Antioxidant Activity of the Extracts from Tussilago farfara.

Xiang Wei¹, Huilan Shang², Aimei Yang*², Zhihui Wang²

¹Bureau of Housing and Urban Rural Construction of Xishan District of Wuxi City, Wuxi, 214101, P. R. China
²School of Life Science and Engineering, Lanzhou University of Technology, Lanzhou, 730050, P. R. China

*Corresponding author’s e-mail: aimeiyang@163.com

Abstract: Objective: The antioxidative properties of Chinese medicine Tussilago farfara were determined by spectrophotometry. Methods: The anti-oxidation activity of petroleum ether, ethyl acetate and n-butanol extracts from ethanol extract of Tussilago farfara were studied, using DPPH free radical scavenging, hydroxyl radical scavenging, and total reducing power determination. Results: Compared with the Vc activity of the positive control, the extracts of ethyl acetate part and the n-butanol part showed significant antioxidant activity, and the antioxidant activity in the ethyl acetate part was higher than Vc. Conclusion: Antioxidant activity of ethyl acetate extract is better than vitamin C, N-butanol extract also has obvious antioxidant activities.

1. Introduction

Tussilago farfara L. belongs to the family Compositae and is the only species of Tussilago genus[1]. The flower buds of T. farfara have been used as Chinese traditional medicine for the treatment of cough, phlegm, bronchitic, and asthmatic disorders[2]. Oxidation is a critical metabolic process by which the body can perform living functions normally. The oxidative metabolism however produces reactive oxygen species (ROS) that in case of highly accumulated, which would create oxidative stress that damages cell structure, contributing to pathogenesis of most inflammatory diseases, cardiovascular diseases, neurodegenerative diseases and cancers by attacking biologically relevant molecules[3,4]. A huge number of natural compounds have been proven to exhibit antioxidant activity and be applicable for treatment of oxidative-damage related diseases. Large number of plants (e.g. Salvia virgate and Silybum marianum), fungi (e.g. Cantharellus cibarius) and alga (e.g. Ecklonia cava and Stoechospermum marginatum) are regarded as major sources of natural antioxidants[5].

In this paper, the antioxidant activities of ethanol extracts of Tussilago farfara, and petroleum ether, ethyl acetate and n-butanol extracts were studied by measuring total reducing power, scavenging DPPH free radical activity and scavenging hydroxyl radical activity, with a view to providing scientific basis for the development of new plant medicines.

2. MATERIALS AND METHOD

2.1. Plant material

Tussilago farfara is collected Weiyuan County, Gansu province and were identified by Professor Zhang Guoliang of Lanzhou University Life Science Institute and saved at Lanzhou University of
Technology. A voucher specimen has been deposited at the Laboratory of Natural Medicine, College of Life and Engineering, Lanzhou University of Technology. No. 20141022.

2.2. Extraction and Isolation
50 g of the crushed *Tussilago farfara* was weighed and extracted three times with ethanol, and the total extract was concentrated under reduced pressure to obtain a total extract, and the sample was taken. The total extract was dissolved in a minimum volume of hot water (60 °C), and extracted with the same volume of petroleum ether, ethyl acetate and n-butanol. The extracts were concentrated under reduced pressure to obtain extracts from various sites[5].

2.3. Instruments and reagents
Vitamin C, DPPH, Anhydrous ethanol, Trichloroacetic acid, Ferrous sulfate, DMSO, 30%-hydrogen peroxide, Salicylic acid, Potassium ferricyanide, Potassium dihydrogen phosphate, Dipotassium hydrogen phosphate, and Ferric chloride were of analytical grade. 712G visible wind photometer (Shanghai Jingke Instrument Co., Ltd.), B-220 constant temperature water bath, pipetting gun, (Xinkang Medical Devices Co., Ltd.), Rotary Evaporator RE-52 (Shanghai Yarong Biochemical Instrument Factory).

3. Determination of Antioxidant Activity of *Tussilago farfara* L.

3.1. Determination of total reducing power
2.5mL of the sample standard solution was placed in the test tube, then add 0.2mol/L phosphate buffer solution (pH=6.6) 2.5mL and 2.5% potassium hydroxide solution containing 1%-potassium ferricyanide solution in a water bath at 50 °C for 20min and cool down. Then, 2.5 mL of a 10%-trichloroacetic acid aqueous solution was added, and after 5 to 3 minutes of standing, 5 mL of the supernatant was accurately aspirated (precipitate was formed and centrifuged at 3000 rpm for 10 min). Further, 4 mL of distilled water and 1 mL of a 0.1%-FeCl₃ aqueous solution were added, and then the volume was adjusted to 10 mL. After shaking for 10 minutes, the absorbance of samples was measured at 700 nm. It indicates that the larger the absorbance, the stronger the reducing ability[7]. The above experiment was repeated with vitamin C as a positive control.

3.2. DPPH radical scavenging activity
Experimental group: 2 mL of 0.1 mg/mL DPPH ethanol solution was added to the test tube in turn, 2 mL of the sample standard solution to be tested, shaken, left at room temperature for 30 min in the dark, and the absorbance A sample was measured at 517 nm of visible light spectrophotometer. Blank group: Absorbance A blank was measured at 517 nm using absolute ethanol as a reference. Control group: Anhydrous ethanol was used instead of DPPH ethanol solution, and the sample standard solution was used as a control group, and the absorbance A control was measured. The results show that the greater the clearance rate, the stronger the ability of the sample to scavenge free radicals and the stronger the antioxidant activity [8]. The DPPH clearance rate of the sample was calculated according to SR (%) = [1 - (A sample - A control) ÷ A blank] × 100% formula.

Note: Sample A: absorbance of DPPH ethanol solution after addition of sample solution;
A control: absorbance after mixing of absolute ethanol and sample solution;
A blank: absorbance of DPPH ethanol solution without added solution.

3.3. Hydroxyl radicals scavenging activity
1 mL of ferrous sulfate solution (6 mmol/L) and 1 mL of salicylic acid-ethanol solution (6 mmol/L) were added to the reaction system. Add the test sample standard solution to the reaction solution, 1 mL of H₂O₂ (0.1%), and add only 1 mL of H₂O₂(0.1%) to the blank group. Add only 1 mL of the sample standard solution to the control group, and make it to a volume of 10 mL. Shake well. thereafter, the mixture was kept at 37°C for 30 min, and distilled water was used as a reference, and the absorbance
A was measured at 510 nm. The clearance rate of the hydroxyl radical to the sample was calculated according to the formula SR (%) = \[1 - (A_{sample} - A_{control}) ÷ A_{blank}\] \times 100%.

Note: Sample A: The absorbance of the solution added to the sample;
A control: absorbance of solution without hydrogen peroxide;
A blank: The absorbance of the solution without the addition solution.

4. RESULTS AND DISCUSSION

4.1. Determination of total reducing power

Fig 1. Determination of total reducing power.

The results are shown in Fig 1. The petroleum ether extract has almost no reducing power, and the other parts have various degrees of reducing ability. The absorbance value has a certain dose-effect relationship with the concentration, and the linear relationship in the test concentration range is good. According to Fig.2, the reducing power of the total extract solution at the concentration of 0.1 mg/mL is equivalent to a 0.0965 mg/mL Vc solution, which is similar to Vc. The reduction ability of ethyl acetate was significantly higher than that of Vc. The absorbance of extract at 0.1mg/mL was equivalent to the reduction ability of 0.118 mg/mL of Vc. It indicates that the reducing power of the extract of *Tussilago farfara* L. is mainly concentrated in the ethyl acetate part.

Fig 2. Vc equivalent of total reducing power of test sample

4.2. DPPH radical scavenging activity

Fig 3. DPPH radical scavenging activity
DPPH scavenging capacity can be assessed by EC_{50}, which is inversely proportional to DPPH scavenging capacity. The results are shown in Fig 3. the total extract site EC_{50} = 0.037 mg/mL, the ethyl acetate fraction EC_{50} = 0.049 mg/mL, the n-butanol fraction EC_{50} = 0.041 mg/mL, the petroleum ether fraction EC_{50} = 0.061 mg/mL, and the Vc EC_{50} = 0.032 mg/mL. The results showed that the EC_{50} of petroleum ether was twice that of Vc, and the DPPH free radical scavenging activity was significantly lower than that of n-butanol and ethyl acetate. The EC_{50} value of the total extract was close to Vc. It is indicated that the single-electron pairing with DPPH scavenges the free radical activity mainly in the n-butanol site, which is superior to the ethyl acetate site, and the scavenging ability is similar to Vc. When the concentration is greater than 0.15 mg/mL, the clearance rate of each test sample is basically stable.

4.3. Hydroxyl radicals scavenging activity

As can be seen from Fig 4, the ethyl acetate site EC_{50} = 0.3351 mg / mL < total extract site EC_{50} = 0.4468 mg / mL < n-butanol site EC_{50} = 0.491 mg / mL < vitamin C EC_{50} = 0.519 mg / mL. According to the EC_{50} value, the weaker the ability to scavenge hydroxyl radicals, the stronger the activity of scavenging hydroxyl radicals in ethyl acetate was obtained, and the activity was higher than that of total extract, n-butanol and Vc. Experiments show that the active substances of scavenging hydroxyl radicals in *Tussilago farfara* L. are mainly concentrated in the ethyl acetate part and n-butanol part, which can replace the research of Vc used to scavenge hydroxyl radical activity.

5. Conclusion

The results of research on the anti-oxidant activity of the samples of *Tussilago farfara* L. showed that the antioxidant active substances were mainly concentrated in the ethyl acetate fraction. The hydroxyl radical scavenging activity of the ethyl acetate moiety was 1.55 times that of Vc, the reducing power was determined to be 1.24 times of Vc, and the DPPH free radical scavenging activity was 1.55 times of Vc. The n-butanol extract also has good antioxidant activity. The main ingredients with antioxidant effects in ethyl acetate extract and n-butanol extract are polyphenols, flavonoids and triterpenes.

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