

Effect of different polarity solvents on total phenols and flavonoids content, and In-vitro antioxidant properties of flowers extract from *Aurea Helianthus*

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ABSTRACT

The total phenols and flavonoids content of different polar solvent extracts from *Aurea Helianthus* flowers, and their antioxidant activity were determined. The ethanol extract of *Aurea Helianthus* flowers were suspended in water and fractionated using different polar solvents; hexane, chloroform, ethyl acetate, butanol and water. The parameters of each extract mentioned above were determined using Folin-Ciocalteu reagent (FCR) method, AlCl₃ colorimetry method, Ferric reducing ability of plasma (FRAP) assay, total antioxidant activity (TAA) assay and DPPH radical scavenging assay. The highest total phenols content (516.21 mg GAE/g) and flavonoids content (326.06 mg QCE /g) were obtained in ethyl acetate extract, the correlation between TPC and TFC assay was found to be 0.967. All polar solvent extracts of *Aurea Helianthus* flowers showed significant antioxidant effects, the highest inhibition was obtained in ethyl acetate and chloroform extracts and the lowest inhibition in the water extract. There is a good correlation of total phenols and flavonoids content with antioxidant activity. This work indicated that the polar solvent extracts of *Aurea Helianthus* flowers contain high phenols and flavonoids content, and exhibited antioxidant activities in vitro, therefore, could be candidates for use as natural antioxidant.

Key words: *Aurea Helianthus*, phenols, flavonoids, extracts, antioxidant activity.

1. Introduction

Plants, as valuable natural resources, provide us with food, food additives, flavours, fragrances, colours, as well as pharmaceuticals in medicine [1]. Approximately 50% plant and their formulation products including antibiotics are all drugs available in clinical use in the world, plants and their formulation products constitute more than 35% of the total drugs in use [2]. Presently, antioxidant medicinal plants, including phenols and flavonoids have been showed a wide range of biological activities including anticarcinogenic actions [3]. Medicinal plants with pharmacological properties have been shown to be rich sources of ingredients with significant potential to prevent incurable diseases [4,5]. Many of the plants used traditionally have received little, the medicinal properties of these are undetermined. There is now an urgent need to search for novel and effective chemical compounds that may prevent cancers and other chronic diseases [6]. So, many studies are currently going to develop inhibitors from medicinal plants to prevent or cure chronic inflammatory conditions for minimal side effects [7]. *Aurea Helianthus* is a annual herb in family *Malvaceae*, and has the most edible, medicinal and health care functions in more than 200 kinds of okra plants, and has high commercial value [8]. The flowers, stems, leaves and seeds of *Aurea Helianthus* are rich in biologically active substances, which have good anti-oxidation, hypolipidemic, hypoglycemic, anti-tumor, analgesic, antipyretic, anti-inflammatory, immune regulation, anti-aging, Liver protection and other functions [9]. However, studies on phenols and flavonoids content, and antioxidant activities of *Aurea Helianthus* flowers have rarely been reported. Therefore, in order to provide theoretical and experimental basis for development and utilization, our present work is to determine the total phenols and flavonoids content of different polar solvent extracts from *Aurea Helianthus* flowers, and their antioxidant activity.

2. Methods

2.1. Materials

Ethanol, hexane, chloroform, ethyl acetate and butanol were procured from Junsei (Tokyo, Japan). 1,1-Diphenyl-2-picrylhydrazyl (DPPH), TPTZ (2,4,6-tripyridyl-s-triazine), gallic acid and quercetin were purchased from Sigma-Aldrich Company, Germany. A UV-visible spectrophotometer from Shimadzu, Japan (Model: UV-2450) was used for measuring the absorbance of different polarity crude extracts.

2.2. Extract sample preparation

Aurea Helianthus flowers were collected from market, 2018. 10. (Beijing, China). The samples were separated and washed with water. The collected samples were dried at room temperature. The samples were ground to powder. In the conventional reflux extraction method, The samples (20.00 g) was extracted three times with 200 mL of 80 % ethanol aqueous solution at 90 °C, 2 h for each time [10]. Then the extracts were combined, filtered, and concentrated under reduced pressure at 45 °C (7.25 g, yield 36.25%). The obtained ethanol extract (5.00 g) was suspended in water until completely dissolved. Next, the extract was transferred into a separatory funnel and fractionated by hexane, ethyl acetate, chloroform and butanol. The process was repeated 3 times. Every extract were combined and concentrated using a rotary evaporator. The remaining water was also evaporated using a rotary evaporator to give a water extract [11].

2.3. Determination of total phenols content

The total phenols content of each extract sample was evaluated using the Foline Ciocalteu reagent (FCR) described by Mc Donald et al [12], with slight modifications. Each extract sample was dissolved in deionized water until 100 µg/mL. The standard curve was obtained using gallic acid (0, 12.5, 25, 50, 100 and 200 µg/mL). Each diluted extract sample or gallic acid (2.0 mL) was added to FCR (0.5 mL, 20 %) and mixed well for 5 minutes. Sodium carbonate (0.5 mL, 10 %) was added to each mixture and all the mixture was kept at room temperature for 1 h. The absorbance of all the mixture was measured at 750 nm using a UV-visible spectrophotometer.

2.4. Determination of total flavonoids content

The total flavonoids content in each extract sample was investigated using AlCl₃ colorimetry method reported by Zhishen et al [13], with some modifications. Each extract sample was dissolved in methanol to a concentration of 150 µg/mL. The standard curve was prepared by quercetin (0, 12.75, 25.5, 50, 100 and 200 µg/mL). Each diluted extract sample or quercetin (2.5 mL) was added to 6% NaNO₂ (0.2 mL) and mixed. After 5 min, 10% AlCl₃ (0.3 mL) was added to each mixture and kept again for 5 min. then, 5% NaOH (1.0 mL) was added to all the mixtures. The absorbance of each mixture was measured at 510 nm using a UV-visible spectrophotometer.

2.5. Antioxidant activity

2.5.1 Ferric reducing ability of plasma (FRAP) assay

The FRAP assay of each extract sample was performed following Benzie & Strain (1996) [14], with minor modifications. The FRAP reagents were mixed with 20 mL of 0.3M acetate buffer (pH 3.6), 2.0 mL of 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) in 40 mM HCl, and 2.0 mL of 20 mM FeCl₃ 6H₂O. The extract sample (100 µL, 0.5 mg/mL) was added to 3 mL of FRAP solution and 300 µL of distilled water and then left for 5 min at 37 °C. The absorbance of the extract sample was measured at 593 nm using a UV-visible spectrophotometer. A standard curve was prepared using solutions of FeSO₄, Ascorbic acid and BHT were used as positive controls. all experiments were carried out in

triplicate, the FRAP values were expressed as mM FeSO₄ per gram of extract (mM FS/g extract).

2.5.2. Total antioxidant activity (TAA)

The total antioxidant activity(TAA) of each extract sample was evaluated using the method described by Prieto, Pineda, and Aguilar (1999)[15]. 1 mL of the extract sample(100-500 µg/mL) was mixed with 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The mixture was incubated for 90 min at 95 °C, then the mixture was cooled to room temperature. The absorbance of this mixture was measured at 695 nm using a UV-visible spectrophotometer, indicated antioxidant activity of each extract sample. BHT was used as a positive control, all experiments were carried out in triplicate.

2.5.3. DPPH radical scavenging assay

DPPH radical scavenging assay of each extract sample was carried out by using a modified method (Gow-Chin Yen and Hui-Yin Chen ,1995)[16]. DPPH solution was prepared by dissolving 10 mg DPPH in 100 mL methanol (about 0.25 mM). 2.0 mL of each extract sample (100-500 µg/mL) was mixed with 2.0 mL of DPPH solution. The mixture was incubated at room temperature in the dark. After 60 minutes, the absorbance was determined at 517 nm and ascorbic acid was used as a positive control. The DPPH radical scavenging ability of each extract sample was calculated using the following equation:

$$\text{DPPH radical scavenging rate(\%)} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100 \%$$

where A_{control} is the absorbance of DPPH solution (without extract sample), A_{sample} is the absorbance of extract sample (containing DPPH solution). The antioxidant activity of each extract sample was expressed as IC₅₀ (mg ml⁻¹), all experiments were carried out in triplicate.

2.5.4. Statistical analyses

All tests were measured in triplicate, and these results were expressed as means ±SD (Standard Deviation). Statistical analysis was performed by SPSS software (version 22.0, USA), and P values < 0.05 was used for statistical significance.

3. Results and discussion

3.1. Extraction yield

Extraction is the main step for recovering and isolating phytochemicals from plant materials, extraction yield is affected by the chemical nature of phytochemicals, the extraction method used, the solvent used, as well as the presence of interfering substances[17]. Each extract sample obtained from ethanol extract (5.00 g) was given to following yields: hexane (0.34 g, yield 6.8%), ethyl acetate (1.12 g, yield 22.4%), chloroform (0.46 g, yield 9.2%), butanol (0.33 g, yield 6.6%) and water(2.62 g, yield 52.4%).

3.2. Total phenols content

The total phenols content values of each extract sample, were determined from the standard curve ($r^2=0.9968$), were expressed as mg gallic acid equivalent per gram of extract (mg GAE/g extract)[12].

As shown in Table 1, the total phenols content of each extract sample followed the order: ethyl acetate (516.21 mg GAE/g) > butanol (246.05 mg GAE/g) > ethanol (225.23 mg GAE/g) > chloroform (212.16 mg GAE/g) > hexane(103.68 mg GAE/g) > water(73.68 mg GAE/g). The highest total phenols content was obtained in ethyl acetate extract, with a trend similar to that of total phenols content obtained from *Z. jujuba* leaf extract [18]. The lowest total phenols content was obtained in water extract, this may be attributable to the content of more nonphenol compounds such as carbohydrate and terpene in water extracts than in other extracts[19].

Table 1Total phenols and flavonoids content in each extract sample of *Aurea Helianthus* flowers

Extract Sample	Total phenols (mg GAE/g)	Total flavonoids (mg QCE /g)
Ethanol	225.23±0.24	141.25±0.34
Hexane	103.68±0.53	32.53±0.22
Ethyl acetate	516.21±0.81	328.06±0.65
Chloroform	212.16±0.36	186.28±0.72
Butanol	246.05±0.62	167.32±0.28
Water	73.68±0.45	51.21±0.31

All values are presented as mean ± SD of triplicates analysis: GAE: gallic acid, QCE: quercetin.

3.3. Total flavonoids content

The total flavonoids content of each extract sample was shown in Table 1, determined from the standard curve ($r^2=0.9983$), was expressed as mg quercetin per gram of extract (mg QCE /g extract) [13]. The total flavonoids content of each extract sample followed the order: ethyl acetate (328.06 mg QCE /g) > chloroform (186.28 mg QCE /g) > ethanol (141.25 mg QCE /g) > butanol (167.32 mg QCE /g) > water (51.21 mg QCE /g) > hexane (32.53 mg QCE /g). The highest flavonoids content was obtained in ethyl acetate extract and the lowest flavonoids content was obtained in hexane extract.

The correlation between TPC and TFC assay was found to be 0.967. This indicates that flavonoids are the dominating phenolic group in *Aurea Helianthus* [20,21]. Some flavonoids that were isolated from *Aurea Helianthus* have been identified by BAI Yun et al [22].

3.4. antioxidant activities

3.4.1 FRAP assay

FRAP assay is often used to evaluate the ability of antioxidant agent to donate electrons, the FRAP values were expressed as mM FeSO₄ per gram of extract (mM FS/g extract) [23]. The FRAP value of each extract sample followed the order: ethyl acetate > chloroform > ethanol > hexane > butanol > water (Table 2).

Table 2FRAP value of each extract sample from *Aurea Helianthus* flowers

Extract Sample	Mean value±SD(mM FS/g)
Ethanol	3.16±0.03 ^a
Hexane	2.53±0.05 ^c
Ethyl acetate	6.54±0.06 ^c
Chloroform	3.68±0.04 ^a
Butanol	2.31±0.05 ^b
Water	1.62±0.04 ^d
Ascorbic acid	12.35±0.08 ^a
BHT	2.83±0.05 ^c

All values are presented as means±SD of triplicates analysis, means in the same column with different letters(^{a-d}) were significantly different from one another ($p < 0.05$).

FRAP values of ethyl acetate extract was 6.54±0.06 mM FS/g, which was the highest of that of all Extract Samples. FRAP values of water extract was 1.62±0.04 mM FS/g, the lowest of them. FRAP values of all extract samples were correlated to total phenols content (0.912) and flavonoids content (0.866).

3.4.2. TAA

The total antioxidant activity (TAA) of each extract sample was calculated based on the formation of the phosphomolybdenum complex, was measured spectrophotometrically at 695 nm using a UV-visible spectrophotometer [6].

As shown in Fig. 1, The TAA of each extract sample increased with increasing concentration. Ethyl

acetate extract showed the highest TAA, which was significantly different from that of the others ($p < 0.05$). The TAA of each extract sample followed the order: ethyl acetate > chloroform > BHT > ethanol > butanol > hexane > water.

3.4.3. DPPH radical scavenging activity

DPPH radical is a stable free radical with a characteristic absorption at 517 nm (Soares et al, 1997)[24]. DPPH radical scavenging activity has been extensively used for screening antioxidant activities of various natural products. The mechanism of the reaction between antioxidant and DPPH radical depends on the structural conformation of the antioxidant (Bondent et al, 1997)[25].

As shown in Fig. 2, DPPH radical scavenging activity of each extract sample followed the order: Ascorbic Acid > ethyl acetate > chloroform > butanol > ethanol > hexane > water, also increased with increasing concentration.

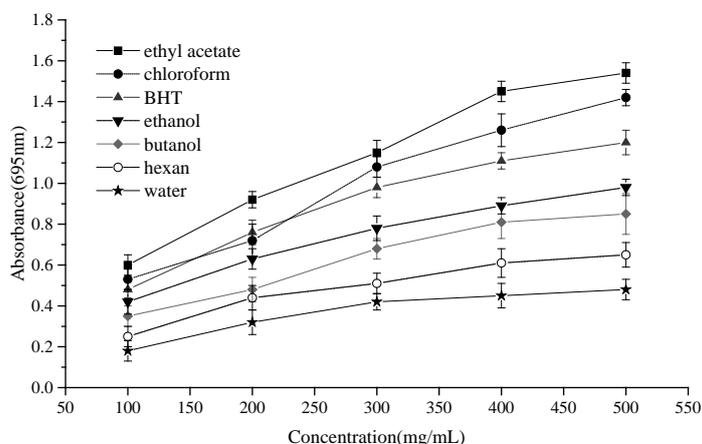


Fig.1. Total antioxidant activity of each extract sample from *Aurea Helianthus* flowers. All results are means±SD of triplicates analysis. ($p < 0.05$)

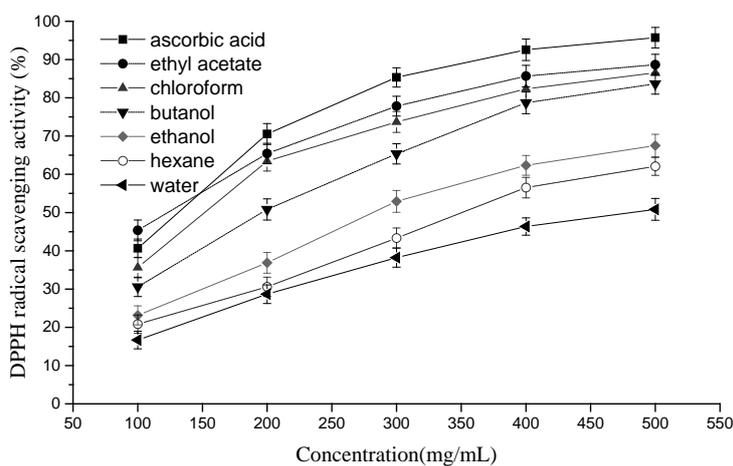


Fig.2. DPPH radical scavenging activity of each extract sample from *Aurea Helianthus* flowers. All results are means±SD of triplicates analysis. ($p < 0.05$)

DPPH radical scavenging activity of Ethyl acetate extract and chloroform extract (except Ascorbic Acid) were significantly different from that of the others ($p < 0.05$). The difference in the DPPH radical scavenging effect of the solvent extracts observed is consistent with research by Bonoli et al. (2004)[26] and Zhao et al. (2006)[27], it was generally found that this antioxidant activity was related to the content of total phenols and total flavonoids of the extracts.

3.4.4. IC50

The IC₅₀ of each extract sample is inversely related to its antioxidant capacity, as it expresses the amount of antioxidant required to decrease the DPPH concentration by 50% [28]. The IC₅₀ values in the DPPH radical scavenging activity assay of each extract sample were showed in Table 3.

As shown in Fig. 3, It was found that ethyl acetate extract(except Ascorbic Acid) possesses the strongest DPPH radical activity(IC₅₀ =132.63µg/mL)and water extract possesses the weakest DPPH radical activity(IC₅₀ =492.36µg/mL). Phenols and flavonoids were the main antioxidant components, and their total contents were directly proportional to their antioxidant activity [28]. IC₅₀ values of all extract samples from *Aurea Helianthus* flowers were correlated to total phenols content (0.776) and flavonoids content(0.843).

Table 3

IC₅₀ values of each extract sample from *Aurea Helianthus* flowers

Extract Sample	IC ₅₀ (µg/mL)
Ethanol	283.61±1.34 ^b
Hexane	353.62±1.86 ^a
Ethyl acetate	132.63±0.82 ^e
Chloroform	151.34 ±1.46 ^d
Butanol	198.53±1.63 ^c
Water	492.36±2.21 ^f
Ascorbic acid	123.12±0.64 ^b

All values are presented as means±SD of triplicates analysis, means in the same column with different letters(^{a-f})were significantly different from one another($p > 0.05$).

This result is different from the results of previous studies [29], the differences may be attributed to different solvents used in extraction [30], method and conditions of extraction[31]and so on.

4. Conclusion

This study indicated that different polarity extracts of *Aurea Helianthus* flowers contain high phenols and flavonoids content, and exhibited antioxidant activities. In this work, maximum amount of total phenols and flavonoids content were obtained in ethyl acetate and chloroform extracts, also showed significant antioxidant activity. The antioxidant activity was determined using the FRAP assay, TAA assay, DPPH radical scavenging assay. There is a good correlation of total phenols and flavonoids content with antioxidant activity. Therefore, the extracts of *Aurea Helianthus* flowers, as a natural antioxidant, could be used for the treatment of diseases related to oxidative stress.

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References

- [1] S. Bahramikia, A. Ardestani, R. Yazdanparast, Protective effects of four Iranian medicinal plants against free radical mediated protein oxidation, *Food Chem.* 115 (2009) 37-42.
- [2] K.M. Nadkarni, *Indian Materia Medica*, third ed., vol. 2, Popular Prakasan, India, 1994, pp. 84-96.
- [3] Bravol, L., 1998. Polyphenols: chemistry, dietary sources, metabolism and nutritional significance. *Nutr. Rev.* 56, 317–333.
- [4] R. Kumar, R.J. Sharma, K. Bairwa, R.K. Roy, A. Kumar, Pharmacological review on natural antidiarrhoeal agents, *Pharm. Chem.* 2 (2) (2010) 66-93.
- [5] S.S. Handa, S.P.S. Khanuja, G. Longo, D.D. Rakesh, Extraction technologies for medicinal and aromatic plants, *Inter. Cent. Sci. High Tech. Trieste* (2004) 21-25.
- [6] Asma Hamood Al-Saeedi, Moza Talib Humaid Al- Ghafri, Mohammad Amzad Hossain, Comparative evaluation of total phenols, flavonoids content and antioxidant potential of leaf and fruit extracts of Omani *Ziziphus jujuba* L,

- Pacific Science Review A: Natural Science and Engineering 18 (2016) 78-83.
- [7] Lampiasi, N., Montana, G., 2016. The molecular events behind ferulic acid mediated modulation of IL-6 expression in LPS-activated RAW 264.7 cell. *Immunobiology* 221, 486–493.
- [8] Lu, D., Jia, R.B., 2015. Research progress on Chinese medicinal material aurea helianthus. *Chin. J. Drug Eval.* 02.
- [9] Hak-Jae Kim, Chun-Geon Park, Rakesh Varghese et al, In-vitro antioxidative, antiinflammatory properties of Aurea helianthus leaf extract a Korean traditional medicinal plant, *Saudi Journal of Biological Sciences* 24 (2017) 1943–1947.
- [10] Peng Huang, Qing Zhang, Hongye Pan, Lianjun Luan, Xuesong Liu, Yongjiang Wu, Optimization of integrated extraction-adsorption process for the extraction and purification of total flavonoids from *Scutellariae barbatae* herba, *J. Separation and Purification Technology* 175 (2017) 203–212.
- [11] Asma Hamood Al-Saeedi, Moza Talib Humaid Al- Ghafri, Mohammad Amzad Hossain, Comparative evaluation of total phenols, flavonoids content and antioxidant potential of leaf and fruit extracts of Omani *Ziziphus jujuba* L, *J. Pacific Science Review A: Natural Science and Engineering* 18 (2016) 78-83.
- [12] Mc Donald S, Prenzler PD, Antolovich M, et al. Phenolic content and antioxidant activity of olive extracts. *Food Chem* 2001;73:73-84.
- [13] Zhishen, J., Mengcheng, T., Jianming, W., 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chem.* 64, 555–559.
- [14] Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Anal Biochem.* 1996 Jul 15;239(1):70-76.
- [15] Pilar Prieto, et al, Spectrophotometric Quantitation of Antioxidant Capacity through the Formation of a Phosphomolybdenum Complex: Specific Application to the Determination of Vitamin E1, *Analytical Biochemistry* 269, 337–341 (1999).
- [16] Gow-Chin Yen, Hui-Yin Chen, Antioxidant Activity of Various Tea Extracts in Relation to Their Antimutagenicity, *J. Agric. Food Chem.* 1995, 43, 27-32.
- [17] Stalikas CD. Extraction, separation, and detection methods for phenolic acids and flavonoids. *J Sep Sci* 2007;30:3268-95.
- [18] Asma Hamood et al, Comparative evaluation of total phenols, flavonoids content and antioxidant potential of leaf and fruit extracts of Omani *Ziziphus jujuba* L, *Pacific Science Review A: Natural Science and Engineering* 18 (2016) 78-83.
- [19] Quy Diem Do et al, Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*, *journal of food and drug analysis* 22 (2014) 296 -302.
- [20] Quy Diem Do et al, Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatic*, *journal of food and drug analysis* 22 (2014) 296 -302.
- [21] Hak-Jae Kim et al, In-vitro antioxidative, antiinflammatory properties of Aurea helianthus leaf extract a Korean traditional medicinal plant, *Saudi Journal of Biological Sciences* 24 (2017) 1943–1947.
- [22] BAI Yun, ZHU Ruichao, LI Jin, MA Lin et al, Simultaneous determination of five flavonoids in the flowers of Aurea helianthus by ultra performance liquid chromatography method, *Tianjin Journal of Traditional Chinese Medicine*, 2018, 35(2); 147-150.
- [23] Jing-En Li et al, Total flavonoids content, antioxidant and antimicrobial activities of extracts from *Mosla chinensis* Maxim. cv. Jiangxiangru, *LWT - Food Science and Technology* 64 (2015) 1022-1027.
- [24] Soares, J. R., Dinis, T. C., Cunha, A. P., & Almeida, L. M. (1997). Antioxidant activity of some extracts of *Thymus zygis*. *Free Radical Research*, 26, 469-478.
- [25] Bondet, V., Brand-Williams, W., & Bereset, C. (1997). Kinetic and mechanism of antioxidant activity using the DPPH free radical methods. *LWT-Food Science and Technology*, 30, 609-615.
- [26] Bonoli, M., Marconi, E., Caboni, M.F., 2004. Free and bound phenolic compounds in barley (*Hordeum vulgare* L.) flour evaluation of extraction capability of different solvent mixtures and pressurized liquid methods by micellar electrokinetic chromatography and spectrophotometry. *J. Chromatogr. A* 1057, 1-12.
- [27] Zhao, H., Dong, J., Lu, J., Chen, J., Li, Y., Shan, L., Lin, Y., Fan, W., Gu, G., 2006. Effects of extraction solvent mixtures on antioxidant activity evaluation and their extraction capacity and selectivity for free phenolic compounds in barley (*Hordeum vulgare* L.). *J. Agric. Food Chem.* 54, 7277-7286.
- [28] Liu SC, Lin JT, Wang CK, et al. Antioxidant properties of various solvent extracts from lychee (*Litchi chinensis* sonn.) flowers. *Food Chem* 2009;114:577-81.
- [29] Pinelo M, Manzocco L, Nunes MJ, et al. Interaction among phenols in food fortification: negative synergism on antioxidant capacity. *J Agric Food Chem* 2004;52:1177-80.
- [30] Pinelo M, Manzocco L, Nunes MJ, et al. Interaction among phenols in food fortification: negative synergism on antioxidant capacity. *J Agric Food Chem* 2004;52:1177-80.
- [31] Robards K. Strategies for the determination of bioactive phenols in plants, fruit and vegetables. *J Chromatogr A* 2003;1000:657-91.