# Synthetic and Natural Coumarins as Antioxidants

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**Abstract:** Coumarins, an old class of compounds, are naturally occurring benzopyrene derivatives. A lot of coumarins have been identified from natural sources, especially green plants. These natural compounds have served as valuable leads for further design and synthesis of more active analogs. The pharmacological and biochemical properties and therapeutic applications of simple coumarins depend upon the pattern of substitution. Coumarins have attracted intense interest in recent years because of their diverse pharmacological properties. Among these properties, their antioxidant effects were extensively examined. In this review, plant derived coumarins and their synthetic analogs will be systematically evaluated based on their plant origin, structure-activity relationship and antioxidant efficacy. Owing their diverse effects and inconclusive results from different *in vitro* studies, the mechanism of their action has not yet been fully understood and the correlation of effects with chemical structure is not conclusive at the moment. It is the objective of this review will be to summarize experimental data for different coumarins used as antioxidant agents, because promising data have been reported for a series of these agents. In addition, their ability to bind metal ions represents an additional means of modulating their pharmacological responses.

**Keywords:** Synthetic and natural coumarins; antioxidants.

## INTRODUCTION

The balance between formation and elimination of free radicals determines the overall stability of a living body. Free radicals originate from large variety of normal and pathological metabolic transformations, from host-defense against undesirable invasion (chemical or biological), and from host-response to a disturbance of the tissues' integrity (due to trauma, cellular damage, etc). Free-radical chain reactions in the body are initiated mostly by Reactive Species (RS - molecules, ions, free-radicals), possessing oxygen (ROS) or nitrogen (RNS) atom with an unpaired electron. If more RS formed than needed for the normal redox-signaling and self-defense of the host, oxidative stress (OS) occurs leading to an oxidative cellular damage, even to cellular death. Free-radicals induce the cell damage by altering the biological activities of lipids, proteins, DNA and carbohydrates [1]. Damaged cells function incorrectly, which may result in a further escalation of the oxidative stress and to an enhanced tissue damage. Simultaneous ionic disbalance, mitochondrial dysfunction and activation of the caspase/calpine cascades, result in a cell death [1]. The negative effects of the RS may be diminished by limiting their synthesis (control over metabolic transformations and enzymes producing RS), by recombination of the RS already formed (e.g. "radicals scavenging"), and by altering the RS-effects. Inhibitors of the RS synthesis (NADPH- oxidases, XO- inhibitors; leukocytes' antibodies) may be administered. Some substances directly recombine free radicals and in this way interrupt the initiation and/or propagation of the free-radical induced chain reactions.

Coumarins comprise a group of natural compounds found in a variety of plant sources. The very long association of plant coumarins with various animal species and other organisms throughout evolution may account for the extraordinary range of biochemical and pharmacological activities of these chemicals in mammalian and other biological systems. The coumarins that were studied have diverse biological properties and various effects on the different cellular systems. A lot of biological parameters should be evaluated to increase our understanding of mechanisms by which these coumarins act. Coumarins have important effects in plant biochemistry and physiology, acting as antioxidants, enzyme inhibitors and precursors of toxic substances. In addition, these compounds are involved in the actions of plant growth hormones and growth regulators, the control of respiration, photosynthesis, as well as defense against infection. The coumarins have long been recognised to possess anti-inflammatory, antioxidant, antiallergic, hepatoprotective, antithrombotic, antiviral, and anticarcinogenic activities. The pharmaceutical use of coumarins therefore has excellent potential. A broad array of medicinal applications of coumarins has been investigated, and several recent reviews summarize advances in these fields, especially concerning their antioxidant properties [1-8]. The hydroxycoumarins are typical phenolic compounds and, therefore, act as potent metal chelators and free radical scavengers. They are powerful chain-breaking antioxidants. The coumarins display a remarkable array of biochemical and pharmacological actions, some of which suggest that certain members of this group of compounds may significantly affect the function of various mammalian cellular systems.

The coumarins are extremely variable in structure, due to the various types of substitutions in their basic structure, which can influence their biological activity. A careful structure-system-activity-relationship study of coumarins with special respect to their antioxidant and cancer-preventing activities should be conducted. A vast majority of coumarins, completely innocuous in plants may be beneficial

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in a variety of human disorders, in spite of some ongoing controversy.

The naturally occurring coumarins will be the primary focus of this review, with occasional reference to synthetic compounds. There has been, in recent years, a major rekindling of interest in pharmacognosy. Coumarins turn out to be present in many natural therapeutically utilised products. They hold a place apart in view of their antioxidant activity. It was suggested that alterations in the chemical structure of coumarins could change their antioxidant and cytotoxic properties.

Coumarin (1,2-benzopyrone) (**1**), the parent molecule of coumarin derivatives, is the simplest compound of a large class of naturally occurring phenolic substances made of fused benzene and α-pyrone rings. The investigation of coumarin compounds revealed that a wide spectrum of medicinal plant extracts that were in use as early as 1000 A.D., which contained a high content of coumarins.

## SYNTHETIC COUMARINS AS ANTIOXIDANTS

Several linear and angular coumarins designed and synthesised as possible anti-inflammatory and antioxidant agents were evaluated for their biological activities, using the carrageenin-induced rat paw edema model [9]. In general, the compounds were found to be potent anti-inflammatory agents. The compounds were found to interact with 1,1-diphenyl-2-picryl-hydrazyl stable free radical (DPPH) whereas most of them were essentially inactive in other tests. The anti-inflammatory activity seemed to be connected with their reducing activity. R(M) values were determined as an expression of their lipophilicity which was also calculated as Clog P. A poor relationship existed between lipophilicity and anti-inflammatory activity, however.

Khan *et al*. [10] reported the modulatory effect of coumarin (1,2-benzopyrone) on potassium bromate ($KBrO_3$) mediated nephrotoxicity in Wistar rats. $KBrO_3$ (125 mg/kg body weight, *i.p.*) enhanced gamma-glutamyl transpeptidase, renal lipid peroxidation, xanthine oxidase and hydrogen peroxide ($H_2O_2$) generation with reduction in renal glutathione content and antioxidant enzymes. It also enhanced blood urea nitrogen, serum creatinine, ornithine decarboxylase (ODC) activity and [$^3$H]-thymidine incorporation into renal DNA. Treatment of rats orally with coumarin (10 mg/kg body weight and 20 mg/kg body weight) resulted in a significant decrease in gamma-glutamyl transpeptidase, lipid peroxidation, xanthine oxidase, $H_2O_2$ generation, blood urea nitrogen, serum creatinine, renal ODC activity and DNA synthesis ($P < 0.001$). Renal glutathione content ($P < 0.01$) and antioxidant enzymes were also recovered to significant level ($P < 0.001$). These results showed that coumarin may be used as an effective chemopreventive agent against $KBrO_3$-mediated renal oxidative stress, toxicity and tumor promotion response in Wistar rats.

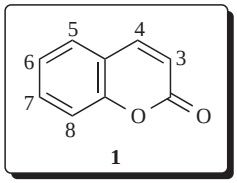
Structurally diverse plant phenolics were examined for their ability to inhibit lipid peroxidation induced either by Fe(II) and Fe(III) metal ions or by azo-derived peroxyl radicals in a liposomal membrane system [11]. The antioxidant abilities of flavonoids were compared with those of coumarin and tert-butylhydroquinone (TBHQ). The antioxidant efficacies of these compounds were evaluated on the basis of their ability to inhibit the fluorescence intensity decay of an extrinsic probe, 3-(p-(6-phenyl)-1,3,5-hexatrienyl)phenylpropionic acid (DPH-PA), caused by the free radicals generated during lipid peroxidation. All the flavonoids tested exhibited higher antioxidant efficacies against metal-ion-induced peroxidations than peroxyl-radical-induced peroxidation, suggesting that metal chelation may play a larger role in determining the antioxidant activities of these compounds than has previously been believed. Distinct structure-activity relationships were also revealed for the antioxidant abilities of the flavonoids and coumarins. The presence of hydroxyl substituents on the flavonoid and coumarin nucleus enhanced activity, whereas substitution by methoxy groups on the same position diminished antioxidant activity.

The antiradical activity of coumarin reductones was investigated by the method of inhibition of Fe(II) induced chemiluminescence of egg-yolk lipoproteins [12]. All coumarins studied exhibited high antioxidant activity. The dependence of chemiluminescence intensity on the antioxidant concentration showed that coumarin reductions resemble their chemical analog-ascorbic acid rather than the lipid antioxidant butylated hydroxytoluene (ionol).

7-Hydroxycoumarin ((**2**), refer Table **1**) is the main coumarin (1,2-benzopyrone) metabolite of coumarin and is a therapeutically active molecule. It exhibits antioxidant properties *in vitro* and may share with other coumarin derivatives vasodilator effects. The aim of the study of Baccard *et al*. [13] was to assess the effects of 7-hydroxycoumarin on isolated perfused and ischemic-reperfused rat heart. After a 10-min perfusion, an increase in the coronary flow was observed with 7- hydroxycoumarin at $10^{-4}$ mol/l ($p = 0.002$) as well as an increase in left ventricular developed pressure with 7- hydroxycoumarin at $10^{-5}$ mol/l ($p = 0.038$). The increase in coronary flow is not solely explained by the increase in inotropism. It appears to be also induced through a direct vasodilator effect which, however, does not involve the release of vasodilator prostaglandins since it was not inhibited by indomethacin. After the 30-min global ischemia and the 45-min reperfusion period, 7- hydroxycoumarin at $10^{-5}$ mol/l induced an increase in left ventricular developed pressure ($p = 0.042$) and in the ratio of heart rate x left ventricular developed pressure over oxygen consumption ($p = 0.036$).

The cytosensor microphysiometer (a biosensing instrument for detecting cellular metabolism) was compared to the established tetrazolium salt assay as a chemosensitivity test. Two coumarin compounds, 7-hydroxycoumarin and esculetin, 6,7-dihydroxycoumarin ((**3**), refer Table **1**), were examined to determine their effect on the cellular metabolism of A431 cells over a 24-h exposure period [14]. In the tetrazolium salt assay, 7-hydroxycoumarin caused suppression of the succinate dehydrogenase activity at concentrations greater than 10 μg/ml. Esculetin exerted a more serious effect on succinate dehydrogenase, with decreases in activity observed at greater than 1 μg/ml. The observed effect was dose-dependent for both compounds examined. The metabolic activities of cells exposed to 100 μg/ml of drug were 90.37 +/- 2.8 and 71.62 +/- 2.96 ($n = 3$), of control values, for 7-hydroxycoumarin and esculetin, respectively. Using the cytosensor

**Table 1. Structures of Coumarins Used as Antioxidants**




**1**

| Compound | | R3 | R4 | R5 | R6 | R7 | R8 |
|---|---|---|---|---|---|---|---|
| **1** | Coumarin | H | H | H | H | H | H |
| **2** | 7-hydroxycoumarin (Umbelliferone) | H | H | H | H | OH | H |
| **3** | 6,7-dihydroxycoumarin (Esculetin) | H | H | H | OH | OH | H |
| **4** | Esculin | H | H | H | OGl | OH | H |
| **5** | 4-hydroxycoumarin | H | OH | H | H | H | H |
| **6** | 7,8-dihydroxy-4-methylcoumarin | H | Me | H | H | OH | OH |
| **10** | 4-methyl-7-hydroxycoumarin (Mendiaxon) | H | Me | H | H | OH | H |
| **12** | 4-methyl-6,7-dihydroxycoumarin | H | Me | H | OH | OH | H |
| **13** | 7,8-dihydroxy-6-methoxycoumarin (Fraxetin) | H | H | H | OMe | OH | OH |
| **14** | 4-methylcoumarin | H | Me | H | H | H | H |
| **15** | 7,8-diacetoxy-4-methylcoumarin | H | Me | H | H | acetoxy | acetoxy |
| **16** | 3-hydroxycoumarin | OH | H | H | H | H | H |
| **17** | 3-aminocoumarin | $NH_2$ | H | H | H | H | H |
| **18** | 3-acetylaminocoumarin | acetylamino | H | H | H | H | H |
| **19** | Coumarin-3-carboxylic acid | COOH | H | H | H | H | H |
| **20** | 3-hydroxyscopoletin | OH | H | H | OMe | OH | H |
| **21** | 3-hydroxyisoscopoletin | OH | H | H | OH | OMe | H |
| **22** | 3,7-dihydroxycoumarin (3-hydroxyumbelliferone) | OH | H | H | H | OH | H |
| **23** | Scoparone | H | H | H | OMe | OMe | H |
| **27** | Scopoletin | H | H | H | OMe | OH | H |
| **28** | 6,7-dihydroxy-4,4-dimethylhydrocoumarin | 2H | 2Me | H | OH | OH | H |
| **29** | Auraptene | H | H | H | H | OGer | H |

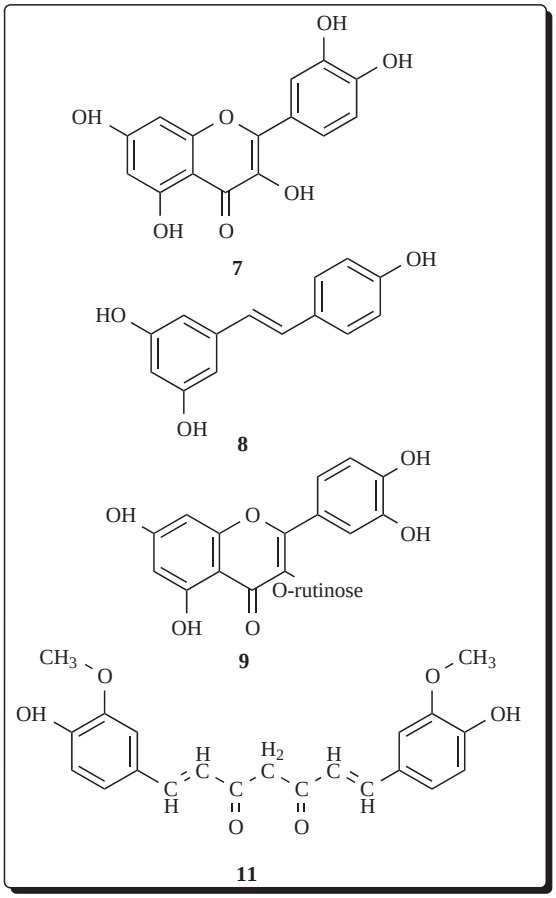
microphysiometer to assess metabolic activities, a similar pattern of inhibition was observed, with esculetin more detrimental to cellular metabolism than 7-hydroxycoumarin. The effect was dose- and time-dependent for both compounds. 7-Hydroxycoumarin (100 μg/ml) caused the cellular metabolic rate to drop to 44.21 +/- 5.34% (n = 4) of the control metabolic rate, while 100 μg/ml esculetin caused the metabolic rate to fall to 21.5 +/- 4.54% (n = 4) of the control rate. The cytosensor method proved to be superior to the tetrazolium salt assay for a number of reasons, which are discussed in [14].

Increasing evidence regarding free radical-generating agents and inflammatory processes suggests that accumulation of reactive oxygen species can cause hepatotoxicity. A short-chain analog of lipid hydroperoxide, t-butyl hydroperoxide (t-BHP), can be metabolised to free radical intermediates by cytochrome P-450 in hepatocytes, which, in turn, can initiate lipid peroxidation, affect cell integrity and result in cell injury. Lin *et al*. [15] used t-BHP to induce hepatotoxicity *in vitro* and *in vivo* and determined the antioxidative bioactivity of esculetin, a coumarin compound. The investigations showed that pretreatment with esculetin (5-20 μg/ml) significantly decreased the leakage of lactate dehydrogenase (LDH) and alanine transaminase (ALT), and also decreased the formation of malondialdehyde (MDA) in primary cultured rat hepatocytes induced by a 30-min treatment with t-BHP. An *in vivo* study in rats showed that pretreatment with esculetin (*i.p.*) at concentrations of 0.5 and 5 mg/kg for 5 days before a single *i.p.* dose of t-BHP (0.1 mmol/kg) significantly lowered the serum levels of the hepatic enzyme markers (ALT and AST) and reduced oxidative stress in the liver. Histopathological evaluation of the rat livers revealed that esculetin reduced the incidence of liver lesions induced by t-BHP, including hepatocyte swelling, leukocyte infiltration, and necrosis. Based on the results described above, the authors speculated that esculetin may play a chemopreventive role *via* reducing oxidative stress in living systems.

The effects of coumarin and its derivatives on rat platelet lipoxygenase and cyclooxygenase activities were studied by Sekiya *et al*. [16]. Esculetin (6,7-dihydroxycoumarin) was found to inhibit the lipoxygenase more strongly than the cyclooxygenase; its concentration for 50% inhibition ($IC_{50}$) was 0.65 μM for platelet lipoxygenase and 0.45 mM for platelet cyclooxygenase. Esculin (the 6-glucoside of esculetin) ((**4**), refer Table **1**) and umbelliferone [7-hydroxycoumarin, (**2**)] also selectively inhibited the lipoxygenase, though less strongly ($IC_{50}$ = 290 and 500 μM, respectively). 4-Hydroxycoumarin ((**5**), refer Table **1**) and coumarin had no inhibitory effect on either enzyme at concentrations up to 1 mM. The mechanism of the lipoxygenase inhibition by esculetin was non-competitive. Other antioxidants (hydroquinone, gallic acid and ascorbic acid) were less inhibitory to both enzymes and showed little selectivity.

The process of childbirth is accompanied by an increase in oxidative aggression. The aim of the study of Zhao *et al*. [17] was to determine DNA damage and oxidative stress in healthy term neonates at birth. A total of 34 healthy full-term neonates, 22 healthy adults and 20 samples of colostrum from mothers of full-term neonates were examined. The malondialdehyde (MDA), DNA damage, GSH/GSSG ratio and total antioxidant capacity (TAC) in mononuclear cells isolated from umbilical blood and adult peripheral blood were measured. Moreover, the TAC of colostrum was also measured. The protective activities of five natural polyphenols against $H_2O_2$-induced DNA damage in mononuclear cells of umbilical blood were studied. A high level of DNA damage ($p<0.001$) accompanied with lower TAC ($p<0.05$) and GSH/GSSG ratio ($p<0.001$) and with higher level of MDA ($p<0.001$) in umbilical blood compared with those of healthy adult peripheral blood were observed with several natural polyphenols. The natural polyphenols, 7,8-dihydroxy-4-methylcoumarin ((**6**), refer Table **1**), quercetin (**7**) and resveratrol (**8**), were able to protect mononuclear cells of umbilical blood from oxidative attack. However, other two polyphenols, rutin (**9**) and 7-hydroxy-4-methylcoumarin ((**10**), refer Table **1**), did not. The TAC of colostrum is significantly higher than that of umbilical blood ($p<0.001$). The DNA oxidative damage in mononuclear cells of umbilical blood as well as other indexes related to redox status provided evidence that a sudden increase in oxygenation exposes the neonate to oxidative stress. Colostrum with a significant high TAC is very important for health care in infants against the oxidative stress.

The protective properties of seven polyphenols against hydrogen peroxide induced DNA damage in human peripheral blood lymphocytes (PBL) were studied using single cell micro-gel electrophoresis [18]. Hydrogen peroxide causes a concentration-dependent increase in single cell DNA strand breakage in human PBL. Quercetin and 7,8-dihydroxy-4-methylcoumarin exhibited the strongest protection, significantly inhibiting 50 μM $H_2O_2$-induced DNA damage at a range of concentrations of 3.1-25 μM. Curcumin (**11**), resveratrol and vanillin protected against DNA damage induced by 50 μM $H_2O_2$ at a range of concentrations of 6.25-25 μM, but rutin and 7-hydroxy-4-methylcoumarin failed to provide any protection even at concentrations up to 50 μM. Quercetin, 7,8-dihydroxy-4-methylcoumarin, curcumin, resveratrol and vanillin are therefore effective in protection of human single cell DNA from oxidative attack.




Protective effects of coumarins against cytotoxicity induced by linoleic acid hydroperoxide were examined in cultured human umbilical vein endothelial cells [19]. When the cells were incubated in medium supplemented with linoleic acid hydroperoxide and coumarins, esculetin (6,7-dihydroxycoumarin) and 4-methylesculetin ((1**2**), refer Table **1**) protected cells from injury by linoleic acid hydroperoxide. Fraxetin ((**13**), refer Table **1**) and caffeic acid showed weak, but significant, protection. Esculin as well as esculetin and 4-methylesculetin were effective for protecting cells against linoleic acid hydroperoxide-induced cytotoxicity in the case of pretreatment for 24 h, however fraxetin and caffeic acid showed no protection. Since esculetin was detected after 24 h treatment with esculin, a sugar moiety in the esculin molecule appears to be hydrolysed during pretreatment. Coumarins such as umbelliferone containing only one hydroxyl group showed no protective effect in pretreatment or concurrent treatment. Esculetin and 4-methylesculetin provided synergistic protection against cytotoxicity induced by linoleic acid hydroperoxide with alpha-tocopherol. Furthermore, the radical-scavenging ability of coumarins was examined in electron spin resonance spectrometry. Esucletin, 4-methylesculetin, fraxetin, and caffeic acid showed the quenching effect on the 1,1-diphenyl-2-picrylhydrazyl radical.

The antioxidative and/or prooxidative activity of 4-methylcoumanrin ((**14**), refer Table **1**), 7-hydroxy-4-methylcoumarin and 7,8-dihydroxy-4-methylcoumarin, respectively, in the peroxidation of human low-density

lipoprotein (LDL) has been studied [20]. The peroxidation was initiated either thermally by the water-soluble initiator 2,2'-azobis(2-amidinopropane hydrochloride) (AAPH), or photochemically by a triplet sensitizer benzophenone (BP) or its water-soluble analog disodium 3,3'-disulfobenzophenonate (DSBP). The reaction kinetics were monitored by the uptake of oxygen and the depletion of alpha-tocopherol (TOH) present in the native LDL. Kinetic analysis of the peroxidation process demonstrated that 7,8-dihydroxy-4-methylcoumarin is a good antioxidant for both the AAPH-initiated and BP- and DSBP-photosensitised peroxidation; 7-hydroxy-4-methylcoumarin is a prooxidant for the AAPH-initiated and DSBP-photosensitised peroxidation, but an antioxidant for the BP-sensitised peroxidation; 4-methylcoumanrin is a prooxidant in all of these initiation conditions. The antioxidative action of the coumarin derivatives may include trapping the initiating radicals, trapping the propagating lipid peroxyl radicals, recycling alpha-tocopherol and/or deactivating the excited photosensitizer.

Twenty-three 4-methylcoumarins bearing different functionalities have been examined by Raj *et al*. for the first time for their effect on NADPH-catalysed liver-microsomal lipid peroxidation with a view to establish structure-activity relationship [21-23]. Dihydroxy- and diacetoxy-4-methylcoumarins produced dramatic inhibition of lipid peroxidation. 7,8-Diacetoxy-4-methylcoumarin ((**15**), refer Table **1**) and 7,8-dihydroxy-4-methylcoumarin (**6**) were found to possess superb antioxidant and radical scavenging activities.

The aim of the paper of Fernandez-Puntero *et al*. [24] was to study the influence of fraxetin (7,8-dihydroxy-6-methoxycoumarin) (**13**) treatment in a Drosophila melanogaster experimental model, analyzing several parameters in normal situations and instances of induced oxidative stress. Fraxetin treatment was introduced at different ages. Antigravity capacity and survival parameters were evaluated as *in vivo* assays, and levels of oxidative status, glutathione and lipid peroxidation, as *ex vivo* assays. The stress situation was induced by negative geotaxis, so physical exercise enhanced its basal metabolism, generating free radicals, which are probably implicated in the molecular damage related to the aging process. In this study, all treatment groups demonstrated a beneficial effect on the evaluated parameters. So, *in vivo* fraxetin protects fruit flies against oxidative stress and improves the survival parameters. Moreover, fraxetin prevents oxidative stress by an important increase in antioxidant reserves of GSH, and peroxidative damage is preserved by fraxetin treatment.

The superoxide-mediated base catalysed autoxidation of alpha-oxo enols is initiated by the deprotonation of the labile hydroxyl group. Thus, the reaction of $O_2^-$ (generated from $KO_2$/crown ether in aprotic media) with 3-hydroxycoumarin ((**16**), refer Table **1**), followed by a $CH_3I$-workup, generates new products *via* a deprotonation-oxidation sequence complicated by a competing saponification of the lactone linkage [25]. The related coumarin reductone (alpha-oxo enediol) is rapidly oxidised by $O_2^-$, $HO^-$ and t-butoxide to the corresponding triketone, which in turn undergoes further oxidation and rearrangement ultimately yielding (upon methyl iodide workup) new products. When the $O_2^-$ mediated oxidation is carried out under argon in completely degassed solutions, large amounts (greater than 20%) of monodeprotonation product accumulate. These results are discussed in light of the differing mechanisms concerning the interaction of $O_2^{\cdot-}$ with the reductone ascorbic acid.

The antioxidant capacity of 3-aminocoumarin ((**17**), refer Table **1**), 3-hydroxycoumarin (**16**), 3-acetylaminocoumarin ((**18**), refer Table **1**), and 3-coumarin carbonic acid ((**19**), refer Table **1**), has been investigated with chemiluminescence measurement and by the accumulation of TBA-active products [26]. All coumarins were found to be antioxidants, with 3-hydroxy-, 3-amino- and 3-acetylamino coumarins being capable of amplifying chemiluminescence at early stages of the process.

First direct 3-hydroxylation of a coumarin ring *via* a purely chemical system, previously only possible using cytochrome P-450, was successfully conducted by a $Cu^{2+}$-ascorbic acid-$O_2$ system; the two 3-hydroxycoumarins obtained were novel compounds, 3-hydroxyscopoletin ((**20**), refer Table **1**) and 3-hydroxyisoscopoletin ((**21**), refer Table **1**) [27]. 5-Lipoxygenase and alpha-D-glucosidase inhibitory activities of coumarins greatly increased through 3-hydroxylation. 3-Hydroxyscopoletin and 3-hydroxyumbelliferone ((**22**), refer Table **1**) had a high inhibitory potency for 5-lipoxygenase and for alpha-D-glucosidase respectively; they serve as lead compounds for new drugs.

Some benzo[*l*]khellactone derivatives and analogs were prepared by Nicolaides *et al*. [28]. All the tested compounds were found to interact with DPPH in a concentration and time dependent manner. All the tested compounds strongly inhibited the soybean lipoxygenase.

Fylaktakidou *et al*. [29] synthesised fused 1,3-dioxolocoumarins and evaluated them as antioxidants and anti-inflammatories. The compounds were tested for their ability to interact with 1,1-diphenyl-2-picrylhydrazyl stable free radical (DPPH), to scavenge superoxide anion radicals, to compete with DMSO for hydroxyl radicals, and to inhibit proteolysis, β-glucuronidase and soybean lipoxygenase activity *in vitro*. These compounds were also tested for their effect on the ferrous ion-stimulated peroxidation of linoleic acid. They showed a potent inhibitory effect (55-57%) against inflammation induced by carrageenan in the rat paw edema model. On the contrary, their reducing ability was found to be low and no inhibition on soybean lipoxygenase was recorded.

A recently developed sensitive fluorimetric assay has been used to examine whether free hydroxyl radicals (HO•) were generated in the immediate vicinity of DNA by Fe(II)-bleomycin [30]. When aqueous solutions of SECCA (the succinimidyl ester of coumarin-3-carboxylic acid) were irradiated with gamma rays or incubated with Fe(II)-bleomycin or Fe (II)-EDTA in the presence of ascorbate and $H_2O_2$, 7-hydroxy-SECCA, a fluorescent product of the interaction of HO• with SECCA was generated. Studies with catalase and several HO• scavengers indicated that the fluorescence induction was mediated by HO• On the contrary, Cu(II)-bleomycin complexes under similar conditions fail to induce 7-hydroxy-SECCA fluorescence. When SECCA is conjugated to DNA *via* SECCA-polylysine-DNA complexes and incubated in the same iron-

containing systems, the relative ability of the scavengers to reduce the fluorescence again demonstrated the generation of 7-hydroxy-SECCA by HO• However, while the fluorescence is practically eliminated by high concentrations of DMSO (100 μmols $dm^{-3}$) in the systems with Fe(II) or Fe(II)-EDTA, it was not possible to reduce it similarly in the case of Fe(II)-bleomycin. These data demonstrated the generation of HO• by Fe(II)-bleomycin in the immediate vicinity of DNA. Because the experiments simulated the lifetime of HO• expected in cells, these data suggest that, if such DNA-associated HO• radicals were also produced *in vivo* by bleomycin, these would not be scavengable by intracellular scavengers and they could interact with chromatin.

The experiments reported by Kennedy *et al*. [31] demonstrated that benzoyl peroxide (BP) can promote radiation induced transformation *in vitro*. BP was shown to be capable of generating free radicals, determined by the kinetics of hydroxylation as measured by fluorescence of coumarin-3-carboxylic acid (**15**). Although the mechanisms involved in the BP enhancement of radiation transformation are unknown, we hypothesize that lipid peroxidation produced by benzoyl radicals in the vicinity of membrane associated unsaturated lipids could contribute to the promotion of transformation *in vitro*.

There is increasing evidence to indicate cardioprotective effects of red wine consumption. Such cardioprotective properties of wine have been attributed to certain polyphenolic constituents of grapes [32]. The purpose of this investigation was to examine whether proanthocyanidins derived from grape seeds possess cardioprotective properties. Rats were randomly divided into two groups: grape-seed proanthocyanidin was administered orally to one group of rats (100 mg/kg/day) for 3 weeks while the other group served as control. After 3 weeks, rats were killed, hearts excised, mounted on the perfusion apparatus and perfused with Krebs-Henseleit bicarbonate (KHB) buffer. After stabilization, the hearts were perfused in the working mode for baseline measurements of contractile functions. Hearts were then subjected to 30 min of global ischemia followed by 2 h of reperfusion. Coronary perfusates were collected to monitor malondialdehyde formation, a presumptive marker for oxidative stress development. At the end of each experiment, the heart was processed for infarct size determination. Peroxyl radical scavenging activity of proanthocyanidin was determined by examining its ability to remove peroxyl radical generated by 2,2'-azobis (2-amidinopropane) dihydrochloride while hydroxyl radical scavenging activity was tested with its ability to reduce 7-hydroxycoumarin-3-carboxylic acid. The results of this study demonstrated that proanthocyanidin-fed animals were resistant to myocardial ischemia reperfusion injury as evidenced by improved recovery of post-ischemic contractile functions. The proanthocyanidin-fed group revealed a reduced extent of myocardial infarction compared to the control group. Fluorimetric study demonstrated the antioxidant property of proanthocyanidin as judged by its ability to directly scavenge peroxyl radicals. Taken together, the results of this study showed that grape seed-proanthocyanidins possessed a cardioprotective effect against ischemia reperfusion injury. Such cardioprotective property, at least in part, may be attributed to its ability to directly scavenge peroxyl and hydroxyl radicals and to reduce oxidative stress developed during ischemia and reperfusion.

The synthesis of several novel coumarin derivatives with a 7-azomethine linkage was carried out starting from 7-formyl-coumarin [33]. The compounds were tested *in vivo* for their anti-inflammatory activity and *in vitro* for their antioxidant ability. The compounds possessed significant protection against carrageenin induced rat paw edema.

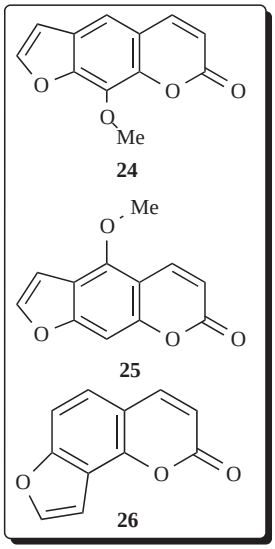
The antioxidant capacity of copper chelates with coumarins has been studied by the method of iron-induced chemiluminescence [34]. All substrates studied were potent antioxidants, comparable to butylated hydroxytoluene. The mechanism of the antioxidant action of these copper-coumarin chelates was similar to that of Cu-Mn-superoxide dismutase, with a coumarin portion of the complex being involved as a free radicals trap.

## NATURAL COUMARINS AS ANTIOXIDANTS

More than 300 coumarins have been identified from natural sources, especially from green plants [35]. The pharmacological and biochemical properties and therapeutic applications of simple coumarins depend upon the pattern of substitution. More complex related compounds based on the coumarin nucleus include the anticoagulants dicoumarol/warfarin, aflatoxins and the psoralens (photosensitizing agents). Coumarin itself (1,2-benzopyrone) has long-established efficacy in slow-onset long-term reduction of lymphoedema in man, as confirmed in recent double-blind trials against elephantiasis and postmastectomy swelling of the arm. The mechanism of action is uncertain, but may involve macrophage-induced proteolysis of edema protein. However, coumarin has a low absolute bioavailability in man (< 5%), due to extensive first-pass hepatic conversion to 7-hydroxycoumarin followed by glucuronidation. It may, therefore, be a prodrug. Scoparone (6,7-dimethoxycoumarin) ((**23**), refer Table **1**) has been purified from the hypolipidemic Chinese herb Artemisia scoparia and was shown to reduce the proliferative responses of human peripheral mononuclear cells, to relax smooth muscle, to reduce total cholesterol and triglycerides and to retard the characteristic pathomorphological changes in hypercholesterolemic diabetic rabbits. Various properties of scoparone were suggested to account for these findings, including the ability to scavenge reactive oxygen species, its inhibition of tyrosine kinases and its potentiation of prostaglandin generation.

Eleven compounds isolated from the heartwood of Mansonia gagei were tested for their antifungal activities against Cladosporium cucumerinum and Candida albicans, as well as for their larvicidal activities against Aedes aegypti and radical scavenging properties in a DPPH assay [36]. Mansonone C was found to be the most interesting compound with antifungal activities against Cladosporium cucumerinum and Candida albicans as well as for its larvicidal properties against Aedes aegypti. Mansonone E was active against Cladosporium cucumerinum and Candida albicans. Two coumarin derivatives, mansorin A and mansorin B were also found to be active against Cladosporium cucumerinum, while mansonone N was the only isolated product to show radical scavenging properties.

A variety of flavonoids, lignans, an alkaloid, bisbenzyl coumarins and terpenes isolated from Chinese herbs were tested for antioxidant activity as reflected in the ability to inhibit lipid peroxidation in rat brain and kidney homogenates and rat erythrocyte hemolysis [37]. The pro-oxidant activities of the aforementioned compounds were assessed by their effects on bleomycin-induced DNA damage. The flavonoids baicalin and luteolin-7-glucuronide-6'-methyl ester, the lignan 4'-demethyldeoxypodophyllo-toxin, the alkaloid tetrahydropalmatine, the bisbenzyl erianin and the coumarin xanthotoxol (**24**) exhibited potent antioxidative activity in both lipid peroxidation and hemolysis assays. The flavonoid rutin and the terpene tanshinone I manifested potent antioxidative activity in the lipid peroxidation assay but possessed no inhibitory activity in the hemolysis assay. The lignan deoxypodophyllotoxin, the flavonoid naringin and the coumarins bergapten (**25**) and angelicin (**26**) slightly inhibited lipid peroxidation in brain and kidney homogenates. It is worth stressing that the compounds with antioxidant effects in this assay, with the exception of tetrahydropalmatin and tanshinone I, have at least one free aromatic hydroxyl group in their structure. Obviously, the aromatic hydroxyl group is very important for antioxidative effects of the compounds. None of the compounds tested exerted an obvious pro-oxidant effect.




**24**




**25**




**26**

Three new compounds, taraxacine-A, taraxacine-B and taraxafolin together with twenty-five known compounds, which include two beta-carboline alkaloids, two indole alkaloids, two chlorophylls, two flavonoids, one coumarin, two triterpenoids, one monoterpenoid, one ionone, four steroids and eight benzenoids, were isolated and characterised from the fresh aerial parts of Taraxacum formosanum [38]. Structures of new compounds were determined by spectral analysis. The compounds were tested for antioxidative activity.

A tyrosinase inhibitor was isolated from the seeds of Euphorbia lathyris L. by bioassay-guided fractionation and purification, using silica gel column chromatography [39]. It was identified as esculetin (**3**) by comparing its physical properties and spectral data with those of an authentic sample. The $IC_{50}$ value of esculetin in the mushroom tyrosinase activity test was 43 μM. The kinetic study indicates that esculetin exhibited competitive inhibition against the oxidation of 3-(3,4-dihydroxyphenyl)-alanine by mushroom tyrosinase. The structure-activity relationships among five esculetin analogs suggest that hydroxyl groups at the C6 and C7 positions of the coumarin skeleton played an important role in the expression of tyrosinase inhibitory activity.

A major constraint to the development of cassava (Manihot esculenta Crantz) as a crop to both farmers and processors is its starchy storage roots' rapid post-harvest deterioration, which can render it unpalatable and unmarketable within 24-72 h [40]. An oxidative burst occurs within 15 min of the root being injured, that is followed by the altered regulation of genes, notably for catalase and peroxidase, related to the modulation of reactive oxygen species, and the accumulation of secondary metabolites, some of which show antioxidant properties. The interactions between these enzymes and compounds, in particular peroxidase and the coumarin, scopoletin ((**27**), refer Table **1**), are largely confined to the vascular tissues where the visible symptoms of deterioration are observed. These, together with other data, are used to develop a tentative model of some of the principal events involved in the deterioration process.

A previously unreported coumarin and a new coumarino-lignan, together with the known compounds scopoletin and cleomiscosins A, C, and D, have been isolated from the root bark of Hibiscus syriacus, and their structures were assigned on the basis of various spectral studies [41]. The coumarin analog and scopoletin inhibited monoamine oxidase with moderate $IC_{50}$ values. The new coumarino-lignan and cleomiscosin C showed lipid peroxidation inhibitory activity comparable to vitamin E.

## COUMARIN ANTIOXIDANTS AND CYTOTOXICITY

Plant phenolics are widely consumed and have received considerable attention as anticarcinogens. Tumor-modulating effects of coumarin antioxidants also have been studied using carcinogens. Protective mechanisms include inhibition of prooxidant carcinogenesis by peroxisome proliferators, antipromotion effects, and induction of detoxifying enzymes. The effects of antioxidants in animal studies are complex, however, and also include tumor promotion, carcinogenic, and co-carcinogenic activities. The most attractive candidate, anticarcinogens, however, are those that suppress the rate at which initiated cells progress through the promotion-progression-metastasis pathway without appreciable toxicity, since their application does not require knowledge of the initiating carcinogen and, by definition, will not have tumor promotional properties.

The ability to control the amount and the rate of production of hydroxyl radicals may prove useful for examining the cytotoxic effects of hydroxyl radicals generated in biological systems. The kinetics of the production of hydroxyl radicals during the autoxidation of ferrous ion complexes at pH 7.4 was investigated using the fluorescent probe coumarin-3-carboxylic acid (**19**) [42]. The data presented indicate the usefulness of autoxidation of ferrous ion complexes for generation of hydroxyl radicals in chemical systems.

Nishiyama *et al.* [43] compared the antioxidative activities of seven hydrocoumarins with those of alpha-

tocopherol for the oxidation of tetralin and linoleic acid in a homogeneous solution. Hydrocoumarins exhibited a higher induction period than that of alpha-Toc in both systems. However, the rate of oxygen absorption during the induction period for alpha-Toc was slower than that of the hydrocoumarins in both systems. In addition, 6,7-dihydroxy-4,4-dimethylhydrocoumarin ((**28**), refer Table **1**) showed less cytotoxicity toward human fibroblasts than did 2,6-di-t-butyl-4-methylphenol.

It is well known that dietary factors play an important role in enhancement of health status and physical strength in humans. Recently, it has been shown that certain foods have a host defense function related to the immune system and anti-oxidation and anti-tumor activity. The immune system plays an important role in physical and chemical carcinogenesis and in tumor-bearing hosts. The role of host immune function has become increasingly important in our understanding of the mechanisms that are involved in the body's ability to prevent cancer. Although the inter-relationship between diet, immune function and carcinogenesis is not clear, there is increasing evidence that dietary alteration of the host's immune functions is a key component of chemoprevention. Macrophages, lymphocytes (T and B cells), dendritic and Langerhans cells and natural killer (NK) cells are important cells for the immune system. Macrophages play a major role in inflammation, repair, humoral and cellular immunity and metabolic and neoplastic disease processes. Cytokines, being messenger molecules of the immune system, modulate natural immunity. It is known that several cancer chemopreventive agents can modulate immune function.

An antioxidant auraptene (7-geranyloxycoumarin) ((**29**), refer Table **1**) isolated from the peel of citrus fruit (Citrus natsudaidai Hayata) has been reported to have chemopreventive effects on chemically induced carcinogenesis. Dietary administration of auraptene significantly increased the activities of detoxification (phase II) enzymes, such as quinone reductase and glutathione *S*-transferase, in the liver and colon of rats. In addition, expression of cell proliferation biomarkers, such as ornithine decarboxylase activity and polyamine biosynthesis, in the colonic mucosal epithelium was significantly inhibited by dietary feeding of auraptene. These biological functions of auraptene may contribute to its anti-tumorigenic effect. However, a modulatory effect of auraptene on immune function has not been investigated. The results in the study [44] clearly indicate that oral administration of auraptene effectively enhances both macrophage and lymphocyte functions in mice. The study suggested involvement of the immune response in chemically induced carcinogenesis. Auraptene is a naturally occurring coumarin-related compound. Coumarin derivatives have been reported to have enhancing effects on lymphocyte mitogen responsiveness. Therefore, the results in the study suggest that the mitogenic activity of auraptene might be due to the coumarin structure, and 200 mg/kg/day might be an appropriate oral dose of auraptene to enhance lymphocyte responsiveness. The results described in [44] may support the hypothesis that auraptene directly activates macrophage activities, whereas it only primes lymphocytes to display a greater immune response following interaction of splenic lymphocytes with another stimulus. The findings suggest that auraptene may exert a part of its cancer chemopreventive activity through enhancement of immune function.

The ingestion of citrus fruit has been reported to be beneficial for the reduction of certain types of human cancer. Several classes of citrus phytochemicals, including monoterpenes, limonoids and flavonoids, have been recognised as effective chemopreventive agents in rodent carcinogenesis models. Auraptene (7-geranyloxycoumarin), a coumarin derivative has been isolated from citrus fruit (*e.g.* grapefruit) and has demonstrated its anti-tumor promoting effect in mouse skin and anti-carcinogenesis activities in the rat tongue, esophagus and colon [45]. Murakami *et al*. [45] reported that auraptene suppressed superoxide anion ($O_2^-$) generation from inflammatory leukocytes in *in vitro* experiments. In the study, they investigated the anti-inflammatory activities of auraptene using a 12-*O*-tetradecanoylphorbol-13-acetate-treated mouse skin model, and compared them with those of Umbelliferone (7-hydroxycoumarin), a structural analog of auraptene that is virtually inactive toward $O_2^-$ generation inhibition. Double pre-treatments of mouse skin with auraptene, but not Umbelliferone, markedly suppressed edema formation, hydrogen peroxide production, leukocyte infiltration, and the rate of proliferating cell nuclear antigen-stained cells. These inhibitory effects by auraptene were attributable to its selective blockade of the activation stage, as revealed by single pre-treatment experiments. Umbelliferone did not show any inhibitory effect. This contrasting activity profile between auraptene and Umbelliferone was rationalised to be a result of their distinct differences in cellular uptake efficiencies, i.e. the geranyloxyl group in auraptene was found to play an essential role in incorporation. Thus, the findings indicate that auraptene is an effective agent to attenuate the biochemical responsiveness of inflammatory leukocytes, which may be essential for a greater understanding of the action mechanism that underlies its inhibition of inflammation-associated carcinogenesis.

Aflatoxin $B_1$ is a potent hepatocarcinogen produced by Aspergillus flavus, a mold that frequently contaminates rice and cereal crops in humid areas of the world. In combination with hepatitis B, Aflatoxin $B_1$ is thought to be largely responsible for the high incidence of hepatocellular carcinoma in southeast China and southern Africa. Like most chemical carcinogens, the mycotoxin requires bioactivation to exert its carcinogenic effects. The ultimate carcinogen of Aflatoxin $B_1$ is the exo-8, 9-epoxide, and once generated by the actions of CYP, it readily forms adducts with DNA. In humans, hepatocellular carcinoma resulting from exposure to Aflatoxin $B_1$ is associated with mutations in codon 249 of the p53 tumor suppressor gene, whereas in the rat it is associated with mutations in codons 12 and 13 of ras oncogenes. Although primates and rats are sensitive to Aflatoxin $B_1$, the mouse can tolerate high levels of the mycotoxin without showing signs of acute liver damage or of developing liver cancer. Because it is highly improbable that Aflatoxin $B_1$ -producing molds can be eradicated from the environment, chemoprevention is an attractive strategy to protect individuals from the risk of liver cancer caused by exposure to the mycotoxin. Structurally diverse compounds can confer resistance to aflatoxin $B_1$ hepatocarcinogenesis in the rat. Treatment with either phytochemical coumarin or synthetic antioxidants and other drugs has been found to

increase hepatic aldo-keto reductase activity toward Aflatoxin $B_1$ -dialdehyde and glutathione *S*-transferase (GST) activity toward Aflatoxin $B_1$ -8,9-epoxide in both male and female rats [46]. Under the conditions used, the natural benzopyrone coumarin was a major inducer of the Aflatoxin $B_1$ aldehyde reductase (AFAR) and the aflatoxin-conjugating class- GST A5 subunit in rat liver, causing elevations of between 25- and 35-fold in hepatic levels of these proteins. Induction was not limited to AFAR and GSTA5: treatment with coumarin caused similar increases in the amount of the class- GST P1 subunit and NAD(P)H:quinone oxidoreductase in rat liver. Immunohistochemistry demonstrated that the overexpression of AFAR, GSTA5, GSTP1, and NAD(P)H:quinone oxidoreductase affected by coumarin is restricted to the centrilobular (periacinar) zone of the lobule, sometimes extending almost as far as the portal tract. This pattern of induction was also observed with ethoxyquin, oltipraz, and trans-stilbene oxide. By contrast, induction of these proteins by ß-naphthoflavone and diethyl maleate was predominantly periportal. Northern blotting showed that induction of these phase II drug-metabolizing enzymes by coumarin was accompanied by similar increases in the levels of their mRNAs. To assess the biological significance of enzyme induction by dietary coumarin, two intervention studies were performed in which the ability of the benzopyrone to inhibit either Aflatoxin $B_1$ -initiated preneoplastic nodules (at 13 weeks) or Aflatoxin $B_1$ -initiated liver tumors (at 50 weeks) was investigated. Animals pretreated with coumarin for 2 weeks prior to administration of Aflatoxin $B_1$, and with continued treatment during exposure to the carcinogen for a further 11 weeks, were protected completely from development of hepatic preneoplastic lesions by 13 weeks. In the longer-term dietary intervention, treatment with coumarin before and during exposure to Aflatoxin $B_1$ for a total of 24 weeks was found to significantly inhibit the number and size of tumors that subsequently developed by 50 weeks. These data suggest that consumption of a coumarin-containing diet provides substantial protection against the initiation of Aflatoxin $B_1$ hepatocarcinogenesis in the rat. This report describes the identification of phytochemicals that are effective inducers of Aflatoxin $B_1$ detoxication enzymes. The study showed that coumarin is highly effective at inducing not only AFAR and GSTA5, but also certain other drug-metabolizing enzymes. On the basis of this information, the hypothesis that enzyme induction by coumarin would confer resistance to Aflatoxin $B_1$ tumorigenesis was tested in the rat. The results from dietary intervention showed that coumarin consumption does indeed provide protection against initiation of Aflatoxin $B_1$ hepatocarcinogenesis. The data presented in this report also reveal the ability of different phytochemicals and synthetic drugs to induce different enzymes in the liver in zone- and sex-specific fashions.

After the consumption of ethanol, acetaldehyde levels increase in the serum, and the serum develops a nondialyzable cytotoxic activity caused by the formation of unstable acetaldehyde-albumin complexes. The concentration of acetaldehyde in the serum and the cytotoxic activity in serum albumin 8.5 hr after six healthy volunteers began to drink 94 g of ethanol were significantly less when the ethanol was consumed as red wine than as white wine [47]. The serum acetaldehyde was measured by a fluorigenic HPLC assay, and the cytotoxic activity in albumin was determined using two different assays based on dissimilar endpoints: detachment of adherent A9 cells and impairment of the ability of A9 cells to reduce tetrazolium. When serum obtained from five other healthy volunteers after the consumption of white wine was incubated at 37 $^0$C for 3 hr with a number of dietary antioxidants at a concentration of 100 µmol/liter, the cytotoxicity of the albumin was markedly reduced. The antioxidants studied consisted of six flavonoids (kaempherol, fisetin, quercetin catechin, taxifolin, and coumarin) and three nonflavonoids (salicylic acid, tannic acid, and alpha-tocopherol). In the cases of alpha-tocopherol, a statistically significant reduction of cytotoxicity was observed at a concentration of 10 µmol/liter. In addition, the cytotoxicity of artificially prepared acetaldehyde-albumin complexes was significantly reduced when such complexes were incubated with 50 to 100 µmol/liter of kaempherol, fisetin, quercetin, coumarin or salicylic acid, or 10 µmol/liter of alpha-tocopherol at 37 $^0$C for 3 hr. Evidently, *in vitro*, flavonoid and nonflavonoid dietary constituents reduce the amount of unstable acetaldehyde-albumin complexes found in both postalcohol serum and in artificially produced acetaldehyde-albumin complexes. The difference in the amount of unstable acetaldehyde-albumin complexes found in serum after the consumption of red and white wine may therefore be caused by the higher concentration of antioxidants, including flavonoids, in red wine than in white wine. Because acetaldehyde and acetaldehyde-albumin complexes have been implicated in the pathogenesis of alcohol-mediated tissue damage, these data suggest that dietary antioxidants may influence the biological consequences of excess alcohol consumption.

Plants have formed the basis for the treatment of diseases in traditional medicine systems for thousands of years, and continue to play a major role in the primary health care of about 80% of the world's inhabitants. While the natural product isolated as the active compound might not be suitable for development as an effective drug, it can provide a suitable lead for conversion into a clinically useful agent.

## CONCLUSION

Coumarins comprise a vast array of biologically active compounds ubiquitous in plants, many of which have been used in traditional medicine for thousands of years. The coumarins constitute an important class of compounds, with several types of pharmacological agents possessing antioxidant, anticancer, anti-HIV, anticoagulant, spasmolytic and antibacterial activity among others. Of the many actions of coumarins, antioxidant and antiproliferative effects stand out. A large number of structurally novel coumarin derivatives have been reported to show substantial antioxidant and cytotoxic activity *in vitro* and *in vivo*. Moreover, the inhibitory action on inflammatory cells appears to surpass any other clinically available compounds. Given that certain substituents are known to be required or increase their actions, the therapeutic potential of select coumarins is fairly obvious.

## ABBREVIATIONS

RS = Reactive Species

OS = Oxidative stress

DPPH = 1,1-Diphenyl-2-picryl-hydrazyl

ODC = Ornithine decarboxylase

TBHQ = Tert-butylhydroquinone

DPH-PA = 3-(p-(6-Phenyl)-1,3,5-hexatrienyl)phenyl-propionic acid

*t*-BHP = *t*-Butyl hydroperoxide

LDH = Lactate dehydrogenase

ALT = Alanine transaminase

MDA = Malondialdehyde

TAC = Total antioxidant capacity

PBL = Peripheral blood lymphocytes

LDL = Low-density lipoprotein

AAPH = 2,2'-Azobis(2-amidinopropane hydrochloride)

BP = Benzophenone

DSBP = 3,3'-Disulfobenzophenonate

TOH = Alpha-tocopherol

SECCA = The succinimidyl ester of coumarin-3-carboxylic acid

KHB = Krebs-Henseleit bicarbonate

NK = Natural killer cells

GST = Glutathione *S*-transferase

AFAR = Aflatoxin $B_1$ aldehyde reductase

## REFERENCES


[1] Traykova, M. and Kostova, I. *Int. J. Pharm*., **2005**, *1*, 29.

[2] Kostova, I. *Curr. Med. Chem. Anti-Canc. Agents*, **2005**, *5*, 29.

[3] Rietjens, I. M.; Martena, M. J.; Boersma, M. G.; Spiegelenberg, W.; Alink, G. M. *Mol. Nutr. Food Res.*, **2005**, *49*, 131.

[4] Fylaktakidou, K. C.; Hadjipavlou-Litina, D. J.; Litinas, K. E.; Nicolaides, D. N. *Curr. Pharm. Des.*, **2004**, *10*, 3813.

[5] Galvano, F.; Piva, A.; Ritieni, A.; Galvano, G. *J. Food Prot.*, **2001**, *64*, 120.

[6] Hayes, J. D.; Pulford, D. J.; Ellis, E. M.; McLeod, R.; James, R. F.; Seidegard, J.; Mosialou, E.; Jernstrom, B.; Neal, G. E. *Chem. Biol. Interact.*, **1998**, *111-112*, 51.

[7] Garnett, W. R. *Am. J. Health Syst. Pharm.*, **1995**, *52*, 1639.

[8] Farmer, J. A.; Gotto, A. M. Jr. *Drug Saf.*, **1994**, *11*, 301.

[9] Kontogiorgis, C. A.; Hadjipavlou-Litina, D. J. *Enzyme Inhib. Med. Chem.*, **2003**, *18*, 63.

[10] Khan, N.; Sharma, S.; Sultana, S. *Redox. Rep*., **2004**, *9*, 19.

[11] Arora, A.; Nair, M. G.; Strasburg, G. M. *Free Radic. Biol. Med.*, **1998,** *24*, 1355.

[12] Vladimirov, Iu. A.; Parfenov, E. A.; Epanchintseva, O. M.; Smirnov, L. D. *Biull. Eksp. Biol. Med.*, **1991**, *112*, 472.

[13] Baccard, N.; Mechiche, H.; Nazeyrollas, P.; Manot, L.; Lamiable, D.; Devillier, P.; Millart, H. *Arzneimittelforschung*, **2000**, *50*, 890.

[14] Cooke, D.; O'Kennedy, R. *Anal. Biochem.*, **1999**, *274*, 188.

[15] Lin, W. L.; Wang, C. J.; Tsai, Y. Y.; Liu, C. L.; Hwang, J. M.; Tseng, T. H. *Arch. Toxicol.*, **2000**, *74*, 467.

[16] Sekiya, K.; Okuda, H.; Arichi, S. *Biochim. Biophys. Acta*, **1982**, *713*, 68.

[17] Zhao, J.; Liu, X. J.; Ma, J. W.; Zheng, R. L. *Early Hum. Dev.*, **2004**, *77*, 89.

[18] Liu, G. A.; Zheng, R. L. *Pharmazie*, **2002**, *57*, 852.

[19] Kaneko, T.; Baba, N.; Matsuo, M. *Chem. Biol. Interact.*, **2003**, *142*, 239.

[20] Liu, Z. Q.; Yu, W.; Liu, Z. L. *Chem. Phys. Lipids*, **1999**, *103*, 125.

[21] Raj, H. G.; Parmar, V. S.; Jain, S. C.; Goel, S.; Poonam; Himanshu; Malhotra, S.; Singh, A.; Olsen, C. E.; Wengel, J. *Bioorg. Med. Chem*., **1998**, *6*, 833.

[22] Raj, H. G.; Parmar, V. S.; Jain, S. C.; Priyadarsini, K. I.; Mittal, J. P.; Goel, S.; Das, S. K.; Sharma, S. K.; Olsen, C. E.; Wengel, J. *Bioorg. Med. Chem*., **1999**, *7*, 2091.

[23] Raj, H. G.; Sharma, S. K.; Garg, B. S.; Parmar, V. S.; Jain, S. C.; Goel, S.; Tyagi, Y. K.; Singh, A.; Olsen, C. E.; Wengel, J. *Bioorg. Med. Chem.*, 1**998**, *6*, 2205.

[24] Fernandez-Puntero, B.; Barroso, I.; Iglesias, I.; Benedi, J.; Villar, A. *Biol. Pharm. Bull.*, **2001**, *24*, 777.

[25] Frimer, A. A.; Marks, V.; Gilinsky-Sharon, P. *Free Radic. Res. Commun.*, **1991**, *12*, 93.

[26] Vladimirov, Iu. A.; Parfenov, E. A.; Epanchintseva, O. M.; Smirnov, L. D. *Biull. Eksp. Biol. Med.*, **1991**, *112*, 358.

[27] Aihara, K.; Higuchi, T.; Hirobe, M. *Biochem. Biophys. Res. Commun.*, **1990**, *168*, 169.

[28] Nicolaides, D. N.; Gautam, D. R.; Litinas, K. E.; Hadjipavlou-Litina, D. J.; Fylaktakidou, K. C. *Eur. J. Med. Chem.*, **2004**, *39*, 323.

[29] Fylaktakidou, K. C.; Gautam, D. R.; Hadjipavlou-Litina, D. J.; Kontogiorgis, C. A.; Litinas, K. E.; Nicolaides, D. N. *J. Chem. Soc., Perkin Trans.*, **2001**, *1*, 3073.

[30] Chakrabarti, S.; Makrigiorgos, G. M.; O'Brien, K.; Bump, E.; Kassis, AI. *Free Radic. Biol. Med.*, **1996**, *20*, 777.

[31] Kennedy, S. H.; Manevich, Y.; Biaglow, J. *Biochem. Biophys. Res. Commun.*, **1995**, *212*, 118.

[32] Sato, M.; Maulik, G.; Ray, P. S.; Bagchi, D.; Das, D. K. *J. Mol. Cell. Cardiol.*, **1999**, *31*, 1289.

[33] Kontogiorgis, C. A.; Hadjipavlou-Litina, D. J. *Bioorg. Med. Chem. Lett.*, **2004**, *14*, 611.

[34] Vladimirov, Iu. A.; Parfenov, E. A.; Epanchintseva, O. M.; Sharov, V. S.; Dremina, E. S.; Smirnov, L. D. *Biull. Eksp. Biol. Med.*, **1992**, *113*, 479.

[35] Hoult, J. R.; Paya, M. *Gen Pharmacol.*, **1996**, *27*, 713.

[36] Tiew, P.; Ioset, J. R.; Kokpol, U.; Chavasiri, W.; Hostettmann, K. *Phytother. Res.*, **2003**, *17*, 190.

[37] Ng, T. B.; Liu, F.; Wang, Z. T. *Life Sci*., **2000**, *66*, 709.

[38] Leu, Y. L.; Shi, L. S.; Damu, A. G. *Chem. Pharm. Bull. (Tokyo)*, **2003**, *51*, 599.

[39] Masamoto, Y.; Ando, H.; Murata, Y.; Shimoishi, Y.; Tada, M.; Takahata, K. *Biosci. Biotechnol. Biochem.*, **2003**, *67*, 631.

[40] Reilly, K.; Gomez-Vasquez, R.; Buschmann, H.; Tohme, J.; Beeching, J. R. *Plant Mol. Biol.*, **2003**, *53*, 669.

[41] Yun, B. S.; Lee, I. K.; Ryoo, I. J.; Yoo, I. D. *J. Nat. Prod.*, **2001**, *64*, 1238.

[42] Kachur, A.V.; Tuttle, S.W.; Biaglow, J.E. *Radiat. Res*., **1998**, *150*, 475.

[43] Nishiyama, T.; Ohnishi, J.; Hashiguchi, Y. *Biosci. Biotechnol. Biochem.*, **2001**, *65*, 1127.

[44] Tanaka, T.; Sugiura, H.; Inaba, R.; Nishikawa, A.; Murakami, A.; Koshimizu K.; Ohigashi H. *Carcinogenesis*, **1999**, *20*, 1471.

[45] Murakami, A.; Nakamura, Y.; Tanaka, T.; Kawabata, K.; Takahashi, D.; Koshimizu, K.; Ohigashi, H. *Carcinogenesis*, **2000,** *21*, 1843.

[46] Kelly, V.P.; Ellis, E.M.; Manson, M.M.; Chanas, S.A.; Moffat, G.J.; McLeod, R.; Judah, D.J.; Neal, G.E.; Hayes, J.D. *Cancer Res.*, **2000,** *60*, 957.

[47] Wickramasinghe, S. N.; Hasan, R.; Khalpey, Z. *Alcohol Clin. Exp. Res.*, **1996**, *20*, 799.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