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In vitro antidiabetic activity of anthocyanin extract of *Asystasia gangetica* (Chinese violet) flower

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ABSTRACT

The main aim of the present study was to investigate the alpha amylase and alpha glucosidase inhibitory effects of extracted anthocyanins from Asystasia gangetica flower. The plant anthocyanins were isolated with ethanol acidified with citric acid. Different concentrations of anthocyanin extracts were evaluated for alpha amylase and alpha glucosidase inhibition activity. Percentage of alpha amylase and alphaglucosidase inhibitory activity was calculated. In this study the anthocyanin extraction showed significant inhibition activity,at the concentration of 400μmg/ml the plant showed appreciable alpha amylase and alpha glucosidase inhibitory activity (71.46 ± 1.21 %, 76.85 ± 0.75 %) with IC50 value of 260 μg/ml and 244μg/ml respectively. In this study we concluded that the crude anthocyanin extract of Asystasia gangetica is a potential source of natural antidiabetic agent. In vitro antidiabetic activity of anthocyanins present in this plant might be due to the presence of anthocyanin phenolic compounds.

Key words: *Asystasia gangetica*, Anthocyanins, alpha glucosidase, alpha amylase, inhibitory activity.

INTRODUCTION

Diabetes mellitus is a metabolic disease characterized by high blood glucose level resulting from defects in insulin secretion, insulin action or both [1]. It is a major public health problem currently affecting 284.6 million people worldwide and according to the latest International Diabetes Federation estimates it is expected to affect 438.4 million adults by 2030 becoming one of the world's main disabler and killer [2]. Type 2 diabetes is complicated by several factors inherent to the disease process, such as insulin resistance, hyperinsulinemia, impaired insulin secretion, reduced insulin mediated glucose uptake, and utilization [3]. One of the therapeutic approaches is to decrease the postprandial hyperglycemia by retarding absorption of glucose by inhibition of carbohydrate-hydrolyzing enzymes, such as α-amylase and α-glucosidases[4]. Oral hypoglycemic agents have been reported as highly effective for glycemic control, but they come with their attendant side effects such as liver disorders, flatulence, abdominal pain, renal tumors, hepatic injury, acute hepatitis, abdominal fullness and diarrhea [5]. Therefore, there is an increasing need for the development of natural drugs from plants. Plant foods have reported to have good enzyme inhibitors in type 2 diabetes [6, 7]. However, previous reports have also indicated that excessive inhibition of pancreatic alpha amylase could result in the abnormal bacterial fermentation of undigested carbohydrates in the colon and therefore mild alpha- amylase inhibition activity is useful [8].According to the World Health Organization (WHO) about 65–80% of the world's population in developing countries depends essentially on plants for their primary healthcare due to poverty and lack of access to modern medicine [9].

Anthocyanins can be useful as non-toxic colorants for foods (giving varieties of orange, red, violet and blue colors), or for human health, as they are antioxidants and free radical scavengers. Potential therapeutic effects include preventive action against cancer [10], and diabetes [11]. Few reports indicate that the natural anthocyanins extract inhibited alpha amylase action, indicating that anthocyanins would have a potential function to suppress the increase in postprandial glucose level from starch [12].

Asystasia gangetica(L).T. (Chinese violet) belongs to Acanthaceae family. Leaves are opposite petioles, flowers are pale purple blue to violet or lime white in colour, and capsules are 2.5-3.5 cm long with wide base and the seeds are 5 mm in diameter. *Asystasia gangetica* is reported to contain biologically active substances such as carbohydrates, proteins, alkaloids, tannins, steroidal aglycones, saponins, flavonoids and triterpenoids. It has been reported that the methanolic extract of *Asystasia gangetica* leaves had α-amylase inhibitory activity [13].The plant has been claimed for anti asthmatic, anthel-mentic and antidiabetic property [14].

The present study is aimed to investigate the antidiabetic activity of extracted anthocyanins from *Asystasia gangetica* flowers by inhibiting alpha glucosidase enzyme.

MATERIALS AND METHODS

Plant materials

The flowers of *Asystasia gangetica* were collected in surrounding areas of Coimbatore district, Tamilnadu, India and identified by Botanical survey of India, Coimbatore. The flowers were kept for shade dry for one week and made it in to powder. The powdered material was stored at 4˚c for further uses.

Extraction

Anthocyanin extraction was carried out according to the standard procedures [15]. The extraction of anthocyanins using ethanol acidified with citric acid (0.01%) instead of hydrochloric acid has been reported. Ethanol would be preferred for food use to avoid the toxicity of methanolic solutions. Citric acid is less corrosive than hydrochloric acid, chelates metals, maintains a low pH, and may have a protective effect during processing [16].

In vitro α-glucosidase inhibition assay

The α-amylase and α-glucosidase inhibitory effect of plant extracts was determined according to the standard method [17].For alpha glucosidase inhibition, yeast α-glucosidase was dissolved in 100 mM phosphate buffer, pH 7.0, containing bovine serum albumin 2 g/liter and sodium azide 0.2 g/liter which was used as enzyme source. Paranitrophenyl- α-d-glucopyranoside was used as substrate. Extract was weighed and serial dilutions of 100µg/ml, 200µg/ml, 300 µg/ml, 400µg/ml were made up with equal volumes of dimethylsulfoxide and distilled water. 10 micro liters of extract dilutions was incubated for 5 min with 50 µl enzyme source. After incubation, 50 µl of substrate was added and further incubated for 5 min at room temperature. The pre substrate and post substrate addition absorbance was measured at 405 nm on a microplate reader. The increase in absorbance on substrate addition was obtained. Each test was performed three times and the mean absorption was used to calculate percentage α-glucosidase inhibition. Acarbose was used as positive control with various concentrations. The concentration of acarbose and plant extract required to inhibit 50% of a-amylase activity under the conditions was defined as the IC_{50} value

Percentage α-glucosidase inhibition was calculated according to the following formula;

$$\text{Percentage of inhibition} = \frac{[(\text{Control 405} - \text{Extract 405})] \times 100}{\text{Control 405}}$$

In vitro alpha amylase inhibition assay

The assay was carried out following the standard protocol with slight modifications [18]. Starch azure (2 mg) was suspended in 0.2 mL of 0.5M Tris-HCl buffer (pH 6.9) containing 0.01 M $CaCl_2$ (substrate solution). The tubes containing substrate solution were boiled for 5 min and then preincubated at 37ºC for 5 min. extract of *Asystasia gangetica* was dissolved in DMSO in order to obtain concentrations of 100, 200, 300, 400 µg/mL. Plant extract of particular concentrations was added to the tube containing the substrate solution. In addition, 0.1 mL of porcine pancreatic amylase in Tris-HCl buffer (2 units/mL) was added to the tube containing the plant extract and substrate solution. The reaction was carried out at 37ºC for 10 min. The reaction was stopped by adding 0.5 mL of 50% acetic acid in each tube. The reaction mixture was centrifuged at 3000 rpm for 5 min at 4ºC. The absorbance of resulting

supernatant was measured at 595 nm using spectrophotometer. Acarbose, a known a-amylase inhibitor was used as a standard drug. The experiments were repeated thrice. The a-amylase inhibitory activity was calculated by using following formula:

Theα-amylase inhibitory activity = (Ac+) - (Ac-) - (As - Ab)/(Ac+) - (Ac-) X 100,

where Ac+, Ac-, As, and Ab are defined as the absorbance of 100% enzyme activity (only solvent with enzyme), 0% enzyme activity (only solvent without enzyme), a test sample (with enzyme), and a blank (a test sample without enzyme), respectively. The concentration of acarbose and plant extract required to inhibit 50% of a-amylase activity under the conditions was defined as the IC_{50} value

RESULTS

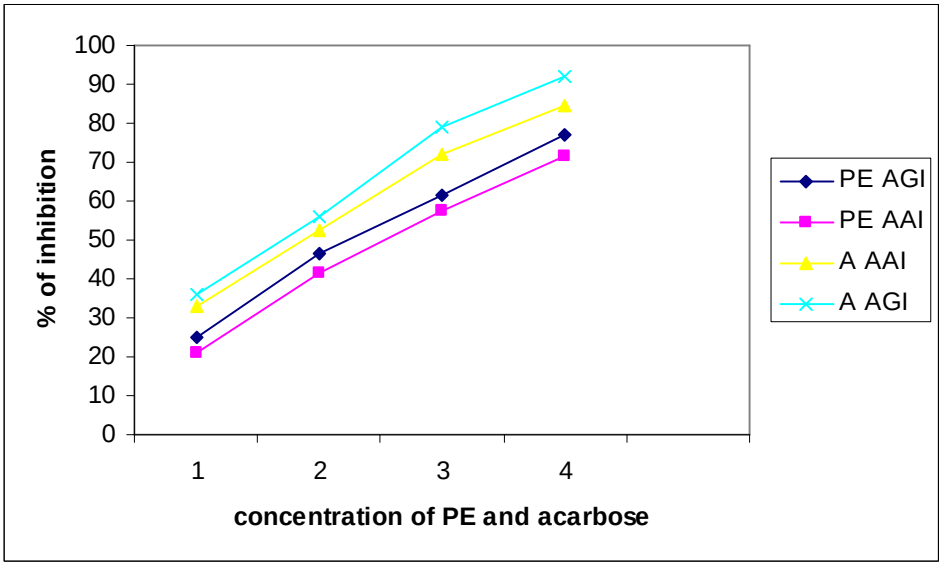
Anthocyanin extract of *Asystasia gangetica* was screened for its *in vitro* antidiabetic activity. The extraction showed significant inhibition activity at the concentration of 400 µg/ml the inhibition activity of alpha amylase and alpha glucosidase was found as 71.46 ± 1.21 %, 76.85 ± 0.75 %with IC_{50} value 260µl/ml and 244µl/ml respectively. Acarbose was used as standard drug with various concentrations. Table 1 showed the dose dependent inhibitory activity of anthocyanin extract. The highest concentration 400µg/ml showed maximum inhibition activity.

Figure 1 showed graphical representation of the comparative results of standard drug and plant extract with various concentrations.

Table 1: Alpha glucosidase and alpha amylase inhibitory activity of anthocyanin extract of *Asystasia gangetica*

Sample concentration	%of alpha Glucosidase inhibition	%of alpha amylase inhibition	%of alpha Amylase inhibition (acarbose)	%alpha glucosidase inhibition (acarbose)
100 µl	24.85±1.22	20.85±1.15	33.13±1.25	35.81±1.05
200µl	46.68 ± 0.92	41.68±0.85	52.45±0.55	56.04±.0.40
300µl	61.42±1.05	57.55±0.75	71.89±1.20	79.24±0.25
400µl	76.85 ± 0.75	71.46±1.21	84.58±0.60	92.16±1.83

Figure 1: % of inhibition of standard Acrbose and *Asystasia gangetica* extract



PE : Plant extract
PE AGI : Plant extract alpha glucosidase inhibition activity
PE AAI : Plant extract alpha amylase inhibition activity
A AAI :Acarbose alpha amylase inhibition activity
A AGI: Acrbose alphaglucosidas inhibition activity

DISCUSSION

Diabetes mellitus is a chronic disease caused by inherited or acquired deficiency in insulin secretion and by decreased responsiveness of the organs to secreted insulin. Such a deficiency results in increased blood glucose level, which in turn can damage many of the body's systems, including blood vessels and nerves[19].The medicinal plants or natural products involve retarding the absorption of glucose by inhibiting the carbohydrate hydrolyzing enzymes. Several α- amylase inhibitors including acarbose, voglibose and miglitol are clinically used for treatment but their prices are high and clinical side effects occur [20]. In contrast to acarbose, plant derived α-amylase and α-glucosidase inhibitors are reported to have lower inhibitory effect against α-amylase activity and stronger inhibitory activity against α-glucosidase[21], an indication that plant extracts and their constituents may be effective therapeutic agents for the management and control of postprandial hyperglycemia with less side effects than acarbose [22]. The most common adverse effect of acarbose is gastrointestinal disturbance such as flatulence, meteorism and abdominal distention which occurs in a dose-dependent manner [20].

Anthocyanins are belonging to the most common class of phenolic compounds, known collectively as flavonoids with more than 8000 flavonoid and 500 anthocyanin structures reported in the year 2000 [23].Numerous publications have reported theantidiabetic effects of anthocyanins [24].Studies performed *in vitro* with extracts of different plant parts, such as roots, stems, leaves, flowers and bark, or with standards of anthocyanins, have shown inhibitory activity of α-glucosidase against maltase and sucrase [25,26].The experimental evidence demonstrating anthocyanin benefits for diabetes and pancreatic disorders have also accumulated in recent years, and again the efficacy is attributed to the multiple, simultaneous biological effects these pigments cause in the body, including prevention of generation of free radicals, decreased lipid peroxidation, reduced pancreatic swelling, and decreased blood sugar concentrations in urine and blood serum [27].

The extraction of anthocyanin showed appreciable alpha amylase inhibition activity. This made proper attempt to isolate the active principles from *Asystasia gangetica* anthocyanins which might help in the findings of new lead compounds in the fields of anti-diabetic drug research.

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