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Full Length Research Paper

Golden rain tree leaf extracts as potential inhibitor of lipid peroxidation and 4-nitroquinoline-1-oxide (4-NQO)-induced DNA damage

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This study was designed to evaluate the peroxyl radical scavenging capacity and deoxyribonucleic acid (DNA) protective effect of extract/fractions of *Koelreuteria paniculata* Laxm. (Golden rain tree) in lipid peroxidation assay and calf thymus DNA protection assay. The leaves of the plant were extracted with different solvents in the order of increasing polarity to obtain methanol extract (KME), chloroform fraction (KCF), ethyl acetate fraction (KEF), n-butanol fraction (KBF) and aqueous fraction (KAF). All the extract/fractions possessed the potential to inhibit lipid peroxidation and 4-nitroquinoline-1-oxide (4NQO)-induced genotoxicity. KEF showed the highest peroxyl radical scavenging activity (80.8 %) with IC_{50} of 36.44 µg/ml while KAF showed the lowest activity (43.20 %). In DNA protection studies, all the extract/fractions showed potential to protect calf thymus DNA against 4-NQO induced DNA damage except KCF which showed slight protection at highest concentration tested (250 µg/ml).

Key words: *Koelreuteria paniculata* Laxm., lipid peroxidation, calf thymus DNA, 4-nitroquinoline-1-oxide.

INTRODUCTION

Various metabolic processes in the human body are responsible for the production of numerous reactive oxygen species (ROS) as byproducts. *In vivo*, some of these ROS play vital role in cell physiology but on the other side, they may also induce oxidation that causes membrane lipid peroxidation, decreased membrane fluidity and deoxyribonucleic acid (DNA) mutations leading to cancer, degenerative diseases etc. (Halliwell, 1994; Niki, 1997; Poulson et al., 1998). Lipids comprise important part of our food and other biological systems in the form of storage lipids. The presence of unsaturation in lipids makes them vulnerable to the attack of oxygen. During oxidative stress, reactive oxygen species possess ability to react with double bonds of polyunsaturated fatty acids. This oxidative degradation of lipids produces lipid hydroperoxides which result in numerous complex changes that ultimately lead to the development of food rancidity and off-flavors. The products so formed during these processes may be harmful to the cell (Halliwell and Chirico 1993). The loss of integrity of lipid bilayers due to peroxidation is one of the most significant effects of oxidative damage (Tappel, 1975). Lipid peroxidation is one of the chief factors that cause degradation during the food storage and processing. Various synthetic antioxidants are known such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and propylgallate (PG) but their use in food stuffs is being under strict regulation. Consumers have also become more vigilant regarding safety and quality of available food additives (Namiki, 1990). So, food enriched with antioxidants, plays a vital role in the prevention of various cardiovascular diseases and cancers (Gerber et al., 2002).

On the other hand, DNA damage caused by environmental mutagens may be responsible for the disability in different organisms, including humans. The accumulation

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Abbreviations: **KME**, Methanol extract; **KCF**, chloroform fraction; **KEF**, ethyl acetate fraction; **KBF**, n-butanol fraction; **KAF**, aqueous fraction; **ROS**, reactive oxygen species; **BHA**, butylated hydroxyanisole; **BHT**, butylated hydroxytoluene; **PG**, propylgallate; **TBARS**, thiobarbituric acid reactive species; **TBA**, 2-thiobarbituric acid; **SDS**, sodium dodecyl sulfate; **LPO**, lipid peroxidation; **MDA**, malondialdehyde; **DNA**, deoxyribonucleic acid.

of mutations is linked with the development of numerous diseases such as cancer, degenerative disorders and genetic defects in new born babies (Cuzzocrea et al., 2001; Migliore and Coppedè, 2002). To check this genotoxic risk, it is necessary to identify the involved environmental mutagens in order to minimize human exposure to them and to enhance the exposure to antimutagenic agents, such as naturally occurring secondary metabolites (Edenharder et al., 2002; Ikuma et al., 2006; Jeong et al., 2006).

Recently, much attention has been paid to finding plant-derived secondary metabolites which provide health benefits. Actually, numerous antioxidant/antimutagenic compounds from natural resources have been demonstrated to be useful for the prevention or attenuation of disease development or progression (Zhang et al., 2006; Ruffa et al., 2002).

Golden rain tree (*Koelreuteria paniculata* Laxm.) belongs to family Sapindaceae, native to China and mostly used for landscape purposes because of its beautiful yellow flowers and reddish papery lantern like fruits. The seed coats of the seeds of this plant are hard, water-impermeable and embryo dormancy has made natural regeneration of this tree more or less impossible. For this reason, in Korea, it is regarded as an endangered plant species.

MATERIALS AND METHODS

Chemicals

Ethidium bromide and 2-thiobarbituric acid (TBA) were procured from Sigma–Aldrich, USA. Calf thymus DNA was obtained from Merck, India. All other chemicals were of analytical grade. All the stock solutions were prepared in double distilled water.

Collection of plant material

The leaves of the plant were collected in the month of November from Botanical garden of Guru Nanak Dev University, Amritsar, Punjab (India). The specimen was identified by the Herbarium of Department of Botanical and Environmental Sciences and Voucher specimen No. 0409/HRB was deposited in herbarium of the same Department.

Extraction and isolation

The leaves were washed with running tap water and dried in shade. The dried leaves were powdered and extracted three times with 80% methanol (3200 ml) and the extract was concentrated using rotary evaporator. The methanol mother extract (35 g) was made aqueous with distilled water and extracted with hexane (900 ml) to separate lipophilic compounds and the remaining extract was fractionated with solvents in the order of increasing polarity viz. chloroform (900 ml), ethyl acetate (900 ml), n-butanol (900 ml) to obtain KCF (chloroform fraction; percentage yield 14.62 %), KEF (ethyl acetate fraction; percentage yield 13.02 %), KBF (n-butanol fraction; percentage yield 29.22 %) and KAF (aqueous fraction; percentage yield 24.14 %). Finally all the fractions were concentrated using rotary vacuum evaporator (Buchi Rotavapor R-210).

Lipid peroxidation assay

A modified thiobarbituric acid reactive species (TBARS) assay (Ohkowa et al., 1979) was used to determine the lipid peroxide produced using egg yolk homogenate as lipid rich media (Ruberto et al., 2000). Different concentrations of test samples (100 to 1000 µg/ml) were added to egg homogenate (0.5 ml of 10% v/v) followed by 50 µl of $FeSO_4$ to induce lipid peroxidation. The mixture was incubated for 30 min followed by addition of 1.5 ml of 20 % acetic acid (pH 3.5) and 1.5 ml of 0.8% of TBA in 1.1 % sodium dodecyl sulfate (SDS) and 50 µl of trichloroacetic acid (20%). The mixture was vortexed and heated at 95°C for 1 h. After cooling, 5 ml of butan-1-ol was added and centrifuged at 1036 × *g* for 10 min. The absorbance was taken at 532 nm (Systronics 2202 UV–Vis Spectrophotometer). Inhibition of lipid peroxidation (%) was calculated as:

$$[(1 - E/C) \times 100]$$

Where, C is the absorbance of fully oxidized control and E is the absorbance in the presence of extract.

DNA protection studies

DNA protection assay was performed using Calf thymus DNA (Lee et al. 2002) with slight modifications. Calf thymus DNA was incubated with 4 NQO (20 µg/ml) and different concentration of extract/fractions (100, 150, 200 & 250 µg/ml) and the final volume of the mixture was raised up to 20 µl. The mixture was then incubated for 30 min at 37°C followed by the addition of loading dye and electrophoresis was carried out in TAE buffer at 50 V for 3 h. DNA was stained by ethidium bromide. Finally, gel was analyzed using Gel Doc XR system (Bio-Rad, USA).

Statistical analysis

All the results were depicted as mean values of triplicate analyses. The data were recorded as mean ± standard error. Regression analysis was carried out by best fit method to determine IC_{50}.

RESULTS AND DISCUSSION

Lipids present in egg yolk undergo quick non-enzymatic peroxidation when incubated with ferrous sulphate. Lipid peroxides are known to be involved in number of pathological events, including inflammation, metabolic disorders and cellular aging (Ames et al., 1993; Wiseman and Halliwell, 1996). Reactive oxygen species along with the metals Fe^{3+}/Fe^{2+} acts as mediators for the induction of lipid peroxidation (LPO), resulting in oxidation of polyunsaturated fatty acids (Cheeseman and Slater, 1993; Esterbauer et al., 1992). Malondialdehyde (MDA) is a secondary product produced as a result of peroxidation of polyunsaturated fatty acids, and reacts with two molecules of thiobarbituric acid which gives pinkish chromogen with an absorbance maximum at 532 nm (Janero, 1990). LPO occurs mainly in biological membranes, where unsaturated fatty acids are comparatively high in amount resulting in the destruction of cell membranes. Various polyphenolics have been shown

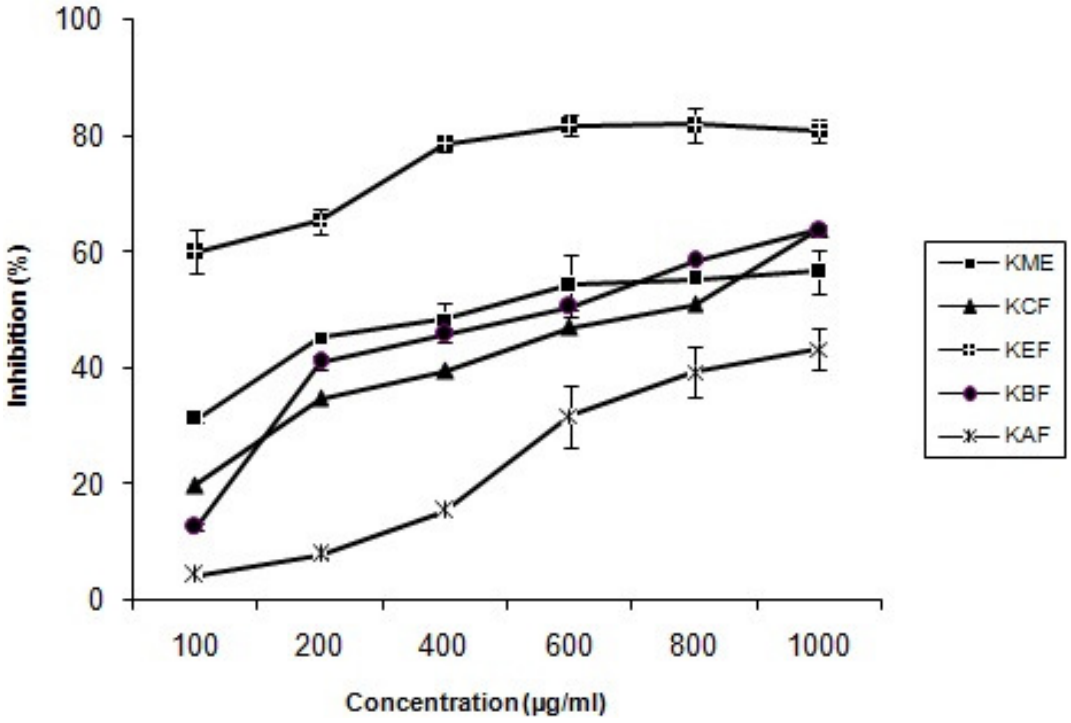

Figure 1. Reduction of TBARS formation by different extracts/fractions of Golden rain tree (*K. paniculata* Laxm.).

to suppress oxidation of lipids and delay the progress of heart diseases (Steer et al., 2002). The hydrogen donating capacity of the polyphenols is responsible for the inhibition of free radical induced LPO (Yen and Duh, 1993). In the lipid peroxidation assay, different extract/ fractions isolated from golden rain tree demonstrated good potential to inhibit lipid peroxides. Among the different fractions, KEF showed the highest potential to inhibit peroxyl radicals with the inhibition of 80.8% (IC_{50} 36.44 µg/ml) and lowest inhibition was showed by KAF (IC_{50} not determined). The order of inhibition was KEF (80.8%) (IC_{50} 36.44 µg/ml) > KCF (63.76%) (IC_{50} 625.38 µg/ml) > KBF (63.64%) (IC_{50} 505.24 µg/ml) > KME (56.49%) (IC_{50} 463.85 µg/ml) > KAF (43.12%) (IC_{50} ND) (Figure 1). KEF was found to be most effective among all extract/fractions. A number of polyphenolic compounds from leaves of *K. paniculata* have been reported including 3''-O-galloyl-4'-O-galloyl-4-O-galloyl-gallic acid, ethyl p-heptagallate, 3''-galloylquercitrin, catechin, galloyle-picatechin, isorhamnetin, kaempferol-3-O-arabinopyran-oside, quercitrin, methyl p-digallate, methyl m-digallate, p-digalloyl acid, m-digalloyl acid, hyperin, kaempferol-3-O-alpha-L-rhamnoside, 6,8-dihydroxy-afzelin, afzelin 3''-O-gallate and quercetin-3'-O-beta-D-arabinopyranoside (Mahmoud et al., 2001; Lin et al., 2002). Earlier, we had reported that chloroform, ethyl acetate, n-butanol and aqueous fractions of *K. paniculata* are rich in phenolic and flavonoid compounds and have shown free radical scavenging activity in different in vitro assays viz DPPH assay, Reducing Power assay, Superoxide radical scavenging assay, ABTS cation radical scavenging assay (Kumar et al., 2011a, b). Mbaebie et al. (2011) evaluated the phytochemical constituents and antioxidant activities of aqueous extract of *Schotia latifolia* bark and reported that there is positive correlation between polyphenol content and free radical scavenging potential. The polyphenols present in the extract/fractions obtained from leaves of golden rain tree may be responsible for the inhibition of lipid peroxidation.

Environmental mutagens/carcinogens are involved in initiation, promotion and progression of various kinds of cancer. Human exposure to these xenobiotics is often inescapable and poses a great risk to human health. The supplementation of diet with chemopreventive agents renders the host organism more resistant to the attack of mutagens and carcinogens. Abundant plant products have been well-known and when included in human diet may affect chronic disease risk (De Flora and Ferguson, 2005). 4-NQO is a known genotoxin responsible for generation of a substantial degree of intracellular oxidative stress. It is believed that 4-NQO undergoes cycling reactions to produce genotoxic ROS resulting in modified bases and DNA strand breaks (Nunoshiba and Demple, 1993). In our studies, all the extract/fraction showed more or less DNA protective activity at the highest concentration tested (250 µg/ml) against the DNA damage induced by 4-NQO except the KCF which showed very less protection only at the highest con-

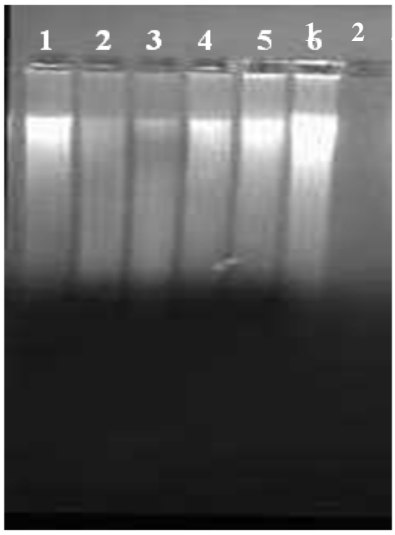

Figure 2. Effect of KME on the protection of calf thymus DNA against 4-NQO induced damage. Lane 1, calf thymus DNA; lane 2: calf thymus DNA + 4-NQO (DNA damage); lane 3: calf thymus DNA + 4-NQO + KME (100 µg/ml); lane 4, calf thymus DNA + 4-NQO + KME (150 µg/ml); lane 5, calf thymus DNA + 4-NQO + KME (200 µg/ml); lane 6, calf thymus DNA + 4-NQO + KME (250 µg/ml).

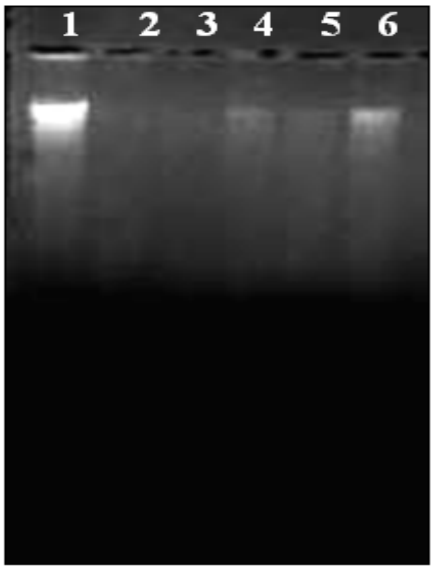

Figure 3. Effect of KCF on the protection of calf thymus DNA against 4NQO induced damage. Lane 1: calf thymus DNA; lane 2, calf thymus DNA + 4-NQO (DNA damage); lane 3, calf thymus DNA + 4-NQO + KCF (100 µg/ml); lane 4, calf thymus DNA + 4-NQO + KCF (150 µg/ml); lane 5, calf thymus DNA + 4-NQO + KCF (200 µg/ml); lane 6, calf thymus DNA + 4-NQO + KCF (250 µg/ml).

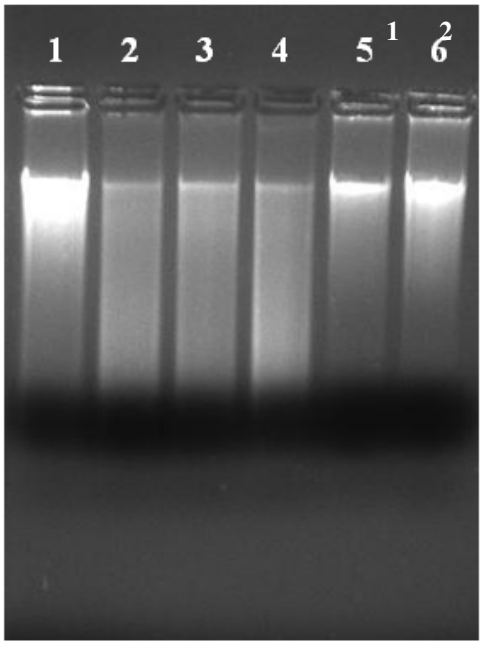

Figure 4. Effect of KEF on the protection of calf thymus DNA against 4NQO induced damage. Lane 1, calf thymus DNA; lane 2, calf thymus DNA + 4-NQO (DNA damage); lane 3, calf thymus DNA + 4-NQO + KEF (100 µg/ml); lane 4, calf thymus DNA + 4-NQO + KEF (150 µg/ml); lane 5, calf thymus DNA + 4-NQO + KEF (200 µg/ml); lane 6, calf thymus DNA + 4-NQO + KEF (250 µg/ml).

centration (Figures 2, 3, 4, 5 and 6). Phenolics reported by earlier workers (Mahmoud et al., 2001; Lin et al., 2002) may in part contribute to DNA protective activity. Further, we already evaluated these extract/fractions for DNA protective activity against hydroxyl radical mediated DNA damage (Kumar et al., 2011a,b). So this radical scavenging activity of extract/fractions may in part be responsible for DNA protective activity against 4-NQO-induced DNA damage.

Conclusions

The results demonstrate that *K. paniculata* leaf extracts have phytoconstituents which efficiently prevent lipid peroxidation and DNA damage and can be exploited for their potential to prevent food rancidity and provide protection from environmental mutagens.

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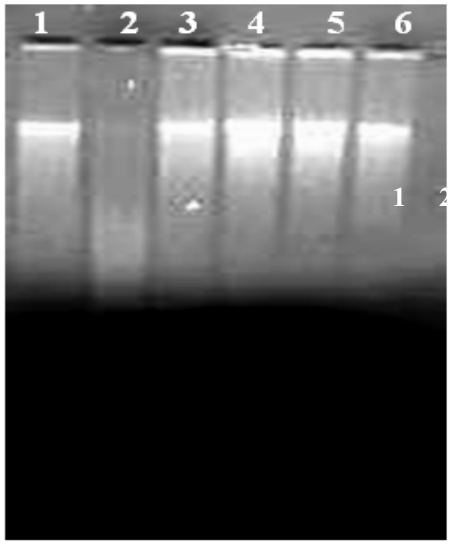

Figure 5. Effect of KBF on the protection of calf thymus DNA against 4NQO induced damage. Lane 1, calf thymus DNA; lane 2, calf thymus DNA + 4-NQO (DNA damage); lane 3, calf thymus DNA + 4-NQO + KBF (100 µg/ml); lane 4, calf thymus DNA + 4-NQO + KBF (150 µg/ml); lane 5, calf thymus DNA + 4-NQO + KBF (200 µg/ml); lane 6, calf thymus DNA + 4-NQO + KBF (250 µg/ml).

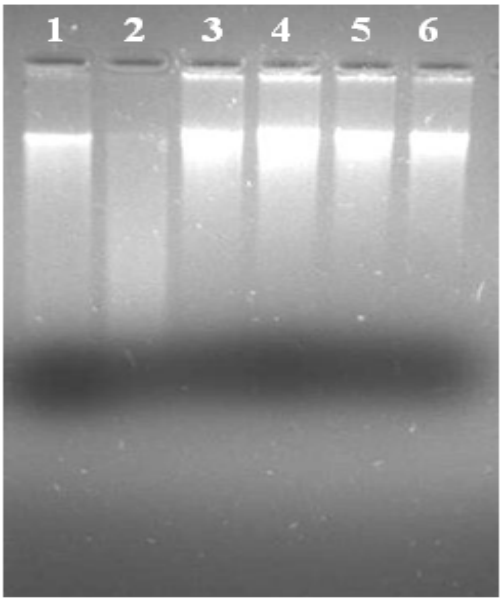

Figure 6. Effect of KAF on the protection of calf thymus DNA against 4NQO induced damage. Lane 1, calf thymus DNA; lane 2, calf thymus DNA + 4-NQO (DNA damage); lane 3, calf thymus DNA + 4-NQO + KAF (100 µg/ml); lane 4, calf thymus DNA + 4-NQO + KAF (150 µg/ml); lane 5, calf thymus DNA + 4-NQO + KAF (200 µg/ml); lane 6, calf thymus DNA + 4-NQO + KAF (250 µg/ml).

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REFERENCES

Ames SN, Shigrenaga MK, Hagen TM (1993). Oxidants, antioxidants and degenerative diseases of aging. Proc. Natl. Acad. Sci. USA., 90: 7915-7922.

Cheeseman KH, Slater TF (1993). An introduction to free radical biochemistry. Br. Med. Bull. 49: 481-493

Cuzzocrea S, Riley PD, Caputi PA, Salvemini D (2001). Antioxidant therapy: a new pharmacological approach in shock, inflammation, and ischemia/reperfusion injury. Pharmacol. Rev. 53: 135-159.

De Flora S, Ferguson LR (2005). Overview of mechanisms of cancer chemopreventive agents. Mutat. Res. 591: 8-15.

Edenharder R, Sager WJ, Glatt H, Muckel E, Platt LK (2002). Protection by beverages, fruits, vegetables, herbs, and Xavonoids against genotoxicity of 2-acetylaminoXuorene and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhPI) in metabolically competent V79 cells. Mutat. Res. 521: 57-72.

Esterbauer H, Waeg G, Puhl H, Dieber-Rotheneder M, Tatzber F (1992). Inhibition of LDL oxidation by antioxidants. EXS. 62: 145-157

Gerber M, Boutron-Ruault MC, Hereberg S, Riboli E, Scalbert A, Siess MH (2002). Food and Cancer: State of the art about the protective effect of fruits and vegetables. B. Cancer, 89: 293-312.

Halliwell B, Chirico S (1993). Lipid peroxidation: its mechanism, measurement, and significance. Am. J. Clin. Nutr. 57: 715-724.

Halliwell B (1994). Free radicals, antioxidants, and human disease: curiosity, cause, or consequence? Lancet, 344: 721-724.

Ikuma NEM, Passoni HM, Biso IF, Longo CM, Cardoso PRC, Campaner SL, Varanda AE (2006). Investigation of genotoxic and antigenotoxic activities of *Melampodium divaricatum* in *Salmonella typhimurium*. Toxicol. Vitro. 20: 361-366.

Janero DR (1990). Malondialdehyde and thiobarbituric acid reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. Free Radic. Biol. Med. 9: 515-540.

Jeong TJ, Moon JH, Park KH, Shin SCh (2006). Isolation and characterization of a new compound from *Prunus mume* fruit that inhibits cancer cells. J. Agr. Food. Chem. 54: 2123-2128.

Kumar M, Chandel M, Kumar S, Kaur SJ (2011a). Protective effects of *Koelreuteria paniculata* Laxm. on oxidative stress and hydrogen peroxide-induced DNA damage. Phytopharmacol. 2011, 1(5) 177-189

Kumar M, Kumar S, Kaur SJ (2011b). Investigations on DNA protective and antioxidant potential of chloroform and ethyl acetate fractions of *Koelreuteria paniculata* Laxm. Afr J Pharm Pharmacol 5(3): 421-427.

Lee J, Kim J, Jang Y (2002). Antioxidant property of an ethanol extract of the stem *of Opuntia ficus-indica* var. Saboten. J. Agr. Food. Chem. 50: 6490-6496.

Lin WH, Deng ZW, Lei HM, Fu HZ, Li J (2002). Polyphenolic compounds from the leaves of *Koelreuteria paniculata* Laxm. J. Asian Nat. Prod. Res. 4: 287-295.

Mahmoud I, Moharram FA, Marzouk MS, Soliman HS, El-Dib RA (2001). Two new flavonol glycosides from leaves of *Koelreuteria paniculata.* Pharmazie, 56: 580-582.

Mbaebie BO, Edeoga HO, Afolayan AJ (2011). Phytochemical analysis and antioxidants activities of aqueous stem bark extract of Schotia latifolia Jacq. Asian Pac J Trop Biomed. pp. 1-7

Migliore L, Coppedè F (2002). Genetic and environmental factors in cancer and neurodegenerative diseases. Mutat. Res. 512: 135-153.

Namiki M. (1990). Antioxidants/antimutagens in foods. Crit. Rev. Food Sci. Nutr. 29: 273-300.

Niki E (1997). Free radicals, antioxidants, and cancer. In: Ohigashi H, Osawa T, Terao J, Watanabe S, Yoshikawa T (Eds.), Food Factors for Cancer Prevention. Springer, Tokyo, pp. 55-57.

Nunoshiba T, Demple B (1993). Potent intracellular oxidative stress exerted by the carcinogen 4-nitroquinoline-N-oxide. Cancer Res. 53: 3250-3252.

Ohkowa M, Ohisi N, Yagi K (1979). Assay for lipid peroxides in animal tissue by thiobarbituric acid reaction. Anal. Biochem. 95: 351-358.

Poulson HE, Prieme H, Loft S (1998). Role of oxidative DNA damage in

cancer initiation and promotion. Eur. J. Cancer Prev. 7: 9-16.
Ruberto G, Baratta MT, Deans SG, Dorman HJD (2000). Antioxidant and antimicrobial activity of *Foeniculum vulgare* and *Crithmum maritimum* essential oils. Planta Med. 66: 687-693.
Ruffa MJ, Ferraro G, Wagner ML, Calgano ML, Campos RH, Cavallaro L (2002). Cytotoxic effect of Argentine medicinal plant extracts on human hepatocellular carcinoma cell line. J. Ethnopharmacol. 79: 335-339.
Steer P, Millgard J, Sarabi DM, Basu S, Vessby B, Kahan T (2002). Cardiac and vascular structure and function are related to lipid peroxidation and metabolism. Lipids, 37: 231-236.
Tappel AL (1975) Lipid peroxidation and fluorescent molecular damage to membranes. In: *Pathobiology of Cell Membranes,* edited by BF Trump and AU Arstila. New York: Academic, pp 145-170.
Wiseman H, Halliwell B (1996). Damage to DNA by reactive oxygen and nitrogen species: Role of inflammatory disease and progression to cancer. Biochem. J. 313: 17-29.
Yen CC, Duh PD (1993). The relationship between antioxidant activity and maturity of peanut hulls. J. Agric. Food Chem. 41: 67-70.
Zhang HY, Yang DP, Tang GY (2006). Multipotent antioxidants: from screening to design. Drug Discov. Today, 11: 749-754.