

ANTIDIARRHOEAL POTENTIALITY OF LEAF EXTRACTS OF *MORINGA OLEIFERA*

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ABSTRACT

Aims: 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.

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

Methodology: Plant extraction, antibacterial analysis and phytochemical analysis was 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 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 in new drugs for diarrhoea as well as for therapy of infectious diseases caused by pathogens. The most active extracts can be subjected to isolation of the therapeutic antimicrobials and undergo further pharmacological evaluation

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

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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], which forms the basis for its traditional use in diarrhea[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 room temperature 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 extract obtained was filtered, concentrated after dryness in rotary flash evaporator maintained at 45°C., percentage yield of each extract was calculated and the dried extract was 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 such as flavanoids, alkaloids, carbohydrates, glycosides, tannins, saponins, steroids, proteins, lipids, oils by standard methods [9, 10].

2.4 Anti-Bacterial analysis

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

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

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

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

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92 % inhibition = 100 -

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$$\left[\begin{array}{c} \text{OD of culture with sample (test)} \\ \hline \text{OD of culture with sample (Control)} \end{array} \right] \times 100$$

2.5 Animals used: Albino wistar rats (150-230g) of either sex were used. The animals were maintained in a well-ventilated room with 12:12 hour light/dark cycle in polypropylene cages. The animals were fed with standard pellet feed and water was given ad libitum. Ethical committee clearance was obtained from the department of Zoology, Ranchi University, Ranchi.

2.6 Castor oil-induced diarrhea: Male wistar rats were divided into four groups of six animals each; diarrhea was induced by administering 1 mL of castor oil orally to rats. Group I treated as control (2 mL/kg, i.p. saline), group II received atropine (3mg/kg, i.p.) served as standard and group III and IV received *M. oleifera* (in order to feed a dose of *M. oleifera* leaf extracts at the rate 100 mg/kg body weight of rat the minimum inhibitory concentration of bacteria was found out and the extract was suitably concentrated to meet the requirement in minimum volume) 1 h before castor oil administration. The number of both wet and dry diarrhoeal droppings were counted every hour for a period of 4 h mean of the stools passed

by the treated groups were compared with that of the positive control group consisted of animals given an intraperitoneal injection of saline (2mL/kg, i.p) [11]

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

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

3. RESULTS AND DISCUSSION

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

The antimicrobial efficacy of the extracts of *M. oleifera* leaves was quantitatively assessed on the basis of inhibition zone (in mm) and the results are shown in Table.1 and 2 following the agar disc diffusion method and minimum inhibitory concentration by broth dilution method. The test organisms were also inoculated with pure antibiotics- Gentamycin to compare the efficacy of leaf extract for their antimicrobial properties and the results are shown in Table.3.

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

Concentration(mg/mL)	<i>E.coli</i>	<i>S.aureus</i>	<i>P.aeruginosa</i>	<i>V. cholerae</i>	<i>S.typhi</i>
0.125	-	-	-	-	-
0.25	-	-	-	-	-
0.50	-	-	-	-	-
1	-	-	-	-	-
2	-	-	-	-	5
4	3	NF*	3	2	8

156	MIC (mg/mL)	4	4	4	2
157	*NF-not found				

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

159	Concentration(mg/mL)	<i>E.coli</i>	<i>S.aureus</i>	<i>P.aeruginosa</i>	<i>V. cholerae</i>	<i>S.typhi</i>
160	0.125	-	-	-	-	-
161	0.25	-	-	-	-	-
162	0.50	-	-	-	-	-
163	1	-	-	-	-	-
164	2	-	-	-	-	3
165	4	2	-	2	-	6
166	MIC (mg/mL)	4	NF*	4	NF*	2
167	*NF-not found					

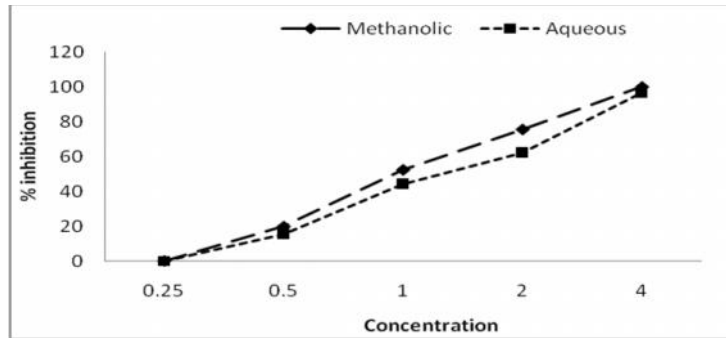
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169 **Table.3: MIC of Gentamycin against the test organisms**

170	Microorganisms	MIC(μG/ML)	ZOI(MM)
171	<i>E.coli</i>	25	18
172	<i>S.aureus</i>	100	1
173	<i>P.aeruginosa</i>	25	13
174	<i>V. cholerae</i>	25	2
175	<i>S.typhi</i>	25	13

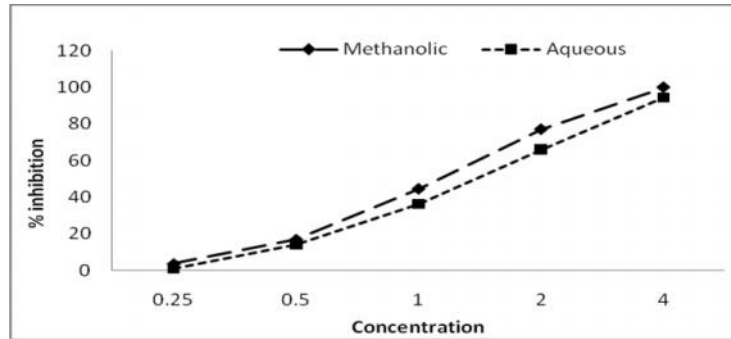
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 178 In the present investigation the extract was found to be effective against all the pathogens.
 179 When the above pathogens were screened by agar disc diffusion method the zone of
 180 inhibition (ZOI) observed for the methanolic and aqueous extract was in the range 2-8mm at
 181 2-4mg/ml concentration of the extract. *S.typhi* was found to be highly susceptible as it
 182 showed an inhibition zone of 5mm and 3mm at 2mg/mL concentration whereas *E.coli*, *P.*
 183 *aeruginosa* and *V.cholerae* were comparatively less sensitive by showing 3mm and 2mm
 184 ZOI at 4mg/mL concentration. *S.aureus* did not show any zone of inhibition reflecting their
 185 insensitiveness towards the methanolic as well as aqueous extract of the leaf.The broth
 186 dilution method of the extracts showed more pronounced antimicrobial activity through 100%
 187 inhibition for all the pathogens in the range of 0.25-64mg/mL concentration. The methanolic
 188 extract proved to be more effective than the aqueous extract. The MIC for *S.typhi* was
 189 2mg/mL (Fig.1) and for *E.coli* and *P.aeruginosa* it was 4 mg/mL(Fig.2 and 3) where as for
 190 *V.cholerae* and *S.aureus* it was 32mg/mL (Fig.4 and 5).



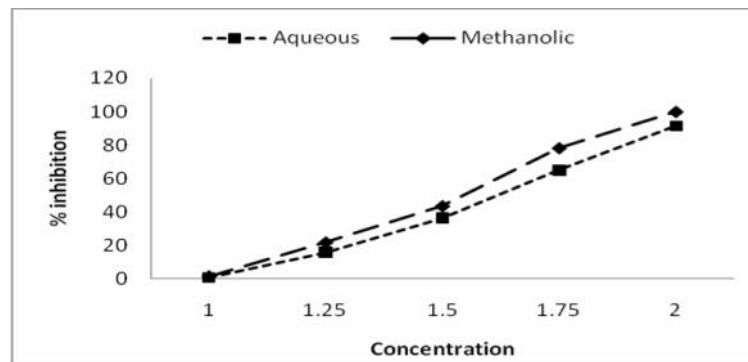
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192 **Fig.1: Inhibition (%) of *E.coli* by methanolic and aqueous extracts of *M. oleifera* in**
 193 **broth medium.**



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



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

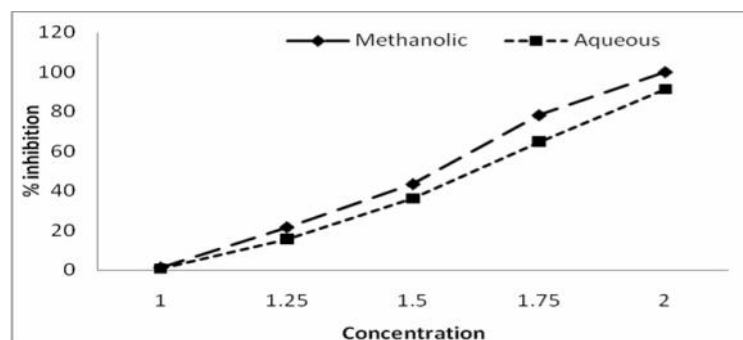


Fig.4: Inhibition (%) of *S.typhi* by methanolic and aqueous extracts of *M. oleifera* in broth medium.

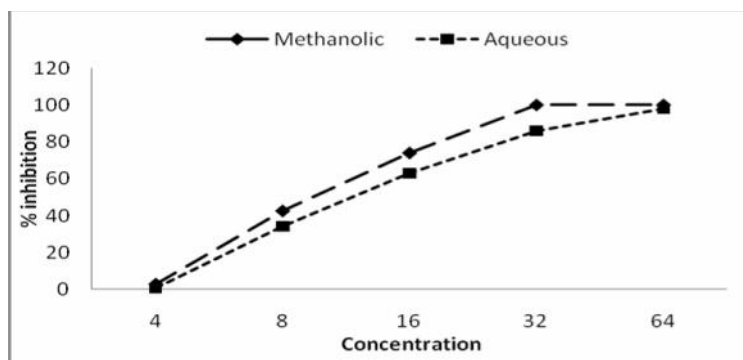


Fig.5: Inhibition (%) of *V.cholerae* by methanolic and aqueous extracts of *M. oleifera* in broth medium.

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).

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

Group	Treatment	Mean defecation in 4hr	%inhibition of defecation
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231	I	Castor oil+saline	14.65±0.67	-----
232	II	Castor oil+atropine	3.45±0.34*	83.75
233	III	Methanolic extract of <i>M. oleifera</i>	6.92±0.81*	49.90
234	IV	Aqueous extract of <i>M. oleifera</i>	7.46±0.45*	56.76
235	Values are expressed as mean ± SEM from the experiments.,n=6 *p<0.001 when compared			
236	with Castor oil + saline-treatedgroup			
237				

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

239	Group	Treatment	Weight of intestinal content	%inhibition of weight of intestinal content
240				
241	I	Castor oil+saline	2.65±0.087	-----
242	II	Castor oil+atropine	1.21±0.64*	44.56
243	III	Methanolic extract of <i>M. oleifera</i>	1.49±0.08*	51.08
244	IV	Aqueous extract of <i>M. oleifera</i>	1.34±0.04*	47.98
245	Values are expressed as mean ± SEM from the experiments.,n=6 *p<0.001 when compared			
246	with Castor oil + saline-treatedgroup			
247				

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

249	Group	Treatment	Total length of intestine(cm)	Distance travelled by marker	% Intestinal transit
250					
251	I	Castor oil+saline	44.20±1.24	39.06±2.26	88.37%
252	II	Castor oil+atropine	48.46±1.07	26.70±2.59*	55.09%
253	III	Methanolic extract of <i>M. oleifera</i>	45.75±1.27	28.66±2.90*	62.64%
254	IV	Aqueous extract of <i>M. oleifera</i>	48.34±1.33	29.87±1.97*	61.24%
255	Values are expressed as mean ± SEM from the experiments.,n=6 *p<0.001 when compared				
256	with Castor oil + saline-treatedgroup.				
257					

258 The results show that *M. oleifera* produced a significant reduction (p<0.001) in the severity
 259 and frequency of diarrhoea produced by castor oil. It is also noted that *M. oleifera*
 260 significantly inhibited castor oil induced intestinal fluid accumulation and the volume of
 261 intestinal content, dose dependently more than atropine. The *M. oleifera* significantly
 262 reduced the castor oil induced intestinal transit. In a similar study the effect of aqueous and
 263 methanolic plant extracts of *Acorus calamus* rhizome, *Pongamia glabra* leaves, *Aegle*
 264 *marmelos* unripe fruit and *Strychnos nux-vomica* root bark for their antidiarrhoeal potential
 265 against castor-oil induced diarrhoea in mice were evaluated and they reported significant
 266 reduction in watery stools and total weight of faeces [22]. The methanolic plant extracts were
 267 more effective than aqueous plant extracts against castor-oil induced diarrhoea. In the
 268 present study the methanolic plant extracts significantly reduced induction time of diarrhoea
 269 and total weight of the faeces and the result obtained establish the efficacy of these plant
 270 extracts as antidiarrhoeal agents.

272 4. CONCLUSION

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 274 In conclusion, the extracts of *M.oleifera* showed marked reduction in the number of
 275 diarrhoea stools and the reduction in the weight and volume of the intestinal contents, as
 276 well as a modest reduction in intestinal transit. The results supports the medicinal usage of
 277 the methanolic leaf extracts of *Moringa oleifera* and suggests that the plant extracts possess
 278 compounds with antibacterial properties that can be used as antimicrobial agents in new
 279 drugs for diarrhoea as well as for therapy of infectious diseases caused by pathogens. The

most active extracts can be subjected to isolation of the therapeutic antimicrobials and undergo further pharmacological evaluation.

5. REFERENCES

- Grover, J.K., Yadav, S., and Vats, V.: Medicinal plants of India with antidiabetic potential. J. Ethnopharmacol., 2002; 81: 81–100.
- Scartezzini, P. and Sproni, E.: Review on some plants of Indian traditional medicine with antioxidant activity. J. Ethnopharmacol., 2000; 71: 23–43.
- Seth, S.D. and Sharma, B.: Medicinal plants of India. Indian J. Med. Res., 2004; 120: 9–11.
- 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.
- Horn R, Perry A and Robinson S. A simple solution Time, 2006 : 42-47.
- 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.
- Dangi SY, Jolly CI, Narayana S. Antihypertensive activity of the total alkaloids from the leaves of *Moringa oleifera*. Pharm Biol 2002; 40: 144–148.
- 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.
- 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.
- Trease, G.E. and W.C. Evans. Pharmacognosy. 15th Ed. Saunders Publishers, London. 2002; 42 p.
- 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.
- Robert A, Nezamis JE, Lancaster C, Hanchar AJ, Klepper MS. Enteropooling assay, a test for diarrhea produced by prostaglandins. Prostaglandins., 1976; 11: 809-828.
- Izzo AA, Mascolo N, Capasso R, Germano MP, DePasquel R, Capasso F. Inhibitory effect of caannabinoid agonists on gastric emptying in the rat. Archives of Pharmacology. 1999; 360: 221-223.
- 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.
- 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.
- 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, 2012a; 2(4), 524-529.
- Choudhury S, Sharan L and Sinha MP . Antibacterial efficacy and Phytochemistry of methanolic leaf extracts of *Mangifera indica* Linn. The Ecoscan, special issue. 2012b; 1: 497-501.
- 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.
- 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.

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