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Research Article

Evaluation of Anti-Diarrhoeal Potential of Methanol Extract of *Ficus bengalensis* Linn. Leaf and *Mangifera indica* Linn. Stem Bark and Root Bark

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ABSTRACT

The aim of the study was to determine the antidiarrhoeal effect of methanolic extract of two commonly used medicinal plants, *Ficus bengalensis* – leaf and *Mangifera indica* – stem bark and root bark using swiss albino mice against castor oil-induced diarrhoea. The extracts were subjected to phytochemical screening and subsequent TLC analysis for the identification of active phytoconstituents. The mice were treated with the extract at a dose of 3, 7.5 and 15 mg/kg b.wt. p.o. Castor oil was administered after 30 minutes. The stool consistency was observed for a period of 4 hours. Phytochemical analysis of the methanol plant extracts proved the presence of steroids, flavonoids, triterpenes, phenols, sugar and tannins. The methanol plant extracts significantly reduced the total number of stool and number of diarrhoeal stool in a dose-dependent manner when compared with the untreated control. The phytoconstituents responsible for antidiarrhoeal activity may have acted by increasing colonic water and electrolyte reabsorption or by inhibiting intestinal motility. Thus the plants have shown to exhibit potent antidiarrhoeal activity proving its ethnomedicinal usage.

Keywords: plant drugs, colonic absorption, gastrointestinal motility, phytochemicals, tannins, flavonoids.

INTRODUCTION

Gastrointestinal disorders are one of the major health problems in developing countries. Among such, diarrhoeal disease is one of the leading causes of illness and death in young children in developing countries. An analysis conducted in 2000, estimates that diarrhoea accounts for 13% of all childhood death, accounting to 1.4 million deaths per year. According to WHO estimation, for the year 1998, there were about 7.1 million deaths due to diarrhoea. Secretory diarrhoea is the most dangerous symptoms of gastrointestinal problems and is associated with excessive defaecation and stool outputs, the stools being of abnormally loose consistency [1]. Infectious diarrhoea is the most common infectious disease world wide. Gastrointestinal infections kill 1.8 million people globally each year. Acute watery bloody diarrhoea may be due to a variety of pathogens – bacterial and viral agents [2]. Diarrhoea is defined as faecal water output greater than 500 ml a day. It may occur when the colons capacity to absorb water and electrolyte is overwhelmed or reduced.

There are a large number of epidemiological and experimental evidence pertaining to world wide acute diarrhoeal diseases. It thus becomes important to identify and evaluate commonly available natural drugs as alternative to currently used antidiarrhoeal drugs, which are not completely free from adverse effects. In recent years there has been a great interest in herbal remedies for treating diarrhoea. Medicinal plants are promising source of antidiarrhoeal drugs. The WHO estimates that around 20,000 species of higher plants are used medicinally throughout the world [3], but over 85% of the plants await scientific investigations for their biological activity and chemical constituents [4]. Indigenous plants such as *Andrographis paniculata, Asparagus racemosus, Butea monosperma, Cassia auriculata* and others are widely used in the treatment of diarrhoea [5]. Plants with astringent properties are particularly used to treat diarrhoea and dysentery. Frequently tannin containing drugs are used to treat diarrhoea and dysentery [6].

Ficus bengalensis Linn. belongs to the family Urticaceae and is commonly known as the banyan tree (Tamil – Alam). It grows wild in lower Himalayas and is also found all over India. The bark is a tonic, astringent, cooling and diuretic. Seeds and fruits are cooling and tonic [7]. The leaf buds were used in traditional medicine to check diarrhoea. The leaves are reported to contain crude protein, crude fibres, CaO, phosphorus, rutin, friedelin, taraxosterol, lupeol, β-amyrin along with psoralen, bergapten and β-sisterol, quercetin-3-galactoside [8].

Mangifera indica Linn. belongs to the family Anacardiaceae and is commonly known as the mango tree (Tamil – Maambazham). It occurs wild or semi-wild nearly throughout India. Various parts of plant are used to treat diarrhea, dysentery, anaemia, asthma, bronchitis, cough, hypertension, insomnia, rheumatism, toothache, leucorrhoea, haemorrhage and piles [9]. The leaves, fruits, stem bark and heartwood and roots have been reported to yield mangiferin. The root bark with n-hexane gave

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Table 1: Qualitative phytochemical analysis

S. No	Compounds	*Ficus bengalensis* (Leaf)	*Mangifera indica* (Stem bark)	*Mangifera indica* (Root bark)
1	Steroids	+	-	-
2	Triterpenes	+	+	+
3	Flavonoids	-	+	+
4	Phenols	+	+	+
5	Sugar	+	+	+
6	Quinones	-	-	-
7	Alkaloids	-	-	-
8	Tannins	+	+	+

'+' represents the presence and '-' represents the absence of phytoconstituents

friedelian-3-□-ol, □-amyrin, □-amyrin, cycloartenol, mangiferonic acid, amangiferolic acid, friedlin and □-sitosterol [10]. The acetone extract of stem bark yielded besides mangiferin, butin, fisertin, euoicycuanidin, gallic acid, protocatechuic acid and quercertin [11].

Hence the present study was done to establish the efficacy of methanol extract of selected parts of the two plants, namely *Ficus bengalensis* leaf and *Mangifera indica* stem bark and *Mangifera indica* root bark in controlling diarrhoea induced by castor oil.

MATERIALS AND METHODS

Plant material: Plant materials were collected locally during the period July – September. All plants in the study were collected from a local source and duly identified by Botany Professor, Voorhees College, Vellore. Plant parts used were *Ficus bengalensis* leaf (FBL), *Mangifera indica* stem bark (MIS) and *Mangifera indica* root bark (MIR).

Preparation of methanol extract: Plant materials were dried in the dark at room temperature. Dried and powdered material (500 g) was taken in round bottom flask and was first defatted with n-hexane twice in the cold. Then the material was soaked in methanol and kept under constant shaking. After 48 hours, the extract was filtered. Nearly 80% of the solvent was removed by distillation on water bath at atmospheric pressure and the last trace was removed under reduced pressure.

Preliminary phytochemical screening: Preliminary phytochemical analysis was carried out for the presence or absence of steroids, triterpenes, flavonoids, phenols, sugar, quinines, alkaloids and tannins. Burchard test for steroids, Salkowski test for triterpenes, Shinoda test for flavonoids, Ferric chloride test for phenols were done for each extract. Sugar test was carried out with anthrone and concentrated sulphuric acid. Test for quinines and tannins were carried out with concentrated sulphuric acid and lead acetate respectively.

TLC Methodology: 4 g of the sample was soaked in 40 ml of rectified spirit with occasional shaking for 18 hours and boiled for 10 minutes and filtered. The filtrate was concentrated and made up to 10 ml in a standard flask. 20 µ1 of the solution was applied on aluminium plate pre-coated with silica gel 60 F_{254} of 0.2 mm thickness using Linomat IV applicator. The plate was developed in n-butanol: acetic acid: water (6.7:2.3:1 v/v). After air drying the plate was visualized in UV 254 nm and UV 366 nm. The plate was then dipped in vanillin-sulphuric acid and heated in air oven at 105°C till spots appeared. The R_f value was determined by using the formula:

R_f = Distance moved by the solute / Distance moved by the solvent

Animals: Male Swiss albino mice were maintained on a standard laboratory commercial feed. The animals were allowed free access to water. They were maintained in the laboratory for a minimum period of 10 days prior to experimentation. All experiments were performed after an overnight fast.

Castor oil-induced diarrhoea in mice: Diarrhoea was induced in mice by castor oil [12]. The bean of the castor plant, *Ricinus communis*, contains two well-known noxious ingredients: an extremely toxic protein ricin and oil composed chiefly of the triglyceride of ricinoleic acid (12-hydroxyoleic acid). Ricinoleic acid, the active component of castor oil produces effects which are traditionally described as those of an intestinal stimulant. Ricinoleic acid can inhibit water and electrolyte absorption from the gastrointestinal tract of animals [13, 14] and men [14, 15]. It does so by a direct effect on mucosal cells.

Experimental design: The animals were divided into control and test group of 10 animals each. Each animal was placed in an individual cage. For each of the plants mentioned above the methanol extracts were given in doses of 3, 7.5 and 15 mg/kg of body weight to various groups of mice.

Group I: Control treated with castor oil only

Group II: Mice treated with 3 mg/kg FBL

Table 2: TLC observation after derivatization in visible light

S. No	*Ficus bengalensis* Leaf		*Mangifera indica* Stem bark		*Mangifera indica* Root bark	
	Colour	R_f	Colour	R_f	Colour	R_f
1	Pale pink	0.15	Orange	--	Orange	0.17
2	Pale pink	0.23	Grey	0.28	Grey	0.28
3	Pale Orange	0.31	Grey	0.41	Grey	0.41
4	Pale Orange	0.76	Pink	0.79	Pink	0.79

Table 3: Antidiarrhoeal activity of the plant extracts on castor oil-induced diarrhoeal mice

Group	Treatment	Dose (mg/kg)	Onset time of diarrhoea (min)	Total weight of wet faeces (mg)
1	Control [a]	0.3 ml	102 ± 18.0	402 ± 34.0
2	*Ficus bengalensis* leaf ME [b]	3 mg/ kg	134 ± 2.6***	210 ± 22.5***
3		7.5 mg/kg	245 ± 16.0***	110 ± 25.3***
4		15 mg/kg	-----	-----
5	*Mangifera indica* stem bark ME [b]	3 mg/ kg	165.6 ± 20.1***	232 ± 20.8***
6		7.5 mg/kg	235 ± 14.0***	163 ± 22.1***
7		15 mg/kg	-----	-----
8	*Mangifera indica* root bark ME [b]	3 mg/ kg	151 ± 14.5***	240 ± 20.1***
9		7.5 mg/kg	220 ± 11.0***	105 ± 27.3***
10		15 mg/kg	-----	-----

*Values are represented as mean ± SD (n=10); ME - methanol extract; [a]Control mice recieving only castor oil; [b]Pre-treatment with extracts 30 min before castor oil administration; *P<0.05, **P<0.01, ***P<0.001 when compared with control group.*

Group III: Mice treated with 7.5 mg/kg FBL
Group IV: Mice treated with 15 mg/kg FBL
Group V: Mice treated with 3 mg/kg MIS
Group VI: Mice treated with 7.5 mg/kg MIS
Group VII: Mice treated with 15 mg/kg MIS
Group VIII: Mice treated with 3 mg/kg MIR
Group IX: Mice treated with 7.5 mg/kg MIR
Group X: Mice treated with 15 mg/kg MIR

30 minutes later each animal received 0.3 ml castor oil orally as per the method described earlier [10]. The following parameters were observed for a period of 4 hours, the time elapsed between the administration of the cathartic agent and the excretion of the first diarrhoeic faeces (wet faeces that leaves halo on the filter paper) and the total weight of diarrhoeal stools in the period of time. A numerical score based on stool consistency was assigned as follows: Normal stool = 1, Semisolid = 2, Watery stool = 3.

Statistical Analysis: Non-parametric analysis was carried out by Kruskal – Wallis test with the level of statistical significance set at 0.05.

RESULT

The general condition of the animals such as alertness, movement and activity were normal. There was neither sedation nor excitation. LD_{50} was calculated. No adverse effects were observed even after 250 mg/kg. Phytochemical analysis of the methanol plant extracts revealed the presence of steroids, triterpenes, phenols, sugar and tannins in FBL, and triterpenes, flavonoids, phenols, sugar and tannins in both MIS and MIR (Table 1). TLC photos were observed for components under UV 254 nm, UV 366 nm and after derivatization in visible light. The appearance of colour and R_f values were noted (Table 2). The thin layer chromatographic observation showed the presence of triterpenes, alkaloids and steroids. The methanol plant extracts significantly reduced the total number of stool and number of diarrhoeal stool in a dose-dependent manner when compared with the untreated control. Even at lower doses of 3 mg/kg administered, the extracts significantly ($p<0.001$) delayed the onset of diarrhoea and reduced the production of wet faeces. However, the methanolic plant extracts of all the three drugs tested were more effective at higher doses of 15 mg/kg inhibiting diarrhoea completely and restraining the appearance of wet faeces (Table 3).

DISCUSSION

Diarrhoea, a national problem especially among children, contributes much morbidity and mortality. Many plants that are conveniently available in India have been reported to be effective against diarrhoea and dysentery as they are used by local people and traditional folklore medicine. In the traditional medicine system, *Ficus bengalensis* and *Mangifera indica* have been used in the management of diarrhoea. Despite its traditional usage as an antidiarrhoeal agent, there is no information regarding the effectiveness of leaves, stem bark and root bark of these plants in controlling diarrhoea. Hence the present study sought to assess their antidiarrhoeal activity.

Our results show that the extracts significantly inhibited castor oil-induced diarrhoea in the tested swiss albino mice. Castor oil-induced diarrhoea is described to be an appropriate model that characterizes secretory diarrhoea [16]. Though several mechanisms have been proposed to explain the diarrhoeal effect of castor oil, it is well known that diarrhoea is initiated by a castor oil metabolite ricinoleic acid through a hypersecretory response which activates intestinal smooth muscles via EP_3 prostaglandin receptors [17]. Ricinoleic acid also reduces active Na^+ and K^+ absorption and decrease Na^+K^+ ATPase activity in the small intestine and colon [15]. The liberation of ricinoleic acid results in irritation and inflammation of intestinal mucosa, leading to release of prostaglandins, which results in stimulation of secretion [18], thereby prevents the reabsorption of NaCl and H_2O and resulting in diarrhoea.

Methanol extract of the plants used in the study significantly reduced the total weight of wet faeces in a dose dependent manner. All the plant parts selected for the present study were more effective at higher doses. The extract probably increased the reabsorption of NaCl and H_2O by decreasing intestinal motility. Secretory diarrhoea is associated with an activation of Cl^- channels, causing Cl^- efflux from the cell. The efflux of Cl^- results in massive secretion of water into the intestinal lumen and profuse watery diarrhoea. The extract may have

inhibited the secretion of water into the lumen by reversing this mechanism [19].

However, for any herbal drug, the phytoconstituents present in it is a contributing factor to overcome the diseased condition. Phytochemical analysis of the extracts revealed the presence of steroids, triterpenes, phenols, sugar and tannins in FBL and triterpenes, flavonoids, phenols, sugar and tannins in MIS and MIR. Among the various medicinal plants, anti-diarrhoea activity was found in plants possessing alkaloids, tannins [20], flavonoids [21] and triterpenes [22]. Tannins and flavonoids are thought to be responsible for antidiarrhoeal activity by increasing colonic water and electrolyte reabsorption. Earlier studies have reported that the bark of *Ficus bengalensis* containing tannins and flavonoids possessed antidiarrhoeal activity and the underlying mechanism appears to be spasmolytic and anti-enteropooling property by which it produced relief from diarrhoea [23]. Antidiarrhoeal activity of flavonoids [24] have been ascribed to their ability to inhibit intestinal motility and hydro-electrolytic secretion [25]. The antidiarrhoeal activity of the extract may also be due to the presence of tannins which denature proteins in the intestinal mucosa by forming protein tannates and subsequently reduce secretion. Studies on the functional role of tannins also reveal that they could also bring similar functions by reducing the intracellular Ca^{2+} inward current or by activation of the calcium pumping system [26]. Additionally, triterpenes have also been reported to show considerable antidiarrhoeal activity [27]. Thus the extracts containing these important phytoconstituents would have very much contributed to its antidiarrhoeal activity.

CONCLUSION

Castor oil is a suitable mode of inducing diarrhoea in mice, since it allows the observation of measurable changes in the weight of diarrhoeal stool. The extract resulted in a marked reduction in the weight of diarrhoeal stool and also increase the onset time of diarrhoea. All the three extracts inhibit diarrhoea at a concentration of 15 mg/kg. Though the exact mechanism is yet to be elucidated, it can be postulated that the extracts directly act on the colon and stimulate the absorption of water and electrolyte and decrease the motility of large intestine or might have mediated by antisecretory mechanism. Thus, the phytocompounds like tannins, flavonoids and triterpenes of the methanolic extract of *Ficus bengalensis* leaf and *Mangifera indica* stem bark and root bark would have contributed to their efficiency and proved to be potential antidiarrhoeal agents.

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