JOURNAL OF CURRENT RESEARCH IN SCIENCE (ISSN 2322-5009)
CODEN (USA): JCRSDJ
2014, Vol. 2, No. 2, pp:264-267
Available at www.jcrs010.com

ORIGINAL ARTICLE

ANTIOXIDANT ACTIVITY AND TOTAL FLAVONOIDS OF SILKWORM EXCREMENT EXTRACTS

Li Xu *, Chuanhong Wang, Chao Liu, Jing Liu, Wei Xiang
College of Biotechnology, Southwest University, Chongqing 400716, China

ABSTRACT: Silkworm excrement, as a traditional Chinese medicine, has the capabilities of antioxidant, hypoglycemic, anti-tumor and other biological activities. The research emphasizes on antioxidant activity of different solvent extracts of silkworm excrement, and the flavonoids content in the extract was also determined. The results showed that strength of antioxidant activity of Silkworm excrement extracts from different solvents can be listed in an order of aqueous extract (AE) > ethanol extract (EE) > chloroform extract (CE) > ethyl acetate extract (EAE) > petroleum ether extract (PEE). The flavonoids content in these different extracts are the following: aqueous extracts (29.498 mg g^{-1}), ethanol extract (15.025 mg g^{-1}), ethyl acetate extract (10.181 mg g^{-1}), chloroform extract (8.506 mg g^{-1}), petroleum ether extract (5.875 mg g^{-1}).

KEYWORDS: Silkworm excrement, Extracts, Antioxidant Activity, Total Flavonoids.

INTRODUCTION

Silkworm excrement has been found by modern researchers to possess the capabilities of antioxidant, hypoglycemic, anti-tumor and other bioactivities, which are mainly attributed to flavonoids, alkaloid, chlorophyll and other bioactive components contained in it (Guo, 2003). Nowadays, studies focusing on hypoglycemia (Liu *et al.*, 2007) and fungistasis (Wu *et al.*, 2006) of Silkworm excrement have been received more attention, while its abilities of antioxidant and scavenging free radicals have been rarely examined. Because of it, the article was going to explore the ability of antioxidant and scavenging free radicals possessed by Silkworm excrement as well as determined the flavonoids content contained in Silkworm excrement, laying a foundation for the future study and development of Silkworm excrement.

MATERIALS AND METHODS

2.1. Materials

Silkworm excrement, of 3 days 5th instar, Beibei, Chongqing Xili worm farm; ABTS, DPPH (Sigma, USA); rutin (Sangon, Shanghai, China); sodium hydroxide (NaOH), ferric chloride ($FeCl_3$), vitamin C (V_C), sodium nitrite ($NaNO_3$), aluminum nitrate ($AlNO_3$), potassium ferricyanide ($K_3Fe(CN)_6$), trichloroacetic acid (TCA), salicylic acid (SA) (Chengdu, China Kelong); iMark microplate reader (Bio-Rad, USA); T6 UV spectrophotometer (Beijing Purkinje); FA2004A electronic balance (Shanghai China); KQ-400KDB ultrasonic cleaner (Kunshan, China); R210 Rotavapor (Buchi, Switzerland).

2.2. Preparation of Silkworm excrement extracts

The precisely weighed 5.0 g Silkworm excrement powder were mixed with 25.0 mL of petroleum ether, chloroform, ethyl acetate, 70% (v/v) ethanol and distilled water respectively in triangular flask and extracted at 50 ℃ for 3 h, 2 times with ultrasound. The filtrates were concentrated to dryness under reduced pressure at the temperature of 40 ℃. 95% (v/v) ethanol dissolved concentrate and adjusted to 100 mL.

2.3. Determination of reducing power

The reducing power assay was according to previous reference (Yen and Chen 1995). 1.0 mL different concentration of the sample was mixed with 1.0 mL 1% (m/v) $K_3Fe(CN)_6$ and 1.0 mL PBS (pH 6.5), the mixture was shaken gently and water bathed in 50 ℃ for 20 min, 1.0 mL 10% (m/v) TCA was added into the mixture, then being shaken gently and allowed to stand for 10 min. Then, 0.1 mL 0.1% (m/v) $FeCl_3$ and distilled water were added into the mixture respectively. After 10 min, the absorbance was determined by microplate reader at 700 nm.

2.4. DPPH radical scavenging activity

The assay was carried out following the previously reference approach with a few modifications (Chu *et al.*, 2000). 200 μL different concentration samples mixed with 20 μL DPPH in 96 well elisa plate, the mixture was shaken vigorously and allowed to stand for 30 min at the temperature of 37℃. After that, the absorbance was measured at 517 nm. The DPPH radical scavenging capacity was calculated by

Corresponding Author: Li Xu, College of Biotechnology, Southwest University, Chongqing 400716, China.
E-mail: mulberry@swu.edu.cn

the following equation.
Scavenging effect (%) = [1− (*As/Ac*)] × 100 .
Where Ac is the absorbance of the control groups and As is the absorbance in the presence of extracts.

2.5. ABTS free radical scavenging activity
According to previous reference with a few modifications (Gülçin, 2010). 2 mmol L^{-1} ABTS was mixed with 2.45 mmol L^{-1} $K_2S_2O_8$ to produce ABTS free radical. 150 μL different concentration samples mixed with 100 mL ABTS, standing for 30 min at the room temperature, the absorbance was measured by microplate reader at 734 nm. The ABTS radical scavenging capacity was calculated by the following equal.
The value of ABTS free radical scavenging (%) = [1− (*As/Ac*)] × 100 .
Where *Ac* is the absorbance of the control groups and *As* is the absorbance in the presence of extracts.

2.6. Hydroxy free radical scavenging activity
According to reference with a few modifications (Jin *et al.*, 2009). 1 mL 4.4 mmol L^{-1} H_2O_2 and 1 mL 4.5 mmol L^{-1} $FeSO_4$ were mixed together, water bath in 37 ℃ for 30 min, hydroxyl radical was generated from Fenton reaction. Then 0.5 mL 20 mmol L^{-1} SA and different concentration samples were mixed with the mixture. The reaction kept in 37 ℃ water bath for 1 h, the absorbance of the mixture was measured at 510 nm. ·OH radical scavenging capacity was calculated by the following equal.
Hydroxy free radical scavenging (%) = [1− (*As/Ac*)] × 100 .
Where Ac is the absorbance of the control groups and As is the absorbance in the presence of extracts.

2.7. Determination of flavonoids
The total flavonoids content assay was based on the previously described way with a few modifications and using rutin as a standard flavonoid (Hairi *et al.*, 1991). 0.5 mL 50 mg mL^{-1} $NaNO_2$ and 0.1 mL extracts were mixed together and shaken vigorously, leaving the mixture at room temperature for 6 min, and then, 0.5 mL $Al(NO_3)_3$ was added into reaction mixture and kept at dark place for 5 min, 2.5 mL NaOH was mixed together with mixture and left at room temperature for 15 min. The absorbance was measured by Spectrophotometer at 510 nm and compared to the rutin calibration curve.

2.8. Statistical analysis
Each experiment was carried out in triplicate, all data were the average of three independent experiments and analyzed by SPSS (version 18.0), and expressed as means ± standard deviation (SD). Results were considered significantly at $P < 0.05$.

RESULTS

3.1. The reducing power
As shown in Figure (1). It can be indicated that the reducing force of Silkworm excrement extracts from different solvents increased with the increasing in concentration. The reducing power of aqueous extract was the strongest among all samples, and what's more, when at the concentration of 40 mg mL^{-1}, the reducing power of aqueous extract was equal to that of Vc. However, the reducing power of extracts from ethanol, chloroform, ethyl acetate and petroleum ether were no significant distinction. In general, the reducing powers of the extracts are in the order of aqueous extract > ethanol extract > chloroform extract > ethyl acetate extract > petroleum ether extract.

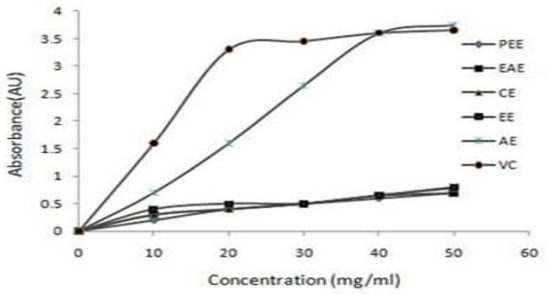

Figure 1: The reduction force of different solvent extracts

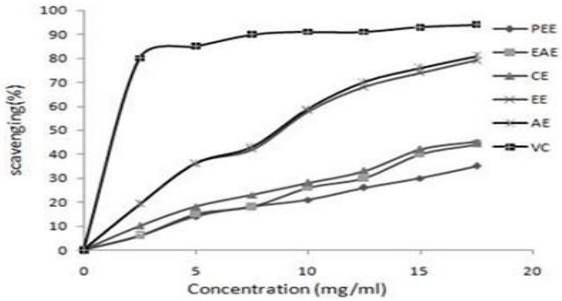

Figure 2: DPPH free radical scavenging ability

3.2 Scavenging DPPH free radical ability
As is shown in Figure (2), the ability of Silkworm excrement extracts from different solvents increased with the increasing in concentration. At the concentration of 17.5 mg mL^{-1}, DPPH free radical scavenging of aqueous and ethanol extracts reached 80%, while the values of extracts from ethyl acetate, chloroform and petroleum ether were comparatively weak. In general, the capabilities of scavenging DPPH free radical of Silkworm excrement extracts from different solvents are in the order of aqueous extract > ethanol extract > ethyl acetate extract > chloroform extract > petroleum ether extract.

3.3 Scavenging ABTS free radical ability

Figure (3) indicates that abilities of scavenging ABTS free radical from different solvents increased with the increasing in concentration. At the concentration of 14 mg mL^{-1}, scavenging ability of aqueous and ethanol extracts reached 70%, significantly stronger than ethyl acetate extract, chloroform extract, and petroleum ether extract. Generally speaking, ABTS free radical scavenging abilities of different extracts can be exhibited in the order of aqueous extract > ethanol extract > ethyl acetate extract > chloroform extract > petroleum ether extract.

3.4 Scavenging hydroxyl free radical ability

Figure (4) demonstrates the abilities of scavenging hydroxyl free radical of extracts from different solvents were different. As is indicated, it was found that all the extracts were capable to scavenge hydroxyl free radical. The extracts from aqueous and ethanol had stronger capability than those from ethyl acetate, chloroform and petroleum ether. Meanwhile, at the concentration of 35 mg mL^{-1}, aqueous and ethanol extract had the similar free radical scavenging rate of 80%, which was proportional to the increasing concentration. In all, hydroxyl free radical scavenging abilities of these different extracts can be exhibited in the order of aqueous extract > ethanol extract > ethyl acetate extract > chloroform extract > petroleum ether extract.

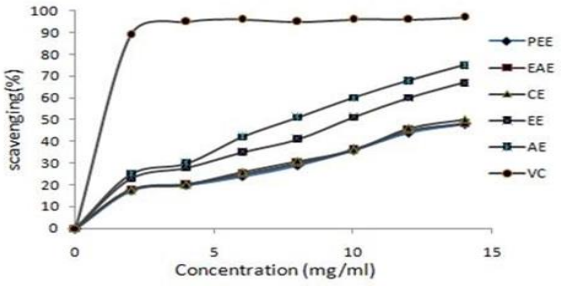

Figure 3: ABTS free radical scavenging ability

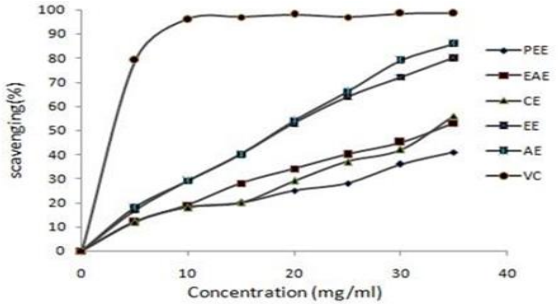

Figure 4: Hydroxyl free radical scavenging ability

3.5 Flavonoids content and IC_{50} from different solvents

The standard curve of determining flavonoids in the extract of silkworm excrement is built by rutin, the standard curve linear regression equation was y = 4.4854x + 0.0007 (R^2 = 0.9985), Table 1 shows the flavonoids content in different solvents were in the range of 5.875 mg mL^{-1} and 29.498 mg mL^{-1}. It can be inferred that the flavonoids content in different extracts had a closely relation with the solvents, aqueous extract containing the highest flavonoids content of 29.498 mg g^{-1} among all the extracts.

Table (1) reveals that the capability of scavenging free radicals of aqueous extract was superior to other extracts, IC_{50} of scavenging DPPH, ABTS and hydroxyl were about 7.827 mg mL^{-1}, 6.860 mg mL^{-1}, 15.51 mg mL^{-1} respectively, and the ethanol extract was weaker than that of aqueous extract. Meanwhile, the following one was petroleum ether extract. What's more, free radical scavenging ability of ethyl acetate and chloroform extract were not so effective. In general, antioxidant activity of these different extracts can be demonstrated in the order of aqueous extract > ethanol extract > ethyl acetate extract > chloroform extract > petroleum ether extract.

Table 1: The flavonoids content and IC_{50} of different phases

	Flavonoids content (mg g^{-1})	DPPH· (mg mL^{-1})	ABTS· (mg mL^{-1})	OH· (mg mL^{-1})
Aqueous extract	29.498 ± 0.040^a	7.827 ± 0.003^a	6.860 ± 0.032^a	15.510 ± 0.001^a
Ethanol extract	15.025 ± 0.050^b	8.067 ± 0.003^a	8.623 ± 0.090^b	16.170 ± 0.001^b
Ethyl acetate extract	10.181 ± 0.030^c	24.563 ± 0.338^b	18.23 ± 0.072^c	36.933 ± 0.028^c
Chloroform extract	8.506 ± 0.030^d	23.337 ± 0.009^c	16.567 ± 0.047^d	36.980 ± 0.005^c
Petroleum ether extract	5.875 ± 0.060^e	75.857 ± 0.228^d	19.917 ± 0.084^e	80.630 ± 0.050^d

Column data marked with different superscripts mean significant difference ($P < 0.05$)

3.6 The relation of flavonoids content and antioxidant activity

Table (2) shows that, within the 95% confidence limits, the abilities of scavenging DPPH, ABTS and hydroxyl radicals were in significant relation with the flavonoids content of Silkworm excrement extracts, and the correlation coefficients were 0.669, 0.851, 0.714 respectively. The ability of clearing DPPH radical and OH radical was in the highest correlation, the correlation coefficient reached 0.996.

Table 2: Flavonoids content and antioxidant activity of correlation coefficient

	DPPH	ABTS	•OH
flavonoids content	0.669*	0.851*	0.714*
DPPH	-	0.743*	0.996*
ABTS	-	-	0.798*

*stand for significant difference（P<0.05）

DISCUSSION

4.1 Analysis of antioxidants in silkworm excrement

Free radicals as universal medium of various biochemical reactions in vivo, whose stability is closely related with health, such as cirrhosis, cancer, aging, cardiovascular and cerebrovascular diseases. Aiming to reduce the damage in human tissues and organs brought by the free radicals, BHT and BHA have been applied in the fields of health care, food, which work significantly effective, however with strong side effect (Botterweck *et al.,* 2000). Flavonoids, as the natural antioxidants, have the function of anti-aging and enhancing immunity, attracting more and more attention (Huang *et al.,* 2007). According to previous research (Liu *et al.,* 2007), Silkworm excrement is rich in flavonoids, chlorophyll, alkaloids, carotenoids, pectin, and lutein compounds. In this study, the author find flavonoids content in extracts are proportional to free radical scavenging and reducing abilities, which indicates that flavonoids may be the main material basis of its antioxidant effects.

4.2 The polarity analysis of the Silkworm excrement antioxidant active ingredients

The antioxidant activity of aqueous extract is the strongest among other extracts which all possess the ability of antioxidant activities. The result reveals that aqueous and ethanol extracts contained the higher content of flavonoids, 29.5 mg g^{-1} and 15.0 mg g^{-1} respectively, which was in consistent with the results measured by other researchers (Yu *et al.,* 2010). It can be found that flavonoids in these extracts played a critical role in antioxidant, scavenging free radical, and main antioxidant components in Silkworm excrement were the water-soluble.

CONCLUSION

Through the comparison between antioxidant activity, free radical scavenging capacity of Silkworm excrement extracts and the determination of flavonoids, which act as the main antioxidant material, it can be found that the capability of antioxidant activity and the flavonoids content of Silkworm excrement extracts from different solvents were difference, and aqueous extract exhibited the strongest ability of antioxidant, the highest content of flavonoids which is the basis of antioxidant substances in Silkworm excrement.

ACKNOWLEDGEMENTS

This work was supported by The Fundamental Research Funds for the Central Universities (Grant no. XDJK2013C053).

REFERENCES

Botterweck AAM, Verhagen H, Goldbohm RA. Intake of butylated hydroxyanisole and butylated hydroxytoluene and stomach cancer risk: results from analyses in the Netherlands cohort study. *Food and Chemical Toxicology* 2000;38(7):599-605.

Chu YH, Chang CL, HSU HF. Flavonoid content of several vegetables and their antioxidant activity. *Journal of the Science of Food and Agriculture* 2000;80(5):561-566.

Gulcin I. Antioxidant properties of resveratrol: A structure–activity insight. *Innovative Food Science and Emerging Technologies* 2010;11(1):210-218.

Guo BX. The medical application of silkworm excrement and Its extract. *Journal of Sichuan Traditional Chinese Medicine* 2003;21(3): 19-21.

Hairi B, Salle G, Andary C. Involvement of flavonoids in the resistance of two popular cultivars to mistletoe (Viscum album L.). *Protoplasma* 1991;162: 20-26.

Huang FY, Gao M, Lou YY. General research situation of flavonoids. *Chinese Archives of Traditional Chinese Medicine* 2007;25(8): 1730-1732.

Jin BJ, Eul WS, Hyung JJ. Effect of extracts from pine needle against oxidative DNA damage and apoptosis induced by hydroxyl radical via antioxidant activity. *Food and Chemical Toxicology* 2009;47(8):2135-2141.

Liu Q, Qiao FX, Ye F. The antidiabetic effect of silkworm excrement extract. *Chinese Journal of New Drugs* 2007;16(19):1589-1592.

Wu L, Li DG, Zh L. Antibacterial Activity of the Different Extracts from Silkworm Feces. *Acta Agriculture Boreali-occidental is Sinica* 2006; 15(6):212-214.

Yen GC, Chen HY. Antioxidant Activity of Various Tea Extracts in Relation to Their Antimutagenicity. *Agric Food Chem* 1995; 43(1): 27-32.

Yu L, Sun W, Xu WQ. Study on flavonoids of Silkworm excrement by ultrasonic extraction. *Guangxi Journal of Light Industry* 2010;6(6): 6-8.