Preliminary Photochemical and Anti-diarrhoeal Studies on Aerial Parts of *Phyllanthus fraternus* Web.

Biradar kalyani*[1],Shekshavali.T[2], Vishwanatha swamy.K.M[3], RamChandra Setty.S[3].

1. Department of Pharmacology, H.K.E.S College Of Pharmacy, Gulbarga, Karnataka, India.
2. Department of Pharmacology, National College of Pharmacy, Shimoga-577201, Karnataka. India.
3. Department of Pharmacology, S.C.S. College of Pharmacy, Harapanahalli-583131, Karnataka.India.

***Corresponding Author:**

E-mail: kalyanibiradar@rediffmail.com

Ph. No. + 91-9620631443

Summary

The effect of aerial parts of *Phyllanthus fraternus* Web 70% Methenolic extract were tested on castor oil induced diarrhea in rats and charcoal meal test in mice. The effect of investigated tested drug was studied at two tested doses of 100 and 200 mg / kg body weight of the animals. The anti diarrhoeal activity of tested drug compared with standard drugs (loparamide 10 mg/kg and atropine 1mg/kg) treated animals. In tested group animals were observed in significantly reduced in mean stools weight and mean latent period in castor oil Induced diarrhoea in rats. In charcoal meal test also markedly reduced in percentage of charcoal movement and the investigated drug was improve the percentage of inhibitions in dose dependent way.

Key words: *Phyllanthus fraternus,* anti-diarrhoeal, charcoal Meal,Loperamide

.

Introduction

Diarrhoea is associated with an increased frequency of bowel movements with the production of soft or watery stools. It may be defined as the passage of more than 300ml of liquid faeces in 24 hours. This results in fluid and electrolyte loss that may lead ultimately to death, particularly in young children [1]. Now a day's several synthetic potent anti-diarrheal agents are very much in clinical practice however very less number of herbal drug researches has been done in relatives with anti-diarrhoeal. *Phyllanthus fraternus,* web, (euphorbiceae) is a widespread tropical genus and has been much employed in traditional medicines. As a part of this concept survey of locally available medicinal plants was undertaken. It was observed that the plant *Phyllanthus fraternus* is grown widely and abundantly. In addition, a native practioner has claimed that this plant is very useful as anti-diarrhoeal agent. The other species of *phyllanthus* are known to posse's antioxidant and Hepatoprotective properties [2]. Keeping all these facts in view the present study was aimed at giving a scientific basis for the native claims and traditional knowledge.

Diarrhoea may be due to a specific disease of the intestines or secondary to a disease outside the intestines. For instance, bacillary dysentery direct affects the gut, while diabetes mellitus causes neuropathy diarrhoeal episodes. Diarrhea can be divided in to acute or chronic forms. Infectious diarrhea is often acute; diabetic diarrhea is chronic. Whether acute or chronic, diarrhea has the same path physiologic causes that help in the identification of specific treatments [3].

Phyllanthus fraternus in a widespread tropical genus and has been much employed in traditional medicines [4]. The plant is distributed throughout India as a weed in cultivated and wasteland, the herb is however the good quality of herb collected in the month of Sept-oct month. It is a branching annual glabrous herb, 30-60 cm light with slender spreading leaf-bearing branch lets leaves numerous, distichous, subsesstile, elliptic oblong, obtuse, base rounded flowers yellowish green or whitish, maxillary, males in groups of 1-3 females solitary fruits globular, trilocular, up to 2mm in diameter, pale green in color occurring in axial of leaves most restricting to the lower side of stem [5].

The study was undertaken to validate its folklore claims, for therapeutic activity scientifically and justify its use in the indigenous systems of medicine.

The plant *Phyllanthus fraternus* Web.(Euphorbiaceae) aerial part has been claimed as anti-diarrhoeal profile in an ancient literature. The present study was designed to investigate anti-diarrhoeal activity of 70% Methanolic extract of aerial parts of *Phyllanthus fraternus* Web on normal healthy Wister albino rats and mice.

Materials and Methods

The plant material and preparation of extracts

Phyllanthus fraternus web aerial parts were collected from the surrounding villages of Harapanahalli in the month of Sept-Oct. The plant was identified and authenticated by Department of Pharmacognosy, S.C.S. College of Pharmacy, Harapanahalli. The plant specimen has been deposited at the herbarium of the college. The aerial part of the plant was shade-dried and crushed at room temperature and pulverized. The powder obtained was subjected to soxhlet extraction with 70% methanol which was used for anti-diarrheal activity the extract were concentrated under reduced pressure and stored in a dedicator until further use

Preliminary phytochemical Test

The preliminary phytochemical Screening was carried out on petroleum ether, chloroform, 95% Methanolic extracts, aqueous extract and 70% Methanolic extract of *Phyllanthus fraternus* Web for qualitative identification of type of phytoconstituents present[6] .

Since it was observed that the extracts prepared from polar solvents like 95% and 70% Methanolic extract and aqueous extract possess flavonoids and saponins, tannins, carbohydrates and protein tests.

The Experimental animals and acute toxicity studies:

Adults Wister albino rats weighing between 140-190gmsand adult Swiss albino mice weighing between 20-25gms of either sex were used for the study. The animals were housed in standard polypropylene cages at room temperature and provided with standard diet (gold Mohr Lipton India Ltd) and water was given *ad labium* under strict hygienic conditions and ethical clearance for animal use was obtained from institutional animal ethical committee prior to the activity.

The acute toxicity for 70% metabolic extracts of *Phyllanthus fraternus* Web. were determined on albino mice, the selected animals were divided in to eight group of six in each .the control group received 2ml per kg of vehicle orally, other groups received the extract as test drug in one of dose 100, 200, 400, 800, 1000, 2000&3000mg per kg in a similar manner, after dosing the animals were observed continuously for first behavioral changes and mortality if any at the end of 24 hrs, 48 hrs and 72 hrs respectively.[7]

Anti-diarrhoeal activities:

a) Castor oil induced diarrhea

The method described by Awouters F. et.al[8]. Was followed here with modification. In the present study albino rats of ether sex weighing 150-180 gm were used. They were divided into 4 groups of 6 animals in each. They were fasted overnight before the test with free access to water. Group one served as control, group two treated with standard drug loparamide (10 mg/kg p.o.), group third and forth received test drug extract in the dosage of 100 and 200 mg /kg body weight respectively.

After 30 minutes of this treatment, each rat received 1 ml of castor oil orally. Each rat was then housed separately in perforated cage over a clean filter paper. Then diarrhoeal episodes were observed for a period of 6 hours. During that period number and weight of diarrhoeal dropping were noted. Using mean weight of stools, percentage of diarrhea and percentage protection was calculated. Anti-diarrhoeal activity was determined in terms of percentage of protection. The percentage of protection was calculated by following formula

$$\text{Percentage of protection (\%)} = \frac{\text{Total weight of stool of Control animals} - \text{Total weight of stool of drug / extract treated animals}}{\text{Total weight of the stool of control animals.}}$$

The data of stool weight and the number of defecation were expressed as Mean ± SEM. The results were subjected to statistical analysis using ANOVA followed by student's t-test. The observations and results are shown in Table No. 1

b)Intestinal motility test in mice (charcoal meal test)[9].

Mice of either sex weighing 20-25 gms were used the animals were divided into four groups each groups containing six animals. They were fasted for 24 hrs. Before the test with free access to water. Group one served as control, group two treated with standard drug atropine (1 mg/kg i.p.), group third and forth received test drug extract in the dosage of 100 and 200 mg /kg body weight respectively.

Ten minutes after drug administration, 0.5 ml of a 5% charcoal suspension in a 10% aqueous suspension of tragacanth mucilage was administered p.o. to each animal. The animals were sacrificed 30 min later and the abdomen opened. The small intestine was removed, and the

Distance (from the pylorus to the caecum) travelled by the charcoal plug was determined and the percentage of inhibition was calculated by following formula.

$$\% \text{ traveled} = \frac{\text{Distance traveled by the charcoal meal}}{\text{Total length of small intestine}} X100$$

$$\% \text{ of inhibition} = \frac{\text{Total length of the small intestine} - \text{Distance traveled by the charcoal meal}}{\text{Total length of small intestine}} x100$$

All results were subjected for the statistical analysis by ANOVA followed by student's t-test to calculate the significance of result. The observations and results are shown in Table No.3

Results and discussion

Preliminary phytochemical studies revealed that the aerial parts contain flavonoids, carbohydrates, saponins, tannins, glycosides, proteins, in 95% methanolic, aqueous and hydro methanolic (70% methanol) extract where as steroids and alkaloids are found to be absent. The results are shown in Table No.1

An attempt was made to identify LD_{50} of 70% methanolic extract of *Phyllanthus fraternus* Web. Aerial parts. Since no mortality was observed at 2000 mg/kg. It was thought that 2000mg/kg was the cut off dose. Therefore $1/10^{th}$ and $1/20^{th}$ dose (i.e. 200mg/kg and 100mg/kg) were selected for the study.

Mean weight of stool and mean latent period are parameter study assess the castor oil induced diarrhoea in rats in control animals mean weight of stools was 3.82 ± 0.42 gm and latent period 21.75 ± 0.08 min. Upon loperamide treatment the mean stool weight 1.30 ± 0.07 gm and latent period was elevated 40.0 ± 2.19 min. Similarly there was significantly reduced of stools weight 1.92 ± 0.08, 1.37 ± 0.13 and mean latent period 29.75 ± 1.93, 28.75 ± 3.25 on 70% methanolic extract of 100 mg/kg and 200 mg/kg treatment on dose dependent manner.

The results are compelled on Table No. 2 and graphically depicted in Fig. No.1 and 2.The movement of the charcoal meal i.e. percentage of charcoal traveled in the intestine in the mice are the parameter study in the anti-gastro intestinal motility effect. In control group, charcoal meal 97% has traveled were as in atropine (1 mg/kg dose) treatment is 58%, similarly treatment with tested extract (100 mg/kg and 200 mg/kg p.o) in significantly reduced in percentageof charcoal movement in dose dependent way 78% and 63% The results are compelled in Table No 3 and graphically depicted on Fig. No. 3 and 4.

It was observed that alkaloids, flavonoids, carbohydrates, saponnins, tannins and proteins are present in aerial parts of the plant. There are reports that flavonoids and polyphenolic compounds like flavonoids and tannins possess very good anti- property.

It is evident from our study that, 70% Methanolic extract of *P. fraternus* possesses anti-diarrhoeal property as indicated by the reduction in the percentage charcoal movement and mean stool weight and mean latent period in castor oil induced diarrhoea. Probably tannins content of the plant may be responsible for this activity.

There is a need to undertake further studies to isolate phytoconstituents responsible for diarrhoeal protection so as to identify the lead molecule responsible for the anti-diarrhoeal activity.

Table No. 1
Preliminary phytochemical studies of different extract of *Phyllanthus fraternus* Web.

Phytochemical constituents	Petroleum ether extract	Chloroform extract	Aqueous extracts	Methanol extract	70% Methanol extract
Alkaloids	–	–	+	+	+
Flavonoids	–	–	+ +	+ + +	+ +
Carbohydrates	–	–	+ +	+ +	+ + +
Saponins	–	–	+ +	+ +	+ + +
Tannins	–	–	+	+ +	+ + +
Glycosides	–	–	–	+	–
Proteins	+	+ +	+ +	+	+ +
Steroids	–	–	–	–	–

– Absent
\+ indicates presence
\+ + more clarity
\+ + + better response

Table No. 2
Effect of 70% Methonolic extract of *Phyllanthus fraternus* Web. Castor oil induced Diarrhoea in rats

Groups	Treatment	Mean wt. of stools ± SEM in gms	Mean latent period ± SEM in min	% of inhibition
I	Control	3.82 ± 0.42	21.75 ± 0.08	–
II	Loperamide (10 mg/kg)	1.30 ± 0.07*	40.00 ± 2.19*	69.90
III	70% Methanolic Extract (100 mg/kg)	1.92 ± 0.08*	29.75 ± 1.93	55.55
IV	70% Methanolic Extract (200 mg/kg)	1.37 ± 0.13*	28.75 ± 3.25*	68.75

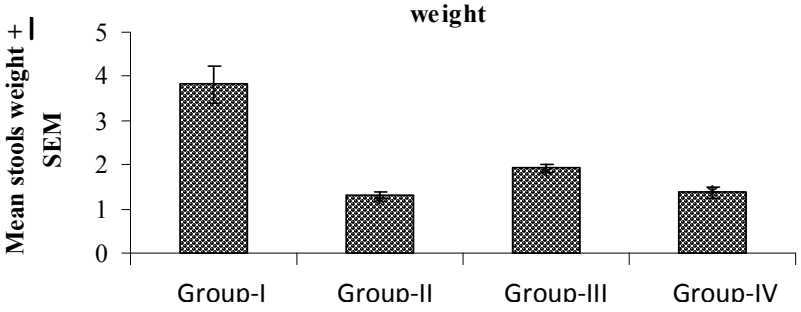
*P<0.001 (vs. Control).

Table No. 3
Effect of 70% alcoholic extracts of *Phyllanthus fraternus* Web. Charcoal meal test in mice

Groups	Treatment	Total length of intestine Mean ± SEM	Total charcoal traveled Mean ± SEM	% of Charcoal traveled	% of inhibition
I	Control	42.66 ± 0.95	42.00 ± 1.03	98.00 %	–
II	Standard	45.50 ± 0.56*	26.83 ± 1.81**	58.96 %	41.03 %
III	Methanolic (100mg/kg)	47.50±0.42**	37.16 ± 1.24	78.23 %	27.82 %
IV	Methanolic (200mg/kg)	43.33 ± 0.76	27.50 ± 0.47**	63.46 %	36.53 %

*P < 0.05, **P<0.001 (vs. Control).

Fig No. 1
Effect of 70% methonolic extract of *Phyllanthus fraternus* Web. in Castor oil induced diarrhoea in rats, mean stools weight



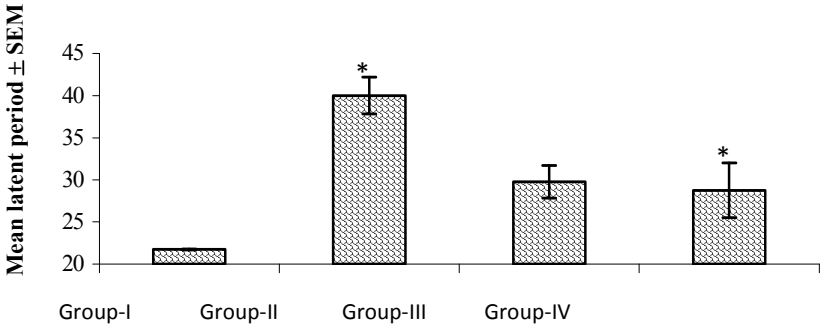
Group-I- Control (0.5 ml of 5% acacia and 1.0 ml of castor oil)

Group-II- Standard (loperamide 10 mg/kg and 1.0 ml of castor oil)

Group-III - 70% Methanolic Extract (100mg/kg) and 1.0 ml of castor oil)

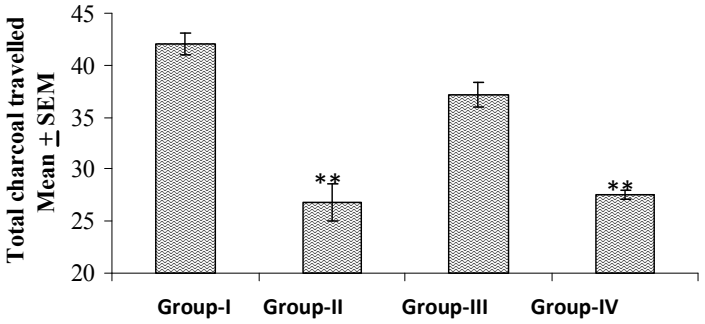
Group-IV - 70% Methanolic Extract (200mg/kg) and 1.0 ml of castor oil)

Fig No. 2
Effect of 70% methonolic extract of *Phyllanthus fraternus* Web. (aerial parts) in Castor oil induced diarrhoea in rats, mean latent period



Group-I - Control (0.5 ml of 5% acacia and 1.0 ml of castor oil)
Group-II - Standard (loperamide 10 mg/kg and 1.0 ml of castor oil)
Group-III - 70% Methanolic Extract (100mg/kg) and 1.0 ml of castor oil)
Group-IV - 70% Methanolic Extract (200mg/kg) and 1.0 ml of castor oil)

Fig No. 3
Effect of 70% alcoholic extract of *Phyllanthus fraternus* Web. (aerial parts) in Charcoal meal test in mice total charcoal travelled



Group-I: Control

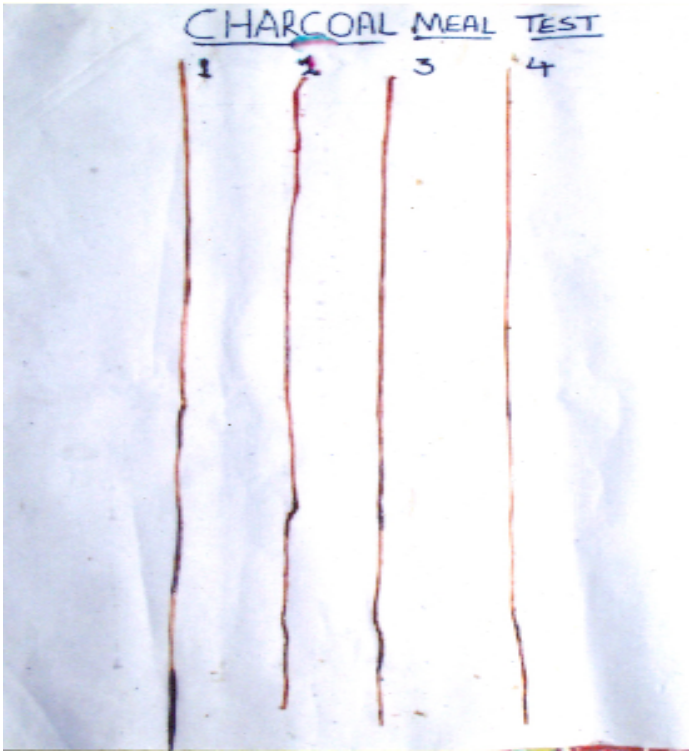
Group: II- Standard (atropine 1mg/kg)

Group-III: 100mg/kg. 70% Methanolic Extract (100mg/kg)

Group-IV: 200mg/kg 70% Methanolic Extract (200mg/kg)

Illustration of Charcoal traveled intestinal motility in mice.

(1,2,3&4 indicates control, standard, test 1 and test 2groups)



References

1. Fordtran JS. Speculations on the pathogenesis of diarrhoea. Fed. Proc., 1967; 26: 1405.
2. Ahmed B, Howiriny and Methew R, Antihepato-toxic activity of Phyllanthus fraternus. Pharmazie 2002; 57(12): 855-856.
3. Rohde J, Northrup RS. Diarrhoea is a nutritional disease. Vol 25. Indian Paediatrics 1988: 914-29.
4. Kurian JC. Plants that heal, Pune. Oriental wat duman publishing house, 1999: 207.
5. Asima Chatterjee, Satyesh C, Pakrashi. The treatise on Indian Medical Plants,New Delhi. Publication and information directorate, CSIR, 1995: 3: 52.
6. Kokate CK. Practical Pharmacognosy, Vallaha Prakashan, 3rd ed., 1991; 107-21.
7. Mrs Prema Veeraraghavan, Expert Consultant, CPCSEA, OECD guideline No. 420. OECD Environment, Health and safety publications environment directorate 2001; 423, 425.
8. Rajeshwara Rao P, Trilochana Y, Chaitanya KK. Anti-diarrhoeal and anti-microbial activities of bark and leaf extracts of *Xylocarpus granatum* koenig. J Nat Rem 2003; 3(2): 155-60.
9. Olajide OA, Awe SO, Makinde JM. Pharmacological studies on the leaf of Psidium guajava. Fitoterapia 1999; 70: 25-31.