REPORT

Anti-glycationand Anti-oxidation properties of *Capsicum frutescens* and *Curcuma longa* fruits: Possible role in prevention of diabetic complication

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Abstract: The accumulation of advanced glycationend products (AGE's) in the body, due to the non-enzymatic glycation of proteins is associated with several pathological conditions like aging and diabetes mellitus. Hence a plant having anti-glycation and anti-oxidation potentials may serve as therapeutic agent for diabetic complications and aging. In this study the anti-glycation and anti-oxidation properties of crude methanolic extracts of fruits of *Capsicum frutescens* and *Curcuma longa* were investigated. Among the two *C. frutescens* had more anti-glycation ability with a minimum inhibitory concentration (MIC_{50}) of 90µg/mlas compared to 324µg/ml MIC_{50} of *C. longa*. *Curcuma longa* had the more anti-oxidation potential i.e. 35.01, 30.83 and 28.08% at 0.5mg, 0.25mg and 0.125mg respectively.

Keywords: AGE's, Anti-glycation, Anti-oxidation, *Capsicum frutescens*, *Curcuma longa.*

INTRODUCTION

Diabetes mellitusis a condition characterized by hyperglycemia. Diabetes affects around a 100 million people worldwide and such individuals are susceptible to long term complications of cataract, neuropathy, atherosclerosis, embropathy, retinopathy and slow wound healing abilities (Nathan, 1993 and Muhammed and Nessar, 2006). Hyperglycemia accelerates the process of glycation of proteins, which is supported by various studies over the past few decades that had generated an overwhelming body of evidence. These studies suggest that glycation is involved in several biochemical abnormalities in diabetes mellitus (Baynes *et al*., 1989). Glycation of proteins is a non-enzymatic reaction (Maillard reaction)that occurs between the carbonyl group of sugar and amino group of proteins forming Amadori products which on further oxidation, rearrangement and reduction reactions result in the production of AGE's such as pentosideine, pyralline and carboxymethllysine (Baynes *et al*., 1989; Bry *et al*., 2001; Sing *et al*., 2001; Ulrich and Cerami, 2001).

Recently scientists have given considerations to agents that possess the ability to inhibit the AGE's formation because glycation is responsible for most of diabetic complications. Amino-guanidine particularly, has been studied extensively and its clinical trials managed to enter phase three (Sing *et al*., 2001, Edelstein and Brownlee, 1992; Freedman *et al*., 1999). However these trials were terminated due to safety concerns associated with it (Sing *et al*., 2001; Freedman *et al*., 1999). Other agents like pyridoxamin, carnosine, phenyl thiazoliumhave also been reported for their anti-glycation abilities however none of these agents shows significant level of anti-glycation (Sing *et al*., 2001; Stitt *et al*., 2002; Vasan *et al*., 1996).

Oxidation reaction is one of the reactions that is involved to covert glycated proteins into AGE's. It is reported in several studies that the formation of AGE's from Amadori products occurs partly because of oxidation (Rashbar and Figaroia, 2003). Therefore agents with anti-oxidation properties that can prevent further oxidation of Amadori products may halt the accumulation of AGE's (Bonnefont, 2002).

C. frutescens (chilies)has been used as food additive and as an anti-microbial agent for years (Siehta *et al*., 1984). Furthermore some studies have also indicated the anti-oxidation potential of *C. frutescens* (Pellegrini *et al*., 2003). *Curcuma longa* (turmeric) has also been an important part of traditional medicines in the subcontinent region. They are used in conditions like nausea, indigestion, inflammation, improving skin complexion, liver diseases and wound healing (Charak, 2002; Jain, 1991; Chemexcil, 1992). Some studies have indicated that *C. longa* can be used as antibacterial, anti-fungal and anti-mutagenic agent (Guddadaranga, 2002). In this study we investigated the anti-glycation and anti-oxidation properties of the crude methanolic extract of the fruits from *C. frutescens* and *C. longa.*

MATERIALS AND METHODS

Plant material

Fruits of *C. frutescens* and *C. longa* were collected from Mardan which were kindly identified by Prof. Dr. Abdur

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Extraction

The fruits of *C.frutescens* and *C. longa* were kept in shade for drying. After drying they were chopped and ground to powder. The powdered fruits were soaked in methanol for 15 days (twice) at room temperature. Each time the mixture was filtered and the filtrates were combined and concentrated to crude methanolic extracts using rotatory evaporator at 40^{o}C.

Materials

The materials used for *in vitro* anti-glycation assay were: Bovine Serum Albumin (BSA), D-glucose, Sodium dihydrogen phosphate (Na_2HPO_4), Potassium dihydrogenphoshpate (KH_2PO_4), Sodium Chloride (NaCl), Potassium Chloride (KCl), Amino guanidine (Merck), Retinoid (Merck) and Tri-Chloro Acetic Acid (TCA)(Sigma). While alkaline PBS (137nM NaCl, 8.1nM Na_2HPO_4, 2.68mM KCl, 1.47mM KH_2PO_4) was prepared and its pH adjusted to 7.4 with 0.25N NaOH.

The material lsused for Nitric Oxide (NO) free radical scavenging assay were: Sodium Nitro prusside (Na_2 [Fe $(CN)_5NO]\cdot 2H_2O$) (SNP), Sulphanilic acid (SA) (0.33% in 20% Acetic acid), [N-(1-Napthyl) Ethylenediamine Dihydrochloride] (NED) (0.1% in H_2O), Phosphate buffer (10mM, pH=7.4), UV double beam spectrophotometer (UV-1800 Shimadzu), micropipettes, test samples (crude methanolic), Methanol (as blank) and Ascorbic Acid (Vitamin-C) as positive control.

In vitro glycation assay

The method of Matsuura *et al* 2002 was followed with little modifications. Stock solutions of the plant extracts were prepared by dissolving 3 mg in 1ml of alkaline PBS. From the stock solution 10, 50, 90 and 130µLas compared to 10, 40, 70, 100µL of Matsuura *et al*2002 solutions were taken using micropipette and were mixed with a solution containing 400µg BSA and 200mM glucose. These reaction mixtures were kept in a water bath at 55^{o}C for 48 hours. BSA and glucose without any inhibitor was used as control. After the incubation time the reaction mixture was transferred into separate Effendorf tubes and 10µL of 100% w/v TCA was added and centrifuged (14500rpm) at 4^{o}C for 4 minutes. Supernatant then discarded and the pellet was re-dissolved in 400µL alkaline PBS.

Using fully automated UV double beam spectrophotometer, the degree of absorbance for both the control and the test reaction mixtures were taken at 350 nm. Percent inhibition was calculated using the following formula:

Percent inhibition = $[1- (A_s-A_o)/(A_b-A_o)] \times 100$

Where A_s is absorbance of test samples, A_b is absorbance of reaction mixture without plant extract and A_o is absorbance of blank control.

Nitric Oxide (NO) free radical scavenging assay

To perform NO free radical scavenging assay the method of Ebrahimzadeh *et al*., 2009 was followed.

Stock solutions of test samples were prepared by dissolving 3mg of the test samples into 1 ml of methanol. Different dilutions i.e. 0.5, 0.25 and 0.125mg/ml of test sample were made from the stock solution and 1ml of each dilution was introduced into separate testubes along with 1ml of SNP to make the reaction mixture. This mixture was then incubated for 90minutes at 27^{o}C. After incubation 0.5ml of the reaction mixture was added to 1ml of SA and incubated at 27^{o}C for 5minutes. Add 1ml of NED to it and again incubate for 30minutes at 27^{o}C.

Results were obtained by taking absorbance at 546nm. Methanol and Vitamin C were used as blank and positive control respectively. Percentage anti-oxidation potential was calculated by the following formula:

Percent inhibition = $[A_o-A_t/A_o] \times 100$

A_o is absorbance of control; reaction mixture without extracts A_t is absorbance of test samples.

RESULTS

Anti-glycation assay

The UV double beam spectro photometric analyses of both the reaction and test mixtures for *C. frutescens* and *C. longa* are shown in the fig 1. It can be seen that as the concentration of the *C. longa* extract increases the absorbance decrease and reaches minimum of 0.024 at 130µL. Same trend is observed for *C. frutescens* too with the exception that on the unexpected rise in absorbance form 90µL to 130µL. Minimum absorbance (0.027nm) for *C. frutescens* occurs at 90µL.

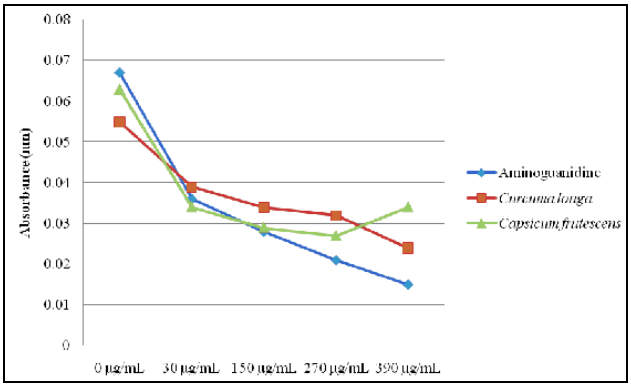

Fig. 1: Anti-glycation properties of amino guanidine, *Curcuma longa* and *Capsicum frutescens*

It can be inferred from fig 1 that the minimum inhibitory concentration (MIC_{50}) of amino guanidine and *C. longa* are 70.14µg/ml and 324µg/ml respectively, while for *C. frutescens*it is 90µg/ml. fig 2 shows the percent inhibition of Millard reaction or glycation by amino guanidine and

the two plants extract verses the amount of test sample used i.e. sample concentration.

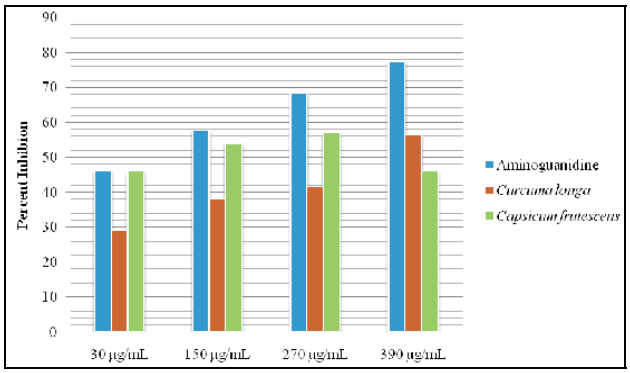

Fig. 2: Percent inhibition of Maillard reaction at different concentration of amino guanidine, *Curcuma longa* and *Capsicum frutescens*

NO free radical scavenging Assay

The anti-oxidation potential of both *C. longa* and *C. frutescens* was compared with ascorbic acid (Vitamin-C) as standard. Results obtained from NO scavenging assay are shown in table 1 and fig. 3.

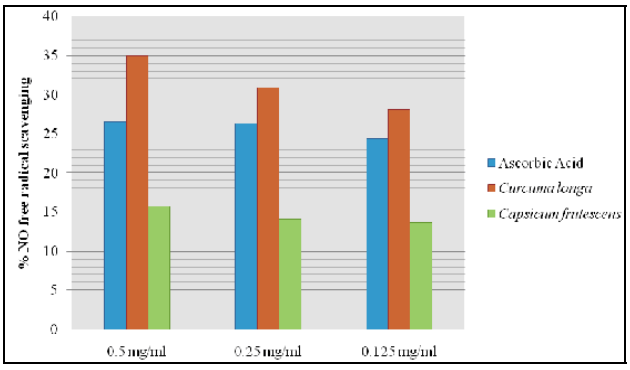

Fig. 3: Anti-oxidation potential of both *Curcuma longa* and *Capsicum frutescens* in comparison with the standard ascorbic acid.

It can be seen from both the fig that *C. longa* has anti-oxidation potential exceeding the anti-oxidation potential of the standard at all the concentration. While *C. frutescens* showed anti-oxidation potential at all the test concentrations but its percent anti-oxidation effect is less than the standard and *Curcuma longa*.

DISCUSSION

As far as we had investigated there has been no anti-glycation study on *C. frutescens* and this study is the first one showing that they do have anti-glycation potentials. Our findings for the anti-glycation in-vitro assay showed that among the two, *C. frutescens* is more effective as an anti-glycation agent. We also found that the percent inhibition of glycationis concentration dependent.

The consistency of our anti-oxidation findings with the findings from other studieson the same plants show that our findings are reliable and applicable for further research (Wiwat and Wallaya, 2009). Like the other studies we also found that both *C. longa* and *C. frutescens* have anti-oxidation potential. However unlike the previous studies we compared the results and found that *C. longa* had more anti-oxidation potential than Ascorbic Acid (standard) and *C. frutescens*. Previously we investigated the anti-glycation and anti-oxidation potential of different fractions of *Fumaria indica* whole plant. Our results indicated that the chloroform fraction of the plant had more anti-glycation ability with a Minimum inhibitory concentration (MIC_{50}) of 148.96µg/mL as compared to the MIC_{50} of 221.96, 270.00, 294.01 and 322.49µg/ml of aqueous, ethyl acetate, *n*-hexane and crude methanolic fractions, respectively, while the results of anti-oxidation assay for Chloroform, ethyl acetate, aqueous, *n*-hexane and crude methanolic fractions were 29.39, 21.41, 15.25, 13.01 and 11.33% at 0.5mg/ml respectively (Haroon and Ibrar, 2012).

Keeping in view the involvement of glycation and oxidation reactions in complication of diabetes and aging, we suggest that both of these plants can be useful therapeutically. However our finding also suggest that a combination therapy of *Curcuma longa* and *Capsicum frutescens* extracts would be more suitable.

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REFERENCES

Baynes JW, Watkins NG, Fisher CI, Hull CJ, Patrick JS, Ahmed MU, Dunn JA and Thorpe SR (1989). The Amadori product on protein: Structure and reactions. *Prog. Clin. Biol. Res.*, **304**: 43-67.

Bonnefont RD (2002). Glucose and reactive oxygen species. *Curr. Opin. Clin. Nutr. Metab. Care*, **5**: 561-568.

Bry L, Chen PC and Sacks DB (2001). Effects of hemoglobin variants and chemically modified derivatives on assays for glycohemoglobin. *Clin. Chem.*, **47**: 153-163.

Charak C (2002). Ch.4.13, Ch. 6/26 vol. 1. Ed. Priyavrat Varma.

Chemexcil (1992). *Curcuma longa* Linn (Haridra) in "111 Indian Medicinal Plants. Bhavan's Swami Prakashananda Ayurveda Research Centre, pp.121-124.

Ebrahimzadeh MA, Nabavi SF and Nabavi SM (2009). Antioxidant activities of methanol extract of *Sambucus ebulus* L. flower. *Pak. J. Biol. Sci.*, **12**: 447-450.

Edelstein D and Brownlee M (1992). Mechanistic studies of advanced glycosylation end product inhibition by aminoguanidine. *Diabetes,* **41**: 26-29.

Freedman BI, Wuerth JP, Cartwright K, Bain RP, Dippe S, Hershon K, Mooradian AD and Spinowitz BS (1999). Design and baseline characteristics for the Aminoguanidine Clinical Trial in Overt Type 2 Diabetic Nephropathy (ACTION II). *Control Clin. Trials,* **20**: 493-510.

Guddadaranga K (2002) Evaluation of Antioxidant activities and anti-mutagenicity of turmeric oil: A byproduct of curcumin production. Z Naturforsch 57c, 828-835.

Ibrar K and Haroon A (2012). Anti-glycation and anti-oxidative properties of fumariaindica variousfractions: Role in reducing diabetic complications and ageing. *Ind. J. Heal Wellbeing.,* **3**(3): 692-694.

Jain SK (1991). Dictionary of Indian Folk Medicine and Ethno botany, pp.65-66.

Matsuura N, Aradate T, Sasaki C, Hiroyuki K, Mitsuharu O, Junichi H and Makoto U (2002). Screening system for the Maillard reaction inhibitor from natural product extracts. *J. Health Sci.,* **48**: 520- 526.

Muhammed SA and Nessar A (2006). Anti-glycation properties of aged garlic extract: Role in prevention of diabetic complications. *J. Nutri.,* **36**: 796-799.

Nathan DM (1993). Long-term complications of diabetes mellitus. *N. Engl. J. Med.,* **328**: 1676-1685.

Pellegrini N, Serafini M, Colombi B, Daniele DR, Sara S, Marta B and Furio B (2003). Total anti-oxidant capacity of plant foods, beverages and oils consumed in Italy assessed by three different *in vitro* assays. J. Nutr., **133**: 2812-2819.

Rashbar S and Figaroia JL (2003). Novel inhibitors of advanced glycation end products. *Arch. Biochem. Biophys.,* **419**: 63-79.

Siehta AS, Magdoub MWI, Fayed EO and Hoft AA (1984). Effect of added salt and *Capsicum tincture* on non lactic acid bacteria in pickled Domiati Cheese. *Annual of Agricultural Science,* **29**: 793-810.

Sing R, Barden A and Mori T (2001). Advanced glycation end products: A review. *Diabetologia,* **44**: 129-146.

Stitt A, Gardiner TA, Alderson NL, Canning P, Frizzell N, Duffy N, Boyle C, Januszewski AS, Chachich M, Baynes JW and Thorpe SR (2002). The AGE inhibitor pyridoxamine inhibits development of retinopathy in experimental diabetes. *Diabetes,* **51**: 2826-2832.

Ulrich P and Cerami A (2001). Protein glycation, diabetes and aging. *Recent Prog. Horm. Res.,* **56**: 1-21.

Vasan S, Zhang X, Zhang X, Kapurniotu A, Bernhagen J, Teichberg S, Basgen J, Wagle D, Shih D, Terlecky I, Bucala R, Cerami A, Egan J and Ulrich P (1996). An agent cleaving glucose-derived protein cross *links in vitro* and *in vivo*. *Nature,* **382**: 275-278.

Wiwat W and Wallaya M (2009). Antioxidant capacity changes of bird chili (*Capsicum frutescens* Linn.) During Hot Air Drying. *Kasetsart J. Nat. Sci.,* **43**: 12-20.