Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology)
ISSN 1673-1581 (Print); ISSN 1862-1783 (Online)
www.jzus.zju.edu.cn; www.springerlink.com
E-mail: jzus@zju.edu.cn

Antioxidant activity of alcohol aqueous extracts of *Crypthecodinium cohnii* and *Schizochytrium* sp.*

Jin-hui YU[1,2,4,5], Yu WANG[3], Jie SUN[1,4], Fei BIAN[5], Gao CHEN[5], Yan ZHANG[5], Yu-ping BI[†‡5], Ying-jie WU[†‡1,4]

([1]*Institute of Genome Engineered Animal Models for Human Disease, Dalian Medical University, Dalian 116044, China*)

([2]*Shandong Center of Crop Germplasm Resources, Jinan 250100, China*)

([3]*School of Life Sciences, Shandong University, Jinan 250100, China*)

([4]*College of Integrative Medicine, Dalian Medical University, Dalian 116044, China*)

([5]*Biotechnology Research Center, Shandong Academy of Agricultural Sciences, Jinan 250100, China*)

[†]E-mail: yupingbi@vip.sina.com; yingjiewu@dmu.edu.cn

Received Aug. 19, 2016; Revision accepted Oct. 31, 2016; Crosschecked Aug. 17, 2017

Abstract: *Crypthecodinium cohnii* (dinoflagellate) and *Schizochytrium* sp. (thraustochytrid) are the main sources for docosahexaenoic acid (DHA). The present study aimed to evaluate the antioxidant activity of petroleum ether, ethyl acetate, *n*-butanol, and water fractions of alcohol aqueous extracts of these two microalgae and to provide a theoretical basis for comprehensive utilization. The antioxidant activity was determined by total antioxidant capacity (TAC) determination, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, ferrous ion-chelating ability (FICA) assay, and reducing power (RP) assay. The total phenolic content (TPC) and total flavonoid content (TFC) were also measured by the Folin-Ciocalteu and spectrophotometry methods, respectively. The results indicated that the extracts from these two microalgae possessed good antioxidant capacity. Analysis showed that most antioxidant performance indicators (TAC, DPPH, and RP) were positively correlated with the TPC of the extracts, suggesting that the phenolics might be the major components in *C. cohnii* and *Schizochytrium* sp., contributing to their antioxidative function. Therefore, the polar fractions of *C. cohnii* and *Schizochytrium* sp. could be further examined and considered for application in health products or cosmetics.

Key words: *Crypthecodinium cohnii*; *Schizochytrium* sp.; Alcohol aqueous extract; Antioxidant activity; Total phenolics
http://dx.doi.org/10.1631/jzus.B1600367 **CLC number:** Q939.9

1 Introduction

Crypthecodinium cohnii and *Schizochytrium* sp. are two important microalgae for docosahexaenoic acid (DHA) production (Fedorova-Dahms *et al*., 2014; Gaffney *et al*., 2014). *C. cohnii*, distributed world-wide, is a marine heterotrophic microalga (dinoflagellate) (Beam and Himes, 1982). Until recently, studies of *C. cohnii* have focused on cultivation (Pleissner and Eriksen, 2012; Hillig *et al*., 2013), DHA production (Gong *et al*., 2015), the biological activity of DHA (Fedorova-Dahms *et al*., 2014), and DHA applications in the breeding and aquatic industries (Schiavone *et al*., 2007; Ganuza *et al*., 2008). *Schizochytrium* sp. belongs to the Stramenopila (thraustochytrid) kingdom and generally lives in marine habitats throughout the world (Lippmeier *et al*., 2009; Fedorova-Dahms *et al*., 2011). Many studies have been concerned with cultivation optimization (Ling

‡ Corresponding authors

* Project supported by the Shandong Academy of Agriculture Sciences (No. 2015YQN32), the Ministry of Science and Technology of China (No. 2014DFA32120), and the National Natural Science Foundation of China (No. 81471000)

ORCID: Jin-hui YU, http://orcid.org/0000-0003-3594-7889

et al., 2015; Guo *et al.*, 2016), safety evaluation of the algal oil (Lewis *et al.*, 2016), and applications in the aquatic product industry (Ganuza *et al.*, 2008; Li *et al.*, 2009). To date, there have been only limited reports examining the antioxidant activity of these two microalgae. Lv *et al.* (2014) explored the antioxidant activity of the microalgae oil of *Schizochytrium aggregatum*. Gaffney *et al.* (2014) reported that flaxseed oil supplementation enhanced the antioxidant status of the microalgae *Schizochytrium* sp. However, extracts of different polarities from these two microalgae have rarely been investigated. Since no reports about harmful compositions in these two microalgae are known (Fedorova-Dahms *et al.*, 2011), it is of interest to investigate the extracts of different polarities for high value-added by-products to reduce the cost of DHA production.

Antioxidants play critical roles in preventing and treating many degenerative diseases such as cancer (Kasala *et al.*, 2016), cardiovascular disease (Sugamura and Keaney, 2011), and schizophrenia (Wu J.Q., *et al.*, 2013) by neutralizing free radicals. However, synthetic antioxidants have side effects and are detrimental to the human body (Pan *et al.*, 2007). Therefore, there is a real need to explore natural compounds with a higher antioxidant activity. Antioxidant capacity, an important index for food and drug efficacy evaluation, is of significant value in the process of screening for antioxidant activity components. There are numerous methods for antioxidant evaluation; for example, 2,2-diphenyl-1-picrylhydrazyl (DPPH), total radical-trapping antioxidant parameter (TRAP), reducing power (RP), oxygen radical absorbance capacity (ORAC), ferric reducing antioxidant power (FRAP), and 2,2-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) assays are commonly used (Alam *et al.*, 2013; López-Alarcón and Denicola, 2013). Since no standardized method has been identified, two or more of the above methods based on different mechanisms can be chosen for evaluating the antioxidant capacity of tested materials.

As far as we are aware, no study has yet been reported which examines the antioxidant activity of the polar compositions of these two microalgae. Hence, we aim to evaluate the antioxidant activity of the alcohol aqueous extracts of *C. cohnii* and *Schizochytrium* sp. Two microalgae were extracted with 70% ethanol and the extracts were further successively extracted by petroleum ether, ethyl acetate, *n*-butanol, and water. Then, the antioxidant activity of the four different polar extracts was measured by total antioxidant capacity (TAC) determination, DPPH radical scavenging assay, ferrous ion-chelating ability (FICA) assay, and RP assay. The total phenolic content (TPC) and total flavonoid content (TFC) were further determined. A correlation analysis between TPC, TFC and indicators of antioxidant activity was also conducted. Our data help to clarify the antioxidant capacity of the aqueous ethanol extracts of *C. cohnii* and *Schizochytrium* sp. and provide a scientific basis for comprehensive utilization of these two microalgae.

2 Materials and methods

2.1 Chemicals

Ethanol, petroleum ether (boiling point (bp) 60–90 °C), ethyl acetate, *n*-butanol, ammonium molybdate, acetic acid, hydrochloric acid, ferrous chloride, ferric chloride, sulfuric acid, sodium phosphate, potassium ferric cyanide, trichloroacetic acid, sodium hydroxide, and sodium carbonate were purchased from local reagent companies in Jinan, China. DPPH, 3-(2-pyridyl)-5,6-bis(4-phenylsulfonic acid) (Ferrozine), and 2,6-di-tert-butyl-4-methylphenol (BHT) were supplied by Sigma-Aldrich (Darmstadt, Germany). Gallic acid and Folin-Ciocalteu reagent were from Aladdin Industries Inc. (Shanghai, China). All chemicals were of analytical grade. Ultrapure water was obtained from the Milli-Q advantage A10 purification system (Massachusetts, USA).

2.2 Cultivation and collection of microalgae

The strains of *C. cohnii* (ATCC30334) and *Schizochytrium* sp. (ATCC20888) were purchased from the American Type Culture Collection (ATCC). *C. cohnii* was maintained on an ATCC460 medium, while ATCC790 medium was used for subculturing *Schizochytrium* sp. The two microalgae were cultured in a 500-ml conical flask containing 200 ml YSG medium (5 g/L yeast, 25 g/L sea salt, and 15 g/L glucose) and shaken at 160 r/min and 25 °C in a vertical shaker. Cells were collected by centrifugation and washed three times with distilled water. The harvest was dried at 40 °C in an oven for at least 24 h

until it reached a constant weight. Then, the dried samples were ground and sieved to obtain a grain size of 0.45 mm powder and stored at −80 °C before use.

2.3 Preparation of the extract

The ground samples ((50±0.01) g) were each mixed with 750 ml of 70% (v/v) ethanol, and subjected to soaking for two days. The supernatant was filtered by filter paper, and re-extracted with the soaking method as before. The extract procedure was repeated six times and all supernatants were combined. The pooled extract was concentrated using a rotary evaporator under vacuum at 50 °C. The concentrated extracts were dried in a vacuum drying oven at 50 °C and weighed. The extraction yield was expressed as the mass percentage of the sample. Then, the residue was extracted with solvents of different polarities (petroleum ether, ethyl acetate, *n*-butanol, and water) and divided into four fractions. The aqueous ethanol extracts of *C. cohnii* were denoted as PE-C, EA-C, NB-C, and W-C, and those of *Schizochytrium* sp. as PE-S, EA-S, NB-S, and W-S. The flow chart is given in Fig. 1. The extract concentration was adjusted to 50 mg/ml with 70% ethanol and stored at 4 °C until further use. The concentrations were diluted to 0.5, 1.0, and 2.0 mg/ml before use.

2.4 Antioxidant capacity assay

The TAC assay method was carried out according to the method described by Pan *et al.* (2007). Briefly, the extract solution of 0.5 ml (0.5, 1.0, and 2.0 mg/ml) was mixed with a 3-ml reagent solution, which contained sulfuric acid (0.6 mol/L), sodium phosphate (28 mmol/L), and ammonium molybdate (4 mmol/L). The reaction mixture was incubated in a water bath for 150 min at 95 °C, and then cooled to room temperature. The absorbance value representing the antioxidant activity of the samples was determined at 695 nm against distilled water as a blank. BHT (0.5 mg/ml) was used as the reference standard.

A DPPH radical scavenging activity assay was performed as described by Narwal *et al.* (2014). DPPH (3 ml, 0.08 mmol/L) was added to the sample solutions and then mixed. After standing at room temperature for 30 min in the dark, the mixtures were measured at 517 nm against ethanol as a blank. BHT (0.5 mg/ml) was used as the reference standard. The radical scavenging activity was calculated according to the equation.

An FICA assay was performed as described by Choochote *et al.* (2014). Distilled water (2.7 ml) was successively added, as well as 0.1 ml $FeCl_2$ (2 mmol/L) and 0.2 ml ferrozine (5 mmol/L) to the sample solutions. After incubation at 25 °C for 10 min, the absorbance was measured at 562 nm, and ethylenediaminetetraacetic acid disodium salt (EDTA-Na_2; 0.05 mg/ml) was used as a reference standard. The FICA was calculated as the reference.

The RP was measured according to the method described by Venuste *et al.* (2013). Sodium phosphate buffer (2.5 ml, 0.2 mol/L, pH 6.6) and 2.5 ml potassium ferric cyanide solution (0.01 g/ml) were successively added to the sample solutions and incubated at 50 °C for 20 min. After cooling to room

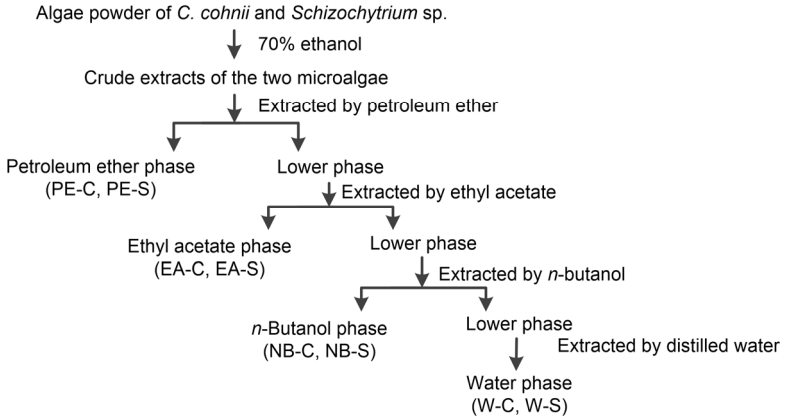

Fig. 1 Extraction flow chart for different polar components of the two microalgae

The aqueous ethanol extracts of *C. cohnii* and *Schizochytrium* sp. were obtained and then extracted with solvents of different polarities (petroleum ether, ethyl acetate, *n*-butanol, and water) and divided into four fractions. Different fractions of *C. cohnii* are denoted as PE-C, EA-C, NB-C, and W-C, and those of *Schizochytrium* sp. as PE-S, EA-S, NB-S, and W-S

temperature, 2.5 ml trichloroacetic acid (0.1 g/ml) was added and mixed, then centrifuged at 5000 r/min for 5 min and 2.5 ml supernatant was obtained, which was mixed with 2.5 ml distilled water and 0.5 ml ferric chloride solution (1 g/L). The mixture was allowed to react for 10 min and the absorbance was read at 700 nm against distilled water as a blank; BHT (0.5 mg/ml) was used as the reference standard.

The sample solution volume used in the above three indices was 1.0 ml in three concentrations (0.5, 1.0, and 2.0 mg/ml).

2.5 Determination of total phenolic and flavonoid contents

TPC was determined according to the method described by Pan *et al.* (2007). The sample volume in this test was 0.5 ml. Results were expressed as milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g DW).

TFC was assessed as described by Saeed *et al.* (2012) with modifications. Briefly, a sample solution (1.0 ml) was mixed with 1.0 ml $NaNO_2$ (0.05 g/ml) and 1.0 ml $AlCl_3$ (0.1 g/ml). The mixture was incubated for 5 min, followed by transferring 10.0 ml NaOH (1 mol/L) to a 25-ml volumetric flask. After mixing, the absorbance was measured at 506 nm after 15 min. Results were expressed as milligrams of quercetin equivalents per gram of dry weight (mg QE/g DW).

2.6 Statistical analysis

Results were presented as mean±standard deviation (SD). Correlation analyses of the relationship between TPC, TFC and different antioxidant indexes were conducted with SPSS Version 16.0 by multiple linear regression. The statistical significance between the correlation curves was according to Pearson's correlation and the correlation was judged to be statistically significant when $P\leq0.05$ or $P\leq0.01$.

3 Results

3.1 Extraction of the microalgae

The extraction yields of the extracts are shown in Table 1. The yield of alcohol aqueous extract of *C. cohnii* was about 4.1% higher than that of *Schizochytrium* sp. The above extracts were successively extracted by petroleum ether, ethyl acetate, *n*-butanol, and water. As the final residue was completely dissolved, the water fractions were relatively high in both microalgae, up to 51.95% and 43.72%, respectively. The ethyl acetate fraction was the lowest for both.

3.2 Total antioxidant capacity

The TAC of the extracts was determined and compared with that of BHT. The optical density indicated that the TAC of the two microalgae increased with increasing concentrations (Fig. 2). The different polar fractions of *C. cohnii* were basically the same, and the capacity was similarly equivalent to that of the positive control at the same concentration (0.5 mg/ml). The ethyl acetate fraction of *Schizochytrium* sp. showed the highest antioxidant capacity, at almost two times that of the highest fraction of *C. cohnii*, followed by the petroleum ether fraction, which all were higher than the TAC of the positive control at 0.5 mg/ml.

3.3 DPPH radical scavenging activity

The comparison of DPPH radical scavenging activity of different polarities of the two extracts is shown in Fig. 3. For *C. cohnii*, water and *n*-butanol fractions possessed nearly the same scavenging activity while the others exhibited a similar capacity. For *Schizochytrium* sp., water extracts exhibited a higher scavenging effect with 81.56%, 42.37%, and 20.58% at 2.0, 1.0, and 0.5 mg/ml, respectively. *n*-Butanol, ethyl acetate, and petroleum ether extract had almost the same scavenging activity.

Table 1 Extraction yields of *C. cohnii* and *Schizochytrium* sp. extracts and different polar components

Microorganism	Extraction yield (%, w/w)				
	Alcohol aqueous extract[a]	Petroleum ether[b]	Ethyl acetate[b]	*n*-Butanol[b]	Water[b]
C. cohnii	21.7±1.2	17.2±3.3	12.2±1.1	17.0±1.9	52.0±5.6
Schizochytrium sp.	17.6±2.4	43.7±2.6	3.6±0.6	10.8±0.4	41.9±2.4

[a] The extraction yield was calculated as the total extract by 70% ethanol in the proportion of the original ground sample. [b] The extraction yield was calculated as the extracts by different polar solvents in the proportion of the alcohol aqueous extract

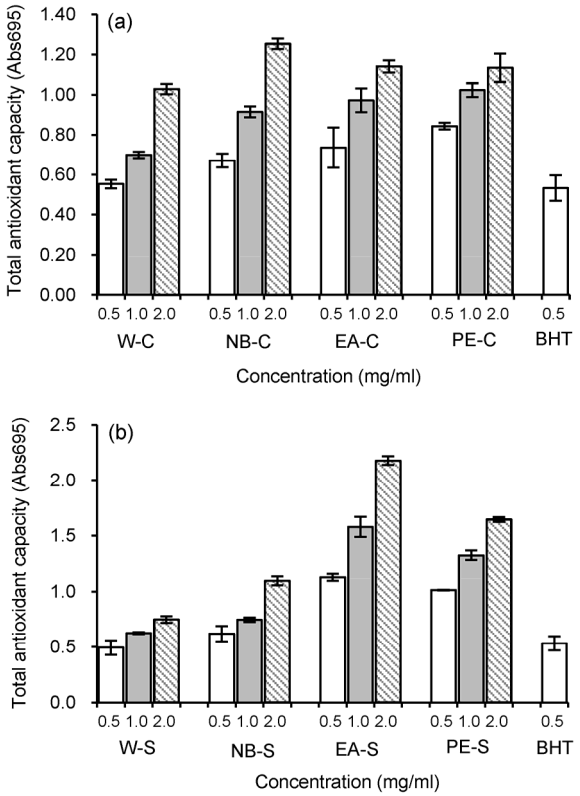

Fig. 2 Total antioxidant capacity (TAC) of *C. cohnii* (a) and *Schizochytrium* sp. (b)
The aqueous ethanol extracts of these two microalgae were extracted successively by petroleum ether, ethyl acetate, *n*-butanol, and water. The four fractions of *C. cohnii* are denoted as PE-C, EA-C, NB-C, and W-C, while those of *Schizochytrium* sp. as PE-S, EA-S, NB-S, and W-S. Samples are diluted into three concentrations (0.5, 1.0, and 2.0 mg/ml) and BHT (0.5 mg/ml) is used as a reference standard. Abs695: absorbance at 695 nm. Results are expressed as mean±SD (n=3)

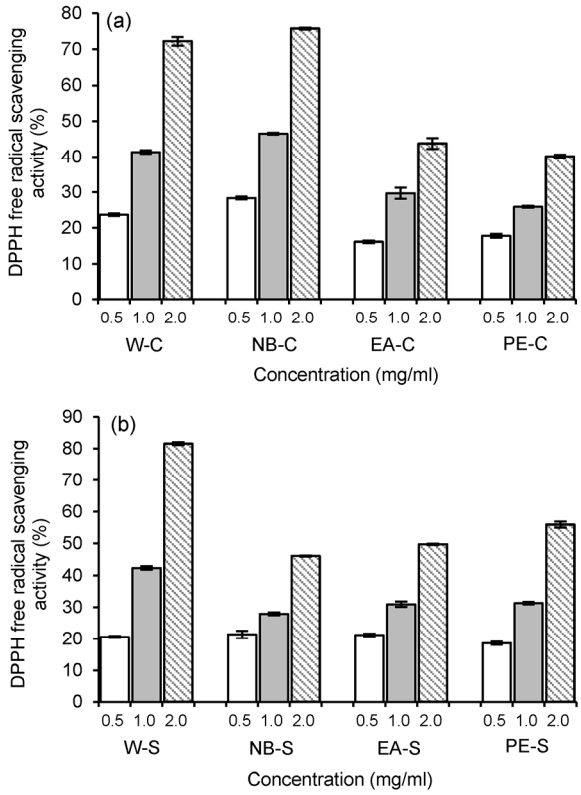

Fig. 3 DPPH free radical scavenging activity of *C. cohnii* (a) and *Schizochytrium* sp. (b)
The aqueous ethanol extracts of these two microalgae were extracted successively by petroleum ether, ethyl acetate, *n*-butanol, and water. The four fractions of *C. cohnii* are denoted as PE-C, EA-C, NB-C, and W-C, while those of *Schizochytrium* sp. as PE-S, EA-S, NB-S, and W-S. Samples are diluted into three concentrations (0.5, 1.0, and 2.0 mg/ml). Results are expressed as mean±SD (n=3)

3.4 Ferrous ion-chelating ability

The FICAs of the different polarity fractions of the extracts were compared with that of EDTA-Na_2. The water fraction of *C. cohnii* exhibited the highest chelating capacity of 63.13% under 2.0 mg/ml. The *n*-butanol fraction of *C. cohnii* possessed the lowest chelating capacity without obvious concentration-dependence (Fig. 4a). The water and petroleum ether fractions of *Schizochytrium* sp. showed significantly higher FICA with 70.59% and 88.10%, respectively, at 2.0 mg/ml, and the *n*-butanol fraction exhibited the lowest activity (Fig. 4b).

3.5 Reducing capacity

The reducing capacities of *C. cohnii* and *Schizochytrium* sp. also exhibited a concentration-dependent effect at different concentrations (Fig. 5). The water fraction of *C. cohnii* had the highest reducing capacity, followed by the *n*-butanol and petroleum ether fractions, and the ethyl acetate fraction was the lowest, all of which were lower than that of the positive control of BHT at the same concentration (0.5 mg/ml). The petroleum ether fraction of *Schizochytrium* sp. possessed the highest RP. The other fractions showed a similar level, which was much lower than that of the positive control at the same level. By contrast, the RP of the extracts of *C. cohnii* was better than that of *Schizochytrium* sp.

3.6 Total phenolic and flavonoid contents

The TPC of the extracts varied from 42.95 to 140.52 mg GAE/g extract and the TFC from 0.31 to

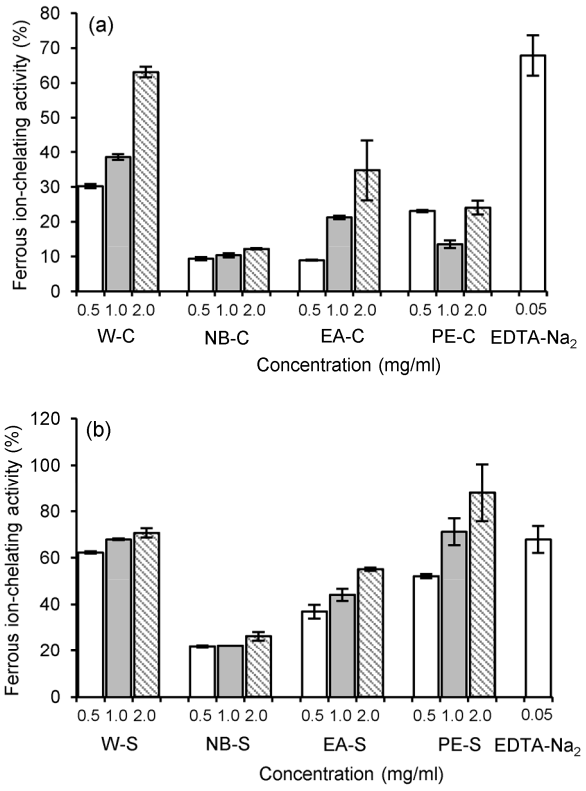

Fig. 4 Ferrous ion-chelating activity (FICA) of *C. cohnii* (a) and *Schizochytrium* sp. (b)

The aqueous ethanol extracts of these two microalgae were extracted successively by petroleum ether, ethyl acetate, *n*-butanol, and water. The four fractions of *C. cohnii* are denoted as PE-C, EA-C, NB-C, and W-C, while those of *Schizochytrium* sp. as PE-S, EA-S, NB-S, and W-S. Samples are diluted into three concentrations (0.5, 1.0, and 2.0 mg/ml) and EDTA-Na_2 (0.05 mg/ml) is used as a reference standard. Results are expressed as mean±SD (n=3)

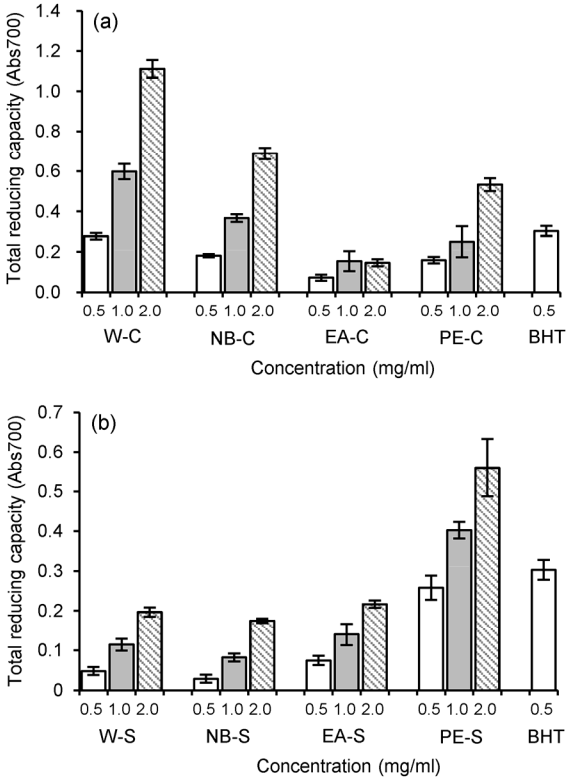

Fig. 5 Reducing power of *C. cohnii* (a) and *Schizochytrium* sp. (b)

The aqueous ethanol extracts of these two microalgae were extracted successively by petroleum ether, ethyl acetate, *n*-butanol, and water. The four fractions of *C. cohnii* are denoted as PE-C, EA-C, NB-C, and W-C, while those of *Schizochytrium* sp. as PE-S, EA-S, NB-S, and W-S. Samples are diluted into three concentrations (0.5, 1.0, and 2.0 mg/ml) and BHT (0.5 mg/ml) is used as a reference standard. Abs700: absorbance at 700 nm. Results are expressed as mean±SD (n=3)

134.05 mg QE/g extract (Fig. 6). The *n*-butanol fractions of *C. cohnii* and *Schizochytrium* sp. had the highest phenolic content, 133.93 and 140.52 mg GAE/g, respectively, while the TFC was higher in the ethyl acetate fraction. The TPC and TFC of the other fractions of the two microalgae had a similar tendency.

3.7 Relationship of total phenolic and flavonoid contents and antioxidant ability

A regression analysis was performed for correlations between TPC, TFC and TAC, DPPH, FICA, and RP of different polar extracts of *C. cohnii* and *Schizochytrium* sp. The results are listed in Tables 2 and 3. For *C. cohnii*, TPC correlated positively with TAC, DPPH, and RP values. Only FICA had no correlation with TPC, while TFC was significantly related to TAC. Therefore, the high antioxidant activity of extracts of *C. cohnii* might closely depend on the contents of the phenolic and flavonoid compounds. For *Schizochytrium* sp., TPC correlated positively but weakly with TAC and DPPH values, but negatively with the FICA value. TFC was significantly related to TAC and RP but not to other indexes. Hence, the TPC parameter represented a suitable measure of the antioxidant activity for *C. cohnii* but might not be the right indicator for *Schizochytrium* sp., which probably depended on TFC.

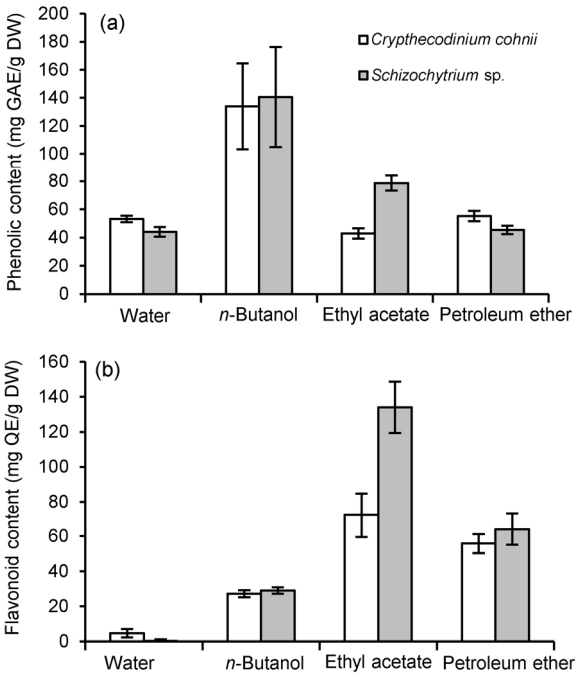

Fig. 6 Total phenolic and flavonoid contents of different polar fractions of *C. cohnii* (a) and *Schizochytrium* sp. (b) The aqueous ethanol extracts of these two microalgae were extracted successively by petroleum ether, ethyl acetate, *n*-butanol, and water. Then, total phenolic and flavonoid contents of different fractions of these two microalgae were measured. Results are expressed as mean±SD (n=3)

Table 2 Correlation coefficients of TPC, TFC and TAC, DPPH, FICA, and RP values of different polar extracts of *C. cohnii*

Component	Correlation coefficient			
	TAC	DPPH	FICA	RP
TPC	0.684**	0.848**	−0.007	0.532*
TFC	0.714**	0.019	−0.163	−0.268

* $P\leq0.05$; ** $P\leq0.01$. TPC: total phenolic content; TFC: total flavonoid content; TAC: total antioxidant capacity; DPPH: DPPH radical scavenging activity; FICA: ferrous ion-chelating activity; RP: reducing power

Table 3 Correlation coefficients of TPC, TFC and TAC, DPPH, FICA, and RP values of different polar extracts of *Schizochytrium* sp.

Component	Correlation coefficient			
	TAC	DPPH	FICA	RP
TPC	0.313	0.359	−0.440	0.004
TFC	0.958**	0.184	0.195	0.642*

* $P\leq0.05$; ** $P\leq0.01$. TPC: total phenolic content; TFC: total flavonoid content; TAC: total antioxidant capacity; DPPH: DPPH radical scavenging activity; FICA: ferrous ion-chelating activity; RP: reducing power

4 Discussion

The results indicated that the extracts of the two microalgae exhibited good antioxidant capacity. For *C. cohnii*, the extracts of different polarities showed a similar antioxidant activity on TAC, while the water and *n*-butanol fractions showed higher activity in the assays for DPPH and RP. For *Schizochytrium* sp., the ethyl acetate and petroleum ether fractions showed higher levels in the assays of TAC and RP, while the water fraction was the highest in the DPPH radical scavenging assay.

Microalgae are an attractive alternative resource as they are rich in bioactive chemicals (Encarnação *et al.*, 2015; Luo *et al.*, 2015; Khozin-Goldberg *et al.*, 2016). Much research on the antioxidant effect of different microalgae has been carried out (Safafar *et al.*, 2015; Singh *et al.*, 2016). Polyphenol and flavonoid contents are commonly measured components referring to antioxidant activity (Pan *et al*., 2007; Saeed *et al.*, 2012; Li *et al.*, 2014; Narwal *et al.*, 2014; Ying *et al*., 2017). Previous studies also verified the relationships between algal phenols and antioxidant capacity (Hajimahmoodi *et al.*, 2010).

In order to identify the effective antioxidant composition in these two microalgae, we measured their total phenolic and flavonoid contents. Correlation analysis showed that TPC was significantly correlated with most antioxidant performance indicators (TAC, DPPH, and RP) for *C. cohnii* extracts, indicating the participation of phenolics as antioxidants. It has been reported that phenolic components may contribute to the antioxidant capacity of supplemented *Schizochytrium* sp. cultures (Gaffney *et al.*, 2014), but poor correlation between TPC and the antioxidant parameters of *Schizochytrium* sp. was observed in this study, indicating that TPC might not be the key antioxidant component present in the alcohol aqueous extract of this species, which is consistent with a previous study (Li *et al.*, 2007). TFC in *Schizochytrium* sp. was significantly correlated with TAC and RP. Although many studies have shown that phenolic compounds have an important effect against reactive oxygen species, other chemicals in the extracts of plants might have promoting or inhibiting effects on the antioxidant process (Podsędek, 2007). In our study, TFC was only significantly correlated with TAC but not with others, which might reflect the

complex relationship among various natural antioxidant compounds. Antioxidants include diverse compositions, including polyphenols, vitamins, alkaloids, saponins, polysaccharides, and active peptides (O'Brien *et al.*, 2006; Samaranayaka and Li-Chan, 2011; Carocho and Ferreira, 2013; Cheok *et al.*, 2014; Zeng *et al.*, 2014). Our preliminary study also found polysaccharides in *C. cohnii* (data not shown). Therefore, whether polysaccharides or other components play the main roles in the antioxidant capacities of the two microalgae still needs to be analyzed.

Recently, the concept of comprehensive utilization, which allows maximization of the value and advantages of resources, has received significant attention (Wu X. *et al.*, 2013). As important materials in DHA, these two microalgae could produce substantial volumes of polar residues with constantly increasing DHA demand (Salem and Eggersdorfer, 2015). Therefore, it is imperative to study the other high value-added products that are left over for cost reduction and sustainable development. Based on the above results, we concluded that the alcohol aqueous extracts of the two microalgae, *C. cohnii* and *Schizochytrium* sp., have relatively high antioxidant activity. To the best of our knowledge, this is the first study to evaluate the antioxidant capacity of the polar components of these two microalgae. The results reported here may give some insight and guidance on the comprehensive and high value-added utilization of these two microalgae, in addition to producing polyunsaturated fatty acids. Further studies need to be conducted to identify the antioxidant compounds present in *C. cohnii* and *Schizochytrium* sp., and to evaluate whether the antioxidant compounds that are present in the described extracts are suitable for dietary or cosmetic application.

5 Conclusions

In conclusion, we defined different polar extracts of the two microalgae that had certain antioxidant effects, which implied that other products could be considered for exploitation in addition to polyunsaturated fatty acids. The correlation analysis between the antioxidant capacity and the total polyphenol and flavonoid contents indicates that there are likely to be different types of antioxidants in these two microalgae. Therefore, we need to conduct further work to isolate and identify the other antioxidant components present in the two microalgae.

Compliance with ethics guidelines

Jin-hui YU, Yu WANG, Jie SUN, Fei BIAN, Gao CHEN, Yan ZHANG, Yu-ping BI, and Ying-jie WU declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

References

Alam, M.N., Bristi, N.J., Rafiquzzaman, M., 2013. Review on in vivo and in vitro methods evaluation of antioxidant activity. *Saudi Pharm. J.*, **21**(2):143-152. http://dx.doi.org/10.1016/j.jsps.2012.05.002

Beam, C.A., Himes, M., 1982. Distribution of members of the *Crypthecodinium cohnii* (Dinophyceae) species complex. *J. Eukaryot. Microbiol*., **29**(1):8-15. http://dx.doi.org/10.1111/j.1550-7408.1982.tb02874.x

Carocho, M., Ferreira, I.C., 2013. A review on antioxidants, prooxidants and related controversy: natural and synthetic compounds, screening and analysis methodologies and future perspectives. *Food Chem. Toxicol*., **51**:15-25. http://dx.doi.org/10.1016/j.fct.2012.09.021

Cheok, C.Y., Salman, H.A.K., Sulaiman, R., 2014. Extraction and quantification of saponins: a review. *Food Res. Int*., **59**:16-40. http://dx.doi.org/10.1016/j.foodres.2014.01.057

Choochote, W., Suklampoo, L., Ochaikul, D., 2014. Evaluation of antioxidant capacities of green microalgae. *J. Appl. Phycol.*, **26**(1):43-48. http://dx.doi.org/10.1007/s10811-013-0084-6

Encarnação, T., Pais, A.A., Campos, M.G., *et al.*, 2015. Cyanobacteria and microalgae: a renewable source of bioactive compounds and other chemicals. *Sci. Prog.*, **98**(2): 145-168. http://dx.doi.org/10.3184/003685015X14298590596266

Fedorova-Dahms, I., Marone, P.A., Bauter, M., *et al.*, 2011. Safety evaluation of DHA-rich Algal Oil from *Schizochytrium* sp. *Food Chem. Toxicol.*, **49**(12):3310-3318. http://dx.doi.org/10.1016/j.fct.2011.08.024

Fedorova-Dahms, I., Thorsrud, B.A., Bailey, E., *et al.*, 2014. A 3-week dietary bioequivalence study in preweaning farm piglets of two sources of docosahexaenoic acid produced from two different organisms. *Food Chem. Toxicol.*, **65**: 43-51. http://dx.doi.org/10.1016/j.fct.2013.12.008

Gaffney, M., O'Rourke, R., Murphy, R., 2014. Manipulation of fatty acid and antioxidant profiles of the microalgae *Schizochytrium* sp. through flaxseed oil supplementation. *Algal Res.*, **6B**:195-200. http://dx.doi.org/10.1016/j.algal.2014.03.005

Ganuza, E., Benítez-Santana, T., Atalah, E., *et al.*, 2008. *Crypthecodinium cohnii* and *Schizochytrium* sp. as potential substitutes to fisheries-derived oils from seabream

(*Sparus aurata*) microdiets. *Aquaculture*, **277**(1-2):109-116. http://dx.doi.org/10.1016/j.aquaculture.2008.02.005

Gong, Y., Liu, J., Jiang, M., *et al.*, 2015. Improvement of omega-3 docosahexaenoic acid production by marine dinoflagellate *Crypthecodinium cohnii* using rapeseed meal hydrolysate and waste molasses as feedstock. *PLoS ONE*, **10**(5):e0125368. http://dx.doi.org/10.1371/journal.pone.0125368

Guo, D.S., Ji, X.J., Ren, L.J., *et al.*, 2016. Development of a real-time bioprocess monitoring method for docosahexaenoic acid production by *Schizochytrium* sp. *Bioresour. Technol.*, **216**:422-427. http://dx.doi.org/10.1016/j.biortech.2016.05.044

Hajimahmoodi, M., Faramarzi, M.A., Mohammadi, N., *et al.*, 2010. Evaluation of antioxidant properties and total phenolic contents of some strains of microalgae. *J. Appl. Phycol.*, **22**(1):43-50. http://dx.doi.org/10.1007/s10811-009-9424-y

Hillig, F., Pilarek, M., Junne, S., *et al*., 2013. Cultivation of marine microorganisms in single-use systems. *In*: Eibl, D., Eibl, R. (Eds.), Disposable Bioreactors II. Advances in Biochemical Engineering/Biotechnology, Vol. 138. Springer, Berlin, Heidelberg, p.179-206. http://dx.doi.org/10.1007/10_2013_219

Kasala, E.R., Bodduluru, L.N., Barua, C.C., *et al.*, 2016. Antioxidant and antitumor efficacy of Luteolin, a dietary flavone on benzo(a)pyrene-induced experimental lung carcinogenesis. *Biomed. Pharmacother.*, **82**:568-577. http://dx.doi.org/10.1016/j.biopha.2016.05.042

Khozin-Goldberg, I., Leu, S., Boussiba, S., 2016. Microalgae as a source for VLC-PUFA production. *In*: Nakamura, Y., Li-Beisson, Y. (Eds.), Lipids in Plant and Algae Development. Subcellular Biochemistry, Vol. 86. Springer, Cham, p.471-510. http://dx.doi.org/10.1007/978-3-319-25979-6_19

Lewis, K.D., Huang, W.F., Zhang, X.H., *et al.*, 2016. Toxicological evaluation of arachidonic acid (ARA)-rich oil and docosahexaenoic acid (DHA)-rich oil. *Food Chem. Toxicol*., **96**(25):133-144. http://dx.doi.org/10.1016/j.fct.2016.07.026

Li, H.B., Cheng, K.W., Wong, C.C., *et al.*, 2007. Evaluation of antioxidant capacity and total phenolic content of different fractions of selected microalgae. *Food Chem.*, **102**(3):771-776. http://dx.doi.org/10.1016/j.foodchem.2006.06.022

Li, M.H., Robinson, E.H., Tucke, C.S., *et al.*, 2009. Effects of dried algae *Schizochytrium* sp., a rich source of docosahexaenoic acid, on growth, fatty acid composition, and sensory quality of channel catfish *Ictalurus punctatus*. *Aquaculture*, **292**(3-4):232-236. http://dx.doi.org/10.1016/j.aquaculture.2009.04.033

Li, N., Shi, J., Wang, K., 2014. Profile and antioxidant activity of phenolic extracts from 10 crabapples (*Malus* wild species). *J. Agric. Food Chem*., **62**(3):574-581. http://dx.doi.org/10.1021/jf404542d

Ling, X., Guo, J., Liu, X., *et al.*, 2015. Impact of carbon and nitrogen feeding strategy on high production of biomass and docosahexaenoic acid (DHA) by *Schizochytrium* sp. LU310. *Bioresour. Technol.*, **184**:139-147. http://dx.doi.org/10.1016/j.biortech.2014.09.130

Lippmeier, J.C., Crawford, K.S., Owen, C.B., *et al.*, 2009. Characterization of both polyunsaturated fatty acid biosynthetic pathways in *Schizochytrium* sp. *Lipids*, **44**(7):621-630. http://dx.doi.org/10.1007/s11745-009-3311-9

López-Alarcón, C., Denicola, A., 2013. Evaluating the antioxidant capacity of natural products: a review on chemical and cellular-based assays. *Anal. Chim. Acta*, **763**:1-10. http://dx.doi.org/10.1016/j.aca.2012.11.051

Luo, X., Su, P., Zhang, W., 2015. Advances in microalgae-derived phytosterols for functional food and pharmaceutical applications. *Mar. Drugs*, **13**(7):4231-4254. http://dx.doi.org/10.3390/md13074231

Lv, J.W., Yang, X.Q., Li, L.H., 2014. Antioxidant activity and chemical constituents of microalgae oil of *Schizochytrium aggregatum*. *Adv. Mater. Res.*, **919-921**:2022-2029. http://dx.doi.org/10.4028/www.scientific.net/AMR.919-921.2022

Narwal, S., Thakur, V., Sheoran, S., *et al.*, 2014. Antioxidant activity and phenolic content of the Indian wheat varieties. *J. Plant Biochem. Biotechnol.*, **23**(1):11-17. http://dx.doi.org/10.1007/s13562-012-0179-1

O'Brien, P., Carrasco-Pozo, C., Speisky, H., 2006. Boldine and its antioxidant or health-promoting properties. *Chem.-Biol. Interact*., **159**(1):1-17. http://dx.doi.org/10.1016/j.cbi.2005.09.002

Pan, Y., Zhu, J., Wang, H., *et al.*, 2007. Antioxidant activity of ethanolic extract of *Cortex fraxini* and use in peanut oil. *Food Chem*., **103**(3):913-918. http://dx.doi.org/10.1016/j.foodchem.2006.09.044

Pleissner, D., Eriksen, N.T., 2012. Effects of phosphorous, nitrogen, and carbon limitation on biomass composition in batch and continuous flow cultures of the heterotrophic dinoflagellate *Crypthecodinium cohnii*. *Biotechnol. Bioeng*., **109**(8):2005-2016. http://dx.doi.org/10.1002/bit.24470

Podsędek, A., 2007. Natural antioxidants and antioxidant capacity of Brassica vegetables: a review. *LWT-Food Sci. Technol*., **40**(1):1-11. http://dx.doi.org/10.1016/j.lwt.2005.07.023

Saeed, N., Khan, M.R., Shabbir, M., 2012. Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts *Torilis leptophylla* L. *BMC Complement. Altern. Med.*, **12**(1):221. http://dx.doi.org/10.1186/1472-6882-12-221

Safafar, H., van Wagenen, J., Moller, P., *et al.*, 2015. Carotenoids, phenolic compounds and tocopherols contribute to the antioxidative properties of some microalgae species grown on industrial wastewater. *Mar. Drugs*, **13**(12):7339-7356. http://dx.doi.org/10.3390/md13127069

Salem, N.Jr., Eggersdorfer, M., 2015. Is the world supply of

omega-3 fatty acids adequate for optimal human nutrition? *Curr. Opin. Clin. Nutr. Metab. Care*, **18**(2):147-154. http://dx.doi.org/10.1097/MCO.0000000000000145

Samaranayaka, A.G., Li-Chan, E.C., 2011. Food-derived peptidic antioxidants: a review of their production, assessment, and potential applications. *J. Funct. Foods*, **3**(4):229-254. http://dx.doi.org/10.1016/j.jff.2011.05.006

Schiavone, A., Chiarini, R., Marzoni, M., *et al.*, 2007. Breast meat traits of Muscovy ducks fed on a microalga (*Crypthecodinium cohnii*) meal supplemented diet. *Brit. Poultry Sci*., **48**(5):573-579. http://dx.doi.org/10.1080/00071660701615796

Singh, P., Baranwal, M., Reddy, S.M., 2016. Antioxidant and cytotoxic activity of carotenes produced by *Dunaliella salina* under stress. *Pharm. Biol*., **54**(10):2269-2275. http://dx.doi.org/10.3109/13880209.2016.1153660

Sugamura, K., Keaney, J.F.Jr., 2011. Reactive oxygen species in cardiovascular disease. *Free Radic. Biol. Med.*, **51**(5):978-992. http://dx.doi.org/10.1016/j.freeradbiomed.2011.05.004

Venuste, M., Zhang, X., Shoemaker, C.F., *et al*., 2013. Influence of enzymatic hydrolysis and enzyme type on the nutritional and antioxidant properties of pumpkin meal hydrolysates. *Food Funct.*, **4**(5):811-820. http://dx.doi.org/10.1039/c3fo30347k

Wu, J.Q., Kosten, T.R., Zhang, X.Y., 2013. Free radicals, antioxidant defense systems, and schizophrenia. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry*, **46**(1):200-206. http://dx.doi.org/10.1016/j.pnpbp.2013.02.015

Wu, X., Wu, F., Tong, X., *et al.*, 2013. Emergy-based sustainability assessment of an integrated production system of cattle, biogas, and greenhouse vegetables: insight into the comprehensive utilization of wastes on a large-scale farm in Northwest China. *Ecol. Eng*., **61**(Part A):335-344. http://dx.doi.org/10.1016/j.ecoleng.2013.09.060

Ying, L., Kong, D., Gao, Y.Y., *et al*., 2017. In vitro antioxidant activity of phenolic-enriched extracts from Zhangping Narcissus tea cake and their inhibition on growth and metastatic capacity of 4T1 murine breast cancer cells. *J. Zhejiang Univ.-Sci. B (Biomed. & Biotechnol.)*, in press. http://dx.doi.org/10.1631/jzus.B1700162

Zeng, W.C., Zhang, Z., Jia, L.R., 2014. Antioxidant activity and characterization of antioxidant polysaccharides from pine needle (*Cedrus deodara*). *Carbohydr. Polym.*, **108**:58-64. http://dx.doi.org/10.1016/j.carbpol.2014.03.022

中文概要

题　目：隐甲藻和裂殖壶藻醇水提物不同极性萃取物的抗氧化活性研究

目　的：评价隐甲藻和裂殖壶藻醇水提物的石油醚萃取相、乙酸乙酯萃取相、正丁醇萃取相和水相的抗氧化活性水平，为综合利用两种微藻提供理论依据。

创新点：隐甲藻和裂殖壶藻是二十二碳六烯酸（DHA）的重要原料，然而提取 DHA 后剩余的藻渣未得到充分利用。本文首次对两种微藻的极性提取物的不同极性部位进行抗氧化活性评价，并初步确定多酚类化合物是其发挥抗氧化作用的主要物质成分，为综合利用两种微藻提供了理论依据和参考。

方　法：用 70%乙醇浸提隐甲藻和裂殖壶藻藻粉，所得粗提物分别使用石油醚、乙酸乙酯、正丁醇和水依次萃取，得到不同极性组分萃取物。采用总抗氧化能力、2,2-二苯基-1-三硝基苯肼（DPPH）自由基清除能力、亚铁离子螯合能力及总还原力等方法对不同极性组分的萃取物进行抗氧化活性评价。采用 Folin-Ciocalteu 方法测定样品中总多酚含量，采用分光光度法测定样品中总黄酮含量。

结　论：本实验结果显示，隐甲藻和裂殖壶藻醇水提物的不同极性组分具有较好的抗氧化作用（图 2~5）。相关性分析结果表明萃取物中的多酚类化合物与其抗氧化水平显著相关（表 2 和表 3），因此，我们推测多酚类化合物是两种微藻的主要抗氧化成分。综上所述，隐甲藻和裂殖壶藻具有综合开发利用潜力，可深入研究。

关键词：隐甲藻；裂殖壶藻；醇水提物；抗氧化；总多酚含量