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Note

Flavonoids from *Saussurea stella* Maxim as superoxide scavengers and antioxidants

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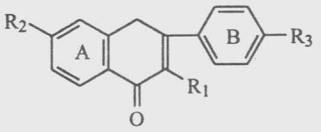
Flavonoids apigenin, acacetin, tilianin, kaempferol and kaempferol-3-*O*-L-rhamnoside have been isolated from the herb *Saussurae stella* Maxim and superoxide anion scavanging and lipid peroxidation inhibitory activities of these flavonoids are reported.

Free radicals have been implicated in the pathophysiology of many diseases status, such as inflammation, ischemia-reperfusion damage, atherosclerosis and carcinogenesis. The diseased or damaged tissues undergo radical reaction much more readily than normal tissues thus exacerbating the primary lesion. The excessive superoxide anion generation and lipid peroxidation can lead to progressive membrane,cellular and tissue damage. Therefore, traditionally natural antioxidants, free radical scavengers, from herbs and foods have played very important roles in medicine and health protection[1]. Flavonoids are also able to scavenge many types of free radicals, including superoxide anions and lipid peroxyl radicals, and to inhibit lipid peroxidation. The therapeutic effects of flavonoids may be related with their free radical scavenging activities[2,3]. Flavonoids are widespread in the plant kingdom and have various physiological activities, some have been used to treat inflammation, diabetes mellitus, allergy, virus infection, oral surgery, stomach duodenal ulcer, cancer cells normalization, pain relief, bleeding cease, smooth muscles relaxation, virus protein coat removal and pressure in eye reduce, etc[4]. Our previous study showed that two flavonoids extracted from *Saussurea involucrata* significantly inhibit DNA synthesis of both ascites hepatoma and S_{180} cells[5].



	R_1	R_2	R_3
Apigenin	H	OH	OH
Acacetin	H	OH	OMe
Tilianin	H	OGluc	OMe
Kaempferol	OH	OH	OH
Kaempferol-3-*O*-L-rhamnoside	ORh	OH	OH

Me = methyl, Gluc = glucosyl, Rh = rhamnoyl

Chart 1

Saussurea stella Maxim is distributed in northwest China including Gansu, Qinghai, Yunnan, Sichuan provinces and the Tibet Autonomous Region and has been used to treat bone fracture and to relieve internal heat, rheumatic pain, food poising[6,7]. We have studied for the first time the chemical composition of this herb and obtained five flavonoids[8]. Chinese medicinal herbs have long been used to treat lots of diseases in China for centuries. In recent year, great progress has been achived in studies on the effects and mechanisms of flavonoids, as well as on the relationship between flavonoids and human health. But the molecular bases of their therapeutic mechanisms have yet to be deeply investigated. In this article, we have reported the superoxide anion scavenging and lipid peroxidation inhibitory activities of five flavonoids extracted from this herb.

Results and Discussion

Flavonoids. The five flavonoids, apigenin, acacetin, tilianin, kaempferol and kaempferol-3-*O*-L-rhamnoside, were isolated from *Saussurea stella* Maxim[8]. Their structures are shown in Chart 1.

Table I–Half inhibition concentrations (IC_{50}, μM) for generation of superoxide and mouse liver microsomal lipid peroxidation by flavonoids

	Superoxide	Lipid peroxidation
Apigenin	52.5 + 3.8	27.4 + 1.3
Kaempferol-3-*O*-L-rhamnoside	72.4 + 4.9	29.9 + 1.6
Kaempferol	85.1 + 3.9	9.0 + 0.5
Tilianin	153.8 + 6.8	/
Acacetin	168.5 + 8.1	11.6 + 0.7

Scavenging on superoxide anions. The half inhibition concentration (IC_{50}) values of the five flavonoids on superoxide anions generation are shown in Table I. All the five flavonoids showed significant scavenging effects. The IC_{50} values of apigenin, kaempferol-3-*O*-L-rhamnoside and kaempferol were within 52.5-85 μM range, and those of acacetin and tilianin were within 154 - 168 μM range.

Inhibition of lipid peroxidation. Among the four flavonoids, kaempferol showed the strongest inhibitory effect on microsomal lipid peroxidation (9 μM), acacetin ranked the second (11.6 μM), while the apigenin and kaempferol-3-*O*-L-rhamnoside were the weakest (27.6 and 29.7 μM) (Table I).

In comparison with the structures and IC_{50} value for superoxide anions generation of the five flavonoids, it seems that the phenolic group on C-4 in B ring is important for the scavenging effect. For example, the scavenging effect is strong for apigenin, kaempferol and kaempferol-3-*O*-L-rhamnoside. While phenolic group substituted by methoxy group, such as acacetin and tilianin, the scavenging effects decreased.

In comparison with the antioxidative and scavenging activities of flavonoids, there is no correlation. This result is in accordance with our previous report[5], and demonstrates our suggestion[9] that the phenolic compounds have more than one mechanism of action for free radicals and lipid peroxidation. For example, phenolic compounds may also be able to suppress the formation of free radicals by binding of heavy metal ions which are known to catalyze many processes leading to the appearance of free radicals[10]. Interestingly, apigenin and kaempferol exhibited the reverting effect on the transformed phenotypes of v-H-*ras* transformed NIH3T3 cells. Treatment with 25 μM of these flavonoids could effectively reverse the transformed morphology of cells into flatter cells with contact inhibition[11]. An increasing amount of experimental evidence inplicates in the involvement of free radicals in the carcinogenesis[5,12]. Although the data do not conclusively prove that the therapeutic effects of flavonoids might be the scavenging of radicals and the inhibition of lipid peroxidation, but it is in support of such a hypothesis. Hopefully, the results may help in the explanation of pharmacological mechanism of this herb.

Experimental Section

Chemicals and flavonoids. NADH and 2-thiobarbituric acid (TBA) were obtained from Sigma. Nitroblue tetrazolium chloride (NBT) was purchased from Fluka. Phenazine methosulphate (PMS) was a product of Shanghai Biochemistry Institute, Chinese Academy of Sciences.

Superoxide anion generation and detection. Superoxide anions were generated in the PMS/NADH system and detected by NBT reduction[18] . Briefly, samples contained in final concentrations: NBT 50 μM, NADH 73 μM, in Tris-HCl buffer pH 8.0, 0.016 *M*. Reaction was started by adding PMS 10 μM. After 2 min of reaction at room temperature, the absorbance of samples reached maximum, read at 560 nm against blank sample which contained no PMS. The flavonoids were dissolved in DMSO as a concentrated solution and added to reach various final concentrations. The final concentration of DMSO in each sample was adjusted to same value and below 3.3% (v/v). Solution with DMSO but without flavonoid was used as control. Half inhibition concentration value (IC_{50}) for the generation of superoxide anions by the tested flavonoids was calculated by regression method.

Lipid peroxidation in mouse liver microsomes. Mouse liver microsomes were prepared according the reference 10. The microsomal pellet was stored at –20°C and resuspended in cold KCl 0.15 *M*. Lipid peroxidation was induced by ferric/ascorbate. The reaction mixtures contained substances in final concentrations: 0.25 mg microsomal protein/mL,

ferric 0.1 *M* and flavonoids of various concentrations in KH_2PO_4-KOH buffer 10 *M*, *p*H 7.4. Ascorbate 0.5 *M* was added to start the reaction. The final volume of reaction mixture was 1.0 mL. Tubes contained the reaction mixture were incubated at 37°C with shaking for 20 min. Reaction was terminated by adding trichloroacetic acid 20% (w/v) 1.0 mL. The mixture was vortexed and cooled in ice water for 10 min. After adding TBA 0.67% (w/v) 1.5 mL, tubes were heated at 100°C for 10 min, then cooled with tap water and centrifuged at 3000x g for 10 min. The absorbance of supernatant at 532 nm against blank sample were read. Sample without flavonoid but with DMSO was used as control. The IC_{50} of each flavonoid was calculated using the regression method. Protein content of microsomes was measured by Lowry method.

Every test has at least three replicates.

Acknowledgement

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