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In-vitro evaluation of the anti-diabetic activity of bottle brush plants

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ABSTRACT

The leaves of 5 Bottle Brush plants belong to family Myrtaceae were exhaustively extracted with hot 80% methanol, under reflux. The dry residues of the alcoholic extracts were tested using Sucrase enzyme inhibitory activity test to evaluate their anti-diabetic activity. The alcholic extract of the leaves of Bottle Brush plants exhibited significant anti-diabetic activity.

KEYWORDS

Callistemon;
Myrtaceae;
Anti-diabetic activity.

INTRODUCTION

Herbal medicines have been used since centuries by different cultures worldwide for treatment of diabetes. Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin action, insulin secretion, or both[20]. One of interesting plants called Bottle brush plants were used in tradition medicine as anti-diabetic. Those plants scientifically named as Callistemon. The genus *Callistemon* is a shrub belonging to the family Myrtaceae. It contains 34 species of beautiful evergreen shrubs and small trees. The majority of the *Callistemon* species is endemic to temperate regions[9]. The species are commonly known as bottle brushes because of their cylindrical brush-like flowers resembling the traditional bottle brush. Some species of this genus are used as a tea substitute and have a refreshing flavor[16]. In folk medicine, the genus *Callistemon* is known for its anticough, antibronchitis, insecticidal, antimicrobial, anti-inflammatory, analgesic, antinociceptive, anticonvulsant and anti-diabetic effects[5,11,12,18,22]. Phytochemical investigations of members of the genus resulted in the identification of flavonoids, flavonoids glycosides, phenolic acids, hydrolysable ellagitannins, triterpenoids, volatile oils and phloroglucinol derivatives[5,8,11-13,21,23]. Control of postprandial hyperglycemia is critical in the early treatment of diabetes mellitus[17] as it could induce non enzymatic glycosylation of various proteins, resulting in the development of chronic complications such as, micro- and macro-vascular diseases[1], and it has also been proposed as an independent risk factor for cardiovascular diseases[3]. Postprandial hyperglycemia can be controlled by decresing the glucose absorption through the inhibition of enzymes responsible for hydrolysis carbohydrate such as α-amylase, α-glucosidase, and sucrase, in the digestive tract[15]. Phytochemicals exhibit their hypoglycemic effect by several mechanisms, such as, inhibition of carbohydrate metabolizing enzymes, manipulation of glucose transporters, β-cell regeneration, and enhancing the insulin releasing activity[19]. The present

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study deals with the investigation of the in-vitro anti-diabetic activity of several Callistemon species through the performance of Sucrase inhibitory activity test. In traditional medicine, people usually use aqueous decoctions to treat patients. That is why in this study we prepared an extract from an aqueous alcoholic decoction.

MATERIALS AND METHODS

Plant material

Leaves of *Callistemon species* (i.e. *Callistemon lanceolatus, Callistemon viminalis, Callistemon rigidus, Callistemon comboynensis and Callistemon viridiflorous)* were collected from Alexandria-Cairo Road, Egypt. The plant was identified by Dr. Trease Labeb, Lecturer of Taxonomy, Department of Floral and Taxonomy, Orman Garden, Cairo, Egypt.

Assay of sucrase inhibitory activity

A crude enzyme solution of rat intestinal sucrose enzyme, prepared according to the method of Dahlqvist[4]. The effect of samples on sucrase activity was assayed according to the method of Honda and Hara[7] The enzyme solution (10 µl) were incubated together for 10 minutes at 37°C, and the volume was made up to 200 µL with maleate buffer (pH 6.0) in case of control or up to 200 µL with buffer solubilized sample (100 µg/ml in maleate buffer with pH 6.0). The enzyme reaction was started by adding 100 µl sucrose solution (60 mM). After 30 minutes, the reaction was terminated by adding 200 µL of 3,5-dinitrosalysilic acid reagent and treating the mixture in a boiling water bath for five minutes. The absorbance of the solution was read at 540 nm. The percent inhibitory activities were calculated using the following formula:

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

Where, *Abs control* is the absorbance of the control reaction (containing all reagents except the test sample), and the *Abs sample* is the absorbance of the test sample. An untreated enzyme solution was used as the control. All the experiments were carried out in triplicate.

Results of the anti-diabetic activity (Sucrase inhibitory activity)

C. rigidus decreases the activity of sucrase enzyme by (63.05±3.43) followed by *C. viminalis* (61.21±3.65), *C. lanceolatus* (54.67±2.34), *C. viridiflorus* (52.54±2.53) then *C. comboynensis* (45.36±2.86) as shown in TABLE 1 and Figure 1

In the present investigation, both untreated and heat-treated aqueous extracts of *Bottle brush extracts* significantly inhibited sucrase enzyme. It is a general opinion that medicinal plants inhibit sucrase activity due to the presence of several possible factors and mechanisms, such as fiber, polyphenolic concentration[14]. In the our present investigation, the effect of various extracts of Bottle brush plants on carbohydrate hydrolyzing enzyme, namely, rat intestinal sucrase, have been studied using *in vitro* model systems. The extracts of *Bottle brush plants* significantly inhibited ($p \leq 0.01$) sucrase activities (Figure 1). The Sucrase inhibitory activity of *Bottle brush plants* supposed to be due to the presence of flavonoid diglycosides[24] hydrolysable tannins. The present investigation reports the Sucrase enzyme (a carbohydrate hydrolyzing enzyme) inhibitory characteristics of Bottle Brush plants. With a constant rise in the incidence of type II diabetes around the world it appears that more anti-diabetic drugs with complementary mechanisms of action should be developed, in order to achieve durable glycemic control by inhibiting, in a reversible way, the hydrolysis of disaccharides and the ultimate steps of the digestion of dietary polysaccharides, to reduce the rise of postprandial blood glucose in diabetics[2]. Rat intestinal sucrase occurs as a

TABLE 1 : Sucrase enzyme activity

Sample	Sucrase I%±SD
C. lanceolatus	54.67±2.34
C. viminalis	61.21±3.65
C. viridiflorus	52.54±2.53
C.comboynensis	45.36±2.86
C. rigidus	63.05±3.43

SD is the standard deviation

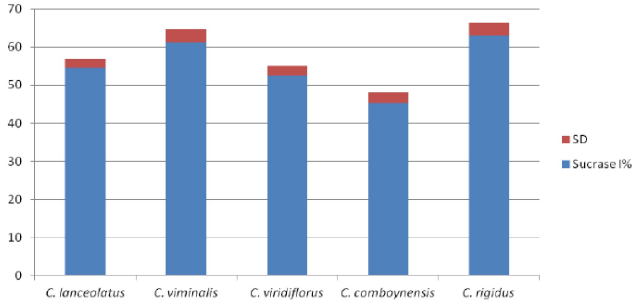

Figure 1 : Sucrase enzyme activity

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complex of sucrase and isomaltase, which converts sucrose into glucose[6].

CONCLUSION

The methanol extract of the leaves of bottle brush leaves exhibited significant in-vitro anti-diabetic activity using Sucrase inhibitory activity test. And we recommend in vitro and in-vivo toxic test to be done to evaluate their safety to be used as complementary drugs to help main medicines in treatments of diabetic patients.

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