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Antiglycation and antioxidative properties of fumaria indica various fractions: Role in reducing diabetic complications and ageing

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Over the centuries medicinal plants have benefited mankind against different diseases. 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 different fraction F. indica whole plant. Our results indicated that the chloroform fraction of B. bulbocastanumfruitshad more antiglycation ability with a Minimum inhibitory concentration (MIC50) of 148.96 µg/mL as compared to the MIC50 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 antioxidation assay showed that chloroform fraction has more antioxidation potential than the standard (ascorbic acid) at 0.5 mg/ml concentration. Chloroform, ethyl acetate, aqueous, n-hexane and crude methanolic fractions had 29.39, 21.41, 15.25, 13.01and 11.33 % antioxidation potential at 0.5 mg / ml respectively.

Keywords: age's, antiglycation, antioxidation, f. indica

Generation of free radicals inside human body takes place from both endogenous and exogenous sources (Young & Woodside, 2001). Endogenous include oxidative enzymes and respiratory chain while exogenous include smoking and other air pollutants (Young & Woodside, 200).These sources create overproduction of free radicals which ultimately leads to various diseases because of oxidative stress (Young & Woodside, 2001; Barnham et al., 2004; Rahimi et al., 2005). Some of the destructive effects of oxidative stress include damaged proteins, nucleic acid and lipids which lead to conditions like diabetes, Alzheimer's, cancer, cardiovascular diseases (Young & Woodside, 2001; Barnham et al., 2004; Rahimi et al., 2005).

This oxidative stress is also an instrumental factor in glycation process (Al-Abed & Bucala, 2000). Glycation is basically a reaction of amino groups in amino acid of proteins and carbonyl group in sugar resulting in formation of Amadori products (Al-Abed & Bucala, 2000; Rojas & Morales, 2004; Wu & Yen, 2005). These intermediate Amadori products then through a series of reactions; including oxidation reactions, ultimately forms advanced glycation end products (AGE's) (Al-Abed & Bucala, 2000; Rojas & Morales, 2004; Wu & Yen, 2005). These AGE's are very dangerous to our health and cause most of diabetic mellitus complications and result in speeding up of ageing process (Wu & yen, 2005; Baral et al., 2000; Parija et al., 2005; Selvaraj et al., 2002). From pathogenesis point of view AGE's accumulation is hazardous to our body cells and results in acceleration of aging and diabetic complications including retinopathy, cataract, neuropathy and atherosclerosis (Ahmed, 2005). Hence any source that has both antioxidation and antiglycation capabilities can be very helpful against such disease conditions.

Fumaria Indica (papdha) is annual, diffuse herb, which is up to 30 cm high. It is found in Europe, Africa and many Asian countries (Gireesh et al., 2011). F. Indica has been used for many years to treat different diseases. Traditionally it is used as aperients, diaphoretic and diuretic. It is also used for aches, pains, diarrhea, fever, influenza and liver complaints (Burmanii, 1768). Furthermore its leaves are also used in udder infection of ruminants (Edmonds & Chweya, 1997). Keeping in view the medicinal potential of F. Indica, the current study was aimed to screen the whole plant of Fumaria Indica for possible antioxidation and antiglycation potential.

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Method and Materials

Plant Material

F. Indica plants 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 F. indica plants 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 40oC.

Fractionation

The crude methanolic extract of F. Indica (170g) was suspended in distilled water (350 ml). It was further partitioned with n-hexane (3x 500 ml), chloroform (3 x 500 ml) and ethyl acetate (3 x 500ml), respectively, to yield the n- hexane (44 g), chloroform (35g), ethyl acetate (31g) and aqueous (51g) fractions. 20 g of the crude methanolic extract of was left for biological/pharmacological activities. All the fractions will only contain particular compounds based on the solubility of these compounds from the crude extract. For instance, the n- hexane fraction will contain only those compounds which are non-polar and soluble in non-polar solvents and so on.

Materials

The materials used for invitro antiglycation assay were: Bovine Serum Albumin (BSA), D-glucose, Sodium dihydrogen phosphate (Na2HPO4), Potassium dihydrogenphoshpate (KH2PO4), Sodium Chloride (NaCl), Potassium Chloride (KCl), Aminoguanidine (Merck, Pakistan), Retinoid (Merck, Pakistan) and Tri-Chloro Acetic Acid (TCA) (Sigma, Pakistan). While alkaline PBS (137nM NaCl, 8.1nM Na2HPO4, 2.68mM KCl, 1.47mM KH2PO4) was prepared and its pH adjusted to 7.4 with 0.25N NaOH.

The materials used for Nitric Oxide (NO) free radical scavenging

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 3mg 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 200mm 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 14500rpm 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 350nm. Percent inhibition was calculated using the following formula: Percent inhibition = [1- (As-Ao)/(Ab-Ao)] ×100

Where As is absorbance of test samples, Ab is absorbance of reaction mixture without plant extract and Ao 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 1ml 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:

Percent inhibition = [Ao-At /Ao] ×100

Where Ao is absorbance of control; reaction mixture without extract At absorbance of test samples.

Results

Antiglycation assay

The UV double beam spectrophotometric analyses of the reaction and test mixtures of F. indica are shown in the Fig 01. It can be seen from the figure that as the concentration of the F. indica fractions increases from 10µg /ml to 390µg/ml the absorbance decrease indicating an increase in antiglycation ability of that fraction. However the relatively slow decrease from 30µg/ml onwards is attributed to the effect of interfering color agents; mainly chlorophyll, present in F. indica fractions

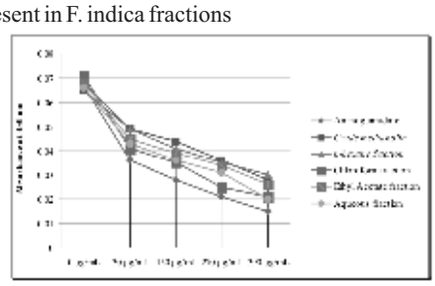

Figure 01. Antiglycation properties of F. indica fractions at different concentrations

The minimum inhibitory concentrations (MIC50) of aminoguanidine and F. indica fractionswere also calculated. We found that the MIC50 of aminoguanidine, crude methanolic, n-hexane, chloroform, ethyl acetate and aqueous extracts were 70.14, 322.49, 294.01, 148.96, 270.00 and 221.96µg/ml respectively. These values can also be inferred from Figure 01. Figure 02 shows the percent inhibition of Millard reaction by aminoguanidine and F. indica whole plant fractionsverses the amount of test sample used i.e. Sample concentration. Hence from the MIC50 and both figure 01 and 02 it is confirmed that the chloroform fraction had most appreciable antiglycation ability with MIC50 of 148.96µg /ml while the n-hexane had least antiglycation potential with MIC50 of 322.49 µg / ml.

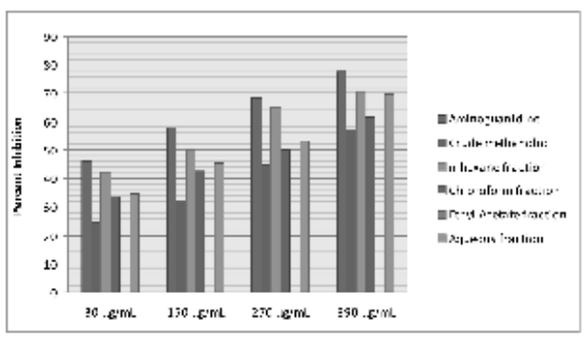

Figure 02. Percentage inhibition of F. indica fractions at different concentrations

No free radical scavenging Assay

The antioxidation potential of F. indica fractions at different concentrations was compared with ascorbic acid (Vitamin-C) as standard. Results obtained from NO scavenging assay are shown in table 01.

It can be seen from the table that among the different fractions of F. indica chloroform fraction had more potential of antioxidation and even exceeds the antioxidation potential (29.39%) of the standard (26.50%) at 0.5mg/ml concentration. Ethyl acetate and aqueous fraction also showed significant antioxidation potential of 21.41 and 15.25% at 0.5mg /ml concentration. However the other two fractions had medium to low antioxidation activity from the standard at all the concentration.

Table01: Percent antioxidation activity of F. indica fractions at 0.5, 0.25and 0.125 mg / ml concentration.

Test Sample	Percent antioxidation activity		
	0.5mg/ml	0.25mg/ml	0.125mg/ml
Ascorbic Acid	26.50	26.28	24.34
Crude methanolic extract	11.33	8.65	7.59
n-hexane fraction	13.01	8.39	6.34
Chloroform fraction	29.39	23.06	16.86
Ethyl acetate fraction	21.41	19.68	19.90
Aqueous fraction	15.25	14.85	13.05

Discussion

As far as we had investigated there have been no antiglycation and antioxidation studies on F. indica plant. This study is the first one that reports that its fractions do have antiglycation and antioxidation potentials. Our findings for the antiglycation in-vitro assay showed that among the test samples chloroform fractionwas the most effective antiglycation agent with MIC50 of 148.96 µg / ml. We also found that the percent inhibition of glycation is concentration dependent.

Our result for the NO free radical scavenging assayshows that as the concentration of test samples increases the percent antioxidation potential increases. Therefore indicating concentration dependence of antioxidation ability in both the standard and test samples. In our study we found that F. indica fractionshave antioxidation potential. The percent antioxidation inhibition of 29.39 and 21.41 % at 0.5 mg / ml for chloroform and ethyl acetate fractions presents an encouraging finding. However in particular the chloroform fraction antioxidation ability of 29.39 % as compared to the standard (Ascorbic acid) value of 26.50% represents its possible therapeutic use as antioxidant.

Previous studies have indicated that extracts of garlic, Acacia nilotica, Khayasenegalensis, Artocarpuslakoocha,Anthemisnobilis and Crataegusoxyacantha that had antiglycation properties could be used as therapeutics (Ahmed & Ahmed, 2006; Nasapon et al., 2010; Waleed et al., 2009; Yoshikazu et al., 2008). Hence the chloroform fraction of F. indica could also be used as antiglycation agent in such therapeutics for reducing the harmful effects of glycation process. While on the other hand the chloroform and ethyl acetate fractions of F. indica can be used as therapeutic agents against harmful oxidation reactions. This claim is supported by other antioxidation studies on different plant extracts like Hyssopus officinalis, Viola odorata, Spondiaspinnata and Nymphaea Alba (Ebrahimzadeh et al., 2010; Hazra, Biswas & Mandal, 2008). These previous studies also claim that their plant extracts could be used therapeutically. Therefore we conclude that F. indicawhole plant fractions can be used therapeutically to reduce diabetic complications and the process of ageing.

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