International Journal of Photochemistry and Photobiology
2017; 1(1): 21-26
http://www.sciencepublishinggroup.com/j/ijpp
doi: 10.11648/j.ijpp.20170101.14

Evaluation of Anti-diarrheal Activity of Crude Extracts and Different Fractions of Stem Bark and Fruits of *Oroxylum indicum*

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To cite this article:
Md. Mamun-Or-Rashid, Md. Shah Amran, Md. Aslam Hossain. Evaluation of Anti-diarrheal Activity of Crude Extracts and Different Fractions of Stem Bark and Fruits of *Oroxylum indicum*. *International Journal of Photochemistry and Photobiology.* Vol. 1, No. 1, 2017, pp. 21-26. doi: 10.11648/j.ijpp.20170101.14

Received: January 4, 2017; **Accepted**: January 17, 2017; **Published**: March 2, 2017

Abstract: Abnormal passage of liquid or unformed stool at an increased frequency is termed as diarrhea: Infectious agents, certain medications, plant and animal toxins, gastrointestinal disorders, and substances that enhance gastrointestinal tract secretions are may be the triggers of diarrhea [1]. In recent times, medicinal plants occupy a considerable position for being the paramount sources of drug discovery irrespective of its categorized groups- herb, shrub or tree [2]. *Oroxylum indicum* (Linn.) Vent is widely used in Ayurvedic system of medicine [3]. The crude methanolic extract of *Oroxylum indicum* bark and fruits with different soluble partitionates were subjected to investigate for the evaluation of analgesic, hypoglycemic, CNS depressant and antidiarrheal activity on mice and thrombolytic, antihelmentic, antimicrobial, antioxidant along with cytotoxicity different in vivo experiment. [4] The antidiarrheal activity of methanolic bark and fruits extract and its different fractions of *Oroxylum indicum* (Ethyl acetate fraction, Dichloromethane fraction, Hexane fraction, Carbon tetrachloride fraction) (400mg/kg) cause reduction of diarrheal feces by 46.5%, 32.6%, 45.3%, 33.7%, 51.2%, respectively compared to standard Loperamide (82.6%). This reveals that methanol extracts of bark and fruits of *Oroxylum indicum* showed significant antidiarrheal activity.

Keywords: *Oroxylum indicum*, Antidiarrheal Activity, Methanolic Extract

1. Introduction

Abnormal passage of liquid or unformed stool at an increased frequency is termed as diarrhea: Infectious agents, certain medications, plant and animal toxins, gastrointestinal disorders, and substances that enhance gastrointestinal tract secretions are may be the triggers of diarrhea. It can also be caused by the ingestion of poorly absorbable resources or inflammatory and dysmotility troubles of the gastrointestinal tract [1].

1 in 9 child deaths worldwide occurs due to Diarrheal diseases, making diarrhea the second leading cause of death among children under the age of 5. Diarrhea kills more than two thousand children every day more than AIDS, malaria, and measles combined [4]. Diarrhea is even more deadly for children with HIV; for these children, the death rate is 11 times greater than that for children without HIV [5].

Diarrheal diseases are a major setback in Third World countries and millions of people are died of it every year [6]. From the ancient time plants have been using as source of new drugs. Many plant species have been screened for substances with therapeutic activity and many of them are found as promising source of antidiarrheal drugs [7]. For this rationale, World Health Organization (WHO) and many other international organizations have encouraged studies pertaining to the treatment and prevention of diarrheal diseases using traditional medical practices [8, 9, 10]. Therefore, study of alternative antidiarrheal drugs has become a burning need. In recent times, medicinal plants occupy a considerable position for being the paramount sources of drug discovery irrespective of its categorized groups- herb, shrub or tree. In addition, herbal drugs can also be scientifically modified for better pharmacological activity and to establish safe and effective drugs [2]. In the current

study, we have attempted to investigate the traditional polyherbal formulation for their antidiarrheal activity.

Oroxylum indicum (Linn.) Vent, the plant used in this study is one among the group of ten drugs named Dasamoola, widely used in Ayurvedic system of medicine [3]. Locally, in Bangladesh, it is known as Khona, Sona, Hona, Nasona. Patti, Dinga, Kanak, Kanaidinga, Bhinga, Thona etc. English names of the plant include, Indian trumpet flower, broken bones plant, Indian calosanthes, Indian Trumpet, Indian trumpet flower, midnight horror, etc [11].

Oroxylum indicum, a deciduous tree, has small or medium size, up to 12 m in height with soft light brown or grayish brown bark with corky lenticels. The leaves are extremely large, 90-180 cm long 2-3 pinnate with 5 or additional pairs of primary pinnate, rachis especially fast, cylindrical, swollen at the connection of branches, leaflets 2-4 pairs ovate or elliptic, acuminate, glabrous. The fresh root bark is malleable and juicy in nature; it's sweet, but becomes bitter later. On drying, the bark shrinks, stick closely to the wood and becomes faintly fissured [11]. The tree has long fruit pods (Figure 1) that curved downward; hang down from the branches, looks like the wings of a large bird or dangling sickles or sword in the night.

Figure 1. *Oroxylum indicum (Fruits).*

Oroxylum indicum grows in India, Bangladesh, Sri Lanka, Philippines, Indonesia, China, Bhutan, Malaysia and Mallaca. In Bangladesh, it is distributed in Chittagong hill tracts, Cox's bazaar and Sylhet. It is mostly sighted along the river banks or slopes of the hills [3, 12, 13].

2. Material and Methods

Principle [14]

The antidiarrheal activity of the crude extract and its different fractions of *Oroxylum indicum* were evaluated using the method of castor oil induced diarrhea in mice [6]. According to this method, each mouse was fed with 10 mm of highly pure analytical grade castor oil which would induce diarrhea. The number of fecal stools was recorded for each individual mouse. The observations of the experiment groups were compared against that of the control to evaluate the antidiarrheal activity of the samples.

Collection and preparation of plant material

The plant samples (bark and fruits) of *Oroxylum indicum* were collected from Teknaf, Cox'sbazar in April 2012. The plant was identified by National Herbarium. Mirpur, Dhaka (Accession No- 37883) Dhaka, for further reference. The bark and fruit were dried under sun and oven, then dried parts were ground in coarse powder using high capacity grinding machine in the Phytochemical Research Laboratory, Faculty of Pharmacy, University of Dhaka.

Extraction of the plant material

About 650 gm of the powdered material of each part was soaked in 2.5 liter of methanol. The concentrated extract solution was air dried to solid residue, where weight obtained from the powdered bark and fruits were 16.75 gm and 14.50 gm respectively. The methanol (crude) extract was later partitioned by Modified Kupchan partition method [15]. For this method, mother solution was prepared by titrating 5 gm of methanol extract with 90 ml of methanol containing 10 ml of distilled water. Next, 100 ml of n-hexane was added to mother solution and organic portion was collected and air dried for solid residue. Mother solution left after partitioning with n-hexane was mixed with 12.5 ml of distilled water and extracted with carbon tetrachloride (CCl4). The carbon tetrachloride fraction was then air dried for solid residue. The aqueous fraction was preserved for the next step, in which 16 ml of distilled water was mixed uniformly and later extracted with dichloromethane (CH2Cl2). The dichloromethane soluble fraction was collected together and air dried. The aqueous fraction was preserved for the next step for extraction with ethyl acetate. The ethyl acetate soluble fraction was collected and air dried. Amount of fractions achieved after partition of methanolic (crude) bark and fruits extract are shown in Table 1 and Table 2 respectively.

Table 1. *Amount of fractions after partition of methanolic (crude) bark extract.*

Fraction	Weight
Hexane soluble fraction	425 mg
Carbon tetrachloride soluble fraction	865 mg
Dichloromethane soluble fraction	1340 mg
Ethyl acetate soluble fraction	1620 mg

Table 2. *Amount of fractions after partition of methanolic (crude) fruits extract.*

Fraction	Weight
Hexane soluble fraction	550 mg
Carbon tetrachloride soluble fraction	925 mg
Dichloromethane soluble fraction	1405 mg
Ethyl acetate soluble fraction	1710 mg

Experimental Animal

Twenty eight adult Swiss albino mice (musdomesticus) of either sex weighing between 30-35g, aged 4-5 weeks obtained from the Jahangirnagar University were used for the experiment. The animals were housed in polypropylene cages (30x20x7 cm) in standard conditions for (21±1°C with a 12 h light and dark cycle) for 3 days before experiment.

Experimental Design

Twenty eight experimental animals were randomly selected and divided into seven groups denoted as group-I, group-II, group-III, group- IV, group –V, group-VI and group-VII consisting of 4 mice in each group. Each group received a particular treatment i.e. control, standard, single dose of Methanolic crude extract and its different fractions of bark and fruits of *Oroxylum indicum*. Prior to any treatment, each mouse was weighed properly and the doses of the test samples and control materials were adjusted accordingly.

Preparation of Test Materials

In order to administer the crude extract at doses of 400 mg/kg body weight of mice, 50 mg of the extract were measured respectively and were triturated unidirectional way by the addition of small amount of suspending agent, Tween-80. After proper mixing of extract and suspending agent, normal saline was slowly added. The final volume of the suspension was made 2.5 ml.

To stabilize the suspension, it was stirred well by vortex mixture. For the preparation of at the dose of 5 mg/kg-body weight, 50mg of loperamide was taken and it was made a suspension in 2.5 ml normal saline.

Table 3. *Test materials used in the evaluation of anti-diarrheal activity of crude extract and its different fraction of Oroxylum indicum.*

Test Samples	Group	Identification	Dose (mg/kg)	Route of administration
1% Tween-80 in normal saline	I	Control Group	400	Oral
LoperamideHCl	II	Standard Group	50	Oral
MEOI	III	Sample group	400	Oral
DCMOI	IV	Sample group	400	Oral
EAOI	V	Sample group	400	Oral
HXOI	VI		400	Oral
CTOI	VII	Sample group	400	Oral
Charcoal (5%)	-	Motility marker	0.3 mL of a 5% charcoal suspension in 5% aqueous suspension of charcoal powder	Oral

Procedure

The method described by (Shoba& Thomas, 2001), was followed for this study with slight modification. The animals were divided into control, positive control and test groups containing four mice in each group. Control group received vehicle (1% Tween 80 in water) at dose of 10ml/kg orally. The positive control group received Loperamide at the dose of 50 mg/kg orally; test groups received methanolic extract, ethyl acetate fraction, dichloromethane fraction, hexane fraction carbon tetrachloride fraction at the dose of 400 mg/kg b.w. orally. Each animal was placed in individual case, the floor of which was lined with blotting paper. The floor lining was changed every hour. Diarrhea was induced by oral administration of castor oil to each mouse, 30 min after above treatments. During an observation period of 4h, the numbers of diarrheic feces excreted by the animals were recorded.

Statistical analysis

All values were expressed as mean+ standard error of mean (SEM) and the results were analyzed statistically by one way analysis of variance (ANOVA) followed btDunnett's test by using SPSS ver 16.

3. Results

3.1. For Bark

The methanolic extract of *Oroxylum indicum* bark showed profound anti-diarrheal property in castor oil induced diarrheal mice (Table 5). The methanolic crude extract and its different fractions (Ethyl acetate fraction, Dichloromethane fraction, Hexane fraction, Carbon tetrachloride fraction) (400mg/kg) cause reduction of diarrheal feces by 50%, 31.4%, 52.3%, 34.9%, 53.551.2%, respectively compared to standard Loperamide (82.6%).

Table 4. *Data representing the total no. of diarrheal feces stool given by each mouse.*

Group	Mice no.	No. of diarrheal feces				Total no. of diarrheal feces	Mean
		1st hour	2nd hour	3rd hour	4th hour		
Control	1	9	7	5	4	25	21.5
	2	8	7	6	3	24	
	3	6	5	4	3	18	
	4	7	5	5	2	19	
Standard	1	2	1	1	2	6	3.75
	2	1	0	1	1	3	
	3	0	2	1	1	4	
	4	0	1	1	0	2	

Group	Mice no.	No. of diarrheal feces				Total no. of diarrheal feces	Mean
		1st hour	2nd hour	3rd hour	4th hour		
Methanolic crude extract	1	4	3	2	1	10	10.75
	2	4	3	3	2	12	
	3	3	3	2	2	10	
	4	4	3	2	2	11	
Ethyl acetate	1	6	5	4	4	19	14.75
	2	5	4	3	3	15	
	3	4	4	3	2	13	
	4	4	3	3	2	12	
Dichloromethane	1	4	4	2	2	12	10.25
	2	4	3	2	2	11	
	3	4	3	1	1	9	
	4	3	3	2	1	9	
Hexane	1	6	4	3	3	16	14
	2	5	4	2	2	13	
	3	4	3	3	2	12	
	4	4	3	3	5	15	
Carbon tetrachloride	1	3	3	2	2	10	10
	2	4	4	2	2	12	
	3	4	2	2	1	9	
	4	3	3	2	1	9	

Table 5. *Effect of methanolic extract and different fractions on castor oil induced diarrhea in mice.*

Treatment	Dose (b.w)	Number of diarrheal feces (Mean + SEM)	% Reduction of diarrhea
Control (Saline)	10 ml/kg	21.5 + 1.76	---
Standard	50 mg/kg	3.75 + 0.853	82.6
Crude	400 mg/kg	10.75 + 0.95	50
Ethyl acetate	400 mg/kg	14.75 + 1.15	31.4
Dichloro methane	400 mg/kg	10.25 + 1.03	52.3
Hexane	400 mg/kg	14 + 1.13	34.9
Carbon tetrachloride	400 mg/kg	10 + 0.885	53.5

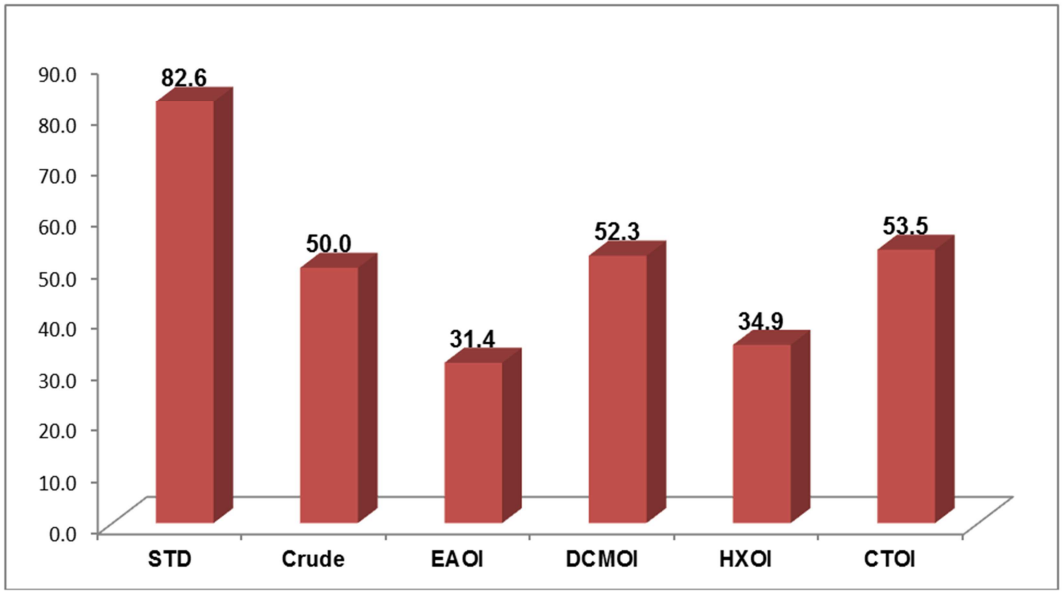

Figure 2. *% reduction of diarrheal feces by different fractions.*

Note: STD= Standard sample, Crude= Methanolic crude extract, EAOI= Ethyl acetatefraction of *Oroxylum indicum*, DCMF= Dichlomethane fraction of *Oroxylum indicum*, CTOI= Carbon tetrachloride fraction of *Oroxylum indicum*

3.2. For Fruits

The methanolic extract of *Oroxylum indicum* fruits showed profound anti-diarrheal property in castor oil induced diarrheal mice (Table 7). The methanolic crude extract and its different fractions (Ethyl acetate fraction, Dichloromethane fraction, Hexane fraction, Carbon tetrachloride fraction) (400mg/kg) cause reduction of diarrheal feces by 46.5%, 32.6%, 45.3%, 33.7%, 51.2%, respectively compared to standard Loperamide (82.6%).

Table 6. *Data representing the total no. of diarrheal feces stool given by each mouse.*

Group	Mice no.	No. of diarrheal feces				Total no. of diarrheal feces	Mean
		1st hour	2nd hour	3rd hour	4th hour		
Control	1	9	7	5	4	25	21.5
	2	8	7	6	3	24	
	3	6	5	4	3	18	
	4	7	5	5	2	19	
Standard	1	2	1	1	2	6	3.75
	2	1	0	1	1	3	
	3	0	2	1	1	4	
	4	0	1	1	0	2	
Methanolic crude extract	1	4	3	2	1	10	11.5
	2	4	3	3	2	12	
	3	5	4	2	2	13	
	4	4	3	2	2	11	
Ethyl acetate	1	6	5	4	4	19	14.5
	2	4	4	3	3	14	
	3	5	4	3	2	14	
	4	4	3	2	2	11	
Dichloromethane	1	6	5	4	2	17	11.75
	2	5	3	2	1	11	
	3	4	3	2	1	10	
	4	3	3	2	1	9	
Hexane	1	6	5	4	3	18	14.25
	2	5	4	3	2	14	
	3	4	4	3	2	13	
	4	4	3	3	2	12	
Carbon tetrachloride	1	4	3	2	2	11	10.5
	2	5	4	2	2	13	
	3	4	2	2	1	9	
	4	3	3	2	1	9	

Table 7. *Effect of methanolic extract and different fractions on castor oil induced diarrhea in mice.*

Treatment	Dose (b.w)	Number of diarrheal feces (Mean + SEM)	% Reduction of diarrhea
Control (Saline)	10 ml/kg	21.5 + 1.76	---
Standard	50 mg/kg	3.75 + 0.853	82.6
Crude	400 mg/kg	11.5 + 1.08	46.5
Ethyl acetate	400 mg/kg	14.5 + 0.408	32.6
Dichloro methane	400 mg/kg	11.75 + 0.288	45.3
Hexane	400 mg/kg	14.25 + 0.408	33.7
Carbon tetrachloride	400 mg/kg	10.5 + 0.707	51.2

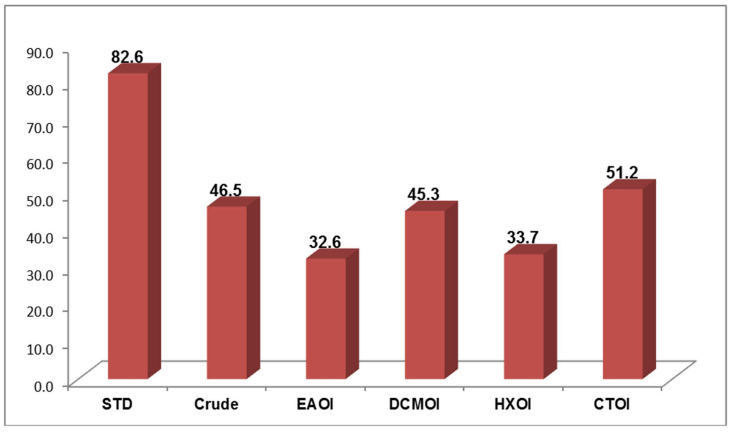

Figure 3. *% reduction of diarrheal feces by different fractions.*

Note: STD= Standard sample, Crude= Methanolic crude extract, EAOI= Ethyl acetatefraction of *Oroxylum indicum*, DCMF= Dichlo methane fraction of *Oroxylum indicum*, CTOI= Carbon tetrachloride fraction of *Oroxylum indicum*

4. Discussion

Castor oil causes diarrhea due to its active metabolite, ricinolic acid [16], which stimulates peristaltic activity in the small intestine, leading to changes in electrolyte permeability

of the intestinal mucosa. Its action also stimulates the release of endogenous prostaglandin [17]. Several other mechanism have been reported to cause diarrhea by castor oil including inhibition of intestinal Na^+/K^+-ATPase activity, activation of adenylcyclase or mucosal cAMP-mediated active secretion and platelet activating factor [18].

The previous reports have demonstrated the antidiarrheal activity of tannin [19], flavonoids [17], alkaloids [6] Saponins, reducing sugars and sterols and or terpenes containing plant extracts.

The present study showed that methanolic crude extract of both bark and fruits and their different fractions (Ethyl acetate fraction, Dichloromethane fraction, Hexane fraction, CCl_4 fraction) (400mg/kg) cause reduction of diarrheal feces compared to standard Loperamide.

5. Conclusion

The result of this study shows that the methanolic extract of both bark and fruits of *Oroxylum indicum* possesses strong antidiarrheal activity. But this study is preliminary type and it would be interesting to carry out further study for isolating the possible phytoconstituent and characterization of the active constituents which may be responsible for the Anti-diarrheal activity.

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