International Journal of ChemTech Research
CODEN (USA): IJCRGG ISSN: 0974-4290
Vol.8, No.12 pp 552-558, 2015

In Vitro Antioxidant Activity of Biphenyl-2,6-diethanone Derivatives

Megha Rikhi[1], Dinesh Kumar Bharadwaj[2]*, Seema Bhatnagar[1]*

[1]Novel molecule synthesis laboratory, Amity Institute of Biotechnology, Amity University, Noida-201303, Uttar Pradesh, India
[2] National Institute of Nutrition (ICMR), Jamai-Osmania, PO, Hyderabad 500 007, India

Abstract: Excessive accumulation of free radicals results in cellular oxidative damage which has been reported to initiate the progression of several diseases such as cancer, alzheimer's disease and parkinson's disease. Antioxidants are free radical scavengers that play an important role in preventing oxidative cell damage and repairing the damage caused by free radicals. Biphenyls have been reported as a promising free radical scavenging scaffold. The objective of the present study was to evaluate the antioxidant activity of biphenyl-2,6-diethanone derivatives. A series of biphenyl derivatives were synthesized by the reported procedures. The antioxidant activity of these derivatives was evaluated using DPPH and lipid peroxidation assay. The *in vitro* antioxidant studies indicated that substituted biphenyl-2,6-diethanones, **1(a-i)** exhibited significant free radical scavenging activity. Compounds **1e** exhibited maximum antioxidant potential with an IC_{50} value of 54.96µg/ml. The present investigation indicated that derivatives containing hydroxyl, amine and methoxy groups on the biphenyl-2,6-diethanone scaffold exhibited significant antioxidant activity.
Keywords: Antioxidant, Biphenyl-2,6-diethanone, DPPH, Lipid peroxidation.

Introduction

Free radicals play a vital role in biological processes of energy production, phagocytosis and signal transduction[1]. However, excessive generation of free radicals is a major problem in metabolic processes since these molecules lead to direct damage to essential biological targets, such as lipids, proteins and DNA[2,3] and is thus a major cause of oxidative stress[4,5,6]. There is increasing evidence to show that active oxygen species may also play a causative role in the development of various diseases such as atherosclerosis, ischemia reperfusion injury, inflammation, carcinogenesis, cataracts, brain dysfunction, immune-system decline, cardiovascular disease, and rheumatoid arthritis[7,8]. Radicals derived from oxygen, Superoxide (O_2^{-2}), hydrogen peroxide (H_2O_2) and hydroxyl (OH^-), represent the most important class of radical species generated in living systems[9]. The hydroxyl radical is known to react with all components of the DNA molecule, damaging both the purine and pyrimidine bases and also the deoxyribose backbone[10]. ROS are also reported to cause rearrangement with the final product of peroxidation process, malondialdehyde (MDA) which is genotoxic[11,12,13,14,15]. The side chains of all amino acid residues of proteins, in particular cysteine and methionine residues of proteins are susceptible to oxidation by the action of ROS[16].

The balance between beneficial and harmful effects of free radicals is detrimental to the health of individuals[17]. Endogenous antioxidant enzymes, catalase, superoxide dismutase and glutathione peroxidase defend against oxidative damage caused by reactive oxygen and related radicals. It has been reported that activation of various transcription factors (nuclear factor/NF-κB, activator proteion/AP-1, hypoxia-inducible factor-1α/HIF-1α, nuclear factor of activated T-cells and NF-E2 related factor-2/Nrf2) by oxidative stress[18] can

be blocked by antioxidants, including l-cysteine,N-acetyl cysteine (NAC), thiols, green tea polyphenols, and vitamin E[17].

Biphenyls have been reported as a pharmacologically important scaffold exhibiting antioxidant potential for scavenging hydrogen peroxide, nitric oxide and lipid peroxidation inhibitory activity[19,20,21]. The above literature prompted us to investigate antioxidant activity of biphenyl-2,6-diethanone derivatives.

The synthesis of novel derivatives of biphenyl-2,6-diethanones has been previously done by our group (unpublished results). Herein we report the antioxidant studies of biphenyl-2,6-diethanone derivatives using DPPH and lipid peroxidation assay.

Experimental

DPPH free radical scavenging activity assay

The free radical scavenging activity of the synthesized molecules was measured in terms of hydrogen donating or radical scavenging ability using the stable radical 1,1-diphenyl-2-picryl-hydrazyl (DPPH). The DPPH radical scavenging model is extensively used to evaluate antioxidant activities faster than with other methods. Radical scavenging activity of solutions at several concentrations of the synthesized compounds were evaluated against stable DPPH (2,2-diphenyl-2-picrylhydrazyl hydrate) spectrophotometrically according to the method described by Blois, 1958[22]. The absorbance decrease was measured at 517 nm. The DPPH solution (0.6mM in ethanol) was freshly prepared. 10 µl of this solution was mixed with 100 µl of solutions of final concentrations of 100-10 µg/ml in a 96-well plate and the final volume was obtained by the addition of Tris buffer (0.1M). The samples were kept in the dark for 30 minutes at room temperature and then the decay of absorption was followed. Vitamin C (1mg/ml in methanol) is known as a natural antioxidant and used as a positive control in the experiment. All experiments were carried out in triplicate and the results were expressed as mean values ± standard deviations. Radical scavenging activity was expressed as the inhibition percentage of free radical by the test compounds using the following formula:

% inhibition= $(A_0 - A_t)/A_0 \times 100$

Where, A_0 is the absorbance of the control (Methanol+DPPH) and $A_t$is the absorbance in the presence of the test samples (Test sample+DPPH).

Lipid peroxidation assay

The degree of lipid peroxidation was assayed by measuring the thiobarbituric acid reactive species (TBARS) using the reported procedure[23]. TBARS are a conventional index for measuring *in vitro*lipid

peroxidation to determine potential antioxidant activity of compounds. In the present experiment, lipid peroxidation was performed using liver homogenate of rabbit. Different concentrations of test compound (10, 20, 50 and 100 µg/ml) were incubated with tissue homogenate (500 µg). Lipid peroxidation was initiated by the addition of 100 µl of 15mM oxidizing agent [Fe^{2+}-H_2O_2] for 60 minutes at 37^0C. Further, 1 ml of TCA (20%) solution was added followed by the addition of 0.5 ml of a 0.67% TBA solution. The mixture was heated at 95^0C for 10 minutes and centrifuged at 3000 rpm for 5 minutes. The supernatant was collected andthe TBARS were measured spectrophotometrically at 532 nm. The results were expressed as percentage inhibition of TBARS against the concentration of test compounds in µg/ml.

Statistical Analysis

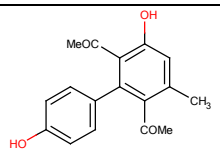
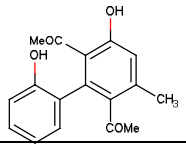
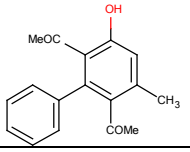
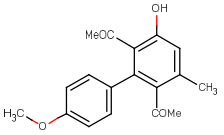
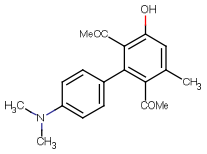
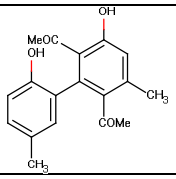
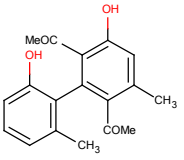
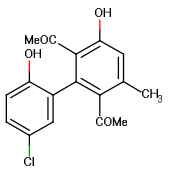
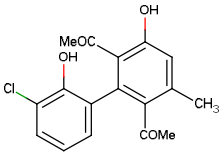
The experimental results were expressed as mean ± standard error of mean of three replicates. Where applicable, the data were subjected to two-way analysis of variance (ANOVA) followed by bonferroni post test. P-value of ≤ 0.05 was regarded as significant. All the statistical calculations were performed using the evaluation version of Graph Pad Prism 5.1 statistical software.

Results

The chemical structures of biphenyl-2,6-diethanone derivatives evaluated for antioxidant activity are depicted in table 1. These derivatives were synthesized using procedures reported previously[24]. *In vitro*

antioxidant activity of compounds **1(a-i)** were evaluated by using different reactive species assay mainly DPPH radical scavenging and lipid peroxidation assay.

Table 1. Substituted Biphenyl-2,6-diethanone derivatives

S.No.	Compound	Structure
1	1a	
2	1b	
3	1c	
4	1d	
5	1e	
6	1f	
7	1g	
8	1h	
9	1i	

The free radical scavenging activity of the synthesized compounds was evaluated through their ability to quench DPPH which was directly measured by the decrease in the absorbance at 517 nm. In the present

study, the DPPH scavenging was exhibited by the test compounds in a dose dependent manner. The percentage of inhibition of free radical is presented graphically in figure 1.

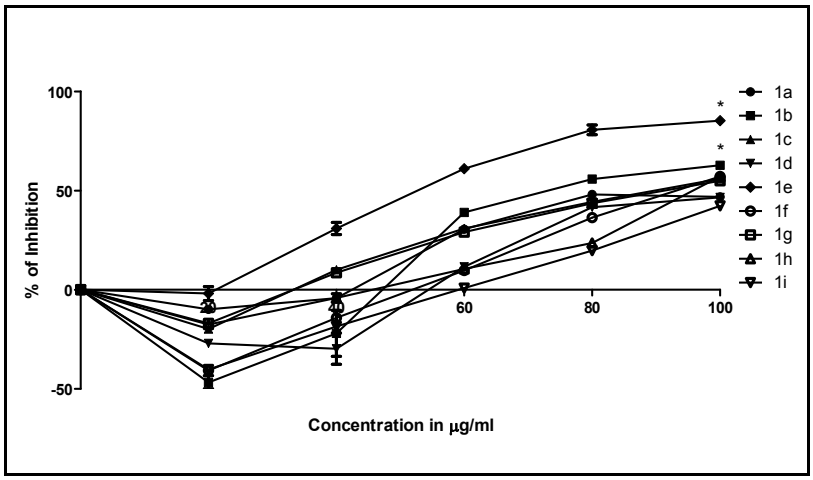


Figure 1. Illustrates percentage inhibition of free radicals by compounds 1(a-i) via DPPH assay. The results are mean±SEM from three samples of each group.

It is well established that the molecules incorporated with electron donating groups are capable of trapping free radicals in the environment. In the series of compounds **1(a-i)**, compound **1e** exhibited maximum percent of inhibition of 85.27% as depicted in figure 1. This can be attributed to the presence of amino alkyl group substituted in the biphenyl ring system, whereas compounds consisting of electron withdrawing group as in the case of compounds **1h** and **1i**, exhibited least antioxidant potential. The quality of the radical scavenging property of the synthesized compounds was determined by calculating the IC_{50} value, a concentration required to scavenge 50% of DPPH radical. The IC_{50} value was calculated using the equation plotted graphically from regression line as represented in figure 2.

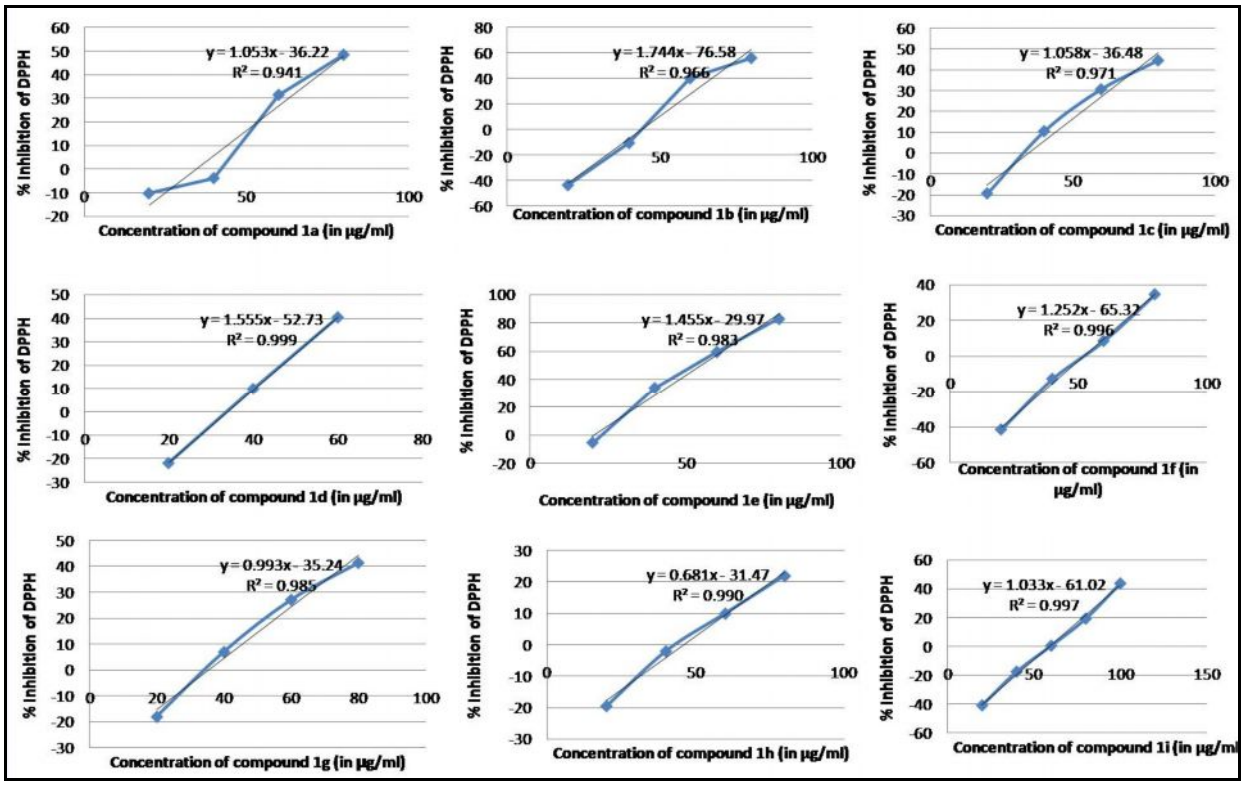


Figure 2. Illustrates percentage inhibition of DPPH radical in presence of Biphenyl-2,6-diethanone derivatives, 1(a-i). The regression line equation was used to calculate the IC_{50} value of respective compounds.

The IC_{50} value for compounds **1(a-i)** are represented in figure 3. It was observed that all compounds exhibited significant antioxidant activity; however, compound **1e** exhibited the most potent antioxidant activity with an IC_{50} value 54.96 µg/ml.

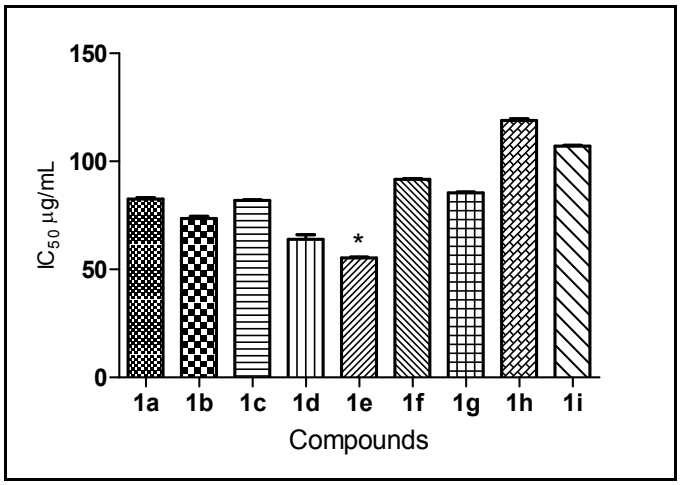


Figure 3. Illustrates IC_{50} value for Biphenyl-2,6-diethanone derivatives, 1(a-i). The results are mean±SEM from three samples of each group.

The DPPH assay measures the radical scavenging property of compounds biochemically whereas lipid peroxidation assay measures the free radical scavenging of compounds in a tissue homogenates. This is important since free radicals are responsible for oxidative damage of lipid membrane via lipid peroxidation. Lipid peroxidation can cause cell injury and can continue as a chain reaction[25,26]. MDA, the genotoxic product of enzymatic and oxygen radical-induced lipid peroxidation[27,28] has been reported as a potentially important contributor towards DNA damage[27]. Therefore, *in vitro* lipid peroxidation assay was also performed to corroborate the results of DPPH assay. Amongst the analogues synthesized, compounds **1a**, **1b**, **1d** and **1e** has exhibited inhibition of TBARS in a concentration dependent manner.The percentage inhibition of TBARS by the synthesized derivatives is depicted in figure 4. Maximum inhibition of TBARS was found in the presence of compound **1e**. The results of lipid peroxidation assay were in agreement with the DPPH assay performed.

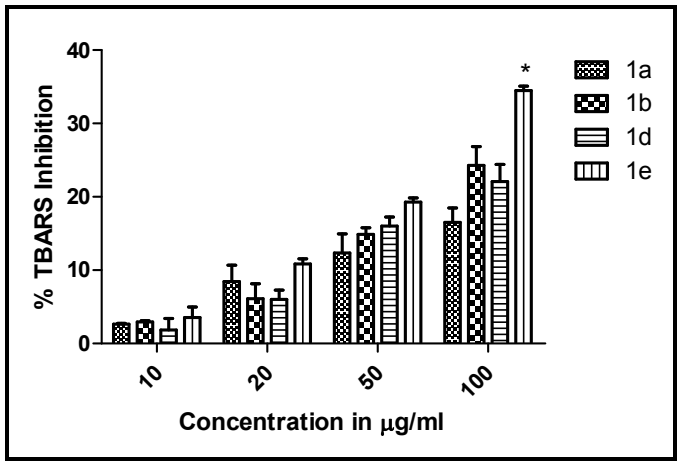


Figure 4. Illustrates percentage inhibition of TBARS by compounds 1a, 1b, 1d and 1e via *in vitro* lipid peroxidation assay. The results are mean±SEM from three samples of each group.

Discussion

Free radicals are unstable chemical entities existing with unpaired electrons which undergoes various chemical reactions in the cellular microenviornment and cause extensive damage[29].

DPPH free radical scavenging method is widely used to determine the antioxidant potential of both natural and synthetic compounds. The method involves the scavenging of DPPH radical by antioxidants via donating protons to form reduced DPPH. From the present study, it may be concluded that the biphenyl-2,6-diethanone and its derivatives have the potency to reduce the DPPH radical.

The peroxidation of lipid membranes initiated by oxygen radical may lead to cell injury[30]. The *in vitro* estimation of lipid peroxidation can be determined using TBARS. The present results depicted significant inhibition of TBARS generated in the process of lipid peroxidation induced experimentally by $FeSO_4$-H_2O_2 mixture in the presence of synthesized biphenyl-2,6-diethanone derivatives.

In our studies it was observed that in all cases of electron donating substituted biphenyl compounds mainly **1e, 1b, 1a** and **1d** exhibited the highest antioxidant activity. On the other hand, the presence of electron withdrawing groups in the biphenyl ring as in the case of **1h** and **1i**, respectively have resulted in least radical scavenging efficiency.

Conclusion

The synthesized compounds exhibited a wide range of potentially promising antioxidant activities. Compounds **1e** exhibited significant scavenging effect against the tested free radicals.

Acknowledgement

The authors gratefully acknowledge Amity University for infrastructure and facilities. Author, MR thanks financial assistance through Amity Science, Technology and Innovation Foundation (ASTIF) fellowship provided by Amity University.

Conflict of interests

Declared None.

References

1. Borek C., Antioxidants and Cancer, Science and Medicine, 1997, 4,51-61.
2. Rama Devi S., and Prasad M.N.V., Copper toxicity in Ceratophyllum demeresum L. (Coontail), a free floating macrophyte: Response of antioxidant enzymes and antioxidants, Plant Sci., 1998, 138,157.
3. Mittova V., Volokita M., Guy M., and Tal M., Activities of SOD and the ascorbate-glutathione cycle enzymes in subcellular compartments in leaves and roots of the cultivated tomato and its wild salt-tolerant relative Lycopersicon pennellii, Physiol. Plant., 2000, 110,45.
4. Kovacic P., and Jacintho J.D., Mechanisms of carcinogenesis: Focus on oxidative stress and electron transfer, Curr. Med. Chem., 2001, 8,773–796.
5. Ridnour L.A., Isenberg J.S., Espey M.G., Thomas D.D., Roberts D.D., and Wink D.A., Nitric oxide regulates angiogenesis through a functional switch involving thrombospondin-1, Proc. Natl. Acad. Sci. U.S.A., 2005, 102,13147–13152.
6. Valko M., Morris H., Mazur M., Rapta P., and Bilton R.F., Oxygen free radical generating mechanisms in the colon: Do the semiquinones of Vitamin K play a role in the aetiology of colon cancer?, Biochem. Biophys. Acta, 2001, 1527,161–166.
7. Ames B.N., Shigenaga M.K., and Hagen T.M., Oxidants, antioxidants, and the degenerative diseases of aging, Proc. Natl. Acad. Sci., 1993, 90,7915-7922.
8. Halliwell B., Free radicals, antioxidants, and human disease: curiosity, cause, or consequence?, Lancet, 1994, 344,721-724.
9. Miller D.M., Buettner G.R., and Aust S.D., Transition metals as catalysts of “autoxidation” reactions., Free Radic. Biol. Med., 1990, 8,95–108.
10. Halliwell B., and Gutteridge J.M.C., Free radicals in biology and medicine(3rd ed.). Oxford University Press, 1999.

11. Fedtke N., Boucheron J.A., Walker V.E., and Swenberg J.A., Vinyl chloride-induced DNA adducts. 2. Formation and persistence of 7-2-oxoethylguanine and 2,3-ethenoguanine in rat-tissue DNA., Carcinogenesis, 1990, 11,1287–1292.
12. Fink S.P., Reddy G.R., and Marnett L.J., Mutagenicity in Escherichia coli of the major DNA adduct derived from the endogenous mutagen malondialdehyde., Proc. Natl. Acad. Sci. U.S.A., 1997, 94,8652–8657.
13. Mao H., Schnetz-Boutaud N.C., Weisenseel J.P., Marnett L.J., and Stone M.P., Duplex DNA catalyzes the chemical rearrangement of a malondialdehyde deoxyguanosine adduct., Proc. Natl. Acad. Sci. U.S.A., 1999, 96,6615–6620.
14. Marnett L.J., Lipid peroxidation—DNA damage by malondialdehyde., Mut. Res-Fund. Mol. Mech Mutagen., 1999, 424,83–95.
15. Wang M.Y., Dhingra K., Hittelman W.N., Liehr J.G., deAndrade M., and Li D.H., Lipid peroxidation-induced putative malondialdehyde–DNA adducts in human breast tissues, Cancer Epidemiol. Biomark Prev., 1996, 5,705–710.
16. Stadtman E.R., Role of oxidant species in aging, Curr. Med.Chem., 2004, 11,1105–1112.
17. Marian V., Dieter L., Jan M., Mark T.D., and Cronin M.M., Free radicals and antioxidants in normal physiological functions and human disease, The International Journal of Biochemistry & Cell Biology, 2007, 39,44–84.
18. Reuter S., Gupta S.C., Chaturvedi M.M., and Aggarwal B.B., Oxidative stress, inflammation, and cancer: How are they linked?, Free Radic. Biol. Med., 2010, 49,1603–1616.
19. Darshan Raja C.G., Sarojini B.K., Sindhu P.E.S., and Khan M.T.H., Effect of new biphenyl chalcone derivatives against gamma radiation induced oxidative stress markers in E .coli K 12 and evaluation of their antimicrobial activities, Der. Pharma. Chemica., 2013, 5,305-317.
20. Nellisara S.D., Ganganaika K., Halehatti B.S., Mayasandra L.R., and Kaginalli J.S., Synthesis of new biphenyl-substituted quinoline derivatives, preliminary screening and docking studies, J. Chem. Sci., 2014, 126,205–212.
21. Maddila S., Damu G.L.V., Oseghe E.O., Abafe O.A., Venakata Rao C., and Lavanya P., Synthesis and Biological Studies of Novel Biphenyl-3,5-dihydro-2Hthiazolopyrimidines Derivatives, Journal of the Korean Chemical Society, 2012, 56,334-340.
22. Blois M.S., Antioxidant determinations by the use of a stable free radical, Nature, 1958, 181,1199–1200.
23. Ohkawa H., Ohishi N., and Yagi K., Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction, Anal. Biochem., 1979, 95,351.
24. Sharma A., Pandey J., and Tripathi R.P., An efficient regioselective synthesis of functionalized biphenyls via sequential reactions of aromatic aldehydes and β-keto esters or ketones, Tetrahedron Letters, 2009, 50,1812-1816.
25. Zieba M., Suwalski M., Kwiatkowska S., Piasecka G., and Grzelewska-Rzymowska I., Comparison of hydrogen peroxide generation and the content of lipid peroxidation products in lung cancer tissue and pulmonary parenchyma, Resp. Med., 2000, 94,800-805.
26. Uzun K., Vural H., Ozturk T., Ozer F., and Imecik I., Diagnostic value of lipid peroxidation in lung cancer, Eastern Journal of Medicine, 2000, 5,48-51.
27. Niedernhofer L.J., Daniels J.S., Rouzer C.A., Greene R.E., and Marnett L.J., Malondialdehyde, a product of lipid peroxidation, is mutagenic in human cells, J. Biol. Chem., 2003, 278,31426-31433.
28. Cighetti G., Allevi P., Anastasia L., Bortone L., and Paroni R., Use of methyl malondialdehydeas an internal standart for malondialdehyde detection: Validation by isotope-dilution gas chromatography-mass spectrometry, Clin. Chem., 2002, 48,2266-2269.
29. Baskar R., Rajeswari V., and Sathish K.T., In vitro antioxidant studies in leaves of Annona species, Indian Journal of Experimental Biology, 2007, 45,480-485.
30. Coyle J.T., and Puttfarcken P., Oxidative stress, glutamate and neurodegenerative diseases, Science, 1993, 291,1184.

*****