

ANTIOXIDATIVE ACTIVITY OF NATURAL PRODUCTS FROM PLANTS

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Summary

A variety of flavonoids, lignans, an alkaloid, a bisbenzyl, coumarins and terpenes isolated from Chinese herbs was tested for antioxidant activity as reflected in the ability to inhibit lipid peroxidation in rat brain and kidney homogenates and rat erythrocyte hemolysis. 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'-demethyldeoxypodophyllotoxin, the alkaloid tetrahydropalmatine, the bisbenzyl erianin and the coumarin xanthoxol 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 no inhibitory activity in the hemolysis assay. The lignan deoxypodophyllotoxin, the flavonoid naringin and the coumarins columbianetin, bergapten and angelicin 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 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.

Key Words: plant, antioxidant, peroxidation, hemolysis

Studies have demonstrated that botany and medicine are related. It is well known that some extracts from Chinese herbs have many pharmacological actions and little cytotoxicity (1). The pharmacological actions of flavonoids, lignans, alkaloids, bisbenzyls, coumarins and terpenes from plants have been extensively studied. It has been found that some terpenoids could inhibit the growth of Ehrlich ascites tumor, sarcoma-180, Lewis lung tumor and P-388 lymphocytic leukemia (2). Alkaloids have also been introduced into clinical use because of their antitumor

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activity and low neurotoxicity (2). Coumarins have been used to treat infections. Recent studies have shown that coumarins have antitumor, immunomodulating and antibacterial activities (3,4). Flavonoids and phenolics also have antitumor, antiviral and antibacterial activities (4,5,6) and their antiradical and antioxidative properties (7,8,9) have been studied extensively. However, up to now, the antioxidative action of lignans, alkaloid, bisbenzyl, coumarins and terpenes has not yet been established.

Free radicals and lipid peroxidation have been suggested as potentially important causative agents of aging and several human diseases (7). Therefore, one of the strategies is to look for natural products from plants for combating free radical-induced pathological status. In the present study, the antioxidative activities of flavonoids, lignans, an alkaloid, a bisbenzyl, coumarins and terpenes were assessed in order to ascertain potential antioxidative drugs.

Methods

Reagents

DNA (Type I, calf thymus), bleomycin sulfate, butylated hydroxyanisole (BHA) and L-ascorbic acid were obtained from Sigma (St. Louis, MO). 2,2'-azo-bis-(2-amidinopropane) dihydrochloride (AAPH) was purchased from Wak. All other chemicals were of the highest quality available.

Animals

Male Sprague-Dawley rats (150-200g) were used in this study. The rats were housed under normal laboratory conditions ($21 \pm 2^\circ\text{C}$, 12/12 h light-dark cycle) with free access to standard rodent chow and water.

Isolation of natural products

The compounds mentioned below were isolated according to published procedures (10) and their structures were elucidated spectroscopically.

Baicalin

The roots of *Scutellaria baicalensis* Georgi, collected in Gansu, China, were cut into thin slices and decocted twice with water (30 min per decoction). The pH of the combined aqueous solution was adjusted with HCl to 1-2. After standing at 80°C for 30 min, it was filtered. The precipitate was rinsed 2-3 times with 95% ethanol under reduced pressure and then dried at 60°C . To the precipitate 10 volumes of water were added, the pH was adjusted to 6.5-7.0 with 40% NaOH, and some activated charcoal was added to the solution. After standing at 80°C for 30 min and then at room temperature for 10 h, the precipitate was collected by filtration and rinsed with ethanol 2-3 times under reduced pressure, dried at 60°C , and the resulting product was baicalin (10) (Table I).

Rutin

The flower buds of *Sophora japonica* L. collected in Hubei, China, were extracted with an ammonium bicarbonate solution in a boiling water bath. The pH of the alkaline extract was adjusted to 5 with HCl at 60°C - 70°C and left to stand for 24 h before it was filtered. The precipitate was recrystallized in water and crystalline rutin was obtained (10) (Table I).

TABLE I

Structures of flavonoids, lignans, alkaloid and bisbenzyl

Flavonoids:	Lignans:
Baicalin	4'-demethyldeoxypodophyllotoxin
Rutin	Deoxypodophyllotoxin
Luteolin-7-glucuronide-6'-methyl ester	Podophyllotoxin
	Bisbenzyl:
Naringin	
Alkaloid:	Erianin
Tetrahydropalmatine	

Luteolin-7-glucuronide-6'-methyl ester

This compound was isolated from *Ixeris denticulata* f. *pinnatipartita* collected in Jiangsu, China. The herb was decocted three times, the combined aqueous decoction was concentrated to a small quantity, and then 95% ethanol was added to precipitate the macromolecular substances. The supernatant was applied to an adsorption resin, which was then eluted with a water-ethanol gradient. The 40% ethanol eluate was repeatedly chromatographed on a silica gel column to obtain luteolin-7-glucuronide-6'-methyl ester which was recrystallized from acetone (Table I).

Naringin

The rhizomes of *Drynaria fortunei* (Kze.) J. Sm. collected in Gansu, China, were extracted with 60% ethanol. The ethanol extract was concentrated and partitioned between water and butanol. The butanolic fraction was chromatographed on a polyamide column that was eluted stepwise with water containing an increasing amount of methanol. From the eluate of water-methanol (7:3, v:v), naringin was obtained and recrystallized (10) (Table I).

Lignans

The three lignans, 4'-demethyldeoxypodophyllotoxin, deoxypodophyllotoxin and podophyllotoxin, were isolated from *Sinopodophyllum emodi* (Wall.) Ying collected in Yunnan, China. The dried fruit was macerated with 95% ethanol and percolated until the percolate was colorless. After recovering the ethanol, the extract was mixed well with some silica and extracted with acetone under reflux in a water bath. The acetone extract was applied to a silica gel column which was then eluted with a petroleum ether-ethylacetate gradient. The lignan positive fractions were separated by repeated chromatography. Podophyllotoxin was recrystallized from ethanol, deoxypodophyllotoxin was recrystallized from acetone, and 4'-demethyldeoxypodophyllotoxin was recrystallized from methanol (Table I).

Tetrahydropalmatine

This product was isolated from *Stephania sinica* Diels collected in Sichun, China. The root was extracted with 80% ethanol containing 0.5% sulphuric acid. The acidic ethanol extract, after adjusting to pH 6-7 with alkali, was concentrated and filtered. The pH of the filtrate was adjusted to 9 with ammonia and crude alkaloid was precipitated. The crude alkaloid was dissolved in 10 volumes of 0.5 N sulphuric acid by heating and filtered. The filtrate was made alkaline by addition of ammonia again and the precipitated alkaloid was recrystallized from chloroform to obtain pure tetrahydropalmatin (Table I).

Erianin

This bisbenzyl derivative was isolated from *Dendrobium chrysotoxum* Lindl. collected in Yunnan, China. The stem was extracted with 95% ethanol and the ethanolic extract was applied to a silica gel column which was eluted with a petroleum ether-chloroform gradient. From the petroleum ether-chloroform (6:4, v:v) eluate, erianin was obtained and recrystallized from petroleum ether-chloroform (Table I).

Coumarins

The fruits of *Cnidium monnieri* (L.) Cuss. collected in Shandong, China, were extracted with 95% ethanol. The ethanolic extract was defatted with petroleum ether and then chromatographed on a silica gel column, which was eluted with a petroleum ether-acetone gradient. The petroleum ether-acetone (9:1, v:v) of eluate was subjected to preparative thin layer chromatography on silica gel to yield angelicin, columbianetin, O-acetylcolumbianetin and a mixture of edultin and cniforin A. The eluate of petroleum ether-acetone (8:2, v:v) was

rechromatographed to produce oroselone, bergapten, xanthotoxin and xanthotoxol. All the coumarins isolated were recrystallized from ethanol (10) (Table II).

Ranunculin

The fresh herb of *Pulsatilla chinensis* collected in Liaoning, China, was extracted with 95% ethanol. The extract, after concentration, was partitioned against petroleum ether, chloroform and n-butanol successively. The n-butanol fraction was added to a silica gel column which was eluted with a chloroform-methanol gradient. From the chloroform-methanol (7:3, v:v) eluate, ranunculin was obtained and recrystallized from methanol (Table III).

Tanshinone I

This phenanthrofurandione was isolated from *Salvia miltiorrhiza* Bunge, collected in Jiangsu, China. The root, in powder form, was extracted with chloroform and the chloroform extract was subjected to a silica gel column which was eluted with a chloroform-acetone gradient. The eluate containing tanshinone I was refined by preparative thin layer chromatography and recrystallized from ethanol (Table III).

Diterpene lactones

The two diterpene lactones, brusatol and brucein D, were isolated from *Brucea javanica* (L) Merr. collected in Guangdong, China. The seeds, in powder form, were defatted with petroleum ether and then extracted with chloroform. The chloroform extract was chromatographed on a silica gel column, which was eluted with a chloroform-methanol gradient. From the fraction of chloroform-methanol (19:1, v:v), brusatol was obtained and recrystallized from acetone. From the fractions of chloroform-methanol (9:1, v:v), brucein D was obtained and recrystallized from acetone (Table III).

Triptolide

This diterpene lactone was isolated from *Tripterygium hypoglaucum* Hutch. collected in Yunnan, China. The methanolic extract of the stem was partitioned with petroleum ether and ethyl acetate successively. The ethyl acetate fraction was subjected to repeated chromatography and triptolide was obtained and recrystallized from dichloromethane-ether (Table III).

Andrographolide

This diterpene lactone was isolated from the herb of *Andrographis paniculata*, collected in Jiangxi, China. The herb was extracted by percolation with 80% ethanol and decolorized by passing through a column of activated charcoal. The eluate was concentrated in vacuum and applied to a silica gel column and eluted with a petroleum ether-acetone gradient. From the petroleum ether-acetone (7:3, v:v) eluate, andrographolide was obtained and recrystallized from methanol (Table III).

Hederagenin

This triterpenoid was isolated from *Aster tartaricus* L.f., collected in Anhui, China. The root was extracted with 95% ethanol and the concentrated extract was partitioned with petroleum ether, chloroform and butanol successively. The chloroform fraction was applied to a silica gel column which was eluted with a chloroform-methanol gradient. From the chloroform-methanol (9:1, v:v) eluate, hederagenin was isolated and recrystallized from ethanol (Table III).

TABLE II

Structures of coumarins

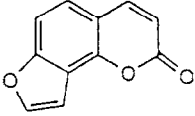
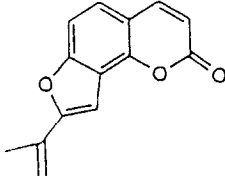
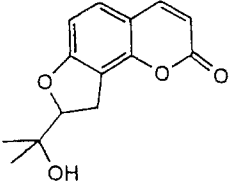
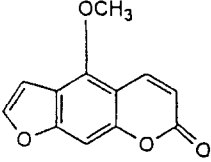
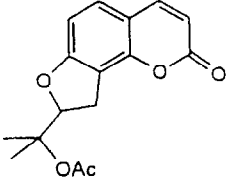
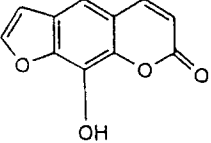
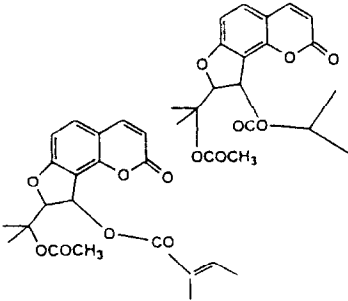
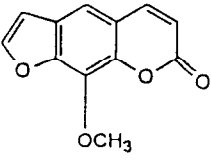
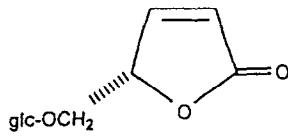
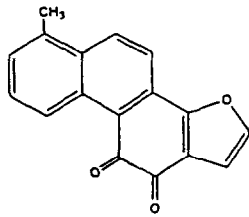
	
Angelicin	Oroselone
	
Columbianetin	Bergapten
	
O-acetylcolumbianetin	Xanthotoxol
	
Edultin+cniforin A	Xanthotoxin

TABLE III

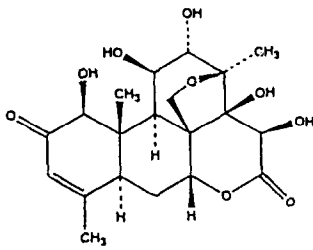
Structures of terpenes



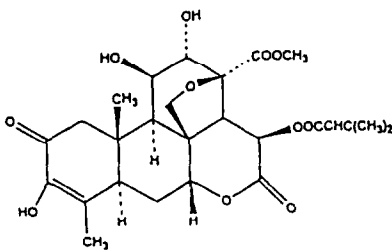
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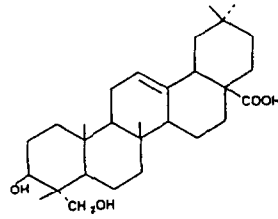
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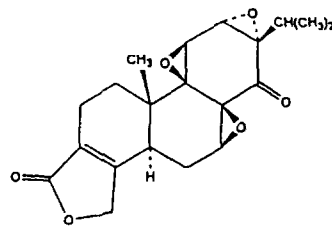
Brucein D



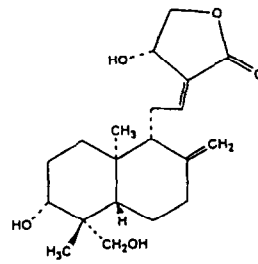
Brusatol



Hederagenin



Triptolide



Andrographolide

Assays of lipid peroxidation using brain and kidney homogenates

For the *in vitro* studies, the brains and kidneys of normal rats were dissected and homogenized with a Polytron (speed setting 7-8) in ice-cold Tris-HCl buffer (20 mM, pH 7.4) to produce a 1/10 homogenate. The homogenate was centrifuged at 14,000 rpm for 15 min. One ml aliquots of the supernatant were incubated with the test samples in the presence of 10 μ M FeSO₄ and 0.1 mM ascorbic acid at 37°C for 1 h. The reaction was stopped by addition of 1.0 ml trichloroacetic acid (TCA, 28%, w/v) and 1.5 ml thiobarbituric acid (TBA, 1%, w/v) in succession, and the solution was then heated at 100°C for 15 min. After centrifugation to remove precipitated protein, the color of the malondialdehyde (MDA)-TBA complex was detected at OD 532 nm using a Milton Roy Spectronic 3000 Spectrophotometer (11). Butylated hydroxyanisole (BHA) was used as a positive control. The inhibition ratio (%) was calculated using the following formula:

$$\text{Inhibition ratio (\%)} = (A - A1)/A \times 100\%$$

where A was the absorbance of the control, and A1 was the absorbance of the test sample.

Assay for erythrocyte hemolysis

Blood was obtained from rats by cardiac puncture and collected in heparinized tubes. Erythrocytes were separated from plasma and the buffy coat and washed three times with 10 volumes of 0.15 M NaCl. During the last washing, the erythrocytes were centrifuged at 2,500 rpm for 10 min to obtain a constantly packed cell preparation (12).

Erythrocyte hemolysis was mediated by peroxy radicals in this assay system (13). A 10% suspension of erythrocytes in pH 7.4 phosphate buffered saline (PBS) was added to the same volume of 200 mM 2,2'-azobis(2-amidinopropane)dihydrochloride (AAPH) solution (in PBS) containing samples to be tested at different concentrations. The reaction mixture was shaken gently while being incubated at 37°C for 2 h. The reaction mixture was then removed, diluted with 8 volumes of PBS and centrifuged at 2,500 rpm for 10 min. The absorbance A of the supernatant was read at 540 nm. Similarly, the reaction mixture was treated with 8 volumes of distilled water to achieve complete hemolysis, and the absorbance B of the supernatant obtained after centrifugation was measured at 540 nm. The percentage hemolysis was calculated by the equation $(1 - A/B) \times 100\%$. The data were expressed as mean \pm standard deviation. L-ascorbic acid was used as a positive control.

Bleomycin-dependent DNA damage

The assay was done according to Aeschlach et al.(14) and Chan et al.(15) with minor modifications. The reaction mixture (0.5ml) contained DNA (0.5mg/ml), bleomycin sulfate (0.05mg/ml), MgCl₂ (5mM), FeCl₃ (50 μ M) and samples to be tested at different concentrations. L-ascorbic acid was used as a positive control. The mixture was incubated at 37°C for 1 h. The reaction was terminated by addition of 0.05 ml EDTA (0.1M). The color was developed by adding 0.5 ml TBA (1%, w/v) and 0.5 ml HCl (25%, v/v) followed by heating at 80°C for 10 min. After centrifugation, the extent of DNA damage was measured by increase in absorbance at 532 nm.

Results

Malondialdehyde (MDA), formed from the breakdown of polyunsaturated fatty acid, serves as a convenient index for determining the extent of lipid peroxidation (16, 17). The effects of flavonoids, lignans, an alkaloid, a bisbenzyl, coumarins and terpenes on lipid peroxidation in the

brain and kidney homogenates are shown in Table IV, V and VI. The percent inhibition due to the flavonoids baicalin, luteolin-7-glucuronide-6'-methyl ester and rutin, the lignan 4'-demethyldeoxydopodophyllotoxin, the alkaloid tetrahydropalmatine and the bisbenzyl erianin was similar to that due to BHA in the brain homogenates, indicating that they were equipotent. The coumarin xanthotoxol and the terpene tanshinone I had a lower potency in counteracting lipid peroxidation in the brain homogenates. Three out of the four flavonoids tested had antioxidative activity, but only one out of the eight coumarins and one out of the eight terpenes tested had antioxidative activity.

The azo compound generates free radicals by its unimolecular thermal decomposition. The rate of generation of peroxy radicals can be easily controlled and measured by adjusting the concentration of AAPH (12). Therefore, the hemolysis induced by AAPH must provide the clearest means for studying the oxidative erythrocyte membrane damage by peroxy radical attack from the outside of the membrane. In the present study, although both lipid peroxidation and hemolysis were caused by the peroxy radical, their reaction mechanisms are different. Table VII shows that two out of the eight compounds that had antioxidative activity, tanshinone I and rutin, could not inhibit lysis of rat erythrocytes. The present study shows that 4'-demethyldeoxydopodophyllotoxin, erianin, tetrahydropalmatine, baicalin, luteolin-7-glucuronide-6'-methyl ester and xanthotoxol tested could inhibit lysis of rat erythrocytes. Among the compounds tested, only aromatic hydroxyl group-bearing compounds exhibited potent antioxidative effects. It is obvious that the aromatic hydroxyl group is closely related to the free radical clearing action.

Damage to DNA in the presence of a bleomycin-Fe complex has been adopted as a sensitive and specific method to examine potential pro-oxidant agents (18). If the samples to be tested are able to reduce the bleomycin-Fe³⁺ to bleomycin-Fe²⁺, DNA degradation in this system will be stimulated, resulting in a positive test for pro-oxidant activity. DNA degradation is accompanied by the formation of a product similar to MDA. L-ascorbic acid, a reducing agent, can reduce Fe³⁺ to Fe²⁺. Table VIII shows that tanshinone I and rutin had very weak stimulatory effects. 4'-Demethyldeoxydopodophyllotoxin, erianin, tetrahydropalmatine, baicalin, luteolin-7-glucuronide-6'-methyl ester and xanthotoxol showed no pro-oxidant action in this system.

Discussion

Halliwell(19) defined antioxidants as substances that, when present at low concentrations compared with that of an oxidizable substrate (carbohydrate, lipid, DNA or protein), significantly delay or prevent the oxidation of that substrate. Antioxidants are often added to foodstuff to minimize lipid peroxidation (20,21). Oxygen radicals and lipid peroxides are implicated in the etiology of aging and diseases including cancer, multiple sclerosis, Parkinson disease, senile dementia, autoimmune disease and asbestosis (22). The potential value of antioxidants has prompted investigators to search for compounds with potent antioxidant activity but low cytotoxicity.

Flavonoid compounds exhibit antioxidative and free radical scavenging activities. Zhou and Zheng (7) tested caffeic acid, vanillin, phloridzin, alizarin yellow R and verbascoside, and found that these compounds were able to suppress formation of superoxide anions and production of lipid peroxides. Aeschlach et al. (14) studied the effects of the phenolic compounds thymol, carvacrol, 6-gingerol, hydroxytyrosol and zingerone. The first four compounds inhibited peroxidation of phospholipid liposomes in the presence of Fe(III) and ascorbate and scavenged

TABLE IV

Effects of flavonoids, lignans, an alkaloid and a bisbenzyl on lipid peroxidation in brain and kidney homogenates

Compounds	Brain		Kidney	
	MDA formation (OD532nm)	Inhibition (%)	MDA formation (OD532nm)	Inhibition (%)
Control (Buffer)	1.952±0.064		0.465±0.011	
BHA	0.021±0.003*	98.94	0.086±0.004*	81.51
Flavonoids				
Baicalin	0.042±0.004*	97.87	0.089±0.004*	80.86
Rutin	0.043±0.002*	97.82	0.104±0.001*	77.63
Luteolin-7-glucuronide- 6'-methyl ester	0.055±0.002*	97.21	0.124±0.003*	73.33
Naringin	1.774±0.004*	10.09	0.376±0.006*	19.14
Lignans				
4'-demethyldeoxyppo- dophyllotoxin	0.062±0.004*	96.86	0.108±0.003*	76.77
Deoxypodophyllotoxin	1.559±0.023*	20.98	0.333±0.001*	28.39
Podophyllotoxin	1.880±0.009	4.71	0.450±0.008	3.23
Alkaloid				
Tetrahydropalmatine	0.042±0.002*	97.87	0.108±0.003*	76.77
Bisbenzyl				
Erianin	0.037±0.004*	98.12	0.112±0.005*	75.91

All compounds were dissolved in DMSO and tested at a final concentration of 1mM. The values are mean±SD (n=3). *: P< 0.05 or less compared with control by Students' t test.

TABLE V

Effects of coumarins on lipid peroxidation in brain and kidney homogenates

Compounds	Brain		Kidney	
	MDA formation (OD532nm)	Inhibition (%)	MDA formation (OD532nm)	Inhibition (%)
Control (Buffer)	1.952±0.064		0.465±0.011	
BHA	0.021±0.003*	98.94	0.086±0.004*	81.51
Angelicin	1.651±0.012*	16.32	0.394±0.014*	15.27
Columbianetin	1.663±0.004*	15.71	0.434±0.007*	6.67
O-acetylcolumbianetin	1.875±0.015	4.97	0.443±0.002	4.73
Edultin+cniforin A	1.783±0.012*	9.63	0.638±0.015	-37.20
Oroselone	1.885±0.016	4.46	0.509±0.010	-9.46
Bergapten	1.536±0.013*	22.15	0.335±0.006*	27.96
Xanthoxol	0.566±0.006*	71.31	0.177±0.001*	61.94
Xanthotoxin	1.808±0.005*	8.36	0.431±0.016	7.31

All compounds were dissolved in DMSO and tested at a final concentration of 1mM. The values are mean±SD (n=3). *: P< 0.05 or less compared with control by Students' t test.

TABLE VI

Effects of terpenes on lipid peroxidation in brain and kidney homogenates

Compounds	Brain		Kidney	
	MDA formation (OD532nm)	Inhibition (%)	MDA formation (OD532nm)	Inhibition (%)
Control (Buffer)	1.952±0.064		0.465±0.011	
BHA	0.021±0.003*	98.94	0.086±0.004*	81.51
Ranunculine	1.924±0.021	2.48	0.454±0.004	2.37
Tanshinone I	0.897±0.009*	54.54	0.116±0.004*	75.05
Brucein D	1.946±0.006	1.37	0.453±0.002	2.58
Brusatol	1.900±0.011	3.70	0.446±0.010	4.09
Triptolide	1.885±0.019	4.46	0.447±0.008	3.87
Andrographolide	1.908±0.013	3.29	0.507±0.008	-9.03
Hederagenin	1.929±0.008	2.23	0.444±0.003	4.52

All compounds were dissolved in DMSO and tested at a final concentration of 1mM. The values are mean±SD (n=3). *: P< 0.05 or less compared with control by Students' t test.

TABLE VII

Effects of natural products on hemolysis of rat erythrocytes

Compounds	Inhibition (%)
Control	
L-ascorbic acid	82.80
Baicalin	79.86
Luteolin-7-glucuronide-6'-methyl ester	69.53
Rutin	16.42
4'-demethyldeoxydophyllotoxin	69.50
Tetrahydropalmatine	80.36
Erianin	50.27
Xanthotoxol	79.85
Tanshinone I	2.29

All compounds were dissolved in DMSO and tested at the final concentration of 1mM. The values are mean±SD (n=3).

TABLE VIII

Pro-oxidant effects of natural products on ferric bleomycin-induced DNA damage

Compounds	Extent of DNA damage (OD 532 nm)
Control	0
L-ascorbic acid	0.426±0.025
Baicalin	0.007±0.003
Luteolin-7-glucuronide-6'-methyl ester	0.003±0.002
Rutin	0.015±0.004
4'-demethyldeoxydophyllotoxin	0.001±0.002
Tetrahydropalmatine	0.001±0.001
Erianin	0.002±0.003
Xanthotoxol	0.007±0.002
Tanshinone I	0.012±0.005

All compounds were dissolved in DMSO and tested at the final concentration of 1mM. The extent of DNA damage is expressed by increase in absorbance at 520 nm. The values are mean±SD (n=3).

peroxyl radicals generated by pulse radiolysis. Afanasv (23) reported that the flavonoids rutin and quercetin inhibited Fe (II)-dependent lipid peroxidation of lecithin liposomes and NADPH and CCl₄-dependent lipid peroxidation in rat liver microsomes. Rutin and quercetin inhibited superoxide formation, hydroxyl radical formation in the Fenton reaction and lipid peroxyl radical generation.

In the present investigation the flavonoids baicalin, rutin and luteolin-7-glucuronide-6'-methyl ester manifested potent antioxidative activity in lipid peroxidation but another flavonoid, naringin, only slightly inhibited lipid peroxidation. Baicalin, rutin and luteolin-7-glucuronide-6'-methyl ester possessing a catechol moiety in their structures were potent inhibitors of lipid peroxidation. Naringin had much weaker antioxidative activity because it lacks this moiety (24). It is noteworthy that the aforementioned flavonoids did not exert a pro-oxidant effect as reflected by the results of the assay for Fe-bleomycin-induced DNA damage.

Paya et al. (25) reported peroxyl radical scavenging by a series of coumarins. Paya et al.(26) also tested 16 plant-derived or synthetic coumarins for their ability to inhibit lipid peroxidation and scavenge free oxygen radicals. Coumarins with two free hydroxyl groups potently inhibited non-enzymatic lipid peroxidation in rat liver microsomes stimulated by FeCl₃-ascorbate, while substitution of hydroxyl with methoxy or glucose led to a dramatic drop in the inhibitory activity. Monosubstituted coumarins were inactive. The mechanism of this protective effect of coumarins against lipid peroxidation is unrelated to chelation of Fe³⁺ ions which are used to initiate peroxidation, but involves the possession, by the coumarins, of a favourable electronic distribution for rapidly reacting with and inactivating alkylperoxy radicals and of lipid solubility for effective partition in lipid bilayers. Ortho-dihydroxylated coumarins were pro-oxidant

(enhanced OH^{\bullet}) in the Fe^{3+} -EDTA- H_2O_2 deoxyribose system but reduced OH^{\bullet} production in the Fe^{3+} -ascorbate- H_2O_2 deoxyribose system, suggesting that these compounds can both chelate iron ions and donate electrons for redox cycling of Fe^{3+} . The metasubstituted coumarin 5,7-dihydroxy-4-methylcoumarin possesses a potentially beneficial biochemical profile. It inhibits lipid peroxidation, lacks pro-oxidant activity and scavenges superoxide anions. Coumarin and 7-hydroxycoumarin have been patented in Germany (27) for the scavenging of free radicals. It is noteworthy that xanthotoxol examined in the present investigation also possesses a hydroxyl group attached to an aromatic ring, and it is effective in inhibiting lipid peroxidation. Similarly, the presence of a hydroxyl group attached to an aromatic ring in the molecule of erianin accounts for its antioxidative activity. The tertiary amine structure present in the alkaloid tetrahydropalmatine contributes to its antioxidative activity.

The well-known diterpenoid 12-tetradecanoyl-phorbol-13-acetate (TPA) is widely used as a biochemical tool to study the mechanism of tumor promotion (28). Epidermal effects correlate with 8-lipoxygenase activity and oxidant species formation, which have been suggested as the basis for the sensitivity or resistance to TPA in mice. Oxygen-derived radicals might play a role in the process of carcinogenesis because tumor promoters initiate the oxidative burst with generation of oxygen-activated species in macrophages, monocytes and neutrophils. Oxygen free radicals do not appear to be involved in TPA effects on normal human bronchial epithelial cells. TPA applied topically to mice inhibits both dermal and epidermal catalase-specific activities, implying that TPA diminishes the capacity of H_2O_2 detoxification by catalase.

Diterpenoid tanshinones I have attracted the attention of medicinal chemists and clinicians because many of them exhibit antibacterial, antidermatophytic, anti-inflammatory, antineoplastic and anti-platelet activities. Tanshinone I is an effective coronary artery dilator (29). It is noteworthy that tanshinone I also exhibits antioxidative activity in this study although it has no aromatic hydroxyl group in its structure. The presence of a furan ring may explain its antioxidative activity. More extensive investigations will be need to corroborate this observation.

The results in the present investigation were derived employing rat tissues and compounds originating from the organic extracts of plant tissues. Future experiments conducted using tissues of other animal species and compounds isolated from the same plants with different extraction procedures would be worthwhile and might provide new insights. The expansion of the present study and perhaps focusing of future studies on a particular class of compounds such as flavonoids will help reinforce current observations and more clearly define structure-function relationships.

The ability of the compounds examined in inhibiting lipid peroxidation in brain homogenate in general correlates with their ability to inhibit lipid peroxidation in kidney homogenate, probably because of the similar mechanisms involved in the two tissues. Rutin potently inhibited lipid peroxidation but only weakly inhibited hemolysis probably due to the slightly different mechanisms involved in the two cases. A similar phenomenon was observed with tanshinone I. However, other than these exceptions there was in general a close correlation between the ability of the selected compounds to inhibit lipid peroxidation in brain and kidney homogenates and their capability to suppress hemolysis. In another study (unpublished data) using aqueous extracts of twelve Chinese medicinal herbs the percent inhibition achieved by a herbal extract on lipid peroxidation in brain and kidney homogenates matched very well, with the exception of only one extract, with the percent inhibition of hemolysis.

To recapitulate, in this study the flavonoids baicalin, rutin and luteolin-7-glucuronide-6'-methyl ester, the alkaloid tetrahydropalmatine, the lignan 4'-demethyldeoxy podophyllotoxin, the bisbenzyl erianin and the coumarin xanthoxol were found to exhibit potent antioxidant activity.

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References

1. Z.T. WANG, T.B. NG, and G.J. XU, *Gen. Pharmac.* **26** 1211-1224 (1995).
2. E.J. LIEN and W.Y. LI *Advances in Chinese Medicinal and Materials Research*. Singapore, World Scientific Press; pp433-454 (1985).
3. G.J. ZLABINGER *Coumarins*. New York: John Wiley and Sons Press; pp103-124 (1997).
4. T.B. NG, J.M.L. LING, Z.T. WANG, J.N. CAI and G.J. XU, *Gen. Pharmac.* **27** 1237-1240 (1996).
5. Z.Z. GRODZINSKA, N.I. ZGORNIK, M. MARCISZEWSKA and A. GATKIEWICZ, *Chem. Abstr.* **86** 37540c (1977).
6. C.V. RAO, A. RIVENSON, B. SIMI and B.S. REDDY, *Cancer Res.* **55** 259-266 (1995).
7. Y.C. ZHOU and R.L. ZHENG, *Bioch. Pharm.* **42** 1177-1179 (1991).
8. L.A. QUINN and H.H. TANG, *J. Am. Oil Chem. Soc.* **73** 1585-1588 (1996).
9. T.A. SEYMOUR, S.J. LI and M.T. MORRISSEY, *J. Agric. Food. Chem.* **44** 682- 685 (1996).
10. T.B. NG, J.M.L. LING, Z.T. WANG, J.N. CAI and C.J. XU, *Gen. Pharmac.* **27** 1237-1240 (1996).
11. T.Y. CHAN and P.L. TANG, *J. Pineal Res.* **20** 187-191 (1996).
12. M. MIKI, H. TAMAI, M. MINO, Y. YAMAMOTO and E. NIKI, *Arch. Biochem. Biophys.* **258** 373-380 (1987).
13. H. SUGIYAMA, K.P. FUNG and T.W. WU, *Life Sci.* **53** 39-43 (1993).
14. R. AESCHLACH, J. LOLIGER, B.C. SCOTT, A. MURCIA, J. BUTLER, B. HALLIWELL and O.I. ARUOMA, *Food Chem. Toxic.* **32** 31-36 (1994).
15. T.Y. CHAN and P.L. TANG, *J. Pineal Res.* **20** 187-191 (1996).
16. H. ESTERBAUER, *Free Radical in Liver Injury*. Oxford: IRL Press, pp 29-48 (1985).
17. K. PRASAD, V.A. LAXDAL, M. YU and B.L. RANEY, *Mol. C. Bioch.* **154** 55-63 (1996).
18. J.M.C. GUTTERIDGE, D.A. ROWLEY and B. HALLIWELL, *Biochem. J.* **199** 263-265 (1981).
19. B. HALLIWELL, *Free Rad. Res. Comms.* **9** 1-32 (1990).
20. B.J.F. HUDSON, *Food Antioxidants*. Elsevier, Amsterdam, (1990).
21. J. LOLIGER, *Free Radicals and Food Additive*, Taylor Francis, London, pp.121-150 (1991).
22. B. HALLIWELL and J.M.C. GUTTERIDGE, *Free Radicals in Biology and Medicine*, Oxford University Press, New York, pp.218-313 (1985).
23. I.B. AFANASV, A.I. DOROZHKO, A.V. BRODSKII, A. KOSTYUK and A.I. POTAPOVITCH, *Bioch. Pharm.* **38** 1763-1769 (1989).

24. Y.H. MIURA, I. TOMITA, T. WATANABE, T. HIRAYAMA and S.FUKUI, *Biol. Pharm. Bull.* **21** 93-96 (1998).
25. M. PAYA, B. HALLIWELL and R.S. HOULT, *Free Rad. Res. Comms.* **17** 293-298 (1992).
26. M. PAYA, B. HALLIWELL and R.S. HOULT, *Bioch. Pharm.* **44** 205-214 (1992).
27. E.F. ELSTNER, P. SCHUFF-WERNER and D. SEIDEL, *Deutsches Patentamt Nr. De 37, 15, 990A1*, Offenlegungstag: 08, 12, (1988).
28. M.J. ALCARAZ and J.L. RIOS, *Ecological Chemistry and Biochemistry of Plant Terpenoids*. Clarendon Press. Oxford, pp. 23-263 (1991).
29. H.M. CHANG, K.P. CHENG, T.F. CHOANG, H.F. CHOW, K.Y. CHUI, P.M. HON, F.W.L. TAN, Y. YANG and Z.P. ZHONG, *J. Org. Chem.* **55** 3537-3539 (1990).