ISSN 2278- 4136

ZDB-Number: 2668735-5

IC Journal No: 8192

Volume 2 Issue 2

Online Available at www.phytojournal.com

Journal of Pharmacognosy and Phytochemistry

In-vitro antioxidant and α-amylase inhibition activity of *Cucurbita maxima*

Shahlah Jihad Ahmed Al-Shaheen[1], Raad A Kaskoos[1,2*], Khitam Jawad Hamad[1], Javed Ahamad[2]

` aCollege of Pharmacy, Hawleer Medical University, Erbil, Iraq.

bPhytochemistry Research Laboratory, Faculty of Pharmacy, Jamia Hamdard, New Delhi-110062, India.

E-mail: raadkaskoos@gmail.com

Cucurbita maxima, belongs to family Cucurbitaceae, is commonly known as pumpkin. Several literature reports suggest it to be antidiabetic, antihypertensive, anticancer, immunomodulators, antibacterial and antihyperlipidaemic. One important approach for the treatment of type 2 diabetes mellitus is by decreasing the postprandial hyperglycemia in effect. This is possible by inhibiting certain carbohydrate hydrolyzing enzyme like α-amylase and α-glucosidase. The objective of present study was to evaluate *in-vitro* antioxidant and α-amylase inhibitory activity of methanolic extract of *C. maxima* leaves. Methanolic extract of leaves of *C. maxima* showed strong antioxidant (DPPH scavenging) and α-amylase inhibition (IC_{50} 125 µg/ml and IC_{50} 2.1 mg/ml), respectively. These results suggest the possible use of pumpkin leaves in the management of diabetes mellitus.

Keyword: *Cucurbita maxima,* Cucurbitaceae, α-amylase, Antioxidant, Antidiabetic.

1. Introduction

Cucurbita maxima Duchesne (Syn. *C. pepo* Linn.; *C. moschata* Duchesne) represent economically important species of Cucurbitaceae family and cultivated worldwide (1). The popularity of pumpkin for use in various traditional medicines for several ailments has attracted scientific attention to this plant. The use of dietary plants and herbal preparations as alternative medicine has recently received considerable attention in the United States and Europe (2). In developing countries all over the world 80% of population continues to use traditional medicine in primary medical problems. Recently, research has been focused on scientific evaluation of dietary plants and preparations of plant origin for the management and treatment of several diseases. Pumpkin is one such plant that has been frequently used as functional food or medicine. *C. maxima* reported to have antidiabetic (3), hepatoprotective (4), anthelmintic (5), immunomodulatory, antihypertensive, anticancer, antibacteria, antihypercholesterolemic and antiinflammatory (1, 6) activities. It is rich in polysaccharides, contains high amounts of amino acids, fatty acids, carotenoids, minerals and vitamin E. The plant is reported to have triglyceride fatty acid mixture, tetrahydro-thiophene, linoleic acid, calotropoleanyl ester, cholesterol and 13(18)-oleanen-3-ol (7). The present study was designed to evaluate *in-vitro* antioxidant and α-amylase inhibition activity of methanolic extract of *C. maxima* leaves.

2. Material And Methods

2.1 Plant Material

The leaves of *Cucurbita maxima* were collected from the Shaqllawa, Iraq. The sample was

identified and a voucher specimen of the plant was submitted to the Phytochemistry Research Laboratory, Jamia Hamdard, New Delhi.

2.2 Chemicals

BHA (3-tert-butyl-4-hydroxyanisole), BHT (butylated hydroxy toulene), Vitamin C and DPPH (2,2-dyphenyl-1-picrylhydrazyl) were obtained from Sigma Chemicals Co., St. Louis, MO, USA. α-Amylase and 3,5-dinitrosalicylic acid (DNS) were purchased from SRL, Bangalore, India. All other solvents and chemicals were of analytical grade.

2.3 Preparation of Extract

The leaves were dried at temperature below 45 ºC in shade and coarsely powdered in grinder. About 10 g of leaf powder was weighed extracted in Ultrasonic water bath at 50 ºC for 30 min in methanol with a solid/solvent ratio of 1:10. Extract was concentrated in rotary evaporator (Hanshin, Korea) and dried in vacuum. The crude residue was reconstituted in methanol and filtered through 0.45 µm membrane filter and stored at 4 ºC in refrigerator for further study.

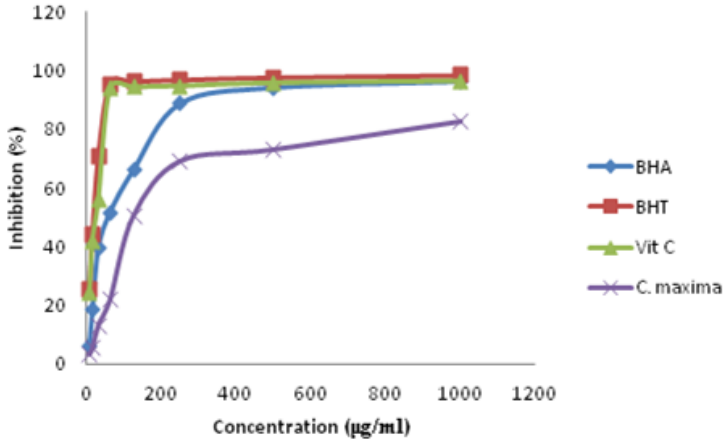

Fig 1: Antioxidant (DPPH scavenging) activity of BHA, BHT, Vit. C and methanolic extract of *C. maxima* leaves

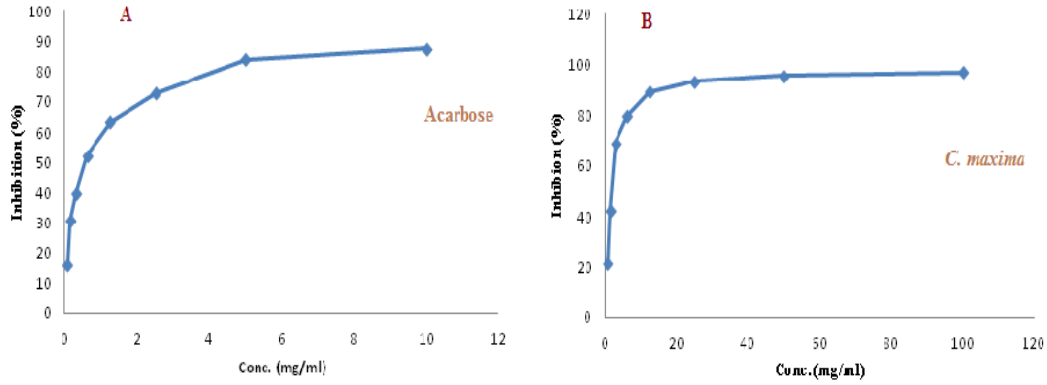

Fig 2: α-Amylase inhibition by (A) Acarbose; (B) Methanolic extract of *C. maxima* leaves

2.4 Evaluation of *in-vitro* Antioxidant Activity

The ability of a substance to scavenge DPPH free radicals was assessed by the standard method (8, 9), adopted with suitable modifications. The stock solution of extract residue was prepared in methanol to achieve the concentration of 1 mg/ml. Dilutions 1000, 500, 250, 125, 62.5, 31.25, 15.62 and 7.81 µg/ml were prepared by serial dilution method. Diluted solutions (1 ml each) were mixed with 1 ml of methanolic solution of DPPH (1 mg/ml). After 30 min incubation in darkness at room temperature (23

°C), the absorbance was recorded at 517 nm. Control sample contained all the reagents except the extract. Percentage inhibition was calculated using equation given below:

$$\% \, Inhibition = \frac{\text{Aco} - \text{A}t}{\text{Aco}} \times 100$$

where, A_{CO} is absorbance of the control and A_t is absorbance of the test samples.

IC_{50} values were estimated from the % inhibition versus concentration plot using a non-linear regression algorithm.

2.5 *In-vitro* α-amylase inhibition activity

The α-amylase inhibitory activity was assessed by the standard method (10, 11), suitable with suitable modification. A 40 µL of sample solution (0.2 mg of sample or Acarbose in 20 mM sodium phosphate buffer, pH 6.9 with 0.006 M sodium chloride) was premixed with 200 µL of α-amylase solution (1.0 U/ml in the pH 6.9 buffer), and incubated at 25 ºC for 30 min. After pre-incubation, 400 µL of a 0.25% starch solution in the pH 6.9 buffer was added to each tube to start the reaction. The reaction was carried out at 37 ºC for 5 min and terminated by addition of 1.0 ml of the DNS reagent (1% 3,5-dinitrosalicylic acid and 12% sodium potassium tartrate in 0.4 M NaOH). The test tubes were then incubated in a boiling water bath for 10 min and cooled to room temperature. The reaction mixture was then diluted after adding 10 ml distilled water and absorbance was measured at 540 nm.

Control incubations represent 100% enzyme activity and were conducted in a similar way by replacing extracts with buffer. For blank incubation (to allow for absorbance produced by the extract), enzyme solution was replaced by buffer solution and absorbance recorded.

The α-amylase inhibitory activity was expressed as inhibition % and was calculated as follows:

$$\% \, Inhibition = \frac{\text{Aco} - \text{A}t}{\text{Aco}} \times 100$$

where, A_{CO} is absorbance of the control and A_t is absorbance of the test samples.

IC_{50} values were estimated from the % inhibition versus concentration plot using a non-linear regression algorithm.

2.6 Result and Discussion

2.6.1 *In-vitro* Antioxidant Activity

The antioxidant activity of methanolic extract of *C. maxima* leaves was determined DPPH scavenging method and was expressed in terms of percentage of inhibition (%). Parallel to examination of the antioxidant activity of the methanolic extract of *C. maxima leaves*, the values for three standard compounds (BHA, BHT and Vitamin C) were obtained and compared with the antioxidant activity of extract. A plot of % inhibition verses concentration, given in Fig 1 was used to calculate IC_{50} values. The examination of antioxidant activity showed a concentration dependant response that varied from 3.28 to 88.91% for 7.81 to 1000 µg/ml, respectively. The IC_{50} value of standard BHT, Vitamin C and BHA and methanolic extract of *C. maxima* was found to be 16 µg/ml, 24 µg/ml, 52 µg/ml and 125 µg/ml, respectively.

2.6.2 α-Amylase Inhibition Study

The methanolic extract of *C. maxima* leaves produced a strong α-amylase enzyme inhibition. *C. maxima* leaves extracts showed a concentration dependent α-amylase inhibition activity that varied from 21.49 to 97.01 % for 0.78 to 100 mg/ml, respectively. The maximum α-amylase inhibition produced by methanolic extract of *C. maxima* leaves was 97.01 % at a concentration of 100 mg/ml. Parallel to examination of the α-amylase inhibition activity of the methanolic extract of *C. maxima* leaves, the value for standard compounds acarbose was obtained and compared with the extract. Acarbose showed a concentration dependant response that varied from 87.76 to 16.23 % for 0.078 to 10 mg/ml, respectively. Figures 2A and 2B show the percentage inhibition of porcine α-amylase of acarbose and methanolic extract of *C. maxima* leaves, respectively. The IC_{50} values for acarbose and methanolic extract of *C. maxima* leaves were 0.62 mg/ml and 2.1 mg/ml, respectively.

3. Conclusion

The results of this *in-vitro* study clearly indicated that methanolic extract of *C. maxima* leaves had strong α-amylase inhibitory and antioxidant activity. These attributes when combined in one plant are potentially useful to manage the glucose-induced hyperglycemia and provide the

biochemical rationale for further animal and clinical studies.

4. References

1. Caili F, Huan S, Quanhong L. A review on pharmacological activities and utilization technologies of pumpkin. Plant Foods for Human Nutrition 2006; 61: 73-80.
2. Ang-Lee MK, Moss J, Yuan CS. Herbal medicines and peri-operative care. JAMA. 2001; 286: 208-216.
3. Sharma A, Sharma AK, Chand T, Khardiya M, Yadav KC. Antidiabetic and antihyperlipidemic activity of Cucurbita maxima Duchense (pumpkin) seeds on streptozotocin induced diabetic rats. J Pharmacog Phytochem. 2013; 1(6): 108-116.
4. Saha P, Mazumder UK, Haldar PK, Bala A, Kar B, Naskar S. Evaluation of hepatoprotective activity of Cucurbita maxima aerial parts. J Herbal Med Toxicol. 2011; 5(1): 17-22.
5. Saha P, Mazumder UK, Haldar PK, Naskar S, Kundu S, Bala A, Kar B. Anticancer activity of methanol extract of Cucurbita maxima against Ehrlich ascites carcinoma. Int. J. Res. Pharm. Sci. 2011; 2(1): 52-59.
6. Iqbal Z, Nadeem QK, Khan MN, Akhtar MS, Waraich FN. In Vitro Anthelmintic activity of Allium sativum, Zingiber officinale, Curcurbita mexicana and Ficus religiosa. Int J Agri Biol. 2001; 3(4): 454-45.
7. Badr-Sherif EA, Shaaban M, Elkholy YM, Helal MH, Hamza AS, Masoud MS, Safty MME. Chemical composition and biological activity of ripe pumpkin fruits (*Cucurbita pepo* L.) cultivated in Egyptian habitats. Nat Prod Res. 2011; 25(16): 1524-1539.
8. Tekao T, Watanabe N, Yagi I, Sakata K. A simple screening method for antioxidant and isolation of several antioxidants produced by marine bacteria from fish and shellfish. Biosci. Biotech. Biochem. 1994; 58: 1780-1783.
9. Roby MHH, Sarhan MA, Selim KAH, Khalel KI. Evaluation of antioxidant activity, total phenols and phenolic compounds in thyme (*Thymus vulgaris* L.), sage (Salvia officinalis L.), and marjoram (Origanum majorana L.) extracts. Ind Crops Prod. 2013; 43: 827-831.
10. Subramanian R., Asmawi MZ, Sadikun A. In vitro α-glucosidase and α-amylase enzyme inhibitory effects of Andrographis paniculata extract and andrographolide. Acta Biochim Pol. 2008; 55(2): 391-398.
11. Dong HQ, Li M, Zhu F, Liu FL, Huang JB. Inhibitory potential of trilobatin from *Lithocarpus polystachyus* Rehd against *α-glucosidase* and *α-amylase* linked to type 2 diabetes. Food Chemistry, 2012; 130: 261-266.