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RESEARCH ARTICLE

A COMPARATIVE STUDY ON THE ANTI-DIABETIC EFFECT OF *BOUGAINVILLEA SPECTABILIS* AND *BOUGAINVILLEA ALBA* FLOWERS

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ABSTRACT

Background: Bougainvillea spectabilis and Bougainvillea alba are used for the treatment of diarrhoea, stomach acidity and fertility control.
Objective: The aim of this research was to examine the anti diabetic activity of Bougainvillea spectabilis and Bougainvillea alba flowers.
Methods: To study the in vitro anti- diabetic effect α-amylase inhibition, glucose diffusion and non-enzymatic glycosylation of hemoglobin assay was performed.
Result: The Bougainvillea spectabilis extract showed good glucose tolerance and exhibit anti diabetic effects than Bougainvillea alba and it is validated by traditional claim of this plant.

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INTRODUCTION

Diabetes mellitus is a complex and diverse group of disorder characterized by a raised blood glucose level due to defect in insulin metabolism and insulin action. Insulin is a hormone secreted from the cells of the pancreas resulting in the conversion of glucose into glycogen, which is involved in maintaining blood glucose level. The insulin deficiency is mainly caused by autoimmune destruction of pancreatic ß - cells, which leads to insufficient insulin secretion. Hyperglycemia is associated with symptoms such as polyuria, polydipsia, and weight loss. Chronic hyperglycemia is also associated with certain infection caused by liability and impairment of growth (Talaviya *et al*., 2013). The long term complication of diabetes mellitus includes failure of various organs, especially eyes(retinopathy), kidney(nephropathy), nerves(neuropathy), Charcot joints, gastrointestinal and heart diseases, stroke and cerebrovascular diseases (Huang *et al*., 2005). Diabetes mellitus is classified as Type I and Type II. Type I diabetes is referred to as juvenile onset diabetes or insulin dependent diabetes mellitus (IDDM) caused by a concomitant resistance of action of insulin on target tissues ex. autoimmune genetic defect and environmental factors associated with it.

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Type II diabetes is otherwise known as adult-onset diabetes and non-insulin dependent diabetes mellitus (NIDDM). So the treatment of diabetes mellitus includes prevention of breakdown a dietary carbohydrate into glucose and preventing of glucose entry into the bloodstream. The postprandial blood glucose level is reduced by inhibition of carbohydrate hydrolyzing enzymes such as α-amylase and α-glucosidase. The starch is converted to form small oligosaccharide which is catalyzed by pancreatic α-amylase. Degradation of oligosaccharide is catalyzed by glucosidase enzyme and. The glucose is diffused into the bloodstream which results in elevated postprandial blood glucose level.The insulin and various oral hypoglycemic agents such as sulfonyl, metformin, glucosidase inhibitors, froglitazone are currently used for diabetes and are reported to induce adverse effects like diarrhoea, liver problem and lactic acidosis (Anbu *et al*., 2012). Many traditional plants are used to cure diabetes throughout the world because the plant drugs are considered to have less-toxic and fewer-side-effect than the action of synthetic drugs. (Mamun *et al*., 2014). *Bougainvillea spectabilis* and *Bougainvillea alba* (Family: Nyctaginaceae) is a woody climber with large thorny stems and long drooping branches and it is commonly known as Paper flower. The flowers are small tubular, inflated midway and varied colors of Red, Purple, Pink, Yellow (or) White. All parts of this plant (leaves, stem, and root) are pharmacologically important in the traditional system of medicine. The decoction of dried flower is used to treat leucorrhea and blood vessels (Mennat Allah

Bahaa and Din Mohamed Abdel Halim, 2016) Various extract of leaves was studied for antibacterial activity against S.aureus, B.subtilis, S. faecalis, M. luteus, E. coli, Psaeruginosa, S typhi and K. pneumoniae. Extract from leaves also inhibited tomato highly spotted wilt tospo virus on capsicum annum (Umamaheswari, 2008). *Bougainvillea spectabilis* is reported high efficiency in reducing okra yellow vein mosaic virus causing okra infection (pun *et al*., 1999). The phytochemical screening of bougainvillea flowers showed the presence of alkaloids, flavonoids, glycosides, tannins and terpenoids (Farzana Rashid *et al*., 2013). In the view of previous facts, the present study was undertaken to evaluate the anti-diabetic activity of *Bougainvillea spectabilis* and *Bougainvillea alba* by in vitro methods such as α-amylase inhibition, glucose diffusion assay and non-enzymatic glycosylation of hemoglobin.

MATERIALS AND METHODS

Plant collection

The flowers of *Bougainvillea spectabilis* and *Bougainvillea alba* were collected from TNAU, Coimbatore, Tamilnadu. The flowers were washed thoroughly in running tap water to remove sand and other dust particles. They are spread over a filter paper and open-air dried under the shade then grounded into a coarse powder stored at room temperature.

Extract preparation

10gm of powdered flowers were successfully macerated with 50% hydroethanol was added and the extraction was performed by triple maceration (72hours process) and this was filtered and evaporated to a cohesive mass using rotary vaccum evaporator.

Evaluation of antidiabetic potential

α-amylase inhibition method

(Narkhede *et al*., 2011) The enzyme solution was prepared by dissolving α-amylase in 20mM phosphate buffer (pH 6.9) at the concentration of 0.5 mg/ml. Different concentrations of flower extract (250, 500, 750, 1000 µg/ml) and 1ml of enzyme solution were mixed together. The solution mixture was incubated at 25^{oc} for 10minutes. After incubation 1ml of starch solution (0.5%) was added to the solution mixture and was further incubated at 25^{oc} for 10minutes. 2ml of coloring reagent (Dinitro salicylic acid) was added to arrest the reaction. The reaction mixture was heated in a boiling water bath for 5 minutes. It was cooled and the absorbance was measured colorimetrically at 565nm. The percentage of α-amylase inhibition was calculated using the following formula.

$$\% \text{ inhibition} = \frac{\text{Absorbance of sample-Absorbance of control}}{\text{Absorbance of sample}} \times 100$$

Non enzymatic glycosylation of haemoglobin method (Gupta daksha *et al*., 2012)

In vitro anti diabetic activity of the hydroethanolic extract of *Bougainvillea spectabilis* and *Bougainvillea alba* flowers were investigated by estimating the degree of non-enzymatic hemoglobin glycosylation and it is measured colorimetrically at 520nm.Glucose (2%), hemoglobin (0.66%) and gentamycin (0.02%) solution were prepared in phosphate buffer 0.01M (pH 7.4). 1ml of flower extracts (12.5,25,50,100µg/ml) were added to the above mixture. The reaction mixture was incubated in dark at room temperature for 72hours. Metformin was used as a standard drug for this assay. The % inhibition was calculated using the formula.

$$\% \text{ inhibition} = \frac{\text{Absorbance of sample-Absorbance of control}}{\text{Absorbance of sample}} \times 100$$

Glucose diffusion assay method (Abirami *et al*., 2014)

Glucose diffusion is a simple method to evaluate the glucose movement in vitro. 2ml of 0.15M NaCl containing 0.22mM D-glucose solution which is transferred to a dialysis tube and mixed with the sample at 1000µg/ml. The other end of the tube was sealed and placed in a centrifuge tube, which contains 45ml of 0.15M NaCl and kept in an orbital shaker at room temperature. The diffusion of glucose was detected by measuring the glucose in the external solution every 30 minutes for a period of 3 hours.

RESULTS

The hydroethanolic extract of Bougainvillea *spectabilis* and *Bougainvillea alba* were studied for in vitro anti-diabetic activity by the inhibition of α-amylase method and the results were tabulated in Table1. From the table it was evident that when the concentration of flower extract increases from 250µg to 1000µg the α-amylase inhibitory activity also increases. Both the flowers of *Bougainvillea spectabilis* and *Bougainvillea alba* exhibited 65.2 and 54.9% of α-amylase inhibition respectively. From the Table 2, it was clear that the percentage inhibition of glycosylation is dose dependent and the highest concentration of 1000µg/ml of *Bougainvillea spectabilis* showed a maximum inhibition of 76.8% when compared to *Bougainvillea alba* showed a maximum inhibition of (60.7%). Metformin, a standard drug used in the present study showed 85.3% of hemoglobin glycosylation. The result of the glucose diffusion inhibitory test are given in Table 3. As inferred from the table, Bougainvillea spectabilis showed a maximum inhibition to the diffusion of glucose. *Bougainvillea alba* also showed continued retardation of movement of glucose with 77.41% of relative movement. The relative movement with respect to *Bougainvillea spectabilis* was only 64.51 % after 180 minutes which showed effective inhibition of glucose diffusion. The result indicate that diffusion of glucose across a membrane is a possible mechanism of anti – diabetic activity of these flowers.

DISCUSSION

Advancing technology and drastic lifestyle changes have associated with increase in various non communicable disorders including Diabetes. Diabetes is due to inadequate secretion of insulin which results in elevated blood glucose level. The body needs insulin to convert sugar, starch into energy. It is one of the major causes of premature death worldwide and every ten second a person dies from diabetes related disease mainly from cardiovascular complications (Das and Rai, 2008). A study showed that 108 plant species belong to 56 families were generally used in therapy of diabetes. In developing and developed countries, people had been growing interest in herbal medicine in the care and management of diabetes, which is due to their natural origin, and less adverse effect.

Table 1. α-amylase inhibitory activity of Bougainvillea flowers

S.No	Concentration (µg/ml)	% Inhibition of α-amylase activity	
		Bougainvillea spectabilis	*Bougainvillea alba*
1	250	11.6	8.6
2	500	20.8	15.7
3	750	40.3	35.2
4	1000	65.2	54.9

The values are expressed as Mean ± SD. (n=3)

Table 2. Effect of Bougainvillea flowers on the inhibitory hemoglobin glycosylation

S.No	Concentration (µg/ml)	% Inhibition of hemoglobin glycosylation		
		Standard drug (Metformin)	*Bougainvillea spectabilis*	*Bougainvillea alba*
1	250	35.1	26.5	19.1
2	500	60.7	55.1	45.8
3	750	74.2	64.3	53.2
4	1000	85.3	76.8	60.7

The values are expressed as Mean ± SD. (n=3)

Table 3. Effect of hydroethanolic extracts of *Bougainvillea spectabilis* and *Bougainvillea alba* on diffusion of glucose.

Time (Minutes)	Control Mean ± SD	*Bougainvillea spectabilis*	Relative Movement %	*Bougainvillea alba*	Relative Movement %
30	0.006 ± 0.04	0.003 ± 0.11	50%	0.004 ± 0.08	66.66%
60	0.03 ± 0.69	0.013 ± 1.02	43.33%	0.028 ± 0.43	93.33%
90	0.115 ± 0.23	0.0145 ± 0.85	12.60%	0.094 ± 0.11	81.73%
120	0.129 ± 0.56	0.0500 ± 0.63	38.75%	0.1 ± 0.58	77.51%
150	0.148 ± 0.62	0.120 ± 0.47	81.08%	0.117 ± 0.33	79.05%
180	0.155 ± 0.93	0.100 ± 0.99	64.51%	0.12 ± 1.24	77.41%

The values are expressed as Mean ± SD. (n=3)

Pancreatic α-amylase is one of the important target enzyme for the treatment of diabetes. It belongs to the class of α-1,4 gluconohydrolases. α-amylase is an enzyme that catalyzes the initial step in the hydrolysis of starch to maltose and glucose. α-amylase inhibitors are agents that bind to the alpha bond of polysaccharide and prevent the conversion of polysaccharide into mono and disaccharide, which inhibit the amylase activity that results in the delay of carbohydrate digestion. It also extends the overall carbohydrate digestion time causing depletion in the rate of glucose absorption and diminish the post parandial plasma glucose rise (Ajithadas aruna *et al*., 2014). Recent studies proved that the activity of intestinal enzymes (α-amylase and α-glucosidase) have induced the development of newer pharmacological agents. The α-amylase inhibitor act as an anti-nutrient that blocks the digestion and absorption of carbohydrate.

Glycosylated hemoglobin (HbA1c) is a form of hemoglobin and it is primarily measured to identify an average plasma glucose concentration. The amount of glycosylated hemoglobin should not be more than 12%. High concentration of glucose in the blood leads to the binding of hemoglobin in red blood cells produced reactive oxygen species. The inhibition of glycosylation is by plant treatment which reduces the formation of glucose-hemoglobin complex and hence the number of free hemoglobin increases (Jijith and Jayakumari, 2017). In recent years, several studies have showed that the influence of different plant extracts on the diffusion of glucose across the semi-permeable membrane or dialysis tube (Gallagher *et al*.,2003). The dialysis tubing method is a simple tool to assess the potential of soluble dietary fiber to retard the diffusion and the movement of glucose in the intestinal tract (Adiotomre *et al*., 1990). This finding clearly indicates that the *Bougainvillea spectabilis* and *Bougainvillea alba* inhibit α-amylase and non-enzymatic glycosylation and reduce the diffusion of glucose.

Conclusion

The result of the present study indicates that the hydroethanolic extracts of Bougainvillea spectabilis showed maximium anti diabetic activity. Hence the flower extraxt may be useful as better therapeutic agent especially for the treatment of Diabetes Mellitus.

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