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EVALUATION OF IN-VITRO GLUCOSE DIFFUSION AND AMYLOLYSIS KINETICS OF *Phyllanthusamarus* STANDARDIZED EXTRACTS

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ABSTRACT

The standardized aqueous and methanolic extract of *Phyllanthusamarus*were studied for their effects on glucose diffusion and amylolysis kinetics using invitro models. The results verified the antidiabetic potential of the standardized aqueous and methanolic extract of *Phyllanthusamarus*.

KEYWORDS: *Phyllanthusamarus*; Glucose Diffusion; Amylolysis Kinetics.

The plants belonging to the genus Phyllanthus (Euphorbiaceae) are widely distributed throughout the world (Calixtoet al ;1998.,Bagalkotkar et al ;2006) .*Phyllanthusamarus* species is used in folk medicine for treatment of several diseases, such as disturbances of kidney and bladder calculi, diabetes and hepatitis B virus (Bagalkotkar et al ;2006.,Patel et al ;2011)**.** The review of the literature suggests that good number of preclinical and clinical studies have confirmed the medicinal use of various *Phyllanthusamarus* (Calixto et al; 1998., Bagalkotkar et al; 2006., Patel et al; 2011).

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

MATERIALS AND METHODS

Extracts of *Phyllanthusamarus*

The standardized water extract of *Phyllanthusamarus* whole plant [PAAE] Reference No: SR/KN/CL/1/2012-L12030241, was procured as a gift sample from Chemiloids Ltd., Vijaywada. Standardized methanolic extract of *Phyllanthusamarus* leaf [PAME] (Methanol extract contains >2.5% of Phyllanthin and Hypophyllanthin) Report No: FP1112042- PA/11LOT05 was procured as a gift sample from Natural Remedies Pvt. Ltd., Bangalore.

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 usedin the study were of extra pure analytical grade.

Evaluation of antidiabetic activity of phyllanthusextracts usingvarious in vitro methods

1. Effect of phyllanthusextracts on in-vitro glucose diffusion

It was performed according to the method stated by(Ahmed and Sairam;2011). A total of 25 mL of glucose solution (20 mmol/L) and the samples of plant extracts (1%) were dialyzed indialysis bags against 200 mL of distilled water at 37 °C in ashaker water bath. The glucose content in the dialysate wasdetermined at 30, 60, 120 and 180 min using glucose oxidaseperoxidase diagnostic kit. A control test was carried outwithout sample. (Table 1)

Table 1: Effect of selected samples 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
PAAE	0.63 ±0.02	1.12 ± 0.02	1.61 ± 0.02	1.77 ± 0.03
GDRI	29.34	17.01	13.14	11.22
PAME	0.68 ± 0.01	1.18 ± 0.01	1.59 ± 0.01	1.81 ± 0.01
GDRI	30.52	15.52	10.16	7.87

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

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2. Effect of phyllanthus extracts on in-vitro amylolysis kinetics (Ou et al;2001)

A total of 40 g of potato starch was added to about 900 mLof 0.05 mol/L phosphate buffer (pH 6.5). The solution afterstirring at 65 °C for 30 min was made up to a final volumeof 1 000 mL to give a 4% (w/v) starch solution. And 25 mL ofthe above starch solution, α-amylase (0.4%), and the plantextracts (1%) were dialyzed in a dialysis bags against 200 mLof 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.(Table 2)

Table 2: Effect of selected samples 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
PAAE	0.0	0.11± 0.02	0.30± 0.02	0.36± 0.02
GDRI	100	45.57	22.25	11.24
PAME	0.0	0.13± 0.02	0.27± 0.02	0.33± 0.02
GDRI	100	35.22	14.15	6.12

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

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

RESULTS

Effect of phyllanthusextracts on in vitro glucose diffusion

The effect of the plant extracts on retarding glucose diffusion across the dialysis membrane is shown in Table 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, both the samples of phyllanthus extracts demonstrated significant inhibitory effects on movement of glucose into external solution across dialysis membrane compared to control.

Effect of phyllanthus extracts on in vitroamylolysis kinetics

The effects of phyllanthus on the amylolysiskinetics are shown in the Table 2. The GDRI was found to be45.57 % and 35.22 % in PAAE and PAMErespectivelyat 60 min which gradually got reduced to 11.24 % and 6.12%respectively at 120 min.

DISCUSSION

The retardation of glucose diffusion may also be due to the inhibition of α-amylaseby the plant extracts thereby limiting the release of glucosefrom the starch. (Ou et al;2001). have mentioned several possiblefactors that may be responsible for α-amylase inhibitionsuch as fiber concentration, the presence of inhibitors onfibers, encapsulation of starch and enzyme by the fiberspresent in the sample, thereby reducing accessibility ofstarch to the enzyme, and direct adsorption of the enzymeon fibers, leading to decreased amylase activity (Ou et al;2001).GDRI is a useful in vitro index to predict theeffect of a fiber on the delay in glucose absorption in thegastrointestinal tract. A higher GDRI indicates a higherretardation index of glucose by the sample. The GDRIwas found to be 29.34% and 30.52% in PAAE and PAME respectively 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 phyllanthusextracts are mediated by the by decreasing glucose diffusion rate.

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