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Short Communication

IN VITRO ANTIDIABETIC ACTIVITY OF ROOTS OF *ACHYRANTHUS ASPERA*

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ABSTRACT

The present study is aimed to evaluate *in vitro* antidiabetic effect of Ethyl acetate and methanolic extracts of roots of *Achyranthus aspera*. 500 gm of the powdered roots of *Achyranthus aspera* were extracted by maceration method using ethyl acetate and methanol. The maceration was continued for seven days after which, the contents were filtered and concentrated by rota evaporator. Both the extracts are evaluated for *in vitro* antidiabetic activity (α-amylase inhibition assay, α-glucosidase inhibition assay). Ethyl acetate extract and methanolic extracts of *Achyranthus aspera* exhibited *in vitro* antidiabetic activity by inhibition of alpha amylase, alpha glucosidase with IC_{50} values of 25±1.83, 27.62±1.83 and 40±2.92, 38.92±1.93 respectively. The study is concluded that methanolic extract of roots of *Achyranthus aspera* showed better *in vitro* antidiabetic activity compared to ethyl acetate extract.

Keywords: *Achyranthus aspera, Invitro* antidiabetic activity.

1. INTRODUCTION

Diabetes mellitus (DM) is a chronic disorder of carbohydrate, protein, and fat metabolism characterized by persistent hyperglycemia secondary to insulin secretion, insulin action, or both [1]. Recently, diabetes is one of the most prevalent diseases in the world which is rapidly increasing worldwide. According to the WHO, the occurrence of diabetes might increase by 35% in the near future. Currently, over 150 million populations in the world are affected by diabetes, which is likely to increase over 300 million or more by the year 2025. In India, the number of diabetic people will increase from 15 million in 1995 to 57 million in the year 2025, which is considered to be the highest number of diabetics in the world [2]. Researchers are developing a number of oral medicines to treat diabetes. However, these drugs demonstrate significant side effects, including weight gain and gastrointestinal distress. Therefore, finding new potential natural products that prevent DM is necessary. *Achyranthes aspera Linn* is very versatile medicinal herb found as a weed throughout India and in tropical environment. Its roots, seeds and flowers are mainly used for various therapeutic activities in traditional system of medicine. *Achyranthes aspera* is also used by traditional healers to treat diabetes [3]. Till date there is no scientific validation of roots of *Achyranthes aspera* against diabetes. The present study is undertaken to evaluate the *in vitro* antidiabetic activity of *Achyranthes aspera*.

2. MATERIAL AND METHODS

2.1. Collection, Identification and Authentication of Plant

Roots of *Achyranthus aspera* were collected in the regions of Ananthapuramu, Anantapur district and authenticated by Dr. B. Ravi Prasad Rao, Professor, Department of Botany, SKU, Ananthapuramu.

2.2. Chemicals and Reagents

All the chemicals and reagents used in the study were obtained from the standard supplier and were of good quality.

2.3. Extraction

500 gm of the powdered roots of *Achyranthus aspera* were extracted by cold maceration method using ethyl acetate and methanol respectively. The maceration was continued for 24 hours, afterwards the contents were filtered and concentrated by rota evaporator. A brownish and resinous greenish extract respectively was obtained which was calculated for the yield and stored in desiccator till further study.

2.4. *In vitro* antidiabetic activity

2.4.1. Alpha-amylase inhibitory assay

α-amylase inhibition activity was measured according to [4]) with slight modifications using a microplate reader (BioTek instruments, USA). 15µl PBS (pH 6.8) was added in all wells of a 96-well plate. 25µl of 0.14 U/ml enzyme and 1-4 mg/ml samples were pre-incubated at 37 °C for 10 min. The reaction was started with the addition of 40µl starch (2 mg/ml) and incubated for 30 min at 37 °C. 20µl 1M HCL was added to stop the reaction, and 90µl iodine reagent was added. The change in color was observed, and absorbance was measured at 620nm after incubation. The control contained all the reagents except inhibitor/sample. Acarbose was used as the standard or positive control. The IC50 values were derived from the percentage inhibition plot. IC50 defines as the concentration at which inhibitor shows 50% of its inhibition activity. The percentage inhibition was calculated according to the following formula:

Inhibitory activity (%) = (1 − As/Ac) ×100

Where Ac was the absorbance of control without inhibitor and As was the absorbance of the sample.

2.4.2. Alpha-Glucosidase Inhibitory assay

The inhibition activity of α-glucosidase was optimized using the method described by Sundar et al. [5]. The reaction mixture contained 25µl of 20mM p-nitrophenyl-α-D- glucopyranoside (PNPG), 69µl PBS (pH 6.8), 5µl sample of different concentration, and 5µl enzyme (0.637 U). The reaction was incubated at 37° c for 30 minutes. NaHCO3 100µl (0.5mM) was used to stop the reaction and change in absorbance was measured at 405nm by using a microplate reader (BioTek instruments, USA). The inhibition activity was measured by using the following formula:

Inhibitory activity (%) = (1 − As/Ac) ×100

Where Ac was the absorbance of control without inhibitor and As was the absorbance of the sample. Acarbose was used as a positive control.

3. RESULTS AND DISCUSSION

Ethyl acetate and methanolic extract of roots of *Achyranthus aspera* is screened for *in vitro* antidiabetic by α-amylase inhibition assay, α-glucosidase inhibition assay. α- amylase produced in saliva begins the digestion of carbohydrates in the mouth and continued in the intestine. Acarbose is the standard drug possesses greatest α-amylase inhibition activity and delays the hydrolysis of starch into oligosaccharides and disaccharides. α-glucosidase is an enzyme that plays a vital role by breaking down α-1,4-glucosidic linkages of disaccharides [6]. The effects of glucosidase inhibitors and their use on delaying the generation of blood glucose after food uptake has been established by various authors. The ethyl acetate and methanolic extract of roots of *Achyranthus aspera* showed the α-amylase inhibition, α-glucosidase inhibition respectively at IC50 = 25±1.83, 27.62±1.83, 40±2.92, 38.92±1.93, respectively. In conclusion ethyl acetate extract of roots of *Achyranthus aspera* showed significant *in vitro* antidiabetic activity compared to methanolic extract, due to presence of non polar constituents and further study is required to isolate the particular chemical constituent with *in vivo* antidiabetic activity.

Table 1: α-amylase inhibition of methanolic and ethyl acetate extract of *Achyranthus aspera*

Extract	Concentration (mg/ml)	Inhibition (%)	IC_{50} (mg/ml)
Ethyl acetate extract	20	45.81 ± 0.847	25 ± 1. 83
	40	31.68 ± 1.409	
	60	35.73 ± 0.606	
	80	26.00 ± 0.588	
	100	27.08 ± 0.499	
Methanolic Extract	20	46.71 ± 0.54	40 ± 2.92
	40	50.68 ± 1.40	
	60	45.73 ± 0.61	
	80	37.00 ± 0.35	
	100	39.07 ± 0.41	

All values are expressed in mean ± SEM of three replicates

Table 2: α-glucosidase inhibition of methanolic and ethyl acetate extract of *Achyranthus aspera*

Extract	Concentration (mg/ml)	Inhibition (%)	IC_{50} (mg/ml)
Ethyl acetate extract	20	46.71 ± 0.547	27.62 ± 1.83
	40	41.68 ± 2.409	
	60	25.73 ± 0.60	
	80	36.00 ± 0.366	
	100	39.08 ± 0.516	
Ethyl acetate extract	20	45.71 ± 0.527	38.92 ± 1.93
	40	51.58 ± 2.409	
	60	26.33 ± 0.51	
	80	35.00 ± 0.36	
	100	40.07 ± 0.51	

All values are expressed in mean ± SEM of three replicates

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