INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 6, No. 3, p. 402-409, 2015

RESEARCH PAPER OPEN ACCESS

Screening of MDR-bacteria from fecal specimens of AAD patient and inhibit them using fruits extracts of *Moringa oleifera* Lam

Md. Sarwar Parvez*, Most. Ferdousi Begum, Farzana Ashrafi Neela, Mohammad Firoz Alam

Department of Botany, University of Rajshahi, Rajshahi-6205, Bangladesh

Key words: Antibiotic-associated diarrhea (AAD), Antibiotic susceptibility, Multidrug-resistant bacteria, *Moringa oleifera* Lam., Antibacterial activities.

http://dx.doi.org/10.12692/ijb/6.3.402-409 Article published on February 18, 2015

Abstract

The present research was undertaken to screen of multidrug resistant bacteria (MDRB) from fecal specimens of Antibiotic-associated diarrhea patient and to evaluate the potentiality of *M. oleifera* fruits extracts on those bacteria with the view to provide scientific evidence for its application in health remedy. MDRB were determined by antibiotic (tetracycline, azithromycin, ciprofloxacin & erythromycin) susceptibility test, using disc-agar diffusion method. Biochemical tests of the MDRB were done according to Bergey's Manual of Determinative Bacteriology for identification of the species. Minimum inhibitory concentration (MIC) as well as minimum bactericidal concentration (MBC) of the extracts was also measured. Among the 13 isolated bacteria only 5 were found resistant to multiple antibiotics. The fruit extract showed a broad-spectrum antibacterial activity against isolated four species (*Escherichia coli*, *Shigella* sp., *Salmonella* sp. & *Klebsiella* sp.) of MDRB. Among used two solvents, methanol possessed better antibacterial activity than ethanol. The highest zone of inhibition (19.86mm) was found in methanol at the concentration of 500mg/ml for *E. coli*. The MIC and MBC values were determined as 300mg/ml and 400mg/ml respectively. The consequences of this investigation suggest that the extracts of *M. oleifera* Lam. can be used to discover antibacterial agent for developing new pharmaceuticals to control different severe illness caused by MRD bacteria.

* **Corresponding Author:** Md. Sarwar Parvez ✉ sarwarparvez@yahoo.com

Introduction

Antibiotic-associated diarrhea is unexplained diarrhea that occurs in association with the administration of antibiotics (Bartlett, 2002). It disturbs the natural balance of "good" and "bad" bacteria in our intestinal tract. World Health Organization (WHO) defines antibiotic-associated diarrhea (AAD) as 3 or more abnormally loose bowel movements in a 24-hour period. The disruption of the normal enteric flora caused by antibiotics may lead to overgrowth of pathogens and functional disturbances of the intestinal carbohydrate and bile acid metabolism, resulting in osmotic diarrhea (Sayeed *et al.*, 2014). The severity of antibiotic-associated diarrhea may range from a brief, self-limiting disease to devastating diarrhea with electrolyte disturbances, dehydration, crampy abdominal pain, pseudomembranous colitis, toxic megacolon, or even death (Bartlett, 1992). Repeated and improper uses of antibiotics are primary causes of the increase in drug-resistant bacteria. The most common culprit broad-spectrum antibiotics which include ampicillin, clindamycin, cephalosporin, sometimes erythromycins, ciprofloxin and tetracycline also can cause antibiotic-associated diarrhea (Akhtar *et al.*, 2012).

Antibiotic resistance is the ability of a microorganism to withstand the effects of an antibiotic. It evolves naturally via natural selection through random mutation but it could also be engineered by applying an evolutionary stress on a population. Once such a gene is generated, bacteria can then transfer the genetic information in a horizontal fashion (between individuals) by plasmid exchange. If a bacterium carries several resistance genes is called multidrug-resistant bacteria (MDRB) or superbug (Arias *et al.*, 2009) which is more dangerous and problematic to people.

The increasing prevalence of multi-drug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raised the specter of 'untreatable' bacterial infections and adds urgency to the search for new infection-fighting strategies (Zy *et al.*, 2005; Rojas *et al.*, 2006).

In Bangladesh and other countries of the world, phytomedicines have been used since time immemorial to treat various ailments long before the introduction of modern medicine. Herbal medicines are still widely used in many parts of the world especially in areas where people do not have access to modern medicines. Moreover in most Asian countries where herbal medicines are still heavily relied upon because of high cost of chemotherapeutic drugs, there is a need for more scientific researches to determine the biological activities of medicinal plants. The findings obtained from such research may lead to the validation of traditionally used medicinally important plants and enable full usage of the properties of these plants (Sinha, 2012).

Moringa oleifera Lam. is one of the best known, widely distributed and grown species of a monogeneric family Moringaceae (Anwar *et al.*, 2007). It is a drought-tolerant plant that thrives best under the tropical climate such as Bangladesh and tolerates different soil types (Fahey, 2005). The plant is highly valued since almost every part of the plant (leaves, roots, barks, fruits/pod, flowers etc.) is used as food with high nutritional value (Anwar *et al.*, 2007). A total of forty four compounds (Chuang *et al.*, 2007), such as, 2-nitrile glycosides, niazirin, niazirinin, moringine, moringinine, 3-mustard oil glycosides, isothiocyanate, niaziminin A and B etc. are isolated from *Moringa oleifera*, which are reported as bio-active compound. In addition the plant has been reported to possess antibacterial properties and this explains the reasons for its wide use in the treatment of human diseases (Rahman *et al.*, 2009; Akhtar *et al.*, 2012; Sayeed *et al.*, 2012; Sinha, 2012).

Therefore, the present study was undertaken specifically to investigate the role of fruits extracts of *M. oleifera* Lam. as a potential antimicrobial agent against MDRB screening from fecal specimens of AAD-patients with the view to provide scientific evidence for its application in health remedy.

Materials and methods

Subjects and Isolation of AAD-bacteria

A total 10 antibiotic-associated diarrheal fecal specimens were collected in sterile screw-capped tube from pediatric ward of Rajshahi Medical College Hospital, Rajshahi, Bangladesh, during March to April, 2011. Those specimens were obtained from diarrhea affected children (1-36 months) during the first two weeks after the starting of the antibiotic treatment, because this period most likely reflects the effect of antibiotic use. The fecal specimens were then cultured following Holt *et al.* (1994) by plated onto MacConkey agar (0.001mg/ml) at 37°C for about 24 h. After the growth of bacteria, the pink color colonies were screened as lactose fermenter.

Antibiotic susceptibility and biochemical tests

MDR bacteria were detected by disc diffusion method according to NCCLS (1997) guidelines. Four commercial prepared antibiotic discs i.e. Tetracycline (30 µg/disc), Azithromycin (15 µg/disc), Ciprofloxacin (5 µg/disc) & Erythromycin (15 µg/disc) were used in this experiment (All disks were obtained from Oxoid Ltd., Basingstoke and Hampshire, England).

Muller Hilton Agar media was used for antimicrobial disc diffusion susceptibility testing. The surface of Muller Hilton agar plate was inoculated by spreader. Then Antimicrobial discs were placed on the surface of the agar plate using forceps. Gently pressed down each disc to ensure complete contact with the agar surface. Discs were distributed in 24 mm gap from center to center. The plates were incubated at 37□C and were examined after 18-24 hours. The zones of inhibition around each disc (including disc) were measured in millimeter (NCCLS, 1997) using a ruler. Antibiotic susceptibility was evaluated by the diameter of inhibition zone (mm) compared with the NCCLS standard manual.

Biochemical tests of the MDRB were done according to Bergey's Manual of Determinative Bacteriology (Holt *et al.*, 1994) using their respective standard strain for identifying the species. Gram staining, oxydase, indole, urease, motility, nitrate, beta-galactosidase, catalase, citrate utilization, methyl red, Voges-Proskauer, Kligler's iron agar and Fermentation tests were done for confirmation of the species.

Preparation of plant extracts

Fresh mature pod/fruits of *M. oleifera* were collected from Saheb Bazar, Rajshahi, Bangladesh, during June, 2011 and authenticated by the plant taxonomist at the Department of Botany, University of Rajshahi, Bangladesh. The freshly collected fruit were cut in ½ inches and shade dried at room temperature (32-35□C) for five days (Doughari, 2006). Dried plant materials were coarsely powdered using a mortar and pestle and were further reduced to powder using an electric blender. After that the fine powder was transferred into airtight glass container for short time preservation.

Two solvents, namely ethanol and methanol, were used for the extracts preparation in present studies. Fifty-gram fine powder was dipped into 250 ml methanol (95%) and 250 ml ethanol (95%) into different conical flask and stirring vigorously with a glass rod for proper extraction. The mixture left for 3 days with constant shaking using orbital shaker at room temperature. After three days, the resulting mixture was than filtered into two stages. First, in Teton cloth followed by Whatman No. 1 filter paper for getting more delicate filtration. Filtrates were taken into glass beaker for evaporating solvents. Semi solid filtrates were dissolved in respective solvent and transferred into airtight screw cap tube and stored at 4 °C (Alo *et al.*, 2012).

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) tests were done according to Doughari *et al.* (2007) with slide modification. The MIC of the extracts was determined for each of the test bacteria in triplicate in test tubes. To 0.5 ml of varying concentrations of the

extracts (100, 200, 300, 400 and 500 mg/ml) in test tubes, LB broth (2ml) was added and then a loopful of the test bacteria, previously diluted to 0.5 McFarland turbidity standard (10^8 CFU/ml), was introduced. A tube containing Nutrient broth only was seeded with the test bacteria to serve as controls. The culture tubes were then incubated at 37°C for 24 h. After incubation the tubes were then examined for microbial growth by observing for turbidity. The least concentration of the samples with no visible growth was taken as the MIC.

To determine the MBC, for each set of test tubes in the MIC determination, a loopful of broth was collected from those tubes that did not show any growth and inoculated onto sterile Nutrient agar by streaking. LB agar plates only were also streaked with the respective test organisms to serve as controls. All the plates were then incubated at 37°C for 24 h. After incubation the concentration at which no visible growth was seen was noted as the MBC.

Results and discussions

Enumeration and isolation of AAD-bacteria

Total numbers of colonies of different AAD-bacteria were enumerated from each sample. The number of colony was ranged from 66 ± 0.7 cfu/plate to 21.5 ± 1.2 cfu/plate (*Data not shown*). Initially, 13 isolates were isolated out of 10 samples screened on MacConkey agar medium. These isolates were subjected to test of antibiotic susceptibility for the determination of multidrug-resistant bacteria.

Table 1. Screening of multi drug-resistant bacteria according to NCCLS.

Isolate No.	Antimicrobial agent and their Standard Zone of inhibition (mm) for resistance according to NCCLS				Identified MDRB
	Azithromycin [R ≤ 13; IS = 14-17 and S ≥ 18mm]	Ciprofloxacin [R ≤ 15; IS = 16-20 and S ≥ 21mm]	Erythromycin [R ≤ 13; IS = 14-22 and S ≥ 23mm]	Tetracycline [R ≤ 14; IS = 15-18 and S ≥ 19mm]	
RMC 1	15 (IS)	17 (IS)	16 (IS)	12 (R)	-
RMC2	13 (R)	20 (IS)	12 (R)	14 (R)	MDRB
RMC 3	17 (IS)	16 (IS)	18 (IS)	12 (R)	-
RMC 4	15 (IS)	18 (IS)	16 (IS)	11 (R)	-
RMC 5	14 (IS)	14 (R)	8 (R)	0 (R)	MDRB
RMC 6	0 (R)	8 (R)	11 (R)	0 (R)	MDRB
RMC 7	12 (R)	16 (IS)	18 (IS)	17 (IS)	-
RMC 8	16 (IS)	19 (IS)	16 (IS)	15 (IS)	-
RMC 9	17 (IS)	16 (IS)	18 (IS)	14 (R)	-
RMC 10	8 (R)	16 (IS)	18 (IS)	10 (R)	MDRB
RMC 11	18 (S)	19 (IS)	16 (IS)	11 (R)	-
RMC 12	9 (R)	16 (IS)	13 (R)	14 (R)	MDRB
RMC 13	16 (IS)	18 (IS)	18 (IS)	15 (R)	-

** according to NCCLS, R = Resistant; IS = Intermediate susceptible and S = susceptible.

Screening of MDRB via Antibiotic susceptibility according to NCCLS

Only 5 out of 13 isolates were found resistant to multiple antibiotics. Among the isolates, RMC-6 was resistant against all the used antibiotics and other 4 isolates were resistant to two or three antibiotics. Almost all of the isolates were sensitive to ciprofloxacin without RMC-5 and RMC-6 (Table 1). The highest resistance was observed in tetracycline (84.61%) followed by Azithromycin (38.46%), Erythromycin (30.76 %) and the lowest was observed in ciprofloxacin (15.38 %) as shown in Fig.1.

Identification of multi drug-resistant bacteria

For identification of multidrug-resistant bacteria different biochemical tests were carried out and the

results are summarized in the Table - 2. On the basis of growth on MacConkey agar, morphological and biochemical test, the selected multidrug-resistant bacteria were identified as *Escherichia coli*, *Shigella* sp., *Salmonella* sp. and *Klebsiella* sp. Many scientists reported the presence of *Escherichia coli* (Vervoort, 2014), *Salmonella* sp. (Ayyagari *et al.*, 2003) and *Klebsiella* sp. (Hoffmann *et al.*, 2010; Gorkiewicz, 2009) in the patients with antibiotic-associated diarrhea. The results of Akhtar *et al.*, (2012) about confirmation the species of MDRB from AAD also support the present studies.

Table 2. Biochemical test for characterization of isolated antibiotic resistant bacteria.

Isolates	Biochemical Test																	Suspected bacteria
	GS	OX	IN	UR	MO	NI	ONPG	CA	CT	MR	VP	KIA			Fermentation			
												Slope	Butt	H_2S	L	M	G	S
RMC 2	-	-	+	-	+	+	+	+	-	+	-	Y	Y	-	+	+	+	+	*Escherichia coli*
RMC 5	-	-	+	+	-	+	+	+	+	+	+	Y	Y	-	+	+	+	-	*Klebsiella sp.*
RMC 6	-	-	+	-	+	+	+	+	-	+	-	Y	Y	-	+	+	+	+	*Escherichia coli*
RMC 10	-	-	-	-	+	+	-	+	+	+	-	R	Y	+	-	+	+	-	*Salmonella sp.*
RMC 12	-	-	-	-	-	+	-	+	-	+	-	R	Y	-	-	+	+	-	*Shigella sp.*

Key: GS = Gram staining, OX = Oxidase, IN = Indole, UR= Urease, MO = Motility, NI = Nitrate, ONPG = beta-galactosidase, CA = Catalase, CT = Citrate test, MR = Methyle Red, VP = Voges-Proskauer, KIA = Kligler Iron Agar , H_2S = Hydrogen sulphide, R = Red, Y = Yellow, L=Lactose, M=Manitol, G=Glucose, S=Sucrose.

Antibacterial assay

The results of the antibacterial assay of the *Moringa oleifera* fruits indicated that these plant exhibited antimicrobial activity against the tested MDR bacteria. The potential sensitivity of the extract was obtained against all the microorganisms tested and the zone of inhibition was recorded and presented in the table 3.

Table 3. Antibacterial activities of *M. oleifera* fruit extracts against identified MDRB.

Extracts (mg/ml)	Solvent	Isolated Bacteria [Zone of inhibition (mm)]			
		Escherichia coli	*Salmonella*	*Shigella*	*Klebsiella*
100	Methanol	+	+	+	+
	Ethanol	+	+	+	+
200	Methanol	11.37±0.55	9.49±0.45	9.72±0.67	10.5±0.95
	Ethanol	+	+	+	+
300	Methanol	13.33±0.66	12.2±0.78	12.6±0.83	12.5±0.33
	Ethanol	9.2±0.34	9.8±0.88	10.4±0.57	9.8±0.54
400	Methanol	17.86±0.43	13.6±0.49	15.4±0.83	12.9±0.65
	Ethanol	13.80±1.04	11.3±0.56	13.6±0.22	10.2±0.33
500	Methanol	19.86±0.43	13.6±0.49	18.4±0.83	12.9±0.65
	Ethanol	15.58±1.04	11.3±0.56	14.6±0.22	10.2±0.33
NC	ME	+	+	+	+
	ET	+	+	+	+

Data are represented as mean ± SEM of triplicate experiments. ET=Ethanol; ME= Methanol; ; NC= Negative control; + = Bacterial growth.

Table 4. Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of *M. oleifera* fruits extracts against selected MDR-bacteria.

Bacterial species	MIC value (mg/ml)		MBC value (mg/ml)	
	Methanol	Ethanol	Methanol	Ethanol
Escherichia coli	300	320	440	460
Salmonella sp.	320	340	400	440
Shigella sp.	340	360	420	440
Klebsiella sp.	320	340	400	460

All the extracts exhibited different degrees of antibacterial activity which were compared with the standard reference (control). Depending on the measured values of the complete inhibition diameter of the zone, the antibacterial activity can be classified as 6-9 mm: week antibacterial activity; 10-15 mm: slight antibacterial activity; 16-20 mm: moderate antibacterial activity and >20: strong antibacterial activity (Arora and Bhardwaj, 1997).

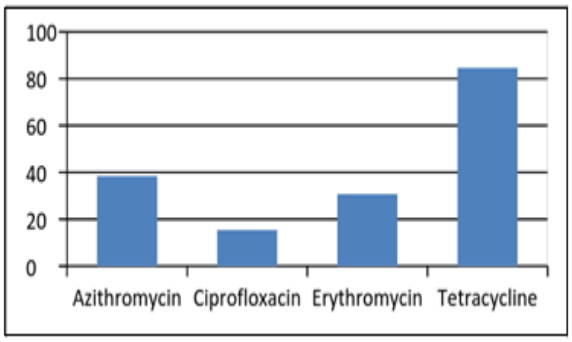

Fig. 1. Antibiotic potential (%) against bacteria isolated from AAD.

Methanol extract showed better antibacterial effect against all of the isolates than ethanol extract. The highest inhibition zone (19.86±0.43 mm) was found against *E. coli* at 500 mg/ml methanol extract. Sayeed *et al.,* (2012) observed that methanol extract of *Moringa oliefera* fruits possessed moderate antibacterial activity against bacterial strains - *Salmonella sp, Shigella sp, Pseudomonas sp, Klebsiella species*. In another study Sinha, (2012) reported about the ability of methanolic extract of *Moringa* plant to inhibit the growth of bacterial strains which is an indication of its antibacterial potential.

MIC and MBC Determination

The MIC values obtained for the two extracts against the isolates varied from one extract to the other (Table 4). The MIC of the methanol extract ranged 300-340 mg/ml, with the 300mg/ml extract demonstrating the lowest values against *E. coli*. Ethanolic fruits extract demonstrated comparatively higher MIC ranged 320-360 mg/ml and the lowest MIC (320 mg/ml) exhibited against *E. coli*. Most of the MIC values were lower indicating the extracts could be bactericidal in action. Lower MIC values and higher zones of inhibition for *Moringa* extracts is the indication of high efficacy which in accordance with the results obtained by many scientists (Rahman *et al.*, 2009; Akhtar *et al.* 2012; Sayeed *et al.*, 2012; Sinha, 2012). MBC value of methanolic and ethanolic extracts ranged 400-460 mg/ml. Antimicrobial properties of substances are desirable tools in the control of undesirable microorganisms in the treatment of infections (Aboaba *et al.*, 2006).

Conclusion

Findings of the present study suggested that methanolic extracts of fruits of *Moringa oleifera* have potential as antibacterial compounds against pathogens and their ability to either block or inhibit resistance mechanisms of bacteria could improve treatment and eradication of bacterial strains. Thus this plant extracts could be used in the treatment of infectious diseases caused by resistant bacteria. Therefore the results laid down a basis for investigation the search of compounds in *M. oleifera fruits* responsible for antibacterial activity.

References

Aboaba OO, Smith SI, Olude FO. 2006. Antibacterial effect of edible plant extract on *Escherichia coli* 0157:H7. Pakistan Journal of Nutrition **5(4),** 325-32.

Akhtar MM, Islam MS, Begum MF, Anisuzzaman M, Alam MF. 2012. Screening of multidrug-resistant bacteria of antibiotic-associated diarrhea and their inhibition by leaf extracts of *Moringa oleifera* Lamk. Journal of Bio-Science **20,** 41-48.

Alo MN, Anyim C, Igwe JC, Elom M, Uchenna DS. 2012. Antibacterial activity of water, ethanol and methanol extracts of *Ocimum gratissimum*, *Vernonia amygdalina* and *Aframomum melegueta*. Advances in Applied Science Research **3(2),** 844-848.

Anwar F, Latif S, Ashraf M, Gilan AH. 2007. *Moringa oleifera*: A food plant with multiple medicinal uses. Phytotherapy Research **21,** 17-25.

Arias CA, Murray BE, Murray BE. 2009. Antibiotic-resistant bugs in the 21st century - a clinical super-challenge. New England Journal of Medicine **360 (5),** 439–443. http://dx.doi.org/10.1056/NEJMp0804651.

Arora DS, Bhardwaj SK. 1997. Antibacterial activity of some medicinal plants. Geobios **24**, 127-131.

Ayyagari A, Agarwal J, Garg A. 2003. Antibiotic associated diarrhoea: infectious causes. Indian Journal of Medical Microbiology **21(1),** 6-11.

Bartlett JG. 1992. Antibiotic-associated diarrhea. Clinical Infectious Diseases **15(4),** 573-581.

Bartlett JG. 2002. Antibiotic-associated diarrhea. New England Journal of Medicine **346(5),** 334-339. http://dx.doi.org/10.1056/NEJMcp011603.

Chuang P, Lee C, Chou J, Murugan M, Shief B, Chen H. 2007. Antifungal activity of crude extracts and essential oil of *Moringa oleifera* Lam. Bioresource Technology **98,** 232-236.

Doughari JH, Pukuma MS, De N. 2007. Antibacterial effects of *Balanites aegyptiaca* L. Drel. and *Moringa oleifera* Lam. on *Salmonella typhi*. African Journal of Biotechnology **6(19)**, 2212-2215.

Doughari JH. 2006. Antimicrobial Activity of *Tamarindus indica* Linn. Tropical Journal of Pharmaceutical Research **5(2),** 597-603.

Fahey JW. 2005. *Moringa oleifera*: A review of the medical evidence for its nutritional, therapeutic, and prophylactic properties. Trees for Life Journal **1,** 5.

Gorkiewicz G. 2009. Nosocomial and antibiotic-associated diarrhoea caused by organisms other than *Clostridium difficile*. International Journal Antimicrobial Agents **33,** 37-41. http://dx.doi.org/10.1016/S0924-8579(09)70015-9.

Hoffmann KM, Deutschmann A, Weitzer C, Joainig M, Zechner E, Högenauer C, Hauer AC. 2010. Antibiotic-associated hemorrhagic colitis caused by cytotoxin-producing *Klebsiella oxytoca*. Pediatrics **125(4),** 960-963. http://dx.doi.org/10.1542/peds.2009-1751.

Holt JG, Krieg NR, Sneath PHA, Staley JT, Williams ST. 1994. Bergey's Manual of Determinative Bacteriology. 9th ed. Philadelphia: Lippincott Williams and Wilkins.

NCCLS. 1997. Performance Standards for Antimicrobial Disk Susceptibility Tests: Approved Standard M2-A7. National Committee for Clinical Laboratory Standards, Wayne, PA, USA.

Rahman M, Mashiar M, Sheikh MI, Sharmin SA, Islam MS, Rahman MA, Rahman MM, Alam MF. 2009. Antibacterial activity of leaf juice and extracts of *Moringa oleifera* lam. against some human pathogenic bacteria CMU. Journal of Natural Sciences **8(2),** 219-227.

Rojas JJ, Ochoa VJ, Ocampo SA, Munoz JF. 2006. Screening for antimicrobial activity of ten medicinal plants used in Colombian folkloric medicine: A possible alternative in the treatment of non-nosocomial infections. BMC Complementary and Alternative Medicine **6,** 2.

Sayeed MA, Hossain MS, Chowdhury MEH, Haque M. 2012. *In vitro* antimicrobial activity of methanolic extract of *Moringa olieifera* Lam. Fruits Journal of Pharmacognosy and Phytochemistry **1(4),** 94-98.

Sayeed MA, Sarker TC, Rahman MM, Parvez MS, Alam MF. 2014. Isolation and identification of antibiotic-associated diarrheagenic resistant bacteria from patients of Rajshahi medical college hospital, Rajshahi, Bangladesh. International Journal of Biosciences **5(1),** 155-160. http://dx.doi.org/10.12692/ijb/5.1.155-160.

Sinha SN. 2012. Phytochemical analysis and antibacterial potential of *Moringa oleifera* Lam. The International Journal of Science Innovations and Discoveries **2(4),** 401-407.

Vervoort J, Gazin M, Kazma M, Kotlovsky T, Lammens C, Carmeli Y, Goossens H, Malhotra-Kumar S, Saturn WP, Mosar WP. 2014. High rates of intestinal colonisation with fluoroquinolone-resistant ESBL-harbouring Enterobacteriaceae in hospitalised patients with antibiotic-associated diarrhoea. European Journal of Clinical Microbiology & Infectious Diseases **33(12),** 2215-2221. http://dx.doi.org/10.1007/s10096-014-2193-9.

Zy EA, Area A, Aam K. 2005. Antimicrobial activity of some medicinal plant extracts in Palestine. Pakistan Journal of Medical Sciences **21,** 187-193.