Various fractions of *Hypericum x moserianum* and *Hypericum ericoides* possess antiglycation, anti-lipid peroxidation, antioxidative activities and non-toxic effects *in vitro*

Ghulam Abbas[1-2], Muhammad Shahzad[3]*, Zeb Saddiqe[4], M Jawad Hassan[5], Sumbal Saba[6], Jamal Rafique[6], Rizwana Malik[6] and Hidayat Hussain[7]

[1] Department of Biochemistry, University of Health Sciences, Lahore, Pakistan
[2]Department of Biological Sciences and Chemistry, University of Nizwa, Nizwa-616, Oman
[3]Department of Pharmacology, University of Health Sciences, Lahore, Pakistan
[4]Department of Botany, Lahore College for Women University, Lahore, Pakistan
[5]ASAB, National University of Sciences and Technology (NUST), Islamabad, Pakistan
[6]International Center for Chemical and Biological Sciences, HEJ RIC, University of Karachi, Karachi, Pakistan
[7]UoN Chair of Oman's Medicinal Plants and Marine Natural Products, University of Nizwa, Nizwa-616, Oman

Abstract: In the present study, two species *Hypericum x moserianum* and *Hypericum ericoides* which belong to genus *Hypericum* were evaluated for their potential antiglycation, antioxidant, anti lipid peroxidation and cytotoxic activities. These species are widely used in folk medicine and to the best of our knowledge there were no previous reports regarding antioxidant, anti-glycation and cytotoxicity studies of these species. Among the crude methanol extracts and fractions of both the species, the ethyl acetate fraction of *H.* x *moserianum* exhibited promising antioxidant activity against 1,1-diphenyl-2-picrylhydrazyl (DPPH) with IC50 129.084±1.215µg/ml, followed by methanol extract (IC50=232.083±1.215µg/ml) and aqueous fraction (IC50=266.962±2.213µg/ml). The ethyl acetate fraction of *H. ericoides* exhibited IC50 value of 295.088±2.320µg/ml. In antiglycation assay, the ethyl acetate fraction of *H*. x moserianum showed 52.096% inhibition at 500µg/ml. For lipid peroxidation assay, the dichloromethane, aqueous and *n*-hexane fractions of *H.* x moserianum showed 67.241, 66.147 and 64.213% inhibition respectively, while aqueous fraction of *H. ericoides* exhibited 67.404% inhibition at 500µg/ml. In cytotoxicity assay, all fractions of both the species were found to be non-toxic on mouse fibroblast 3T3 cells with IC50 value greater than 30µg/ml as compared to cycloheximide with IC50 value 0.073±0.1µg/ml used as a standard. It was concluded from the study that among the two species, crude methanolic and ethyl acetate fractions were more active regarding the antioxidant, anti-glycation activities while dichloromethane, aqueous and n-hexane fractions possessed anti-lipid peroxidation activity.

Keywords: Protein Glycation, oxidative stress, antioxidants, lipid peroxidation, cytotoxicity and genus *Hypericum.*

INTRODUCTION

There is a complex link between the glycation process and oxidation during diabetes mellitus while lipid peroxidation and malondialdehyde (MDA) accumulation is also observed in diabetics. Various substances such as aminoguanidine (AG) and L-arginine have been reported to prevent the formation of advanced glycation endproducts (AGEs) and lipid peroxidation (Jakug *et al*., 1999). The difference in the production of reactive oxygen species and quantities of antioxidants present in the body plays an important role in multiple diseases. Various reactive species of oxygen, nitrogen and chlorine are generated *in vivo* through various mechanisms (Halliwell and Gutteridge, 1999). The antioxidants whether synthetic or natural origins can effectively eliminate free radicals by averting their formation, scavenging them or increasing their decomposition rate. (Heinecke, 2003; Kaur and Kapoor 2002; Cesquini *et al*., 2003). Antioxidants protect against glycation derived free radicals and may have therapeutic potential (Ceriello *et al*., 1991). Vitamin E which is a well known antioxidant blocks the protein glycation by inhibiting MDA formation thus long-term complications can be reduced by reducing oxidative stress in patients with diabetes (Soon, 2004). Due to increased understanding of the damaging effects of glycation process, it is highly desirable to manage this process effectively either by prevention or by managing the consequences of glycation, which could be useful for millions of affected people (Khalifa *et al*., 1999; Rahbar *et al*., 2000). Antioxidant and anti glycation agents obtained from natural products have great therapeutic potential. Moreover, recent study discovered that compounds with combined antioxidant and anti-glycation properties are more efficient for the management of diabetes mellitus (Duraisamy *et al*., 2003).

The genus *Hypericum* (Hypericaceae) comprising of more than 450 species is well known for its use in folk medicine to treat wounds, eczema, burns, bruises, arthritis, hepatitis, herpes, and the disorders of central nervous system and alimentary tract in many parts of the

**Corresponding author:* e-mail: gapakland@gmail.com

world (Barnes *et al*., 2001; Benkikia *et al*., 2003; Saddiqe *et al*., 2010). In a number of studies, several *Hypericum* species have exhibited *in vitro* antioxidant activity (Cakir *et al*., 2003; Conforti *et al*., 2002; Valentão *et al*., 2002 Radulovic *et al*., 2007). The extracts possessing antioxidant activity are rich in phenolic contents particularly flavonoids (Zou *et al*., 2004).

The two *Hypericum* species, *H. ericoides* and *H. x moserianum*, selected for the present study are used in folk medicine in various parts of the world. The leaves, stems and flowers of *H. ericoides* are used in Valentian folk medicine (Cardona and Seoan, 1982). Several flavonoids, xanthonoids and terpenes have been reported from the two species (Cardona and Seoan, 1982, 1983; Saddiqe *et al*., 2011; Alain *et al*., 2006). However, only limited literature is available regarding the phytochemical and biological studies of these two plants. The use of medicinal plants is increasing worldwide and there is a strong and sustained public support for traditional herbal treatments (Motlhanka *et al.*, 2011).

To the best of our knowledge, there are no reports regarding antioxidant, anti-glycation and cytotoxicity studies of these species. Keeping in view the literature available for other *Hypericum* species, crude methanol extracts and various fractions of the two *Hypericum* species were evaluated for antioxidant, anti-glycation and cytotoxicity studies.

MATERIALS AND METHODS

The two *Hypericum* species used in the present study were obtained from Perryhill Nurseries and were grown in the green house of University of Portsmouth, UK, for one year. The aerial parts of the two species were used for the study. *Herbarium* specimens of both the species were lodged in the *Herbarium* of Hampshire County Council Museum Service, Winchester, Hampshire, UK (Index Herbarium code HCMS; accession number Bi 2000 16. 371 and 372 for*, H. ericoides,* and *H. x moserianum*, respectively).

Extraction

The plant material of the two *Hypericum* species was air-dried at room temperature. The dried material was ground into small pieces by using a crushing machine. The powdered plant material was extracted with methanol at room temperature for 15 days with occasional stirring. The process was repeated three times and the combined extracts were concentrated under reduced pressure in rotary evaporator to give the crude methanol extract as a gummy residue. A part of this gummy residue was stored for further analysis and the remaining extract was dissolved and suspended in distilled water. The suspension was partitioned between *n*-hexane, dichloromethane, ethyl acetate and *n*-butanol sequentially (three times each) to give non-polar (*n*-hexane and dichloromethane) and polar (ethyl acetate, *n*-butanol and aqueous) fractions. The organic fractions were condensed down under vacuum while the aqueous fractions were concentrated in the freeze drier. All the fractions were stored at 4°C.

Reagents for DPPH and superoxide assay

1,1-Diphenyl-2-picryIhydrazylradical (DPPH), naphthyl ethylenediamine, potassium dihydrogen phosphate, dipotassium hydrogen phosphate, 3- (2-pyridyl)-5,6-di(*p*-sulfophenyl)-1,2,4-triazine (disodium salt or ferrozine), reduced β-nicotinamide adenine dinucleotide (NADH), 5-methylphenazium methyl sulfate (PMS), nitro blue tetrazolium salt (NBT), and standard radical scavengers propyl gallate *(*propyl 3,4,5-trihydroxybenzoate) were purchased mainly from Sigma Chemical Co.

Reagents for antiglycation assay

Bovine serum albumin (BSA) was purchased from Research Organics, anhydrous D-glucose from Fisher Scientific, sodium azide and trichloro acetic acid (TCA) from Scharlau. Phosphate buffer (pH 7.4), phosphate bubuffer saline (pH 10) and rutin were purchased from Carl Roth.

Reagents for lipid peroxidation (TBARS) assay

Phosphotidyl choline (substrate), thiobarbituric acid (TBA), quercetin, trichloro acetic acid (TCA), butylated hydroxyanisole (BHA) were purchased from Sigma Aldrich while ferrous sulphate was purchased from Roth, Tris-HCL buffer pH.7.1 and double distilled water (DDW) were also used in this assay.

Reagents for cytotoxicity assays

The mouse fibroblast (3T3) cells were purchased from European American Culture Collection (EACC), minimal essential medium (MEM) and fetal bovine serum (FBS) from GIBCO-BRL, MTT (3-[4,5-dimethylthiazole-2-yl]-2,5- diphenyl- tetrazolium bromide) from Amresco, penicillin and streptomycin from Sigma- Aldrich.

DPPH radical scavenging assay

Free radical scavenging activities of the test samples were determined by measuring the change in absorbance of DPPH at 515 nm by the spectrophotometric method described by Lee (1998). However, in this assay reaction mixture comprised of 95µL (0.3mM) of ethanolic solution of DPPH and 5µL of the plant fraction (500µg/ml) dissolved in dimethyl sulfoxide (DMSO).

Superoxide anion assay

The reaction was performed in triplicate in a 96-well plate and the absorbance was measured on multiplate reader (Spectra Max 340). The reaction mixture contained 40µL (0.2mM) of NADH, 40µL (0.018mM) NBT, 90µL of phosphate buffer (100mM) pH 7.4 and 10µL (500µg/ml)

of the test samples (plant extracts) pre-read at 560 nm. The reaction was initiated by the addition of 20µL (0.008mM) of PMS. Plates were incubated at room temperature for 5 min. Formation of blue color formazan dye was measured at 560 nm. The control contained 10 µL of DMSO, instead of the test samples. The solutions of NBT, NADH and PMS were prepared in phosphate buffer, while the test fractions were dissolved in DMSO (Lee, 1998). The radical scavenging activities (%) were calculated by using the following formula:

% RSA = [100-(AS/AC * 100)]

Where, RSA is radical scavenging activity, AS is the absorbance of radicals and formazan dye in the presence of test sample, and AC is the absorbance of formazan dye without sample (control).

Anti-glycation assay protocol

BSA-fluorescent assay was used as described previously by Choudhary *et al*. (2011). In this assay, 500µg/ml of each unknown inhibitor (fraction) was dissolved in DMSO, along with standard inhibitor. The comparison of fluorescence intensity at 370 nm excitations and emission at 440 nm was obtained by using spectrofluorimeter (Matsuda *et al*., 2003; Matsuura *et al*., 2002). Rutin, a standard inhibitor, showed IC50 value 98.01±2.03 µM.

Anti lipid peroxidation assay

Lipid peroxidation, a marker of tissue injury is measured as thiobarbituric acid reactive substance (TBARS). Thiobarbituric acid assay (TBA) described by Buege and Aust (1978) was used to measure tissue TBARS.

Briefly, 20µL of substrate (phosphotidyl choline), 5µL of Tris-HCL buffer (pH 7.1), 5µL of Ferrous sulphate (1 mM), and 20µL (500µg/ml) of sample inhibitor and 30µL of double distilled water were added in 96 well plate and incubated at 37°C for 15 min. Finally, 50µL of TCA (50%) and 100µL of TBA (0.35g) were added to the reaction mixture. It was then incubated for 15 min in boiling water-bath and pink colour chromogen appeared. Readings were taken at 532 nm (spectra Max-340). DMSO sample was used as a control in this assay. The following formula was used to calculate percent lipid peroxidation inhibition activity. Quercetin (500 µM) was used as a standard inhibitor for the anti lipid peroxidation assay which showed 85.025% inhibition.

% Inhibition =100- {(OD test compound/OD control) × 100}

Cytotoxicity assay

Cytotoxicity of the samples was evaluated in 96-well flat-bottom microplates using the standard MTT (3-[4,5-

Table 1: Radical (DPPH) and anion (superoxide) scavenging capacity in terms of scavenging (%) of different extracts of two *Hypericum* species.

Plant Species	Extractant	Scavenging conc. (µg mL^{-1})	Radical Scavenging (%)	IC_{50} (µg mL^{-1})	Anion Scavenging (%)
H x moserianum	MeOH	500	70.96043	232.083	20.554
	n-Hexane	500	44.8681	nd	12.722
	DM	500	53.80256	nd	11.171
	EtOAc	500	92.52885	129.084	36.686
	n-BuOH	500	45.8986	nd	5.021
	Aqueous	500	70.63067	266.962	7.364
H. ericoides	MeOH	500	51.596	nd	21.867
	n-Hexane	500	23.1657	nd	14.771
	DM	500	23.9798	nd	1.932
	EtOAc	500	73.73248	295.088	11.349
	n-BuOH	500	47.339	nd	9.807
	Aqueous	500	55.09068	nd	17.281

nd = not detected

Table 2: Antiglycation activity (%) of various fractions of *Hypericum* species

Plant species	MeOH	*n*-Hexane	DM	EtOAc	*n*-BuOH	Aqueous
H. x moserianum	39.753	4.795	5.705	52.096	14.286	46.487
H. ericoides	45.298	13.249	14.069	20.972	48.765	37.905

Table 3: Anti lipid peroxidation activity (%) of various fractions of *Hypericum* species

Plant species	MeOH	*n*-Hexane	DM	EtOAc	*n*-BuOH	Aqueous
H. x moserianum	23.045	64.213	67.241	58.158	37.010	66.147
H. ericoides	-	51.196	10.608	24.248	-	67.404

dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide) colorimetric assay as described by Choudhary *et al*. (2011). However, in this case, 3T3 cells (mouse fibroblasts) were cultured in MEM, supplemented with 5% (FBS), by using a 75cm2 flask in a 5% CO2 incubator at 37°C. Cycloheximide was used as a standard (IC50 = 0.3±0.089 µM).

STATISTICAL ANALYSIS

The results were expressed as mean ± SEM and the EZ-fit software (Perrella Scientific Inc., Amherst, U.S.A.) was used to calculate the IC50 values (µg/ml). IC50 values were measured by using different concentrations of the active samples.

RESULTS

The results obtained in the present study suggest that the two *Hypericum* species (*H. x moserianum and H. ericoides*) possess antioxidant, anti lipid peroxidation and anti-glycation activities. In radical (DPPH) scavenging assay, ethyl acetate (EtOAc) fraction of *H. x moserianum* showed significant antioxidant activity with IC50 value 129.084±1.215µg/ml, crude methanol extract (MeOH) was active with IC50 value 232.083±1.530µg/ml while aqueous fraction exhibited moderate activity with IC50 value 266.962±2.213µg/ml as compared to standard antioxidant propyl gyllate with IC_{50} value 37.890±1.512. The crude methanol extract and fractions of *H. ericoides* did not exhibit significant antioxidant activity and only the EtOAc fraction showed moderate activity with IC50 value 295.088±2.320 µgml-1 as shown in (fig. 1). For anion scavenging assay, all fractions exhibited poor anion scavenging activity (table 1).

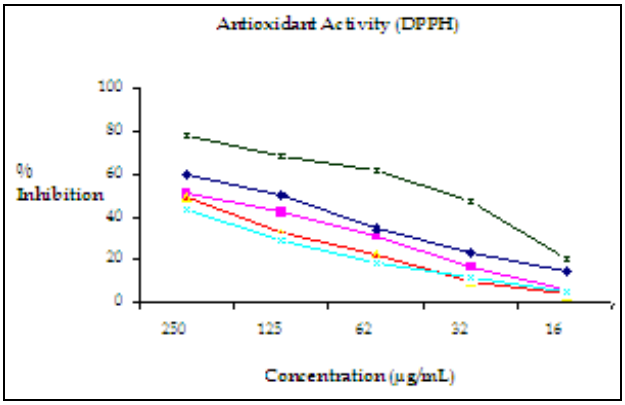

Fig. 1: Antioxidant activity of various fractions at different concentration (IC50 values calculation)
---- --*----- (Propyl gyllate); -- ---●----- (*H. x moserianum* Ethyl Acetate); --- -■--- (*H. x moserianum* Methanol); --- ▼----- (*H. x moserianum* Aqueous)-- ---**x**----- (*H. ericoides* Ethyl Acetate).

Among various fractions of *H. x moserianum* subjected to anti-glycation assay, only ethyl acetate and aqueous fractions showed mild anti-glycation activity with 52.096 and 46.487% for *H. ericoides*; The crude methanol extract and *n*-butanol fractions exhibited 45.298 and 48.765%, respectively as compared to the rutin with 82.50% activity at the same concentration as shown in (table 2).

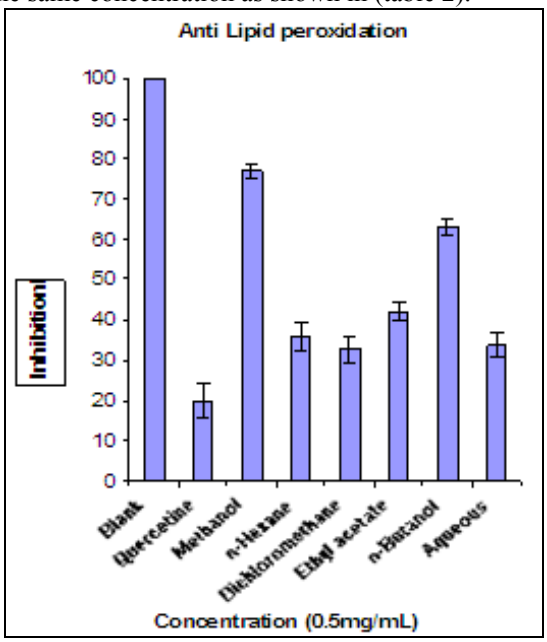

Fig. 2: Percentage anti-lipid peroxidation activity of various fractions of *H. x moserianum*

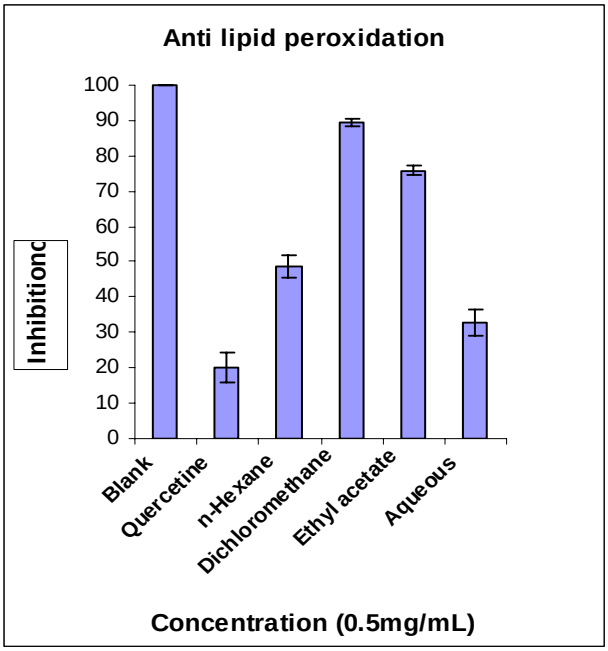

Fig. 3: Percent anti lipid peroxidation activity of various fractions of *H. ericoides*

TBARS assay was performed to investigate anti lipid per oxidation potential of these fractions. Among various fractions of *H. x moserianum*, the dichloromethane,

aqueous, *n*-hexane, and ethyl acetate fractions showed moderate activity with 67.241, 66.147, 64.213 and 58.158% inhibition respectively at 500µg/ml as shown (fig. 2). For *H. ericoides,* the aqueous and *n*-hexane fractions showed 67.404 and 51.196% anti lipid peroxidation activity at same concentration (table 3). Quercetin (500µg/ml) was used as a standard inhibitor in anti lipid peroxidation assay with 85.025% inhibition.

All fractions from both the species were also subjected to cytotoxicity test on mouse fibroblast 3T3 cells. The tested samples of both the species were non-toxic with cytotoxicity values more than 30µg/ml. Cycloheximide with IC_{50} value 0.073±0.1µg/ml was used as a standard in this assay.

DISCUSSION

A large number of traditionally used plants have been studied previously to explore their potential bioactivities against different diseases. Discovery of plant fractions and their active components with combined antioxidant and anti-glycation properties could be beneficial in the treatment of various disorders with low toxicity (Jia *et al*. 2003). Despite the availability of the current therapies to prevent glycation, and oxygen stress related diseases they are still a threat to human health. In this situation, the search for new and more effective anti-glycation and anti-lipid peroxidation agents of natural origin is rather timely and appropriate.

In this study, the anti-glycation, antioxidant, anti lipid peroxidation and cytotoxicity activities of the crude extracts and solvent soluble fractions of *H. x moserianum* and *H. ericoides* were investigated.

Different fractions of both species have various classes of compounds which included phenolic contents particularly flavonoids, xanthonoids and terpenes, which have different biological activities. Since, mechanism of advanced glycation end product (AGEs) formation involved Schiff bases, formation of Amadori products after rearragements. These Amadori products give 1, 2-dicarbonyl compounds, such as glyoxal, after transition-transition-metal-ion-catalyzed glycoxidations and finally cross linking protein AGEs formation. Anti-glycation agents have different mechanism of inhibition. Therefore different anti-glycation agents may be inhibited at any step of the cascade. Some samples with antioxidant activity also exhibited anti-glycation activity via radical scavenging mechanism.

CONCLUSION

In conclusion, the results from this study suggest that the bioactive extracts/fractions of *H. x moserianum* and *H. ericoides* have compounds that are responsible for anti-oxidative, anti-lipid peroxidation and anti-glycation activities. These results may support the use of these bioactive extracts/fractions of the titled plants in folk medicine to prevent the protein glycation, and oxygen stress related diseases. However, a further *in vivo* study would help in exploring other pharmacological properties of these plants. Further studies are required to isolate and characterize the active components of the extracts and also to discover their mechanisms of action in preventing various biological disorders.

ACKNOWLEDGEMENT

The authors are thankful to Higher Education Commission (HEC) Pakistan, for providing funds under HEC-IPFP Program, and HEC-BC Link Project and to University of Portsmouth for providing green house facilities for this research work.

REFERENCES

Alainml, Justin K, Fernande NN, Jean GT, David L, Ajaz A, Choudhary MI., Beiban LS and Atta -ur-Rahman (2006). Antioxidant benzophenones and xanthones from the root bark of *Garcinia smeathmannii*. *Bull. Chem. Soc. Eth.,* **20**(2): 247-252**.**

Barnes J, Anderson L and Phillipson J (2001). A review of its chemistry, pharmacology and clinical properties. *J. Pharm. Pharmacol.,* **53**(5) 583-600.

Benkikia N, Kaboucheb Z, Tillequinc F, Vérité P, Chossondand E and Seguind E (2003). A New polyisoprenylated phloroglucinol derivative from *Hypericum perfoliatum* (Clusiaceae). *Z. Naturforsch.,* **58**: 655-658.

Buege JA and Aust SD (1978), Microsomal lipid peroxidation. *Methods Enzymol.,* **52**: 302-310.

Cakir A, Mavi A, Yildirim A, Duru ME, Harmandar M and Kazaz C (2003), Isolation and characterization of antioxidant phenolic compounds from the aerial parts of *Hypericum hyssopifolium* L. by activity-guided fractionation. *J. Ethnopharmacol.,* **87**: 73-83.

Cardonaml and Seoan E (1982a), Flavonoids and xanthonolignoids of Hypericum ericoides. *Phytochemistry,* **21**: 2759-2760.

Cardonaml and Seoan E (1982b), Xanthone constituents of *Hypericum ericoides*. *J. Nat. Prod.,* **45**: 134-136.

Cardonaml and Seoan E (1983). Flavonoids and xanthones of *Hypericum ericoides* L. Anales de Quimica Serie C: *Quimica Organica y Bioquimica.,* **79**: 144-148.

Ceriello A, Giugliano D, Quatraro A, Donzella C, Dipalo G and Lefebvre PJ (1991). Vitamin E reduction of protein glycosylation in diabetes. New prospect for prevention of diabetic complications. *Diabetes Care,* **14**: 68-72.

Cesquini M, Torsoni MA, Stoppa GR and Ogo SH (2003). t-BuOH-induced oxidative damage in sickle

red blood cells and the role of flavonoids. *Biomed. Pharmacother.,* **57**: 124-129.

Choudhary MI, Abbas G, Ali S, Shuja S, Khalid N, Khan KM, Atta-ur-Rahman and Basha FZ (2011). Substituted benzenediol Schiff bases as new promising antiglycation agents. *J. Enzyme Inhib. Med. Chem.,* **26**(1): 98-103.

Conforti F, Statti GA, Tundis R, Menichini F and Houghton P (2002). Antioxidant activity of methanolic extract of *Hypericum triquetrifolium* Turra aerial part. *Fitoterapia,* **73**: 479-483.

Duraisamy Y, Gaffney J, Slevin M, Smith CA, Williamson K and Ahmed N (2003). Aminosalicylic acid reduces the anti-proliferative effect of hyperglycaemia, advanced glycation endproducts and glycated basic fibroblast growth factor in cultured bovine aortic endothelial cells: Comparison with aminoguanidine. *Mol. Cell. Biochem.,* **246**: 143-153.

Halliwell B and Gutteridge JM (1999). Free radicals in biology and medicine, 3rd ed., Oxford, pp.258-265.

Heinecke JW (2003). Oxidative stress: New approaches to diagnosis and prognosis in atherosclerosis. *Am. J. Cardiol.,* **91**: 12-16.

Jakug V, Eiarova MH, Sky J, Krahulec B and Rietbrock N (1999). Inhibition of non-enzymatic protein glycation and lipid peroxidation by drugs with antioxidant activity. *Life Science,* **65**:1991-1993.

Jia W, Gao W and Tang L (2003). Anti-diabetic herbal drugs officially approved in China. *Phytother. Res.,* **17**: 1127-134.

Kaur C and Kapoor HC (2002). Antioxidant activity and total phenolic content of some Asian vegetables. *Int. J. Food Sci. Tech.,* **37**: 153-162.

Khalifa RG, Baynes JW and Hudson BG (1999), Amadorins: Novel post-Amadori inhibitors of advanced glycation reactions. *Biochem. Biophys. Res., Commun.,* **257**: 251-258.

Lee SK, Mbwambo ZH, Chung H, Luyengi L, Gamez EJ, Mehta RG, Kinghorn D and Pezzuto JM (1998), Evaluation of the antioxidant potential of natural products. *Comb. Chem. High Throughput Screen,* **1**: 35-46.

Matsuda H, Wang T, Managi H and Yoshikawa M (2003). Structural requirements of flavonoids for inhibition of protein glycation and radical scavenging activities. *Bioorg Med Chem.,* **11**: 5317-5323.

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

Motlhanka DM, Makhabu SW (2011). Medicinal and edible wild fruit plants of Botswana as emerging new crop opportunities, *J. Med. Plants Res.,* **10**, 1836-1842.

Radulovic N, Stankov-Jovanovic V, Stojanović G, Smelcerović A, Spiteller M and Asakawa Y (2007), Screening of *in vitro* anti-microbial and antioxidant activity of nine Hypericum species from the Balkans. *Food Chem.,* **103**: 15-21.

Rahbar S, Natarajan R, Yerneni K, Scott S, Gonzales N and Nadler JL (2000). Evidence that pioglitazone, metformin and pentoxifylline are inhibitors of glycation. *Clin Chim. Acta,* **301**: 65-77.

Saddiqe Z, Naeem I and Maimoona A (2010). A review of anti-bacterial activity of *Hypericum perforatum* L. *J. Ethnopharmacol.,* **131**: 511-521.

Saddiqe Z, Naeem I, Maimoona A, Patel AV and Hellio C (2011). Assay of flavonoid aglycones with HPLC in four species of genus Hypericum. *J. Med. Plants Res.,* **5**(9): 1526-1530.

Soon YT, Matthew W, Zhao FP, Andrew J, Eu LY and Barry H (2004). Characterization of antioxidant and antiglycation properties and isolation of active ingredients from traditional Chinese medicines. *Free Radic. Biol. Med.,* **36**: 1575-1587.

Valentão P, Fernandes E, Carvalho F, Andrade PB, Seabra RM and Lourdes MB (2002), Antioxidant activity of Hypericum and rosaemum infusion: Scavenging activity against superoxide radical, hydroxyl radical and hypochlorous acid. *Biol. Pharm. Bull.,* **25**: 1320-1323.

Zou Y, Lu Y and Wei D (2004). Antioxidant activity of flavonoid--rich extract of *Hypericum perforatum* L. *in vitro. J. Agric. Food Chem.,* **52**: 5032-5039.