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Evaluation of anti-diabetic effect of ethanolic extract Of *andrographis echioides* by alpha glucosidase and alpha amylase assay

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ABSTRACT

Andrographis echioides ,otherwise called as false water willow is a medicinal plant which was used as the remedy for curing fever,headache,constipation etc.This plant is mainly found in countries like India and Srilanka. The above study involves evaluation of anti diabetic activity of ethanolic extract of *Andrographis echioides* plant (gopuram Thaangi) using alpha glucosidase assay and alpha amylase assay. The collected whole plant material was extracted using ethanol solvent and was further concentrated under reduced pressure. This was subjected to an *in vitro* alpha glucosidase and alpha amylase inhibition activity for anti diabetic effect whose absorbance was measured using spectrophotometery. Acarbose, an approved antidiabetic drug was used as a positive control. Alpha glucosidase inhibition activity of ethanolic extract of *Andrographis echioides* was 75.7% with 50% inhibitory concentration (IC50) of 500 mcg/ml and that of Alpha amylase inhibition activity was 66.6% with IC50 of around 500 mcg/ml. Acarbose (reference) exhibited 94.6 % inhibition with IC50 of 250 mcg/ml. The assay results show that the "*Andrographis echioides*" has inhibitory activity on alpha glucosidase enzyme and alpha amylase enzyme. The alpha glucosidase inhibition is more compared to alpha amylase inhibition but their antiglucosidase and antiamylase activity is less when compared to the control drug Acarbose.

KEY WORDS: Ethanolic, Alpha glucosidase, Alpha amylase, Acarbose, *Andrographis Echioides*

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INTRODUCTION

Various medicinal plants have been focussed for the development of new drugs thereby contributing global healthcare.[1] In developing country like India ,infectious diseases play an important role in public health concern and it is also famous for traditional medicine practices like siddha,ayurvedha and unani[2,3]. Andrographis which was popularly called as Kalmegh, or "King of Bitters" has been widely used for various conditions like common cold, flu,goiter,liver diseases, fertility problems, Bacterial and fungal disorders. It is widely found in South Asian countries such as India and Sri Lanka. Among them *Andrographis paniculata* play a vital role in controlling URI, fever. Furthermore it was used as an antidote for snakes and insects poisoning. This plant has been reported to exhibit various mode of biological activities *in vivo* as well as *in vitro* such as antibacterial / anti viral, anti-inflammatory,anti HIV, Immunomodulating / immunostimulatory and anticancer activity[4]. *Andrographis echioides* which is also called as Indoneesiella echioides L. Nees (False Water Willow) exhibits various bioactivities such as antimutagenic, anticarcinogenic, antioxidant, antimicrobial and anti inflammatory properties. The leaf juice of this plant is used as a remedy for fever. Although it shows many bioactivities, the anti diabetic activity has not been evaluated so far. Moreover this plant is rich in phytochemical constituent named flavonoids which is responsible for the anti diabetic activity[5].Alpha glucosidase and alpha amylase enzyme inhibition helps in reducing the rate of digestion of carbohydrates thereby reducing absorption of glucose and consequently resulting in decrease in post prandial blood glucose level[6]. Hence in this study the anti diabetic activity is evaluated from the ethanolic extract of *Andrographis echioides* plant (gopuram Thaangi) using alpha glucosidase assay and alpha amylase assay since these two enzyme inhibition play an important strategy in *in vitro* research on drugs for type 2 diabetes[7] .

MATERIALS AND METHODS

Authentification and preparation of plant extract

The whole plant was collected locally and was authentified by qualified botanist Professor sasikala, Siddha institute of research, Chennai and the specimen voucher was dated on 17.01.2014. This was shade dried and powdered mechanically. About 5g of powdered plant sample was taken and subjected to soxhlet extraction with 100ml of solvent (Ethanol). The extract was concentrated under reduced pressure in rotatory vacuum evaporator and was reconstituted with ethanol to obtain crude extract[8].The control used was ethanol.

In vitro methods for anti diabetic activity

Alpha amylase assay

A starch solution (0.1% w/v) was prepared by mixing 0.1g of starch in 100 ml of 16 mM of sodium acetate buffer. By adding 27.5 mg of alpha-amylase to 100 ml of distilled water, the enzyme solution was obtained. The colorimetric reagent is then prepared by mixing 3, 5 di nitro salicylic acid and sodium potassium tartarate solution at 96 mM. Under alkaline conditions, the starch solution was left to react with alpha- amylase enzyme after adding both control and sample at 25ºC. The reaction was measured over 3 minutes and the quantification of generation of maltose was measured by the reduction of 3, 5 dinitro salicylic acid to 3- amino-5- nitro salicylic acid. This was then detected at 540 nm using spectrophotometer[9,10]. Here Acarbose at various concentration (0.1-0.5 mg/ml) was used as the reference alpha –amylase inhibitor. Acarbose was procured from sigma labs,Chennai. The percentage of inhibition of both control and sample was then calculated by using the following formula.

Alpha glucosidase assay

The assay was initiated by incubating 1 ml of starch substrate (2 % w/v maltose or sucrose) with 0.2 M Tris buffer in the pH of 8.0 at various concentration of Sample for 5 min at 37°C and by adding 1 ml of alpha-glucosidase enzyme (1U/ml) to this solution. This was again allowed to incubation for 40 min at 35°C.Then the reaction ends by measuring the intensity of the colour at 540 nm using spectrophotometer after the addition of 2 ml of 6N HCl to it. Here also Acarbose was used as the reference Alpha -glucosidase inhibitor and the percentage of inhibition is calculated as mentioned below[11].

50% Inhibitory concentration(IC 50) calculation

IC 50 is the concentration of the plant compound at which it inhibits 50% of the enzymes. This was calculated by using the following formula:

$$I\ \% = (Ac-As)/Ac\ X\ 100,\ (Shai\ et\ al.,\ 2010)[12].$$

where I % is the percentage inhibition and Ac stands for absorbance of the control and As for the absorbance of the sample.

RESULTS

Alpha amylase assay showed maximum inhibitory activity of 66.6% of that enzyme with 50% inhibitory concentration(IC50) at around 617.67 mcg/ml whereas the acarbose which was used as the control exhibited 92.3% inhibition with IC50 value of 425.2 mcg/ml for amylase enzyme (Table no.1). The graph was then plotted having concentration of sample on 'X' axis and percentage of inhibition of amylase enzyme on 'Y' axis (graph.1). Whereas the alpha glucosidase inhibition activity of ethanolic extract of *andrographis echioides* was 75.7% with IC50 value of 535.93 mcg/ml with acarbose (reference) exhibiting 94.6 % inhibition with IC50 value of 332.87 mcg/ml for glucosidase enzyme (Table no.2).Similarly the graph between concentration of sample on 'X' axis and percentage of inhibition of glucosidase enzyme on 'Y' axis was plotted (graph.2).

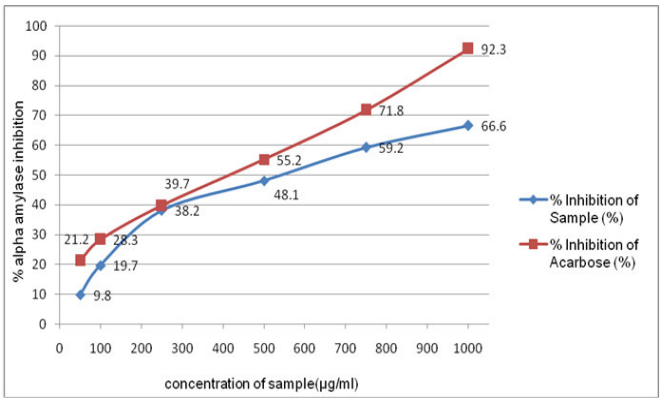
Table 1
In vitro anti diabetic activity by alpha-amylase method and IC 50 Calculation of both sample and reference (Acarbose)

S.No	Concentration of test (µg/ml)	% Inhibition of Sample (%)	Concentration of Control(acarbose) (µg/ml)	% Inhibition of Acarbose (%)
1	1000	66.6	1000	92.3
2	750	59.2	750	71.8
3	500	48.1	500	55.2
4	250	38.2	250	39.7
5	100	19.7	100	28.3
6	50	9.8	50	21.2
IC 50		617.67(µg/ml)		425.2(µg/ml)

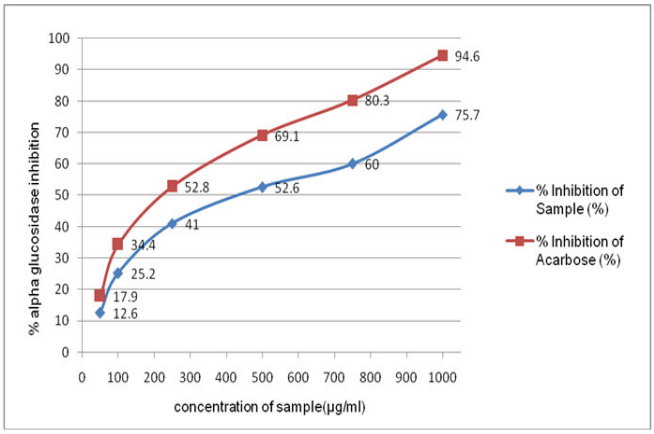
Table 2
In vitro anti diabetic activity by alpha-glucosidase method and IC 50 calculation of both sample and reference (Acarbose)

S.No	Concentration of test (µg/ml)	% Inhibition of Sample (%)	Concentration of control(acarbose) (µg/ml)	% Inhibition of Acarbose (%)
1	1000	75.7	1000	94.6
2	750	60.0	750	80.3
3	500	52.6	500	69.1
4	250	41.0	250	52.8
5	100	25.2	100	34.4
6	50	12.6	50	17.9
IC 50		535.93(µg/ml)		332.87(µg/ml)

Graph 1
In vitro anti diabetic activity by alpha-amylase method of both sample and reference (Acarbose)



Graph 2
In vitro anti diabetic activity by alpha-glucosidase method of both sample and reference (Acarbose)



DISCUSSION

All over the world, diabetes mellitus play a key role among the metabolic disorders with increasing incidence every year. Although there are many hypoglycaemic agents which were in practice, the management of diabetes mellitus without any side effect is the challenging part to the practitioners. Moreover there are several inherent factors such as hyperinsulinemia,impaired insulin secretion ,insulin resistance complicating type 2 diabetes mellitus[13].The main cause is due to insulin deficiency which affects the metabolism of carbohydrates, fat and proteins[14].The present study involves the evaluation of anti diabetic activity of ethanolic extract of the plant *Andrographis echioides* by the inhibition of alpha amylase and alpha glucosidase enzyme which was already proposed in previous studies to reduce the degradation of carbohydrate, thereby decreasing the absorption of glucose and hence the elevation of post prandial blood glucose level is controlled[15,16]. The reason why we had selected this plant is due to the potential of having anti diabetic effect in other studies for *andrographis* genus belonging to different species[17]. It also possess flavonoids which is responsible for regulating different signaling pathways in pancreatic β-cells, hepatocytes, adipocytes and skeletal myofibers. The anti diabetic effect of flavonoids is due to insulin secretion enhancement and by regulation of glucose metabolism in hepatocytes[18]. Andrographis acts by increasing the GLUT 4 expression and by inhibiting the nuclear factor kappa B activation which impairs the beta cell function [19,20,21].The reason for selecting acarbose which is a synthetic hypoglycaemic agent as a control drug is that it inhibits both alpha glucosidase and alpha amylase enzymes[22]. Other in vitro methods to evaluate the anti diabetic activity are non enzymatic glycosylation of haemoglobin assay, glucose diffusion inhibitory test, sucrose inhibitory activity[23]. The assay results show that the "*Andrographis echioides*" has inhibitory activity on alpha glucosidase enzyme and alpha amylase enzyme. The alpha glucosidase inhibition is more compared to alpha amylase inhibition. But their inhibition of alpha glucosidase and amylase enzymes is less when compared to the control drug Acarbose. Further studies are needed to elucidate the mechanism of action by *in vitro* and *in vivo* experiments of this plant to find out if it can be a good adjuvant to be used in the treatment of diabetes mellitus with less toxicity.

LIMITATIONS

Although we have evaluated the potential for antidiabetic effect in this plant further studies need to be conducted to elucidate the same by different in vitro methods.

CONCLUSION

This study reveals that the *Andrographis echioides* have an anti diabetic potential activity by inhibiting alpha glucosidase and amylase enzymes. Its anti diabetic activity can further be evaluated by using other assays mentioned above.

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CONFLICT OF INTEREST

Conflict of interest declared none.

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