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Antioxidant activities of flavidin in different in vitro model systems

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Abstract—Flavidin was isolated from *Orchidaceae* species and purified by silica gel column chromatography. The structure was identified using physical and spectral (^{1}H, ^{13}C NMR, and mass) data. Antioxidant potency of flavidin was investigated employing various established in vitro model systems viz., β-carotene-linoleate, 1,1-diphenyl-2-picryl hydrazyl (DPPH), phosphomolybdenum method, and scavenging of hydrogen peroxide methods. Flavidin showed very good antioxidant activity (90.2%) and almost equivalent to that of BHA at 50 ppm level by β-carotene-linoleate method. Radical scavenging activity of flavidin was compared with BHA at 5, 10, 20, and 40 ppm concentration and flavidin showed more radical scavenging activity than BHA at all the tested concentrations. Furthermore, flavidin showed very good antioxidant capacity by the formation of phosphomolybdenum complex method. Besides this, flavidin showed effective hydrogen peroxide scavenging activity. The data obtained in the in vitro models clearly establish the antioxidant potency of flavidin. However, comprehensive studies need to be conducted to ascertain the in vivo safety of flavidin in experimental animal models. This is the first report on antioxidant activity of 9,10-dihydro-5H-phenanthro-(4,5 bcd)-pyrans/flavidin type of compounds.

1. Introduction

Oxidative damage to membrane lipids (i.e., lipid peroxidation in biological systems) has been studied for many years. Lipid peroxidation is a primary event produced by oxidative stress or as a consequence of tissue damage, which can exacerbate tissue injury, due to the potential cytotoxicity and genotoxicity of the end products of lipid peroxidation. Membrane lipids with double bonds are most susceptible to oxidation. Lipid peroxidation can reduce membrane fluidity, leading to increased rigidity throughout the hydrophobic space of membranes, decreased permeability, osmotic fragility and altered activity of certain membrane-bound enzymes and transport systems.[1]

Antioxidants protect the quality of foods by retarding oxidative breakdown of the lipid components.[2] Commercial antioxidants are generally synthetic compounds and there has been a growing interest in replacing them with natural ingredients due to possible toxicity of synthetic antioxidants[3–5] as shown by clinical studies.[6]

Natural antioxidants such as nonenzymatic dietary components are not specific but can scavenge organic and inorganic radicals. These agents are found in numerous plant materials and commonly include an aromatic ring as part of their molecular structure. There are a variety of cyclic ring structures that are generally associated with one or more hydroxyl groups to provide labile hydrogen and a basis for free radical formation. These antioxidants can be classified as water soluble or lipid-soluble, depending on whether they act primarily in the aqueous phase or in the lipophilic region of cell membranes. Hydrophilic antioxidants include ascorbic acid and urate. Ubiquinols, retinoids, carotenoids, flavonoids, and tocopherol are representative lipid-soluble antioxidants. Plasma proteins, glutathione, and urate are endogenous, whereas ascorbic acid, carotenoids, retinoids, flavonoids, and tocopherols constitute some of the dietary antioxidants. These compounds possess the potential to scavenge and quench various radicals and ROS. Certain radical scavengers are not recyclable, however, others are recycled through the intervention of a series of enzyme systems or other nonenzymic antioxidant systems.[1,6]

Flavanoids with potential antioxidant activity are of particular interest among naturally occurring substances.[7] Based on safety and other pragmatic reasons, the number of active substances used as antioxidants is

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restricted to a few phenolic substances. The flavonoids are an unusually large group of naturally occurring phenolic compounds ubiquitously distributed in the plant kingdom. These aromatic compounds are formed in plants from the aromatic amino acids, phenylalanine, tyrosine, and acetate units. Phenylalanine and tyrosine are converted to cinnamic acid and *p*-coumaric acid that condense with acetate units to form the cinnamoyl structure of the flavonoid.[8] The use of natural antioxidants in food is limited due to the lack of knowledge about their molecular compositions, the content of active compounds in the raw materials and the availability of relevant toxicological data. Hence, the search for newer natural antioxidants, especially of plant origin has ever since increased.[9]

Orchids constitute the largest family of the flowering plants. A number of physiologically active alkaloids such as dendrobine and its structural analogues were isolated from the genes *Dendrobium*. 9,10-Dihydro-5H-phenanthro-(4,5 bcd)-pyrans and pyrones were isolated from a number of species like *Coelogyne*, *Pholidota*, and *Otochilus*.[10] Recently, *trans*-3,4,3′,5′-tetrahydroxystilbene is reported to possess a promising chemopreventative agent with anti-leukemic activity and is being extensively studied in various cancers including colorectal and lung cancer as well as in cardiovascular disease *trans*-3,4,3′,5′-tetrahydroxystilbene is structurally similar to the anti-cancer stilbene resveratrol also found in red wine, and preliminary data suggest that resveratrol is metabolized to piceatannol via cytochrome P4501B1.[11] Bioautographic tests with *Cladosporium herbarum* displayed antifungal activity for stilbenoids. Ten derivatives stilbenoids were found to inhibit five microfungi using the microdilution technique linked with digital image analysis of germ tubes.[12]

Besides this, hydroxylated stilbenes viz., *trans*-resveratrol has been reported to provide protection against cardiovascular diseases by having lipid-lowering activity and by inhibiting lipid peroxidation in humans. It was found to be a potent inhibitor of tyrosine kinase (p56lck) and has been widely reported to possess antifungal properties. Resveratrol and piceid exert anticarcinogenic effects and piceid has been shown to improve blood microcirculation. These effects may not only be the outcome of stilbenes' antioxidant capacity but also be due to their structural resemblance to tyrosine.[13] Literature review revealed that, there is no reports on antioxidant activity of flavidin/2,7-dihydroxy-9,10-dihydrophenanthro-4,5-bcd-pyran (Fig. 1) type of compounds. Hence, the present work investigates the possible antioxidative effects of flavidin. In the present study, the antioxidant activity of flavidin [2,7-dihydroxy,9,10-dihydro-phenanthro-4,5-bcd-pyran] employing various in vitro assay systems is reported. This is first report on the antioxidant activity of this class of compounds.

2. Results and discussion

Flavidin was isolated from orchid and it was purified on silica gel column chromatography. TLC analysis of isolated compound showed single spot indicated that the compound is pure. The structure of the isolated compound was identified using ^{1}H NMR spectral data. It showed signals at 6.46 (d, 2.0 Hz, 1H), 6.33 (d, 2.2 Hz), 6.31 (d, 2.0 Hz, 1H), 6.60 (d, 2.0 Hz, 1H), 2.85 (s, 4H), and 5.08 (s, 2H). ^{13}C NMR chemical shifts are as follows, 108.7 (C-1), 154.8 (C-2), 101.5 (C-3), 156.4 (C-4), 112.5 (C-4a), 119.9 (C-4b), 132.8 (C-5), 109.5 (C-6), 153.6 (C-7), 115.7 (C-8), 138.7 (C-8a), 27.2 (C-9), 27.4 (C-10), 138.6 (C-10a), and 66.8 (CH_2). Mass spectrum showed fragments (*m*/*z*) at 240 (M^+, 88%), 239 (100%), 238 (7%), 181 (10%), 152 (8%), 120 (15%). On the basis of above spectral data the isolated compound was characterized as flavidin.

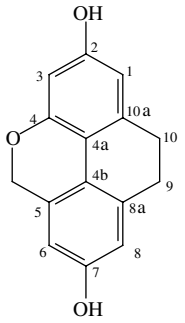

Figure 1. Structure of flavidin.

The antioxidative activity of flavidin has been demonstrated earlier in one model system.[14] However, the ability of flavidin to scavenge free radicals in chemical and biological systems has not been comprehensively investigated. Accordingly, this study primarily aims to elucidate the antioxidant attributes of flavidin, employing a wide range of well-established in vitro systems to gain mechanistic insights.

The antioxidant activity of flavidin and BHA at different concentrations was estimated by bleaching of β-carotene as model systems are shown in Figure 2. The controls (no additive) are decolorized within 120 min, indicating that rapid oxidation occurred. The addition of flavidin and BHA at different concentrations enhanced the bleaching time of β-carotene. Flavidin at 10 ppm concentration showed 68.5% antioxidant activity and at

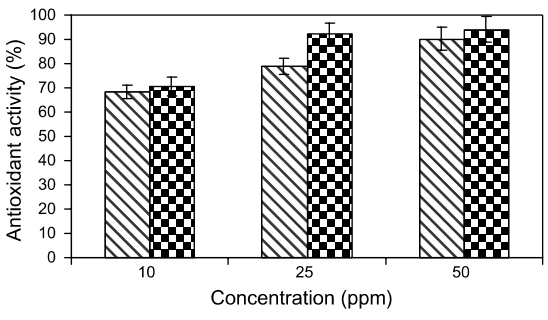

Figure 2. Antioxidant activity of flavidin and BHA at different concentrations by β-carotene-linoleate model system; ▨ flavidin, ▩ BHA.

50 ppm showed maximum activity of 90.2% in 120 min. The high antioxidant activity of flavidin may be due to the presence of two phenolic hydroxyl groups along with three methylenes, which can scavenge the radical oxygen species.

Free radical scavenging potentials of flavidin and BHA at different concentrations were tested by DPPH method and the results are depicted in Figure 3. Antioxidant reacts with DPPH, which is a nitrogen-centered radical with a characteristic absorption at 517 nm and convert it to 1,1,-diphenyl-2-picryl hydrazine, due to its hydrogen donating ability at a very rapid rate.[15] The degree of discoloration indicates the scavenging potentials of the antioxidant. At 40 ppm, flavidin and BHA exhibited 95.6% and 93.3% free radical scavenging activity, respectively, by this method. The activity of the flavidin is attributed to their hydrogen donating ability.[16] It is well known that free radicals cause autooxidation of unsaturated lipids in food.[17] On the other hand, antioxidants are believed to intercept the free radical chain of oxidation and to donate hydrogen from the phenolic hydroxyl groups, thereby forming stable end product, which does not initiate or propagate further oxidation of lipid.[18] The data obtained reveal that the flavidin is free radical inhibitor and primary antioxidant that react with DPPH radical, which may be attributed to its hydrogen donating ability.

Free oxygen centered and nitrogen-centered radicals ($ROO^{\cdot}$, $RO^{\cdot}$, DPPH radical) react with phenols (ArOH) via two different mechanisms such as a direct abstraction of phenol hydrogen atom by DPPH radical and an electron-transfer process from AROH or ArO^{-} to $X^{\cdot}$.[19–21] Therefore, the antioxidative property of flavidin may be attributed not only to the hydrogen donating ability but also to other physical properties, that is oxidation potential. As intrinsically electron rich compounds such as flavidin is prone to enter into electron-donation reaction with oxidizing agent such as DPPH. Typically, an electron is transferred from flavidin to the unfilled orbital of a one electron oxidant such as peroxyl radical, followed by rapid proton transfer. The net result is the equivalent of a hydrogen atom transfer from the flavidin hydroxyl groups to the free radical. The product phenoxyl radical such as 9,10-dihydro-5H-phenanthro-(4,5 bcd)-pyran radical is stabilized by resonance delocalization of the unpaired electron to the *ortho* and other positions of the ring. In order to act as an effective antioxidant, the phenoxyl radical must not be reactive enough to initiate further free radical reactions on its own. Whereas phenol itself is a rather ineffective antioxidant, when other radical-stabilizing features are accentuated, extremely potent antioxidants can result. In addition to the intrinsic stability of the radical that results from resonance properties, these characteristics can be intensified by various structural features, such as steric and inductive effects of ring substituents. The various structural features of different compounds impart very different reactivities toward oxidants of moderate activity.

The phosphomolybdenum method is based on the reduction of Mo(VI) to Mo(V) by the antioxidant compounds and the formation of a green Mo(V) complex, which has a maximal absorption at 695 nm. Flavidin and propyl gallate exhibited various degrees of antioxidant capacity. The antioxidant capacity of flavidin and propyl gallate showed 5993.8 ± 410 and 5158.4 ± 934 μmol/g (as equivalent to ascorbic acid), respectively, at 25 ppm.

Hydrogen peroxide is generated in vivo by several oxidase enzymes and by activated phagocytes and it is known to play an important role in the killing of several bacterial and fungal strains.[22] There is increasing evidence that, hydrogen peroxide, either directly or indirectly via its reduction product, $OH^{\cdot}$, can act as a messenger molecule in the synthesis and activation of several inflammatory mediators.[23] When a scavenger is incubated with H_2O_2 using a peroxidase assay system, the loss of H_2O_2 can be measured. Figure 4 shows the scavenging ability of flavidin and ascorbic acid on hydrogen peroxide at different concentrations. Flavidin was capable of scavenging hydrogen peroxide in an amount dependent manner at all the tested concentrations. Hydrogen peroxide itself is a rather weak oxidant and most organic compounds (except for some sulfur-containing molecules) are virtually inert to attack by it at ordinary environmental or cellular concentrations and temperatures. In the presence of reduced transition metal ions, however, hydrogen peroxide is converted to

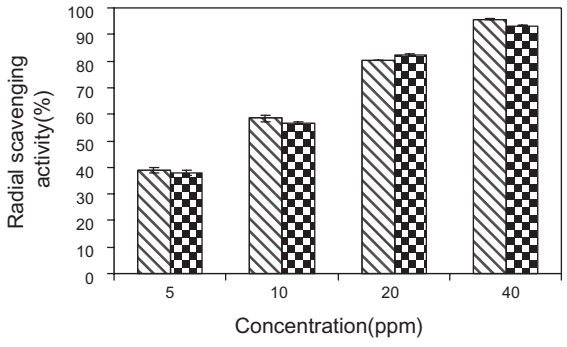

Figure 3. Radical scavenging activity by flavidin and BHA at different concentrations (ppm) by DPPH method; ▨ flavidin, ▩ BHA.

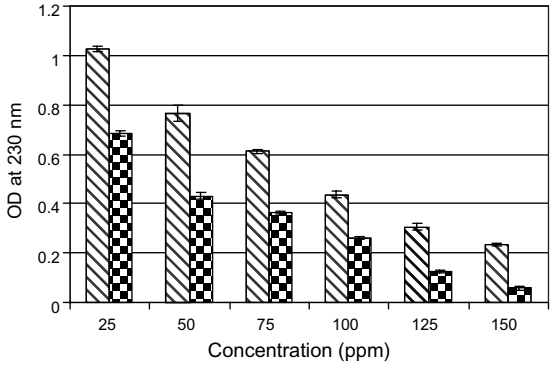

Figure 4. Scavenging of hydrogen peroxide using flavidin and ascorbic acid at different concentrations; ▨ flavidin, ▩ ascorbic acid.

the much more reactive oxidant, hydroxyl radical in the cells by Fenton reaction. Besides this, studies have shown that other transition metals such as copper(I), cobalt(II), and nickel(II) also take part in the process.[22] Thus, the removing is very important for antioxidant defense in cell or food systems.

The presence of this class of compounds in *Orchidaceae* plants,[10] indicate that extracts of these plants may play an important role as natural antioxidants. Incidentally, stilbenols precursors of 9,10-dihydrophenanthrene in the biosynthesis[24] exhibited antioxidant activity in thermoplastics.[25] Matsuda et al.,[26] reported the structural requirements of stilbenes for their antioxidant activity. It was reported that, the antioxidant activity of stilbenes due to the presence of phenolic hydroxyl groups. Kereem et al.,[13] reported antioxidant activity of resveratrol, 5,4′-dihydroxy-3-methoxystilbene and 3,5-dihydroxy-4′-methoxystilbene using scavenging of the radical cation of 2,2-azinobis-3-ethylbenzthiazoline-6-sulfonic acid (ABTS). It was observed that, 3,5-dihydroxy-4′-methoxystilbene found to possess low activity as compared to other compounds. It was concluded that, the high activity of other two compounds is due to the presence of 4′-hydroxyl groups. Flavidin is related to the analogues of above compounds structurally. It must have derived biogenetically from 9,10-dihydrophenanthrenes, which are formed biosynthetically from stilbenols. Stilbeneols are abundant in the species of *Orchidaceae*. The results obtained in the present study clearly demonstrate that the flavidin, which can effectively scavenge various reactive oxygen species/free radicals under in vitro conditions. This may be due to the number of stable oxidized products that it can form after oxidation or radical scavenging.

3. Experimental

β-Carotene, DPPH, and butylated hydroxyanisole (BHA) were obtained from Sigma Chemical Co., (St. Louis, MO). Tween 40 was obtained from Himedia Ltd, India. Silica gel (mesh size 60–120), ascorbic acid, and H_2O_2 were obtained from Merck, India. All solvents/chemicals used were of analytical grade and obtained from Merck, Mumbai, India. UV–visible spectra measurements were done using Genesys-5 UV–visible spectrophotometer (Milton Roy, NY, USA). ^1H and ^{13}C NMR spectra were recorded at 400 and 100 MHz, respectively, on a Bruker AMX 400 FT instrument (Bruker, Rheinstetten, Germany). TMS was used as internal standard. Mass spectrum was recorded using a QP-5000 (Quadrapole) Mass Spectrometer (Shimadzu, Kyoto, Japan). Elemental analyses were recorded on Varioel elemental analyzer (Elementar Americas, Inc. NJ, USA).

4. Isolation and purification of flavidin

Flavidin was isolated and identified as per procedure of Veerraju et al.[10] The fraction containing flavidin was further purified by column chromatography. Fraction (400 mg) containing flavidin was impregnated with silica gel (mesh size 60–120) (1.0 g) and loaded on to a 10 g silica column and eluted with chloroform. First two fractions 200 mL each contains other minor compounds. Flavidin was eluted with 1% MeOH in chloroform. Eluates were evaporated *under vacuum* and crystallized from chloroform (305 mg).

5. Identification

Mp 210 °C exhibited violet color with ferric chloride. TLC was carried out using hexane–EtOAc as a developing solvent and spot was developed when plate was sprayed with 10% sulfuric acid in methanol followed by heating at 110 °C. Analyzed for C, 74.89; H, 5.05 $C_{15}H_{12}O_3$ requires C, 74.99; H, 5.03. Further, the structure of isolated compound was identified from NMR and mass spectral data.

6. Antioxidant assay by β-carotene-linoleate model system

The procedure of Hidalgo et al.[27] was followed for testing of antioxidant activity of the flavidin with minor modification. The assay reagent was prepared as follows. β-Carotene (0.2 mg) in 0.5 mL of chloroform was added to 20 mg of linoleic acid and 200 mg of Tween 40 (polyoxyethylene sorbitan monopalmitate) were mixed. Tween 40 was warmed in a water bath at 50 °C before use. The chloroform was removed at 40 °C under vacuum using a rotary evaporator. The resulting solution was immediately diluted with 10 mL of triple-distilled water and the emulsion was mixed well for 1 min. The emulsion was further diluted with 40 mL of oxygenated water before using. Aliquots 4 mL of this reagent was transferred into different tubes containing 0.2 mL of the desired amount of antioxidant in ethanol. A control consisting of 0.2 mL of ethanol and 4 mL of emulsion was prepared. Readings of all samples were taken immediately ($t = 0$) and at 15 min intervals for 2 h ($t = 120$). The tubes were placed in a water bath at 50 °C between measurements. All determinations were performed in duplicate. Measurement of color was recorded until the color of β-carotene disappears. The antioxidant activity (AA) of the isolated compound was evaluated in terms of bleaching the β-carotene using the formula of Hidalgo et al., (1994). $AA = 100[1 - (A_0 - A_t)/(A_0^\circ - A_t^\circ)]$, where A_0 and A_0° is the absorbance measured at the beginning of the incubation for flavidin and control, respectively. A_t and A_t° are the absorbance measured for flavidin and control after incubation for 120 min, respectively.

7. Radical scavenging activity using DPPH method

Different concentrations (5, 10, 20, and 40 μL equivalent to 5, 10, 20, and 40 ppm) of flavidin and BHA were taken in different test tubes. The volume of the sample/BHA was adjusted to 100 μL by adding MeOH. Methanolic solution (5.0 mL) of DPPH (100 μM) was added to these tubes and shaken vigorously. The tubes

were allowed to stand at 27 °C for 20 min.[28] The control was prepared as above without flavidin and MeOH was used for the baseline correction. The changes in the absorbance of the flavidin and BHA were measured at 517 nm. Radical scavenging activity was expressed as the inhibition percentage and was calculated using the following formula: %Radical scavenging activity = (control OD − sample OD/control OD) × 100.

8. Evaluation of antioxidant capacity by phosphomolybdenum method

The total antioxidant capacity of flavidin was evaluated by the method of Prieto et al.[29] An aliquot of 0.1 mL of sample solution/propyl gallate (equivalent to 25 ppm) was combined with 1 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). In case of blank 0.1 mL of methanol was used in place of sample. The tubes were capped and incubated in a boiling water bath at 95 °C for 90 min. After the samples had cooled to room temperature, the absorbance of the aqueous solution of each was measured at 695 nm against blank in Genesys-5-UV–visible spectrophotometer (Milton Roy, New York, USA). For samples of unknown composition, water soluble antioxidant capacity was expressed as equivalents of ascorbic acid (μmol/g).

9. Scavenging of hydrogen peroxide

The ability of flavidin to scavenge hydrogen peroxide was determined according to the method of Ruch et al.[30] A solution of hydrogen peroxide (20 mM) was prepared in phosphate buffered saline (PBS; pH 7.4). Hydrogen peroxide concentration was determined spectrophotometrically from absorption at 230 nm by using the molar absorptivity of $81\,M^{-1}cm^{-1}$. Different concentrations of flavidin (25, 50, 75, 100, 125, and 150 ppm) in ethanol was added to hydrogen peroxide and absorbance at 230 nm was determined after 10 min against a blank solution that contained extracts in PBS without hydrogen peroxide.

10. Conclusion

The results obtained in the present study clearly demonstrate that the flavidin, which can effectively scavenge various reactive oxygen species/free radicals under in vitro conditions. This may be due to the number of stable oxidized products that it can form after oxidation or radical scavenging. The broad range of activity of the flavidin suggests that multiple mechanisms are responsible for the antioxidant activity. The multiple antioxidant activity of flavidin demonstrated in this study clearly indicates the potential application value of the orchids. However, the in vivo safety of flavidin needs to be thoroughly investigated in experimental rodent models prior to its possible application as an antioxidant ingredient, either in animal feeds or in human health foods. The above results showed that, 9,10-dihydrophenathropyrans could exhibit antioxidant properties approximately comparable to commercial synthetic antioxidants. Further studies, on the use of *Orchidaceae* plants for their antioxidant role in various systems may provide potential natural antioxidants.

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References and notes

1. Chan, H. W. S. The Mechanism of Autoxidation, Autoxidation of Unsaturated Lipids. In *Food Science and Technology, A Series of Monographs*; Chan, H. W. S., Ed.; Academic: London, 1987; pp 1–16.
2. Shahidi, F.; Wanasundra, U. N.; Amarowicz, R. *Food Res. Int.* **1994**, *27*, 489.
3. Chang, S. S.; Ostric-Matyasevic, B.; Hsieh, O. A. L.; Huang, C. L. *J. Food Sci.* **1977**, *42*, 1102.
4. Ito, N.; Fukushima, S.; Tsuda, H. *CRC Crit. Rev. Toxicol.* **1985**, *15*, 109.
5. Ito, N. S.; Fukushima, S.; Tamano, M.; Horoe, A.; Hagiwara, A. *J. Natl. Cancer Inst.* **1986**, *77*, 1261.
6. Barlow, S. M. Toxicological Aspects of Antioxidants Used as Food Additives. In *Food Antioxidants*; Hudson, B. J. F., Ed.; Elsevier: London, 1990; pp 253–307.
7. Milovanovic, M.; Picuric-Jovanovic, K.; Vucelic-Radovic, B.; Vrbaski, Z. *J. Am. Oil Chem. Soc.* **1996**, *73*, 773.
8. Kandaswami, C.; Middleton, E. Free Radical Scavenging and Antioxidant Activity of Plant Flavonoids, Free Radicals in Diagnostic Medicine. In *A Systems Approach to Laboratory Technology, Clinical Correlations, and Antioxidant Therapy, Advances in Experimental Medicine and Biology*; Armstrong, D., Ed.; Plenum: New York, 1994; Vol. 366, pp 351–376.
9. Amarowicz, R.; Wanasundara, U. N.; Karamac, M.; Shahidi, F. *Nahrung* **1996**, *40*, 261.
10. Veerraju, P.; Prakasa Rao, N. S.; Jagan Mohan Rao, L.; Jagannadha Rao, K. V.; Mohana Rao, P. R. *Phytochemistry* **1989**, *28*, 3031.
11. Wolter, F.; Clausnitzer, A.; Akoglu, B.; Stein, J. *J. Nutr.* **2002**, *132*, 298–302.
12. Pacher, T.; Seger, C.; Engelmeier, D.; Vajrodaya, S.; Hofer, O.; Greger *J. Nat. Prod.* **2002**, *65*, 820–827.
13. Kereem, Z.; Regev-Shoshani, G.; Flaishman, M. A.; Sivan, L. *J. Nat. Prod.* **2003**, *66*, 1270.
14. Jaganmohan Rao, L.; Jayaprakasha, G. K.; Sakariah, K. K. U.S. Patent No. 6,503,552, 7th January 2003.
15. Yamaguchi, T.; Takamura, H.; Matoba, T.; Terao, J. *Biosci. Biotechnol. Biochem.* **1998**, *62*, 1201.
16. Shimada, K. K.; Fujikawa, K. Y.; Nakamura, T. *J. Agric. Food Chem.* **1992**, *40*, 945.
17. Kaur, H.; Perkins, J. The Free Radical Chemistry of Food Additives. In *Free Radicals and Food Additives*; Aruoma, O. I., Halliwell, B., Eds.; Taylor and Francis: London, 1991, pp 17–35.
18. Sherwin, E. R. *J. Am. Oil Chem. Soc.* **1978**, *55*, 809.
19. Avila, D. V.; Ingold, K. U.; Lusztyk, J. *J. Am. Chem. Soc.* **1995**, *117*, 2929.
20. Banks, J. T.; Ingold, K. U.; Lusztyk, J. *J. Am. Chem. Soc.* **1996**, *118*, 6790.
21. MacFaul, P. A.; Ingold, K. U.; Lusztyk, J. *J. Org. Chem.* **1996**, *61*, 1316.

22. Halliwell, B.; Aeschbach, R.; Lolliger, J.; Aruoma, O. I. *Food Chem. Toxicol.* **1995**, *33*, 601.
23. Sprong, R. C. A.; Winkelhuyzen-Jansen, C.; Aarsman, J.; Van Oirschot, T.; Van deer Bruggen; Van Asbeck, B. *J. Am. J. Crit. Care Med.* **1998**, *157*, 1283–1293.
24. Luckner, M. *Sceondary Metabolism in Microorganism, Plants and Animals*; Springer: Berlin, 1984; p 452.
25. Minn, J.; Daly, W. H.; Negulescu, I. I.; McMurtrey, K. D.; Schultz, T. P. *J. Agric. Food Chem.* **1996**, *44*, 2946.
26. Matsuda, H.; Morikawa, T.; Toguchida, I.; Park, J. Y.; Harima, S.; Yoshikawa, M. *Bioorg. Med. Chem.* **2001**, *9*, 41.
27. Hidalgo, M. E.; Fernandez, E.; Quiehot, W.; Lissi, E. *Phytochemistry* **1994**, *37*, 1585.
28. Blios, M. S. *Nature* **1958**, *181*, 1199.
29. Prieto, P.; Pineda, M.; Aguilar, M. *Anal. Biochem.* **1999**, *269*, 337.
30. Ruch, R. J.; Cheng, S. J.; Klannig, J. E. *Carcinogensis* **1989**, *10*, 1003.