

ANTIDIARRHOEAL POTENTIALITY OF LEAF EXTRACTS OF *MORINGA OLEIFERA*

Sushmita Choudhury^{1*}, Latika Sharan² and M. P. Sinha¹

¹ University Department of Zoology, Ranchi University Ranchi, Jharkhand-834008, INDIA.

² Department of Botany, Ranchi Women's College, Ranchi, Jharkhand-834001, INDIA.

ABSTRACT

Aims: The present study aims to evaluate the antidiarrhoeal activity of aqueous and methanolic extracts from *M. oleifera* leaves by evaluating its potential as antibacterial (against pathogens like *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Vibrio cholerae*), antisecretory and antipropulsive agent.

Place and Duration of Study: Department of Zoology, Ranchi University, Ranchi, between January 2012-July 2012

Methodology: Plant extraction, antibacterial analysis and phytochemical analysis were done. Albino wistar rats (150-230g) of either sex were used. The antidiarrhoeal effects were investigated.

Results: The result showed that, the methanolic and aqueous extracts exhibited high activity against the common gastrointestinal pathogens. The minimum inhibitory concentration (MIC) of the methanolic extract was in the range 4-64 mg/mL with *S. typhi* being the most susceptible showing a zone of inhibition (ZOI) of 5mm at 2mg/mL. The phytochemical analysis carried out revealed the presence of carbohydrates, proteins, oils, lipids, glycosides, alkaloids, triterpenoids and steroids and absence of tannins, flavanoids and saponins. The weight and volume of intestinal content induced by castor oil were studied by enteropooling method. Standard drug atropine (3mg/kg, i.p) showed significant reductions in fecal output and frequency of droppings whereas the methanolic and aqueous extracts of *Moringa oleifera* at the dose of 100 mg/kg i.p significantly retarded the castor-oil induced enteropooling and intestinal transit. The gastrointestinal transit rate was expressed as the percentage of the longest distance travelled by the charcoal (marker) divided by the total length of the small intestine. *Moringa oleifera* at the dose of 100 mg/kg significantly inhibited ($P<0.001$) weight and volume of intestinal content.

Conclusion: The extracts of *M.oleifera* showed marked reduction in the number of diarrhoea stools and the reduction in the weight and volume of the intestinal contents, as well as a modest reduction in intestinal transit. The results supports the medicinal usage of the methanolic leaf extracts of *Moringa oleifera* and suggests that the plant extracts possess compounds with antibacterial properties that can be used as antimicrobial agents.

Keywords: *Moringa oleifera*, antidiarrhoeal, antipropulsive, antisecretory.

* Tel.: +91 9431360645.

E-mail address: sushmita.choudhury7683@gmail.com.

1. INTRODUCTION

In the last few years there has been an exponential growth in the field of herbal medicine and these drugs are gaining popularity both in developing and developed countries because of their natural origin and less side effects. Many traditional medicines in use are derived from medicinal plants, minerals and organic matter [1]. A number of medicinal plants, traditionally used for over 1000 years named “rasayana” are present in herbal preparations of Indian traditional health care systems [2]. In Indian systems of medicine most practitioners formulate and dispense their own recipes [3].

Diarrhoea is characterized as rapid movement of faecal matter through intestine resulting in poor absorption of water, nutritive elements and electrolytes producing abnormal frequent evacuation of watery stools. It is the one of the most common cause of morbidity and mortality in many developing countries effecting mainly the infants and children [4]. It is often caused by enterotoxins which are produced by bacteria such as *Escherichia coli*, *Salmonella typhi*, *Salmonella typhimurium*, *Clostridium difficile*, *Clostridium freundii*, *Aeromonas hydrophila*, *Campylobacter jejuni* and *Vibrio cholerae*, to name a few [5]. These bacteria cause the influx of water and ions to the intestinal lumen and thus increase the intestinal motility, thereby causing watery stools. Such secretory diarrhoea is treated by the administration of oral rehydration salts to reduce the loss of essential electrolytes and maintain the body fluids osmolality [5]. Thus diarrhoea is caused due to toxicity of bacteria and even other chemicals either cause hyper secretion in lumen and/or hyper propulsive activity. *Moringa* leaves have been extensively studied pharmacologically and it has been found that the ethanol extract and its constituents exhibit antispasmodic effects possibly through calcium channel blockade [6,7]. The antispasmodic activity of the ethanol extract of *M. oleifera* leaves has been attributed to the presence of 4-[α -[L-rhamnosyloxy] benzyl]-o-methyl thiocarbamate [trans][6].

Moreover, spasmolytic activity exhibited by different constituents provides pharmacological basis for the traditional uses of this plant in gastrointestinal motility disorder [8]. The present study aims to evaluate the antidiarrhoeal activity of *M. oleifera* leaf extracts by evaluating its potential as antibacterial (against pathogens like *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Vibrio cholerae*), antisecretory and antipropulsive agent and a natural source of treatment.

2. MATERIAL AND METHODS

2.1 Collection of plant material: The fresh and tender leaves were collected, dried in a shade under $28\pm 2^\circ\text{C}$ (for six to seven days and then crushed into coarse powdery substance by using electric grinder. The coarse powdery substance was dried again and was then sieved to get fine powder using the fine plastic sieve, which was then stored in an air tight bottle in the laboratory until required.

2.2 Extract preparation: 50 g of the sieved powder was weighed accurately and subjected to extraction in a soxhlet apparatus at room temperature using ~350 mL methanol and distilled water separately. The extracts obtained were filtered, concentrated in rotary flash evaporator and maintained at 45°C the percentage yield of each extract were calculated and the dried extracts were stored in air tight containers at room temperature for further studies.

2.3 Phytochemical analysis: Freshly prepared extracts of the powdered leaves were subjected to phytochemical analyses to find the presence of the following phyto constituents

67 such as flavanoids, alkaloids, carbohydrates, glycosides, tannins, saponins, steroids,
68 proteins, lipids, oils by standard methods [9, 10].

69 2.4 Anti-Bacterial analysis

70 **2.4.1 Test Microorganisms:** The organisms namely *Staphylococcus aureus*, *Pseudomonas*
71 *aeruginosa*, *Escherichia coli*, *Salmonella typhi*, *Vibrio cholerae* used during the present
72 experiment were procured from Hi-media Laboratories (Mumbai, India) which are potential
73 causative pathogen for different diseases.

74 **2.4.2 Concentrations screened:** 0.0625, 0.125, 0.25, 0.5, 1.0 and 2.0 mg for agar diffusion
75 method and for broth dilution method up to 64 mg/mL concentrations were used according to
76 the sensitivity of samples.

77 **2.4.3 Agar diffusion method:** Media Used: Peptone-10 g, NaCl-10g and Yeast extract 5g,
78 Agar 20g in 1000 mL of distilled water. Initially, the stock cultures of bacteria were revived by
79 inoculating in broth media and grown at 37°C for 18 h. The agar plates of the above media
80 were prepared and wells were made in the plate. Each plate was inoculated with 18 h old
81 cultures (100 µL, 104 cfu) and spread evenly on the plate. After 20 min, the wells were filled
82 with different concentrations of samples. The positive control wells were filled with
83 Gentamycin along with solvent. All the plates were incubated at 37°C for 24 h and the
84 diameter of inhibition zones were noted.

85 **2.4.4 Broth dilution method:** Media Used: Peptone-10 g, NaCl-10g and Yeast extract 5g, in
86 1000 ml of distilled water. Initially, the stock cultures of bacteria were revived by inoculating
87 in broth media and grown at 37°C for 18 h. The tubes containing above media were
88 prepared, autoclaved and respective concentrations of the samples were added. Each tube
89 was inoculated with 18 h old cultures (100 µL, 104 cfu). A control tube with inoculums and
90 without any sample was prepared along with a sterile media tube as blank. All the tubes
91 were incubated at 37°C on a shaker with 140 rpm for 24 h and the growth was measured at
92 660 nm. The % of inhibition was calculated by using the formula below and the minimum
93 inhibitory concentration (MIC- the minimum concentration at which the test organism gets
94 100% inhibited) was depicted graphically.

95

96

97 % inhibition = 100 -

98

99

100

$$\left[\frac{\text{OD of culture with sample (test)}}{\text{OD of culture with sample (Control)}} \times 100 \right]$$

101 **2.5 Animals used:** Albino wistar rats (150-230g) of either sex were used. The animals were
102 maintained in a well-ventilated room with 12:12 hour light/dark cycle in polypropylene cages.
103 The animals were fed with standard pellet feed and water was given ad libitum. Ethical
104 committee clearance (process no: 46, page no.137) was obtained from the department of
105 Zoology, Ranchi University, Ranchi.

106 **2.6 Castor oil-induced diarrhea:** Male wistar rats were divided into four groups of six
107 animals each; diarrhoea was induced by administering 1 mL of castor oil orally to rats. Group
108 I treated as control (2 mL/kg, i.p. saline), group II received atropine (3mg/kg, i.p.) served as
109 standard and group III and IV received *M. oleifera* extracts (extracts were concentrated to
110 meet the 100mg/kg body weight dose, based on MIC for the bacteria was given) 1 h before
111 castor oil administration. The number of both wet and dry diarrhoeal droppings were counted
112 every hour for a period of 4 h mean of the stools passed by the treated groups were
113 compared with that of the positive control group consisted of animals given an intraperitoneal
114 injection of saline (2mL/kg, i.p) [11]

115 **2.7 Castor oil-induced enteropooling:** Intraluminal fluid accumulation was determined by
116 the method of Robert et al., [12]. Overnight fasted rats were divided into four groups of six
117 animals each. Group I received normal saline (2 ml/kg, i.p.) served as a control, group II
118 received atropine (3mg/kg, i.p.) and groups III and IV received *M. oleifera* extracts
119 intraperitoneally respectively 1h before the oral administration of castor oil. Two hours later
120 the rats were sacrificed, the small intestine was removed after tying the ends with thread and
121 weighed. The intestinal contents were collected by milking into a graduated tube and their
122 volume was measured. The intestine was reweighed and the difference between full and
123 empty intestines was calculated.

124 **2.8 Small intestinal transit :** Rats were fasted for 18 h divided into five groups of six
125 animals each, Group I received 2 ml normal saline orally, group II received 2 ml of castor oil
126 orally with saline 2 ml/kg intraperitoneally, group III received atropine (3 mg/kg, i.p.), group
127 IV and V received *M. oleifera* intraperitoneally respectively, 1h before administration of
128 castor oil. One ml of marker (10% charcoal suspension in 5% gum acacia) was administered
129 orally 1 h after castor oil treatment. The rats were sacrificed after 1h and the distance
130 traveled by charcoal meal from the pylorus was measured and expressed as percentage of
131 the total length of the intestine from the pylorus to caecum [13].

132 133 **3. RESULTS AND DISCUSSION**

134
135 Anti-dysentric and antidiarrhoeal properties of medicinal plants were found to be due to
136 tannins, alkaloids, saponins, flavonoids, sterols and/or triterpenes and reducing sugars [14,
137 15, 16, 17, 18]. Fresh leaf juice and aqueous extract from the seeds of *M. oleifera* were
138 found to inhibit the growth of *P. aeruginosa*, *S. aureus* and *B. subtilis*. A compound 4 (α -L-
139 rhamnosyloxy) benzylisothiocyanate, isolated from the seeds and roots is reported to act on
140 several bacteria and fungi [19, 20]. The roots as well as ethanol extract of the leaves of *M.*
141 *oleifera* showed antispasmodic action, possibly through calcium channel blockade.
142 Spasmolytic activity exhibited by the constituents of the plant provides a scientific basis for
143 the traditional uses of the plant in gastrointestinal motility disorders [6, 7]. There are several
144 reports on the antimicrobial activity of different herbal extracts in different regions of the
145 world [21]. The constituents listed above may mediate the anti-diarrhoeal property of *M.*
146 *oleifera* which could make the plant useful for treating different ailments and having a
147 potential of providing useful and safe drugs and drug leads for human use.

148 The antimicrobial efficacy of the extracts of *M. oleifera* leaves were quantitatively assessed
149 on the basis of inhibition zone (in mm) and minimum inhibitory concentration(MIC- the
150 minimum concentration at which the test organism gets 100% inhibited). The results are
151 shown in Table.1 and 2. The test organisms were also inoculated with pure antibiotics-
152 Gentamycin to compare the efficacy of leaf extract for their antimicrobial properties and the
153 results are shown in Table.3.
154

155 **Table.1: Zone of inhibition (in mm) of methanolic leaf extracts of *M. oleifera***

156	Concentration(mg/mL)	<i>E.coli</i>	<i>S.aureus</i>	<i>P.aeruginosa</i>	<i>V. cholerae</i>	<i>S.typhi</i>
157	0.125	-	-	-	-	-
158	0.25	-	-	-	-	-
159	0.50	-	-	-	-	-
160	1	-	-	-	-	-
161	2	-	-	-	-	5
162	4	3	-	3	-	8
163	MIC (mg/mL)	4	~*	4	~*	2
164	* = no zone of inhibition found					

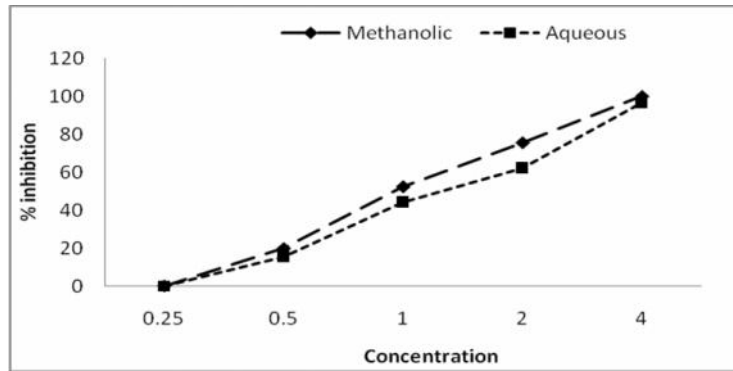
165 **Table.2: Zone of inhibition (in mm) of aqueous leaf extracts of *M. oleifera***

166	Concentration(mg/mL)	<i>E.coli</i>	<i>S.aureus</i>	<i>P.aeruginosa</i>	<i>V. cholerae</i>	<i>S.typhi</i>
167	0.125	-	-	-	-	-
168	0.25	-	-	-	-	-
169	0.50	-	-	-	-	-
170	1	-	-	-	-	-
171	2	-	-	-	-	3
172	4	2	-	2	-	6
173	MIC (mg/mL)	4	~*	4	~*	2
174	* = no zone of inhibition found					

175 **Table.3: MIC of Gentamycin against the test organisms**

176	Microorganisms	MIC(μG/ML)	ZOI(MM)
177	<i>E.coli</i>	25	18
178	<i>S.aureus</i>	100	1
179	<i>P.aeruginosa</i>	25	13
180	<i>V. cholerae</i>	25	2
181	<i>S.typhi</i>	25	13

184 In the present investigation the extracts were found to be effective against all the pathogens.
 185 When the above pathogens were screened by agar disc diffusion method the zone of
 186 inhibition (ZOI) observed for the methanolic and aqueous extracts were in the range 2-8mm
 187 at 2-4mg/mL concentration. *S.typhi* was found to be highly susceptible as it showed an
 188 inhibition zone of 5mm and 3mm at 2mg/mL concentration whereas *E.coli*, *P. aeruginosa*
 189 and *V.cholerae* were comparatively less sensitive by showing 3mm and 2mm ZOI at 4mg/mL
 190 concentration. *S.aureus* did not show any zone of inhibition reflecting their insensitiveness
 191 towards the methanolic as well as aqueous extract of the leaf. The broth dilution method
 192 showed more pronounced antimicrobial activity through 100% inhibition for all the pathogens
 193 in the range of 0.25-64mg/mL concentration. The methanolic extract proved to be more
 194 effective than the aqueous extract. The inhibitory impact of methanolic extract was
 195 significantly ($p>0.001$) higher than the aqueous extract. The MIC for *S.typhi* was 2mg/mL
 196 (Fig.1) and for *E.coli* and *P.aeruginosa* it was 4 mg/mL(Fig.2 and 3) where as for *V.cholerae*
 197 and *S.aureus* it was 32mg/mL (Fig.4 and 5).

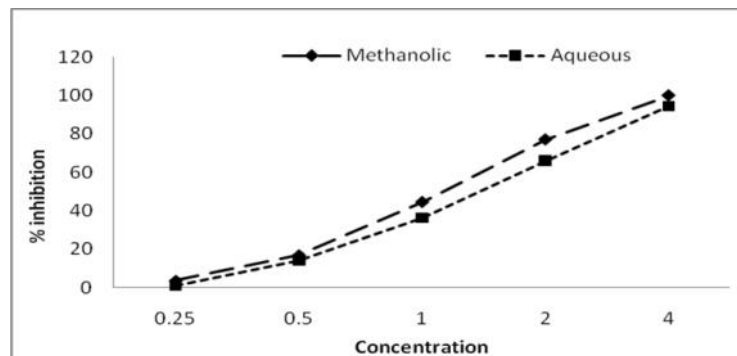


198

199 **Fig.1: Inhibition (%) of *E.coli* by methanolic and aqueous extracts of *M. oleifera* in**
 200 **broth medium.**

201

202

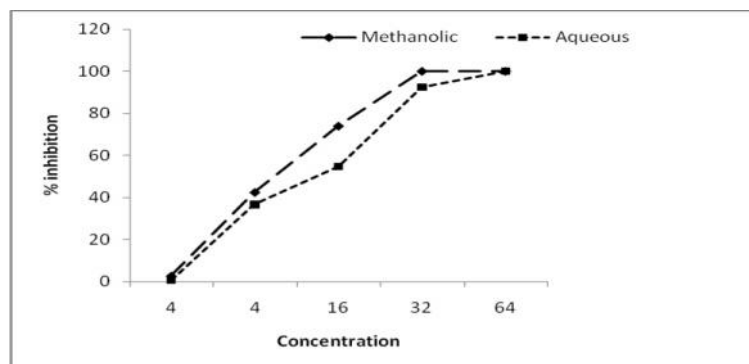


203

204 **Fig.2: Inhibition (%) of *P.aeruginosa* by methanolic and aqueous extracts of *M.***
 205 ***oleifera* in broth medium.**

206

207



208

209 **Fig.3: Inhibition (%) of *S.aureus* by methanolic and aqueous extracts of *M. oleifera* in**
 210 **broth medium.**

211

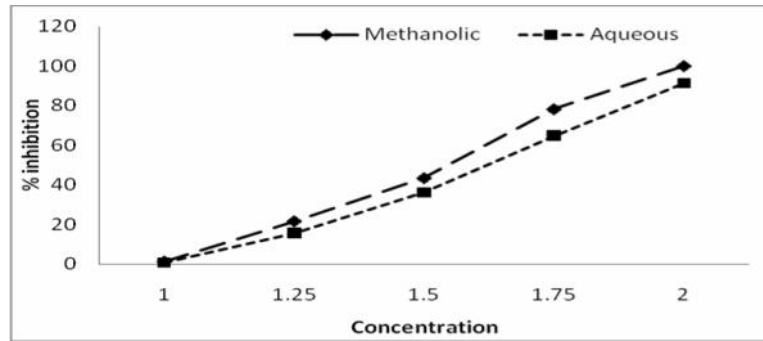


Fig.4: Inhibition (%) of *S.typhi* by methanolic and aqueous extracts of *M. oleifera* in broth medium.

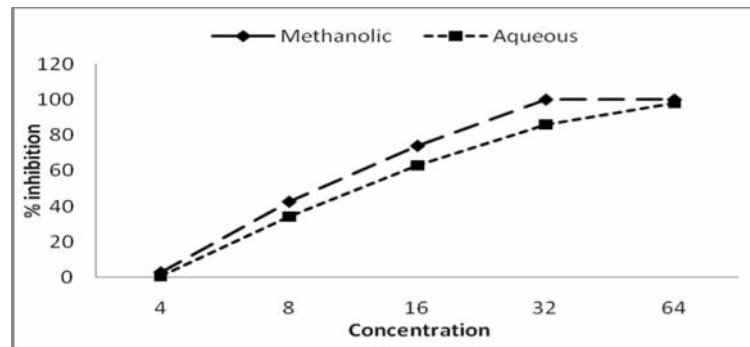


Fig.5: Inhibition (%) of *V.cholerae* by methanolic and aqueous extracts of *M. oleifera* in broth medium.

3.1 CASTOR OIL-INDUCED DIARRHOEA

30 min after administration of castor oil the diarrhoea was clinically apparent in all the animals of control group, for the next 4 hour. This was markedly reduced by the intraperitoneal injection of atropine, 3 mg/kg (83.75%). A similar marked reduction in the number of defecations over four hours was achieved with methanolic and aqueous extracts of *M. oleifera* at the dose of 100 mg/kg i.p. *M. oleifera* significantly inhibited the defecation (49.90% and 56.76%). *M. oleifera* extracts delayed the onset of diarrhoea and only 30% of animals showed diarrhoea at first hour ($p < 0.001$) (Table 4).

3.2 CASTOR OIL-INDUCED ENTEROPOOLING

Castor oil caused accumulation of water and electrolytes in intestinal loop. Castor oil-induced enteropooling is not influenced by atropine in rats at the dose of 3 mg/kg, i.p. *M. oleifera* produced a dose-dependent reduction in intestinal weight and volume. *M. oleifera* dose produced 51.08% (methanolic) and 47.98% (aqueous) inhibition of volume of intestinal content respectively with significance ($p < 0.001$). The weight of intestinal content was also reduced significantly at both the doses (Table 5).

3.3 SMALL INTESTINAL TRANSIT

The percent intestinal transit was increased with castor oil (88.37%), but it was reduced in both extracts, and much more markedly by atropine (55.09%). Methanolic extract of *M. oleifera* produced 62.64% intestinal transit induced by castor oil. Whereas, aqueous extract produced 61.44% of castor oil induced charcoal meal transit (Table 6).

242 **Table- 4: Effect of *M. oleifera* on castor oil-induced diarrhoea in rats.**

243	Group	Treatment	Mean defecation in 4hr	%inhibition of defecation
244	I	Castor oil+saline	14.65±0.67	-----
245	II	Castor oil+atropine	3.45±0.34*	83.75
246	III	Methanolic extract of <i>M. oleifera</i>	6.92±0.81*	49.90
247	IV	Aqueous extract of <i>M. oleifera</i>	7.46±0.45*	56.76

248 Values are expressed as mean ± SEM from the experiments.,n=6 *p<0.001 when compared
249 with Castor oil + saline-treatedgroup
250

251 **Table- 5: Effect of *M. oleifera* on castor oil-induced enteropooling in rats**

252	Group	Treatment	Weight of intestinal content	%inhibition of weight of intestinal content
253	I	Castor oil+saline	2.65±0.087	-----
254	II	Castor oil+atropine	1.21±0.64*	44.56
255	III	Methanolic extract of <i>M. oleifera</i>	1.49±0.08*	51.08
256	IV	Aqueous extract of <i>M. oleifera</i>	1.34±0.04*	47.98

258 Values are expressed as mean ± SEM from the experiments.,n=6 *p<0.001 when compared
259 with Castor oil + saline-treatedgroup
260

261 **Table- 6: Effect of *M. oleifera* on castor oil-induced small intestinal transit in rats**

262	Group	Treatment	Total length of intestine(cm)	Distance travelled by marker	% Intestinal transit
263	I	Castor oil+saline	44.20±1.24	39.06±2.26	88.37%
264	II	Castor oil+atropine	48.46±1.07	26.70±2.59*	55.09%
265	III	Methanolic extract of <i>M. oleifera</i>	45.75±1.27	28.66±2.90*	62.64%
266	IV	Aqueous extract of <i>M. oleifera</i>	48.34±1.33	29.87±1.97*	61.24%

268 Values are expressed as mean ± SEM from the experiments.,n=6 *p<0.001 when compared
269 with Castor oil + saline-treatedgroup.
270

271 The results show that *M. oleifera* produced a significant reduction (p<0.001) in the severity
272 and frequency of diarrhoea produced by castor oil. It is also noted that *M. oleifera*
273 significantly inhibited castor oil induced intestinal fluid accumulation and the volume of
274 intestinal content, dose dependently more than atropine. The *M. oleifera* significantly
275 reduced the castor oil induced intestinal transit. In a similar study the effect of aqueous and
276 methanolic plant extracts of *Acorus calamus* rhizome, *Pongamia glabra* leaves, *Aegle*
277 *marmelos* unripe fruit and *Strychnos nux-vomica* root bark for their antidiarrhoeal potential
278 against castor-oil induced diarrhoea in mice were evaluated and they reported significant
279 reduction in watery stools and total weight of faeces [22]. The **methanolic leaf extract of**
280 ***M.oleifera* is more effective than aqueous leaf extract against** castor-oil induced diarrhoea. In
281 the present study the methanolic plant extracts significantly reduced induction time of
282 diarrhoea and total weight of the faeces and the result obtained establish the efficacy of
283 these plant extracts as antidiarrhoeal agents.
284

285

286

287 **4. CONCLUSION**

288

289 In conclusion, the extracts of *M.oleifera* showed marked reduction in the number of
diarrhoea stools and the reduction in the weight and volume of the intestinal contents, as

well as a modest reduction in intestinal transit. Therefore, the results support the medicinal usage of the methanolic leaf extracts of *Moringa oleifera*

5. REFERENCES

1. Grover, J.K., Yadav, S., and Vats, V.: Medicinal plants of India with antidiabetic potential. J. Ethnopharmacol., 2002; 81: 81–100.
2. Scartezzini, P. and Sproni, E.: Review on some plants of Indian traditional medicine with antioxidant activity. J. Ethnopharmacol., 2000; 71: 23–43.
3. Seth, S.D. and Sharma, B.: Medicinal plants of India. Indian J. Med. Res., 2004; 120: 9–11.
4. Fernando C, Ramon A, Halley P. Effect of plants used in Mexico to treat gastrointestinal disorders on charcoal gum acacia induced hyperperistalsis in rats. J. Ethnopharmacol. 2010; 28:49-51.
5. Horn R, Perry A and Robinson S. A simple solution Time, 2006 : 42-47.
6. Gilani AH, Aftab K, Shaheen F et al. Antispasmodic activity of active principle from *Moringa oleifera*. In Natural Drugs and the Digestive Tract, Capasso F, Mascolo N [eds]. EMSI: Rome, 1992; 60–63.
7. Dangi SY, Jolly CI, Narayana S. Antihypertensive activity of the total alkaloids from the leaves of *Moringa oleifera*. Pharm Biol 2002; 40: 144–148.
8. Gilani AH, Aftab K, Suria A et al. Pharmacological studies on hypotensive and spasmodic activities of pure compounds from *Moringa oleifera*. Phytother Res 8: 87–91.
9. Sofowora, A. 2008. Screening Plants for Bioactive Agents. In: Medicinal Plants and Traditional Medicinal in Africa. 3rd Ed. Spectrum Books Ltd, Sunshine House, Ibadan, Nigeria, 1994; 134 p.
10. Trease, G.E. and W.C. Evans. Pharmacognosy. 15th Ed. Saunders Publishers, London. 2002; 42 p.
11. Awouters F, Niemegeers CJE, Lenaerts FM, Janseen PAJ. Delay of castor oil diarrhoea in rats; a new way to evaluate inhibitors of prostaglandin biosynthesis. Journal of Pharmacy Pharmacology. 1978; 30: 41-45.
12. Robert A, Nezamis JE, Lancaster C, Hanchar AJ, Klepper MS. Enteropooling assay, a test for diarrhea produced by prostaglandins. Prostaglandins., 1976; 11: 809-828.
13. Izzo AA, Mascolo N, Capasso R, Germano MP, DePasquel R, Capasso F. Inhibitory effect of cannabinoid agonists on gastric emptying in the rat. Archives of Pharmacology. 1999; 360: 221-223.
14. Vimala, R., S. Nagarajan, M. Alam, T. Susan and S. Joy. Anti-inflammatory and antipyretic activity of *Michelia champaca* Linn., (white variety), *Ixora brachiata* Roxb. and *Rhynchosia cana* (Willd.) D.C. flower extract. Indian. J. Exp. Biol. 1997; 35(12): 1310-1314.
15. Pampattiwar, S.P. and N.V. Advani. Evaluation of anti-diarrhoeal activity of *Ficus glomerata* in castor oil induced diarrhoea in rats. J. Sci. 2011; 1(1): 26-30.
16. Choudhury S, Sharan L., Sinha MP. Phytochemical and Antimicrobial Screening of *Psidium Guajava* L. Leaf Extracts against Clinically Important Gastrointestinal Pathogens. J. Nat. Prod Plant Resour, 2012; 2(4), 524-529.
17. Choudhury S, Sharan L and Sinha MP . Antibacterial efficacy and Phytochemistry of methanolic leaf extracts of *Mangifera indica* Linn. The Ecoscan, special issue. 2012; 1: 497-501.
18. Choudhury S, Sharan L and Sinha MP. Phytochemical and antimicrobial standardization of the methanolic leaf extracts of *Murraya koenigii* Linn. Archives des Sciences. 2013; 66(3), 67-80.
19. Caceres A, Cabrera O, Morales O, Mollinedo P, Mendia P. Pharmacological properties of *Moringa oleifera* 1: Preliminary screening of antimicrobial activity. J Ethnopharmacol. 1991; 33: 213-216.

- 342 20. Eilert U, Wolters B, Nahrstedt A. The antibiotic principle of seeds of *Moringa oleifera* and
343 *Moringa stenopetala*. Planta Med. 1981; 42, 55-61.
- 344 21. De Boer HJ, Kool A, Broberg Mziray A W, Hedberg I. Antifungal and antibacterial activity
345 of some herbal remedies from Tanzania. J. Ethnopharmacol., 2005; 96, 461-469.
- 346 22. Shoba F.G and Thomas M. Study of antidiarrhoeal activity of four medicinal plants in castor-oil
347 induced diarrhoea. J. Ethnopharmacol., 2001;76(1): 73-76.