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ANTIOXIDANT ACTIVITIES OF METHYL AND CHLORINE SUBSTITUTED 2-(2-ALKYL/ARYLAMINOTHIAZOL-5-OYL)-N-METHYLBENZIMIDAZOLES

S. Sangeetha*[1] and T. F. Abbs Fen Reji[1]

[1*]Department of Chemistry and Research Centre, Nesamony Memorial Christian College, Marthandam, Tamilnadu, India.

ABSTRACT

The term antioxidant is used for two groups of substances, industrial chemicals and natural chemicals found in body tissue and foods which are said to have vital health effects. The antioxidant activities of methyl and chlorine substituted 2-(2-alkyl/arylaminothiazol-5-oyl)-N-methylbenzimidazoles are studied using BHA as standard. The standard solution and the title compound were prepared with different concentration and a graph is plotted and from the graph the percentage reduction and IC_{50} value is calculated.

KEYWORDS

Antioxidant, Benzimidazole, Delocalization, Control absorbance, Inhibition, Concentration, Sample absorbance, DPPH and Free radical.

Author for Correspondence:

Sangeetha S, Reg No. 11806,

Department of Chemistry and Research Centre,

Nesamony Memorial Christian College,

Marthandam, Tamilnadu, India.

Affiliated to Manonmanium Sundaranar University,

Tirunelveli-627 012.

Email: sangeethasss1982@gmail.com

INTRODUCTON

An antioxidant is a molecule which inhibits the oxidant of other molecules. Oxidation is a chemical reaction that can produce free radicals leading to chain reactions that may damage cells[1]. Antioxidant such as thiols terminates these chain reactions by removing free radical intermediates and inhibits other oxidation reactions[2]. The term "antioxidant" is used for two groups of substances one is industrial chemicals which are added to products to prevent oxidation, and other is natural chemicals found in food and body tissues which are said to have beneficial health effects[1,2]. To balance the oxidative state, plants and animals maintain complex systems of overlapping antioxidants, such as glutathione and enzymes produced internally[3,4]. Antioxidant dietary supplements do not improve health nor are they

effective in preventing diseases by randomized clinical trials including supplements of beta-Carotene. Supplementation with selenium does not reduce the risk of cardiovascular disease[5,6]. Oxidative stress can be considered as either a cause or consequence of some disease, an area of research stimulating drug development for antioxidant compounds for use as potential therapies[7].

Relation to diet

Although certain levels of antioxidant vitamins in the diet are required for good health, there is considerable debate on whether a whether antioxidant rich foods have anti-disease activity[8-10]. Many polyphenols may have non - antioxidant roles in minute concentrations that affect cell-to-cell signaling, receptor sensitivity, inflammatory enzyme activity or gene regulation[11-13]. Although dietary antioxidants have been investigated for potential effects on neurodegenerative diseases such as Alzheimer's disease, Parkinsons disease and amyotrophic lateral Sclerosis[14,15] these studies have been inconclusive[16-18.] As of November 2014, other antioxidants are being studied as neuroprotectants[19]. Common properties may interface with the efficacy of certain anticancer meditation and radiation[20,21].

The evolution of angiosperm plants between 50 and 200 million years ago resulted in the development of many antioxidant pigments[22]. Particularly during the Jurassic period as chemical defenses against reactive oxygen species that are byproducts of photosynthesis[23]. Originally the term antioxidant specially referred to a chemical that prevents the consumption of oxygen[24]. In the late 19th and early 20th centuries, extensive study concentrated on the use of antioxidant in important industrial processes, such as the prevention of metal corrosion, the vulcanization of rubber, and the polymerization of fuels in the fouling of internal combustion engines[25-27]. Early research on the role of antioxidants in biology focused on the use in preventing the oxidation of unsaturated fats, which is the cause of rancidity. Antioxidant activity could be measured simply by placing the fat in a closed container with oxygen and measuring the rate of oxygen consumption[27]. However it was the identification of vitamins A, C and E as antioxidants in the biochemistry of living organisms. The possible mechanisms of action of antioxidants were first explored when it was recognized that a substance with anti-oxidative activity is likely to be one that it if self readily oxidized. Research into have vitamins prevents the process of lipid per oxidation led to the identification of antioxidants as reducing agents that prevent oxidative reactions often by scavenging reactive oxygen spices before they can damage cells[28, 29].

Toxicity associated with high doses of water soluble antioxidants such as ascorbic acid are less of a concern, as these compounds can be excreted rapidly in wine. More seriously, very high doses of some antioxidants may have harmful long- term effects[30]. These harmful effects may also be seen in non-smokers, as a recent meta-analysis including data from approximately 230,000 patients showed that β-Carotene, Vitamin A supplementation is associated with increased mortality. While antioxidant supplementation is widely used in attempts to prevent the development of cancer, antioxidant may interface with cancer treatments[31], since the environment of cancer cells causes high levels of oxidative stress, making these cells more susceptible to the further oxidative stress induced by treatments[32,]. As a result by reducing the redox stress in cancer cells, antioxidant supplements could decrease the effectiveness of radiotherapy and chemotherapy[33,34]. On the other hand, other reviews have suggested that antioxidants could reduce side effects or increase survival times[35].

Organisms contain a complex network of antioxidant metabolites and enzymes that work together to prevent oxidative damage to cellular compounds such as DNA, proteins and lipids[36,37]. In general, antioxidant systems either prevent these reactive species from being formed or remove them before they can damage vital components of the cell[37]. However, reactive oxygen species also have useful cellular functions, such as redox signaling[38]. Thus the function of antioxidant systems is not to remove oxidants entirely, but instead to keep them at an optimum level[38]. The reactive oxygen species produced in cells include hydrogen, peroxide,

hypochlorous acid and free radical such as hydroxyl radical and the superoxide anion[39]. The hydroxyl radical is particularly unstable and will react rapidly and non- specifically with most biological molecules[39]. This species is produced from hydrogen peroxide in metal catalyzed redox reactions such as the Fenton reaction[39]. These oxidants can damage cells by starting chemical chain reactions such as lipid per oxidation or by oxidizing DNA or proteins. Damage to DNA can cause mutation and possibly cancer if not reversed by DNA repair mechanisms, while damage to protein causes enzyme inhibition denaturation and protein degradation[40-42]. The use of oxygen as part of the process for generating metabolic energy produces reactive oxygen species[43].

Metabolites

Antioxidants are classified into two broad divisions, depending on whether they are soluble in water or in lipids. In general water soluble antioxidants react with oxidants in the cell cytosol and the blood plasma, while lipid soluble antioxidants protect cell membranes from lipid peroxidation[44]. These compounds may be synthesized in the body or obtained from the diet[45]. The different antioxidants are present at a wide range of concentrations in body fluids and tissues[45]. Some antioxidants are only found in a few organisms and these compounds can be important in pathogens and can be virulence factors[46]. The relative importance and interactions between these different antioxidants is a very complex question, with the various metabolites and enzyme systems having synergistic and interdependent effects on one another[47]. The action of one antioxidant depends upon the proper functioning of other antioxidant system. The amount of protection provided by any one antioxidant will depends on its concentration, its reactivity towards the particular reactive oxygen species being considered and the status of the antioxidants with which it interacts.

Some compounds contribute to antioxidant defense by chelating transition metals and preventing them from catalyzing the production of free radial in the cell. Particularly important is the ability to sequester iron, which is the function of iron-binding proteins such as transferring and ferritin. Selenium and Zinc are commonly referred to as antioxidant nutrients but these chemical elements have no antioxidant action themselves and are instead required for the activity of some antioxidant enzymes[48]. Glutathione is a cysteine containing peptide found in most forms of aerobic life[49]. Glutathione has antioxidant properties since the thiol group in its cysteine moiety is a reducing agent and can be reversibility oxidized and reduced. In cells, glutathione is maintained in the reduced from by the enzyme glutathione reductase and in turn reduces other metabolites and enzyme systems. Such as ascorbate in the glutathione, ascorbate cycle, glutathione peroxidases directly with oxidants[50]. Due to its high concentration and its central role in maintaining the cell's redox state, glutathione is one of the most important cellular antioxidants[51,52]. In some organisms glutathione is replaced by other thiols, such as by mycothil in the Actinomycetes, bacillithiol in some Gram-positive bacteria or by trypanothione in the Kinetoplastids[53,54].

Melatonin is a powerful antioxidant[55]. Melatonin easily crosses cell membranes and the blood brain barrier[56]. Unlike other antioxidants, melatonin does not undergo redox cycling, which is the ability of the molecule to undergo reduction and oxidation. Redox cycling may allow other antioxidants to act as pro-oxidants and promote free radical formation. Melatonin forms numerous stable end products when reacts with free radicals. But once melatonin is oxidized it cannot be reduced to its former state. Therefore, it has been referred to as a terminal (or suicidal) antioxidant[57]. Antioxidants that are reducing agents can also act as pro-oxidants[58]. For instance, vitamin C has excellent antioxidant activity when it reacts with oxidizing substances such as hydrogen peroxide. However, it will also reduce metal irons that generate free radical through the Fenton reaction[59].

$$2Fe^{3+} + \text{Ascorbate} \rightarrow 2Fe^{2+} + \text{Dehydroascorbate}$$

$$2Fe^{2+} + 2H^2O^2 \rightarrow 2Fe^{3+} + 2OH. + 2OH^-$$

Some antioxidant supplements may promote disease and increase mortality in humans under certain conditions[60]. Free radicals may sometimes increase life span. This increase may be prevented by

antioxidants, providing direct evidence that toxic radicals may mitohormetically exerts life extending and healthy promoting effects[61]. As with the chemical antioxidants cells are protected against oxidative stress by an interacting network of antioxidant enzymes[61,62]. Here the superoxide released by processes such as oxidative phosphorylation is first converted to hydrogen peroxide and then further reduced to give water[62]. This detoxification pathway is the result of multiple enzymes, with superoxide dismutases catalyzing the various peroxidases removing hydrogen peroxide[62]. As with antioxidant metabolites, the contributions of these enzymes to antioxidant defenses can be hard to separate from one another[62].

Many antioxidants are used as food additives to help guard against food deterioration[63]. Antioxidants are an especially important class of preservatives as, unlike bacterial or fungal spoilage, oxidation reactions still occur relatively rapidly in frozen or refrigerated food[63]. These preservatives include natural antioxidants such as ascorbic acid (AA, E300) and tocopherols (E306), as well as synthetic antioxidants such as propyl gallate (PG, E310), tertiary butylhydroquinone (TBHQ), butylated hydroxyanisole (BHA,E320) and butylated hydroxytoluene (BHT,E321)[64,65]. The most important common molecules attacked by oxidation are unsaturated fats; oxidation causes them to turn rancid. Since oxidized lipids are often discolored and usually have unpleasant taste such as metallic or sulfurous flavours, it is important to avoid oxidation in fat-rich foods. Such foods are preserved by drying; rather than they are preserved by salting, smoking or fermenting. Since oxidation is catalyzed by metals, fats such as butter should not be wrapped in aluminium foil. Some fatty foods such as olive oil are partially protected from oxidation by their natural content of antioxidants, but remain sensitive to photo oxidation[65]. Antioxidant preservatives are also added to fat based cosmetics such as lipstick and moisturizers to prevent rancidity[65]. Substituted phenols and derivatives of phenylenediamine are common antioxidants used to inhibit gum formation in gasoline (petrol)[65].

EXPERIMENT

Measurement and invalidation of ORAC

Measurement of antioxidant content in food is not a straight forward process, as antioxidants collectively are a diverse group of compounds with different reactivities to various reactive oxygen species. DPPH is a stable free radical due to delocalization of the spare electron over the whole molecule[65]. The delocalization restricts from dime rising as would be the case with most other free radicals[64,65]. The delocalization also gives rise to the deep violet colouration characterized by a strong absorption band at 517 nm. DPPH free radical can accept an electron or hydrogen radical and can be converted into a stable, diamagnetic molecule with a loss of the violet colour. This paired radical can undergo further reaction, which control the overall stoichiometry and the absorption decreases with respect to the number of electron take up[64, 64].

DPPH 1mg in 10^{-5} mol methanol was prepared in 250ml standard flask (control 208ml of this solution +0.05ml methanol). Benzimidazole solutions of different concentrations (0.1, 0.25, 0.5, 0.75, 1mM) were prepared. BHA (standard) solutions of different concentrations (0.1, 0.25, 0.5, 0.75, 1mM) were prepared. The absorbance of the control and the test solutions were recorded out at 517nm. By following similar procedure the absorbance will be measured for BHA solutions. From the absorbance values, % of inhibition was calculated. Then the % of inhibition was plotted against concentrations for different samples as well as BHA. The % reduction and IC_{50} were calculated. The IC_{50} values indicate that less IC_{50} is a more antioxidant capacity. The standard BHA shows the IC_{50} values of 624µM.

$$\% \text{ inhibition} = \frac{\text{Control absorbance} - \text{sample absorbance}}{\text{Control absorbance}} \text{ X } 100$$

The antioxidant properties of all new compounds were studied and the results were correlated to the nature of the heterocyclic moiety attached to the C-5 of the thiazole and the substituent present in C-5 of the thiazole and the substituent present in C-2 position.

RESULTS AND DISCUSSION

Effect of C-2 substituents

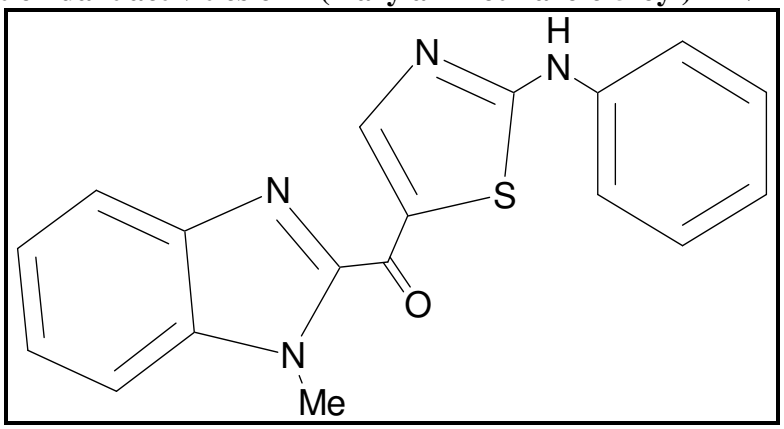
The compound containing unsubstituted phenylamino group 1a, exhibit an IC_{50} value 191 μM, 3a has an IC_{50} value of 406μM and 5a has 132 μM. The compounds containing 4-chlorophenyl ring 1b and 5b shows an IC_{50} value below the value of BHA (standard) and the compound 3b has an IC_{50} value 310μM. The compound, which consists of 4-methylphenyl group attached to the ring 1c, 3c, and 5c shows an IC_{50} value below the value of BHA (standard). The compound with 4-methoxyphenyl group attached to the ring 1d has an IC_{50} value of 103 μM, but the compound 3d has an IC_{50} value of 124 μM. The compounds containing 4-ethoxyphenyl group 1e shows an IC_{50} value of 179 μM and the compound 3e exhibits an IC_{50} value of 528 μM.

The compound containing ethyl group 2a exhibits an IC_{50} value of 132 μM and the compound 4a exhibits an IC_{50} value 357 μM. The compound 6a exhibits an IC_{50} value at 520 μM.

The compounds containing n-propylamino group 2b, 4b and 6b shows an IC_{50}values of 521, 810 and 659 μM respectively. The compound containing isopropylamino group 2c and 4b shows an IC_{50} values of 570 and 810 μM respectively. The compounds with n-butylamino substituent 2d, 4d and 6d shows an IC_{50} values of 357, 241 and 524μM. From these results we observed that the compounds with 4-chlorophenylamino group are more powerful antioxidants than those with 4-methylphenylamino group, which in turn more powerful than those with phenylamino group.

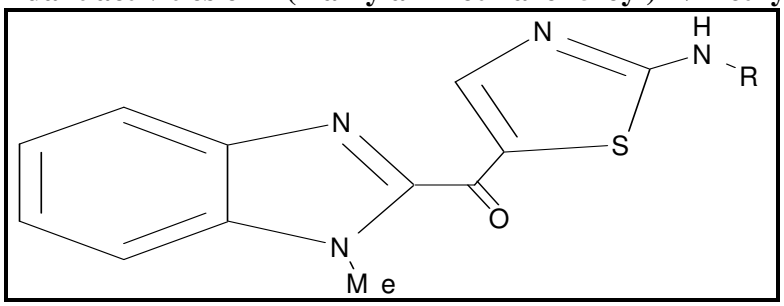
The benzimidazoles with aryl substituents are better antioxidants than those with alkyl substituents. Among the aryl series compounds compounds, the compounds with methoxyamino group is more powerful than with 4-methylphenylamino group than those with unsubstituted phenylamino group which is more powerful than with 4-chlorophenylamino group. In the alkyl series, the compounds with n-propylamino group are more powerful antioxidants than ethylamino group than butylamino group than isopropylamino group.

Table No.1: Antioxidant activities of 2-(2-arylaminothiazole-5-oyl)-1-N-methylbenzimidazoles 1



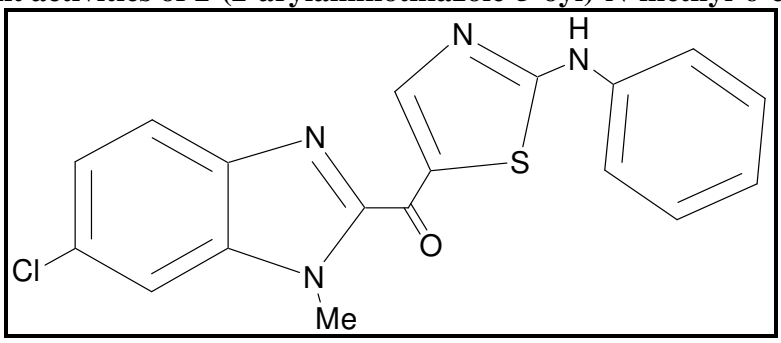
S.No	Compound	IC_{50} Value (μM)
1	1a (phenyl)	191
2	1b (chlorophenyl)	132
3	1c (methylphenyl)	166
4	1d (methoxyphenyl	103
5	1e (ethoxyphenyl)	179
6	BHA (Standard)	624

Table No.2: Antioxidant activities of 2-(2-alkylaminothiazol-5-oyl)-N-methylbenzimidazoles 2



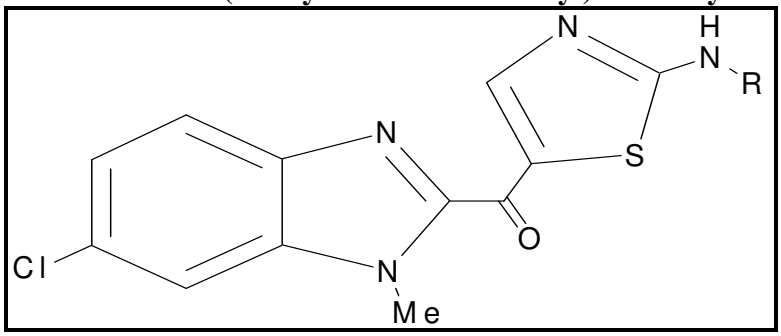
S.No	Compound	IC_{50} Value (µM)
1	2a (ethyl)	132
2	2b (propyl)	521
3	2c (isopropyl)	570
4	2d (butyl)	357
5	BHA (Standard)	624

Table No.3: Antioxidant activities of 2-(2-arylaminothiazole-5-oyl)-N-methyl-6-chlorobenzimidazoles 3



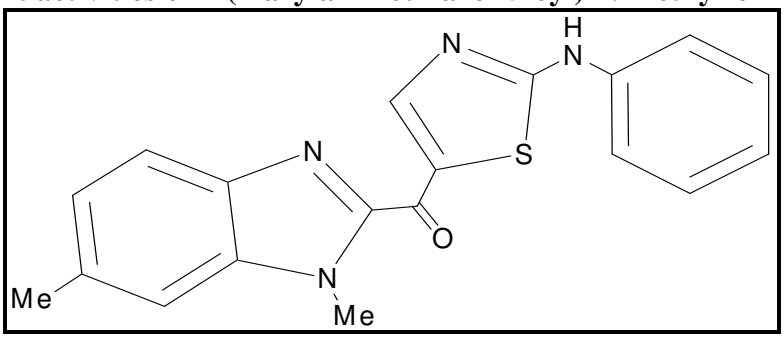
S.No	Compound	IC_{50} Value (µM)
1	3a (phenyl)	406
2	3b (chlorophenyl)	310
3	3c methylphenyl)	121
4	3d (methoxyphenyl)	124
5	3e (ethoxyphenyl)	528
6	BHA (Standard)	624

Table No.4: Antioxidant activities of 2-(2-alkylaminothiazol-5-oyl)-N-methyl-6-chlorobenzimidazoles 4



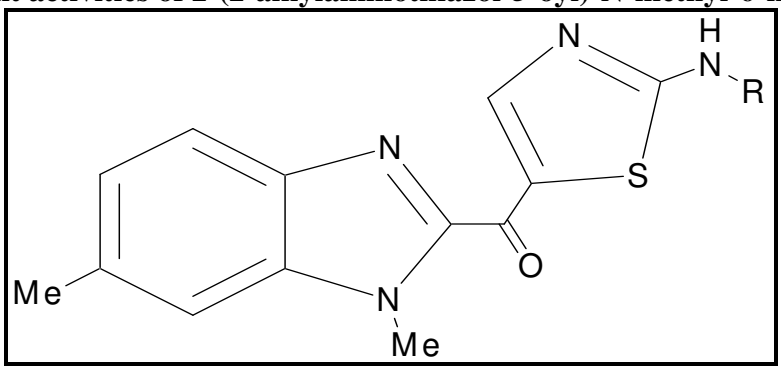
S.No	Compound	IC_{50} Value (µM)
1	4a (ethyl)	357
2	4b (propyl)	810
3	4c (isopropyl)	659
4	4d (butyl)	241
5	BHA (Standard)	624

Table No.5: Antioxidant activities of 2-(2-arylaminothiazol-5-oyl)-N-methyl-6-methylbenzimidazoles 5



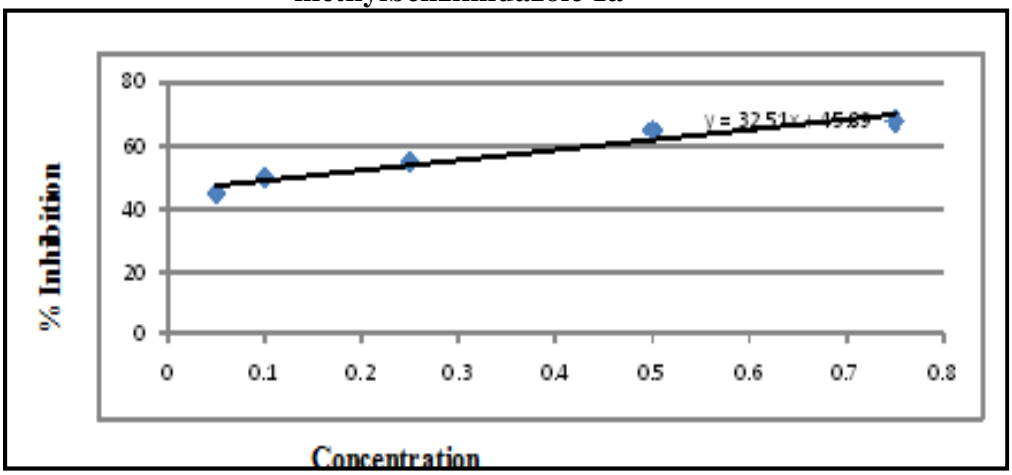
S.No	Compound	IC_{50} Value (µM)
1	5a (phenyl)	132
2	5b (chlorophenyl)	51
3	5c (methyl phenyl)	189
4	5d (methoxyphenyl)	357
5	5e (ethoxyphenyl)	241
6	BHA (Standard)	624

Table No.6: Antioxidant activities of 2-(2-alkylaminothiazol-5-oyl)-N-methyl-6-methylbenzimidazoles 6

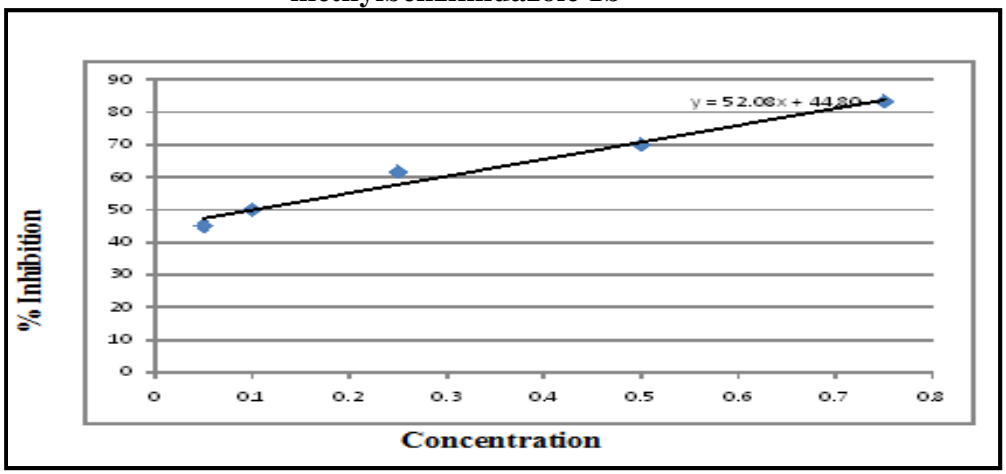


S.No	Compound	IC_{50} Value (µM)
1	6a (ethyl)	520
2	6b (propyl)	659
3	6c (isopropyl)	810
4	6d (butyl)	524
5	BHA (Standard)	624

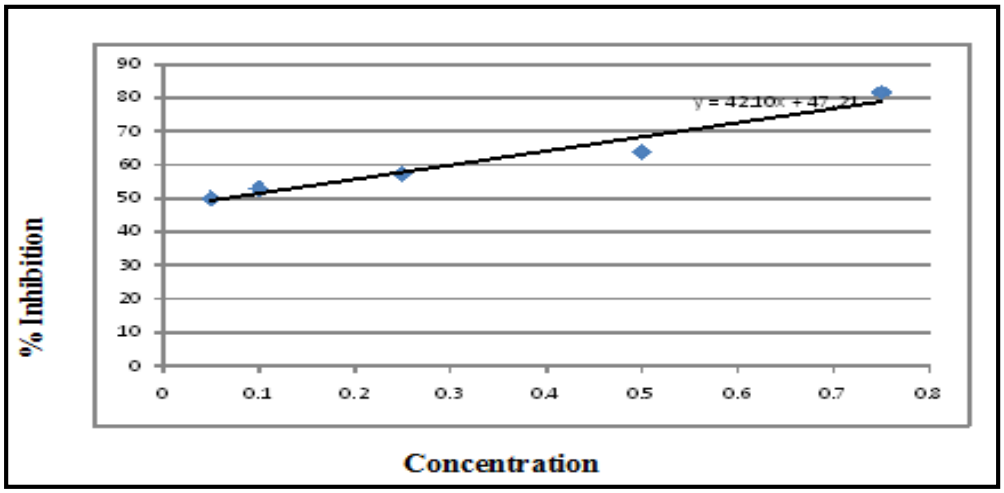
Plot of % of inhibition Vs concentration of 2-(2-phenylaminothiazol-5-oyl)-N-methylbenzimidazole 1a



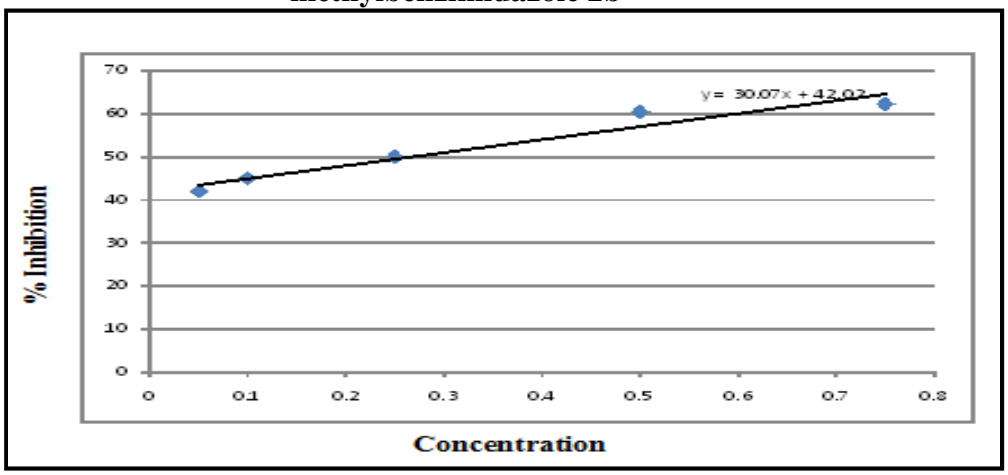
Plot of % of inhibition Vs concentration of 2-(2-chlorophenylaminothiazol-5-oyl)-N-methylbenzimidazole 1b



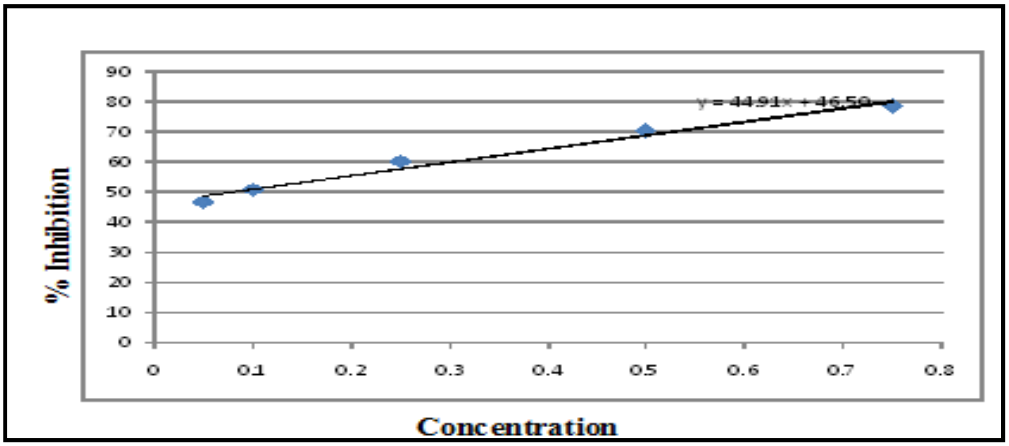
Plot of % of inhibition Vs concentration of 2-(2-ethylaminothiazol-5-oyl)-N-methylbenzimidazole 2a



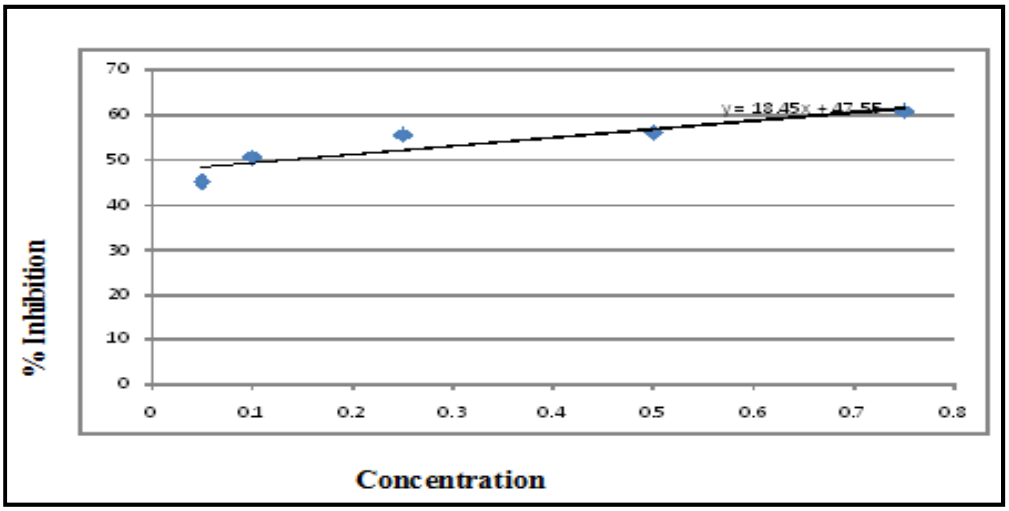
Plot of % of inhibition Vs concentration of 2-[2-(n-propylamino) thiazol-5-oyl]-N-methylbenzimidazole 2b



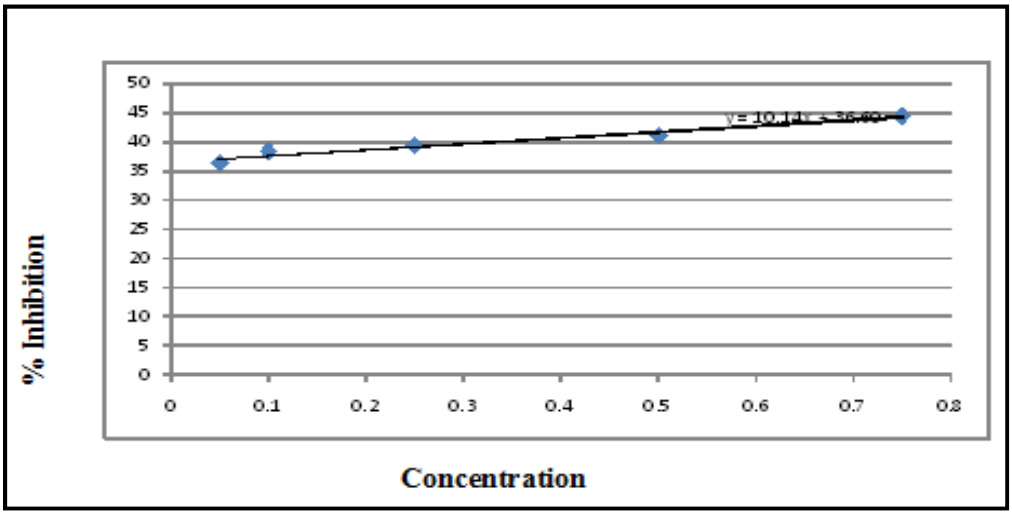
Plot of % of inhibition Vs concentration of 2-(2-phenylaminothiazol-5-oyl)-N-methyl-6-chlorobenzimidazole 3a



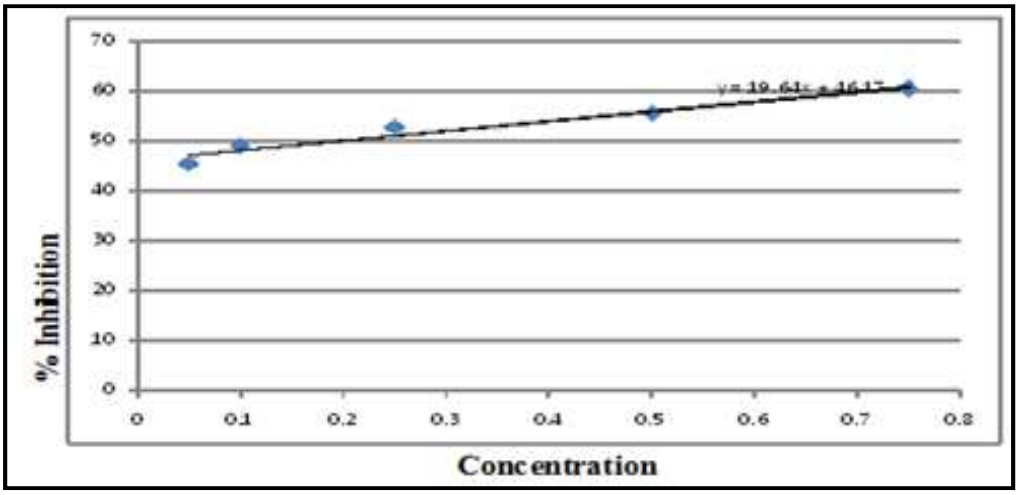
Plot of % of inhibition Vs concentration of 2-(2-chlorophenylaminothiazol-5-oyl)-N-methyl-6-chlorobenzimidazole 3b



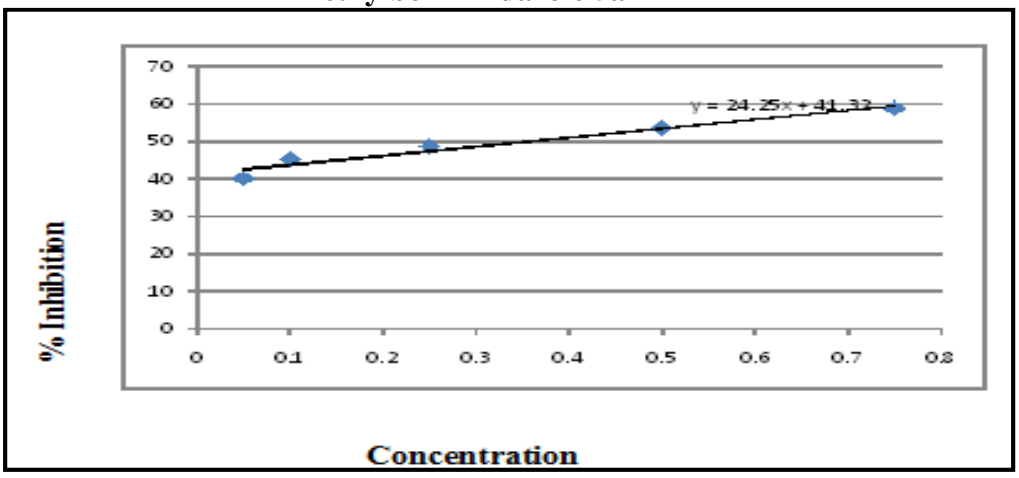
Plot of % of inhibition Vs concentration of 2-(2-ethylaminothiazol-5-oyl)-N-methyl-6-chlorobenzimidazole 4a



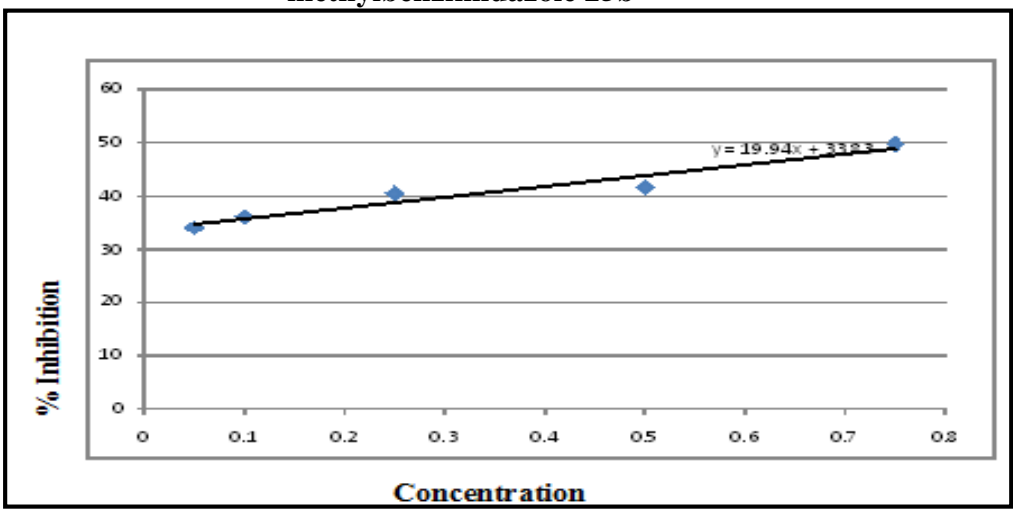
Plot of % of inhibition Vs concentration of 2-[2-(n-propylamino) thiazol-5-oyl]-N-methyl-6-chlorobenzimidazole 4b



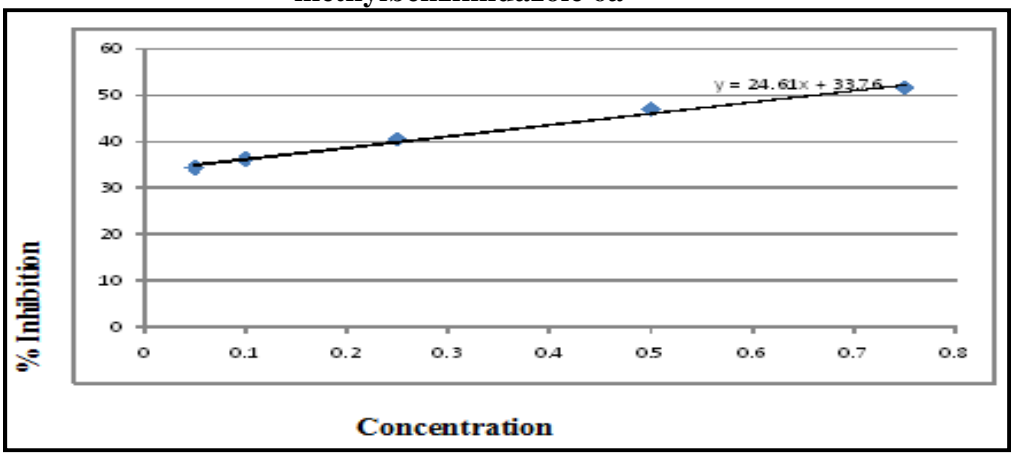
Plot of % of inhibition Vs concentration of 2-(2-phenylaminothiazol-5-oyl)-N-methyl-6-methylbenzimidazole 5a



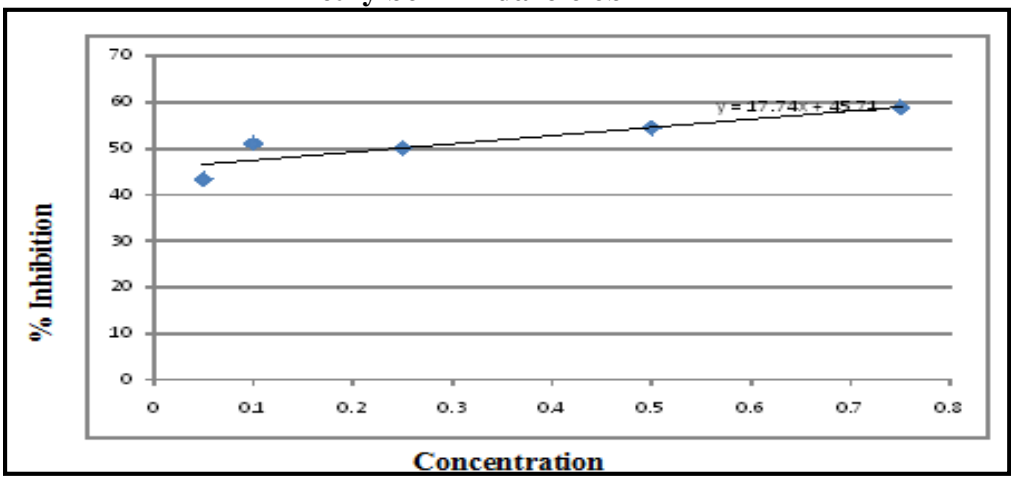
Plot of % of inhibition Vs concentration of 2-(2-chlorophenylaminothiazol-5-oyl)-N-methyl-6-methylbenzimidazole 25b



Plot of % of inhibition Vs concentration of 2-(2-ethylaminothiazol-5-oyl)-N-methyl-6-methylbenzimidazole 6a



Plot of % of inhibition Vs concentration of 2-[2-(n-propylamino) thiazol-5-oyl]-N-methyl-6-methylbenzimidazole 6b



CONCLUSION

In the antioxidant results, the compounds containing phenylamino group 1d have low IC $_{50}$ value. The compounds 1b, 2a and 2d have intermediateIC_{50} value. The compound 1c have average IC_{50} value. The compound 1a have average IC_{50} value. The compounds containing 4-chlorophenylamino group 3c and 4d have very low IC_{50} value than that of BHA (standard). The compound 4d has low IC_{50} value. The compounds 3d and 4a have low IC_{50} values. The compounds containing 4-methylphenylamino group have very low IC_{50} values. The compound 5c have a low IC_{50} value. The compounds 6a and 6b have intermediate IC_{50} values.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

BIBLIOGRAPHY

1. Shapiro H M. Practical Flow Cytometry, 2nd Edition, 1988, 129.
2. Hawley, Robert C, Teresa S, Hawley. Flow Cytometry protocols [Methods in Molecular Biology], 2nd Edition, 263, 2004.
3. Jiang L, Yang K H, Tian J H, Guan Q L, Yao N, Cao N, Mi D H, Wu J, Ma B and Yang S H. 'Efficacy of antioxidant vitamins and selenium supplement in prostate cancer prevention: a meta-analysis of randomized controlled trials', *Nutrition and cancer,* 62(6), 2010, 719-727.
4. Rees K, Hartley L, Day C, Flowers N, Clarke A and Stranges S. 'Selenium supplementation for the primary prevention of cardiovascular disease', *The Cochrane Database of Systematic Reviews,* 1(1), CD009671, 2013.
5. Shekelle P G, Morton S C, Jungvig L K, Udani J, Spar J, Tu W J, Suttorp M, Coulter I, Newberry S J and Hardy M. 'Effect of supplemental vitamin E for the prevention and treatment of cardiovascular disease', *Journal of General Internal Medicine,* 19(4), 2004.
6. Dabelstein W, Reglitzky A and Reders K. 'Automotive Fuels', *Ullmann's Encyclopedia of Industrial Chemistry,* 6, 2007, 341-344.
7. Stanner S A, Hughes J, Kelly C N and Buttress J. 'A review of the epidemiological evidence for the 'antioxidant hypothesis'', *Public Health Nutrition,* 7(3), 2004, 407-22.
8. Shenkin A. 'The key role of micronutrients', *Clinical Nutrition,* 25(1), 2006, 1-13.
9. Food, Nutrition, Physical Activity, and the Prevention of Cancer: a Global Perspective, *World Cancer Research Fund,* 2007.
10. Hail N, Cortes M, Drake E N and Spallholz J E. 'Cancer Chemoprevention: a radical perspective', *Free Radical Biology and Medicine,* 45(2), 2008, 97-110.
11. 'Flavanoids' Linus Pauling Institute, *Oregon State University, Corvallis,* 2016.
12. Csepregi K, Neugart S, Schreiner M and Hideg Eva. 'Comparative Evaluation of Total Antioxidant Capacities of Plant polyphenols', 21(2), 2016, 208.
13. Williams R J, Spencer J P and Rice-Evans C. 'Flavanoids: antioxidants or signaling molecules', *Free Radical Biology and Medicine,* 36(7), 2004, 838-49.
14. Aggarwal B B and Shishodia S. 'Molecular targets of dietary agents for prevention and therapy of cancer', *Biochemical Pharmacology,* 71(10), 2006, 1397-421.
15. Virgili F and Marino M. 'Regulations of Cellular signals from nutritional molecules: a specific role for phytochemicals, beyond antioxidant activity', *Free Radical Biology and Medicine,* 45(9), 2008, 1205-16.
16. Di Matteo V and Esposito E. 'Biochemical and therapeutic effects of antioxidants in the treatment of Alzhemiers disease, Parkinsons

disease and amyotrophic lateral sclerosis', Current Drug Targets, *CNS and neurological disorders,* 2(2), 2003, 95-107.

17. Rao A V and Balachandran B. 'Role of oxidative stress and antioxidants in neurodegenerative diseases', *Nutritional Neuroscience,* 5(5), 2002, 291-309.
18. Crichton G E, Bryan J and Murphy K J. 'Dietory antioxidants, cognitive function and dementia-a systematic review', *Plant Foods for Human Nutrition,* 68(3), 2013, 279-92.
19. Takeda A, Nyssen O P, Syed A, Jansen E. Bueno-de-Mesquita B and Gallo V. 'Vitamin A and carotenoids and the risk of Parkinsons disease: a systematic review meta-analysis', *Neuroepidemiology,* 42(1), 2014, 25-38.
20. Harrison F E. 'A critical review of vitamin C for the prevention of age-related cognitive decline and Alzheimers disease', *Journal of Alzheimers Disease,* 29(4), 2012, 711-26.
21. Sena E, Wheble P, Sandercock P and Macleod M. 'Systematic review and meta-analysis of the efficacy of tirilazad in experimental stroke', *A Journal of Cerebral Circulation,* 38(2), 2007, 388-94.
22. Zhang S, Wang L, Liu M and Wu B. 'Tirilazad for aneurismal subarachnoid haemorrhage', *The Cochrane Database of Systematic Reviews,* 2, 2010, CD006778.
23. Andrea G M. 'Use of antioxidants during chemotherapy and radiotherapy should be avoided', *CA: A Cancer Journal for Clinicians,* 55(5), 2005, 319-321.
24. Dekkers J C, van Doornen L I and Kemper H C. 'The role of antioxidant vitamins and enzymes in the prevention of exercise – induced muscle damage', *Sports Medicine,* 21(3), 1996, 232-238.
25. Takanami Y, Iwane H, Kavai Y and Shimoitsu T. 'vitamin E supplementation and endurance exercise: are there benefits', *Sports Medicine*, 29(2), 2000, 73-83.
26. German J B. 'Food processing and lipid oxidation', *Advances in Experimental Medicine and Biology,* 459, 1999, 23-50.
27. Jacob R A. 'Three eras of vitamin C discovery', *Sub-Cellular Biochemistry,* 25, 1996, 1-16.
28. Knight J A. 'Free radicals: their history and current status in aging and disease', *Annals of Clinical and Laboratory Science,* 28(6), 1998, 331-346.
29. Moureu C and Dufraisse C. 'Sur l'autoxydation: Les antioxygens', *Comptes Rendus des Seances et Memoires de la Societe de Biologie (in French),* 86, 1922, 321-322.
30. Albanes D. 'Beta-carotene and lung cancer: a case study', *The American Journal of Clinical Nutrition,* 69(6), 1999, 1345s-13450s.
31. Lawenda B D, Kelly K M, Ladas E J, Sagar S M, Vickers A and Blumberg J B. 'Should supplemental antioxidant administration be avoided during chemotherapy and radiation therapy?', *Journal of the National Cancer Institute,* 100(11), 2008, 773-783.
32. Block K I, Koach A C, Mead M N, Tothy P K, Newman R A and Gyllenhaal C. 'Impact of antioxidant supplementation on chemotherapeutic toxicity: a systematic review of the evidence from randomized controlled trials', *International Journal of Cancer. Journal International Du Cancer,* 123(6), 2008, 1227-1239.
33. Block K I, Koach A C, Mead M N, Tothy P K, Newman R A and Gyllenhaal C. 'Impact of antioxidant supplementation on chemotherapeutic toxicity: a systematic review of the evidence from randomized controlled trials', *Cancer Treatment Reviews,* 33(5), 2007, 407-418.
34. Davis K J. 'Oxidative Stress: the paradox of aerobic life', *Biochemical Society Symposium,* 61, 1995, 1-31.
35. Sies H. 'Oxidative Stress: oxidants and antioxidants', *Experimental Physiology,* 82(2), 1997, 291-295.

36. Rhee S G. 'The cell signaling, H202, a necessary evil for cell signaling', *Science,* 312(5782), 2006, 1882-1883.
37. Valko M, Leibfritz D, Moncol J, Cronin M T, Mazur M and Telser J. 'Free radicals and antioxidants in normal physiological functions and human disease', *The International Journal of Biochemistry and Cell Biology,* 39(1), 2007, 44-84.
38. Stohs S J and Bagchi D. 'Oxidative mechanisms in the toxicity of metal ions', *Free Radical Biology and Medicine,* 18(2), 1995, 321-336.
39. Nakabeppu Y, Sakumi K, Sakamoto K, Tsuchimoto D, Tsuzuki T and Nakatsu Y. 'Mutagenesis and carcinogenesis caused by the oxidation of nucleic acids', *Biological chemistry,* 387(4), 2006, 373-379.
40. Valko M, Izakovic M, Mazur M, Rhodes C J and Telser J. 'Role of oxygen radicals in DNA damage and cancer incidence', *Molecular and Cellular Biochemistry,* 266(1-2), 2004, 37-56.
41. Raha S and Robinson B H. 'Mitochondria, oxygen free radicals, disease and ageing', *Trends in Biochemical Sciences,* 25(10), 2000, 502-508.
42. Lenaz G. 'The mitochondrial production of reactive oxygen species; mechanisms and implications in human pathology', *IUBMB Life,* 52(3-5), 2001, 159-164.
43. Finkel T and Holbrook N J. 'oxidants, oxidative stress and the biology of ageing', *Nature,* 408(6809), 2000, 239-247.
44. Chaudiere J and Ferrari-Iliou R. 'Intracellular antioxidants: from chemical to biochemical mechanisms', *Food and Chemical Toxicology,* 37(9-10), 1999, 949-962.
45. Sies H. 'Strategies of antioxidant defense', *European Journal of Biochemistry/FEBS,* 215(2), 1993, 213-219.
46. Ames B N, Cathcart R, Schwiers E and Hochstein P. 'Uric acid provides an antioxidant defense in humans against oxidant and radical- caused aging and cancer: a hypothesis', *Proceedings of the National Academy of Sciences of the United States of America,* 78(11), 1981, 6858-6862.
47. Khaw K T and Woodhouse P. 'Interrelation of vitamin C, infection haemostatic factors and cardiovascular disease', *BMJ,* 310(6994), 1995, 1559-1563.
48. Krieger-Liszkay A. 'Singlet oxygen production in photosynthesis', *Journal of Experimental Botany,* 56(411), 2005, 337-346.
49. Gaballa A, Newton G L, Antelmann H, Parsonage D, Upton H, Rawat M, Claiborne A, Fahey R C and Helmann J D. 'Biosynthesis and functions of bacillithiol, a major low molecular weight thiol in Bacilli', *Proceedings of the National Academy of Sciences of the United States of America*, 107(14), 2010, 6482-6486.
50. Newton G L, Rawat M, La Clair J J, Jothivasan V K, Budiarto T, Hamilton C J, Claiborne A, Helmann J D and Fahey R C. 'Bacillithiol is an antioxidant thiol produced in Bacilli', *Nature Chemical Biology,* 5(9), 2009, 625-627.
51. Fahey R C. 'Novel thiols of prokaryotes', *Annual Review of Microbiology,* 55(1), 2001, 333-356.
52. Fairlamb A H and Cerami A. 'Metabolism and functions of trypanothione in the Kinetoplastida', *Annual Review of Microbiology,* 46(1), 1992, 695-729.
53. Tan D X, Manchester L C, Terron M P, Flores L I and Reiter R J. 'One molecule many derivatives: a never ending interaction of a melatonin with reactive oxygen and nitrogen species?', *Journal of Pineal Research,* 42(1), 2007, 28-42.
54. Reiter R J, Parades S D, Manchester L C and Tan D X. 'Reducing oxidative/nitrosative stress: a newly discovered genre for melatonin', *Critical Reviews in Biochemistry and Molecular Biology,* 44(4), 2009, 175-200.
55. Tan D X, Manchester L C, Reiter R J, Qi W B, Karbownik M and Calvo J R.

'Significance of melatonin in antioxidative defense system: reactions and products', *Biological signals and receptors,* 9(3-4), 2000, 137-159.

56. Herrara E and Barbas C. 'Vitamin E: action, metabolism and perspectives', *Journal of Physiology and Biochemistry,* 57(2), 2001, 43-56.
57. Packer L, Weber S U and Rimbach G. 'Molecular aspects of alpha-tocotrienol antioxidant action cell signaling', *The Journal of Nutrition,* 131(2), 2001, 369s-373s.
58. Schneider C. 'Chemistry and Biology of vitamin E', *Molecular Nutrition and Food Research,* 49(1), 2005, 7-30.
59. Halliwell B. 'Are polyphenols antioxidants or pro-oxidants? What do we learn from cell culture and in vivo studies?', *Archives of Biochemistry and Biophysics,* 476(2), 2008, 107-112.
60. Ho Y S, Magnenat J L, Gargano M and Cao J. 'The nature of antioxidant defense mechanisms: a lesson from transqenic studies', *Environmental Health Perspectives,* 106(Suppl. 5), 1998, 1219-1228.
61. Zelko I N, Mariani T J and Folz R J. 'Superoxide dismutase multigene family', *Free Radical Biology and Medicine,* 33(3), 2002, 337-349.
62. Bannister J V, Bannister M H and Rotilio G. 'Aspects of the structure, function and applications of superoxide dismutase', *CRC Critical Reviews in Biochemistry,* 22(2), 1987, 111-180.
63. Robards K. 'Rancidity and its measurement in edible oils and snack foods', *A Review', The Analyst,* 113(2), 1988, 213-214.
64. Kerr A F and Patsalides E. 'Rancidity and its measurement in edible oils and snack foods, A review', *The Analyst,* 113(2), 1988, 213-214.
65. Del Carlo M, Sacchetti G, Di Mattia C, Compagnone D, Liberatore L and Cichelli A. 'Contribution of the phenolic fraction to the antioxidant activity and oxidative stability of olive oil', *Journal of Agriculture and Food Chemistry,* 50(13), 2004, 4072-4079.

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