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In vitro evaluation of antidiabetic potential of leaf and stem extracts of *Solanum xanthocarpum* and *Solanum nigrum*

Selvi. R[1] and Yogananth, N[2*]
[1]Research and Development Centre, Bharathiar University, Coimbatore-641 046
[2]PG & Research Department of Biotechnology, Mohamed Sathak College of Arts & Science, Chennai, Tamilnadu, India
*Corresponding author: *bioyogaa@gmail.com*

Abstract

Diabetes mellitus is a metabolic disease characterized by high blood glucose level resulting from defects in insulin secretion, insulin action or both. Now a days, diabetes is considered as a big killer and is among the most significant diseases in the developed world. The incidence of diabetes is increasing every day and this indicates the increasing need for the treatment of diabetes. The blood glucose level can be regulated by various mechanisms. In the present study *S. xanthocarpum* and *S. nigrum* leaf and stem extracts were screened for antidiabetic activity in *invitro*. *In vitro* antidiabetic assays such as glycosylation of hemoglobin assay, glucose uptake by yeast cells and alpha amylase inhibition assay and are performed. Inhibition of glycosylation of haemoglobin and α- amylase inhibition was in a dose dependent manner and glucose transport differs with the sample and glucose concentration. The results of the work indicate that the both plant extracts possessed considerable *in vitro* anti diabetic activity and can be applied as alternative in the treatment of diabetes and diabetic induced complication

Keywords: *Solanum xanthocarpum*, *Solanum nigrum*, *In vitro* assay, antidiabetic activity.

Introduction

Diabetes mellitus results from the defects in the insulin secretion and action, this may be characterized by chronic hyperglycemia, which is connected with the carbohydrates, protein and lipid metabolism (WHO, 1999). It is a major public health problem currently affecting 284.6 million people worldwide and according to the latest International Diabetes Federation estimates it is expected to affect 438.4 million adults by 2030 becoming one of the world's main disabler and killer (IDF, 2009). Currently, the management of diabetes without any side effect is still a challenge to the medical community. The use of the drugs is restricted by their pharmacokinetic properties, secondary failure rates and accompanying side effects (Vishwakarma *et al*., 2010). Thus searching for a new class of compounds is essential to overcome diabetic problems. There is continuous search for alternative drugs (Syamsudin, 2010).

Herbal preparations are used to treat diabetes, as an alternative therapy but their reported hypoglycemic effects are multifarious. There are more than 200 compounds from plant sources that have been reported to show blood glucose lowering effect. The wide variety of chemicals classes indicates that a variety of mechanisms of action are likely to be involved in lowering blood glucose levels (Kim *et al* ., 1998). The Literature survey revealed that several thousands of plants showed the antidiabetic activity but the study

lacks the proper scientific validation and systematic evaluation (Jia *et al*., 2009).

S. xanthocarpum (Solanaceae) is one of the important medicinal plants distributed throughout India. It is one of the major medicinal plants used in other Indian traditional medicines as well. The leaves are used for treatment of piles, rheumatism. applied locally to relieve pain, a decoction of the plant is used in cases of gonorrhoea. The fruit is bitter, digestible; improve appetite, good in disease of heart, prutitus, asthma, fever, anthelmintic, anaphrodiasiac, causes biliousness, laxative, good in inflammations, chronic bronchitis, muscular pains, dysuria, stone bladder, sterility in wome (Manandhar, 1996). *Solanum nigrum* L. (Black night shade) a member of the Solanaceae, has a wide range of medicinal values. The herb is antiseptic, antidysenteric and antidiuretic used in the treatment of cardiac, skin disease, psoriasis, herpsvirus and inflammation of kidney. The root bark is laxative, useful in the treatment of ulcers on the neck, burning of throat, inflammation of liver and chronic fever. Berries are bitter and pungent useful in the heart disease, piles, dysentery (Yogananth *et al*., 2009).
In the view of the abovementioned facts, the present investigation is directed to the exploration of the antidiabetic activity based on the study of the leaf and stem extracts of *S. xanthocarpum* and *S. nigrum* which show inhibitory effect of glucose utilization glucose uptake by yeast cells and alpha amylase inhibition, are in use as hypoglycemic agent in traditional system of medicine.

Materials and Methods

The fresh stem and leaves of *S. xanthocarpum* and *S. nigrum* were collected locally and shade dried. The plant parts were powdered mechanically and stored in an air tight container. The extraction was carried out by hot percolation method using Soxhlet apparatus. The solvent used was ethanol. About 40 gm of powder was extracted with 200 ml of ethanol. The extract was concentrated to dryness under controlled temperature 40-50°C. The extract was preserved in refrigerator till further use.

Non-enzymatic glycosylation of haemoglobin method - (Acharya *et al*., 1980)

Invitro Antidiabetic activity of ethanol extracts of *S. xanthocarpum* and *S. nigrum* stem and leave were investigated by estimating degree of non-enzymatic haemoglobin glycosylation, measured colorimetrically at 520nm. Glucose (2%), haemoglobin (0.06%) and Gentamycin (0.02%) solutions were prepared in phosphate buffer 0.01 M, pH 7.4. 1 ml each of above solution was mixed. 0.5 and 1.0ml of each concentration was added to above mixture. Mixture was incubated in dark at room temperature for 72 hrs. The degree of glycosylation of haemoglobin was measured colorimetrically at 520nm. Metformin was used as a standard drug for assay. % inhibition was calculated

$$\% \text{ inhibition} = \frac{As - Ac}{As} \times 100$$

b) Glucose uptake in Yeast cells method- (Cirillo, 1962)

The commercial baker's yeast was washed by repeated centrifugation (3,000×g; 5 min) in distilled water until the supernatant fluids were clear and a 10% (v/v) suspension was prepared in distilled water. Various concentrations of both plant extracts (1–5 mg) were added to 1ml of glucose solution (5, 10 and 25 mM) and incubated together for 10 min at 37 °C. Reaction was started by adding 100µl of yeast suspension, vortex and further incubated at 37°C for 60 min. After 60 min, the tubes were centrifuged (2,500 × g, 5 min) and glucose was estimated in the supernatant. GLINOSE was taken as standard drug.

c) α- Amylase Inhibition method – (Nickavar and Yousefiana, 2009).

1ml of substrate-potato starch (1% w/v), 1ml of drug solution (GLINIL drug/ethanol extract of both plant leaf and stem) of 4 different concentrations such as 250, 500, 750 and 1000µg/ml. 1ml of α- amylase enzyme (1% w/v) and 2ml of acetate buffer (0.1 M, 7.2pH) was added. The mixture was incubated for 1hr.then 0.1 ml iodine-iodide indicator (635mg iodine and 1gm potassium iodide in 250ml distilled water) was added in the mixture. Absorbance was taken at 565nm in UV-Visible spectroscopy. The percentage increase in glucose uptake by yeast cells and % of α-amylase inhibition were calculated using the following formula

Increase in glucose uptake (%) =

$$\frac{\text{Abs sample} - \text{Abs control}}{\text{Abs sample}} \times 100$$

Where, Abs control is the absorbance of the control reaction (containing all reagents except the test sample), and Abs sample is the absorbance of the test sample. All the experiments were carried out in triplicates.

Results and Discussion

Human bodies possess enzymatic and non- enzymatic antioxidative mechanisms which minimize the generation of reactive oxygen species, responsible for many degenerative diseases including diabetes. Increased concentration of glucose in the blood leads to its binding to hemoglobin which may result in the formation of the reactive oxygen species (Bailey and Day, 1989).

Non enzymatic glucosylation of haemoglobin method

The plant extracts significantly inhibited the haemoglobin glycosylation which is indicated by the presence of increasing concentration of haemoglobin. The leaf extracts of *S. xanthocarpum* and *S. nigrum* exhibited higher inhibition of glycosylation (90% and 88% in 1 µg/ml, respectively) as compared with the standard drug (36% in 1 µg/ml). The plant extracts also displayed the inhibition of haemoglobin glycosylation at different physiological concentrations of the glucose over the period of 72 hrs, indicating that the plant extracts decreases the formation of the glucose- haemoglobin complex and thus amount of free haemoglobin increases (Table 1)

Table1: Non enzymatic glucosylation of haemoglobin method

Con (µg/ml)	Blank (control)	Standard		Ethanol extract of *S. xanthocarpum* leaf		Ethanol extract of *S. xanthocarpum* stem		Ethanol extract of *S. nigrum* leaf		Ethanol extract of *S. nigrum* stem	
		Abs	Inh %	Abs	Inh%	Abs	Inh%	Abs	Inh%	Abs	Inh%
0.5	0.078 ±0.003	0.092±0.005	21%	0.608±0.031	88%	0.533±0.068	76%	0.607±0.025	88%	0.365 ±0.04	79%
1.0		0.112±0.005	36%	0.743±0.046	90%	0.598±0.008	89%	0.639±0.039	88%	0.538 ±0.008	87%

values are expressed as mean ± SEM

Glucose uptake in yeast cells

This assay is based on the movement of glucose across the membrane of yeast cells, with the help of the cc fractioned plant extract. The yeast cells were suspended in plant extract and various concentrations of glucose (1ml to 5 µg/ml). The plant extract enhances the yeast cells to take in the glucose. The amount of glucose remaining in the solution after incubation was observed. From the results, it was found that the percentage increase in glucose uptake by yeast cells at 2 µg/ml glucose concentration with ethanolic extract ranges from 50 – 76% and minimum uptake of glucose at 1 µg/ml glucose concentration (21 – 50%). The result suggests that ethanol extract of *S. nigrum* leaf exhibited maximum level inhibition was recorded than other extracts tested (Table 2).

Table-2: Glucose uptake in yeast cells

Conc (µg/ml)	Blank (control)	Standard		Ethanol extract of *S. xanthocarpum*		Ethanol extract of *S. xanthocarpum* stem		Ethanol extract of *S. nigrum* leaf		Ethanol extract of *S. nigrum* stem	
		Abs	Inh%	Abs	Inh%	Abs	Inh%	Abs	Inh%	Abs	Inh%
1.0	0.105± 0.020	0.092± 0.005	21%	0.113± 0.005	36%	0.136± 0.008	47%	0.097± 0.006	24%	0.147± 0.008	50%
2.0		0.153± 0.020	50%	0.208± 0.041	63%	0.138± 0.031	43%	0.315± 0.047	76%	0.159± 0.030	50%
3.0		0.171± 0.011	58%	0.133± 0.015	44%	0.139± 0.014	47%	0.213± 0.041	64%	0.194± 0.041	59%
4.0		0.155± 0.003	57%	0.166± 0.021	55%	0.149± 0.037	46%	0.086± 0.006	14%	0.216± 0.058	62%
5.0		0.162±0. 020	54%	0.149± 0.008	51%	0.103± 0.010	28%	0.149± 0.008	51%	0.189± 0.037	58%

values are expressed as mean ± SEM

Alpha amylase inhibition method

α-amylase is an enzyme that converts starch to glucose in its presence. When α- amylase, glucose, plant extract are taken together as a solution, the plant extract causes the inhibition of enzyme activity (Suhashini *et al*., 2014). The percentage inhibition of α amylase increases from 37 to 88% with increasing concentration of cc fractioned plant extract (750 and 1000 µl) (table 3). The standard drug of Glinil exhibited the rate of glucose inhibition maximum level 51% and minimum level 26%.

Table 3: Alpha amylase inhibition method

S no	Conc (ml)	Blank (control)	Standard		Ethanol extract of *S. xanthocarpum* leaf		Ethanol extract of *S. xanthocarpum* stem		Ethanol extract of *S. nigrum* leaf		Ethanol extract of *S. nigrum* stem	
			Abs	Inh %	Abs	Inh %	Abs	Inh %	Abs	Inh %	Abs	Inh %
1	250 µl	0.09±0.003	0.165±0.008	51%	0.119±0.038	56%	0.766±0.035	71 %	0.286±0.026	87 %	0.230±0.035	63 %
2	500 µl		0.114±0.018	26%	0.502±0.046	83%	0.580±0.124	55 %	0.203±0.050	86 %	0.453±0.069	81 %
3	750 µl		0.135±0.006	41%	0.651±0.070	87%	0.580±0.100	71 %	0.546±0.021	84 %	0.540±0.105	84 %
4	1000 µl		0.119±0.010	32%	0.642±0.035	87%	0.710±0.030	71 %	0.526±0.017	88 %	0.536±0.073	84 %

In this present study we evaluated *in vitro* Non enzymatic glucosylation of haemoglobin method, Glucose uptake in yeast cells and alpha amylase inhibition of crude ethanol extracts of *S. xanthocarpum* and *S. nigrum*. However further pharmacological and biochemical investigations will clearly elucidate the mechanism of action and helpful in projecting both plant as a therapeutic target in diabetes research.

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