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Research Article

ANTI DIABETIC ACTIVITY *(INVITRO)* OF THE RHIZOMES OF *ANAPHYLLUM WIGHTII* SCHOTT

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ABSTRACT
Diabetes is one of the major causes of premature death worldwide. The major therapeutic approach is to Diabetes is to reduce gastro intestinal glucose production and absorption through the inhibition of carbohydrate digesting enzymes such as α- amylase and α-glucosidase . In this study the *invitro* antidiabetic activity of the methanolic extract of the rhizomes were evaluated. The antidiabetic action of *Anaphyllum wightii* can be attributed to the intestinal alpha amylase and alpha glucosidase inhibitory activity.

Keywords: Anti-diabetic activity, *invitro, Anaphyllum wightii* , alpha amylase, alpha glucosidases

INTRODUCTION

According to WHO, it is estimated that 3% of the world's population have diabetes and the prevalence is expected to double by the year 2025 to 6.3% (M.Abdalla *et.al*.,2012). Diabetes mellitus is a metabolic disorder with increasing incidence throughout the world. Management of diabetes without side effects is still challenge to the medical community. There comes the importance of Natural remedies. The genus *Anaphyllum* from Araceae family comprises of two species like; *Anaphyllum beddomei* Engl. and *Anaphyllum wightii* Schott. Tribal communities and villagers of various parts of Kerala use these plants as antidote to snake bite (V.J.Dominic.,2010).

MATERIALS AND METHODS

Plant material

The Fresh rhizomes of *Anaphyllum wightii* Schott were collected from the Ghatt section of Sakleshpur, Karnataka. The plant material was identified and and a voucher specimen was deposited in the Department of Pharmacognosy of Academy of Pharmaceutical Sciences, Pariyaram, Kerala.

Extraction of rhizome

The shade dried rhizomes were powdered mechanically and sieved through sieve no 20 and stored in an air tight container. The extraction was carried out by hot percolation method using Soxhlet apparatus. The solvent used was methanol. About 100 gm of powder was extracted with 500 ml of methanol. The extract was concentrated to dryness under controlled temperature 45- 55°C. The percentage yield was found to be 12.50%. The extract was preserved in refrigerator till further use.

Invitro methods employed in antidiabetic studies (Hamdan II *et.al*.,2004)

Inhibition of alpha amylase enzyme

A total of 500 µl of test samples and standard drug (100-1000µg/ml) were added to 500 µl of 0.20 mM phosphate buffer (pH 6.9) containing α-amylase (0.5mg/ml) solution and were incubated at 25°C for 10 min. After these, 500 µl of a 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added to each tube. The reaction mixtures were then incubated at 25°C for 10 min. The reaction was stopped with 1.0 ml of 3, 5 dinitrosalicylic

acid colour reagent. The test tubes were then incubated in a boiling water bath for 5 min, cooled to room temperature. The reaction mixture was then diluted after adding 10 ml distilled water and absorbance was measured at 540 nm. Control represent 100% enzyme activity and were conducted in similar way by replacing extract with vehicle (N.R.Thalapaneni *et.al*.,2008).

Inhibition of alpha glucosidases enzyme

The inhibitory activity was determined by incubating a solution of starch substrate (2 % w/v maltose or sucrose) 1ml with 0.2 M Tris buffer pH 8.0 and various concentration of plant extract for 5 min at 37°C. The reaction was initiated by adding 1ml of α-glucosidase enzyme (1U/ml) to it followed by incubation for 10 min at 37°C. Then, the reaction mixture was heated for 2 min in boiling water bath to stop the reaction. The amount of liberated glucose is measured by glucose oxidase peroxidase method (N.W.Tietz *et.al* .,1999).

Calculation of 50% Inhibitory Concentration (IC_{50})

The concentration of the plant extracts required to scavenge 50% of the radicals (IC_{50}) was calculated by using the percentage scavenging activities at five different concentrations of the extract. Percentage inhibition (I %) was calculated by;

I % = (Ac-As)/Ac X 100, (L J Shai *et.al*.,2010)

* Ac is the absorbance of the control .
* As is the absorbance of the sample.

RESULTS

Evaluation of *in vitro* α-amylase inhibitory activity using *Anaphyllum wightii* rhizome extract

There was a dose-dependent increase in percentage inhibitory activity against α-amylase enzyme. At a concentration 100µg/ml of extract showed a percentage inhibition 33.52 ± 0.2664 and for 1000 µg/ml it was 81.70 ± 0.6171 . The extract gave an IC_{50} value of 762.11 ± 2.67µg/ml. (Table 1).

Table 1: α-amylase inhibition by *Anaphyllum wightii* methanol extract

S. No	Concentration Micro grams/ml	Percentage Inhibition	IC $_{50}$ Micro grams/ml
1	100	33.52 ± 0.2664	762.11 ± 2.67
2	200	46.77 ± 0.1620	
3	400	51.76 ± 0.2861	
4	800	64.37 ± 0.7815	
5	1000	81.70 ± 0.6171	

All determinations were carried out in triplicate manner and values are expressed as the mean ± SEM.
The IC_{50} value is defined as the concentration of inhibitor to inhibit 50% of its activity under the assayed conditions.

Evaluation of in vitro α-glucosidase inhibitory activity using Anaphyllum wightii rhizome extract

The methanolic extract of the rhizomes revealed a significant inhibitory action on α-glucosidase enzyme. The percentage inhibition at 100-1000 µg/ml concentrations of the extract showed a concentration dependent increase in percentage inhibition. The percentage inhibition varied from 80.10 ± 0.5350 to 30.44 ± 0.7674 for highest concentration to the lowest concentration of 100 µg/ml. The concentration required for 50% inhibition (IC_{50}) was found to be 608.51 ± 3.25 µg/ml .(Table 2).

Table 2: α-glucosidase inhibition by *Anaphyllum wightii* methanol extract

S. No	Concentration Micro grams/ml	Percentage Inhibition	IC $_{50}$ Micro grams/ml
1	100	30.44 ± 0.7674	608.51 ± 3.25
2	200	41.50 ± 0.3455	
3	400	50.24 ± 0.2570	
4	800	61.77 ± 0.8237	
5	1000	80.10 ± 0.5350	

All determinations were carried out in triplicate manner and values are expressed as the mean ± SEM. The IC_{50} value is defined as the concentration of inhibitor to inhibit 50% of its activity under the assayed conditions.

DISCUSSIONS

Insulin is a key player in the control of glucose homeostasis. Lack of insulin affects carbohydrate, fat and protein metabolism (Rajiv Gandhi *et.al*.,2012). The intestinal enzymes like α-amylase and α-glucosidase are found to be very important in carbohydrate digestion and glucose absorption. The suppression of the activity of such digestive enzymes would delay the degradation of

starch and oligo saccharides, which would in turn cause a decrease in the absorption of glucose and consequently the reduction of postprandial blood glucose level elevation (S.N.Davis *et.al*.,2001). Alpha glucosidase inhibitor retards the digestion of carbohydrates and slows down the absorption. The therapeutic approaches for reducing postprandial blood glucose levels in patient with diabetes mellitus is to prevent absorption of carbohydrate after food intake. Inhibition α-amylase and α-glucosidases reduced the high postprandial blood glucose peaks in diabetes (F.Conforti *et.al.,*2005)

CONCLUSION

The present finding reveals that methanolic extract of the rhizomes of *Anaphyllum wightii* efficiently inhibits both alpha amylase and alpha glucosidase enzymes *in vitro* in a dose dependent manner. The antidiabetic action of *Anaphyllum wightii* can also be attributed to the intestinal alpha amylase and alpha glucosidase inhibitory activity. In this present study we evaluated *invitro* alpha amylase and alpha glucosidase activity of crude methanol extract of the rhizomes of *Anaphyllum wightii*. The plant showed significant inhibition activity, so further the compound isolation, purification and characterization which is responsible for inhibiting activity, has to be done for the usage of an antidiabetic agent.

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