Pak. J. Bot., 46(4): 1469-1471, 2014.

ANTIGLYCATION AND ANTIOXIDATION PROPERTIES OF *BERBERIS LYCEUM* AND *TERMINALIA CHEBULA*: POSSIBLE ROLE IN CURING DIABETES AND SLOWING AGING

IBRAR KHAN*, HAROON AHMAD, BASHIR AHMAD AND SADIQ AZAM

[1]*Centre of Biotechnology and Microbiology,*
University of Peshawar, Khyber Pukhtoon Khwa, Pakistan
**Corresponding author: ibrarkhan1984@upesh.edu.pk ,*

Abstract

Plants have been shown to possess a great potential to benefit mankind. Extracts from plants that have antiglycation and antioxidation abilities can be of great therapeutic value in reducing complication of diabetes and slowing down aging. Advanced glycation end-products (AGE's) formation due to non-enzymatic glycation and oxidative stress has been demonstrated in the pathogenesis of diabetic complications and aging processes. In this study we investigated the antiglycation and antioxidation potential of methanolic extracts of *Berberis lyceum* and *Terminalia chebula.* Results indicated that the methanolic extract of *B. lyceum* had more antiglycation ability with a Minimum inhibitory concentration (MIC_{50}) of 123 µg/mL as compared to the MIC_{50} of 110 µg/ml of *T. chebula*. While the results of antioxidation assay showed that *T. chebula* has more antioxidation potential than *B. lyceum*. *T. chebula* had 46.55, 35.01 and 32.81% antioxidation potential at 0.5 mg, 0.25 mg and 0.125 mg respectively as compared to the 16.53, 16.09 and 15.10 % oxidation inhibition at same mass values by *B. lycium*.

Introduction

Maillard reaction involves the non-enzymatic glycation of proteins to form advanced glycation end-products (AGE's). Some of the recent studies have shown that this reaction not only involves non-enzymatic glycation but oxidation as well (Chetyrkin *et al*., 2008; Argirova & Ortwerth, 2003; Prince, 2001). Therefore agents that have antiglycation and antioxidation abilities could be used to prevent this reaction.

Accumulation of AGE's in different parts of the body like heart, muscles and large blood vessels, results in the promotion and progression of diabetic complication like nephropathy, neuropathy, cardiovascular disease and atherosclerosis (Sugimoto *et al*., 2008; Fukami *et al*., 2008; Yamagishi *et al*., 2008; Nathan, 1993; Muhammed & Nessar, 2006). During diabetes mellitus AGE's are formed at an accelerated rate because of the hyperglycemic condition in the body (Kumar *et al*., 2004; Kumar *et al*., 2004). Therefore any drug or agent that could stop AGE's formation can be of therapeutic value.

Glycation of proteins and its further oxidation alters its conformation, stability and induces protein aggregation and immobilization through cross-linkage (Kumar *et al*., 2004; Kumar *et al*., 2004; Watala *et al*., 1996). Collagen elasticity is responsible for the proper shape of the body. Glycation results in loss of collagen elasticity and results in arterial stiffness decreased myocardial compliance and hence aging (Sing *et al*., 2001; Aronson, 2003).

One of the ways to reduce diabetic complication and slowing down aging is to stop glycation and oxidation process. This is only possible by using compounds or agents that have antiglycation and antioxidation properties (Siddiqui *et al.,* 2014). Plants have been major sources of drugs in the world and the drugs derived from them have fewer side effects (Samina *et al*., 2008; Mahmood *et al.,* 2011). In the subcontinent, various plants have been used to treat diabetes and various other human diseases. *Galenga officialis* has been used for diabetes throughout history (Oubre *et al*., 1970). It is also believe by folk medicine experts (Hakims) that certain plants like *B. lyceum* have anti-ageing effect and keeps the skin fresh. Hence in this particular study we have tried to find out antiglycation and antioxidation effect of *B. lyceum* and *T. chebula*.

Materials and Methods

Plant material: Stem of *Berberis lyceum* and fruits of *Terminalia chebula* were collected from Mardan and kindly identified by Prof. Dr. Abdur-Rasheed, Plant taxonomist, Department of Botany, University of Peshawar, Khyber Pukhtunkhwa, Pakistan.

Extraction: The stem of *Berberis lyceum* and fruits of *Terminalia chebula* were kept in shade for drying and were then chopped and grounded to powder. The powdered materials were soaked in methanol (twice) for 15 days at room temperature. Each time the filtrate was filtered and the filtrates were combined and concentrated to crude methanolic extracts using rotatory evaporator at 40°C.

Materials: The materials used for invitro antiglycation assay were: Bovine Serum Albumin (BSA), D-glucose, Sodium dihydrogen phosphate (Na_2HPO_4), Potassium dihydrogen phoshpate (KH_2PO_4), Sodium Chloride (NaCl), Potassium Chloride (KCl), Aminoguanidine (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 materials used for Nitric Oxide (NO) free radical scavenging assay were: Sodium Nitroprusside (Na2[Fe(CN)5NO]·2H2O) (SNP), Sulphanilic acid (SA)(0.33% in 20% Acetic acid), [N-(1-Napthyl) Ethylene diamine Dihydrochloride] (NED) (0.1% in H2O), Phosphate buffer (10mM, pH=7.4), UV double beam spectrophotometer, micropipettes, test samples (crude methanolic extract), 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 130 µL solutions were taken using micropipette and were mixed with a solution containing 400 µg BSA and 200 mM glucose. These reaction mixtures were kept in a water bath at 55°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 10 µL of 100% w/v TCA was added and centrifuged at 14500 rpm at 4°C for 4 minutes. Supernatant then discarded and the pellet was re-dissolved in 400 µL 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:

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

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 in 1 ml of methanol. Different dilutions i.e. 0.5, 0.25 and 0.125 mg/ml of test sample were made from the stock solution and 1ml of each dilution was introduced into separate test tubes along with 1ml of SNP to make the reaction mixture. This mixture was then incubated for 90 minutes at 27°C. After incubation 0.5ml of the reaction mixture was added to 1ml of SA and incubated at 27°C for 5 minutes. Add 1ml of NED to it and again incubate for 30 minutes at 27°C.

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

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

where A_o is absorbance of control; reaction mixture without extract

A_t absorbance of test samples.

Results

Antiglycation assay: The UV double beam spectrophotometric analyses of both the reaction and test mixtures for *B. lyceum* and *T. chebula* are shown in the (Fig. 1). It can be seen from the figure that as the concentration of the *B. lyceum* extract increases the absorbance decrease from 0.079 nm and reaches minimum of 0.022 nm at 390 µg/ml. The same trend is observed for *T. chebula* too which reaches it minimum of 0.030 nm at concentration of 390 µg/ml from maximum absorbance of 0.074nm.

It can also be inferred from the Fig. 1 that the minimum inhibitory concentration (MIC_{50}) of aminoguanidine and *B. lyceum* are 70.14 µg/ml and 123 µg/ml respectively, while for *T. chebula* it is 330µg/ml. Fig. 2 shows the percent inhibition of Millard reaction or glycation by aminoguanidine and the two plants extract verses the amount of test sample used i.e., Sample concentration.

NO free radical scavenging assay: The antioxidation potential of both *B. lyceum* and *T. chebula* was compared with ascorbic acid (Vitamin-C) as standard. Results obtained from NO scavenging assay are shown in Table 1 and Fig. 3.

Table 1. Percent antioxidation activity of ascorbic acid, *Berberis lyceum* and *Terminalia chebula* at 0.5, 0.25 and 0.125 mg per ml concentration.

	0.5mg/ml	0.25mg/ml	0.125mg/ml
Ascorbic acid	26.50	26.28	24.34
Berberis lyceum	16.53	16.09	15.10
Terminalia chebula	46.55	35.01	32.81

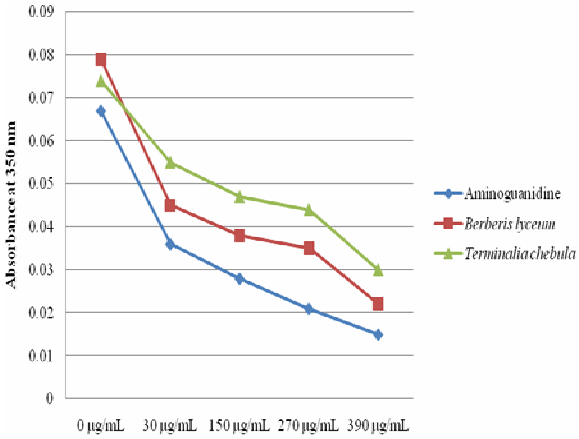

Fig. 1. Antiglycation properties at different concentration of *Berberis lyceum* and *Terminalia chebula*.

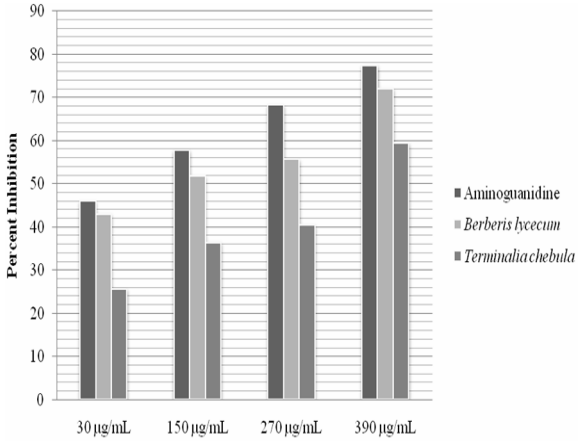

Fig. 2. Percentage inhibition at different concentration of *Berberis lyceum* and *Terminalia chebula*.

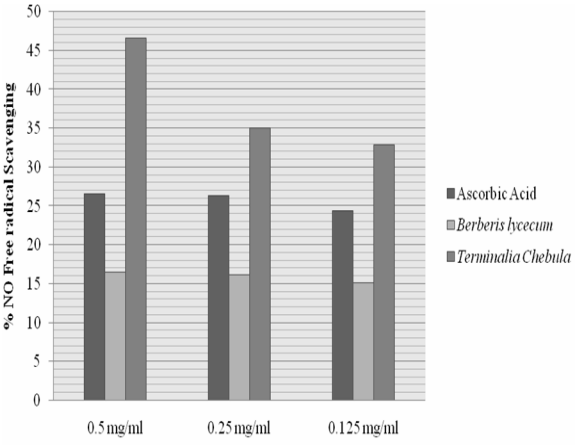

Fig. 3. Antioxidation potential of both *Berberis lyceum* and *Terminalia chebula* in comparison with the standard.

It can be seen from both the figures that *T. chebula* among the two had more potential of antioxidation and even exceeds the antioxidation potential of the standard i.e. Ascorbic acid, at all the concentration. While *B. lyceum* shows antioxidation potential at all the test concentrations but its percent antioxidation effect is less than the standard and *T. chebula.*

Discussion

As far as we had investigated there has been no antiglycation study on both *B. lyceum* and *T. chebula* and this study is the first one that reports that both of them do have antiglycation potentials. Our findings for the antiglycation *In vitro* assay showed that among the two, *B. lyceum* is more effective as an antiglycation agent. We also found that the percent inhibition of glycation is concentration dependent.

The consistency of our antioxidation findings for *T. chebula* with the findings from other studies on the same plants shows that our findings are reliable and applicable for further research (Hua-Yew *et al*., 2003). In our study we found that both *B. lyceum* and *T. chebula* have antioxidation potential. However when we compared the results it was found that *T. chebula* had more antioxidation potential than Ascorbic Acid (standard) and *B. lyceum*.

Keeping in view the involvement of glycation and oxidation reactions in complication of diabetes and aging effects we suggest that both of these plants can be useful therapeutically for the problem. However our finding also suggest that a combination therapy of both *B. lyceum* and *T. chebula* extracts would be most suitable.

References

Argirova, M.D. and BJ. Ortwerth. 2003. Activation of protein bound copper ions during early glycation: study on two proteins. *Arch. Biochem. Biophys*., 420(1): 176-184.

Aronson, D. 2003. Cross-linking of glycated collagen in the pathogenesis of arterial and myocardial stiffness of aging and diabetes. *J. Hypert*., 21: 3-12.

Chetyrkin, S.V., M.E. Mathis, A.J. Ham, D.L. Hachey, B.G. Hudson and P.A. Voziyan. 2008. Propagation of protein glycation damage involves modification of tryptophan residues via reactive oxygen species: Inhibition by pyridoxamine. *Free Radic. Biol. Med*., 44(7): 1276-1285.

Ebrahimzadeh, M.A., S.F. Nabavi and S.M. 2009. Nabavi. Antioxidant activities of methanol extract of *sambucus ebulus* L., flower. *Pak. J. Biol. Sci*., 12(5): 447-450.

Fukami, K., S. Yamagishi, S. Ueda and S. Okuda S. 2008. Role of AGE's in diabetic nephropathy. *Curr. Pharm. Des*., 14: 946-952.

Hua-Yew, C., T.C. Lin, Y.U. Kuo-Hua, Y. Chien-Min and C.C. Lin. 2003. Antioxidant and free radical scavenging activities of *Terminalia chebula*. *Biol. Pharm. Bull*., 26(9): 1331-1335.

Kumar, M.S., P.Y. Reddy and PA. Kumar. 2004. Effect of diacarbonyl induced browning on α- Crystallin Chaperone-like acitivity: physiological significance of caveats of invitro aggregation assays. *Biochem J*., 379: 273-282.

Kumar, M.S., T. Mrudula and N. Mitra. 2004. Enchancedproteolytic degradation and altered ATP binding of methylglyoxal modified α-crystallin. *Exp. Eye. Res*., 79: 577-583.

Mahmood, A., R.N. Malik, Z.K. Shinwari and A. Mahmood. 2011. Ethnobotanical Survey of Plants from Neelum, Azad Jammu & Kashmir, Pakistan. *Pak. J. Bot*., 43(Special Issue): 105-110.

Matsuura, N., T. Aradate, C. Sasaki, H. Kojima, M. Ohara, J. Hasegawa and M. Ubukata. 2002. Screening system for the maillard reaction inhibitor from natural product extracts. *J. Health Sci*., 48(6): 520-526.

Muhammed, S.A. and A. Nessar. 2006. A. Antiglycation properties of aged garlic extract: Role in prevention of diabetic complications. *J. Nutri*., 3: 3.

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

Oubre, A.Y., T.J. Carlson, S.R. King and G.M. Reaven. 1970. From plant to patient, an Ethanomedical approach to the identification of new drugs for the treatment of NIDDM. *Diabetologia*., 40(5): 614-617.

Prince, D.L., P.M. Rhett, S.R. Thorpe and J.W. Baynes. 2001. Chelating activity of advanced glycation end-products inhibitors. *J. Biol. Chem*., 276(52): 48967-48972.

Samina, K., A.S. Munir, M. Ashar and R. Robina. 2008. Effect of onion (*Allium cepa* L.) extract on millard reaction under *In vitro* conditions. *Pak. J. Agri. Sci*., 45(1): 103-106.

Siddiqui, B.S., M. Hasan, F. Mairaj, I. Mehmood, R.M. Hafizur, A. Hameed and Z.K. Shinwari. 2014. Two new compounds from the aerial parts of Bergenia himalaica Boriss and their anti-hyperglycemic effect in streptozotocin-nicotinamide induced diabetic rats. *Journal of Ethnopharmacology*. http://www.sciencedirect.com/science/article/pii/S0378874114001032.

Sing, R., A. Barden, T. Mori and L. Beilin. 2001. Advanced glycation end products: *Rev. Diabetologia*., 44: 129-146.

Sugimoto, K., M. Yasujima and S. Yagihashi. 2008. Role of advanced glycation end products in diabetic neuropathy. *Curr. Pharm. Des*., 14: 953-961.

Watala, C., K. Gwozdzinski, E. Pluskota, C. Pietrucha, B. Walkowiak, Z. Trojanowski and C.S. Cierniewski. 1996. Diabetes mellitus alters the effect of peptides and protein ligands on membrane fluidity of blood platelets. *Thromb. Haemost*., 75: 147-153.

Yamagishi, S., S. Ueda, T. Matsui, K. Nakamura and S. Okuda. 2008. Role of advanced glycation endproducts (AGE's) and oxidative stress in diabetic retinopathy. *Curr. Pharm. Des*., 14: 962-968.

(Received for publication 14 February 2013)