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Free radical-scavenging activity of *Aloe vera* (*Aloe barbadensis* Miller) extracts by supercritical carbon dioxide extraction

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Abstract

The free radical-scavenging activities of extracts of *Aloe vera* of leaf skin by supercritical CO_2 extraction and solvent extraction were determined. An orthogonal array design matrix of L_9 (3^4) was considered to optimize supercritical carbon dioxide extraction processing at a CO_2 flow rate of 12–36 l h^{-1}, 35–45 MPa and 32–50 °C. The optimum extracted yield of 1.47% was provided at 50 °C 36 l h^{-1}, 35 MPa and 20% of modifier of methanol. These four factors were all demonstrated to be significantly crucial in the supercritical carbon dioxide extraction operation, as two-variable interactions. The extracts of *A. vera* rind by supercritical carbon dioxide extraction and solvent extracts provided significantly higher free radical-scavenging activities of 33.5% and 39.7%, respectively, than extracts of *A. vera* gel extracted by ethanol with a free radical-scavenging activity of 14.2%. The inhibition percentage of extracts of *A. vera* and reference antioxidants followed the decreasing order: Trolox (76.8%) > ethanol extracts of *A. vera* skin (39.7%) > BHT (35.9%) > the extract of supercritical carbon dioxide extraction (33.5%) > α-tocopherol (25.6%) > ethanol extracts of *A. vera* pulp (14.2%). Compared to BHT and α-tocopherol, the extracts of *A. vera* skin, by supercritical carbon dioxide extraction and ethanol, showed stronger antioxidant activities. Components in the rind of *A. vera* are responsible for the higher antioxidant activity of *A. vera* extracts.
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Keywords: Extracts of *Aloe vera*; Antioxidant activity; Supercritical CO_2 extraction

1. Introduction

Growing apprehensiveness about the safety of synthetic commercial antioxidants has prompted great efforts to screen active and stable antioxidants obtained from natural sources. *Aloe vera* leaves, which are used traditionally for their therapeutic properties, have been studied for their potential antioxidant activity (Reynolds & Dweck, 1999). In the previous research from our laboratory, the growth period played a vital role in the composition and antioxidant ability of ethanol extracts derived from *A. vera* leaf (Hu, Xu, & Hu, 2003). In contrast to the freeze-dried whole leaf powder and freeze-dried leaf skin powder, the boiled leaf skin powder displayed a stronger level of DPPH Trolox radical-scavenging activity (Beppu et al., 2003). Furthermore, various highly polar and thermally unstable compounds have been isolated from the organic extracts of *A. vera* leaf and their obvious antioxidant capacity has been confirmed by some in vitro experiments (Lee, Weintraub, & Yu, 2000).

Nevertheless, attention should be paid to the inevitable defects of solvent extraction, such as toxity, harm to the environment and persistance. By contrast, the supercritical CO_2 extraction technique is considered as

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non-toxic, free of residues, moderate and comparatively environmentally harmless among liquid–solid extraction operations. Because of its peculiarity, supercritical CO_2 extraction has been widely performed to extract plant-oil, pesticides and natural antioxidants. A high yield of α-tocopherol extraction (97.1%) from olive tree was obtained by supercritical CO_2 extraction under optimal operative conditions (de Lucas, Martinez de la Ossa, Rincón, Blanco, & Gracia, 2002). A greatly enhanced recovery of carbamates was observed by adding a small volume (10%) of methanol to CO_2 in an orthogonal array design (Sun & Lee, 2003). An antioxidant rosemary extract, with minimum rosemary aroma and colour was produced from rosemary leaves by supercritical CO_2 extraction, based on the Taguchi experimental design (López-Sebastián et al., 1998).

It is of great interest to discover whether supercritical CO_2 extraction can effectively increase the antioxidant activity of *A. vera* extracts compared to conventional solvent methods. The purpose of this study was to evaluate and compare free radical-scavenging activities of *A. vera* extracts by optimized supercritical CO_2 extraction with solvent extraction and reference antioxidants.

2. Materials and methods

2.1. Reagents

The following chemicals were used: α,α-diphenyl-β-picrylhydrazyl (DPPH); α-tocopherol (Sigma Chemicals Co., St. Louis, MO, USA); butylated hydroxytoluene (BHT) (Nanjing Chemical Industry, Nanjing, China); (R)-(+)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) (Aldrich Co., Milwaukee, WI, USA); 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid ammonium salt) (ABTS) and 2,2′-azobis-(2-amidino-propane) dihydro-chloride (AAPH) (Wako Pure Chemical Industries, Ltd., Osaka, Japan.). All other chemicals used were of analytical grade.

2.2. Plant material

Four-year old *A. barbadensis* Miller was supplied by the Institute of New Century Horticulture of Nanjing, Jiangsu Province. Immediately after harvest, the outer layer and inner gel of whole *A. vera* leaves were immediately cut apart, minced and then lyophilized in vacuo for 36 h. After fragmentation, the lyophilized powder of aloatic epidermis and pulp were stored frozen prior to further use.

2.3. Supercritical CO_2 extraction of *A. vera*

Extractions were conducted in 1l stainless steel vessels with a Hua'an supercritical fluid extractor (Hua'an Corp., Nantong, China). On the basis of a previous study of supercritical fluid extraction, pressure, temperature, CO_2 flow rate and modifier were four important factors affecting the extraction yields (Hailong, Chun, Xianyi, Zhengliang, & Ceng, 1999; Kuibin et al., 1999; Songgang et al., 1998). The parameters considered in optimization of the supercritical fluid extraction conditions are shown in Table 1. About 45 g of lyophilized aloatic epidermis powder were packed into the extraction cell. CO_2, pure or premixed with 10% or 20% methanol (v/w), was used as the extraction fluid. The extraction process started after the extraction vessel attained working conditions. After equilibration for 15 min, the supercritical fluid was passed through the extraction cell containing the sample. Extracts obtained from two separators were combined and evaporated at 40 °C under reduced pressure. After being weighed, the residue was diluted with the mixed solvent, of acetone and ethanol (7:3), for further antioxidant assay.

Table 1
Main variables and their level settings considered in the supercritical CO_2 extraction optimization process

Level setting	Main variables			
	Extraction pressure *A* (MPa)	Extraction temperature *B* (°C)	Flow rate of CO_2 *C* ($l\,h^{-1}$)	Modifier *D* (%)
I	35	32	12	0
II	40	41	24	10
III	45	50	36	20

2.4. Preparation of Aloatic epidermis extracts by solvent extraction

Two 5 g lots of lyophilized powder of aloatic epidermis were extracted with 200 ml ethanol and hexane, respectively, sonicated three times at room temperature for 60 min, and then filtered. Evaporation of the filtrate under reduced pressure at 40 °C gave the ethanol extracts (0.866 g; 17.3%) and the hexane extracts (0.0766 g; 1.53%), respectively. Two (about 1 g) samples of lyophilized powder of leaf pulp were subjected to the same (above) procedures and the extraction yields were 23.6% and 0.7% for ethanol and hexane, respectively. All these extracts were diluted with solvents (ethanol or hexane) before their antioxidant capacity assay.

2.5. The dispensing of *A. vera* extracts and reference antioxidant

The final concentration of all samples in solvents was 2.6 mg ml^{-1} when used for evaluation of their ability to scavenge free radicals. Trolox was diluted in distilled water while BHT and α-tocopherol were in anhydroethanol at the same concentrations.

2.6. Assay of ABTS radical anion scavenging activity of A. vera extracts

The ABTS assay of free radical-scavenging capacity of *A. vera* extracts was performed according to Kim, Lee, Lee, and Lee (2002). 1.0 mM AAPH was mixed with 2.5 mM ABTS as the diammonium salt in phosphate buffered saline (PBS) solution (100 mM potassium phosphate butter (pH 7.4) containing 150 mM NaCl). The mixture was diluted to an absorbance of 0.65 ± 0.02 at wavelength of 734 nm and pre-incubated at 68 °C for 13 min. 1.0 mM trolox was prepared in PBS for use as a stock standard. Fresh working standards and ABTS/AAPH stock solution were prepared daily. The sample solution of 60 μl was added to the radical solution of 2940 μl. The mixture was incubated in a water bath at 37 °C for 10 min and kept from light. The absorbance at 734 nm was measured at the endpoint of 10 min and was used for calculating the trolox equivalent radical-scavenging activity. The blanks (the decrease in absorption of solvents without the compound added) for each measurement were recorded. Trolox equivalent antioxidant capacity value of each compound was calculated according to a trolox dose–response curve.

2.7. Assay of DPPH radical-scavenging activity of A. vera extracts

The DPPH scavenging activity of *A. vera* extracts was determined by the method of von Gadow, Joubert, and Hansmann (1997). The DPPH was prepared in absolute ethanol and the concentration of the resulting solution was 6×10^{-5} M. The stock solution was kept in the refrigerator. The sample solution of 0.1 ml was added to 29 ml of ethanolic DPPH solution. The mixture was shaken vigorously and the absorbance was monitored at 517 nm immediately, until the steady state was reached. A blank test without any compounds was conducted. The inhibition of the DPPH radical by the sample was calculated according to the formula of Yen and Duh (1994).

2.8. Statistics analysis

Each data point was presented as mean ± standard deviation ($n = 3$) and the significance of differences among treatment means was done by one-way analysis of variance (ANOVA), followed by the *t*-test (LSD) at the $p < 0.05$ level. All computations were made by employing the statistical software SAS system for windows V8.

3. Results and discussion

3.1. Analysis for extraction yield of supercritical CO_2 extraction optimization

An orthogonal array design, L_9 (3^4), was performed to optimize the extract temperature, pressure, CO_2 flow rate and modifier. Table 2 shows the results of extraction yield obtained under the experimental conditions tested. Analyses on the orthogonal array design indicated that variable *B*, namely extraction temperature, had the highest *R* value of 0.57 and exerted the largest effect on extraction field. The extent of the impact of variables on extraction yields followed the order: variable *B* (extraction temperature) < *C* (flow rate) < *A* (extraction pressure) < *D* (modifier). We also concluded that extraction temperature and pressure were the two major factors affecting extraction yield. The optimum extraction yield was provided at 50 °C (*B*), 36 $\mathrm{l\,h^{-1}}$ (*C*), 35 MPa (*A*) and 20% modifier (*D*).

Table 2
Results obtained under the experimental conditions using L_9 (3^4) orthogonal array design

Run	Extraction pressure	Extraction temperature	Flow rate of CO_2	Modifier	Extraction yield (%)
1	I	I	I	I	0.15
2	I	II	II	II	0.41
3	I	III	III	III	1.5
4	II	I	II	III	0.09
5	II	II	III	I	0.46
6	II	III	I	II	0.18
7	III	I	III	II	0.13
8	III	II	I	III	0.13
9	III	III	II	I	0.43
I[a]	0.68	0.12	0.15	0.35	
II[a]	0.24	0.33	0.31	0.24	
III[a]	0.23	0.69	0.69	0.56	
R value[b]	0.45	0.57	0.54	0.32	

[a] Average response of each level about extraction yield.
[b] *R* value means average range between three average responses of each level about extraction yields.

Extraction yields increased with the increasing temperature at the same pressure except at 40 MPa. This result has been previously reported by (Eggers, 1996; Lucas et al., 2002; Oghaki, Tsukahara, Semba, & Katayama, 1989).According to the basic principles of supercritical fluid extraction, however, the higher the temperature, the lower is the density and hence solvent strength of the fluid, and the influences of extraction pressure and flow rate on yield are very complicated. On average, the extraction yield increased with the increasing flow rate, which could be explained by assuming that extraction yield is limited by diffusion of the analyte from the matrix to the bulk fluid when threshold pressure is reached. For this reason, a further increase in extraction pressure, beyond the threshold point, results in a higher viscosity of the fluid and a lower diffusion coefficient and therefore lower extraction yield (Oghaki et al., 1989). However, the modifier seems to have little effect on the extraction yield in this study. This result was not in accord with the reports that methanol could increase the extraction power of non-polar CO_2 (Cháfer, Berna, Montón, & Muñoz, 2002; Eggers, 1996; Sun & Lee, 2003).

3.2. Evaluation of antioxidant activity of extracts by optimized supercritical CO_2 extraction

Table 3 displays the effects of variables on the antioxidant activity of SC-CO_2 extracts. Variable *A* (extraction pressure), variable *B* (extraction temperature), variable *C* (flow rate of CO_2), variable *D* (modifier) and six two-variable interactions are all statistically significant at $p < 0.01$. This result suggested that, both, main variables alone and the interactions, which are not considered in supercritical CO_2 extraction optimization systems may noticeably affect the extraction of antioxidant components derived from *A. vera*.

The extents of effect of variable on extract antioxidant activities followed in the order: Variable *C* (flow rate) > *D* (modifier) > *A* (extraction pressure) > *B* (extraction temperature).The SC-CO_2 extract with the highest antioxidant activity will be obtained at 12 l h^{-1}, 24% of modifier, 45 MPa and 32 °C. Generally, the antioxidant activities of extract increased with the growing extraction pressure while temperature had an adverse effect on the extract antioxidant activities. This can be explained in that higher pressure contributed to the diffusion of polar components which showed the most active antioxidant ability among *A. vera* extracts. Moreover, the tendency that the antioxidant activity was enhanced with decrease of extraction temperature suggested a heat-sensitive property of these polar antioxidant extracts. However, the non-collinear changes of the average response about main factors *C* and *D* may be explained by considering the statistically significant two-variable interactions among main factors (Table 4).

3.3. Antioxidant activity of *A. vera* exudates by the ultrasonic extraction

The radical-scavenging activities of organic extracts of *A. vera Exudates* were also determined using the $ABTS^{\cdot +}$ method and DPPH radical-scavenging method (Table 5). Extracts of leaf skin of *A. vera* had a significantly higher radical-scavenging capacity than those from the pulp extracted either with ethanol or with hexane. This suggested that the more active free radical scavengers were in the skin. On the other hand, the significantly higher levels of antioxidant activity in ethanol

Table 3
The radical-scavenging activities of extracts of supercritical CO_2 extraction of *A. vera* under the experimental condition using L_9 (3^4) orthogonal array design by DPPH assay

Run	*A*	*B*	*C*	*D*	Inhibition[A](%)
1	I	I	I	I	13.4 ± 0.84[d]
2	I	II	II	II	8.89 ± 0.32[f]
3	I	III	III	III	10.3 ± 0.23[e]
4	II	I	II	III	10.2 ± 0.77[e]
5	II	II	III	I	10.7 ± 0.32[e]
6	II	III	I	II	23.2 ± 0.16[c]
7	III	I	III	II	33.5 ± 0.25[a]
8	III	II	I	III	27.3 ± 0.74[b]
9	III	III	II	I	2.79 ± 0.08[g]
I[a]	10.9	19.0	21.3	8.97	
II[a]	14.7	15.6	7.29	21.9	
III[a]	21.2	12.1	18.2	15.9	
R value[b]	10.32	6.93	14.01	12.90	

[A] Values are means of three determinations ± SD. Values followed by different letters are different ($P < 0.05$) from one another.
[a] Average response of each level about inhibition percentage using DPPH assay.
[b] *R* value means average range between three average responses of each level about the radical-scavenging activities of extracts.

Table 4
ANOVA table including inhibition percentage for the antioxidant capacity evaluation using the DPPH assay in the L_9 (3^4) matrix

Parameter	*A*	*B*	*C*	*D*	*A***B*	*A***C*	*A***D*	*B***C*	*B***D*	*C***D*
SS	488.36	216.00	972.55	749.46	1722.01	965.46	1188.55	1237.82	146.91	704. 36
df	2	2	2	2	4	4	4	4	4	4
MS	244.18	108.00	486.28	374.73	430.50	241.37	297.14	309.46	365.23	176. 09
F-value	995.0	440.09	1981.53	1526.99	1754.26	983.54	1210.81	1261.00	1288. 27	717.55
P-value	<0.0001**	<0.0001**	<0.0001**	<0.0001**	<0.0001**	<0.0001**	<0.0001**	<0.0001**	<0.0001**	<0.0001**

Table 5
The free radical-scavenging activities of extracts of leaf skin and gel of *A. vera* by methods of DPPH and ABTS assay

Samples	Extraction method	Inhibition[A] (%)	Radical-scavenging activity (mM trolox equivalents mg^{-1})[B]
Extracts of leaf skin of *A. vera*	Ethanol	39.7 ± 0.07[a]	2.60 ± 0.32[a]
	Hexane	26.4 ± 0.14[b]	1.13 ± 0.12[b]
Extracts of leaf gel of *A. vera*	Ethanol	14.2 ± 0.42[c]	1.08 ± 0.02[b]
	Hexane	5.18 ± 0.32[d]	0.50 ± 0.01[c]

[A] Inhibition is the inhibition percentage counted at 30th minute by the DPPH assy.
[B] The radical-scavenging activity was evaluated by the ABTS method.
[a] Values are means of three determinations ± SD. Values followed by different letters are different ($P < 0.05$) from one another.

extracts of *A. vera* indicated the polar nature of these compounds. The same conclusion could be drawn from the apparently lowest values of both inhibition percentage and the trolox equivalent antioxidant activity of the hexane exudates of *A. vera* mesophyll.

3.4. Comparison of antioxidant activities of Aloe extracts and reference antioxidants

Table 6 shows comparisons of antioxidant activities between SC-CO_2 extracts, solvent extracts and reference antioxidants. The inhibition percentage of *Aloe* extracts and commercial antioxidants followed the decreasing order: Trolox (76.8%) > ethanol extracts of *A. vera* skin (39.7%) > BHT (35.9%) > the extract of No. 7 sample of supercritical CO_2 extraction (33.5%) > α-tocopherol (25.6%) > ethanol extracts of *A. vera* pulp (14.2%). It can be noted that the green rind exudates obtained by supercritical CO_2 extraction and ultrasonic extraction displayed significantly higher levels of free radical-scavenging activity than those obtained by ethanol. Compared to the commercial antioxidants, both ethanol and supercritical CO_2 extraction extracts derived from skin showed apparently stronger levels of antioxidant capacity than α-tocopherol and the activity of ethanol exudates was even greater than that of BHT. Significances were also observed among the values of the percentage inhibitions of all the samples.

Table 6
The free radical-scavenging activities of *A. vera* extracts and reference antioxidants by the DPPH assay

Reference	Inhibition (%)	*A. vera* extracts	Inhibition (%)
Trolox	76.8 ± 0.24[a]	Ethanol extracts of *A. vera* skin	39.7 ± 0.07[b]
BHT	35.9 ± 0.90[c]	Ethanol extracts of *A. vera* pulp	14.2 ± 0.42[f]
α-tocopherol	25.6 ± 1.32[e]	Extracts of supercritical CO_2 extraction of *A. vera* skin	33.5 ± 0.25[d]

[a] Values are means of three determinations ± SD. Values followed by different letters are different ($P < 0.05$) from one another.

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