SUMMARIZATION OF FREE RADICALS AND ANTIOXIDANT THERAPY

Chalerm Saiin[1*], Khesorn Nantachit[2], Dammrong Santiarwornt[3]

[1]Department of Pharmaceutical Chemistry and Pharmacognosy, Pharmaceutical Sciences Faculty, Naresuan University, Muang Phitsanulok; [2, 3]Department of Pharmaceutical Science, Faculty of Pharmacy, Chiang Mai University, Chiang Mai, THAILAND.

[1]Chalerms@nu.ac.th

ABSTRACT

The aim of the paper is to show that (1) the relation between free radicals and pathology in the human, (2) the roles of endogenous and exogenous antioxidants in antioxidant therapy and (3) the future direction of antioxidant therapy. Lipid peroxidation is a free radical-mediated process. The peroxidation of polyunsaturated fatty acids is implicated in several pathologic conditions including atherosclerosis, myocardial infraction, inflammation, aging, and cancer. There are defence mechanisms presenting in cells and extracellular fluid for controlled over production of free radicals and reactive oxygen species. The important defence mechanisms are enzymatic and non-enzymatic mechanism. When endogenous antioxidative defence machanisms can not work or imbalance, the excess free radicals cause pathophysiological process. Thus, antioxidant therapy is useful for diseases relating to lipid peroxidation. In selection of diseases that we candidate for antioxidant therapy, we should differential between those that have oxidative damage as a primary mechanism at the core of their pathogenesis on the one hand and diseases in which oxidative damage represents a late consequence of primary mechanisms. Cancer and neurodegenerative diseases could probably be included in the list of such diseases.

Keywords: free radicals, lipid peroxidation, antioxidant therapy

A. FREE RADICALS

A free radical is defined as any species (molecule, fragment complex, or atom) that has one or more unpaired electrons. The unpaired electron causes the species highly reactive. In biology, oxygen free radicals such as superoxide radical (O_2^-) and hydroxyl radical ($OH^·$) are dangerous species. Superoxide radical is formed in almost all aerobic cells, one important source being the "respiratory burt" of phagocytic cells when they contact foreign particles or immune complexes. Phagocytic cells known to produce superoxide radical include neutrophils, monocytes, macrophages and eosinophiles. Addition of an electron to superoxide radical gives the peroxide ion (O_2^{2-}), which has no unpaired electrons and is not a radical. Peroxide ion formed at physiological pH will immediately protonate to give hydrogen peroxide (H_2O_2). Homolytic fission of the oxygen-oxygen (O-O) bond in hydrogen peroxide produces two hydroxyl radicals ($OH^·$). This homolysis can be achieved by heat or ionizing radiation. In addition, a mixture of hydrogen peroxide and an iron (II) can form the hydroxyl radical via "Fenton reaction".

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + OH^{\bullet} + OH^-$$

An iron (III) occuring in "Fenton reaction" can react with superoxide radical to form an iron (II) that in turn reacts with hydrogen peroxide in "Fenton reaction". As a result, more hydroxyl radicals are produced.

$$Fe^{3+} + O_2^- \longrightarrow Fe^{2+} + O_2$$

In general, superoxide radical, hydrogen peroxide and hydroxyl radical are often called as "Reactive oxygen species" (ROS). Among reactive oxygen, hydroxyl radical is the most reactive specie. (Pryor, 1966; Hay, 1974; Nonhebel and Walton, 1974; Slater, 1984; Halliwell and Gutteridge, 1984 & 1991; Andersson et al., 1996).

B. LIPID PEROXIDATION AND PATHOLOGY

Lipid peroxidation is a free radical-mediated process. The peroxidation of polyunsaturated fatty acids is implicated in several pathologic conditions including atherosclerosis, myocardial infraction, inflammation, aging, and cancer. As shown in figure 1, the first step of peroxidation process is an initiation reaction. The initiator such as hydroxyl radical abstracts hydrogen from α-methylene group of the lipid chain leading to formation of a lipid radical. The lipid radical is stabilized by a molecular rearrangement to form a conjugated diene. This then reacts rapidly with oxygen to form the peroxyl radical, which in turn attacks another α-methylene group yielding a lipid hydroperoxide and a new lipid radical which propagated the chain reaction. The hydroperoxide, which is the first product of peroxidation, is rather unstable and easily decompose into other product.

Peroxidation of low-density lipoprotein (LDL) plays a significant role in the development of atherosclerosis. Oxidized low-density lipoprotein can enter the macrophage through a scavenger receptor pathway, thereby producing lipid-rich foam cells. In addition, circulating monocyte are attracted to endothelial and smooth mucle cells by chemotatic protein 1, a chemoattractant that is augmented by the oxidatively modified lipoproteins. With continued uptake of the oxidatively modified lipoproteins by the macrophage scavenger receptor, foam cells form and progress to the next phase of atherosclerosis, development of the fatty streak. Simultaneously, smooth muscle cells migrate into the subendothelial space and begin proliferating within the intima. During the next phase of atherogenesis, lesions continue to grow by increases in both smooth muscle cell proliferation and collagen synthesis. Then, necrosis of the foam cell and formation of an extracellular lipid core occur and progress as long as plasma low-density lipoprotein is elevated. Thus, atherosclerosis leads to the blockage of an essential artery. Moreover, severe restriction of blood flow leads to myocardial infraction. Finally, An autoimmune inflammatory is occurred.[12] Moreover, malodialdehyde which reacts with DNA, is the major mutagenic and carcinogenic products generated by lipid peroxidation. It can attack amino groups on protein molecule to form both intramolecular cross links and intermolecular cross link (McBrien and Slater, 1982; Lunec and Griffiths, 1990; Harman, 1990; Gutteridge, 1991; Halliwell and Gutteridge, 1991; Elliott and Kandaswami, 1993; Carter et al., 1995).

C. ENDOGENOUS ANTIOXIDANT DEFENCE MECHANISMS

There are defence mechanisms presenting in cells and extracellular fluid for controlled over production of free radicals and reactive oxygen species. The important defence mechanisms are enzymatic and non-enzymatic mechanism.

1. Enzymatic Mechanisms

The enzymes, superoxide dismutase, catalase and glutathione peroxidase are the antioxidants within the cells. Superoxide dismutase acts as a scavenger of superoxide anions by catalysing their dismutation reaction into hydrogen peroxide and molecular oxygen. Catalase and glutathione peroxidase reduce hydrogen peroxide as well as alkyl hydroperoxide into water and alcohols, respectively (Ferrari et al., 1991).

Leena and Luna International, Chikusei, Japan.
(株) リナアンドルナインターナショナル, 筑西市,日本
ISSN: 2186-8476, ISSN: 2186-8468 Print
www.ajsc. leena-luna.co.jp

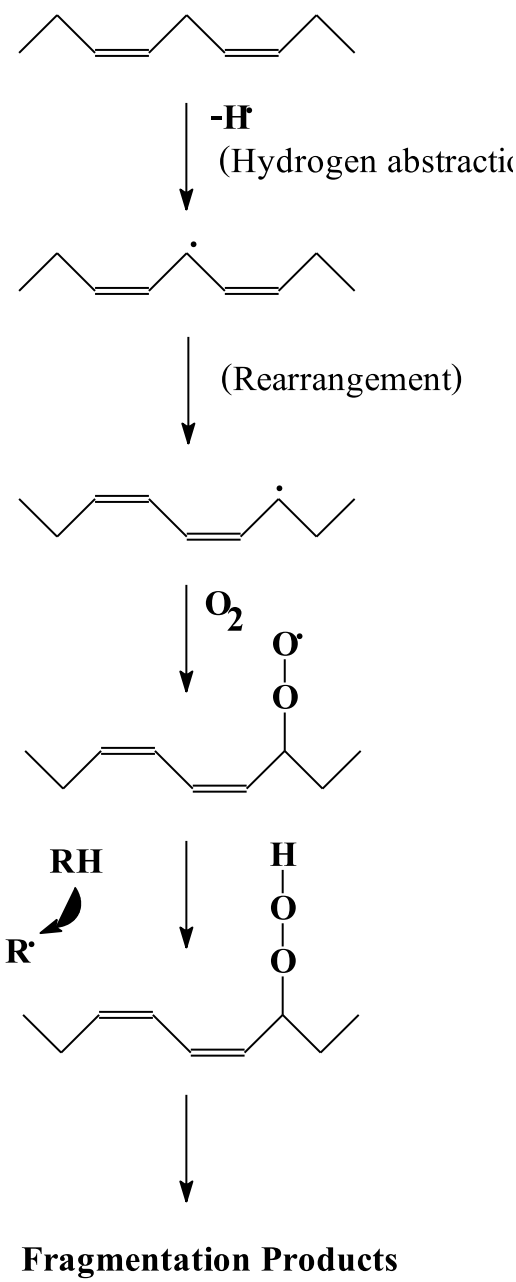
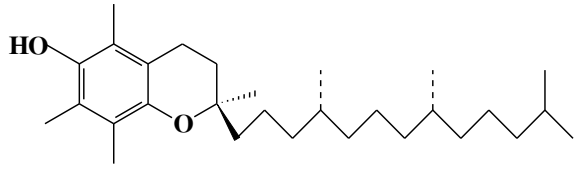


Figure 1: Schemetic representation of the initiation and propagation reaction of lipid peroxidation

2. Non-Enzymatic Mechanisms

2.1 *Hydrophobic protection mechanism*

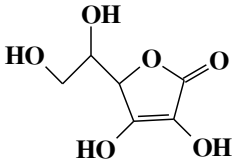
Hydrophobic protection mechanism controls free radicals in the hydrophobic regions within the cell. Vitamin E, mainly (R,R,R)-α-tocopherol, is the major lipid-soluble chain-breaking antioxidant in this mechanism (Andersson et al., 1996).



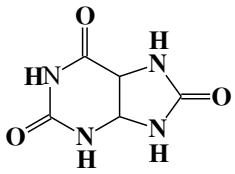
R,R,R-α-tocopherol

2.2 Hydrophilic protective mechanism

Hydrophilic mechanism controls free radicals in the hydrophilic regions within the cell. Ascorbic acid and uric acid are the major antioxidants in this mechanism (Andersson et al., 1996).



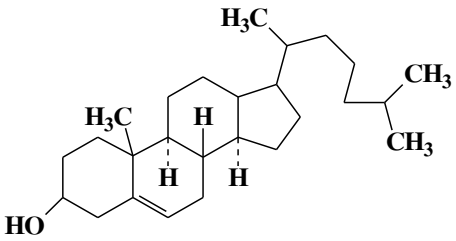
Ascorbic acid



Uric acid

2.3 Structural mechanism

The protective mechanisms are associated with the structural integreity of living cells. Disruption of this structural integrity leads very quickly to the peroxidative free radical induced reaction within polyunsaturated lipids. Cholesterol, by its structural and size, protect living cell from peroxidative injury (Del Maestro, 1980).



Cholesterol

D. STRATEGIES FOR ANTIOXIDANT THERAPY

When endogenous antioxidative defence machanisms can not work or imbalance, the excess free radicals cause pathophysiological process. Thus, antioxidant therapy is useful for diseases relating to lipid peroxidation (Andersson et al., 1996).

The most commonly strategies for antioxidant therapy must include the following:

Leena and Luna International, Chikusei, Japan.
(株) リナアンドルナインターナショナル, 筑西市,日本
ISSN: 2186-8476, ISSN: 2186-8468 Print
www.ajsc. leena-luna.co.jp

1. Enhancement of tissue endogenous antioxidant levels through administration of antioxidant vitamins or glutathione precursors.

2. Prevention of initiation events by scavenging (commonly discussed for hydroxyl radical scavengers).

3. Chelation of metal ion by introducing multidentate complexing agents.

4. Decomposition of peroxide and superoxide into non-radical products (using antioxidant enzyme or synthetic superoxide dismutase or glutathione peroxidase mimics).

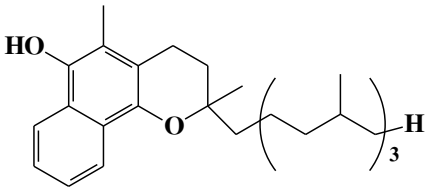
5. Termination of free radical peroxidation (through administration of generally lipophilic chain-breaking antioxidants).

A large number of compounds included semisynthesis analogues of endogenous antioxidant and natural products are widely developed for antioxidant therapy.

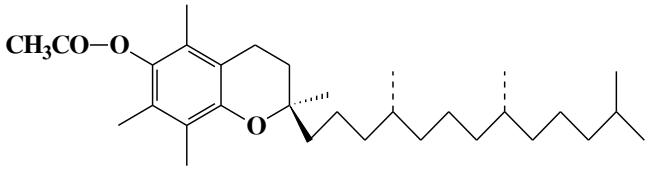
1. Semisynthesis analogues of endogenous antioxidant

1.1 Tocopherol analogues

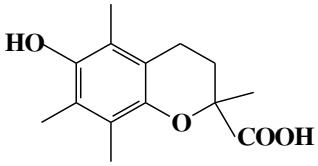
To improve antioxidant effects, lipophilic and hydrophilic tocopherol analogues are developed (Andersson et al., 1996; Mukai, 1994). Examples are vitamin K_1-chromanol, vitamin E acetate and trolox.



Vitamin K_1 - chromanol



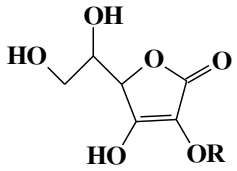
Vitamin E acetate



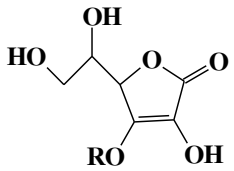
Trolox

1.2 Ascorbic acid analogues:

Ascorbic acid was rapidly oxidized with loss of activity in time, and very limited transcellular potency owing to its being hydrophilic. In order to solve these problems, ascorbic acid analogues were synthesized (Tojo and Lee, 1987; Kato et al., 1988; Luckewicz and Saccaro, 1990; Maeda and Fukada, 1991; Nihro et al., 1991). Examples are 2-O-Alkylascorbic acids and 3-O-Alkylascorbic acids.



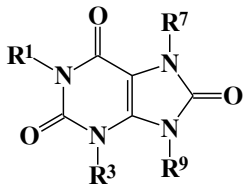
2-O-Alkylascorbic acids



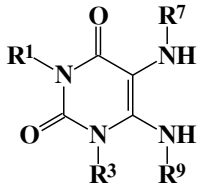
3-O-Alkylascorbic acids

1.3 Uric acid analogues

A new series of N-alkylated uric acids (2,6,8-purinetrione and 5,6-diaminouracils (5,6-diamino-2,4-pyrimidinedione) were synthesized, and their activities against free radicals were evaluated (Fraisse et al., 1993).



N-Alkylated uric acids

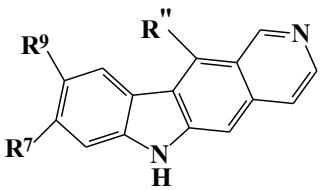


N-Alkylated diaminouracils

2. Natural products

2.1 Alkaloids

Some alkaloids have antioxidant activity. Structure-activity relationship in a series of ellipticine analogues, were investigated with respect to antioxidant activity (Andersson et al., 1996).

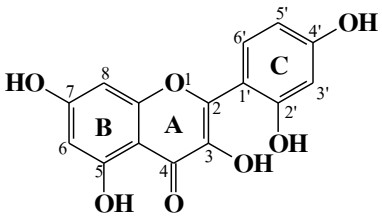


Ellipticine analogues

Leena and Luna International, Chikusei, Japan.
(株) リナアンドルナインターナショナル, 筑西市,日本
ISSN: 2186-8476, ISSN: 2186-8468 Print
www.ajsc. leena-luna.co.jp

2.2 Flavonoids

Several flavonoids have been reported to inhibit either enzymatic or non-enzymatic lipid peroxidation (Bindoli et al., 1977; Havsteen, 1983; Valenzuela et al., 1985; Robak and Gruglewski, 1988; Afanas'ev et al., 1989). In addition, the studies have shown that morin (2′,3,4′,5,7-pentahydroxy flavone) has the important pharmacological activities such as anti-inflammatory, anti-atherosclerosis and anticarcinogenic activity. Structure-activity relationship studies of morin were demonstrated that (i) two hydroxy groups on C-ring and one hydroxyl group at 3-position and (ii) C2-C3 unsaturated bond (C=C) increase antioxidative activity. Moreover, the antioxidative mechanisms of morin were considered. First, morin acted as free radical scavenger. Second, morin could chelate some metal ions such as iron(II) ion in Fenton reaction (Tauber et al., 1984; Pagonis et al., 1986; Hodnick et al., 1986; Mora et al., 1990; Decharneux et al, 1992; Limasset et al., 1993; Krol et al., 1994; Wu et al., 1994; Wu et al., 1995):



Morin (2′,3,4′,5,7-pentahydroxy flavone)

E. FUTURE DIRECTIONS

In selection of diseases that we candidate for antioxidant therapy, we should differential between those that have oxidative damage as a primary mechanism at the core of their pathogenesis on the one hand and diseases in which oxidative damage represents a late consequence of primary mechanisms. We should therefore be able identify diseases that antioxidant therapy has a higher probability of success. Cancer and neurodegenerative diseases could probably be included in the list of such diseases (Aliev et al, 2008, Halliwell, 2009 & Firuzi et al, 2011). The recent studies have shown that morin has the important pharmacological activities such as antiangiogenic, *in vivo* anti-inflammatory, antinociceptive activities (Jung et al, 2010) and it has a potential as an anticancer agent (Manna et al, 2007; Yuan et al, 2012; Karimi et al, 2013). The method for preparation of morin from the wood of Thai *Artocarpus heterophyllus* was developed by Saiin (2017). Therefore, the investigation of chemical constituents of *A. heterophyllus* and pharmacological evaluation of morin may bring to the new anticancer drug.

REFRENCES

[1] Afanas'ev, I.B., Dorozhko, A.I., Brodskii, A.V., Kostyuk, V.A., & Potapovitch, A.I. (1989). Chelating and free radical scavenging mechanisms of inhibitory action of rutin and quercetin in lipid peroxidation. *Biochemical Pharmacology*, *38*(11), 1763-1769.

[2] Aliev, G., Obrenovich, M.E., Reddy, V.P., Shenk, J.C., Moreira, P.I., Nunomura, A., Zhu, X., Smith, M.A., & Perry, G. (2008). Antioxidant therapy in Alzheimer's disease: Theory and practice. *Mini Rev Med Chem*, *8*(13), 1395-406.

[3] Andersson, C.M., Hallberg, A., & Högberg, T. (1996). *Advances in drug research*. London: Academic Press.

[4] Bindoli, A., Cavallini, L., & Siliprandi, N. (1977). Inhibitory action of silymarin of lipid peroxide formation in rat liver mitochondria and microsomes. *Biochemical Pharmacology*, *26*, 2405-2409.

[5] Carter, B.L., Angaran, D.M., Lake, K.D., & Raebel, M. (1995). *Pharmacotherapy self-assesment program: Module I, cardiovascular*. USA: American Collage of Clinical Pharmacy.

[6] Decharneux, T., Dubois, F., Beauloy, C., Coninck, S.W.D., & Wattiaux, R. (1992). Effect of various flavonoids on lysosomes subjected to an oxidative or an osmotic stress. *Biochemical Pharmacology*, *44*(97), 1243-1248.

[7] Del Maestro, R.F. (1980). An approach to free radical in medicine and biology. *Acta Physiological Scandinavica*. 492, 153-168.

[8] Elliott, M.J.R., & Kandaswami, C. (1993). *The flavonoids: advances in research since 1986*. Great Britain: Chapman and Hall.

[9] Ferrari, R., Ceconi, C., Curello, S., Cargnoni, A., Alfieri, O., Pardini, A., Marzollo, P., & Visioli, O. (1991). Oxygen free radicals and myocardial damage: protective role of thiol containing agents. *The American Journal of Medicine*, *91*, 95s-105s.

[10] Firuzi, O., Miri, R., Tavakkoli, M., & Saso, L. (2011). Antioxidant therapy: current status and future prospects. *Current Medicinal Chemistry*, *18*(25), 3871-3888.

[11] Fraisse, L., Verlhac, J. B., Roche, B., Rascle, M.C., Rabion, A., & Seris, J.L. (1993). Long-chain-substituted uric acid and 5, 6-diaminouracil derivatives as novel agent against free radical processes: synthesis and *in vitro* activity. *Journal of Medicinal Chemistry*, *36*, 1465-1473.

[12] Gutteridge, J.M.C. (1991). Use of the thiobarbituric acid reaction in oxygen radical chemistry. *Analytical Proceedings*, *27*, 219-221.

[13] Halliwell B. (2009). The wanderings of a free radical. *Free Radic Biol Med*, *46*(5), 531-42.

[14] Halliwell, B., & Gutteridge, J.M.C. (1984). Oxygen toxicity, oxygen radicals, transition metals and disease. *Biochemical Journal*, *219*, 1-14.

[15] Halliwell, B., & Gutteridge, J.M.C. (1991). *Free radicals in biology and medicine*. Great Britain: Clarendon Press.

[16] Harman, D. (1990). Free radicals in aging processes. *Analytical Proceeding*, *27*, 213-214.

Leena and Luna International, Chikusei, Japan.
(株) リナアンドルナインターナショナル, 筑西市,日本
ISSN: 2186-8476, ISSN: 2186-8468 Print
www.ajsc. leena-luna.co.jp

[17] Havsteen, B. (1983). Flavonoids, a class of natural products of high pharmacological potency. *Biochemical Pharmacology*, *32*(7), 1141-1148.

[18] Hay, J.M. (1974). *Reactive free radicals*. London: Academic Press.

[19] Hodnick, W.F., Kung, F.S., Roettger, W.J., Bohmont, C.W., & Pardini, R.S. (1986). Inhibition of mitochondrial respiration and production of toxic oxygen radicals by flavonoids: a structure-activity study. *Biochemical Pharmacology*, *35*(14), 2345-2357.

[20] Jung, H.J., Kim, S.J., Song, Y.S., Park, E.H., & Lim, C.J. (2010). Evaluation of the antiangiogenic, anti-inflammatory, and antinociceptive activities of morin. *Planta Med*, *76*, 273-275.

[21] Karimi, R., Parivar, K., Roudbari, N.H., Sadeghi, S.V., Hashemi, M., & Hayat, P. (2013). Anti-proliferative and apoptotic effects of morin in human leukemia cell lines (HUT-78). *International Journal of Cellular & Molecular Biotechnology, 2013*, 1-13.

[22] Kato, K., Terao, S., Shimamoto, N., & Hirata, M. (1988). Studies on scavengers of active oxygen species. 1. synthesis and biological activity of 2-O-alkylascorbic acids. *Journal of Medicinal Chemistry*, *31*, 793-798.

[23] Krol, W., Czuba, Z., Scheller, S., Paradowski, Z., & Shani, J. (1994). Structure-activity relationship in the ability of flavonols to inhibit chemiluminescence. *Journal of Ethnopharmacology*, *41*, 121-126.

[24] Limasset, B., Doucen, C.L., Dore, J.C., Ojasso, T., Damon, M., & Paulet, A.C.D. (1993). Effects of flavonoids on the releases of reactive oxygen species by stimulated human neutrophils. *Biochemical Pharmacology*, *46*(7), 1257-1271.

[25] Luckewicz, W., & Saccaro, R. (1990). Determination of ascorbyl dipalmitate in cosmetic whitening powders by differential scanning calorimetry. *Journal of the Society of Cosmetic Chemists*, *41*, 359-367.

[26] Lunec, J., & Griffiths, H. (1990). Oxygen radicals: Their role in disease processes. *Analytical Proceeding*, *27*, 212-213.

[27] Maeda, K., & Fukada, M. (1991). In vitro effectiveness of several whitening cosmetic component in human melanocytes. *Journal of the Society of Cosmetic Chemists*, *42*, 361-368.

[28] Manna, S.K., Aggarwal, R.S., Sethi, G., Aggarwal, B.B., & Ramesh, G.T. (2007). Morin (3,5,7,2',4'-pentahydroxyflavone) abolishes nuclear factor-kappa B activation induced by various carcinogens and inflammatory stimuli, leading to suppression of nuclear factor-kappa B-regulated gene expression and up-regulation of apoptosis. *Clin Cancer Res.*, *13*(7), 2290-2297.

[29] McBrien, D.C., & Slater, T.F. (1982). *Free radical, lipid peroxidation and cancer*. London: Academic Press.

[30] Mora, A., Paya, M., Rios, J.L., & Alcaraz, M.J. (1990). Structure-activity relationship of polymethoxyflavones and other flavonoids as inhibitors of non-enzymic lipid peroxidation. *Biochemical Pharmacology*, *40*(4), 739-797.

[31] Mukai, K. (1994). *Vitamin E in health and disease*. USA: Marcel Dekker, Inc.

[32] Nihro, Y., Miyataka, H., Sudo, T., Matsumoto, H., & Satoh, T. (1991). 3-*O*-Alkylascorbic acids as free-radical quenchers: synthesis and inhibitory effect on lipid peroxidation. *Journal of Medicinal Chemistry*, *34*, 2152-2157.

[33] Nonhebel, D.C., & Walton, J.C. (1974). *Free-radical chemistry; structure and mechanism*. Great Britain: Cambridge University Press.

[34] Pagonis, C., Tauber, A.I., Pavlotsky, N., & Simons, E.R. (1986). Flavonoid impairment of neutrophil response. *Biochemical Pharmacology*, *35*(2), 237-245.

[35] Pryor, W.A. (1966). *Introduction to free radical chemistry*. USA: McGraw Hill, Inc.

[36] Robak, J., & Gruglewski, R.J. (1988). Flavonoids are scavengers of superoxide anions. *Biochemical Pharmacology*, 37(5), 837-841.

[37] Saiin, C. (2017). Isolation of morin from the wood of Thai *Artocarpus heterophyllus*. *Asian Journal of Natural & Applied Sciences*, *6*(4), 85-89.

[38] Slater, T.F. (1984). Free radical mechanisms in tissue injury. *Biochemical Journal*, *222*, 1-15.

[39] Tauber, A.I., Fay, J.R., & Marletta, M.A. (1984). Flavonoid inhibition of the human neutrophil NADPH-oxidase. *Biochemical Pharmacology*, *33*(8), 1367-1369.

[40] Tojo, K., & Lee, Ae-Ri C. (1987). Bioconversion of a provitamin to vitamin C and E in skin. *Journal of the Society of Cosmetic Chemists*, *38*(5), 333-340.

[41] Valenzuela, A., Lagos, C., Schmidt, K., & Videla, L.A. (1985). Silymarin protection against hepatic lipid peroxidation induced by acute ethanol intoxication in the Rat. *Biochemical Pharmacology*, *34*(12), 2209-2212.

[42] Wu, T.W., Fung, K.P., Zeng, L.H., Wu, J., Hampel, A., Grey, A.A., & Camerman, N. (1995). Molecular properties and myocardial salvage effects of morin hydrate. *Biochemical Pharmacology*, *49*(4), 537-543.

[43] Wu, T.W., Zeng, L.H., Wu, J., & Fung, K.P. (1994). Morin: A wood pigment that protects three types of human cells in the cardiovascular system against oxyradical damage. *Biochemical Pharmacology*, *47*(6), 1099-1103.

[44] Yuan, C.H., Filippova, M., Tungteakkhun, S.S., Duerksen-Hughes, P.J., & Krstenansky, J.L. (2012). Small molecule inhibitors of the HPV16-E6 interaction with caspase 8. *Bioorg Med Chem Lett*, *22*(5), 2125-2129.

Leena and Luna International, Chikusei, Japan.
(株) リナアンドルナインターナショナル, 筑西市,日本
ISSN: 2186-8476, ISSN: 2186-8468 Print
www.ajsc. leena-luna.co.jp