Research Square

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Component content and antioxidant activity of different extracts of cigarette ethanol extract

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Research Article

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Abstract

In this paper, the contents of total flavonoids, total phenols, and antioxidant activity of different extracts of cigarette ethanol extract were studied, and their correlation was to be analyzed. The contents of total flavonoids were determined by $Al(NO_3)_3$-$NaNO_2$ colorimetric method, and the contents of total phenols were determined by the Folin-phenol method. 1,1-diphenyl-2-picrylhydrazyl(DPPH) method and pyrogallol autoxidation method were used to study the antioxidant activities of each extract. Last, the correlation between antioxidant activities and the contents of each extract was analyzed. The results showed that the contents of total flavonoids (28.34 ± 0.20mg/g, 29.49 ± 0.14mg/g) and total phenols (24.89 ± 0.06mg/g, 24.44 ± 0.08mg/g) in ethyl acetate and n-butanol fractions were the highest. The scavenging rates of DPPH and superoxide anion of ethyl acetate and n-butanol fractions in IC_{50} were also the best. Correlation analysis showed that there was a positive correlation between the antioxidant activity and its content. Different extracts of cigarette ethanol extract contain total phenols, total flavonoids and antioxidant activity, among which the ethyl acetate extract is the best.

Introduction

Tobacco is the second largest cash crop in the cauldron of Solanaceae, which is rich in protein [] and alkaloids []. As far as the Chinese tobacco monopoly is concerned, although smoking harms people's health, restraining smoking threatens social stability and government income, which may be more serious problems for any government []. Although smoking cigarettes is very harmful to the human body, with the spread of cigarettes becoming more and more widespread, people have discovered more and more value of cigarettes: early records show that cigarettes can treat beriberi, the poison of snakes and adder, and treat malaria, etc []. According to the earliest medical book of tobacco in China, Materia Medica, "This medicine is very bitter, so that the smoke is burned and inhaled into the throat, which can greatly protect the cold of frost, dew, wind and rain, and avoid the gas of mountain ghosts and evil spirits. Children can eat this medicine to kill malnutrition, and women can eat this medicine to eliminate disease and ruffian" []. "National Compilation of Chinese Herbal Medicine" records: cigarette warm, with swelling, detoxification, insecticide and other effects. In recent years, there are more and more studies on cigarettes, which are widely used in many research fields such as pharmacology, chemistry and biology. Researchers use genetic engineering means to use cigarettes as bioreactors to produce safe and high-quality drugs with high efficiency and low cost [–]. At present, the drug development related to cigarettes mainly includes transgenic cigarette medicinal protein, antibody and vaccine []. Flavonoids are the general name of a class of natural compounds. As one of the main active components of antioxidant, flavonoids have a variety of pharmacological effects and biological activities such as lowering blood sugar, lowering blood pressure, preventing arteriosclerosis, anti-aging, antibacterial, anti-inflammatory, antiviral and so on []. Phenols are important secondary metabolites in plants, which not only play an important role in plant resistance to disease and insect pests and stress resistance [], but also have antioxidant, free radical scavenging, anti-aging, anti-cancer and anti-cancer, anti-radiation and prevention of cardiovascular and

cerebrovascular diseases [-]. In this experiment, the content of components in different extraction parts of cigarette ethanol extract was detected and the antioxidant activity of each component was studied by different antioxidant methods. It provides the experimental basis for further comprehensive utilization of tobacco, and also provides a reference for enriching plant antioxidant libraries.

Materials And Methods

Materials and reagents

(1) Materials: The cigarettes come from tian Xia Xiu cigarettes of Sichuan China Tobacco Industry Co., LTD; Rutin was purchased from Shanghai Luyuan Biotechnology Co., LTD.

(2) Reagents: Analytically pure (AR) : Salicylic acid, gallic acid, potassium dihydrogen phosphate, ascorbic acid (Vc), ferrous sulfate, anhydrous sodium carbonate, sodium nitrite, aluminum nitrate, pyrogallic acid, 30% hydrogen peroxide, ethanol absolute.

Guaranteed reagent (GR): Sodium hydroxide, hydrochloric acid.

Biological reagent (BR) : folinol,1,1-diphenyl-2-picrylhydrazyl.

(3) Instrument: GL-20G-□ bench high speed refrigerated centrifuge (Shanghai Anting Scientific Instrument Factory); KQ5200 CNC ultrasonic cleaner (Kunshan Ultrasonic Instrument Co., LTD); KW-1000DC Constant temperature Water bath (Guohua Electric Appliance Co., LTD); ME204E Electronic balance (Mettler Toledo Instrument (Shanghai) Co., LTD); L6S UV-Visible Spectrophotometer (Shanghai Precision Scientific Instrument Co., LTD); Microwave chemical reaction system (Microwave synthetic extraction system) (Shanghai Yiyao Instrument Technology Development Co., LTD); SHB-□ Circulating water multi-purpose vacuum pump (Zhengzhou Great Wall Science, Industry and Trade Co., LTD); DGG-9140A Type Electric Heating Constant Temperature Air Blowing Drying Oven (Shanghai Senxin Experimental Instrument Co., LTD); 750T multi-functional mill (Platinum Ou hardware Factory); FDU-1200 Vertical freeze dryer (Japan); DC-0506 Low temperature constant temperature tank (Shanghai Shunyu Hengping Scientific Instrument Co., LTD); Model C 30 Glass instrument quick dryer (Great Wall Science, Industry and Trade Co. LTD); RV10 digital Rotary evaporator (IKA).

Pretreatment of raw materials

The cigarettes are dried (60℃) to constant weight, crushed, screened, and stored in refrigerator at -20℃ for later use. 200 g cigarette powder was accurately weighed, soaked in 95% ethanol, and repeatedly extracted by microwave (300 W, 6 min) for three times. Then, brewer funnel was used for extraction and combined with filtrate, and ethanol extract (A, 53.0 g) was condensed under pressure. 2 g ethanol extract was taken for use, and the rest was dissolved and suspended in water. Petroleum ether extract (B, 5.3 g), ethyl acetate extract (C, 1.5 g), n-butanol extract (D, 10.9 g) and water extract (E, 35.0 g) were obtained by rotary evaporation and concentration of each organic extraction layer and the last water layer.

Experimental methods

The standard curve of total flavonoids and the content determination of total flavonoids in different extraction parts

$Al(NO_3)_3$-$NaNO_2$ colorimetric method was used to determine the content of total flavonoids . 10.0mg of rutin standard was accurately weighed, dissolved in anhydrous ethanol and dissolved in a 25 mL volumetric flask. The standard solution of rutin (0.4 mg /mL) was obtained by shaking well. Absorb 0.00 0.50 1.00 2.00 2.50 3.00 and 3.50 mL rutin standard solution in seven 10 mL volumetric bottles, each adding 0.40 mL 5% $NaNO_2$ solution, shake well, stand for 6 min; Then 0.40 mL 10% $Al(NO_3)_3$ solution was added, shaken well, and stood for 6 min; Then add 4.00mL 4% NaOH solution, anhydrous ethanol to the scale, shake well, stand for 15 min, absorb 0.00 mL rutin standard solution as blank control, measure the absorbance at 510 nm wavelength, three times in parallel. A standard curve was drawn with mass concentration as x-coordinate (X) and absorbance as y-coordinate.

2.00 mL of the liquid to be tested with different mass concentrations was accurately absorbed, and the absorbance of different extraction parts was measured according to the above method and substituted into the standard curve equation to calculate the total flavonoid content of each component of the sample.

Standard curve of total phenol and determination of total phenol content in different extraction parts

Folin-phenol method was used to determine total phenols . 10.0 mg standard gallic acid was accurately weighed and dissolved in distilled water in a 50 mL volumetric flask. The standard gallic acid solution (0.2 mg /mL) was obtained by shaking well. Accurately absorb 0.00 0.10 0.20 0.30 0.40 and 0.50 mL gallic acid standard solutions into six 10 mL volumetric bottles, add 5.00 mL distilled water, add 0.50 mL Folin-phenol reagent, shake well, stand for 2 min. 2.00 mL 7.5% Na_2CO_3 solution was added, distilled water was kept constant volume to scale, shaken well, constant temperature water bath at 75℃ for 10 min, dark room temperature for 20 min, and the absorbance was measured at 760 nm. A standard curve was drawn with mass concentration as x-coordinate (X) and absorbance as y-coordinate.

Accurately absorb 1.00 ml of the solution to be tested with different mass concentrations, as the above method to determine the absorbance of different extraction parts and substitute into the standard curve equation, calculate the total phenol content of each component of the sample.

Study on antioxidant activity of cigarette

DPPH clearance rate was determined

On the basis of Zhang Ming et al. , the clearance rate of DPPH was optimized and determined. 2.00 mL solution of different mass concentrations to be tested was accurately absorbed (mass concentration was

modified according to clearance rate), and 2.00 mL 0.15 mmol /L DPPH solution was added, shaken well, dark for 20 min at room temperature, and its absorbance was measured at 517 nm wavelength (A1). 0.15 mmol/L DPPH solution was replaced with an equal amount of anhydrous ethanol, and its absorbance was measured under the same conditions (A_2). The absorbance (A_0) was measured under the same conditions by replacing the samples of different concentrations with anhydrous ethanol. Vc was taken as the control, and determined 3 times in parallel.

DPPH free radical scavenging rate (%) $=[1-\frac{\left(A_1-A_2\right)}{A_0}]\times 100$

Determination of hydroxyl radical scavenging rate

According to the hydroxyl radical scavenging rate of different extraction parts of cigarette ethanol extract, the concentration of extracts obtained from different extraction parts of cigarette extract was configured, 1.5 mL sample solution was taken, 1.0 mL 2.5 mmol/L salicylic acid solution, 1.0 mL 5 mmol/L $FeSO_4$ solution and 2.0 mL distilled water were successively added to mix thoroughly, and 1.0 mL 5 mmol/L H_2O_2 was added, and placed in a 37℃ constant temperature water bath for reaction for 30 min. The absorbance was measured at 510 nm. Vc was taken as the control and determined in parallel three times.

hydroxyl radical scavenging rate (%) $=[1-\frac{\left(A_2-A_1\right)}{A_0}]\times 100$

Note

A_0 is blank control absorbance; A_2 is the absorbance of sample solution (plus H_2O_2); A_1 is the absorbance of sample solution (without H_2O_2).

Determination of superoxide anion clearance

Pyrogallol autoxidation method was adopted . 5.00 mL 50 mmol/L phosphate buffer solution was taken, and 2 mL of sample solution to be tested with different mass concentrations (according to the superoxide anion clearance rate of different extraction parts of cigarette ethanol extract) was added, then 1.00 mL 5 mmol/L pyrogallol solution was added, and mixed. After dark for 5 min at room temperature (26℃), 1.00 mL 10 mol/L hydrochloric acid solution was added to stop the reaction, and the absorbance was measured at 320 nm (A). An equal amount of distilled water was used to replace the sample solution, and the absorbance (A_0) was similarly determined. 5 mmol/L pyrogalechol solution was replaced by an equal amount of distilled water, and the absorbance was measured similarly (A_1). Vc was used as the control.

Superoxide anion radical scavenging rate (%) $=[1-\frac{\left(A-A_1\right)}{A_0}]\times 100$

Data processing

Microsoft Office Excel 2010 and Origin 2019 were used for data analysis and mapping to obtain antioxidant clearance rates of different extraction parts of cigarette ethanol extract. The correlation between antioxidant activity and content of cigarette components was obtained by USING IBM SPSS Statistics software.

Results And Analysis

Analysis of total flavonoids and phenolic content

Using mass concentration as abscissa (X) and absorbance as ordinate (Y), the standard curve was drawn, and the linear equation of Rutin was obtained: Y = 10.656x + 0.0258 (R^2 = 0.9971), and the linear range was 0.02–0.14 mg /mL; Gallic acid linear equation: Y = 90x + 0.0153 (R^2 = 0.998), linear range of 0.002–0.010 mg/mL; According to the experimental results, there were total flavonoids and total phenols in different extraction parts of cigarette ethanol extract, but the contents were significantly different (as shown in Fig. 1 and Fig. 2). The total flavonoid content was D > C > A > B > E. Total phenol content: C > D > A > E > B. The contents of total flavonoids and total phenols in n-butanol and ethyl acetate fractions were very high, indicating that flavonoids and phenolic compounds could highly exist in n-butanol and ethyl acetate fractions.

Note

Ethanol extract: A; Petroleum ether extract: B; Ethyl acetate extract: C; N-butanol extract: D; Water phase: E; The same below.

Analysis of antioxidant activity

Effect on DPPH free radical scavenging

It can be seen from Figs. 3 and 4 that different extraction parts of cigarette ethanol extract have different degrees of influence on DPPH free radical scavenging. Within a certain range of mass concentration, the scavenging rate of DPPH free radical of each part is positively correlated, and there is an obvious dose-effect relationship when the mass concentration of extract (dewatering phase) is 0.0-0.1 mg/mL. As shown in the figure, when the mass concentration was 0.05 mg/mL, the influence of extraction parts on DPPH radical scavenging rate was as follows: Vc > C > D > A > E > B. Among all extraction parts of cigarettes, ethyl acetate extract had the highest scavenging rate (83.99%), while petroleum ether extract

had the lowest scavenging rate (28.57%). As can be seen from Table 1, the IC_{50} of ethyl acetate (0.013 ± 0.010 mg/mL) was the lowest, while the IC_{50} of n-butanol (0.015 ± 0.016 mg/mL) was not much different from that of n-butanol. Both of them had stronger antioxidant capacity than others. The results showed that the main substances of DPPH radical scavenging activity of cigarette mainly existed in ethyl acetate and n-butanol fractions.

Table 1

IC_{50} of different extracts of tobacco ethanol extract on DPPH free radical($\bar{X} \pm SD$)

The sample	A	B	C	D	E	Vc
IC_{50}(mg/mL)	0.049 ± 0.009	0.124 ± 0.049	0.013 ± 0.010	0.015 ± 0.016	0.049 ± 0.045	0.005 ± 0.017

Effect on hydroxyl radical scavenging

The hydroxyl radical scavenging capacity of different extraction parts of cigarette ethanol extract was shown in Fig. 5. Within a certain range of mass concentration, the hydroxyl radical scavenging rate of each extraction part increased with the increase of concentration. At 0.1–0.2 mg/mL, the hydroxyl radical scavenging rate of petroleum ether and ethyl acetate extract was greater than that of Vc. As shown in the figure, when the mass concentration was 0.5 mg/mL, the influence of extraction sites on hydroxyl radical scavenging rate was as follows: B > C > A > D. As can be seen from Table 2, the IC_{50} of ethyl acetate (0.100 ± 0.057 mg/mL) was the lowest, followed by that of petroleum ether (0.124 ± 0.085 mg/mL), indicating that the main substances in tobacco hydroxyl radical scavenging activity mainly existed in ethyl acetate and petroleum ether.

Table 2

IC_{50} of different extracts of tobacco ethanol extract on hydroxyl radical scavenging activity($\bar{X} \pm SD$)

The sample	A	B	C	D	E	Vc
IC_{50}(mg/mL)	1.117 ± 0.993	0.124 ± 0.085	0.100 ± 0.057	1.411 ± 0.244	2.980 ± 1.607	0.135 ± 0.120

Effect on superoxide anion scavenging

Cigarette ethanol extract of different extraction parts to the ultra oxygen anion removal ability as shown in Fig. 6, in the range of certain concentration, the extraction of parts to the ultra oxygen anion removal ability increases as the rise of the concentration and, under the low concentration, the extraction of parts to the ultra oxygen anion scavenging effect are less than Vc for the role. When the mass concentration is 1.0 mg/mL, the influence of each extraction site on superoxide anion is as follows: C > D > B > A > E, the scavenging rate of ethyl acetate extract was the highest (95.70%), n-butanol extract was the second (92.05%), and water extract was the lowest (29.14%). It could be seen that both ethyl acetate extract and n-butanol extract had high scavenging effect on superoxide anion, and their scavenging ability was similar. As can be seen from Table 3, The IC_{50} values of ethanol extract were 1.127 ± 0.404mg/mL.The IC_{50} values of superoxide anion scavenging of petroleum ether extract, ethyl acetate extract, n-butanol extract and water phase were 0.878 ± 0.110 mg/mL, 0.349 ± 0.136 mg/mL, 0.420 ± 0.208 mg/mL and 1.942 ± 1.297 mg/mL, respectively. The scavenging ability of superoxide anion in different parts of cigarette was as follows: C > D > B > A > E. However, there was a significant difference between Vc and positive control.

Table 3

IC_{50} of superoxide anion scavenging effect of different extracts of tobacco ethanol extract($\bar{X} \pm SD$)

The sample	A	B	C	D	E	Vc
IC_{50}(mg/mL)	1.127 ± 0.404	0.878 ± 0.110	0.349 ± 0.136	0.420 ± 0.208	1.942 ± 1.297	0.059 ± 0.062

Correlation analysis of antioxidant activity and content of extracts

Table 4 shows that antioxidant activity of different extracts of cigarette ethanol extract is positively correlated with its extract content. Remove DPPH free radicals and petroleum ether extracts only significant correlation on the 0.05 level (bilateral), ability to remove hydroxyl radicals with ethanol extraction and petroleum ether extract, n-butanol extract and aqueous phase on the 0.01 level (double side) significantly correlated, and the ability to remove hydroxyl radicals and ethyl acetate extract on the 0.05 level (double side) significantly correlated; The scavenging ability of superoxide anion was significantly correlated with ethanol extract, ethyl acetate extract, n-butanol extract and water phase at 0.01 (bilateral) level, while the scavenging ability was significantly correlated with petroleum ether extract at 0.05 (bilateral) level.Among them, DPPH radical scavenging ability was most correlated with petroleum ether extract, hydroxyl radical scavenging ability was most correlated with ethanol extract, superoxide anion scavenging ability was most correlated with n-butanol extract.

Table 4
Correlation coefficients between the contents of different extracts and antioxidant activities of tobacco ethanol extracts

The sample	DPPH free radical scavenging ability	Scavenging ability of hydroxyl radical	Ability to remove superoxide anion
A	0.800	0.999**	0.981**
B	0.904*	0.995**	0.933*
C	0.596	0.956*	0.980**
D	0.610	0.989**	0.996**
E	0.769	0.994**	0.969**

Note: *: there was significant correlation at the level of 0.05 (bilateral); **: significant correlation at 0.01 level (bilateral).

Conclusions

Through this experimental study, it can be concluded that: Different extracts of cigarette ethanol extract contained total phenols and flavonoids and had antioxidant activity. The contents of total flavonoids (28.34 mg/g, 29.49 mg/g) and total phenols (24.89 mg/g, 24.44 mg/g) in ethyl acetate extract and n-butanol extract were the highest. The clearance rates of DPPH radical, hydroxyl radical and superoxide anion in ethyl acetate fraction were 0.013 ± 0.010 mg/mL, 0.100 ± 0.057 mg/mL and 0.349 ± 0.136 mg/mL, respectively. The IC_{50} of DPPH radical scavenging rate and superoxide anion scavenging rate of n-butanol fraction were 0.015 ± 0.016 mg/mL and 0.420 ± 0.208 mg/mL, respectively. According to correlation analysis, the contents of different extracts of cigarette ethanol extract were positively correlated with antioxidant activity. The purpose of this study was to explore the application prospect of DPPH radical, hydroxyl radical and superoxide anion scavenging in cigarette, and to provide theoretical basis for the development of antioxidant functional products.

Declarations

Funding information

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Authors Contributions

W. N. L. participated in the data collation, data analysis, visualization of experimental results, experimental exploration and paper writing of this study, while G. W. R. carried out experimental thinking,

paper review, experimental supervision and leadership. All authors are responsible for the final manuscript.

Disclosure statement

Ethical Approval and Consent to participate Not applicable

Consent to Publish Not applicable

Availability of data and materials Not applicable

Competing Interests The authors declare no competing interests.

Figures

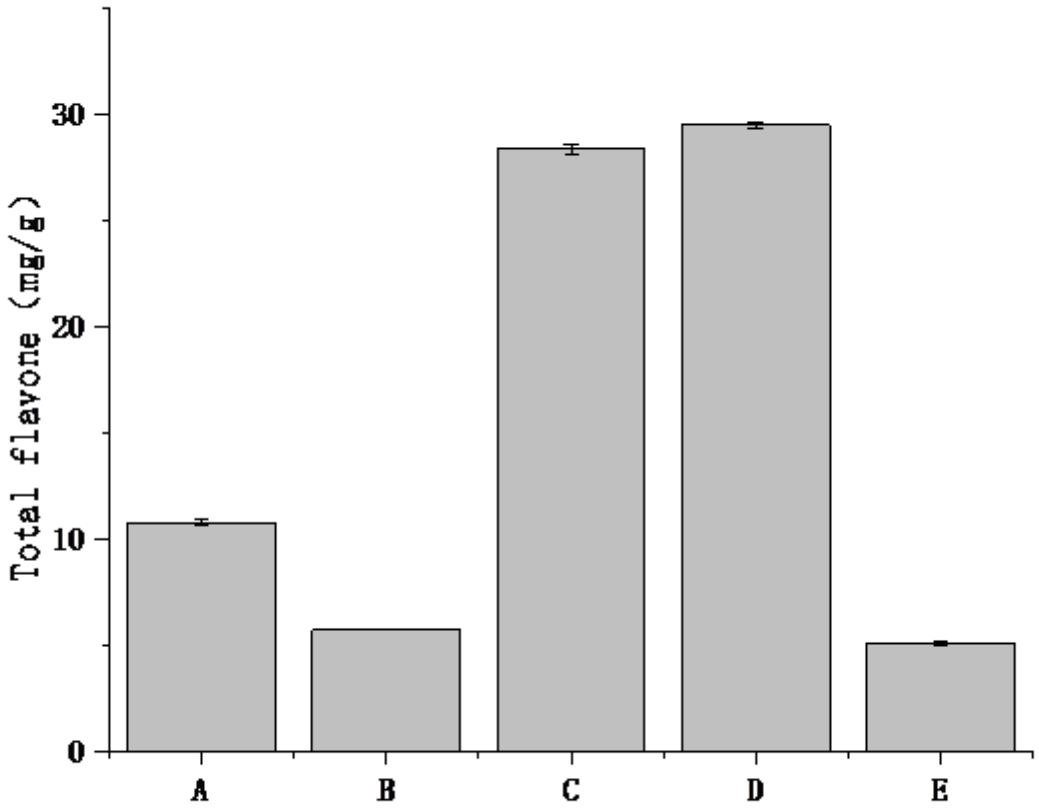

Figure 1

Total flavonoids content in different parts of tobacco ethanol extract

Note: Ethanol extract: A; Petroleum ether extract: B; Ethyl acetate extract: C; N-butanol extract: D; Water phase: E; The same below.

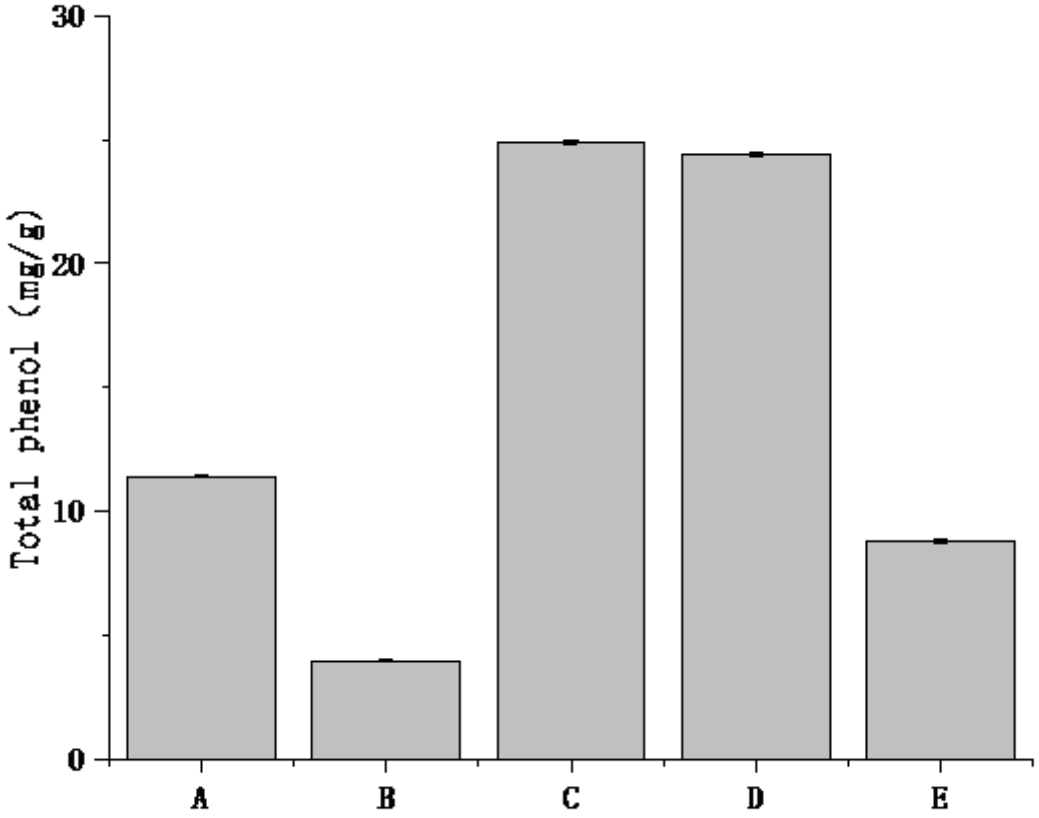

Figure 2

Total flavonoids content in different parts of tobacco ethanol extract

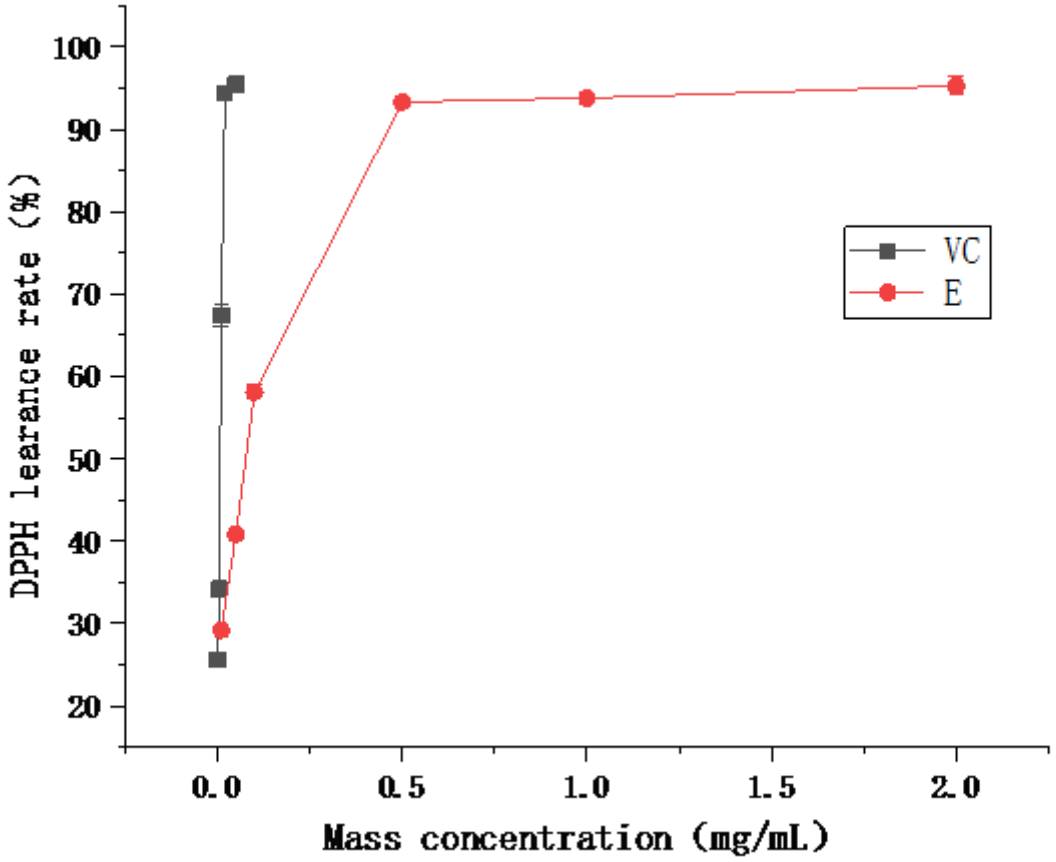

Figure 3

DPPH scavenging effect of different extracts of tobacco ethanol extract (water phase)

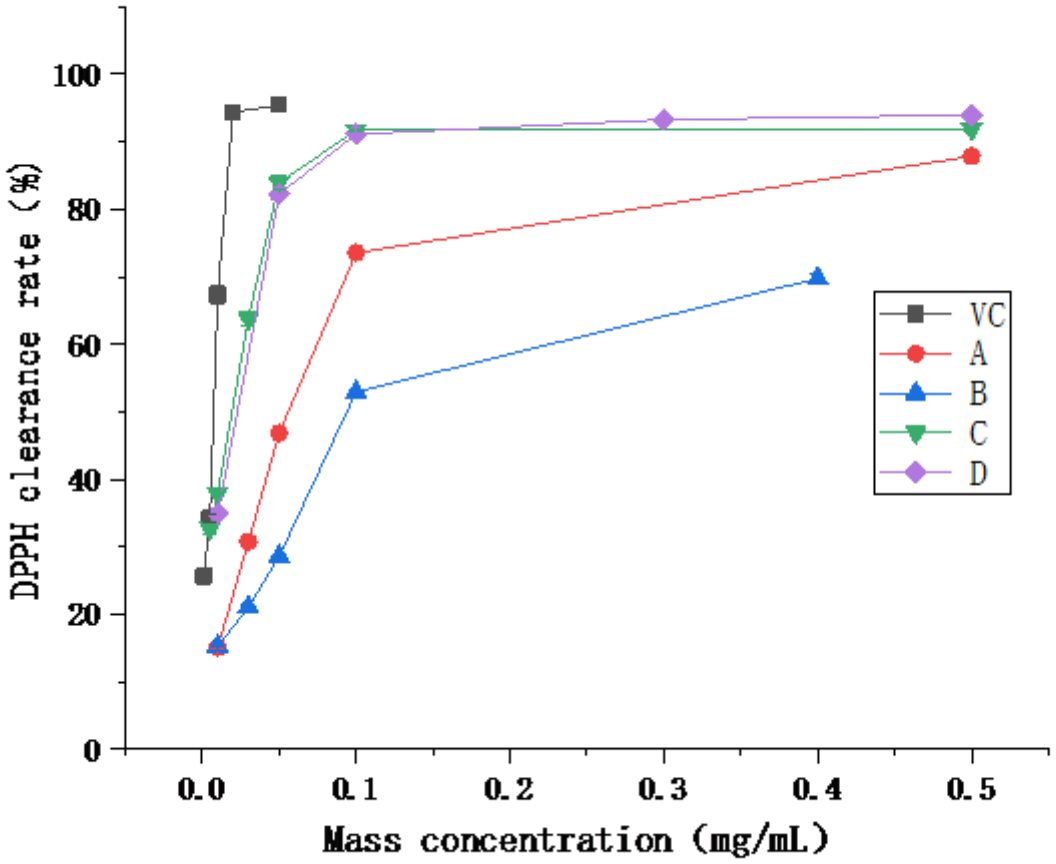

Figure 4

DPPH scavenging effect of water phase extract of tobacco ethanol extract (in addition to the water phase)

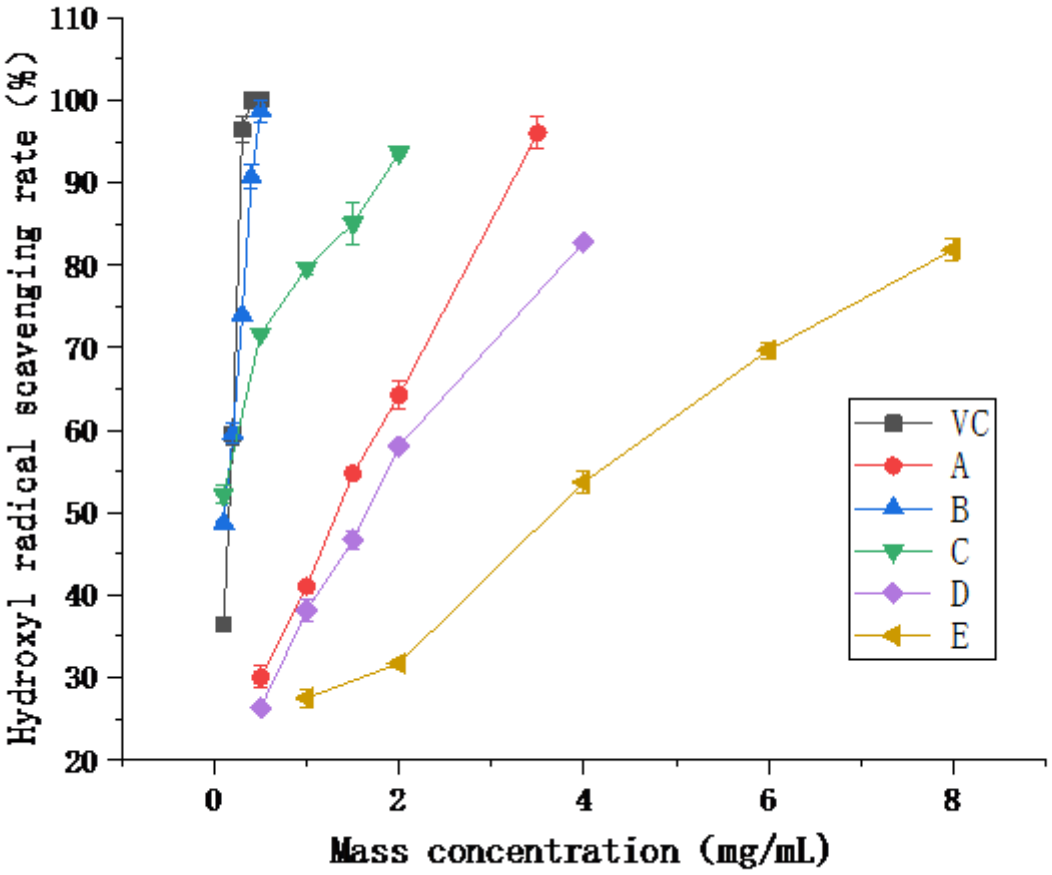

Figure 5

Hydroxyl radical scavenging effect of different extracts of tobacco ethanol extract

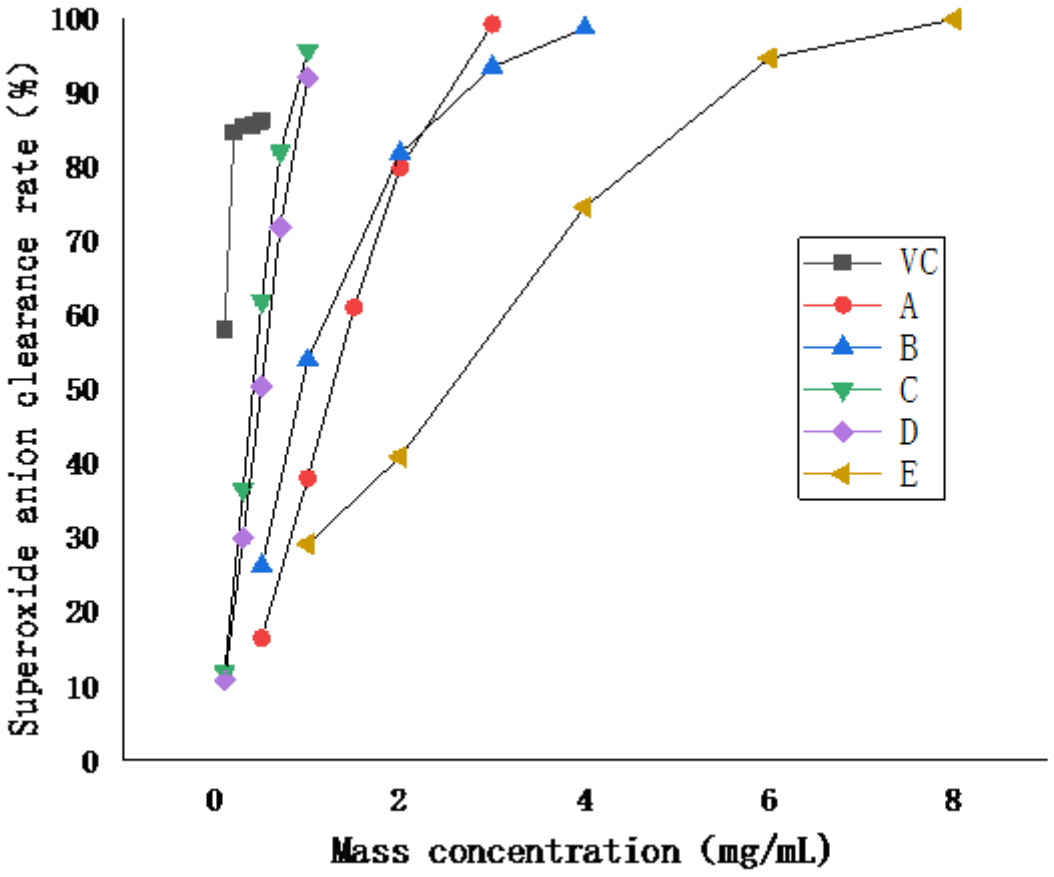

Figure 6

Removal of superoxide anion by different extracts of tobacco ethanol extract