

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

Mohamed I.S.Abdelhady^{1,2*}, Mahmoud Youns^{3,4}

¹Department of Pharmacognosy, Faculty of Pharmacy, Umm Al-Qura University, Makkah 21955, (SAUDIARABIA)

²Pharmacognosy Department, Faculty of Pharmacy, University of Helwan, Ain Helwan, Cairo, (EGYPT)

³Department of Biochemistry and Molecular Biology, Faculty of Pharmacy, University of Helwan, Ain Helwan, Cairo, (EGYPT)

⁴Division of Functional Genome Analysis, German Cancer Research Center (DKFZ), Heidelberg, (GERMANY)

E-mail : mohibrahem@yahoo.com; miabdelhady@uqu.edu.sa

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 alcoholic extract of the leaves of Bottle Brush plants exhibited significant anti-diabetic activity.

© 2014 Trade Science Inc. - INDIA

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^[6]. 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 decreasing 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

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 viridiflorus*) were collected from Alexandria-Cairo Road, Egypt. The plant was identified by Dr. Trease Labebe, Lecturer of Taxonomy, Department of Floral and Taxonomy, Orman Garden, Cairo, Egypt.

Assay of sucrase inhibitory activity

A crude enzyme solution of rat intestinal sucrase 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 μ l) were incubated together for 10 minutes at 37°C, and the volume was made up to 200 μ L with maleate buffer (pH 6.0) in case of control or up to 200 μ L with buffer solubilized sample (100 μ g/ml in maleate buffer with pH 6.0). The enzyme reaction was started by adding 100 μ l sucrose solution (60 mM). After 30 minutes, the reaction was terminated by adding 200 μ L 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:

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

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 \pm 3.43) followed by *C. viminalis* (61.21 \pm 3.65), *C. lanceolatus* (54.67 \pm 2.34), *C. viridiflorus* (52.54 \pm 2.53) then *C. comboynensis* (45.36 \pm 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% \pm SD
<i>C. lanceolatus</i>	54.67 \pm 2.34
<i>C. viminalis</i>	61.21 \pm 3.65
<i>C. viridiflorus</i>	52.54 \pm 2.53
<i>C. comboynensis</i>	45.36 \pm 2.86
<i>C. rigidus</i>	63.05 \pm 3.43

SD is the standard deviation

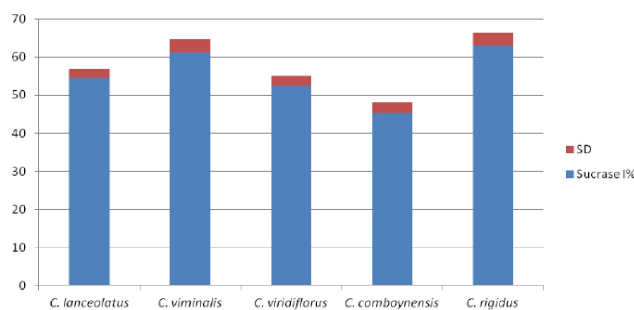


Figure 1 : Sucrase enzyme activity

Regular Paper

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.

REFERENCES

- [1] A.D. Baron; Postprandial hyperglycemia and α -glucosidase inhibitors. *Diabetes Res. Clin. Pract.*, **40**, 51-5 (1998).
- [2] J.F. Blicke, E. Andres, J.M. Brogard; Current status of the treatment of type 2 diabetes mellitus: α -glucosidase inhibitors. *Food Chem.*, **106**, 247-252 (2008).
- [3] A. Ceriello; The emerging role of post-prandial hyperglycaemic spikes in the pathogenesis of diabetic complications. *Diabetic Med.*, **15**, 188-193 (1998).
- [4] A. Dahlqvist; Method for assay of intestinal disaccharides. *Anal. Biochem.*, **7**, 18-25 (1964).
- [5] S.A. El-Batran, S.S. Mahmoud, F.A. Moharram, M.S. Marzouk, M.I. Mohamed; Evaluation of some pharmacological effects of *Callistemon viminalis* and *Callistemon lanceolatus* extracts. *J. Union Arab. Biol. Cairo.*, **17**, 17-28 (2002).
- [6] H.P. Hauri, H. Wacker, E.E. Rickli, B. Bigler-Meier, A. Quaroni, G. Semenza; Biosynthesis of sucrase-isomaltase: Purification and NH₂-terminal amino acid sequence of the rat sucrase-isomaltase precursor (pro-sucrase-isomaltase) from fetal intestinal transplants. *J. Biol. Chem.*, **257**, 4522-8 (1982).
- [7] M. Honda, Y. Hara; Inhibition of rat small intestinal sucrase and α -glucosidase activities by tea polyphenols. *Biosci Biotechnol Biochem.*, **57**, 123-4 (1993).
- [8] F. Huq, L.N. Misra; An alkenol and C-methylated flavones from *Callistemon lanceolatus* leaves. *Planta. Med.*, **63**, 369-70 (1997).
- [9] P.C. Kanjilal, A. Das; *Flora of Assam*, Omsons Publications, New Delhi, (1992).
- [10] I.I. Mahmoud, F.A. Moharram, M.S. Marzouk, M.W. Linscheid, M.I. Abdelhady; Polyphenolic constituents of *Callistemon lanceolatus* leaves. *Pharmazie*, **57**, 494-6 (2002).
- [11] Mohamed I.S. Abdelhady, Amel M. Kamal, Madiha R. Mahmoud, N.F. Tawfik, S.M. Abdelkhalik; Antinociceptive and antischistosomal activities of aqueous methanolic extract of *Callistemon viridiflorus* leaves in addition of detection of new polyphenolic metabolite. *Journal of Pharmacy Research*, **5(7)**, 3777-3783 (2012a).
- [12] Mohamed I.S. Abdelhady, Amel M. Kamal, Nashwa F. Tawfik, Soad M. Abdelkhalik; Polyphenolic constituents of the methanolic extract of *Callistemon viridiflorus* leaves and its antimicrobial activity. *Pharmacognosy, Journal*, **4(31)**, 47-53, Sep-Oct (2012b).
- [13] Mohamed I.S. Abdelhady, Hamdy A.H. Aly; The antioxidant and the antimicrobial activities of the essential oil of *Callistemon comboynensis*. *Journal of Free Radicals and Antioxidants*, **2(1)**, 35-39, Jan-Mar (2012).
- [14] S. Ou, K. Kwok, Y. Li, L. Fu; In vitro study of possible role of dietary fiber in lowering postprandial serum glucose. *J. Agric. Food Chem.*, **49**, 1026-9 (2001).
- [15] R. Rhabasa-Lhoret, J.L. Chiasson; International textbook of diabetes mellitus. 3rd Edition, UK: John Wiley and Sons Ltd., **1**, 901-914 (2004).
- [16] R.K. Sharma, R. Kotoky, P.R. Bhattacharya; Volatile Oil from the leaves of *Callistemon lanceolatus* D.C. Grown in Northeastern India, *Flavonoid and Fragrance Journal*, **21**, 239-40 (2006).
- [17] Y.J. Shim, H.K. Doo, S.Y. Ahn, Y.S. Kim, J.K. Seong, I.S. Park et al.; Inhibitory effect of aqueous extract from the gall of *Rhus chinensis* on α -glucosidase activity and postprandial blood glucose. *J. Ethnopharmacol.*, **85**, 283-7 (2003).
- [18] M. Sudhankar, Ch. V. Rao, A. R. Lakshman, D. B. Raju; Antinociceptive and anti-inflammatory effects of the standardized oil of Indian *Callistemon lanceolatus* leaves in experimental animals. *Acta Pharm. Turc.*, **46**, 131-9 (2004).
- [19] A.K. Tiwari, J.M. Rao; Diabetes mellitus and multiple therapeutic approaches of phytochemicals: Present status and future prospects. *Curr. Sci.*, **23**, 30-3 (2002).
- [20] C.C. Teixeira, C.A. Rava, P.M. Da Silva, R. Melchior, R. Argenta, F. Anselmi et al.; Absence of antihyperglycemic effect of jambolana in experimental and clinical models. *J. Ethnopharmacol.*, **71**, 343-7 (2000).
- [21] R.S. Varma, M.R. Parthasarathy; Triterpenoids of *Callistemon lanceolatus*. *Phytochemistry*, **14**, 1676 (1975).
- [22] G.S. Wheeler; Maintenance of a narrow host range by *Oxypos vitiosa*: A Biological Control Agent of *Melaleuca*, *Biochem. Syst. Ecol.*, **33**, 365-83 (2005).
- [23] E. Wollenweber, R. Wehde, M. Dorr, G. Lang, J.F. Stevens; C-methyl flavonoids from the leaf waxes of some Myrtaceae. *Phytochemistry*, **55**, 965-70 (2000).
- [24] L.L. Yang, C.V. Lee, K.Y. Yen; Induction of apoptosis by hydrolysable tannins from *Eugenia jambos* on human leukemia cells. *Cancer Lett*, **57**, 65-75 (2000).