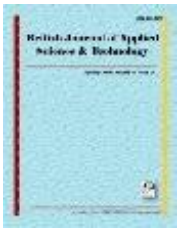

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Antidiarrhoeal Potentiality of Leaf Extracts of *Moringa oleifera*

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Authors' contributions

This work was carried out in collaboration between all authors. Author SC designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript and managed literature searches. Authors SC, LS, MPS managed the analyses of the study and literature searches. All authors read and approved the final manuscript.

Research Article

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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. Male albino wistar rats (150-230g) were used. The antidiarrhoeal effects were investigated by inducing diarrhoea with the help of castor oil.
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

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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* extracts (both methanolic and aqueous) 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.

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 that have been traditionally used for over 1000 years as “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. MATERIALS AND METHODS

2.1 Collection of Plant Material

The fresh and tender leaves were collected, dried in a shade under 28±2ºC (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 45ºC 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 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 Laboratories (Mumbai, India) 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 37ºC 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 positive control wells were filled with Gentamycin along with solvent. All the plates were incubated at 37ºC 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 37ºC 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 37ºC 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 and the minimum inhibitory concentration (MIC- the minimum concentration at which the test organism gets 100% inhibited) was depicted graphically.

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

2.5 Animals used

Male albino wistar rats (150-230g) were uesd. 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 (process no: 46, page no.137) 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; diarrheoa 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* extracts (100mg/kg bw) 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* extracts 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 of *M. oleifera* were 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 of *M. oleifera* 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 were quantitatively assessed on the basis of inhibition zone (in mm) and minimum inhibitory concentration (MIC- the minimum concentration at which the test organism gets 100% inhibited). The results are shown in Tables 1 and 2. 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)	*E.coli*	*S.aureus*	*P.aeruginosa*	*V. cholerae*	*S.typhi*
0.125	-	-	-	-	-
0.25	-	-	-	-	-
0.5	-	-	-	-	-
1	-	-	-	-	-
2	-	-	-	-	5
4	3	-	3	-	8
MIC (mg/mL)	4	-*	4	-*	2

*= *no zone of inhibition found*

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

Concentration (mg/mL)	*E.coli*	*S.aureus*	*P.aeruginosa*	*V. cholerae*	*S.typhi*
0.125	-	-	-	-	-
0.25	-	-	-	-	-
0.5	-	-	-	-	-
1	-	-	-	-	-
2	-	-	-	-	3
4	2	-	3	-	6
MIC (mg/mL)	4	-*	4	-*	2

**= no zone of inhibition found*

Table 3. MIC of Gentamycin against the test organisms

Microorganisms	MIC(µg/mL)	ZOI(mm)
E.coli	25	18
S.aureus	100	1
P.aeruginosa	25	13
V. cholerae	25	2
S. typhi	25	13

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

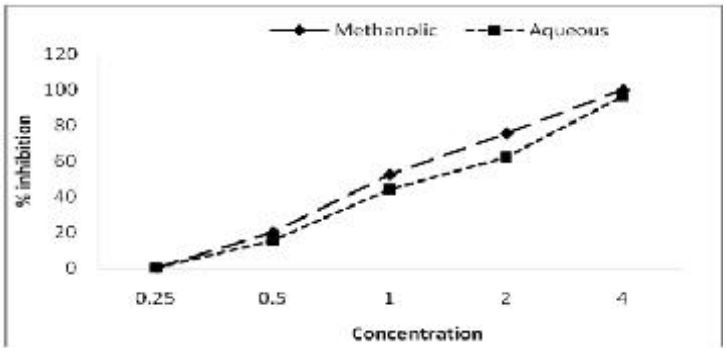

Fig. 1. Inhibition (%) of *E. coli* by methanolic and aqueous extracts of *M. oleifera* in broth medium

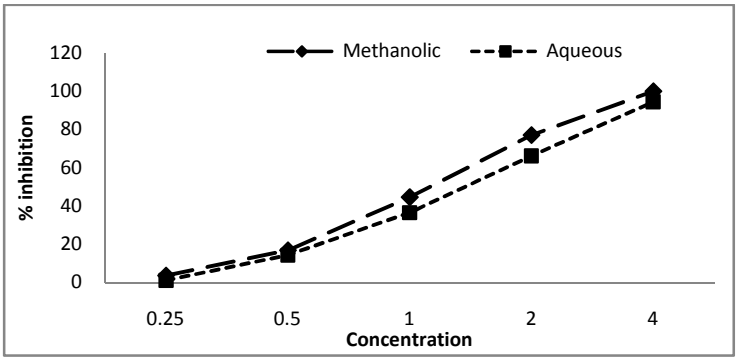

Fig. 2. Inhibition (%) of *P. aeruginosa* by methanolic and aqueous extracts of *M. oleifera* in broth medium

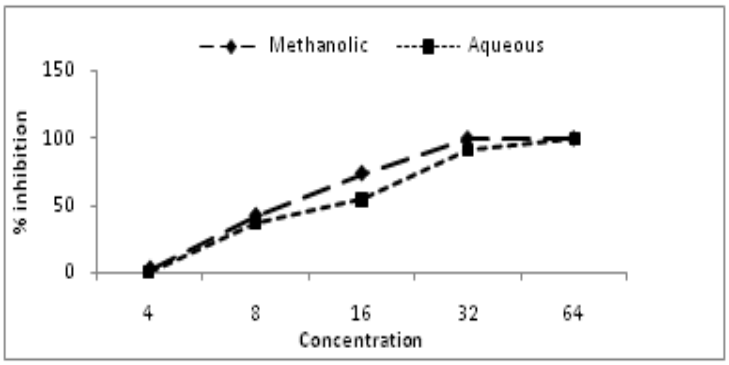

Fig. 3. Inhibition (%) of *S. aureus* by methanolic and aqueous extracts of *M. oleifera* in broth medium

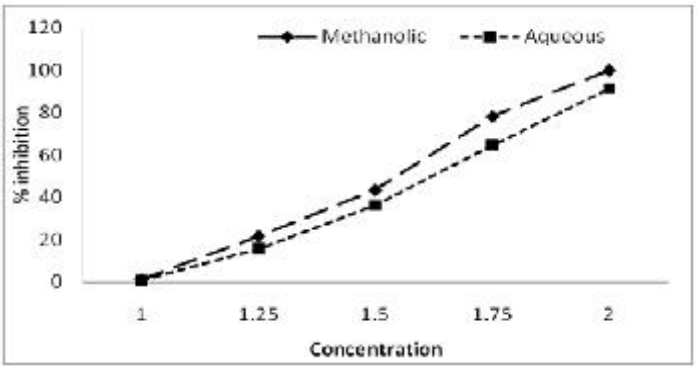

Fig. 4. Inhibition (%) of *S. typhi* by methanolic and aqueous extracts of *M. oleifera* in broth medium

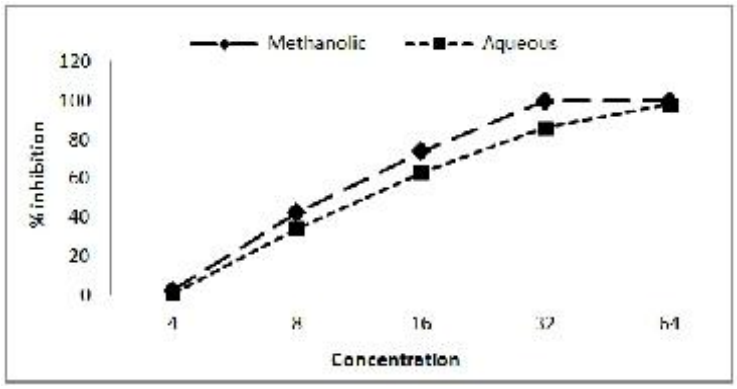

Fig. 5. Inhibition (%) of *V. cholerae* by methanolic and aqueous extracts of *M. oleifera* in broth medium

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
I	Castor oil+saline	14.65±0.67	--------
II	Castor oil+atropine	3.45±0.34*	83.75
III	Methanolic extract of *M. oleifera*	6.92±0.81*	49.90
IV	Aqueous extract of *M. oleifera*	7.46±0.45*	56.76

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

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

Group	Treatment	Mean defecation in 4hr	%inhibition of weight of intestinal content
I	Castor oil+saline	2.65±0.087	--------
II	Castor oil+atropine	1.21±0.64*	44.56
III	Methanolic extract of *M. oleifera*	1.49±0.08*	51.08
IV	Aqueous extract of *M. oleifera*	1.34±0.04*	47.98

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

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

Group	Treatment	Total length of intestine (cm)	Distance travelled by marker	% intestinal weight
I	Castor oil+saline	2.65±0.087	39.06±2.26	88.37%
II	Castor oil+atropine	1.21±0.64*	26.70±2.59*	55.09%
III	Methanolic extract of *M. oleifera*	1.49±0.08*	28.66±2.90*	62.64%
IV	Aqueous extract of *M. oleifera*	1.34±0.04*	29.87±1.97*	61.24%

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

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

4. CONCLUSION

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.*

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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