



International Journal of Pharmacology

ISSN 1811-7775

science
alert

ansinet
Asian Network for Scientific Information

Inhibitory Effects of Methonolic Pericarp Extract of *Feronia limonia* on *in vitro* Protein Glycooxidation

¹Eswar Kumar Kilari, ¹Swathi Putta, ¹Rohini Koratana, ²Neelakanta Rao Nagireddy and ³Absar Ahmad Qureshi

¹A.U. College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, India

²147 Wind Breeze dr; Memphis TN 38109, United States of America

³Department of Pharmacology, College of Pharmacy, King Khalid University, ABHA, Kingdom of Saudi Arabia

ARTICLE INFO

Article History:

Received: August 07, 2014

Accepted: December 20, 2014

Corresponding Author:

K. Eswar Kumar,
A.U. College of Pharmaceutical
Sciences, Andhra University,
Visakhapatnam, 530003, Andhra
Pradesh, India
Tel: +91944063272

ABSTRACT

The involvement of free radicals and oxidative reactions in protein glycooxidation processes, compounds with antioxidant activity have been tested in order to reduce or to stop glycooxidation. In this study, the antioxidant potential of methonolic pericarp extract of *Feronia limonia* (MPFL) was evaluated using different *in vitro* assays including the scavenging activities of super oxide radical, hydroxyl radical, hydrogen peroxide, DPPH radical, Nitric oxide radical and Fe⁺³ radical scavenging activity. The extract was evaluated for antioxidant potential by the phosphomolybdenum method, Fe⁺² chelating activity, Ferric Reducing Ability Power (FRAP) and 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid (ABTs) radical scavenging assay. The results indicated that the MPEF possesses the highest antioxidant activity. To establish the link between glycation and oxidation processes, further the extract have been evaluated for its *in vitro* antiglycation activities like the inhibitory activities on Bovine Serum Albumin (BSA) and protein oxidation markers including Protein Carbonyl Formation (PCO). The MPFL extract at different concentrations (25-100 µg mL⁻¹) has significantly quenched the fluorescence intensity of glycated BSA and the glycooxidation measured in terms of advanced glycation end products (AGEs). Furthermore, the study demonstrate that the inhibitory effects of MPFL extract in preventing oxidative protein damages including effect on PCO formation which are believed to form under the glycooxidation processes. These results clearly demonstrate that the MPFL is capable of suppressing the formation of AGEs and protein oxidation *in vitro* might be due to the presence of active principles like volatile flavour and free fatty acids.

Key words: Advanced glycated end products, protein glycooxidation, *Feronia limonia*

INTRODUCTION

Over the past years reactive oxygen species production and oxidative stress have been linked to disorders such as diabetes, cancer, cardiovascular diseases and some degenerative diseases. Diabetic patients are more susceptible to oxidative attack than the normal people because of higher production of ROS. Several mechanisms are involved in the hyperglycemia mediated oxidative stress, such as glucose auto oxidation, protein glycation and formation of advanced glycation end products. Some natural antioxidants play an

important role in interfering with the oxidation processes by reacting with free radicals and chelating the catalytic metals in biological system.

The MPFL (Rutaceae) consists of 2, 6-dimethoxybenzoquinone and osthenol and Three volatile flavour components like methyl hexanoate, ethyl 3-hydroxyhexanoate and butanoic acid. Free fatty acids like palmitic, oleic, linoleic, linolenic acid, palmitoleic and stearic acids; β-sitosterol, β-amyrin (Saima *et al.*, 2000), unsaponifiable matter like lupeol and stigmasterol (MacLeod and Pieris, 1981).

So, the present study was designed to evaluate the antioxidant potential of methanolic pericarp extract of *Feronia limonia* (MPEL) by using different *in vitro* assays.

MATERIALS AND METHODS

Materials: Nitroblue Tetrazolium (NBT), Bovine Serum Albumin (BSA) were obtained from Merck, Mumbai. 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,4 dinitrophenylhydrazine(DNPH), trichloroacetic acid (TCA) were obtained from Sigma (St. Louis, MO, USA). 2,4,6-Tri-(2'-pyridyl)-1, 3, 5-triazine (TPTZ), 2, 2'-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and Trolox were obtained from Sigma Aldrich Chemical Co., Ltd. (England). All other reagents were of Analytical Reagent (AR) grade.

Plant material preparation: The ripened wood apple (*Feronia limonia*) were obtained from local market in Visakhapatnam. The pericarps were manually separated and shade dried. The pericarps were powdered in a grinder to get 40-mesh size powder. The moisture content of pericarp powder was found to be 13.5%. The powder was suspended in 2% gum acacia and used in the experimental studies.

Super oxide radical scavenging activity: The assay was based on the capacity of the extract to inhibit formazan formation by scavenging the superoxide radicals generated in riboflavin-light-NBT system (Beauchamp and Fridovich, 1971). The reaction mixture contained 58 mM phosphate buffer, pH 7.6, 20 μM riboflavin, 6 mM EDTA and 50 μM NBT, final volume made up to 3 mL, added in that sequence. Initiated with the reaction (Kumaran and Karunakaran, 2007) the reaction mixture with the different concentrations was exposed to 40 V under fluorescence lamp for 15 min to initiate the reaction. Immediately after illumination, the absorbance was measured at 560 nm. The entire reaction assembly was enclosed in a box lined with aluminium foil. Identical tubes, with reaction mixture, above were kept in the dark and served as blanks. The percentage inhibition of superoxide anion generation was calculated using the following equation:

$$\text{Inhibition (\%)} = \frac{A_0 - A_1}{A_0} \times 100$$

where, A_0 was the absorbance of the control and A_1 was the absorbance of extract/standard. All experiments were performed in triplicate.

Hydroxyl radical scavenging activity: Scavenging activity of hydroxyl radical was measured by the method of Halliwell and Gutteridge (1985). Hydroxyl radicals were generated by a Fenton reaction (Fe^{3+} -ascorbate-EDTA- H_2O_2 system) and the scavenging capacity of the extract and standard towards the hydroxyl radicals was measured by using deoxyribose method. The reaction mixture contained 2-deoxy-2-ribose (2.8 mM), phosphate buffer (0.1 mM,

pH 7.4), ferric chloride (20 μM), EDTA (100 μM), hydrogen peroxide (500 μM), ascorbic acid (100 μM) and various concentrations (10-1000 μg mL⁻¹) of the test sample in a final volume of 1 mL. The mixture was incubated for 1 h at 37°C. After the incubation an aliquot of the reaction mixture (0.8 mL) was added to 2.8% TCA solution (1.5 mL), followed by TBA solution (1% in 50 mM sodium hydroxide, 1 mL) and sodium dodecyl sulphate (0.2 mL). The mixture was then heated (20 min at 90°C) to develop the colour. After cooling, the absorbance was measured at 532 nm against an appropriate blank solution. All experiments were performed in triplicates. The percentage of inhibition was expressed, according to the following equation:

$$\text{Inhibition (\%)} = \frac{A_0 - A_1}{A_0} \times 100$$

where, A_0 was the absorbance of the control without a sample, A_1 is the absorbance in the presence of the sample.

Hydrogen peroxide radical scavenging activity: The hydrogen peroxide scavenging assay was carried out following the procedure of Ruch *et al.* (1989). The principle of this method is that there is a decrease in absorbance of H_2O_2 upon oxidation of H_2O_2 . A solution of 43 mM H_2O_2 was prepared in 0.1 M phosphate buffer (pH 7.4). The MPFL of different concentrations were prepared in 3.4 mL phosphate buffer were added to 0.6 mL of H_2O_2 solution (43 mM) and absorbance of the reaction mixture was recorded at 230 nm. All experiments were performed in triplicates. The percentage of inhibition was expressed, according to the following equation:

$$\text{Inhibition (\%)} = \frac{A_0 - A_1}{A_0} \times 100$$

where, A_0 was the absorbance of the control without a sample, A_1 is the absorbance in the presence of the sample.

DPPH radical scavenging activity: The potential of extract and AA was determined on the basis of the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical. Aliquots of 1 mL of a methanolic solution containing each concentration of extract were added to 3 mL of 0.004% MeOH solution of DPPH. Absorbance at 517 nm, against blank methanol without DPPH, was determined after 30 min (UV, Perkin-Elmer-Lambda 11 spectrophotometer) and the percent inhibition activity was calculated (Braca *et al.*, 2001). The percentage of inhibition was expressed, according to the following equation:

$$\text{Inhibition (\%)} = \frac{A_0 - A_1}{A_0} \times 100$$

where, A_0 was the absorbance of the control without a sample, A_1 is the absorbance in the presence of the sample. All tests were run in triplicate and averaged.

Nitric oxide radical scavenging activity: Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH interacts with oxygen to produce nitrite ions which were measured by the Griess reaction (Ebrahimzadeh *et al.*, 2010). The reaction mixture (3 mL) containing sodium nitroprusside (10 mM) in Phosphate Buffered Saline (PBS) and MPFL and the AA in different concentrations were incubated at 25°C for 150 min. After incubation 1.5 mL of the Griess reagent (1% sulphanilamide and 0.1% naphthyl ethylene diamine dihydrochloride in 2% H₃PO₄) was added. The absorbance of the chromophore formed was measured at 546 nm. The percentage of inhibition was expressed, according to the following equation:

$$\text{Inhibition (\%)} = \frac{A_0 - A_1}{A_0} \times 100$$

where, A₀ was the absorbance of the control without a sample, A₁ is the absorbance in the presence of the sample.

Reducing power: The reducing power of the extract was determined according to the method of Oyaizu (1986). Various concentrations of the extracts (mg mL⁻¹) in distilled water were mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and 1% of potassium ferricyanide water solution (2.5 mL, K₃[Fe(CN)₆]). The mixture was incubated at 50°C for 20 min. Aliquots of trichloroacetic acid (2.5 mL, 10% aqueous solution) were added to the mixture which was then centrifuged at 3000 rpm for 10 min. The supernatant (2.5 mL) was mixed with distilled water (2.5 mL) and a freshly prepared FeCl₃ solution (0.5 mL, 0.1%). The absorbance was measured at 700 nm. The percentage of inhibition was expressed, according to the following equation:

$$\text{Inhibition (\%)} = \frac{A_0 - A_1}{A_0} \times 100$$

where, A₀ was the absorbance of the control without a sample, A₁ is the absorbance in the presence of the sample.

Phosphomolybdenum method: The antioxidant activity of MPFL extract was evaluated by the phosphomolybdenum method of Prieto *et al.* (1999). An aliquot of 0.1 mL of sample solution (equivalent to 100 µg) was combined with 1 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). In the case of the blank, 0.1 mL of methanol was used in place of sample. The tubes were capped and incubated in water bath at 95°C for 90 min. After the samples were cooled to RT, the absorbance of the aqueous solution of each was measured at 695 nm.

The percentage of inhibition was expressed, according to the following equation:

$$\text{Inhibition (\%)} = \frac{A_0 - A_1}{A_0} \times 100$$

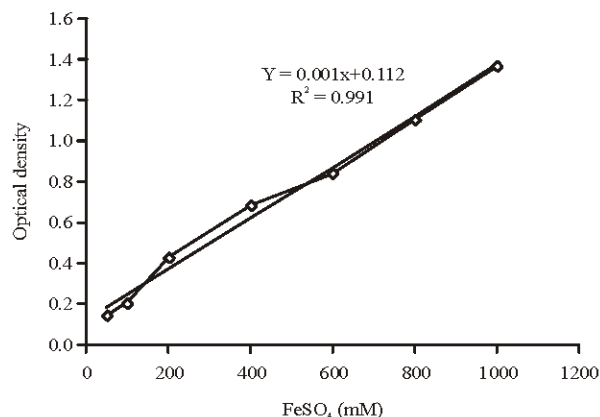


Fig. 1: Standard curve of Ferrous sulphate at different concentrations

where, A₀ was the absorbance of the control without a sample, A₁ is the absorbance in the presence of the sample.

Fe²⁺ chelating activity: The chelating activity of the extracts for ferrous ions (Fe²⁺) was measured according to the method of Dinis *et al.* (1994). To 0.5 mL of extract, 1.6 mL of deionized water and 0.05 mL of FeCl₂ (2 mM) was added. After 30 sec, 0.1 mL ferrozine (5 mM) was added. Ferrozine reacted with the divalent iron to form stable magenta complex species that were very soluble in water. After 10 min at room temperature, the absorbance of the Fe²⁺/Ferrozine complex was measured at 562 nm. The percentage of inhibition was expressed, according to the following equation:

$$\text{Inhibition (\%)} = \frac{A_0 - A_1}{A_0} \times 100$$

where, A₀ was the absorbance of the control without a sample, A₁ is the absorbance in the presence of the sample.

Ferric reducing ability power: The FRAP method measures the absorption change that appears when the TPTZ (2,4,6-tripyridyl-s-triazine)-Fe³⁺ complex is reduced to the TPTZ-Fe²⁺ form in the presence of antioxidants (Benzie and Strain, 1996). An intense blue colour develops with absorption maximum at 595 nm. The FRAP reagent contained 2.5 mL of 10 mM tripyridyltriazine (TPTZ) solution in 40 mM HCl plus 2.5 mL of 20 mM FeCl₃ and 25 mL of 0.3 M acetate buffer, pH 3.6, was freshly prepared. The extracts were dissolved in ethanol at a concentration of 1 mg mL⁻¹. An aliquot of 0.2 mL of solution was mixed with 1.8 mL of FRAP reagent and the absorption of the reaction mixture was measured at 595 nm. Ethanolic solutions of known Fe (II) concentration, in the range of 50-1000 µM (FeSO₄), were used for obtaining the calibration curve (Fig. 1). The FRAP value represents the ratio between the slope of the linear plot for reducing Fe³⁺-TPTZ reagent by plant extract compared to the slope of the plot for FeSO₄.

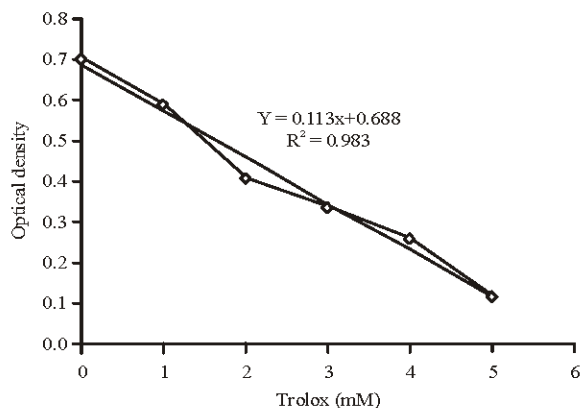


Fig. 2: Effect of various concentrations of trolox in ABTS⁺ radical scavenging activity

ABTS⁺ assay: ABTS assay was based on the method of Re *et al.* (1999) with slight modifications. ABTS radical cation (ABTS⁺) was produced by reacting 7 mM ABTS solution with 2.45 mM potassium persulphate and allowing the mixture to stand in the dark at room temperature for 12-16 h before use. The ABTS⁺ solution was adjusted to an absorbance 0.70 ± 0.02 by diluting with ethanol at 734 nm. The 25 μ L of sample or standard Trolox was added to 2 mL of diluted ABTS⁺ solution and the absorbance was measured after 6 min. The decrease in absorption with the addition of different concentrations of extract was used for calculating TEAC values (Fig. 2). A standard curve was prepared by measuring the reduction in absorbance of ABTS⁺ solution at different concentrations of Trolox. Appropriate blank measurements were carried out and the values recorded. The reduction in the absorbance of different concentrations of extract was measured from the trolox standard graph a TEAC values. Results were expressed as Trolox Equivalent Antioxidant Capacity (TEAC).

In vitro protein glycoxidation method: This assay was adopted from the literature by Wu and Yen (2005) and used as an *in vitro* model. In brief, 5 g BSA and 14.4 g D-glucose were dissolved in phosphate buffer (1.5 M, pH 7.4) to obtain the control solution with 50 mg mL⁻¹ BSA and 0.8 M D-glucose. Two milliliter of the control solution was incubated at 37°C for 21 days in the presence or absence of 1 mL of MPFL and AA in phosphate buffer (1.5 M, pH 7.4) (the final concentration of extract in the 3 mL test solution was 500 ppm). The test solution also contained 0.2 g L⁻¹ Na₃ to assure an aseptic condition. After 21 days of incubation, fluorescent intensity (excitation, 370 nm; emission, 440 nm) was measured for the test solutions. Percent inhibition of AGE formation by MPFL and AA was calculated using the following equation:

$$\text{Inhibition (\%)} = 1 - \frac{\text{Fluorescence of the solution with inhibitors}}{\text{Fluorescence of the solution without inhibitors}} \times 100$$

Estimation of protein carbonyl content: The effects of MPFL extract on oxidative modification of BSA during

glycoxidation process were carried out according to method Ardestani and Yazdanparast (2007). For determination of protein carbonyl content in the samples, 1 mL of 10 mM 2, 4-dinitrophenylhydrazine (DNPH) in 2 M HCl was added to the samples (1 mg). Samples were incubated for 30 min at RT. Then, 1 mL of cold TCA (10%, w/v) was added to the mixture and centrifuged at 3000 g for 10 min. The protein pellet was washed three times with 2 mL of ethanol/ethyl acetate (1:1, v/v) and dissolved in 1 mL of guanidine hydrochloride (6 M, pH 2.3). The absorbance of the sample was read at 370 nm. The carbonyl content was calculated based on the molar extinction coefficient of DNPH. The data was expressed as nmol mg⁻¹ protein.

RESULTS AND DISCUSSION

Oxidative stress is defined as a situation of serious imbalance between the production of free radicals (ROS) and antioxidant defense mechanisms, leading to potential tissue dysfunction and damage (Halliwell and Gutteridge, 1990). These reactive oxygen species are generated in the early and advanced glycation processes. The glycation phenomenon corresponds to the non enzymatic and non-oxidative covalent attachment of glucose molecule to protein. This process classically proceeds through early and advanced stages. In the early stage, reducing sugars such as glucose react with the amino groups of lysine side chains and the terminal amino group of proteins to form unstable Schiff bases and through rearrangement, Amadori products. Then, this product undergoes slow and complex series of chemical reactions to form Advanced Glycation End products (AGEs) (Pennathur and Heinecke, 2007). Extensive modification to lysine side chains and minor modification to arginine side chains have been found to occur during the AGE formation process with glucose (Cohen *et al.*, 2003). The accumulation of the reaction products of protein glycation in living organisms leads also to functional modifications of tissue proteins. From cross linking of proteins and interaction of AGE with their receptors and/or binding proteins which leads to enhanced formation of reactive oxygen species with subsequent activation of nuclear factor- κ B and release of pro-inflammatory cytokines, growth factors and adhesion molecules (Lehmann and Schleicher, 2000).

Oxidative stress in diabetes could originate from various processes, such as excessive production of oxygen radicals from the autoxidation of glucose, glycoxidized proteins and glycoxidation of antioxidative proteins (Brownlee, 1994).

The maintenance of protein redox state is fundamental for cell functions, whereas, structural changes in proteins are considered to be among the molecular mechanism leading to progression and development of many pathological conditions such as diabetes and its complications (Telci *et al.*, 2000). Free radicals can induce protein modifications including losses of protein functions such as the activity of enzymes, receptors and membrane transporters, resulting in biological dysfunctions (Stadtman and Levin, 2000). Proteins can also be

modified by glucose through the glycation processes. Oxidative reactions considerably participate in the process of the AGEs formation, indicating that oxidative stress can modify proteins to AGEs (Yokozawa and Nakagawa, 2004). The probable mechanism of protein damage by glycation reactions is based on generation of ROS either from autoxidation of glucose or in the process of oxidative degradation of Amadori intermediates (Mossine *et al.*, 1999; Wolff and Dean, 1987).

The superoxide radical (O_2^-) scavenging activity of the MPFL, as measured by the riboflavin-NBT-light system *in vitro*. Superoxide radical is known to be very harmful to cellular components as a precursor of more reactive oxygen species (Halliwell and Gutteridge, 1985). During the electron transport chain in mitochondria one of the carrier electrons was replaced by oxygen, so produce superoxide radical. To prevent the superoxide radical mediated injury, cells contain Superoxide Dismutase (SOD) as a cellular antioxidant enzyme which removes this ubiquitous superoxide metabolic product by converting it into hydrogen peroxide and oxygen and this hydrogen peroxide radical readily decomposed into hydroxyl radical in the presence of catalase in biological systems (Chan *et al.*, 1996; Lehmann and Schleicher, 2000; Giorgio *et al.*, 2007). The MPFL were shown similar activity like AA in scavenging the superoxide and hydroxyl radicals. Hydrogen peroxide itself is not very reactive but reacts with transitional metal ion dependent OH radical mediated oxidative damage to the DNA. The MPFL were shown better activity than AA in scavenging the hydrogen peroxide radical (Table 1). Hydroxyl radicals can occasionally produced as a byproduct of immune action by macrophages. They can damage virtually all types of macromolecules like carbohydrates, nucleic acids, lipids and amino acids. The hydroxyl radicals cannot be eliminated by enzymatic reactions, only endogenous antioxidants can scavenge these radicals (Sies, 1993; Reiter *et al.*, 1995). The DPPH known to abstract labile hydrogen and the ability to scavenge the DPPH radical is related to the inhibition of lipid peroxidation. The AA was shown better activity than MPFL in scavenging the DPPH radical (Table 1). The antioxidant products are capable of scavenging free radicals at pH 7.4 via electron or hydrogen donating mechanisms and thus should be able to prevent the initiation of deleterious free radical mediated chain reactions. Nitric oxide is a free radical in terms its unpaired electron. It reacts with O_2^- in termination reactions in the mitochondrial matrix, yielding peroxynitrite ($ONOO^-$). These oxyradical and peroxynitrite induce oxidative damage to mitochondrial DNA damage and protein inactivation and ATP synthesis (Wink and Mitchell, 1998). The antioxidant capability of MPFL was found to have better than ascorbic acid because of presence of active principles like tannins, phenols and anthocyanidins.

Iron is essential for life due to its unusual flexibility to serve as both an electron donor and acceptor. Among the transition metals, iron is known as the most important lipid oxidation. In nature it can be found as either ferrous (Fe^{2+}) or ferric (Fe^{3+}). If iron is free within the cell it can catalyze the

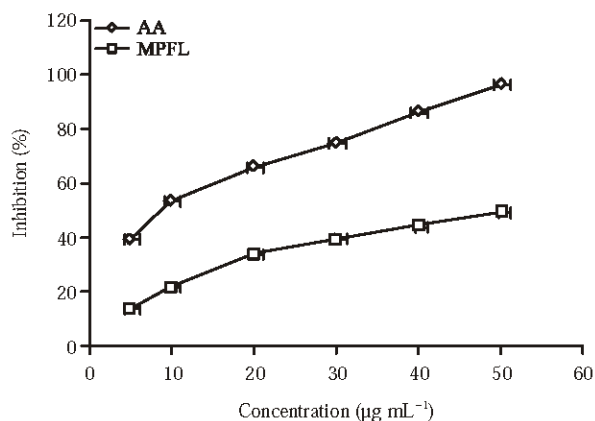


Fig. 3: Effect of various concentrations of ascorbic acid and MPFL on Fe^{2+} chelating activity

Table 1: Effect of Ascorbic acid and MPFL on IC_{50} values of different *in vitro* models

Method	Ascorbic acid	MPFL
Superoxide radical	04.27±0.31	05.40±0.23
Hydroxyl radical	29.70±0.11	41.11±0.15
Hydrogen peroxide radical	05.08±0.03	03.39±0.04
DPPH radical	53.92±5.22	91.94±3.47
Nitric oxide radical	16.93±0.07	29.43±0.06
Reducing power	05.52±0.01	13.31±0.02
Phosphomolybdenum method	03.19±0.03	04.72±0.09

conversion of hydrogen peroxide into free radicals (Andrews, 1999; Conrad and Umbreit, 2000). Minimizing these ions may afford protection against oxidative damage by inhibiting production of ROS. Reducing power is often used as an indicator of electron donating activity which is an important mechanism of phenolic antioxidant action, can be strongly correlated with other antioxidant properties. In the reducing power assay, the presence of antioxidants in the sample would result in the reduction of Fe^{3+} to Fe^{2+} by donating an electron. The presence of reductants such as antioxidant substances in the MPFL causes the reduction of the Fe^{3+} /ferricyanide complex to the ferrous form (Fe^{2+}). In the phosphomolybdenum method is based on the reduction of Mo(VI) to Mo(V) by the antioxidant compounds and the formation of green Mo(V) complexes (Halliwell and Gutteridge, 1985). In the metal chelating assay, ferrozine can quantitatively form complexes with Fe^{2+} . In the presence of other chelating agents, the complex formation is disrupted with consequent decrease in the intensity of the red color of the complex. The present results indicated that the MPFL showed a dose dependent effect with an increase in the concentration (Fig. 3). FRAP assay measures the reducing ability of antioxidant that react with ferric tripyridyltriazine (Fe^{3+} -TPTZ) complex and produce a coloured ferrous tripyridyltriazine (Fe^{2+} -TPTZ) (Benzie and Strain, 1996). Using this assay, the FRAP value of MPFL shown to have dose dependent effect with an increase in the concentration (Fig. 4).

The ABTS⁺ radical assay can be used to measure the substances, i.e., both aqueous phase radicals and lipid peroxy radicals. The scavenging activity of the extract on the radical

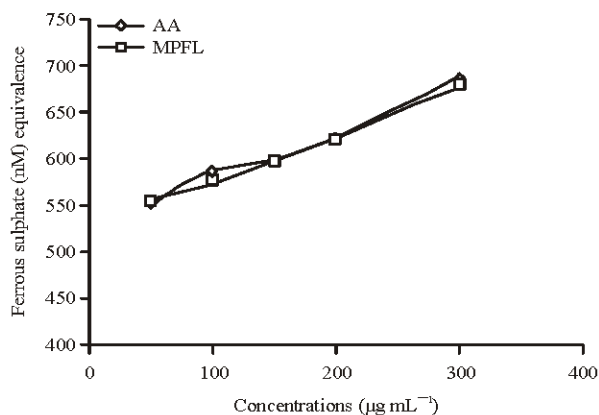


Fig. 4: Effect of various concentrations of ascorbic acid and MPFL on Ferric Reducing Ability Power (FRAP)

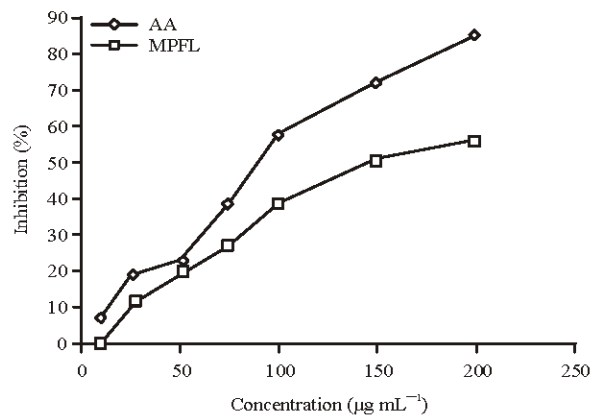


Fig. 6: Effect of various concentrations of ascorbic acid and MPFL on *in vitro* glycation of bovine serum albumin

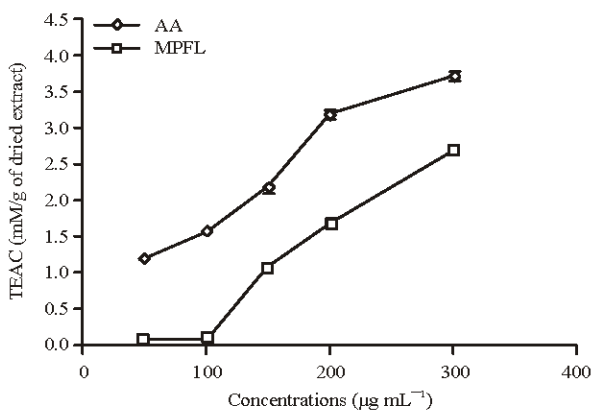


Fig. 5: Effect of various concentrations of ascorbic acid and MPFL on ABTS⁺ assay

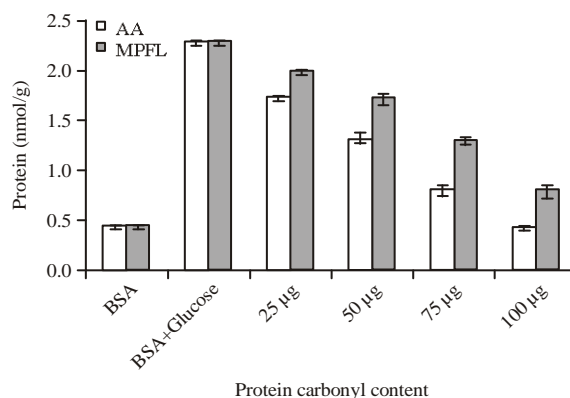


Fig. 7: Effect of various concentrations of ascorbic acid and MPFL on Protein Carbonyl Content (PCO)

ABTS, generated by potassium persulfate was compared with a standard amount of trolox. In general, medicinal plants may play an important role in chemical protection from oxidative damage by possessing endogenous antioxidants such as phenolic compounds. Hagerman *et al.* (1998) have reported that the high molecular weight phenolics (tannins) have more ability to quench free radicals (ABTS⁺) (Hagerman *et al.*, 1998). The TEAC value of MPFL was found to be better than AA (Fig. 5).

The incubation of BSA with D-glucose following 21 days incubation induced a loss of helical structure in BSA. However, the presence of plant extract alone induced an increase in the helicity of BSA (more negative ellipticity values). Therefore, MPFL may stabilize the native protein structure. The presence of plant extract in the protein/glucose from reducing the degree of secondary structure in BSA. These data confirms the data obtained from fluorescent spectroscopy and show that MPFL may stabilize the native structure of BSA. Fluorescence intensity of glycosylated collagen was significantly higher after incubation with glucose. The AA and MPFL inhibited the glycation of BSA and subsequent formation of fluorescent glycation (Fig. 6).

To evaluate whether extracts can reduce the protein glycation in glycation process, the extent of protein carbonyl formation after 21 days by DNPH reagent was evaluated. As shown in Fig 4, glycation elicited a significant increase of carbonylation of BSA in the presence of glucose compared to the control sample without reducing sugar. However, a significant effect on the inhibition of protein oxidation due to glycation was exerted in a dose-dependent manner with increasing the concentrations of MPFL (Fig. 7). *In vitro* analysis reveals that the MPFL showed dose dependant effect with an increase in the concentration in all the above parameters.

Thus, it was proposed that antioxidative effect of *Feronia limonia* might be due to the presence of volatile flavours and free fatty acids in pericarp are involved in the AGEs inhibitory mechanisms.

CONCLUSION

It is concluded that, using different *in vitro* models for estimation the antioxidant potential *Feronia limonia* showed better effect in scavenging the different free radicals. In

addition to antioxidant activity it also showed better activity against the formation of protein carbonyl content and protect the structural changes in BSA during glycation processes. All the activities might be due to high levels of volatile flavours and free fatty acids in pericarp extract of *Feronia limonia*.

ACKNOWLEDGMENT

The author acknowledges the financial support for completion of research work from Council for Scientific Industrial research, New Delhi (Ref: 111177/2K11/1).

REFERENCES

- Andrews, N.C., 1999. Disorders of iron metabolism. N. Engl. J. Med., 341: 1986-1995.
- Ardestani, A. and R. Yazdanparast, 2007. Antioxidant and free radical scavenging potential of *Achillea santolina* extracts. Food. Chem., 104: 21-29.
- Beauchamp, C. and I. Fridovich, 1971. Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. Anal. Biochem., 44: 276-287.
- Benzie, I.F.F. and J.J. Strain, 1996. The Ferric Reducing Ability of Plasma (FRAP) as a measure of antioxidant power: The FRAP assay. Anal. Biochem., 239: 70-76.
- Braca, A., N. de Tommasi, L. di Bari, C. Pizza, M. Politi and I. Morelli, 2001. Antioxidant principles from *Bauhinia terapotensis*. J. Nat. Prod., 64: 892-895.
- Brownlee, M., 1994. Glycation and diabetic complications. Diabetes, 43: 836-841.
- Chan, P.C., R.C. Sills, A.G. Braun, J.K. Haseman and J.R. Bucher, 1996. Toxicity and carcinogenicity of Δ^9 -tetrahydrocannabinol in fischer rats and B6C3F1 mice. Fundam. Applied Toxicol., 30: 109-117.
- Cohen, M.P., E. Shea, S. Chen and C.W. Shearman, 2003. Glycated albumin increases oxidative stress, activates NF- κ B and Extracellular signal-Regulated Kinase (ERK) and stimulates ERK-dependent transforming growth factor- β 1 production in macrophage RAW cells. J. Lab. Clin. Med., 141: 242-249.
- Conrad, M.E. and J.N. Umbreit, 2000. Disorders of iron metabolism. N. Engl. J. Med., 342: 1293-1294.
- Dinis, T.C.P., V.M.C. Madeira and L.M. Almeida, 1994. Action of phenolic derivatives (acetaminophen, salicylate and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Arch. Biochem. Biophys., 315: 161-169.
- Ebrahimzadeh, M.A., S.F. Nabavi, S.M. Nabavi, B. Eslami and H. Asgarirad, 2010. *In vitro* antioxidant and free radical scavenging activity of *Leonurus cardiaca* subsp. *Persicus*, *Grammosciadium platycarpum* and *Onosma demawendicum*. Afr. J. Biotech., 9: 8865-8871.
- Giorgio, M., M. Trinei, E. Migliaccio and P.G. Pelicci, 2007. Hydrogen peroxide: A metabolic by-product or a common mediator of ageing signals? Nat. Rev. Mol. Cell Biol., 8: 722-728.
- Hagerman, A.E., K.M. Riedl, G.A. Jones, K.N. Sovik, N.T. Ritchard, P.W. Hartzfeld and T.L. Riechel, 1998. High molecular weight plant polyphenolics (Tannins) as biological antioxidants. J. Agric. Food Chem., 46: 1887-1892.
- Halliwell, B. and J.M.C. Gutteridge, 1985. Free Radicals, Ageing and Disease. In: Free Radicals in Biology and Medicine, Halliwell, B. and J.M.C. Gutteridge (Eds.). Clarendon Press, Oxford, pp: 279-315.
- Halliwell, B. and J.M.C. Gutteridge, 1990. Role of free radicals and catalytic metal ions in human disease: An overview. Methods Enzymol., 186: 1-85.
- Kumaran, A. and R.J. Karunakaran, 2007. *In vitro* antioxidant activities of methanol extracts of five *Phyllanthus* species from India. LWT-Food Sci. Technol., 40: 344-352.
- Lehmann, R. and E.D. Schleicher, 2000. Molecular mechanism of diabetic nephropathy. Clin. Chim. Acta, 297: 135-144.
- MacLeod, A.J. and N.M. Pieris, 1981. Volatile flavor components of wood apple (*Feronia limonia*) and a processed product. J. Agric. Food Chem., 29: 49-53.
- Mossine, V.V., M. Linetsky, G.V. Glinsky, B.J. Ortwerth and M.S. Feather, 1999. Superoxide free radical generation by Amadori compounds: The role of acyclic forms and metal ions. Chem. Res. Toxicol., 12: 230-236.
- Oyaizu, M., 1986. Studies on products of browning reaction: Antioxidative activities of products of browning reaction prepared from glucosamine. Jpn. J. Nutr., 44: 307-315.
- Pennathur, S. and J.W. Heinecke, 2007. Mechanisms for oxidative stress in diabetic cardiovascular disease. Antioxid. Redox. Signal., 9: 955-969.
- Prieto, P., M. Pineda and M. Aguilar, 1999. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. Anal. Biochem., 269: 337-341.
- Re, R., N. Pellegrini, A. Proteggente, A. Pannala, M. Yang and C. Rice-Evans, 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biol. Med., 26: 1231-1237.
- Reiter, R.J., D. Melchiorri, E. Sewerynek, B. Poeggeler and L.R. Barlow-Walden *et al.*, 1995. A review of the evidence supporting melatonin's role as an antioxidant. J. Pineal Res., 18: 1-11.
- Ruch, R.J., S.J. Cheng and J.E. Klaunig, 1989. Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogenesis, 10: 1003-1008.
- Saima, Y., A.K. Das, K.K. Sarkar, A.K. Sen Sr. and P. Sur, 2000. An antitumor pectic polysaccharide from *Feronia limonia*. Int. J. Biol. Macromol., 27: 333-335.
- Sies, H., 1993. Strategies of antioxidant defense. Eur. J. Biochem., 215: 213-219.
- Stadtman, E.R. and R.L. Levin, 2000. Protein oxidation. Ann. N.Y. Acad. Sci., 899: 191-208.

- Telci, A., U. Cakatay, S. Salman, I. Satman and A. Sivas, 2000. Oxidative protein damage in early stage Type 1 diabetic patients. *Diabetes Res. Clin. Pract.*, 50: 213-223.
- Wink, D.A. and J.B. Mitchell, 1998. Chemical biology of nitric oxide: Insights into regulatory, cytotoxic and cytoprotective mechanisms of nitric oxide. *Free Radic. Biol. Med.*, 25: 434-456.
- Wolff, S.P. and R.T. Dean, 1987. Glucose autoxidation and protein modification. The potential role of autoxidative glycosylation in diabetes. *Biochem. J.*, 245: 243-250.
- Wu, C.H. and G.C. Yen, 2005. Inhibitory effect of naturally occurring flavonoids on the formation of advanced glycation endproducts. *J. Agric. Food Chem.*, 53: 3167-3173.
- Yokozawa, T. and T. Nakagawa, 2004. Inhibitory effects of Luobuma tea and its components against glucose-mediated protein damage. *Food Chem. Toxicol.*, 42: 975-981.