Evaluation of the antidiarrhoeal potential of *Citrus limon* (Linn.) leaves

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ABSTRACT

The objective of the present study was to evaluate in vivo anti-diarrheal potential of *Citrus limon* leaves. The sample was analyzed for quantitative estimation of phytochemical and anti-diarrheal activity of ethanol extract, at 150 and 300 mg/kg body weight (b.w) was investigated using castor oil-induced model. Study reveals that protein was maximum in leaves (4.54%) followed by fiber, starch, oil, sugar, alkaloid, tannin, Flavanoids, and Phenolics. At dosages of 150 and 300 mg/kg b.w extract showed significant ($p<0.01$) activity, as compared to control. In the course of observation for 4 hr, oral dose of 300mg/kg b.w showed a remarkable dose-dependent delay in onset of copious diarrhea, decrease in the frequency of purging, the weight of wet stools, the weight of total stool and percentage protection. The study thus signifies the potential clinical effect of the extract in disease and needs to be extended further for activity guided extraction/isolation of the active chemical moiety.

Keywords: Castor oil, Diarrhea, Loperamide.

1. Introduction

Citrus limon (Rutaceae) are small trees that are believed to be native to the Indian sub-continent. Europeans were introduced to the lemon only in the 2nd century AD; the lemon is by now a major cultivated fruit tree in all Mediterranean and subtropical climates around the world. Diarrhea is a major health problem, especially for children under the age of 5 and up to 17% of infected children die off with this disease. Several parts of the species were used in tribal/traditional medicine for diseases like antiscorbutic, astringent, and febrifuge. In Italy, the sweetened juice is given to relieve gingivitis, stomatitis, etc. *Limon* contains various phytochemicals, some of which are of high interest because of their medicinal values; in particular, this plant is rich in a fairly unique group of Flavonoid, phenol and volatile oils. In developing countries, a majority of people living in rural areas almost exclusively use traditional medicine in treating all sorts of diseases, including diarrhea, which is a very common and recurring disease in the community **[1-3]**. Worldwide distribution of diarrhea accounts for more than 5-8 million deaths each year in infants and children below five years, especially in developing countries **[4]**. According to W.H.O. estimates, about 7.1 million deaths were caused by diarrhea **[5]**. It is therefore important to identify and evaluate available natural alternatives to currently used anti-diarrheal drugs, which are not always free from adverse effects**[6]**. Literature suggested that some parts of this species had potential as an anti-diarrheal agent. A study on the anti diarrheal activity of *Citrus limon* peel had reported earlier **[1]**. In other experiments, anti-diarrheal activity in methanol, aqueous and hydroalcoholic extract of *C.limon fruits* were also evaluated **[7, 8]**. Some studies were also carried out on antibacterial activity of various parts of species **[9-11]**, which includes assay on coliform bacteria (major causative microorganism for

diarrhea), also **[12]**. In similar series of action, an attempt has been made to analyze the various phytochemicals present in the leaves and to evaluate the activity potential in ethanol extract of *C.limon* leaves through castor oil-induced model in experimental animals.

2. Materials and Methods

2.1 Drugs

Doses of the extract (Test) was selected as 150 mg/kg (T1) and 300 mg/kg (T2) b.w on the basis of toxicity studies, which was observed at a maximum dose of 5000 mg/kg b.w. Loperamide hydrochloride is used as a standard drug.0.5% Carboxy Methyl Cellulose (CMC) in distilled water is used as a vehicle to make suitable dilutions of standard and extract**[13, 14]**. All the chemicals and reagents (AR grade) are purchased from Sigma-Aldrich.

2.2 Plant material

Fresh leaves of *Citrus limon* were collected from Lucknow cantonment, authenticated and deposited in institute's herbarium [LWG 97847.]. The sample was shade dried, grinded and sieved (40 mesh) to get a uniformly coarse powder.

2.3 Phytochemical characterization

Quantification of various phytochemicals viz. Sugar & Starch**[15]**, Oil**[15]**, Tannin**[16]**, Phenolic & Flavonoid**[17]**, Fiber**[18]**, Total protein**[19]** and Crude alkaloid content**[20]** were determined in *C.Limon* leaves.

2.4 Preparation of extract and preliminary phytochemical screening

Powdered sample (500 g) was initially macerated for 7 hr with petroleum ether (40-60 °C) to remove the fatty component and then subjected to alcohol (absolute ethanol, 99.99%) as a solvent for 7days (Temp. 27±2 °C) with intermittent shaking. The crude extract was filtered and dried under reduced pressure at 40 °C. Qualitative estimation of carbohydrates, proteins, alkaloids flavonoids, tannins, saponins, steroids, anthraquinones, and cyanogenic glycosides were performed as per standard procedures. **[21, 22]** in ethanol extract of leaves.

2.5 Experimental animals

Animals (Sprague-Dawley rats) of 125-175 g were selected and randomly divided into six groups (n=6) for screening. Two groups for test doses (150 and 300 mg/kg) of ethanol extract, while one each for standard drug and control respectively. Animals were placed in cages, fed with standard diet and water (Temp 27±2 °C). Before treatment, animals fasted overnight of food but not water **[23]**. Conditions were maintained as per animal ethical committee guidelines.

2.6 Castor oil-induced model

The animals were divided into four groups of 6 animals each. Group 1 served as the control and received 0.5% CMC suspended in distilled water. The next three groups received castor oil (Paras Chemicals) in the dose of 1 ml per animal p.o. **[24]**. Half an hour after castor oil administration, group 2 and three receive extract at a dose of 150 and 300 mg/kg body weight, p.o. and the group 4 receive Loperamide (3 mg/kg; p.o.) respectively. Following their administration, the animals were placed separately in acrylic cages with filter paper, which was changed every hour. The severity of diarrhea was assessed each hour until four h. The total

number of feces (dry and wet stool) and diarrhoeal feces (wet stool) excreted in record time were scored and compared with the control group. The total score of diarrhoeal feces of the control group was considered that of 100%. The results were expressed in percentage of inhibition **[23, 25, 26]**

2.7 Statistical analysis:

Results obtained from the study were expressed as mean ± SEM. The data were analyzed using one-way ANOVA, followed by Dunnett's test. Results were considered significant when $P<0.01$.

3. Results

The study supports the presence of various phytochemicals in *C. limon* leaves as shown in figure 1, and their quantification reveals that protein content was found to be highest (4.54%) followed by alkaloids, protein, Flavonoids, Phenolics, tannins, starch, and sugar. 4.44,4.49,0.49,0.19,4.54,1.27% respectively. This depicts the nutritional importance and socioeconomic use of leaves apart from edible fruits, more in rural and under-nourished part of the world. *C.limon* leaves when treated with ethanol, yielded 4.7% of the extract on a weight basis. Qualitative estimation of phytochemicals in ethanol extract of leaves as summarized in table 1 shows the presence of carbohydrate, protein, steroid, flavonoids, tannin, alkaloid, and glycosides. **[28,29]**

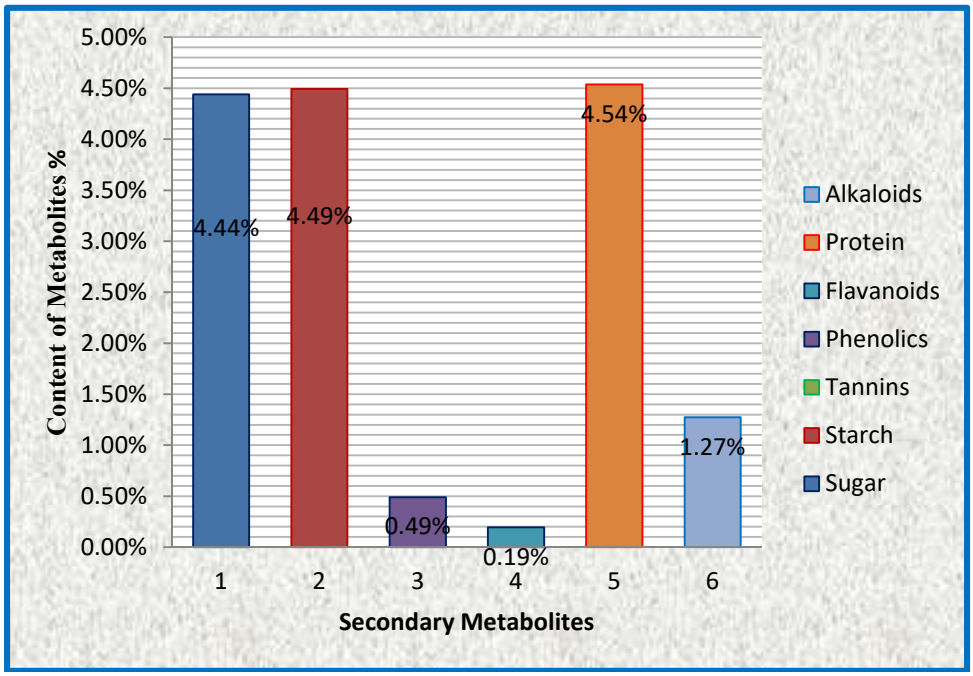

Fig: Quantitative estimation of phytochemicals present in C.limon

Table 1: Preliminary phytochemical screening of *Moringa* leaves (alcoholic)

Chemical tests	***C. limon* leaves (extract)**
Carbohydrate	+
Protein	+
Steroid	+
Flavonoids	+
Tannin	+
Alkaloids	+
Glycosides: a)Coumarin b) Saponin c) Cardiac d) Cynogenetic	 - + - + +

In vivo screening, as in table 2 showed that ethanol extract of *C.limon* leaves produced a statistically significant ($p < 0.01$) antidiarrheal activity. The onset of diarrhea is delayed, after administration of doses, i.e. 52 and 62 min for 150 and 300 mg/kg b.w respectively and is significant to that of control, which shows diarrheal symptom only after 40 min. In the course of observation for 4 hrs after castor oil administration, all the animals in tested groups which previously produces copious diarrhea show decrease in the frequency of purging (reduction in no. of wet stool and total no. of stools), the weight of wet stools and weight of total stool. Mean no of wet stool in control, standard and test doses were shown in Figure 2 and are observed that the frequency of wet stool decreases as dose increases. In selected doses of 150 and 300 mg/kg b.w percentage inhibition was found to be 35.5 and 42.75% respectively as compared to control (100%) and standard Loperamide, having 80% inhibition respectively as represented in figure 3)

Groups	Dose (mg/kg)	Onset of Diarrhea (min)	Mean Number of total stools	Weight of wet stools (gm)	Weight of total stools (gm)
Control	----	After 40	9.5±0.35	0.295	0.330
Standard	3	After 67	2±0.70	0.070	0.110
Test group	150(T1)	After52*	8±0.80	0.085	0.135
	300 (T2)	After 62*	6.5±0.35	0.140	0.200

Values are expressed as mean ± S.E.M. (N=6). *P< 0.01 when compared with control

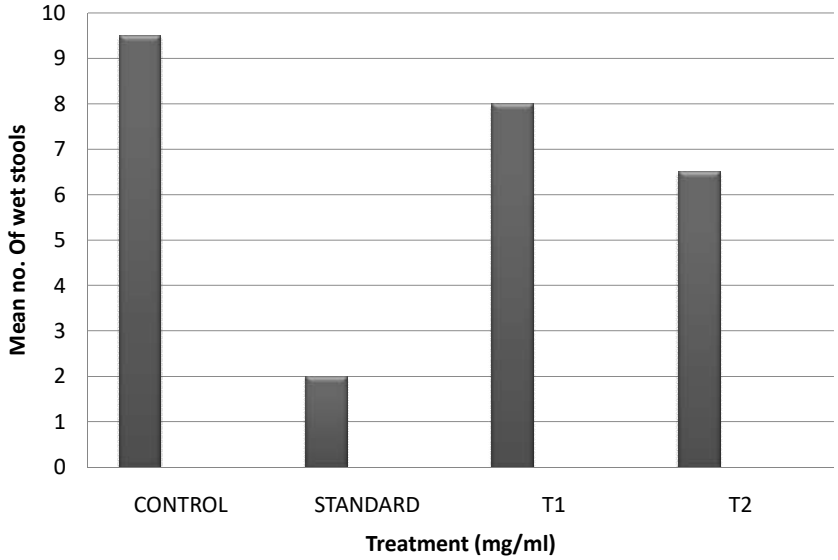

Fig 2: Graphical representation of mean no. of wet stools for all groups.(Test doses were compared with control at P<0.01)

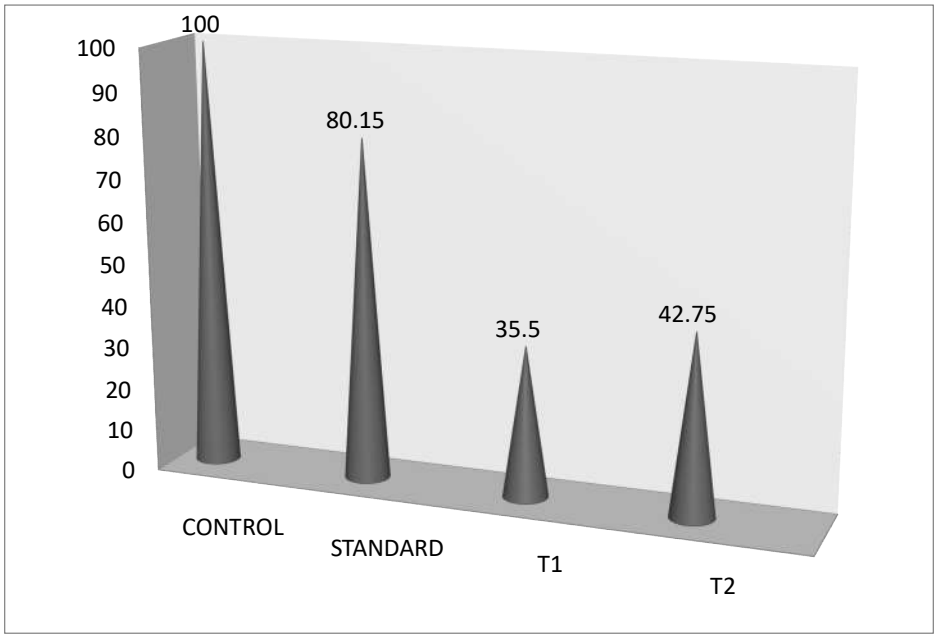

Fig 3: Percentage inhibition of diarrhea in control, standard, and test doses. (*P<0.01, when compared with control).

4. Discussion

The result of the present study would suggest that ethanolic extract of *C.limon* leaves exhibit significant action against castor oil-induced diarrhea. Castor oil causes diarrhea due to its active metabolite, ricinoleic acid [**27, 28]**, which stimulate peristaltic activity in the small intestine, leading to changes in the electrolytic permeability of the intestinal mucosa and thus increases the volume of intestinal content by preventing the reabsorption of water. The liberation of ricinoleic acid also results in irritation and inflammation of intestinal mucosa, leading to the release of prostaglandins and autocoids **[13, 29]**. Loperamide at present is one of the most efficacious and widely employed anti-diarrheal agents and effectively antagonizes the action of castor oil due to its antimotility and antisecretory property. Since the alcoholic extract successfully inhibits the castor oil-induced diarrhea, the action might be via the anti-secretary mechanism. The significant reduction in the frequency of defecation, number of wet stool, mean stool count, the weight of wet stool and weight of total stool signifies the efficacy of ethanolic extract of *C.limon* leaves as an effective anti-diarrheal agent. Traditionally heelers and common man uses an alcoholic decoction of various herbs to cure the disease, and this is, in fact, the reason to analyze the antidiarrheal activity in ethanol extract. In addition to this, it is well reported that several group/classes of phytochemicals have anti dysenteric and antidiarrhoeal property, i.e. tannins, alkaloids, saponins, flavonoids, sterols/triterpenoids, reducing sugars and anthraquinone glycosides, especially known for their laxative effect. Thus the presence of tannin, alkaloid, flavonoids, sugar, and anthraquinone may be the underlying cause of mechanism. Besides this, the potentiating action may also be due to denatured proteins, which form protein tannates; these

complexes of tannin make the intestinal mucosa more resistant and therefore reduces the secretion **[30]**. Thus the overall possible mechanism of action may be due to the antisecretory mechanism.

5. Conclusion

The present investigation revealed that alcoholic extract of *C.limon* contains pharmacologically active Phyto molecule(s) with potential anti-diarrhoeal properties and can be used as a non-specific antidiarrhoeal agent. Since the extract contains a range of compounds, the observed activity may be due to single chemical moiety and group of therapeutically active components like protein, flavonoids, tannin, etc. which may add to the underlying cause of the action. Hence, further extensive and elaborated studies are needed to extract and isolate the bioactive compound (s) for a better understanding of such actions in a more scientific manner**.[29]**

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