Control of coliform bacteria detected from diarrhea associated patients by extracts of *Moringa oleifera*

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ABSTRACT

The aims of this study were to determine the total population of coliform bacteria in the samples collected from diarrhea associated patients from the local area of Bangladesh and to examine the antibacterial efficacy of leaf extracts of *Moringa oleifera* (Moringaceae) against the isolated coliform bacteria. The coliform bacteria detected in these samples by some microbial-biochemical tests such as *Escherichia coli, Shigella dysenteriae, Salmonella* sp., *Enterobacter* sp., *Klebsiella pneumoniae* and *Serratia marcescens*. The total isolation rate of coliform bacterial species was ranged from 38.01-3.51%. At the concentration of 300 ìg/disc, the organic extracts of hexane, chloroform, ethyl acetate and methanol extracts of *Moringa oleifera* leaf exhibited a remarkable antibacterial effect against all the tested bacterial pathogens. The zones of inhibition against all the tested bacterial pathogens were found in the range of 8.0 to 23.2 mm, along with their respective minimum inhibitory concentration (MIC) values ranging from 62.5-1000 ìg/mL. The results obtained in this study suggest that the extracts from *Moringa oleifera* leaf can be a source of natural antimicrobials with potential applications in pharmaceutical industry to control coliform bacteria.

Keywords: *Moringa oleifera*, coliform bacteria, diarrhea, antibacterial activity, MIC.

INTRODUCTION

Detection of the etiological agents of diarrhea is important for therapeutic aspects and for implementing appropriate control strategies. In developing countries, the bacterial pathogen most commonly associated with endemic form of diarrhea is diarrheagenic *E. coli*. Providing modern healthcare to rural people in Bangladesh is still a far-reaching goal due to economic constraints. Hence, people mainly depend on the locally available plant materials to cure various health disorders. Plant possesses components which render beneficial properties.[1] Therefore, currently attention is being drawn towards exploring plant sources for substances that provide nutritional and pharmaceutical advantages to humans. Green leafy vegetables are a good source of minerals and vitamins.

M. oleifera is rich in compounds containing the simple sugar, rhamnose and a fairly unique group of compounds called glucosinolates and isothiocyanates.[2] Sulaiman et al [3] has evaluated of *M. oleifera* aqueous extract for antinociceptive and anti-inflammatory activities in animal models. Mahajan *et al*[4] has reported seed extract of *M. oleifera* has effect on toluene diisocyanate-induced immune-mediated inflammatory responses in rats. Almost all the parts of this plant have various effects such as cardiovascular activity, gastrointestinal activity, hematological activity, hepatorenal disorders inhibitory activity.[5,6] *M. oleifera* have been extensively studied pharmacologically and it has been found that the ethanol extract and its constituents exhibit antispasmodic, antitumor activity antiulcer and hepatoprotective activities.[7,8,9] It *has* also been reported to exhibit other diverse activities antiurolithiatic, antihypertensive, diuretic and cholesterol lowering activities.[10,11]

M. oleifera Lam. (Moringaceae), commonly referred to simply as *Moringa*, is the most widely cultivated variety of the genus *Moringa*. It is native to the sub-Himalayan tracts of India, Pakistan, Bangladesh, and Afghanistan. It is an exceptionally nutritious vegetable with a variety of potential uses. In the Philippines, *M. oleifera* is commonly grown for its leaves, which are used in soup. The flowers, leaves, and roots are used in folk remedies for tumors; the seeds are used for abdominal tumors treatment.[12] In developing tropical countries, *M. oleifera* have been used to combat malnutrition, especially among infants and nursing mothers. Its bark is regarded as an antiscorbic and it exudes a reddish gum sometimes used for diarrhea. The roots of *M. oleifera* are bitter, act as a tonic to the body and lungs, and an expectorant. The leaf tea is used to treat gastric ulcers and diarrhea while

Table-1: Different types of samples from the study families, incidence of history of diarrhea of three villages of Rajshahi

Types of samples		Number of samples
HRC[a]	PBC[a1]	39
	EBC[a2]	29
HRM[b]	PBM[b1]	39
	EBM[b2]	29
LF[c]		59
FU[d]		59
DW[e]		59
Total number of samples		313

[a]Hand ringing children, [a1]Partially breastfed children, [a2]Exclusively breastfed children, [b]Hand ringing mother, [b1]Partially breast fed mother, [b2]Exclusively breast fed mother, [c]Leftover foods, [d]Feeding utensils, [e]Drinking water

flower juice improves the quality and flow of mothers' milk when breast feeding and is also useful for urinary problems as it encourages urination. The seeds are used for their antibiotic and anti-inflammatory properties to treat arthritis, rheumatism, gout, cramp, sexually transmitted diseases, and as a relaxant for epilepsy; the powder from whole plant can be used as a quick and simple method for cleaning dirty river water.[13] Therefore, the present study was undertaken to demonstrate sources of coliform bacterial contamination of infants and their mothers in a rural area of Rajshahi region of Bangladesh and their control by *M. oleifera* leaf extracts.

MATERIAL AND METHODS

Samples collection: For samples collection, 68 infants (aged between 0-12 months) and their mothers were selected from three villages namely Meherchondi, Buthpara and Dasmari of Rajshahi district, Bangladesh in June 2007. A total of 313 samples were collected from diarrhea affected hand rinsing children (aged 0-12 months) of their feeding utensils, leftover food and drinking water and their mothers. All samples were transferred immediately into the laboratory for analyses. Of the 313 samples, 29 samples were collected from exclusively breastfeeding children's and their mothers.

Culture of bacteria: Selective and differential solid media as well as enrichment broth were used for the primary isolation of coliform bacteria. Those include MacConkey agar, Luria-Bertani media, Simmon's citrate agar and an enrichment broth. Samples of leftover food were vortexed for homogenous mixture before culture. Each sample (20 µL) was spread on separate Petri dish and incubated at 37°C for 24 h. The growth of bacteria was observed through the development of colored colonies.

Biochemical tests for the characterization of bacteria

Gram stain: Gram staining of the cultured bacteria was performed as described previously by Rahman *et al*.[14] The bacterial smear was stained by the primary stain crystal violet and iodine (Gram's iodine) solution (1.0% iodine, 2.0% potassium iodide in water). To prevent over-decolorisation, it was rinsed immediately with water. Finally, the secondary stain safranin was added and incubated for 1 min, and then washed with water for a maximum of 5 sec. The slide was observed under a microscope.

Indole test: For detection of *E. coli*, indole test was done as described previously by Rahman et al.[16] Briefly, the bacteria of colored colonies were primarily cultured in tryptophan containing medium. The culture medium was incubated at 35 ± 2ºC for up to 48 h. 0.5 mL of Kovac's reagent (4(p)-dimethylamino-benzaldehyde) was added in the medium with gentle shaking and observed for a bright pink color in the top layer.

Oxidase test: Oxidase test was done as previously described by Rahman et al.[14] A piece of filter paper was placed in a clean Petri dish and 2 or 3 drops of freshly prepared oxidase reagent was added. The colony of indole-positive bacteria was smeared on filter paper.

Catalase test: This test was performed according to procedure depicted by Rahman et al.[14] One drop of hydrogen peroxide was placed on a microscope slide. Using a sterile needle, a single colony of indole-positive bacteria was removed and immersed in the hydrogen peroxide solution for the development of an immediate froth of bubbles.

Urease test: Urease test was done as described previously by Chomvarin et al.[15] The test organism was inoculated on the stab medium of the tubes (just touching the surface of an individual colony) and incubated at

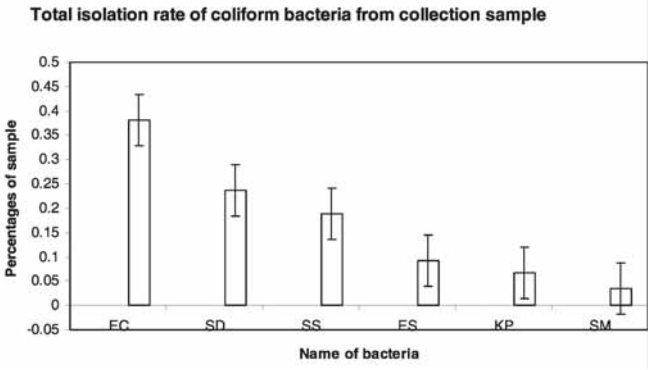

Fig. 1. The total isolation rate of coliform bacteria from collected samples. EC, *Escherichia coli*; SD, *S. dysenteriae*; SS, *Salmonella* sp.; ES, *Enterobacter sp.*; KP, *K. pneumoniae*; SM, *S. marcescens*

Table-2: List of bacteria identified by biochemical tests.

Colony type	Gram stain	Cell Shape	Indole test	Oxidase test	Catalase test	Urease test	MR- VP		Citrate test	TSI				Bacteria
							MR	VP		Slant	Butt	H_2S	Gas	
Smooth pink	-	ShortRod	+	-	+	-	+	-	-	Y	Y	-	+	*E.coli*
Mucoid pink	-	ShortRod	-	-	+	+	-	+	+	Y	Y	-	+	*K. .pneumoniae*
Mucoid pink	-	Rod	-	-	+	-	+	+	+	Y	Y	-	+	*Enterobacter* sp.
Red pink	-	ShortRod	-	-	+	-	-	+	+	R	Y	-	+	*S. marcescens*
Pale colored	-	Rod	+	-	+	_	+	-	+	R	Y	+	+	*Salmonella sp.*
Pale coloredwith 1-2 mm diameter	-	Rod	-	-	+	-	+	-	-	R	Y	-	-	*S. dysenteriae*

+ = Positive, - = Negative, Y= Yellow, R= Red,

37°C for 24 h and observed at every half an hour intervals. Fermentation of red-pink color throughout the medium indicated the urease production (positive test) and no color change indicated absence of urease production (negative test).

MR-VP (Methyl Red-Vogues Proskauer) test: This test was done as described previously by Barry and Feeney.[16] Briefly These tests were performed by inoculating a single tube of MRVP media with a transfer loop of an isolate and then allowed the culture to grow at 35ºC for 3-5 days. After the culture was grown, about half of the culture was transferred to another sterilized tube. One tube of culture used to conduct the MR **(methyl red)** test, the second tube served as the VP **(Vogues Proskauer)** test. Color was observed.

Citrate utilization test: This test was done as described previously by Simmons.[17] Simmon's citrate agar is a medium containing citrate as the sole carbon source and ammonium salts as the sole nitrogen source and the indicator bromophenol blue. A Simmon's citrate agar plate was streaked with the organism and incubated at 37°C for 48 h to examine the bacterial growth.

Triple sugar iron agar (TSI): This test was done as described previously by Schneid et al.[18] Triple sugar iron agar (TSI) is a differential medium that contains lactose, sucrose, glucose (dextrose), ferrous sulfate, and the pH indicator phenol red. The slant was inoculated with a pure culture by streaking over the entire surface of the slant (zig-zag to cover surface) and incubated at 37ºC for 24 h followed by stabbing deep into the butt.

Plant materials: The leaves of *M. oleifera* were collected from a local area of Kushtia region of Bangladesh in June 2007. The plant was identified by a senior taxonomist Md. Habibur Rahman, National Herbarium, Mirpur, Dhaka 1216, Bangladesh, where a voucher specimen (DACB 32494) has been deposited.

Preparation of extracts: The air dried leaves were pulverized into powdered form. The dried powder (50 g) was dipped into 200 mL hexane, chloroform, ethyl acetate, and 70% methanol into a conical flask separately at room temperature for 7 days. The solvents from the extracts were filtered through Whatman no. 1 filter papers and evaporated by vacuum rotary evaporator at 50°C. The extraction process yielded in hexane (7.2 g), chloroform (5.6 g), ethyl acetate (5.6 g) and methanol (6.5 g) extracts. Solvents (analytical grade) for extraction were obtained from commercial sources (Sigma–Aldrich, St. Louis, MO, USA). The extracts were then stored in a refrigerator at 4ºC until further antibacterial activity test.

Antibacterial activity assay: The disc diffusion method was used for antibacterial assay [19] using 0.1 mL of standardized inoculum suspension (10^7 CFU/mL) of bacteria. A Whatman no. 1 sterile filter paper disc (6 mm diameter) was impregnated with 10 µL of 30 mg/mL (300 ìg/disc) of *M. oleifera* leaf extracts of hexane, chloroform, ethyl acetate and methanol. Negative controls were prepared using the same solvent employed to dissolve the samples. Standard reference antibiotic tetracycline (10 ìg/disc for each), were used as positive controls for the tested bacteria. The plates were incubated at 37ºC for 24 h and at the end of the period; the inhibition zones formed around each disk were evaluated in millimeters. Each assay in this experiment was replicated three times.

Minimum inhibitory concentration (MIC): Minimum inhibitory concentrations (MICs) of various leaf extracts of hexane, chloroform, ethyl acetate, and methanol were determined by a two-fold serial dilution method.[20] The tests of leaf extracts were incorporated into Luria-Broth medium to get a concentration of 2000 ìg/mL and further, serially diluted to achieve 1000, 500, 250 125, 62.5, and 31.25 ìg/mL, respectively. A 10 ìL of standardized suspension of each tested organism (10^7 CFU/mL) was transferred to each tube. The control tubes containing only bacterial suspension were incubated at 37ºC for 24 h. The lowest concentration of the test samples at which

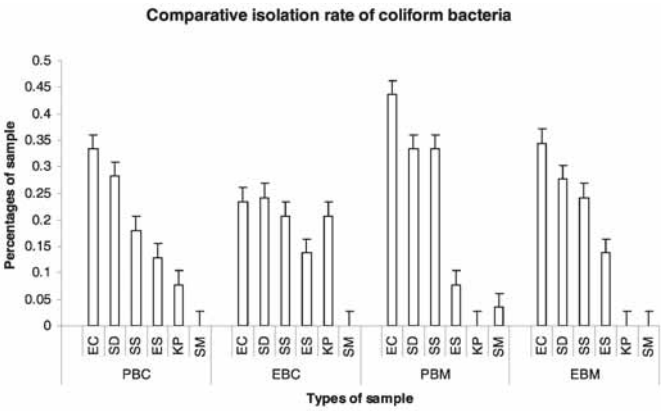

Fig. 2. The comparative isolation rate of coliform bacteria from the samples of partially and exclusively breast-fed children and their mothers

the tested organism did not demonstrate visible growth was determined as MIC, which was expressed in ìg/mL.

RESULTS

Isolation and characterization of bacteria: A total of 313 samples collected from hand rinsing of the study children (HRC) ages between 0-12 months and their mothers (HRM), swabs from feeding utensils, leftover food and drinking water from three villages of Rajshahi are shown in Table-1. The coliform bacteria such as *E. coli*, *K. pneumoniae*, *Enterobacter* sp., *S. marcescens*, *Salmonella* sp. and *S. dysenteriae* were isolated and identified by color detection and colony size on MacConkey agar plates and the results of biochemical tests are shown in Table-2. The difference between Gram-positive and Gram-negative bacteria lies in the ability of the cell wall of the organism to retain the crystal violet. Gram-negative bacteria do not retain crystal violet dye in the Gram staining protocol. Gram-positive bacteria retain the crystal violet dye when washed in a decolorizing solution. All isolated strains were rod shaped and Gram-negative as it was retaining the safranine color. Smooth pink colony showed positive indole, MR, catalase test and negative oxidase, urease, VP and citrate test whereas in TSI test produced yellow colored slant and butt with gas evolved without production of H_2S indicating *E. coli*. The indole test was used for the differentiation of genera and species, e.g., *E. coli* (+) from *Enterobacter* sp. (-); *Proteus mirabilus* (-) and from other *Proteus* sp. Positive indole test produced red surface layer while positive catalase test produced catalase enzyme; this enzyme converted hydrogen peroxide to water and oxygen gas was evolved. The evolution of gas caused bubbles to form, which indicated a positive catalase test. Methyl red test showed red color represents a positive test as it is a pH indicator. It is red in pH under 4.4, yellow in pH over 6.2, and orange in between. Production of yellow slant and yellow butt indicate acid reaction resulting in the fermentation of lactose, sucrose. Mucoid pink colony were catalase, urease, VP and citrate positive as they produced gas caused bubbles, dark blue, red-pink and green to deep blue colour, respectively while TSI test produced yellow slant and yellow butt with gas production. The VP test is used to differentiate between genera, e.g., *K. pneumonia*e (+) and *Enterobacter* (+) from *E. coli* (-). Mucoid pink colony was also indicated a positive catalase, urease, citrate and MR tests but not VP. The positive MR test used to differentiate between genera e.g., *E. coli* (+) from *Enterobacter* sp. (-) and *Yersinia* sp. (+). Red pink colony under the TSI test produced red slant and yellow butt with bubbles identifying the bacterium as *S. marcescens*. Pale colored colony indicated Red slant, yellow butt with bubbles and black precipitate in the medium on TSI test confirmed the bacterium as *Salmonella* sp. Pale colored colony (1-2 mm diameter) also noticed red slant and yellow butt without evolution of gas and H_2S identifying the bacterium as *S. dysenteriae*. Furthermore, a total of 313

Table-3: The percentage distribution of *Escherichia coli*, *Shigella dysenteriae*, *Salmonella* sp., *Enterobacter* sp., *Klebsiella pneumoniae* and *Serratia marcescens* isolated from various samples

Types of Samples		Samples Examined(n)	Samples Positive for Bacteria					
			Escherichia coli	***S. dysenteriae***	***Salmonella* sp.**	***Enterobacte* sp.**	***K. pneumoniae***	***S. marcescens***
HRC[a]	PBC[a1]	39	13(33.33%)	11(28.20%)	7(17.95%)	5(12.82%)	3(7.69%)	nd
	EBC[a2]	29	7(23.33%)	6(24.14%)	6(20.69%)	4(13.79)	6(20.68%)	nd
HRM[b]	PBM	39	17(43.58%)	13(33.33%)	5(33.33%)	3(7.69%)	nd	1(2.56%)
	EBM	29	10(34.48%)	8(27.59%)	7(24.14%)	4(13.79)	nd	nd
LF[c]		59	31(52.54%)	11(18.64%)	10(16.95%)	nd	4(6.77%)	3(5.08%)
FU[d]		59	16(27.11%)	15(25.42%)	11(18.64%)	6(10.16)	4(6.77%)	7(11.86%)
DW[e]		59	25(42.37%)	10(16.95%)	13(22.03%)	7(11.86)	4(6.77%)	nd
Total		313	119(38.01%)	74(23.64%)	59(18.84%)	29(9.26%)	21(6.70%)	11(3.51%)

Table-4: Antibacterial activity of *Moringa oleifera* L. leaves juice and extracts against isolated coliform bacteria.

Bacteria	Fresh leaf juice		Aqueous extract [b]				Ethanol extract [b]		Positive control
	Juice[a]	Powdered from fresh leaf juice[b]	Fresh Leaf		Dried Leaf		Fresh Leaf	Dried Leaf	Tetracycline (30 ìg disc^{-1})
			Cold	Hot	Cold	Hot			
Escherichia coli	17.7±0.2	40.45±0.37	16.8± 0.12	nd	nd	nd	21±0.16	nd	19.03±0.44
S. dysenteriae	23.1±0.08	35.4±0.08	14.9±2.20	nd	nd	nd	19±0.48	nd	17.0±2.24
Salmonella sp.	18.0±0.04	37.55±0.44	8.0±0.20	nd	nd	nd	23±0.40	nd	22.5±0.04
Enterobacter sp.	18.0±0.04	39.25±0.2	14.5±0.40	nd	nd	nd	19±0.24	nd	19.16±1.02
K. pneumoniae	19.4±2.16	35.15±0.12	11.86±0.04	nd	nd	nd	18±0.25	nd	12.5±0.20
S. marcescens	18.0±0.04	35.15±0.12	11.0±0.40	nd	nd	nd	17±0.12	nd	11.16±1.02

Values are represented as mean ± S.E. of triplicate experiments. nd: no detection, [a] Diameter of inhibition zone including diameter of disc 6 mm (tested at a volume of 10 µl disc^{-1}), [b] Diameter of inhibition zone including diameter of disc 6 mm (tested at a volume of 1000µg disc^{-1})

samples were examined including hand rinsing children (partially breastfed children and exclusively breastfed children) and their mothers (partially breast fed mother, exclusively breast fed mother), feeding utensils, leftover foods and drinking water. The total isolation rate of *E. coli*, *S. dysenteriae*, *Salmonella* sp., *Enterobacter* sp., *K. pneumoniae* and *Serratia marcescens* from collected samples was found to be 38.01, 23.64, 18.84, 9.26, 6.70 and 3.51%, respectively (Table 3). The highest isolation rate of *E. coli*, *S. dysenteriae* and *Samonella* sp., *Enterobacter* sp., *K. pneumoniae*, and *S. marcescens* were noticed in leftover food, PBM, EBC and their mother, EBC, and feeding utensils, respectively. On the other hand, lowest isolation rates of *E. coli*, *S. dysenteriae* and *Samonella* sp. were observed in FU, DW, LF, respectively, whereas *Enterobacter* sp., *K. pneumoniae* and *S. marcescens* were completely absent in LF, HRM, and HRC, EBM and DW. The higher isolation rate of *E. coli* and *S. dysenteriae* was found in PBC in comparison to EBC, whereas the higher isolation rate of *Samonella* sp., *K. pneumoniae* and *Enterobacter* sp. were noticed in EBC than PBC. On the other hand, the higher percentage distribution of *E. coli*, *S. dysenteriae*, *Samonella* sp. was observed in PBM in comparison to EBM, whereas the higher isolation rate of *Enterobacter* sp. was found in EBM than the PBM.

Antibacterial activity: The *in vitro* antibacterial activity of various leaf extracts of *M. oleifera* against the employed bacteria were qualitatively assessed by the presence or absence of inhibition zones. Based on the result shown in Table 4, methanol, ethyl acetate, chloroform and hexane extracts of *M. oleifera* leaf revealed a promising antibacterial activity against all the isolates of coliform bacteria. The methanol extract exhibited a great potential of antibacterial activity against all the tested bacteria such as *E. coli*, *S. dysenteriae*, *Salmonella* sp, *Enterobacter* sp., *K. pneumoniae*, and *S. marcescens* with their respective zones of inhibition of 21.2, 19.1, 23.2, 19.5, 18.4 and 17.0 mm. The ethyl acetate extract also showed strong antibacterial effect against all the coliform isolates with their inhibition zones ranging from 13.2 and 20.2 mm. Chloroform extract exerted moderate antibacterial activity against all the coliform isolates (inhibition zones: 12.1 to 15.5 mm), while hexane extract had moderate to low antibacterial effect (inhibition zones: 8.0 to 14.5 mm). The activity of the extracts was compared with standard antibiotic tetracycline. In this study, in all cases, methanol and ethyl acetate extracts exhibited higher antibacterial activity as compared to reference antibiotic tetracycline. The blind control did not inhibit the growth of any of the bacteria tested.

Minimum inhibitory concentration (MIC): As shown in Table 5, the methanol extract of *M. oleifera* leaf found more susceptible to all tested bacteria such as *E. coli*, *S. dysenteriae*, *Salmonella* sp., *Enterobacter* sp., *K. pneumoniae*, and *S. marcescens* with their MIC values ranging from 62.5 to 250 µg/mL. The MIC values of ethyl acetate extract against the employed isolates of coliform bacteria were recorded in the range of 62.5-500 µg/mL. On the other hand, chloroform and hexane extract showed inhibitory activity against all the tested bacteria as MIC values ranging from 250 to 1000 µg/mL. In this study, methanol extract showed the highest antibacterial activity against the tested isolates as compared to other extracts by MIC values.

DISCUSSION

In this study, the presence of coliform bacteria in collected samples was an indication of bacterial contamination as a potential source of diarrhea pathogens. Incidence of diarrhea resulted from epidemic of coliform bacterial transmission. The percentage distribution of coliform bacterial species show that *E.*

Table-5: Minimum inhibitory concentration of *Moringa oleifera* L. leaves extracts

Bacteria	Minimum inhibitory concentration (MIC)							
	Fresh leaf sap		Aqueous extract (mgml^{1})				Ethanol extract (µg ml^{1})	
	Liquid juice (µl/disc)	Powdered from fresh leaf juice (µg ml^{1})	Fresh leaf		Dried leaf		Fresh Leaf	Dried Leaf
			Cold	Hot	Cold	Hot		
E. coli	1.25	195	25	nd	nd	nd	390	nd
S. dysenteriae	2.5	390	50	nd	nd	nd	780	nd
Salmonella sp.	1.25	195	25	nd	nd	nd	390	nd
Enterobacter sp.	1.25	195	25	nd	nd	nd	390	nd
K. pneumoniae	2.5	390	50	nd	nd	nd	780	nd
S. marcescens	2.5	390	50	nd	nd	nd	780	nd

nd: no detection.

coli is one of the major indicators of coliform bacterial contamination while *S. dysenteriae* and *Salmonella* sp. are moderate could be used for searching for the sources of potential contamination of infants and their foods by diarrhea pathogens. Pinfold [21] discussed the advantages of *E. coli* as an indicator of faeco-oral contamination and disease. This is because during weaning infants are being exposed to food-borne germs for the first time and they are losing the protection of breast milk which has anti-infective properties. High levels of contamination are often found in animal milk and traditional weaning foods, especially cereal gruels. *E. coli*, which causes at least 25 percent of all diarrheas in developing countries, is commonly found in weaning food. Leftover foods have a strikingly high rate of *E. coli* contamination, 31 out of the 59 samples (52.5%), whereas drinking water are more polluted by *E. coli*, 25 out of the 59 samples (42.4%). The contamination of LF and DW by *S. dysenteriae* were 11 out of the 59 samples (18.6%) and 10 of the 59 samples (16.9%), respectively, that were least in the comparison of other samples positive for *S. dysenteriae*. The reason was that storing food at room temperature caused bacteria in the food to multiply rapidly. Infant-rearing practices, more particularly infant-feeding habits were similar throughout the families. Early complementary feeding of infants was also common. Safe preparation and storage of food is crucial to good health. Up to 70.0% of all diarrhea episodes are caused by germs that can be carried in food and swallowed. It is estimated that improvements in food hygiene could decrease the incidence of diarrhea between 15 and 70.0%. Drinking water resulted significant rate of *E. coli* contamination at a rate of 42.4% after the leftover food contamination by *E. coli*. *Salmonella* sp., *S. dysenteriae*, *Enterobacter* sp. and *K. pneumoniae* contaminated drinking water at a rate of 22.03, 16.95, 11.86, and 6.7%, respectively. The reason was that the water sources were from tube well for drinking and cooking, pond for bathing and washing utensils and clothes among the studied families. Mother's hands exhibited higher rate of *E. coli*, *S. dysenteriae* and *Salmonella* sp. contamination at a rate of 39.03, 30.46, and 28.7% than their children at a rate of 28.33, 26.17, and 19.4%, respectively, whether exclusively breastfed or receiving complementary feeding both suffer from similarly defective personal hygiene and lack of environmental sanitation. Mother's hands also showed *Enterobacter* sp. and *K. pneumoniae* contamination at a rate of 10.74 and 0%, whereas children exhibited 13.30 and 4.2%, respectively. On the contrary, *S. marcescens* contaminated mother's hands at a rate of 1.3% only but not children. Mothers' hands are important sources of coliform contamination than the infants as food can be contaminated through contact with dirty hands of food handlers. This gain emphasizes the importance personal hygiene, particularly frequent hand washing of mothers and their infants. The similar view was reflected in earlier studies.[22, 23] Partially breast-fed children are at high risk of *E. coli* and *S. dysenteriae* contamination than the exclusively breast-fed children. The reason is that breast milk is the ideal food for infants as it remains a major source of nutrients meets the full term infant's complete nutritional needs and has low risk of bacterial contamination.[24] The immunological benefits of breastfeeding are reduces the risk of infectious diseases such as gastroenteritis, respiratory infections and ear infections because maternal antibodies are passed to the infant [24] and also reduces the risk of food allergy and the risk of sudden infant death syndrome (SIDS).[25] Not doubt it, feeding utensils are also contaminated by *E. coli*, *S. dysenteriae*, *Salmonella* sp. and *S. marcescens*. Feeding bottles and rubber teats, which are particularly difficult

to clean, are often breeding grounds for germs. Our findings suggest that mothers are willing to practice prolonged exclusive breast feeding, washing of hands, using of water from a safe source, feeding gruel with a cup and spoon rather than a bottle and giving infants leftover foods should be discouraged and infant feeding with freshly prepared food encouraged. Mathur and Reddy [25] reported to the same conclusion. It is important for health workers to work with local communities to identify and encourage safe weaning practices and to improve infants' nutrition to increase their resistance to infections such as diarrhea.[26] The heavy pollution of leftover food, drinks and utensils might, at least in part, explain that in our study community the risk of diarrhea is two times higher in partially breastfed babies than in exclusive breastfed ones. Still, one should not forget that the root causes are crushing poverty and as one of its many consequences, maternal illiteracy. In conclusion, our study showed the heavy exposure of infants to coliform bacterial contamination by their mothers and by other sources in rural area. The growing resistance of microorganisms to convectional antimicrobial agents is a source of concern to clinical microbiologists all over the world. As a result, efforts are being made to develop antimicrobial agents from local sources for better chemotherapeutic effects. The demand for more natural antimicrobials has driven scientists to investigate the effectiveness of inhibitory compounds such as extracts from plants.[27] Various publications have documented the antimicrobial activities of plant extracts.[28, 29] Thus plant extracts are promising natural antimicrobial agents with potential applications in pharmaceutical industry for controlling the pathogenic bacteria. Most antibacterial medicinal plants attack Gram-positive strains, while few are active against Gram-negative bacteria.[30, 31] In this study, we found that methanol, ethyl acetate, chloroform and hexane extracts of *M. oleifera* leaf were active against all the isolated Gram-negative bacteria tested. In comparison with commercial antibiotics, the significant antibacterial activity was observed from most of the extracts. The antimicrobial activity of seeds of *M. oleifera* has been investigated.[32] Also, the antibiotic principles of *M. oleifera* have been isolated by others.[33, 34]

The results of this study suggest that *M. oleifera* leaf extracts possess potential antibacterial activity against coliform bacteria and this activity is highly comparable with the commercial antibiotic tetracycline. Some pharmacological activities of aqueous and ethanol extract of *M. oleifera* root-wood on calcium oxalate urolithiasis in male Wistar albino rats have been reported earlier.[35] Thus, *M. oleifera* could become an alternative to synthetic bactericides for using in pharmaceutical industry to control some coliform bacteria. Our further study will focus on the purification of the active ingredients from the plant and observe their effects in animal model after and before pathogen infections to clarify whether those active ingredients are preventive or treatable medicine, find the toxicity level whether they could be any use for the babies.

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