

EVALUATION OF IN-VITRO HYPOGLYCEMIC ACTIVITY OF *Phyllanthus fraternus*

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ABSTRACT

The standardized hydroethanolic extract of *Phyllanthus fraternus* was studied for its effects on glucose diffusion and amylolysis kinetics using invitro models. The results verified the antidiabetic potential of the standardized extract of *Phyllanthus fraternus*.

KEYWORDS: *Phyllanthus fraternus*, Glucose Diffusion, Amylolysis Kinetics.

Phyllanthus fraternus Webster Linn (family, Euphorbiaceae) is an annual herb, whose stem is non-erect and 30 cm long, the leafy shoots are 5–10 cm long, oblong and joined to the brachlets of the stem, six sepals in the flower and well distributed in India. It is used as a folklore remedy for the treatment of various diseases of liver by traditional healers and tribals. (Calixto et al., 1998, Binay Sen et al., 2011, Sharma et al., 2012).

The present study was undertaken to verify the antidiabetic potential of *Phyllanthus fraternus* using various in vitro techniques and also as an attempt to predict its mechanism of action.

MATERIALS AND METHODS

Plant Material

Phyllanthus fraternus Webster was obtained from different places in Karad western Maharashtra. The plant was identified and authenticated by Botanical survey of India, Pune.

Preparation of the *Phyllanthus fraternus* Extract [PFE]

The dried leaves, stems and roots of *Phyllanthus fraternus* was minced and extracted with 70% ethanol-water in the proportion of 70:30, being stirred and macerated at room temperature (22-28°C) for 15 days. The ethanol was evaporated and the extract (yield 5-7%) was concentrated to the desired level and stored in a refrigerator. The extracts were dissolved in 0.9% NaCl solution or distilled water to the desired concentration just before use.

Chemicals

Glucose oxidase peroxidase kit was procured from Pathozyme Diagnostics, Kagal, India. Dialysis bags (12 000 MW cutoff; Himedia laboratories, India) were

used. All the chemicals used in the study were of extra pure analytical grade.

Evaluation of Antidiabetic Activity of *Phyllanthus fraternus* Extract Using Various in Vitro Methods

1. Effect of *Phyllanthus fraternus* extract on in-vitro glucose diffusion

It was performed according to the method stated by (Ahmed et al., 2011). A total of 25 mL of glucose solution (20 mmol/ L) and the samples of plant extracts (1%) were dialyzed in dialysis bags against 200 mL of distilled water at 37 °C in a shaker water bath. The glucose content in the dialysate was determined at 30, 60, 120 and 180 min using glucose oxidase peroxidase diagnostic kit. A control test was carried out without sample.

2. Effect of *Phyllanthus fraternus* extract on in-vitro amylolysis kinetics (Ou et al .,2001)

A total of 40 g of potato starch was added to about 900 mL of 0.05 mol/L phosphate buffer (pH 6.5). The solution after stirring at 65 °C for 30 min was made up to a final volume of 1 000 mL to give a 4% (w/v) starch solution. And 25 mL of the above starch solution, α -amylase (0.4%), and the plant extracts (1%) were dialyzed in a dialysis bags against 200 mL of distilled water at 37 °C (pH 7.0) in a shaker water bath. The glucose content in the dialysate was determined at 30, 60, 120 and 180 min. A control test was carried out without sample.

Statistical analysis- All the determinations were carried out in triplicates and data were analyzed by ANOVA followed by students T test. Values were considered at P<0.05.

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RESULTS

Effect of *Phyllanthus fraternus* Extracts on in Vitro Glucose Diffusion

The effect of the PFE extracts on retarding glucose diffusion across the dialysis membrane is shown in T. 1. The rate of glucose diffusion was found to

increase with time from 30 to 180 min. In the present study, the movement of glucose across the dialysis membrane was monitored once in 30 min till 180 min and it was found that PFE demonstrated significant inhibitory effects on movement of glucose into external solution across dialysis membrane compared to the control.

Table 1: Effect of *Phyllanthus fraternus* on glucose diffusion and GDRI.

Sample	Glucose content in dialysate (mmol/L)			
	30 min	60 min	120 min	180 min
Control	0.90 ± 0.01	1.27 ± 0.01	1.77 ± 0.01	1.95 ± 0.01
PFE	0.71 ± 0.02	1.14 ± 0.02	1.61 ± 0.02	1.50 ± 0.03
GDRI	26.22	18.77	13.22	10.13

Mean values (n=3) significantly from each other (P < 0.05)

Effect of *Phyllanthus fraternus* Extracts on in Vitro Amylolysis Kinetics

The effects of PFE on the amylolysis kinetics are shown in the T. 2. The GDRI was found to be 47.23 % at

60 min which gradually got reduced to 13.43% at 120 min.

Table 2: Effect of *Phyllanthus fraternus* on starch digestibility and GDRI.

Sample	Glucose content in dialysate (mmol/L)			
	30 min	60 min	120 min	180 min
Control	0.0	0.21 ± 0.01	0.29 ± 0.01	0.37 ± 0.01
PFE	0.0	0.13 ± 0.01	0.33 ± 0.02	0.38 ± 0.01
GDRI	100	47.23	24.13	13.43

Mean values (n=3) significantly from each other (P < 0.05)

DISCUSSION

(Ou et al., 2001) have mentioned several possible factors that may be responsible for α -amylase inhibition such as fiber concentration, the presence of inhibitors on fibers, encapsulation of starch and enzyme by the fibers present in the sample, thereby reducing accessibility of starch to the enzyme, and direct adsorption of the enzyme on fibers, leading to decreased amylase activity (Ou et al., 2001). GDRI is a useful in vitro index to predict the effect of a fiber on the delay in glucose absorption in the gastrointestinal tract. A higher GDRI indicates a higher retardation index of glucose by the sample. The GDRI for PFE was found to be 26.22 % at 30 min.

Amylolysis kinetic experimental model the rate of glucose diffusion was found to increase with the time from 30 to 180 mins and both the extracts demonstrated significant inhibitory effects on movement of glucose into external solution across dialysis membrane as compared to control.

To conclude, the results of the present study suggest hypoglycemic effect of *Phyllanthus fraternus* extracts are mediated by the by decreasing glucose diffusion rate.

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