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Antioxidant Activity of Flavonoids on Superoxide Anion Free Radical ($O_2^{\bullet-}$)

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The objective of the present investigation was to study the antioxidant capacity of quercetin, catechin, luteolin, scutellarein, genistein and baicalein on superoxide anion free radical ($O_2^{\bullet-}$) and explored the relationship between their structure and clearing ability. In this paper, the antioxidant capacity and IC_{50} were determined by cyclic voltammetry. Result showed that six kinds of flavonoids have strong scavenging ability to $O_2^{\bullet-}$. The removal ability turn out to be quercetin (IC_{50}, 0.4659) > catechin (IC_{50}, 0.5867) > luteolin (IC_{50}, 0.7085) > scutellarein (IC_{50}, 1.3066) > baicalein (IC_{50}, 1.4836) > genistein (IC_{50}, 1.7695). The results indicate that clearing ability was closely related to their structure. The number and location of hydroxyl in A, B, C rings, *meta*-hydroxy, pro-hydroxyl and double bond of the formatting are all important influencing factors to antioxidant activity.

Keywords: Flavonoids, Antioxidant activity, Cyclic voltammetry, Antioxidant capacity.

INTRODUCTION

There has been a tremendous increased in research related to reactive oxygen species (ROS), such as superoxide anion ($O_2^{\bullet-}$), hydroxyl radical ($OH^{\bullet}$), single oxygen ($^1O_2$) and hydrogen peroxide H_2O_2, because they are involved in a number of biochemical processes. A lot of research shows that free radical reactions are implicated in the pathology of many human diseases including atherosclerosis, ischemic heart disease, ageing and others [1]. Among all the reactive oxygen species, the superoxide anion ($O_2^{\bullet-}$), is the most aggressive and toxic oxygen radical known to date, which can initiate radical chain reactions and has been suggested to play a central role in many pathological processes [2,3]. Therefore the importance of removing excessive active oxygen species, especially $O_2^{\bullet-}$ from living organisms, is becoming increasingly recognized.

The flavonoids are a group of polyphenolic compounds, which have gained recent interest because of their broad pharmacological activity. Large number of studies showed that flavonoids have free radical scavenging, antioxidant, antimutation, antitumor, antibacterial, antiviral and immunoregulation function [4-7]. So it was significant to find natural antioxidants with low toxic or non-toxic. Electrochemical method expressed its unique advantages in detecting of antioxidant capacity, for example high sensitivity, small sample, simple instrument and real-time on-line analysis. And besides, flavonoids showed specific electrochemical activity. For these reasons, cyclic voltammetry [8] as the optimal method has been established for determination antioxidant activity of natural antioxidant. At last, the mechanism of antioxidant activity for flavonoids was inferred.

The main aim of this work was to detect the antioxidant properties of quercetin, catechin, luteolin, scutellarein, genistein and scutellarein on $O_2^{\bullet-}$, through cyclic voltammetry *in vitro* assay system and provide insight information with reference to medicinal value of natural antioxidant.

EXPERIMENTAL

Quercetin, catechin, luteolin, scutellarein, genistein and baicalein were purchased from Chengdu Must Bio-Technology Co., Ltd (Chengdu, China, HPLC, purity ≥ 98 %). Pyrogallol and other chemical reagents were obtained from Tianjin Chemical Reagent Company (Tianjin, China). All other aqueous solutions were prepared in distilled water. CHI600E electrochemical workstation was obtained from Shanghai Chen-hua Instruments Co., Ltd. Electronic balance-AL204 from Mettler-Toledo Instruments Co., Ltd.

Electrochemical measurement system: All the experiments of detection were carried out in CHI600C electrochemical workstation, glassy carbon electrode as working electrode, saturated calomel electrode as reference electrode, platinum electrode as the counter electrode. Experimentally determined the optimum pH of PBS (NaH_2PO_3-Na_2HPO_3,

50 mmol/L) and scanned rate. The optimal pH value and scanned rate were 7.5 and 90 mV s^{-1}.

Sample preparation: Accurately weigh quercetin, catechin, baicalein, scutellarein, luteolin and genistein, 0.1 mg, 0.2 mg, 0.4 mg, 0.6 mg, 0.8 mg and prepared to 0.01 mg/mL, 0.02 mg/mL, 0.04 mg/mL, 0.06 mg/mL, 0.08 mg/mL with methanol, keep at 4 °C in fridge. Pyrogallol prepared to reserve liquid and dilution before use.

Experimental procedure: $O_2^{\bullet-}$: The oxidation of pyrogallol under alkaline conditions produced $O_2^{\bullet-}$. Six kinds of flavonoids solution prepared in a series of concentration. Control group: sample + methanol; Experience group: sample + $O_2^{\bullet-}$.

Add 10 mL 50 mmol/L phosphate buffer solution into electrolytic cell, pH was 7.4, scanned rate was 90 mV s^{-1}, sweeping the potential between +2V and -2V, pass into the high purity nitrogen removal of oxygen before experiment. Then recorded the oxidation peak current and peak potential of experimental group and control group, calculation the inhibition rate of antioxidants on free radical and count IC_{50} of every samples, The scavenging activity was calculated by the following formula:

$$\text{Inhibition ratio (\%)} = \frac{(I_{P0} - I_P)}{I_{P0}} \times 100$$

where, I_P: the oxidation peak current value of reaction system without radicals; I_{P0}: the oxidation peak current value of reaction system after adding the radicals; IC_{50}: antioxidant concentrations of superoxide anion free radical scavenging to half.

RESULTS AND DISCUSSION

Different concentrations of flavonoids (quercetin, catechin, baicalein, scutellarein, luteolin and genistein) reacting with free radical, the concentration changed from 0 to 0.08 μg/mL, as showed in Fig. 1. When reaction take place between antioxidants and free radical, oxidation reduction was occured and exist electronic gains and losses, then the current value of cyclovoltametry in system was changed (Fig. 1). When the

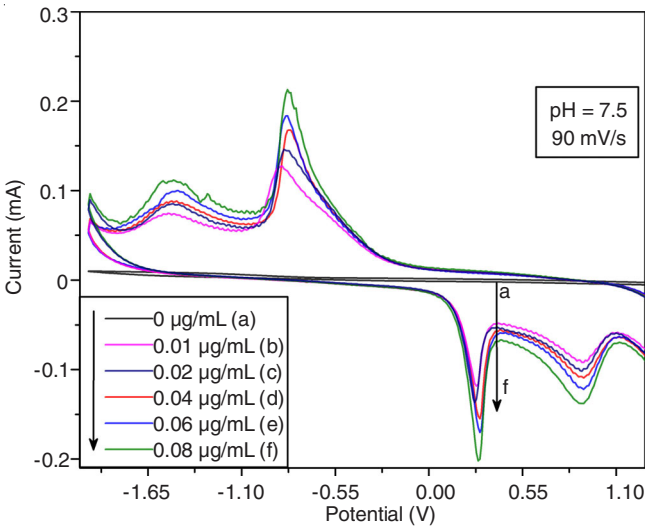

Fig. 1. Linear sweep voltammetry curve of different concentrations of flavonoids (quercetin, catechin, baicalein, scutellarein, luteolin and genistein)

TABLE-1
SCAVENGING EFFECTS OF SIX NATURAL FLAVONOIDS ON FREE RADICAL

Antioxidants	Concentration (μg/mL)	$I_P \times 10^{-5}$ A	$I_{P0} \times 10^{-5}$ A	Inhibition ratio (%)	IC_{50} (μg/mL)
Catechin	0.01	-2.530	-3.210	21.6	0.5867
	0.02	-3.232	-4.650	30.5	
	0.04	-3.481	-5.871	40.6	
	0.06	-3.346	-7.029	52.4	
	0.08	-3.648	-9.142	60.1	
Quercetin	0.01	-2.740	-3.581	23.5	0.4659
	0.02	-2.942	-4.417	33.4	
	0.04	-2.852	-6.017	52.6	
	0.06	-2.945	-7.853	62.5	
	0.08	-3.122	-8.721	64.2	
Luteolin	0.01	-2. 976	-3.318	10.3	0.7085
	0.02	-3.127	-4.061	23.0	
	0.04	-3.206	-4.895	34.5	
	0.06	-2.961	-5.760	48.6	
	0.08	-3.472	-7.013	50.5	
Genistein	0.01	-2.863	-3.253	12.0	1.7695
	0.02	-3.242	-3.827	15.3	
	0.04	-3.519	-4.302	18.2	
	0.06	-3.723	-4.905	24.1	
	0.08	-3.925	-5.451	28.0	
Baicalein	0.01	-2.787	-3.211	13.2	1.4836
	0.02	-2.879	-3.419	15.8	
	0.04	-3.074	-3.847	20.1	
	0.06	-3.160	-4.235	25.4	
	0.08	-3.579	-5.302	32.5	
Scutellarein	0.01	-2.552	-2.985	14.5	1.3066
	0.02	-2.797	-3.466	19.3	
	0.04	-2.952	-4.005	26.3	
	0.06	-2.957	-4.381	32.5	
	0.08	-3.073	-4.621	33.5	

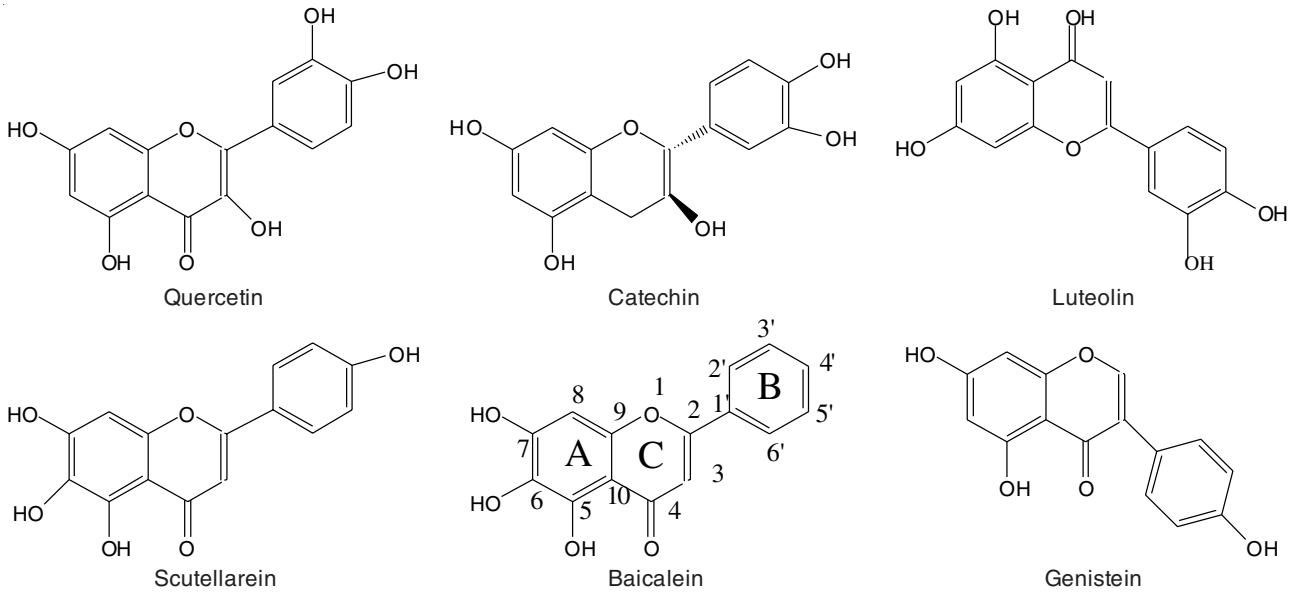

Fig. 2. Six kinds of flavonoids structure

concentration of flavonoids was 0, there has no electronic gains and losses, so the cyclovoltametric curve was horizontal, along with the concentration increased the more vigorous of redox reaction, the more electronic gains and losses increased, the oxidation peak current of cyclovoltametry performed larger. For the oxidation peak current of cyclovoltametry have been used to evaluate the antioxidant activity [9,10]. So it indicated that six kinds of flavonoids all have $O_2^{\bullet-}$ scavenging capacity. However, the removal ability made a difference about flavonoids, as showed in Table-1.

Table-1 showed that six kinds of flavonoids all have $O_2^{\bullet-}$ radicals scavenging capacity and exist dose dependence, with the increase of concentration the scavenging effect were enhanced, but the removal effect made a difference about flavonoids. The order of scavenging ability on $O_2^{\bullet-}$ radicals act as quercetin (IC_{50}, 0.4659) > catechin (IC_{50}, 0.5867) > luteolin (IC_{50}, 0.7085) > scutellarein (IC_{50}, 1.3066) > baicalein (IC_{50}, 1.4836) > genistein (IC_{50}, 1.7695). While difference is related to the structure of compounds (Fig. 2).

Quercetin, catechin and luteolin all have *meta*-hydroxy on A ring and adjacent hydroxyl on B ring, which performed strong free radical scavenging capacity as showed in Table-1. While the structure of genistein is similar to luteolin, but has not adjacent hydroxyl on B ring, the results showed different, that its scavenging ability much smaller than luteolin. So it is easy to find that the adjacent hydroxyl more significant than *meta*-hydroxy in clearing $O_2^{\bullet-}$ radicals.

Simultaneously, the number and location of hydroxyl on the ring also have a great impact on scavenging capacity. Scutellarein and baicalein have the parallel structure, however the scavenging ability of scutellarein is better than baicalein, for a hydroxyl on B ring. At the same time, double bond and conjugated structure are all important factors in enhanced antioxidant capacity.

Conclusion

The paper used cyclic voltammetry and compared electrochemical behaviour of six kinds of flavonoids on $O_2^{\bullet-}$ elimination. The method is sensitive, rapid credible and simple operation. Results showed, six kinds of flavonoids all performed good clearing capacity on $O_2^{\bullet-}$ radicals and closely related to their structure.

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