015) and Bhattarai (2015) who find that the city dwellers of Bangladesh and Nepal are willing to pay for getting improved solid waste management service.

The study respondents argue that improper management of solid wastes by the municipality invites their sufferings from various diseases. Such finding is similar to the finding of Ahmed and Quader (2011) and Sarker et al. (2012) who find that improper management of municipal solid waste is a root cause behind spreading diseases among the city dwellers.

There is scope to improve the waste management system in the city. The municipality authority is not familiar with any modern waste management system. In this connection, some innovative civic authorities of non-governmental organizations (NGOs) and the communities such as 'Pradipan' in Khulna have been successful in developing participatory community-based solid waste management (Murtaza, 2002), which may be explored by the municipality under consideration.

Conclusion

The capacity of the municipality in terms of manpower, vehicle and waste disposal center is not sufficient for managing the generated solid waste properly. Only 16 dustbin and one truck is not sufficient to serve more than 0.1 million people of the municipality. There is no 'door to door' collection system of waste in the study area. More than half of the people in the area are not aware

about proper management of municipal solid waste. A serious mismatch is observed regarding time of waste throwing by the households and collecting by the municipal authority. Most of the respondents are dissatisfied with the current municipal solid waste management service. More than half of them are willing to pay for an improvement of the service.

Therefore, this study suggests for creating awareness among the people for proper management of solid wastes. Some other recommendations, as derived from the study findings include time management of waste disposal, establishment of more disposal points, employment of more manpower and vehicles. Introduction of the 'door to door' waste collection mechanism might go a long way in solving the waste management problem. Since a majority of the respondents are willing to pay for improving the waste disposal system, the municipal authority might think to impose a monthly fee to raise fund for managing solid wastes in a better way. Steps are needed to segregate industrial and clinical wastes and dump those with special attention. A better managed solid waste disposal system may be achieved through joint involvement and participation of all the stakeholders including local and national government, private organizations, individuals and local community.

This study covers only one purposively selected municipality of Bangladesh which is the main limitation of this study. Future study might extend the area coverage to get better insight on the study topic. An attempt to collect latest data will definitely help to understand the overall situation. Special effort to collect financial and willingness related data might help to validate the findings of this study.

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