Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6, Issue 6, 2014

Original Article

PRELIMINARY QUALITATIVE PHYTOCHEMICAL SCREENING AND IN VITRO HYPOGLYCEMIC POTENTIAL OF ACANTHUS ILICIFOLIUS AND EVOLVULUS EMERGINATUS

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Received: 28 Apr 2014 Revised and Accepted: 27 May 2014

ABSTRACT

Objective: The present study was planned to explore phytoconstituents and hypoglycemic potential of the leaf extracts of *Acanthus ilicifolius* and *Evolvulus emerginatus* by invitro method.

Methods: The aqueous, ethanol and methanol leaf extracts were prepared by soxhlation method. Qualitative phytochemical screening was done by using Horborne method. Methanol leaf extracts and standard drug acarbose were analyzed for their inhibition on α-amylase and α-glucosidase by standard DNSA method. Glucose diffusion inhibitory activity was analyzed by using dialysis membrane. IC_{50} values were calculated from the dose response curve of standard drug acarbose.

Results: Phytochemical analysis revealed that the methanol leaf extract contains maximum phytoconstituents than aqueous and ethanol extract. Methanol leaf extracts showed maximum inhibition on α-amylase and α-glucosidase inhibitory effect in a concentration dependant manner. Inhibitory activity of *A. ilicifolius* on α-amylase was 82.32 ± 0.02% (IC_{50} 33.13 ± 0.030µg /ml) and on α-glucosidase was 79.35 ± 0.02% (IC_{50} 39.42 ± 0.01µg/ml). E.*emerginatus* extract inhibitory effect on α- amylase was 78.26 ± 0.014% (IC_{50} 29.76 ± 0.07µg/ml) and on α-glucosidase was 80.8 ± 0.08% (IC_{50} 28.04 ± 0.01µg/ml).The inhibitory activity of acarbose on α-amylase was 84.11 ± 0.57% (IC_{50} 44.16 ± 0.15µg/ml) and on α-glucosidase was 85.0 ± 0.22% (IC_{50} 39.86 ± 0.34µg /ml). Methanol leaf extracts of these two plants effectively retarded the glucose diffusion across the dialysis membrane.

Conclusion: The *invitro* analysis proves the function of extracts in lowering glucose absorption and decrease post prandial blood glucose concentration. Hence these plant extracts can be used for *invivo* animal studies and development of a new drug without any harmful side effects.

Keywords: Phytochemistry, Medicinal plants, Hypoglycemic agent, *Acanthus ilicifolius*, *and Evolvulus emerginatus*.

INTRODUCTION

Diabetes mellitus is a metabolic chronic disorder characterized by increased blood glucose level. It is a major health problem which causes considerable morbidity and mortality due to micro and macro vascular complications [1]. The prevalence of diabetes is increasing globally and the maximum is in India, where the number of diabetics will rise from 19 million in 1995 to 57 million in 2025. India is thus designated as the diabetic capital of the world [2]. Some synthetic oral hypoglycemic drugs like acarbose and miglitol are used currently to treat diabetes. But these synthetic drugs are non specific in their action and fail to reduce diabetic complications. Medicinal plants are considered as a resource of new drugs. They are more potent in the management of diabetes, as they have less side effects, enhanced bioavailability and low cost when compared to the synthetic drugs [3]. Herbal medicines have not gained much importance due to the lack of scientific evidence for their mechanism of action. Inhibition of enzymes like α-amylase and α-glycosidase which involved in the digestion and absorption of carbohydrates is one of the therapeutic approaches for reducing postprandial hyperglycemia [4]. Pancreatic α-amylase is a key enzyme in the digestive system which catalyses the first step in digestion of dietary starch. It converts starch into a mixture of small oligosaccharides which consists of maltose, maltotriose and a number of α (1-6) and α (1-4) oligoglycans. These are further acted upon by α-glucosidase and degraded as glucose. After absorption, glucose enters the blood stream, which leads to post-prandial hyperglycemia. Hence retardation of starch digestion by inhibition of these enzymes plays a major role in the treatment of diabetes [5]. These inhibitors obtained from medicinal plants can be used for effective treatment of diabetes [6]. Natural products containing viscous and complex polysaccharides also decrease post-prandial blood glucose (PPBG) concentration by inhibiting glucose diffusion across intestinal membrane during digestion and absorption [7]. *Acanthus ilicifolius,* a member of Acanthesea family, is lesser known but important folklore medicine which is commonly known as sea-Holy, Holy-mangrove and Hargoza [8]. The plant *Evolvulus emerginatus* is known as mooshikakarnee in Sanskrit belongs to *Convulecea* family and is mainly found in Andhra Pradesh. The present plan is to explore these plants for their hypoglycemic potential by *in vitro* method.

MATERIALS AND METHODS

Chemicals and reagents

Chemicals such as α-amylase, soluble starch, α-glucosidase, Para nitro phenyl glucopyranoside, DNSA and acarbose were purchased from SRL PVT LTD, Mumbai, India. Dialysis membrane, ethanol, methanol solvents were purchased from Hi media laboratories Mumbai, India. All other chemicals, reagents used in this study were AR grade from local manufacturer.

Preparation of various extracts and qualitative phytochemical screening

The leave of A*canthus ilicifolius,* and *Evolvulus emerginatus* were collected from the Morappur forest area, Dharmapuri District, Tamil Nadu during the month of November 2013. It was authenticated and the voucher specimens were prepared and deposited in the Forest department, Dharmapuri district, Tamilnadu, India. Leaves of A*canthus ilicifolius,* and *Evolvulus emerginatus* were washed with distilled water, shade dried, powdered and stored in an air-tight container. 10 g of two plant leaves were soxhleted with 250 ml of different high polar solvents like aqueous, ethanol, and methanol for 24 hours [9]. This was then filtered through a Whatman No. 2 filter paper and kept in vacuum rotary evaporator to get crude extract powder for further use. These leaves extracts were analyzed for identification of phytoconstituents present in them by standard method [10].

Glucose diffusion inhibition assay

Four different concentrations (100µg/ml, 200 µg/ml, 300µg/ml, and 400µg/ml) of methanol leaf extract of two plants and were prepared

by soxhlation. 1 ml of extract was placed in a dialysis membrane (12000 MV Hi media Laboratories Mumbai, India) and 1 ml of 0.22mM glucose in 0.15M NaCl was added. Then the dialysis membrane was tied at both ends and immersed in a beaker containing 40ml 0.15M NaCl and 10ml of distilled water. For control 1ml of 0.22mM glucose in 0.15M NaCl was added in dialysis membrane bag along with 1 ml of distilled water, and immersed in a beaker (40ml 0.15M NaCl + 10ml distilled water). The beakers were kept at room temperature. The glucose movement from internal solution to external solution (beaker solution) was measured every half an hour by DNSA method. Three replications were done for every half an hour for 3 hours [11].

Inhibition assay for α-amylase activity (DNSA method)

Four different concentrations (100µg/ml, 200 µg/ml, 300µg/ml, and 400µg/ml) of methanol leave extracts of two plants and standard drug acarbose were prepared and taken in different test tubes and made up to 1 ml with DMSO. From this 500 µl sample was premixed with equal volume of 0.02M sodium phosphate buffer (pH 6.9 with 0.006M NaCl) containing α-amylase (0.5mg/ml), and incubated for 10 minutes at 25° C. Then 500 µl of 1% soluble starch solution in 0.02M sodium phosphate buffer was added to each concentration sample and incubated for 10 minutes at 25°C. 1 ml of DNSA reagent was added to all the test tubes and kept in a boiling water bath for 10 minutes, cooled and absorbance was taken at 540 nm [12].

% of Inhibition = [(A540 control – A540 sample) / A540 control] X 100

Inhibition assay for α-glucosidase activity:

Four different concentrations of methanol leave extracts (100-400µg/ml) of two plants were prepared. Same concentration of standard drug acarbose was prepared to compare inhibitory activity of these plants. These samples were taken in different test tubes and premixed with α-glucosidase (0.075 units). The substrate, p-nitro phenyl glucopyranoside (3mM), was added in each test tube to start the reaction. The test tubes were incubated at 37°C for 25 minutes and 1 ml of 0.02M Na_2CO_3 was added to stop the reaction. Triplicates are done for each sample at different concentrations. The activity of α-glucosidase was measured by the amount of p-nitro phenol released from the substrate, at 400nm. % of inhibition was calculated by using the formula [13].

% of Inhibition = [(A400 control – A400 sample) / A400 control] X 100

IC_{50} calculation

IC_{50} Value represents the required concentration of inhibitor which inhibits 50% of the enzyme activity. Acarbose was used as positive control and standard dose response curve plotted at different concentrations. From this, IC_{50} values of two plants were calculated using regression analysis and graph pad prism software 5.0 version [14].

Statistical analysis

Data were subjected to one way ANOVA using origin of SPSS software 16.0 version. IC_{50} values were calculated by using graph pad prism software 5.0 version. Values were considered significant at $P \leq 0.05$. All values are expressed as mean ± SEM (n = 3).

RESULTS

Qualitative phytochemical screening

The results of preliminary qualitative phytochemical screening are presented in Table 1. Out of three extracts, methanol leaf extracts showed the maximum number of phytoconstituents for both plants. Tannins, glycosides, flavonoids, proteins, carbohydrates, terpenoids, phenol, steroids and saponins were present in methanolic leaf extract of these two plants. Alkaloids were absent the prepared extract of *Acanthus ilicifolius* and present in aqueous and ethanol leaf extracts of *Evolvulus emerginatus*.

Table 1: Qualitative Phytochemical Analysis

Test	*Acanthus ilicifolius*			*Evolvulus emerginatus*		
	Aqueous extract	Ethanol extract	Methanol extract	Aqueous extract	Ethanol extract	Methanol extract
Flavonoids	–	–	+	–	+	+
Tannins	–	+	+	+	+	+
Alkaloids	–	–	–	+	+	–
Steroids	–	–	+	–	+	+
Saponins	–	+	+	+	+	+
Glycosides	+	+	+	+	+	+
Gums	–	–	–	+	+	+
Proteins	–	+	+	–	+	+
Resin	–	–	–	–	–	–
Carbohydrates	+	+	+	+	+	+
Terpenoids	+	+	+	–	+	+
Cardio glycosides	–	–	+	–	–	–
Phenol	–	+	+	–	+	+

+ Present; – Absent

Table 2: Effect of methanolic leaves extract of *Acanthus ilicifolius* and *Evolvulus emerginatus* on diffusion of glucose through a bio-membrane at different time intervals

Time(Min)	Control	*Acanthus Ilicifolius*		*Evolvulus Emerginatus*	
	Glucose concentration (mg/ml)	Glucose concentration (mg/ml)	Relative movement (%)	Glucose concentration (mg/ml)	Relative movement(%)
30	3.57 ± 0.02**	0.71 ± 0.07	12.49 ± 0.01	0.08 ± 0.01	17.85 ± 0.06
60	4.00 ± 0.09	0.86 ± 0.01	18.59 ± 0.02*	0.71 ± 0.01**	20.23 ± 0.04
90	4.57 ± 0.03	0.57 ± 0.03	19.99 ± 0.05	1.28 ± 0.02	23.99 ± 0.05
120	5.00 ± 0.05	1.00 ± 0.01	21.43± 0.09	1.43 ± 0.09	28.11 ± 0.01
150	5.51 ± 0.09	1.14 ± 0.03	20.00 ± 0.05	1.14 ± 0.09*	28.50 ± 0.03*
180	6.14 ± 0.04*	1.14 ± 0.02	20.50 ± 0.04	1.00 ± 0.09	28.57 ± 0.01

Values are mean±SEM for groups of 3 observations. *p < 0.05 **p<0.01

Glucose diffusion inhibitory test

The results of the glucose diffusion inhibitory test are given in Table 2. Concentration of glucose in control (without plant extract) was considered as 100% relative movement of glucose. The diffusion of glucose was time dependent and maximum diffusion was found at the end of third hour. The inhibitory effect on the relative movement of glucose by *Acanthus ilicifolius* extract was 20.50 ±.05% and *Evolvulus emerginatus* was 28.56 ± 1.00%. It shows that the both plant extracts effectively retards the movement of glucose from dialysis bag into the external solution.

α- amylase inhibition study

The results of inhibition study of α-amylase are summarized in Table 3. α amylase inhibitors offers an effective therapeutic approach for the control of diabetes [14]. These two methanolic leaf extracts show maximum inhibition in a concentration dependant manner. The maximum inhibition of *Acanthus ilicifolius* was 82.32 ± 0.02% at 400µg/ml concentration, inhibition by *Evolvulus emerginatus* was 79.35 ± 0.025% and acarbose shows 84.11 ±0.05% inhibitions on α-amylase.

α - glucosidase inhibition study

The results are tabulated in table 3. *Acanthus ilicifolius* shows maximum inhibition 79.35 ± 0.02% on α-glucosidase at 400µg/ml and *Evolvulus emerginatus* shows slightly higher value of 80.79 ± 0.08%. The standard drug acarbose showed 85.0 ± 0.22% inhibition on α-glucosidase. The *in vitro* α-glucosidase inhibitory studies confirmed that both plant extracts had α-glucosidase inhibitory activity. IC_{50} values for these two plants extract are compared with acarbose and given in table 4.

Table 3: % Inhibition of α-amylase and α-glucosidase by varying concentrations of methanolic extract of *Acanthus ilicifolius* and *Evolvulus emerginatus*

Concentration (µg/ml)	% Inhibition of α-amylase			% Inhibition of α-glucosidase		
	Acanthus ilicifolius	***Evolvulus emerginatus***	**Std drug Acarbose**	***Acanthus ilicifolius***	***Evolvulus emerginatus***	**Std drug Acarbose**
100	66.3 ± 0.07*	63.0 ± 0.07*	65.6±0.99*	73.9 ± 0.02	65.9 ± 0.02	67.06±0.99*
200	75.7 ± 0.05	66.6 ± 0.05	74.03 ±0.40	75.0 ± 0.02	69.6 ± 0.09	76.09 ±0.40
300	79.4 ± 0.02	73.9 ± 0.03	82.07 ± 0.07**	77.5 ± 0.05	75.0 ± 0.03**	83.10± 0.16**
400	82.4 ± 0.02	78.3 ± 0.01	84.11 ± 0.57	79.4 ± 0.03	80.8 ± 0.08	85.0 ± 0.22

Values are Means ± SEM for groups of 3 observations. *p < 0.05* *p<0.01

Table 4: IC_{50} values of *Acanthus ilicifolius* and *Evolvulus emerginatus* on α-amylase and α-glucosidase activity.

Extract	IC_{50} (µg/ml)	
	α-amylase	**α –glucosidase**
Acarbose	44.16 ± 0.15*	39.86 ± 0.34
Acanthus ilicifolius	33.13 ± 0.03	39.42 ± 0.01**
Evolvulus emerginatus	29.76 ± 0.07	28.04 ± 0.01

Values are Means ± SEM for groups of 3 observations. *p < 0.05* *p<0.01

DISCUSSION

Drugs that reduce post-prandial hyperglycemia by suppressing hydrolysis of starch such as carbohydrate hydrolyzing enzyme inhibitors have been found useful in the control of diabetes mellitus [15, 16]. Many herbal extracts have been reported for their anti-diabetic activities and are currently being used in Ayurveda and homeopathy for the treatment of diabetes. However, such medicinal plants have not gained much importance as medicines due to the lack of sustained scientific evidence. An Ethanobotonical study indicates that India is rich in medicinal plants and more than 800 plants are used to control diabetes [17]. But still only few of them were explored scientifically for their hypoglycemic activity [18].

Many modern medicines like aspirin are produced from medicinal plants. Medicines for diabetes from the plants are presently under limited use to popularize it, new set of drugs from the plant origin to fit the present day habits and life style should be developed [19]. The inhibition by natural products is safer than synthetic drugs. Synthetic drugs like metformin causes lactic acidosis, gastrointestinal upset and weight loss [20]. Sulphonyl ureas, metiglinides and TZD cause weight gain, hypoglycemia and heart failure [21]. Therefore, there is a need to search for inhibitors of α-glucosidase and α-amylase from natural resources, which will become an attractive approach for the management of diabetes.

In the present study, two anti diabetic medicinal plants explored for their α-amylase and α-glucosidase inhibitory potential with their mechanism of action which is similar to that of synthetic drug, acarbose. Several studies performed on these plants state them to be hypoglycemic, but none of these plants have been studied or tested for carbohydrate hydrolyzing enzyme inhibitors in order to justify their hypoglycemic property.

The *invitro* studies demonstrated that both *Acanthus ilicifolius* and *Evolvulus emerginatus* effectively reduces glucose passage across dialysis membrane and having appreciable inhibitory activity on α-amylase and α-glucosidase. The percentage inhibition at 100, 200, 300, 400µg/ml concentrations of both plant extracts on α-amylase and α-glucosidase showed a concentration dependant reduction in their activity. The highest concentration (400µg/ml) of *Acanthus ilicifolius* showed a maximum inhibition of nearly 82% and 79% of α-amylase and α-glucosidase respectively. The *Evolvulus emerginatus* showed only slight different inhibition on α-amylase 78% and α-glucosidase activity 80%. These plant extracts showed inhibition on carbohydrate hydrolyzing enzymes more or less similar to that of synthetic drug acarbose. Therefore, these two plant extracts can be used to retard the digestion and absorption of carbohydrate to control sudden rise of post-prandial rise in blood glucose. Up to our knowledge this is the first report for hypoglycemic potential effect of these plants.

CONCLUSION

The results indicated that methanol leaf extracts of these plants prove their function in lowering the rate of glucose absorption and decrease postprandial hyperglycemia. Hypoglycemic potential of plant extracts are due to presence of secondary metabolites, hence further studies are planned for isolation and purification of bioactive constituents from these plants and also perform *in vivo* animal studies to confirm these observations obtained in the present study, which will lead to development of new novel antidiabetic drug.

ACKNOWLEDGEMENTS

We would like to thank the management of VIT University for supporting this study.

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