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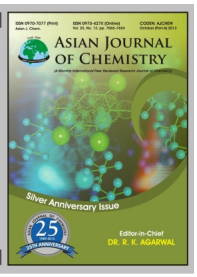

Determination of Antioxidant Activity of Total Flavonoids from *Tussilago farfara* by Flow Injection Chemiluminescence

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The antioxidant activity of total flavonoids from *Tussilago farfara in vitro* was determinated by flow injection analysis chemiluminescence. The determination was based on the inhibition effect of their natural antioxidants on the free radical-induced luminol chemiluminescence. Ascorbic acid was used as antioxidant standard. At the optimum operational conditions, the antioxidant activity was measured by plotting the inhibition percentage *versus* the content of samples to calculate the IC_{50}. The results revealed that the flavonoids from *Tussilago farfara* showed stronger free radical scavenging activity on superoxide ($O_2^{-\bullet}$) than ascorbic acid. It is suggested that *Tussilago farfara* is a good natural antioxidant and has the potential to be used in the manufacturing of foods and medicines.

Key Words: Flow injection chemiluminescence, Flavonoids from *Tussilago farfara*, Antioxidation.

INTRODUCTION

Tussilago farfara L. is a member of the Asteraceae family and flower buds of this plant have been used as a traditional Chinese herbal medicine for the treatment of bronchitic and asthmatic conditions[1]. *T. farfara* has been reported to have inhibitory activity against nitric oxide synthase[2], antimicrobial activity [3] and antagonistic activity on platelet-activating factor receptor[4]. It contains polysaccharides[5], alkaloids[6], flavonoids[7] substances.

It is well known that flavonoids in plants can be applied in medical field with its antioxidant activitives. The role of antioxidants seems contribute to the interaction between flavonoids in plants, which are present in different combinations[8]. The synergistic interaction of flavonoids may be an important factor of the overall antioxidant activity of plant-derived products, which could be more distinct than the activity of individual compounds. Antioxidant activity of *T. farfara* has already been studied[7,9], but none of the previously described antioxidant capacity of the total flavonoids. Therefore, in this study, we attempted to characterize the antioxidant and free radical scavenging activities of the total flavonoids from *T. farfara in vitro*.

To evaluate the potential antioxidant effects of substances, many *in vitro* procedures are employed[10,11]. In the present study, we evaluate the antioxidative effect of the total flavonoids from *T. farfara* by using a flow injection analysis method (FIA), which is a fast, accurate system for monitoring chemiluminescence (CL) reactions induced by reactive oxygen species (ROS)[12]. The ascorbic acid was used to positive control.

EXPERIMENTAL

The flower buds of *T. farfara* was purchased from Taian Pharmacy in Shandong Province, China and were identified by pharmacist LI Tong-De. Voucher specimens have been deposited in the College of Pharmacy, Taishan Medical University. Luminol was purchased from Sigma. Rutin (reference standards) was purchased from National Institutes for Food and Drug Control. Water was prepared with doubly distilled. Other chemicals were of analytical-reagent grade and were used as supplied unless otherwise stated.

Buffer solutions, pyrogallol solution, potassium ferrocyanide solution, luminol solution and H_2O_2 solution, were stored at -4 ºC. Prior to use they were allowed to reached room temperature. All the luminol solutions were protected from light by a foil wrapper. All the antioxidants solutions were freshly prepared and diluted in doubly distilled water.

The ultra weak luminescence analyzer (IFFM-D, Remex Electronic Institute Limited Co., Xi'an, China), equipped with an eight-channel rotary injection valve and two peristaltic pumps was used to measure chemiluminescence. The photomultiplier tube was used to detect chemiluminescence signal in the flow cell. An IFFL-D flow-injection chemiluminescence

analysis software system (Xi'an Remex Electronic equipment Corporate, Xi'an, China) was used to analysis chemiluminescence signal in the flow cell.

Procedures: The flow injection manifold used has been previously described[13] as shown in Fig.1. According to this configuration, the oxidant stream was merged with a water carrier immediately, and then the combined flow was merged with luminol immediately before the flow cell. Thus, a constant chemiluminescence signal was continuously detected when only water was flowing on the S channel (maximum intensity (I_{max}). When antioxidant was injected into S channel, the attenuation of the chemiluminescence emission signal (minimum intensity (I_{min}) was detected.

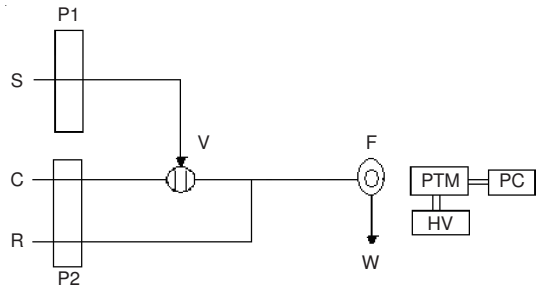

Fig. 1. Diagram of the flow system used in the experiments. S, sample (antioxidant or water); C,oxidant ; R, luminol; P, Peristaltic pump; V, injection valve; F, chemiluminescence flow cell; PMT, photomultiplier tube; HV, negative high-voltage supply; PC, computer; W, waste solution

The chemiluminescence is measured as the photomultiplier output in millivolt; the effects of antioxidants were measured by the depression of the signal from its uninhibited level and were expressed as a percentage attenuation of the chemiluminescence maximum due to the antioxidant. By plotting the chemiluminescence intensity against the time, the maximum and minimum intensity were determined and the percentage of inhibition was determined in each case using the following equation:

$$\text{Inhibitiion}\,(\%) = \left[\frac{(I_{max} - I_{min})}{I_{max}}\right] \times 100\,\%$$

Inhibition curves are represented by plotting the content of samples in each solution against the % Inhibition. These curves are used to determine the IC_{50} that represents the amount of sample that produced a chemiluminescence inhibition of 50 %.

Results are the mean values of three replicates of the same sample. Statistical analysis was performed using analysis of variance.

Superoxide ($O_2^{-\bullet}$) scavenging method: Superoxide radicals were generated by the autoxidation of pyrogallol which reacted with luminous and gave the chemiluminescence. The concentration of the radicals was not known and is represented by the chemiluminescence intensity. The optimal working conditions were 0.5 mmol/L pyrogallol in 1 mmol/L hydrochloric acid, 0.40 mmol/L luminol and 0.10 mol/L carbonate buffer at pH 10.

Hydroxyl radicals ($^{\bullet}OH$) scavenging method: Hydroxyl radicals were generated by Fenton reaction. Luminol is luminous synergistic agent. The concentration of the radicals was not known and is represented by the chemiluminescence intensity. The optimal working conditions were 0.26 mmol/L potassium ferrocyanide, 0.10 mmol/L luminol, 0.20 % hydrogen peroxide and KH_2PO_4-NaOH buffer at pH 7.20.

Hydrogen peroxide (H_2O_2) scavenging method: In alkaline conditions, hydrogen peroxide oxidates luminol to shine and the luminous intensity is proportional to the content of hydrogen peroxide, the decrease chemiluminescence intensity can reflect the scavenging ability of samples. Hydrogen peroxide was prepared daily and protected from light by a foil wrapper. The optimal working conditions were 0.20 % H_2O_2, 0.20 mmol/L luminol and 0.10 mol/L carbonate buffer at pH 9.60.

Sample pre-treatment of *T. farfara*: Air-dried flower buds of *T. farfara* (2.0 g) were extracted by 50 % ethanol (40 mL) at 80 ºC for 1 h and then filtrated immediately. After cooling at room temperature, the extracts were diluted to 100 mL. The total flavonoids content of the samples was determined spectrophotometrically, A calibrating curve was plotted using rutin as standard. The total flavonoids content of *T. farfara* extract was 6.3 %, the content of sample solution was 1.26 g/L.

RESULTS AND DISCUSSION

Superoxide: Fig. 2 shows a typical chart recorder trace of a continuous chemiluminescence signal from pyrogallol and luminol (pH 10) and the effect of a sequence of injections of water and the extract of *T. farfara*.

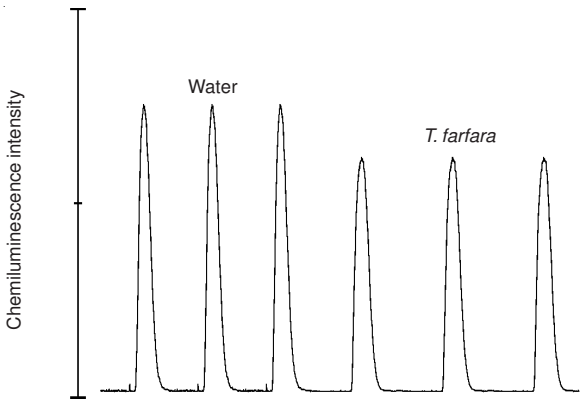

Fig. 2. Effect of water and the extract of *T. farfara* on chemiluminescence intensity

The chemiluminescence intensity was inhibited by the extract of *T. farfara* and the total flavonoids from *T. farfara* had the scavenging activity on $O_2^{-\bullet}$ (Fig. 2). Curves in Fig. 3 were used to determine the IC_{50}. Low values of IC_{50} implied higher antioxidant capacity. The IC_{50} value were 9.93 and 42.20 µg/mL for the extract of *T. farfara* and ascorbic acid, respectively (Table-1). Thus the antioxidant potency order is *T. farfara* extract > ascorbic acid.

TABLE-1
ANTIOXIDATION OF *T. farfara* AND ASCORBIC ACID

Samples	IC_{50} (mg/L)		
	$O_2^{-\bullet}$	$^{\bullet}OH$	H_2O_2
Ascorbic acid	42.20	12.42	0.34
T. farfara	9.93	14.15	0.92

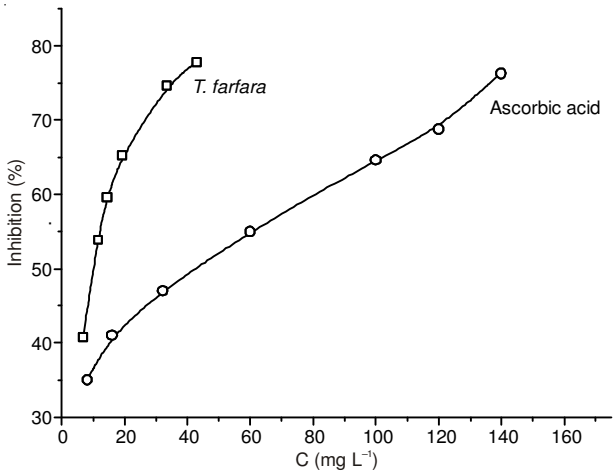

Fig. 3. Scavenging ability on $O_2^{-\bullet}$ of ascorbic acid and *T. farfara*. LSD at $p < 0.05$

Hydroxyl radical: Ferrous iron-induced chemiluminescence was inhibited by *T. farfara* extract and ascorbic acid in a concentration-dependent manner, as shown in Fig. 4. The IC_{50} value were 14.15 and 12.42 µg/mL for *T farfara* extract and ascorbic acid, respectively (Table-1). The antioxidant potency order is ascorbic acid > *T. farfara* extract.

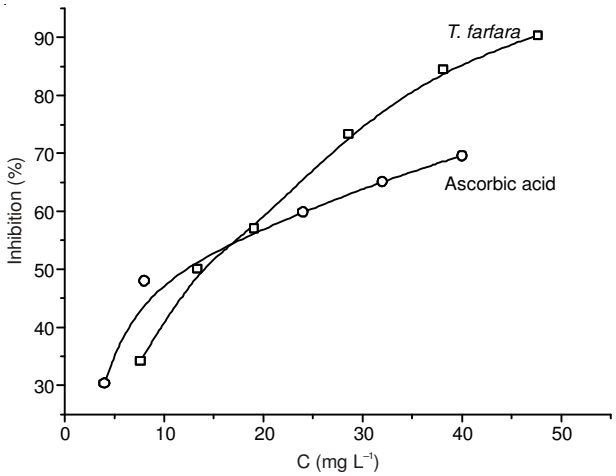

Fig. 4. Scavenging ability on $^{\bullet}OH$ of ascorbic acid and *T. farfara*. LSD at $p < 0.05$

Hydrogen peroxide: As summarized in Fig. 5, the H_2O_2-dependent chemiluminescence signal was inhibited by either *T. farfara* extract or ascorbic acid. The scavenging activity of *T. farfara* extract or ascorbic acid was found in a concentration-dependent manner. The results show that the activity of ascorbic acid was stronger than those of *T. farfara* extract. The antioxidant potency order is ascorbic acid > *T. farfara* extract. The IC_{50} value were 0.92 and 0.34 µg/mL for *T. farfara* extract and ascorbic acid (Table-1).

The above results demonstrate that *T. farfara* extract can inhibit and scavenge the ROS such as $O_2^{-\bullet}$, $^{\bullet}OH$ and H_2O_2 effectively. This suggests that *T. farfara*, a good resource of natural antioxidant, has a potential to be used in the manufacturing of foods and medicines. It is worth noting that *T. farfara* extract exhibited stronger scavenging activity than ascorbic acid on $O_2^{-\bullet}$, though those were weaker than ascorbic acid on $^{\bullet}OH$ and H_2O_2.

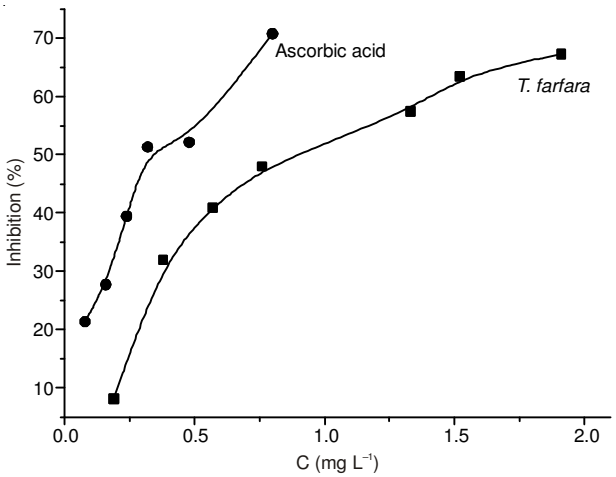

Fig. 5. Scavenging ability on H_2O_2 of ascorbic acid and *T. farfara*. LSD at $p < 0.05$

It is known that the superoxide radical is quite harmful to cellular components as a precursor of the more ROS, contributing to the various diseases such as inflammation, mutagenesis, carcinogenesis and tissue injury by circulatory disturbance and aging. In this study, we found that the total flavonoids from *T. farfara* significantly inhibited superoxide radical generation. This may be attribute partly to its phenolic compounds. Because of their redox properties, hydrogen donors and single oxygen quenchers, phenolics in plants can inhibit free radicals generation and are favorable to human health. Modern pharmacological studies indicated that 75 % alcohol *T. farfara* extract had antiinflammatory effects and its flavonoids had the role of inhibiting mouse lung adenocarcinoma cells[14], the great potent antioxidant may be an important factor.

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REFERENCES

1. T. Namba, The Encyclopedia of Wakan-Yaku, Hoikusha, Tokyo, Vol. 2, p. 525 (1980).
2. J.H. Ryu, Y.S. Jeong and D.W. Sohn, *J. Nat. Prod.*, **62**, 1437 (1999).
3. L. Kokoska, Z. Polesny, V. Rada, A. Nepovim and T. Vanek, *J. Ethnopharmacol*, **82**, 51 (2002).
4. S.-B. Hwang, M.N. Chang, M.L. Garcia, Q.Q. Han, L. Huang, V.F. King, G.J. Kaczorowski and R.J. Winquist, *Eur. J. Pharmacol*, **141**, 269 (1987).
5. E. Haaland, A. Hankey, U. Svanholm, A.M. Duffield, A.T. Balaban and J.C. Craig, *Acta. Chem. Scand.*, **23**, 2546 (1969).
6. P.P. Fu, Y.C. Yang, Q. Xia, M.C. Chou, Y.Y. Cui and G. Lin, *J. Food Drug Anal.*, **10**, 198 (2002).
7. M.R. Kim, J.Y. Lee, H.H. Lee, D.K. Aryal, Y.G. Kim, S.K. Kim, E.R. Woo and K.W. Kang, *Food Chem. Toxicol.*, **44**, 1299 (2006).
8. X. Yao, X. Xu, G. Fan, Y. Qiao, S. Cao and S. Pan, *Eur. Food Res. Technol.*, **229**, 743 (2009).
9. C.T. Li, Y.P. Liu, F.C. He and Y. Li, *Chin. J. Nat. Med.*, **10**, 260 (2012).
10. B.N. Singh, B.R. Singh, R.L. Singh, D. Prakash, R. Dhakarey, G. Upadhyay and H.B. Singh, *Food Chem. Toxicol.*, **47**, 1109 (2009).
11. D. Prakash, B.N. Singh and G. Upadhyay, *Food Chem.*, **102**, 1389 (2007).
12. M. Sariahmetoglu, R.A. Wheatley, I. Çakici, I. Kanzik and A. Townshend, *Anal. Lett.*, **36**, 749 (2003).
13. C.H. Liu, B.X. Jia, Y.Q. Li, Y.X. Qi, K. Li, Y.R. Guo, *Asian J. Chem.*, **25**, 7401 (2013).
14. K.Y. Liu, H.J. Liu, J.Z. Wu and T.J. Zhang, *J. Fudan Univ.*, **48**, 125 (2009).