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Antidiabetic Potential of the Flowers of *Aquilaria agallocha* Roxb.

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Abstract

In the present study the *in vitro* antidiabetic activity of the ethanolic extract of the flowers were evaluated. The finding reveals that ethanolic extract of the flowers of *Aquilaria agallocha* efficiently inhibits both alpha amylase and alpha glucosidase enzymes *in vitro* in a dose dependent manner. The antidiabetic action of *Aquilaria agallocha* can be attributed to the intestinal alpha amylase and alpha glucosidase inhibitory activity.

Keywords: Anti-diabetic activity, *in vitro*, *Aquilaria agallocha*, alpha amylase, alpha glucosidases

Introduction

Diabetes mellitus is a metabolic disorder characterised by increase blood glucose level associated with discharge of glucose in urine. There are two major types of diabetes mellitus like insulin dependant diabetes mellitus and non insulin dependent diabetes mellitus. Insulin dependant diabetes mellitus is also called type 1 diabetes and non insulin dependent diabetes mellitus is also called type 2 diabetes which is due to insulin resistance. Insulin resistance is developed due to the defects at the receptor level or insulin signalling at the post receptor level. This defect may be in the effector cells such as the skeletal muscle, the adipose tissue or in the beta islet cells. A large number of drugs including herbs and minerals with suspected anti-diabetic activity have been successfully tested in the laboratory (S.S.Agarwal and M.Paridhavi, 2012). There are several synthetic drugs which are available in the market to control this disorder which can also cause severe side effects in long time administration. Hence in the present study an attempt has been carried out to screen the flowers of *Aquilaria agallocha* extract for its antidiabetic potential.

Aquilaria agallocha Roxb. is an endangered economic plant used for production of agar wood having large greenish white coloured flowers (S.Nesamony, 2001). *Aquilaria* is a genus in the family Thymeleaceae and class Magnoliopsida. The plant can be found in all most all rain forest of Kerala, Karnataka and Tamil Nadu. The plant have central nervous system antidepressant activities (H.Okugwa et al, 1981).

Materials and methods

Plant material

The Flowers of *Aquilaria agallocha* were collected from the Ghatt section of Sakleshpur village of Karnataka state. The plant material was identified and a voucher specimen was deposited in the department of Pharmacognosy of Academy of Pharmaceutical Sciences, Pariyaram, Kerala.

Preparation of extracts

The shade dried flowers 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 ethanol. About 500 gm of powder was extracted with 500 ml of ethanol and the extract was concentrated to dryness under controlled temperature 45- 50°C. The percentage yield was found to be 9.75%.

Phytochemical screening

Phytochemical screening of the flowers were carried out as per the standard procedure (C.K.Kokate, 1999 and K.R.Khandelwal, 2000).

Invitro methods of antidiabetic screening

Inhibition of alpha amylase enzyme activity

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. There after, 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 (Hamdan et al, 2004 and N.R.Thalapaneni et al, 2008).

Inhibition of alpha glucosidases enzyme activity

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 (Hamdan et al, 2004 and N.W.Tietz, 1999).

Calculation of IC₅₀ value

The IC₅₀ value is defined as the concentration of inhibitor to inhibit 50% of its activity under the assayed conditions. The concentration of the plant extracts required to scavenge 50% of the radicals (IC₅₀) was calculated by using the percentage scavenging activities at five different concentrations of the extract. Percentage inhibition (I %) was calculated by the following formula; (L.J. Shai et al, 2010)

$I\% = (Ac - As) / Ac \times 100$, where Ac is the absorbance of the control and As is that of sample.

Results

Phytochemical screening

Phytochemical screening of the flower extract shows the presence of Polyphenolic compounds, flavonoids, essential oil, resins and glycosides.

Discussion

Diabetes mellitus, a metabolic disorder characterized by hyperglycemia associated with impairment in insulin secretion, insulin action or both and alteration in intermediary metabolism of carbohydrate, fat and protein. In past there have been many medicinal plants which have been used for traditional medicines for their blood sugar lowering properties without any scientific support. Diabetes mellitus is a group of metabolic disorders that result in hyperglycemia due to decreased insulin production or inefficient insulin utilization (K.Rajaram, 2013)

The present finding reveals that ethanolic extract of the flowers of *Aquilaria agallocha* efficiently inhibits both alpha amylase and alpha glucosidase enzymes *invitro* in a dose dependent manner. The antidiabetic action of

Aquilaria agallocha can also be attributed to the intestinal alpha amylase and alpha glucosidase inhibitory activity. The intestinal enzymes like α-glucosidase and α-amylase 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.Confori et al, 2005).

Evaluation of *in vitro* α-amylase inhibitory activity

Table 1: α-amylase inhibition by *Aquilaria agallocha* flower extract

Sl no	Concentration (Micro gms/ml)	Percentage Inhibition	IC ₅₀ (Micro gms/ml)
1	50	27.14 ± 0.3768	801.30 ± 1.59
2	100	44.09 ± 0.4734	
3	200	60.78 ± 0.3221	
4	400	87.49 ± 0.3985	
5	800	99.01 ± 0.5776	

All determinations were carried out in triplicate manner and values are expressed as the mean ± SEM.

Evaluation of *in vitro* α-glucosidase inhibitory activity

Table 2: α-glucosidase inhibition by *Aquilaria agallocha* flower extract

Sl no	Concentration (Micro gms/ml)	Percentage Inhibition	IC ₅₀ Micro (gms/ml)
1	50	47.16 ± 0.8920	701.22 ± 2.39
2	100	57.30 ± 0.2714	
3	200	61.14 ± 0.3919	
4	400	77.67 ± 0.1418	
5	800	92.26 ± 0.3075	

All determinations were carried out in triplicate manner and values are expressed as the mean ± SEM.

Conclusion

The present study reveals the antidiabetic potential of the flowers of *Aquilaria agallocha*. There is a need to explore the plant for possible isolation of the active constituents responsible for the said activity for the usage of *Aquilaria agallocha* as an antidiabetic agent.

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