Food Control 30 (2013) 48–53

Contents lists available at SciVerse ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Antimicrobial and antioxidant activities of the essential oil from onion (*Allium cepa* L.)

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ARTICLE INFO

Article history:
Received 9 April 2012
Received in revised form
13 July 2012
Accepted 21 July 2012

Keywords:
Allium cepa
Essential oil
Antioxidant activity
Antimicrobial activity

ABSTRACT

The aims of this study were to test the efficacy of essential oil of *Allium cepa* against food spoilage and food-borne pathogenic microorganisms and its antioxidant activity. The essential oil revealed an interesting antimicrobial effect against the tested microorganisms with the MIC and MBC values in the ranges of 0.18–1.80 mg/mL and 0.54–3.6 mg/mL, respectively. The antioxidant activities of the essential oil were investigated and the oil showed moderate antioxidant activities in ABTS assay (0.67 mg/mL as IC_{50} value), DPPH test (IC_{50} value = 0.63 mg/mL) and metal chelating assay (IC_{50} value of 0.51 mg/mL). Furthermore, the reducing power of the oil was dose dependent, and the reducing capacity of the oil was inferior to butylated hydroxytoluene, which is known to be a strong reducing agent. It was suggested that the essential oil from *A. cepa* may be a new potential source as natural antimicrobial and antioxidant agents applied in food systems.

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1. Introduction

Lipid peroxidation and microbial contamination in foods not only result in food deterioration and shelf life reduction, but also lead to disease and economic losses. At present, the food industry is facing an enormous pressure coming from food deterioration caused by the two factors above. The growth of microorganisms in food products may cause intestinal disorders, vomiting and diarrhoea (Friedman, Henika, & Mandrell, 2002). It has been reported that as many as 30% of people in industrialized countries suffer from a food-borne disease each year. With the environment pollution, more population will suffer from food-borne diseases (Loizzo et al., 2010).

Adding antioxidants is an effective means for storing food to slow down oxidation of food or even deter corruption (Stich, 1991). The use of synthetic antioxidants is restricted because of their carcinogenicity (Guyton et al., 1991; Kimmel, Kimmel, & Frankos, 1986). Thus, there has been increasing interest in finding natural, effective, and safe antioxidants, since they can protect the human body from free radicals and retard the progress of many chronic diseases (Kinsella, Frankel, German, & Kanner, 1993; Nandita & Rajini, 2004). Antioxidants that retard the oxidation process may additionally exhibit antimicrobial activity (Cutter, 2000; Puupponen-Pimiä et al., 2001). The antimicrobial compounds found in plants are of interest because antibiotic resistance is becoming a worldwide public health concern especially in terms of food-borne illness and nosocomial infections (Hsueh, Chen, Teng, & Luh, 2005; Mora et al., 2005). One such possibility is the use of essential oils as antioxidant and antimicrobial additives. So far, a variety of studies had been carried out to evaluate the antimicrobial and antioxidant activities of essential oils, and the results indicated that essential oil did have noticeable antimicrobial and antioxidant activities (Burt, 2004; Castilho, Savluchinske-Feio, Weinhold, & Gouveia, 2012; Sacchetti et al., 2005). Thus, essential oils isolated from plants are a potential source of food additives in the food industry.

Onions (*Allium cepa* L.) possess strong, characteristic aromas and flavours, which have made them important ingredients of food. Onions and onion flavours (essential oil) are important seasonings widely used in food processing. Recent research has demonstrated that onions possess several biological properties, such as antibacterial (Griffiths, Trueman, Crowther, Thomas, & Smith, 2002), antimutagenic (Singh et al., 2009) and antioxidant activities (Dini, Tenore, & Dini, 2008). The medicinally most significant components of onion oil are the organosulfur-containing compounds (Dron, Guyeru, Gage, & Lira, 1997; Sinha, Guyer, Gage, & Lira, 1992). These compounds are reactive, volatile, odour producing and lachrymatory (Block, Naganathan, Putman, & Zhao, 1992). To the best of our knowledge, the antimicrobial activity of onion oil against a wide range of food-associated microorganisms (bacteria, moulds, and yeasts) and its potential antioxidative properties have not been studied. The aim of this study was to determine the

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http://dx.doi.org/10.1016/j.foodcont.2012.07.033

antimicrobial activity of the essential oil, extracted by supercritical CO_2 extraction, against food-associated microorganisms, and to evaluate its antioxidative activities on ABTS radicals, DPPH radicals, metal chelating and reducing power.

2. Materials and methods

2.1. Chemicals

The essential oil was extracted by supercritical CO_2 extraction in our lab as described by Ye and Lai (2012). Chemical composition of the oil was analysed by GC–MS and the most representative compounds of the essential oil were organosulfur-containing compounds, such as methyl 5-methylfuryl sulfide (18.30%), methyl 3,4-dimethyl-2-thienyl disulfide (11.75%) and 1-propenyl propyl disulfide (9.72%) (Fig. 1). 1,1-Diphenyl-2-picrylhydrazyl (DPPH) was purchased from Fluka Biochemika AG (Buchs, Switzerland). 2, 2′-Azino-bis (3-ethylbenzthiazoline-6-sulfonic) acid (ABTS), Butylated hydroxytoluene (BHT) and ascorbic acid (Vc) were purchased from Sigma (St Louis, MO, USA). All other chemicals were of analytical grade.

2.2. The tested microorganisms

The following food spoilage and food-borne microorganisms including *Escherichia coli* ATCC 25922, *Bacillus subtilis* ATCC 21216, *Staphylococcus aureus* ATCC 25923, *Rhodotorula glutinis* ATCC 16740, *Saccharomyces cerevisiae* ATCC 9763, *Candida tropicalis* ATCC 13801, *Aspergillus niger* ATCC 16404, *Monascus purpureus* ATCC 36928, and *Aspergillus terreus* ATCC 20542, were used as the tested microorganisms. Bacteria, yeasts and moulds were maintained on nutrient agar (NA), yeast extract peptone dextrose agar (YPD) and potato dextrose agar (PDA) culture medium at 4 °C, respectively. All the tested microorganisms were sub-cultured on appropriate medium at 37 °C (yeasts and moulds at 28 °C) for 24 h.

2.3. In vitro antimicrobial assay

2.3.1. Inhibitory zone assay

The essential oil was diluted with sterile water to a final concentration of 150 mg/mL and sterilized by filtrating through 0.22 μm Millipore filters. The determination of the inhibitory effect of the essential oil on test microorganisms was carried out by Oxford plate method (Beverlya, Janes, Prinyawiwatkula, & No, 2008). In brief, all the microbial cultures (sub-cultured before assay) were diluted with sterile water to obtain a microbial suspension of 10^6 CFU/mL. Petri plates containing 20 mL of culture medium were inoculated with 200 μL of microbial suspension and allowed to dry in sterile chamber. The Oxford plates (6 mm in diameter) were impregnated with 100 μL of 150 mg/mL sample and placed on the inoculated culture medium. The sterile water was also used as the negative control. Norfloxacin (100 μg/disk) was used as positive control for bacteria and yeasts. Miconazole Nitrate (100 μg/disk) was used as positive control for moulds. The plates of bacteria inoculated were incubated at 37 °C for 52 h, while moulds at 28 °C for 52 h, and yeasts at 28 °C for 48 h. The antimicrobial activity was evaluated by measuring the inhibition zone against the tested microorganisms.

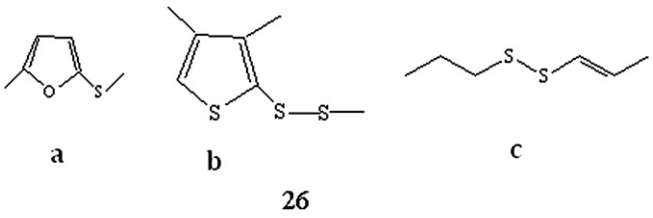

Fig. 1. Structures of major bioactive compounds of the essential oil from *Allium cepa*. (a) methyl 5-methylfuryl sulfide; (b) methyl 3,4-dimethyl-2-thienyl disulfide; and (c) 1-propenyl propyl disulfide.

2.3.2. Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) was determined by broth dilution method (Barry, 1976). Each strain was tested with sample that was serially diluted in broth to obtain concentrations ranging from 0.09 to 9 mg/mL. The sample, previously sterilized with Millipore filter of 0.20 μm, was stirred and inoculated with 50 μL suspension of 10^6 CFU/mL of the tested microorganisms, and incubated for 24 h at 37 °C for bacteria, at 28 °C for yeasts and moulds. Another culture medium without adding microorganisms suspension was prepared as the negative control. The MIC value was determined as the lowest concentration of the sample at which the tested microorganisms did not demonstrate any visible growth after incubation.

2.3.3. Determination of minimum microbicidal concentration (MMC)

The minimum microbicidal concentration (MMC), which includes minimum bactericidal (MBC) and minimum fungicidal concentrations (MFC), of the essential oil was determined according to the MIC values. The samples showing no increases in turbidity were streaked on nutrient agar medium and incubated for 24 h at 37 °C for bacteria, 28 h at 28 °C for yeasts and 24 h at 28 °C for moulds. The lowest concentration in the medium which had fewer than five colonies was taken as the minimum microbicidal concentration (MMC) (Li et al., 2012; Tenore et al., 2011).

2.4. In vitro antioxidant assay

2.4.1. ABTS radical scavenging assay

The radicals scavenging activity of the essential oil against radical cation ($ABTS^+$) was estimated according to the previously reported procedure with some modifications (Re et al., 1999). $ABTS^+$ was produced by reacting 7 mmol/L of $ABTS^+$ solution with 2.45 mmol/L of potassium persulphate, and the mixture would be kept in the dark at room temperature for 16 h. In the moment of use, the $ABTS^+$ solution was diluted with ethanol to an absorbance of 0.70 ± 0.02 at 734 nm. Each sample (0.2 mL) with various concentrations (0.2–1.0 mg/mL) were added to 2 mL of $ABTS^+$ solution and mixed vigorously. After reaction at room temperature for 6 min, the absorbance at 734 nm was measured. The $ABTS^+$ scavenging effect was calculated by the following formula:

$$\text{scavenging effect}(\%) = \left(1 - \frac{A_{\text{sample}}}{A_{\text{control}}}\right) \times 100\%$$

where A_{control} is the absorbance of control without sample and A_{sample} is the test sample with $ABTS^+$.

2.4.2. Radical scavenging activities on DPPH

The free radical scavenging activity of the essential oil was measured using DPPH by the method of Blois (Blois, 1958). A 0.1 mM solution of DPPH in methanol was prepared and 1 mL of this solution was added to 3 mL of various concentrations (0.2–1.0 mg/mL) of sample dissolved in methanol to be tested. After 30 min, absorbance was measured at 517 nm. Vc was used as a reference material. All tests were performed in triplicate. The scavenging activity was calculated as follows:

$$\text{scavenging activity}(\%) = \frac{A_0 - (A_s - A_c)}{A_0} \times 100\%$$

where A_s, with the presence of DPPH and test essential oil; A_0, with the presence of DPPH but without test essential oil; and A_c, with the presence of test essential oil but without DPPH.

2.4.3. Metal chelating assay

Chelating ability was determined according to the previously reported procedure with a slight modification (Dinis, Madeira, & Almeida, 1994). The different concentrations of samples (0.2–1.0 mg/mL, 1 mL) were mixed with 3.7 mL of methanol and 0.1 mL of 2 mM ferrous chloride. The reaction was initiated by the addition of ferrozine (0.2 mL, 5 mM). After 10 min at room temperature, the absorbance of the mixture was determined at 562 nm against a blank. EDTA was used for comparison. The ferrous ion-chelating activity was given by the following equation:

$$\text{chelating ability}(\%) = \left(1 - \frac{A_{\text{sample}}}{A_{\text{control}}}\right) \times 100\%$$

where A_{control} was the absorbance of the control and A_{sample} was the absorbance in the presence of the sample extracts and standards. The control contained $FeCl_2$ and ferrozine, with complex formation molecules.

2.4.4. Reducing power

The reducing power of the prepared essential oil was determined according to the method of Oyaizu (1986). Briefly, 1.0 mL of different concentration sample (0.2–1.0 mg/mL) was mixed with 2.5 mL of a 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of a 1% (w/v) solution of potassium ferricyanide. The mixture was incubated in a water bath at 50 °C for 20 min. Afterwards, 2.5 mL of a 10% (w/v) trichloroacetic acid solution was added and the mixture was then centrifuged at 3000 rpm for 10 min. A 2.5 mL aliquot of the upper layer was combined with 2.5 mL of distilled water and 0.5 mL of a 0.1% (w/v) solution of ferric chloride, and absorbance was measured at 700 nm. BHT was used as a reference material. All tests were performed in triplicate and the graph was plotted with the average of the three determinations.

2.5. Statistical analysis

Data are reported as the mean ± SD of three measurements. The scientific statistic software GraphPad Prism 3.03 was used to evaluate the significance of differences between groups. Comparisons between groups were done using Kruskal–Wallis test followed by Dunn's post hoc test. $P < 0.05$ was regarded as significant. The IC_{50} (the concentration of antioxidant at which 50% of the reaction was inhibited) was determined using the statistics program SPSS for Windows, version 13.0.

3. Results

3.1. In vitro antimicrobial activity

The in vitro antimicrobial activity of the essential oil from *A. cepa* against the tested microorganisms was qualitatively and quantitatively assessed by the inhibition zones. According to the results shown in Table 1, the essential oil exhibited a potent inhibitory effect against all bacteria (*E. coli*, *B. subtilis* and *S. aureus*), yeasts (*R. glutinis*, *S. cerevisiae* and *C. tropicalis*) and moulds (*A. niger*, *M. purpureus* and *A. terreus*) with diameter of inhibition zones ranging from 4.1 to 19.3 mm, while it was 3.9–28.7 mm for the positive control. The essential oil exerted a broad antimicrobial spectrum and showed a high antimicrobial effect on *B. subtilis*, *C. tropicalis* and *M. purpureus* with the diameter of inhibition zones of 19.3, 15.1 and 13.2 mm, respectively.

3.2. Minimum inhibitory concentration (MIC) and minimum microbicidal concentration (MMC)

MIC and MMC values of essential oil against the employed microbicidal strains are shown in Table 1. These results demonstrated that this oil displayed potential antibacterial and microbicidal property. In general, the MIC values of the essential oil against the tested microorganisms ranged from 0.18 mg/mL to 1.80 mg/mL and MBC from 0.54 mg/mL to 3.6 mg/mL, respectively. By considering the results of inhibition zone assay in Table 1, *B. subtilis* was the most sensitive one in the tested microorganisms.

3.3. Scavenging effect on ABTS radical

ABTS assay is often used in evaluating total antioxidant power of single compounds and complex mixtures of various plants (Huang et al., 2008; Katalinic, Milos, Kulisic, & Jukic, 2006). Specific absorbance at 734 nm can be used in both organic and aqueous solvents as an index reflecting the antioxidant activity of the extracted essential oil (Ćavar, Maksimović, Vidic, & Parić, 2012; Tel, Öztürk, Duru, Harmandar, & Topçu, 2010). In the experiment, the scavenging ability of the essential oil on ABTS free radical was shown in Fig. 2. Its scavenging powers correlated well with

Table 1
Antimicrobial activity of the essential oil from *Allium cepa*.

Microorganisms	Strain	Inhibition zone (mm)			Essential oil (mg/mL)	
		Essential oil[a]	Norfloxacin[b]	Miconazole Nitrate[c]	MIC[d]	MMC[e]
E. coli	ATCC 25922	13.4 ± 0.9[f]	26.1 ± 1.2		0.27 ± 0.03	0.54 ± 0.05
B. subtilis	ATCC 21216	19.3 ± 1.2	28.7 ± 1.4		0.18 ± 0.02	0.54 ± 0.03
S. aureus	ATCC 25923	17.4 ± 1.1	26.9 ± 1.1		0.18 ± 0.03	0.54 ± 0.03
R. glutinis	ATCC 16740	11.4 ± 0.8	8.1 ± 0.6		0.36 ± 0.03	1.80 ± 0.08
S. cerevisiae	ATCC 9736	12.9 ± 0.7	3.2 ± 0.2		0.36 ± 0.03	1.80 ± 0.11
C. tropicalis	ATCC 13801	15.1 ± 0.8	1.3 ± 0.1		0.36 ± 0.02	0.90 ± 0.05
A. niger	ATCC 16404	4.1 ± 0.3		3.9 ± 0.3	0.54 ± 0.04	3.60 ± 0.23
M. purpureus	ATCC 36928	13.2 ± 0.7		8.3 ± 0.4	0.36 ± 0.03	2.52 ± 0.18
A. terreus	ATCC 20542	12.7 ± 0.4		10.8 ± 0.5	1.80 ± 0.09	2.88 ± 0.18

[a] The concentration of essential oil was 15 mg/disk.
[b] Norfloxacin (100 μg/disk) was used as positive control.
[c] Miconazole Nitrate (100 μg/disk) was used as positive control.
[d] Minimum inhibitory concentrations.
[e] Minimum microbicidal concentrations.
[f] Each value is expressed as means ± SD ($n = 3$).

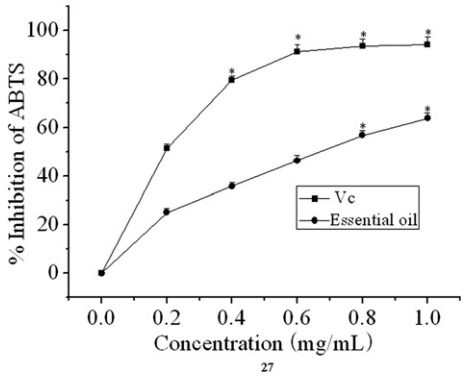

Fig. 2. Inhibition effects of the essential oil and Vc on ABTS radical. Data are mean ± SD for three measurements. *$P < 0.05$ compared with control (% inhibition by control = 0%; Kruskal–Wallis test followed by Dunn's post hoc test).

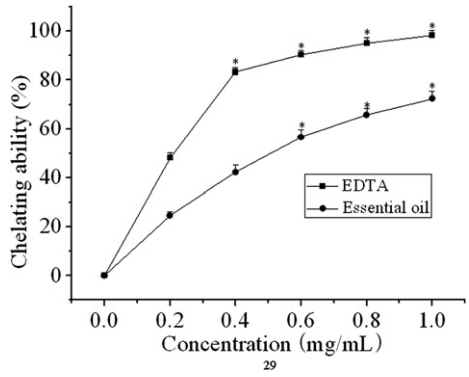

Fig. 4. Chelating effects of the essential oil and EDTA. Data are mean ± SD for three measurements. *$P < 0.05$ compared with control (% inhibition by control = 0%; Kruskal–Wallis test followed by Dunn's post hoc test).

increasing concentrations, but were lower than ascorbic acid, with the IC_{50} of the essential oil and Vc 0.67 and 0.19 mg/mL, respectively.

3.4. DPPH radical scavenging activity

The DPPH free radical is a stable free radical, which has been widely accepted as a tool for estimating the free radical-scavenging activity of antioxidant (Nagai, Inoue, Inoue, & Suzuki, 2003). In the DPPH test, the antioxidants were able to reduce the stable DPPH radical to the yellow-coloured diphenylpricryhydrazine. The effect of antioxidant on DPPH radical scavenging was conceived to their hydrogen-donating ability (Chen, Xie, Nie, Li, & Wang, 2008).

The result of DPPH free radical-scavenging ability of the essential oil is shown in Fig. 3 and compared with ascorbic acid as control standards. As can be seen from Fig. 3, the DPPH radical scavenging increased from 33.1% to 61.6%, when the concentration of the essential oil increased from 0.2 to 1.0 mg/mL. The IC_{50} of the essential oil and Vc were 0.63 and 0.27 mg/mL, respectively. The results indicated that the essential oil exhibited a potential DPPH radical scavenging activity.

3.5. Metal chelating activity

As shown in Fig. 4, the metal chelating activity of the essential oil increased with increasing concentrations used in the test. Compared with EDTA, which is known to be a strong metal chelating agent, the chelating ability of the samples on ferrous ion was weaker. The IC_{50} of the essential oil and EDTA in this reaction system were 0.51 and 0.21 mg/mL, respectively.

It has been recognized that the metal chelating ability might be involved in antioxidant activity and affected other functions that contribute to the antioxidant activity (Moure, Dominguez, & Parajo, 2006). Therefore, at least partly, the chelating effect of essential oil from *A. cepa* on ferrous ions might affect the other activities of scavenging free radicals to protect organism against oxidative damage.

3.6. Reducing power

The reducing capacity of a compound, which may serve as a significant indicator of its potential antioxidant activity (Meir, Kanner, Akiri, Hadas, & Hadas, 1995), was determined using a modified Fe^{3+}-to-Fe^{2+} reduction assay. In this assay, the yellow colour of the test solution changes to various shades of green and blue depending on the reducing power of extracts or compounds. The presence of reductants in the solution causes the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form and the Fe^{2+} can be monitored by measuring the formation of Perl's Prussian blue at 700 nm.

Fig. 5 showed the reducing abilities of the essential oil compared with BHT. Both of them had strong reducing powers; however, as anticipated, the reducing power of the essential oil was inferior to BHT, which is known to be a strong reducing agent. The reducing power of the essential oil was dose dependent. The reducing powers (absorbance at 700 nm) of the essential oil and BHT were

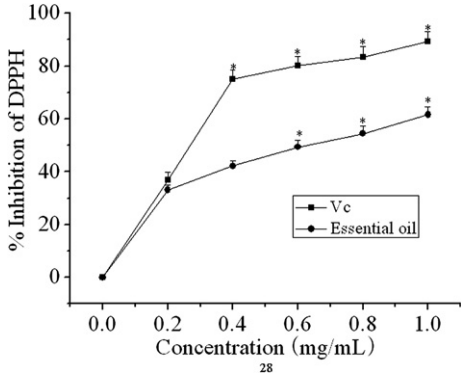

Fig. 3. Inhibition effects of the essential oil and Vc on DPPH radical. Data are mean ± SD for three measurements. *$P < 0.05$ compared with control (% inhibition by control = 0%; Kruskal–Wallis test followed by Dunn's post hoc test).

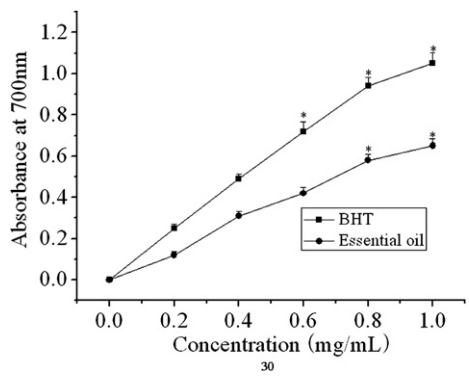

Fig. 5. Reducing power of the essential oil and BHT. Data are mean ± SD for three measurements. *$P < 0.05$ compared with control (% inhibition by control = 0%; Kruskal–Wallis test followed by Dunn's post hoc test).

0.65 and 1.05 at a dose of 1.0 mg/mL, showing that the essential oil can act as electron donors and can react with free radicals to convert them to more stable products and thereby terminate radical chain reactions.

4. Discussion

Preservation of food materials from degradation during production, storage and marketing is an important issue in the food industries. Also food-borne diseases pose a considerable threat to human health. Concern over pathogenic and spoilage microorganisms in foods is increasing due to the increase in outbreaks of food-borne disease. Although increasing use of chemical preservatives can effectively prevent the growth of most foodborne microorganism, safety problems related to chemical preservatives are receiving growing attention (Deba, Xuan, Yasuda, & Tawata, 2008). Therefore, much effort has been expended in the search for new types of effective and nontoxic antimicrobial compounds from natural materials. One such possibility is the use of essential oils as antimicrobial additives (Holley & Patel, 2005). In the present study, the results of in vitro antimicrobial assay showed that the essential oil from *A. cepa* had high antimicrobial activity against some representative spoilage microorganisms. When the result is considered together with the composition of the essential oil, it is likely that antimicrobial activity could be mainly due to the presence of organosulfur-containing compounds, such as methyl 5-methylfuryl sulfide (18.30%), methyl 3,4-dimethyl-2-thienyl disulfide (11.75%) and 1-propenyl propyl disulfide (9.72%). These findings are in agreement with previous reports (Hughes & Lawson, 1991; Kim, Huh, Kyung, & Kyung, 2004). From a comparison of our results with values reported in the literature, it is interesting the oil showed an antimicrobial effect in the concentration range as the most active essential oils (Gutierrez, Barry-Ryan, & Bourke, 2009; Hyldgaard, Mygind, & Meyer, 2012). Essential oils, which are odorous and volatile products of plant secondary metabolism, have wide applications in the food flavouring and preservation industries (Smith-Palmer, Stewart, & Fyfe, 2001).

Owing to the complex reactive facets of phytochemicals, the antioxidant activities of plant extracts cannot be evaluated by only a single method, but at least two test systems have been recommended for the determination of antioxidant activity to establish authenticity (Schlesier, Harwat, Bohm, & Bitsch, 2002). For this reason the antioxidant activity of *A. cepa* essential oil was determined by four spectrophotometric methods, ABTS, DPPH, metal chelating and reducing power tests. The reduction of DPPH and ABTS absorption is indicative of the capacity of the oils to scavenge free radicals, independently of any enzymatic activity, the metal chelating method is due to the capacity of the essential oil to break down hydrogen peroxide and lipid peroxides and the reducing power test is used to determine the capacity of reductant in a sample. Our results suggested that the oil exhibited moderate antioxidant activity with a dose–response in four assays. These experimental data are consistent with previous reports (Takahashi & Shibamoto, 2008).

5. Conclusion

Due to the undesirable problems and side effects arisen from the consumption of artificial chemical compounds, the essential oils and extracts from various plant species, especially edible and medicinal ones have attained appreciable interest among the research community. Our data indicate that *A. cepa* essential oil shows a moderate antimicrobial activity against referenced strains and also possess an interesting antioxidant activity. Taken together, these results show that the essential oil could be considered as a natural alternative to traditional food preservatives and be used to enhance food safety and shelf life.

Acknowledgement

This project has been financially supported by the Science and Technology Department of Zhejiang Province of the People's Republic of China (No. 2008C32024).

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