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Evaluation of Antidiabetic Activity of *Cipadessa baccifera* (Roth.) Miq.

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Abstract

Diabetes is a serious chronic, complex metabolic condition associated with impairment of insulin secretion and /or insulin action where the body cells are unable to utilize glucose resulting in inappropriately high level of blood glucose. Type 1 diabetes is an auto immune disorder while Type 2 diabetes is the most common one where the is either unable to produce sufficient insulin or cannot respond to insulin. Diabetes is classically characterized by frequent urination, excessive thirst, weight loss and dryness of skin. However,uncontrolled diabetes can lead to complications like retinopathy, nephropathy, neuropathy, cardiac problems etc. A stress free life with healthy eating habits, moderate exercise and weight management programme aid immensely in controlling hyperglycemia. A considerable number of medicinal plants possess antidiabetic properties and are widely used in diabetes management throughout the world. Several species of family Meliaceae due to their hypoglycemic potentialhave either been used as dietary adjuncts or phytotherapeutic agents to control diabetes. The present study reports the antidiabetic properties of *Cipadessa baccifera,*an ethnobotanically important shrub growing in the tropical forests of southern India. Being rich in phytochemicals it is extensively exploited by tribal folks. Leaf extract of *Cipadessa baccifera* is found to inhibit α amylase and α glucosidase activity thus making the plant a potential therapeutic agent for diabetes management.

Keywords- Diabetes, *Cipadessa baccifera*, hyperglycemia, α amylase, α glucosidase

Introduction

Diabetes mellitus is one of the most common and prevalent chronic metabolic disorder affecting both the developing and the developed countries. According to the World Health Organization projection, by the year 2025 there would be a dramatic increase (64%) in diabetes incidences worldwide i.e., a whopping 300 million people will be diabetic (Sy *et al.,* 2005; Rowley, 2012). In India it is more pronounced with the diabetic population expected to rise from the present 40.9 million to 69.9 million according to the Diabetes Atlas published by the International Diabetes Federation (Sicree *et al.,* 2006). This emerging enormity of the diabetic scenario has earned India the dubious distinction of being the 'Diabetic Capital of the World' (Joshi *et al*., 2007).

Diabetes mellitus is an endocrine metabolic disorder caused by the abnormality or dysfunction of carbohydrate metabolism where in the pancreas does not produce or properly use the hormone insulin (Maiti, 2004). Genetic and environmental factors are significant contributors to the development of diabetes (Murea M *et al.,* 2012). Among the types of diabetes, the most prevalent are Type 1 DM (T1DM) and Type 2 DM (T2DM). Type 1 diabetes mellitus is caused due to insufficiency of insulin whereas, Type 2 diabetes mellitus is a result of ineffectiveness of insulin in the body. Chronic uncontrolled diabetes could result in microvascular (retinopathy, neuropathy and nephropathy) and macrovascular (heart attack, stroke, and peripheral vascular disease) complications leading to significant morbidity and mortality (Patel, 2011).

The increase in diabetes risk is influenced by ethnicity, family history of diabetes, and previous gestational diabetes, older age, overweight and obesity, unhealthy diet, physical inactivity, and smoking (Folorunso O and Oguntibeju O, 2013). T2DM diabetes (90%) is mostly observed in adults, but these days it is also seen increasingly affecting children also (Sy GY *et al*., 2005). Demographic studies indicate that by 2030, the increase in diabetes cases among people older than 64 years will be greater in developing countries (≥82 million) than in developed countries (≥48 million) (Narayan *et al.,* 2006). In the past 3 decades there has been a steep increase in the prevalence of diabetes in the low- and middle-income countries when compared to high-income countries.

The currently available diabetic therapy includes administering insulin and various other oral anti-diabetic compounds such as sulfonylureas, biguanides and glinides etc. Notwithstanding the progress made in diabetic treatment, due to limitations, adverse side effects, increase risk of hypoglycemia (Turner *et al.,* 1999) and the challenges posed by the

disease, scientists all over the world are once again seeking newer therapeutics in indigenous and traditional medicinal plants. Nearly 1200 medicinal plants have been reported to be used in the treatment of diabetes owing to their ability to help in enhancing the insulin secretion by pancreas and fight problems of insulin resistance (Kavishankar *et al*., 2011). Metformin, one of the well-known approved drugs today, used in the treatment of non-insulin dependent Diabetes mellitus is derived from the ethno-medicinal plant *Galega officinalis*, (Shukla *et al*., 2000).

Although synthetic oral hypoglycemic drugs along with insulin have been the primarily used for controlling diabetes, they have been unable to reverse the complications arising due to diabetes and are also said to cause side effects. This forms a compelling force for the scientific community to discovering alternative natural sources of antidiabetic agents which offer great potential for discovery and design of anti-diabetic drugs. (Rao *et al*., 2010).

Medicinal plants such as,*Catharanthus roseus, Gymnema sylvestre* and *Ocimum sanctum* has been reported to have the most potent blood sugar lowering effect and have been considered a vital source of potent antidiabetic drugs for centuries. Severalspecies of family Meliaceae such as, *Azadirachta indica* and *Melia dubia*have played an important role in controlling the blood sugar levels, in preventing or delaying the onset of diabetes mellitus, thereby helping in the management of diabetes mellitus (Khosla *et al.* Shukla *et al.,* Valentina *et al.,* 2013). Due to hypoglycemic potential these plants have either been used as dietary adjuncts or phytotherapeutic agents to control diabetes.

Medicinal plants are a rich source of phytochemicals such as flavonoids, alkaloids, glycosides, galactomannan, hypoglycans, steroids, terpenoids, phenols and dietary fibres which have shown anti-diabetic properties (Grover *et al.,* 2002). These phytoconstituents show antioxidant, hypolipidemic and anticataract properties. In addition they are also involved in regeneration of pancreatic islets and alleviation of liver and renal damage (Mukherjee *et al.,* 2006). Understanding the combined biological action and beneficial activities of the repository of bio active compounds present in medicinal plants (i.e., polyphenols, carotenoids, lignans, coumarins, glucosinolates, etc.) is vital. (Durazzo *et al*., 2018).

*Cipadessa baccifera*is a perennial shrub widely distributed in the tropical forests of Asia. It is an ethnobotanically important plant of family Meliaceae and used in folk medicine for treating different maladies like diabetes, dysentery, malaria, rheumatism, piles, headache

and psoriasis (Chinese Materia Medica, 1999, Malarvannan *et al.*, 2009).Several bioactive compounds such as limonoids like, tetratriterpenoids, terpenoids, glucosides, have been reported in *Cipadessa* species (Liang *et al.*, 1991). Despite the presence of potential phytochemicals, literature shows that there are not many scientific studiesvalidatingthe ethnopharmacological properties of this plant. Hence the present investigation is an attempt to evaluate the antidiabetic potential of *Cipadessa baccifera* (Roth.) Miq.

Material and Methods

In Vitro Anti-diabetic activity

In vitro anti-diabetic activity of methanolic extracts of leaves and roots were evaluated by α-Amylase and α-Glucosidase inhibition bio-assays using standard protocols (Gella *et al.*, 1997).

Chemicals

The chemicals; $CNPG_3$ (2-chloro-4-nitrophenyl-α-D-maltotrioside), acarbose, sucrose, sodium dihydrogen phosphate (NaH_2PO_4. $2H_2O$), disodium hydrogen phosphate dihydrate (Na_2HPO_4. $2H_2O$), Dimethyl sulfoxide (DMSO) were obtained from Merck Ltd., Mumbai, India. Glucose reagent kit (EC. 3.2.1.1) Type VI-B: from porcine pancreas, 500,000 units [15.8 units/mg solid at pH 6.9], α-glucosidase isolated from small intestine of rat, were purchased from HiMedia Laboratories Ltd., Mumbai. The CNPG3 reagent, α-amylase procured were stored at 2-8°C in the lab.

Alpha-amylase inhibition assay

The α-amylase inhibitory activity of the methanolic extracts of the leaves and roots was evaluated by standard spectrophotometric assay with acarbose as the reference compound as described by Gella *et al.*, (1997) with slight modifications. The aliquots of extract samples were prepared in DMSO to obtain various concentrations ranging from 12.5, 25, 50, 100, 200, and 400 µg/mL. Standard acarbose of 2.5 mg/mL concentration was prepared and further dilutions of 0.5, 1, 2, 4, 8 and 16 µg/mL were made using phosphate buffer (pH 7). The α-amylase enzyme solution (0.5 U/mL) was prepared by mixing 3.246 mg of α-amylase enzyme (EC 3.2.1.1) in 100 mL of 40 mM phosphate buffer (pH 6.9). The phosphate buffer, 60 µL of enzyme along with standard acarbose/plant extract

concentrations were mixed and pre-incubated at 37°C for 10 min. Then, 125 µL of the substrate, 2-Chloro-4-Nitrophenyl-α-D-Maltotrioside ($CNPG_3$) was added, mixed and incubated at 37°C for 8 min. The reaction was then arrested by heating on boiling water bath for 2 minutes. After cooling the mixture, the absorbance was measured at 405 nm. Similarly, a positive control reaction was performed without the plant extract.

Pancreatic α-amylase hydrolyses $CNPG_3$ to release 2-chloro-4-nitrophenol and form 2-chloro-p-4-nitrophenyl-α-D–maltoside ($CNPG_2$), maltotriose and glucose. The rate of formation of $CNPG_2$ which is measured at 405 nm is a direct measurement of amylase present in the sample. Percentage inhibition of α-amylase was calculated using the formula:

$$\text{Percentage inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

Alpha-glucosidase inhibition assay

The effect of leaf and root methanolic extracts of *C. baccifera* on α-glucosidase activity was assayed according standard protocol given by Matsui *et al*., (2001) with slight modifications. About 250 µL of 0.1 M phosphate buffer (pH 7.0), 50 µL of 0.5 mg protein equivalent of crude α-glucosidase enzyme was incubated with different concentrations of methanolic extracts of leaf and root (12.5, 25, 50, 100, 200, and 400 µg/mL) at 37°C for 30 min. The reaction was initiated by adding 500 µL of substrate sucrose (37 mM). The reaction mixture was incubated for 20 min at 37°C. Further, the reaction was arrested by adding 1.0 mL of Tris base. Standard acarbose of 0.5, 1, 2, 4, 8 and 16 µg/mL concentrations were prepared and used as positive control. The α-glucosidase activity was determined by measuring the glucose released from sucrose by glucose oxidase. To 100 µL of the reaction mixture, 500 µL of glucose reagent kit was added and incubated at room temperature for 10 min. Then the absorbance was measured at 510 nm.

Enzyme inhibitory activity was expressed as percentage inhibition. Finally the data were expressed as IC_{50} value (The concentration of methanolic extract of leaf and root required to inhibit 50% of α- amylase/α-glucosidase activity).

$$\text{Percentage of inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of cont rol}} \times 100$$

Results

Anti-diabetic activity of methanolic extracts

The inhibitory activity of methanolic extracts of leaf and root of *Cipadessa baccifera* on α-amylase and α-glucosidase was assayed and the results are shown in Table 1.

Alpha amylase inhibition

The *in vitro*α-amylase inhibition potential of methanolic extracts of the leaf and root of *C. baccifera* was tested. At a concentration of 400 µg/mL the α-amylase inhibition by the leaf and root extracts was significant and measured 43.68% and 39.26% respectively. The results revealed that α-amylase inhibition increased with increase in the concentration of the extracts. The percentage of inhibition of methanolic extract of the leaf at all concentrations was found to be higher than that of the root extract. The standard reference acarbose at 16 µg/mL concentration showed 93.45% α amylase inhibitory activity and IC_{50} value of 2.46 µg /mL.

Table 1. *In vitro* α-amylase and α-glucosidase inhibitory activity of methanolic extracts of leaf and root of *C. baccifera*

Concentration (µg/mL)	**Percentage Inhibition**			
	Alpha amylase		**Alpha glucosidase**	
	ME of leaf	**ME of root**	**ME of leaf**	**ME of root**
12.5	7.97±0.13	4.11±0.22	3.03±0.24	4.12±0.21
25	11.80±0.21	7.53±0.18	5.92±0.27	6.85±0.23
50	18.28±0.23	11.29±0.31	10.42±0.25	9.52±0.18
100	21.59±0.19	19.16±0.28	18.53±0.19	16.91±0.20
200	29.47±0.18	25.79±0.25	24.51±0.31	22.65±0.28
400	43.6±0.24	39.26 ±0.19	31.52±0.38	28.12±0.26

Values are Mean ±SD, n=9; ME- methanolic extract

Alpha glucosidase inhibition

The methanolic extracts of leaf and root were evaluated for *in vitro*α-glucosidase inhibition activity. The percentage of glucosidase inhibition ranged from 3.03-31.52% for leaf and 4.12-28.12% for the root extract (Table 1). It was seen that there was a dose-dependent increase in the percentage inhibitory activity against α-glucosidase. The glucosidase inhibition activity of leaf and root extracts at 400µg/mL concentration was 31.52% and 28.12% respectively. The standard reference acarbose showed an IC_{50} value of 11.07 µg/mL.

It was inferred from the study that the leaf extract of *C. baccifera* had higher potential for α-amylase and α-glucosidase inhibition activity than the root extract. However the IC_{50} values of both extracts of *C. baccifera* were not significant and exceeded 400 µg/mL.

Discussion

***In vitro* anti-diabetic activity**

In the present study, the hypoglycemic effect of the methanolic extracts of leaf and root of *Cipadessa baccifera* was assessed through *in vitro* enzymatic inhibition of α-amylase and α-glucosidase with reference to commercially prescribed anti-diabetic compound acarbose. Acarbose is a pseudo tetrasaccharide responsible for the inhibition of the α-glucosidase enzyme. The results revealed an increase in α-amylase and α-glycosidase inhibition with increase in the concentration of the leaf and root extracts of *C. baccifera,* however the inhibitory activity was not significant when compared to earlier findings reported in *Eugenia jambolana* and *Azadirachta indica* (Pardeep *et al*., 2013).

The α-amylase and α-glycosidase inhibitory activity of the root methanolic extract of *C. baccifera* was found to be lower than the leaf although it contained a higher amount of flavonoids which have been reported to be the bioactive anti-diabetic agents with enhanced α-glycosidase and α-amylase inhibitory properties (Kim *et al*., 2000; Mcdougall and Stewart, 2009). This difference in inhibition percentage of the root and leaf extracts may not be totally dependent on the quantity of the flavonoids alone. Instead, the bioactive compounds involved in the inhibitory action of α-amylase and α-glucosidase could be flavonoids, tannins, saponins or phenolic acids, as literature survey shows a clear relationship between these compounds and anti-diabetic activity of herbal extracts (Kazeem *et al.*, 2013).

Conclusion

Though *Cipadessa baccifera* has been used in traditional medicines for its anti-diabetic potential, no anti-diabetic studies have been carried out (Sandhya *et al*., 2011). Thus, appreciable α amylase and α glucosidase inhibitory activity of leaf extract at higher concentrations substantiates the use of this plant in the traditional medicines for treatment of diabetes. Hence the present study serves as a promising platform for further investigations.

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