

# Antibacterial Activity and Antioxidant Properties of Water Extract from the Residue of Jelly Fig (*Ficus awkeotsang* Makino) Achenes

YING-JANG LAI, JIAN-NAN CHEN AND JAMES SWI-BEA WU

Graduate Institute of Food Science and Technology, National Taiwan University, Taipei, Taiwan (R.O.C.)

(Received: April 23, 2007; Accepted: September 7, 2007)

## ABSTRACT

The water extract from the residue of jelly fig (*Ficus awkeotsang* Makino) achenes (WERJFA) was found to inhibit the growth of *Bacillus cereus* and *Escherichia coli* O157:H7 effectively. The antioxidant activity of WERJFA was evaluated in radical scavenging activity, reducing capacity, ferrous ions chelating activity, total antioxidant activity, and hydrogen peroxide scavenging activity. The reducing capacity of WERJFA at 150 µg/mL and higher was found to be better than those of  $\alpha$ -tocopherol and BHA at the same concentration. The ferrous ion chelating activity of WERJFA was higher than that of citric acid. The antioxidant activity, the superoxide anions radical scavenging activity and the hydrogen peroxide scavenging activity of WERJFA at 200 µg/mL were equivalent to those of BHA at 50 µg/mL. The experiment results suggest the possibility of using WERJFA as an additive from natural source to extend the shelf-life and to improve the safety of food.

Key words: jelly fig, antibacterial, antioxidant, water extract

## INTRODUCTION

Synthetic chemicals are often used as preservatives in food processing and storage to inhibit food-borne pathogens and to extend shelf life. Consumer awareness and concerns over the potential risks of synthetic food additives to human health have renewed the interests in using naturally occurring alternatives.

Jelly fig (*Ficus awkeotsang* Makino) is a native woody vine in Taiwan. Pectin jelly curd prepared from jelly fig achenes by repeated extraction with tap water is an ingredient in a popular local summer drink. The achenes were found to contain pectinesterase (PE)<sup>(1)</sup>, chitinase<sup>(2,3)</sup>, thaumatin-like protein<sup>(4)</sup>, and pectinesterase inhibitor<sup>(5)</sup> as well. The extracted jelly fig achenes as a waste in the process retain noticeable activity of the pectinesterase inhibitor. Wu *et al.*<sup>(6)</sup> reported the use of pectinesterase inhibitor in the water-extract from the residue of jelly fig achenes (WERJFA) to reduce the methanol content in carambola wine. Moreover, WERJFA has been reported to effectively inhibit the proliferation of human leukemic U937 cells although the active component remains unknown<sup>(7)</sup>.

Active oxygen species and free radical-mediated reactions are involved in the degenerative or pathological processes such as aging<sup>(8,9)</sup>, cancer, coronary heart disease

and Alzheimer's disease<sup>(10-13)</sup>. Meanwhile, there are many epidemiological surveys results revealing an association between the people, who have a diet rich in fresh fruit and vegetable, and a decrease in the risk of cardiovascular diseases and certain forms of cancer<sup>(14)</sup>. There were also reports concerning natural compounds in fruit and vegetable, such as echinacoside<sup>(15)</sup>, anthocyanin<sup>(16)</sup> and phenolic compounds<sup>(17)</sup>, for their antioxidant activities.

The phenolics are common to many plants, and have evolved as antibacterial and antioxidant agents against environmental stress due to a variety of oxidizing and potentially harmful free radicals. As a consequence, the market of healthy and herbal nutraceuticals constantly addresses its attention to new plant sources offering functional efficacy. Nowadays, the resistance of pathogens against antibiotics develops much faster than ever. The search for new antimicrobial and antioxidant substances from nature is on great demand. The present study was to investigate the antibacterial and antioxidant properties of WERJFA in the evaluation of its potential to be a preservative from natural source.

## MATERIALS AND METHODS

### I. Chemicals

Butylated hydroxyanisole (BHA),  $\alpha$ -tocopherol,

\* Author for correspondence. Tel: +886-2-33664117;  
Fax: +886-2-33664117; E-mail: jsbwu@ntu.edu.tw

the stable free radical 1,1-diphenyl-2-picryl-hydrazyl (DPPH•), citric acid, ferric chloride ( $\text{FeCl}_2$ ), 3-(2-pyridyl)-5,6-bis(4-phenyl-sulfonic acid)-1,2,4-triazine (Ferrozine), nicotinamide adenine dinucleotide (NADH), linoleic acid, nitroblue tetrazolium (NBT), phenazine methosulphate (PMS), trichloroacetic acid (TCA) and polyoxyethylenesorbitan monolaurate (Tween-20) were obtained from Sigma (Sigma-Aldrich GmbH, Sternheim, Germany). Ammonium thiocyanate was purchased from Merck (Merck KGaA, Darmstadt, Germany). All other chemicals used were of analytical grade and obtained from either Sigma-Aldrich or Merck.

## II. Preparation of WERJFA

Jelly fig achenes were purchased from a farm in Fengshan City of Kaohsiung Prefecture. The achenes were repeatedly washed in tap-water to deplete the pectin and PE, homogenized with a blender (Model 4127, Osterizer, Tijuana, Mexico) for 2 min, extracted with distilled water (1/15, w/v) for 5 hr, and centrifuged (10000  $\times$ g, 10 min at 4°C). The supernatant was then filtered and freeze-dried to obtain WERJFA in powder form. The powder was re-dissolved in distilled water according to the specific assay conditions as described below.

## III. Antibacterial Assays

*Bacillus cereus* (BCRC 10603) and the *Escherichia coli* O157:H7 (ATCC 43895) were used for antibacterial assays. Both bacteria were streaked on the nutrient agar plates, and then incubated at 37°C for 24 hr to grow colonies. A single colony was picked up, seeded in nutrient broth, and then cultivated at 37°C for 12 hr. The cultured broth was diluted with 0.1% peptone water for the following antibacterial assays. Each 0.5-mL aliquot of the diluted broth ( $10^5$ - $10^6$  CFU/mL) was added to 4.5 mL nutrient broth containing 0, 1, 5, 10 or 20 mg/mL of WERJFA, and then incubated at 37°C for 12 hr or 24 hr. Samples of 1.0 mL aliquots were taken, spread onto a nutrient agar plate, and incubated at 37°C for 24 hr for colony counting. Mean values  $\pm$  SD of triplicates were calculated.

## IV. DPPH Free Radical Scavenging Activity

The DPPH free radical scavenging activities of WERJFA, using BHA and  $\alpha$ -tocopherol as standards, were measured by the method described by Shimada *et al.*<sup>(18)</sup> with modifications. Briefly, 1 mL ethanolic DPPH solution (0.1 mM) was added to 3 mL aqueous WERJFA solution or the ethanolic standard solution of BHA or  $\alpha$ -tocopherol at concentrations of 25 to 200  $\mu$ g/mL. The mixture was shaken vigorously and incubated at room temperature for 30 min. The absorbance at 517 nm was then measured using a spectrophotometer (Model 7800UV/VIS, Jasco, Tokyo, Japan). The free radical scavenging activity was calculated using the following equation:

$$\text{DPPH scavenging effects (\%)} = (A_0 - A_1) / A_0 \times 100$$

where  $A_0$  and  $A_1$  were the absorbance values of the blank and the sample, respectively.  $\text{IC}_{50}$ , the concentration required for 50% reduction in scavenging activity, was also calculated.

## V. Reducing Capacity

The reducing capacities of WERJFA and standards were quantified by the method of Yen and Chen<sup>(19)</sup>. An 1 mL aliquot of aqueous WERJFA solution (25-200  $\mu$ g/mL) was mixed with 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide [ $\text{K}_3\text{Fe}(\text{CN})_6$ ] solution. The mixture was incubated at 50°C for 20 min, added with 2.5 mL of 10% TCA, and centrifuged for 10 min at 4000  $\times$ g (Sorvall RC 5C, Dupont, Wilmington, DE) to obtain the upper layer. Each 2.5 mL aliquot of the upper layer was mixed thoroughly with 2.5 mL of distilled water and 0.5 mL of 0.1%  $\text{FeCl}_3$  solution. The absorbance at 700 nm of the reaction mixture was then measured.

## VI. Ferrous Ion-chelating Activity

The ferrous ion-chelating activity was evaluated by the method of Dinis<sup>(20)</sup>. Briefly, a 0.4 mL aliquot of the sample (25-200  $\mu$ g/mL) was added with 0.05 mL of 2 mM  $\text{FeCl}_2$  solution, 0.2 mL of 5 mM ferrozine, and deionized water to the total volume of 4 mL. The mixture was shaken vigorously and set aside at room temperature for 10 min. Absorbance at 562 nm was then measured. The ferrous ion-chelating activity was calculated using the following equation:

$$\text{ferrous ion chelating activity (\%)} = (A_0 - A_1) / A_0 \times 100$$

where  $A_0$  and  $A_1$  were the absorbance values of the blank and the sample, respectively.

## VII. Total Antioxidant Activity

The antioxidant activity was determined according to the ferric thiocyanate method of Mitsuda<sup>(21)</sup>. Linoleic acid (3.1  $\mu$ g/mL) was emulsified in 40 mM potassium phosphate buffer (pH 7.0) containing 0.351% Tween-20. A 2.5 mL aliquot of the emulsion was mixed with 2.5 mL of 40 mM potassium phosphate buffer (pH 7.0) that contained WERJFA (50-200  $\mu$ g/mL) or the standard (BHA or  $\alpha$ -tocopherol, 50  $\mu$ g/mL). The mixture (5 mL) was added with 0.1 mL of 20 mM  $\text{FeCl}_2$  in 3.5% HCl solution and 0.1 mL 30% ammonium thiocyanate solution and incubated at 37°C. Samples were taken periodically to measure the maximum absorbance at 500 nm. The buffer solution that contained neither WERJFA nor the standard was used as the blank. The inhibition of lipid peroxidation in the linoleic acid emulsion was calculated using the following equation:

inhibition of lipid peroxidation (%) =  $100 - (A_1/A_0) \times 100$

where  $A_0$  and  $A_1$  were the absorbance values of the blank and the sample, respectively.

#### VIII. Superoxide Anion Radical Scavenging Activity

Measurement of superoxide anion radical scavenging activity was based on the method described by Liu *et al.*<sup>(22)</sup>. A solution of 16 mM Tris-HCl (pH 8.0) containing NADH (78  $\mu$ M), NBT (50  $\mu$ M), or PMS (10  $\mu$ M) was prepared. A 3 mL aliquot of the mixture was added with 1 mL WERJFA (50, 100, 200  $\mu$ g/mL), and incubated at 25°C for 5 min before the absorbance at 560 nm was measured. BHA or  $\alpha$ -tocopherol, 50  $\mu$ g/mL, was used as the control. The superoxide anion radical scavenging activity was calculated using the following equation:

$$\text{superoxide anion radical scavenging activity (\%)} = (A_0 - A_1) / A_0 \times 100$$

where  $A_0$  and  $A_1$  were the absorbance values of the blank and the sample, respectively.

#### IX. Hydrogen Peroxide Scavenging Activity

The hydrogen peroxide scavenging activity of WERJFA was determined according to the method of Ruch *et al.*<sup>(23)</sup>. A solution of 40 mM  $H_2O_2$  in phosphate buffer (pH 7.4) was prepared. A 3.4 mL aliquot of aqueous WERJFA solution (50, 100, or 200  $\mu$ g/mL), ethanolic solution of BHA or  $\alpha$ -tocopherol (50  $\mu$ g/mL) was added to 0.6-mL  $H_2O_2$  solution. The mixture was allowed to react for 10 min before measuring the absorbance at 230 nm. The

phosphate buffer that contained no  $H_2O_2$  was used as the blank. The scavenging activity was calculated as:

$$H_2O_2 \text{ scavenging activity (\%)} = (A_0 - A_1) / A_0 \times 100$$

where  $A_0$  and  $A_1$  were the absorbance values of the blank and the sample, respectively.

#### X. Total Phenolics

The total content of phenolic compounds in WERJFA was determined with Folin-Ciocalteu reagent according to the method of Slinkard and Singleton<sup>(24)</sup>. Gallic acid was used as the standard compound. WERJFA (0.1 g) was dissolved in 5 mL of 0.3% HCl in methanol/water (60:40, v/v). The solution (100  $\mu$ L) was added to 2%  $Na_2CO_3$  (2.0 mL). After 2 min, 50% Folin-Ciocalteu reagent (100  $\mu$ L) was added to the mixture, and set aside for 30 min. Absorbance at 750 nm was then measured.

#### XI. Statistical Analysis

All tests and analyses were run in triplicates. Statistical analysis was performed using SAS Statistical Software, Version 6.11 (SAS Institute). The difference between treatment means was analyzed using General Linear Model Procedure and Duncan's multiple range test. A  $P$  value of < 0.05 was considered statistically significant.

## RESULTS AND DISCUSSION

#### I. Antibacterial Activity

The inhibitory effect of WERJFA on *B. cereus* and *E. coli* O157:H7 is presented in Table 1. There was

**Table 1.** Antibacterial activity of WERJFA against *Escherichia coli* O157:H7 and *Bacillus cereus*

Bacteria	WERJFA concentration (mg/mL)	Colony count (log CFU/ mL)		
		Incubated with WEJRFA for		
		0 hr	12 hr	24 hr
<i>Bacillus cereus</i>	0	5.41 $\pm$ 0.10 <sup>a</sup>	7.31 $\pm$ 0.06 <sup>a</sup>	7.49 $\pm$ 0.09 <sup>a</sup>
	1	5.43 $\pm$ 0.05 <sup>a</sup>	4.93 $\pm$ 0.04 <sup>b</sup>	3.87 $\pm$ 0.05 <sup>b</sup>
	5	5.40 $\pm$ 0.06 <sup>a</sup>	1.45 $\pm$ 0.21 <sup>c</sup>	1.00 $\pm$ 0.00 <sup>c</sup>
	10	5.40 $\pm$ 0.04 <sup>a</sup>	1.00 $\pm$ 0.00 <sup>d</sup>	0
	20	5.41 $\pm$ 0.06 <sup>a</sup>	0	0
<i>Escherichia coli</i> O157:H7	0	5.84 $\pm$ 0.12 <sup>a</sup>	7.81 $\pm$ 0.04 <sup>a</sup>	7.93 $\pm$ 0.10 <sup>a</sup>
	1	5.91 $\pm$ 0.01 <sup>a</sup>	2.88 $\pm$ 0.07 <sup>b</sup>	0.83 $\pm$ 0.57 <sup>b</sup>
	5	5.91 $\pm$ 0.08 <sup>a</sup>	2.57 $\pm$ 0.02 <sup>c</sup>	0.83 $\pm$ 0.57 <sup>b</sup>
	10	5.89 $\pm$ 0.05 <sup>a</sup>	1.88 $\pm$ 0.29 <sup>d</sup>	0.33 $\pm$ 0.65 <sup>c</sup>
	20	5.88 $\pm$ 0.03 <sup>a</sup>	0	0

Means  $\pm$  S.D. (n = 3). Means in the same column, for a specific parameter, that do not share the same superscript are significantly different at 5% level in Duncan's multiple range test.

dosage effect in the administration of WERJFA to inhibit these bacteria.

Saadoun and Hameed<sup>(25)</sup> reported that the parasitic plant *Orobancha cernua* extract exhibited antibacterial activity against *B. cereus* and *E. coli* (ATCC 25922) with the same minimum inhibitory concentration (MIC) value of 50 mg/mL for either microbe. Cruz *et al.*<sup>(26)</sup> reported that the polyphenol extracts from hemicellulosic hydrolysates (barley bran, corn cobs, and corn leaves) exhibited antibacterial activity against *E. coli* (CECT 434) with an MIC value near 10 mg/mL. Voravuthikunchai<sup>(27)</sup> reported the antibacterial properties of aqueous extracts of 38 medicinal plant species against *E. coli* O157:H7 (RIMD 0509952) with MIC values ranging from 0.09 to 6.25 mg/mL. In the present study, the MIC values of WERJFA at 24 hr incubation against *B. cereus* and *E. coli* O157:H7 were found to be 5–10 mg/mL and 10–20 mg/mL respectively, fall somewhere in-between the reported extremities.

In the absence of WERJFA, the numbers of *B. cereus* and *E. coli* O157:H7 increased steadily in incubation. With the addition of WERJFA at 1 mg/mL and an incubation period of 12 hr, the numbers of *B. cereus* and *E. coli* O157:H7 were decreased from 5.41 log CFU/mL and 5.84 log CFU/mL in the controls to 4.93 log CFU/mL and 2.88 log CFU/mL, respectively. As the concentration of WERJFA increased to 20 mg/mL, no survival was detected. Similar trend was observed in the samples incubated for 24hr.

Nasar-Abbas<sup>(28)</sup> reported that the aqueous extract of sumac (*Rhus coriaria* L.) at 1.0 mg/mL reduced the numbers of *B. cereus* and *E. coli* O157:H7 by 4.30 and 2.58 log CFU/mL, respectively, in 1-hr incubation. Sumac appeared more effective in the inhibition of *B. cereus* than that of *E. coli* O157:H7. Calculated from the data in Table 1, WERJFA at the same concentration was able to reduce the numbers of *B. cereus* and *E. coli* O157:H7 by 0.5 and 3.03 log CFU/mL in 12-hr incubation. WERJFA appeared more effective in the inhibition of *E. coli* O157:H7 than that of *B. cereus* instead. Nevertheless, the experiment data suggested the potential of WERJFA as an antibacterial food additive from natural source.

## II. Antioxidant Properties

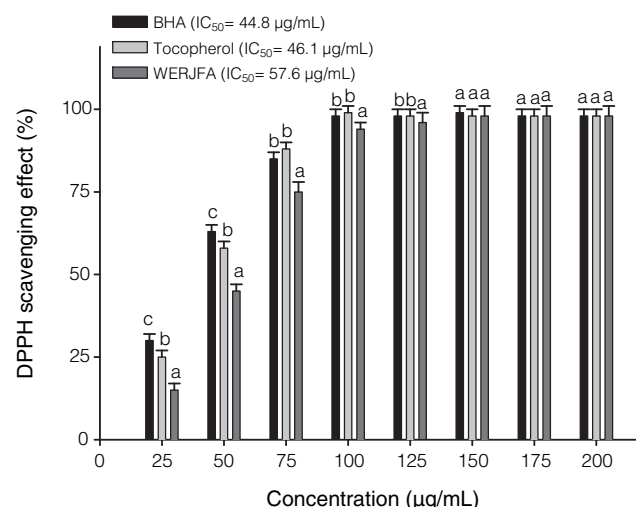
Many studies have revealed that natural antioxidants in plants are closely related to their biofunctionalities such as the reduction of chronic diseases and the inhibition of pathogenic bacteria<sup>(29)</sup>. In this study, the antioxidant activities of the WERJFA, BHA and  $\alpha$ -tocopherol were evaluated in several parameters, including DPPH free radical scavenging activity, reducing capacity, ferrous ion chelating activity, total antioxidant activity, superoxide anion radical scavenging activity and hydrogen peroxide scavenging activity.

### (I) DPPH Free Radical Scavenging Activity

Antioxidant properties, especially radical scavenging activities, are very important due to the deleterious role of free radicals in foods and in biological systems. Excessive formation of free radicals accelerates the oxidation of lipids in foods and impairs food quality and consumer acceptance<sup>(30)</sup>.

DPPH method has been widely employed to evaluate the free radical scavenging effectiveness of various antioxidant substances in food systems<sup>(31)</sup>. The method is based on the reduction of alcoholic DPPH in the presence of a hydrogen-donating antioxidant to form the non-radical form, DPPH-H<sup>(32)</sup>. The DPPH scavenging effect of WERJFA is illustrated in Figure 1 with BHA and  $\alpha$ -tocopherol as references. The scavenging effect of WERJFA, BHA, or  $\alpha$ -tocopherol, each at 75  $\mu$ g/mL, on DPPH radical was 75%, 85%, or 88%, respectively, and in descending order:  $\alpha$ -tocopherol>BHA>WERJFA.

Gülçin<sup>(33)</sup> reported that the DPPH scavenging effect of the boiling water extract of black pepper (WEBP) at 75  $\mu$ g/mL was 55%, while the scavenging effect of the ethanol extract (EEBP) was only 48%. Hou *et al.*<sup>(34)</sup> reported the scavenging effect of 80% methanolic extracts from SL (small leaf), BL (big leaf), and TL (thin leaf) cultivars of *Liriope spicata* L. (Mai-Men-Dong) against DPPH radicals. The IC<sub>50</sub> values in DPPH radical scavenging by these extracts were 81.08, 96.76, and 53.78  $\mu$ g/mL, respectively. The IC<sub>50</sub> by hot water extracts of the SL, BL, and TL cultivars were 378.97, 171.12, and 95.84 mg/mL, separately. The DPPH scavenging effect of WERJFA in the present study was found to be better than those of the black pepper and Mai-Men-Dong extracts.



**Figure 1.** Comparison in DPPH radical scavenging activity among WERJFA, BHA and  $\alpha$ -tocopherol at various concentrations. Data are expressed as means and standard deviations of three independent experiments, each used triplicate samples. Means in the same concentration that don't bear the same superscript were significantly different at 5% level according to Duncan's multiple range test.



## (II) Reducing Capacity

It has been reported that the antioxidant activity is concomitant with the development of reducing capacity<sup>(35)</sup>. Reducing capacities (shown by absorbance at 700 nm in the ferricyanide reduction test) of all samples increased with increasing concentrations (Figure 2). At the concentration of 25 µg/mL, WERJFA was inferior to BHA and  $\alpha$ -tocopherol in reducing capacity. At the concentration of 100 µg/mL, however, the reducing capacities of the three samples were close to one another. When the concentration further reached to 150 µg/mL, WERJFA became the most potent among three samples in the reduction of ferricyanide ions.

The increase in absorbance of the reaction mixture indicated the increase in reducing power. Gülçin<sup>(33)</sup> showed that the reducing capacity values of WEBP and EEBP at 75 µg/mL were 0.665 and 0.855, respectively. The reducing capacity of WERJFA at the same concentration was 1.055, which was higher than that of black pepper extracts.

## (III) Ferrous Ion Chelating Activity

Iron stimulates lipid peroxidation via Fenton reaction, and also accelerates peroxidation by decomposing lipid hydroperoxides into peroxy and alkoxy radicals that in turns can abstract hydrogen and perpetuate the chain reaction of lipid peroxidation<sup>(36)</sup> by themselves. Therefore, ferrous ion chelating activity is a good measure of the antioxidant activity.

The ferrous ions chelating activity of WERJFA was higher than that of citric acid, although lower than that

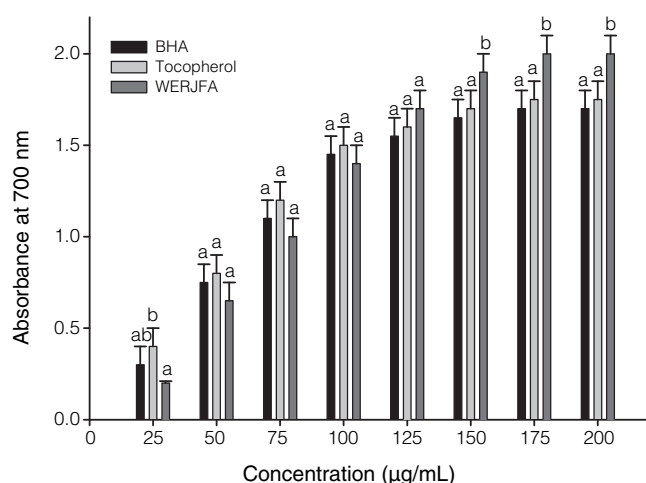
of EDTA (Figure 3). Since ferrous ions are the most effective and commonly found pro-oxidant in the food system<sup>(37)</sup>, the ferrous ion chelating activity of WERJFA proves its potential to be used as a natural antioxidant.

Gülçin<sup>(33)</sup> reported that the ferrous ions chelating activity of WEBP or EEBP, at 75 µg/mL, was 84% or 83%, respectively. WERJFA is somewhat inferior as it takes approximately 150 µg/mL to reach the same ferrous ions chelating activity. Yang *et al.*<sup>(38)</sup> reported that cold water and hot water extracts from *Glossogyne tenuifolia* at 1.0 mg/mL concentration chelated 61.73 and 45.76% of ferrous ions, respectively. Their performance is surpassed by WERJFA.

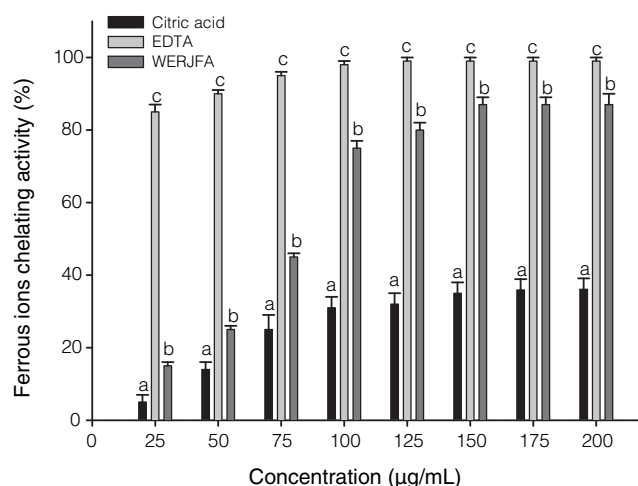
## (IV) Total Antioxidant Activity

Lipid peroxidation, which leads to the rapid development of rancid and stale flavors, is considered as a primary mechanism of quality deterioration in lipid foods and oils<sup>(37)</sup>. The total antioxidant activities, or the percentage of inhibition in lipid peroxidation, of 50 µg/mL of BHA,  $\alpha$ -tocopherol, and WERJFA in the emulsified linoleic acid system in 24-hr incubation were found to be 95%, 86%, and 78%, respectively (Figure 4). It was also shown in Figure 4 that the total antioxidant activity increased with the increase in WERJFA concentration. The total antioxidant activity of WERJFA at 200 µg/mL equaled to that of BHA at 50 µg/mL.

Gülçin<sup>(23)</sup> reported that the total antioxidant activity of WEBP or EEBP, at 75 µg/mL, was 95% or 93% respectively. WERJFA was somewhat inferior as it takes approximately 200 µg/mL to reach the same total antioxidant activity.



**Figure 2.** Comparison in reducing capacity of WERJFA, BHA and  $\alpha$ -tocopherol at various concentrations. Reducing capacity is shown by absorbance at 700 nm in the ferricyanide reduction test. Data are expressed as means and standard deviations of three independent experiments, each used triplicate samples. Means in the same concentration that don't bear the same superscript were significantly different at 5% level according to Duncan's multiple range test.



**Figure 3.** Comparison in ferrous ion chelating activity among citric acid, EDTA and WERJFA at various concentrations. Data are expressed as means and standard deviations of three independent experiments, each used triplicate samples. Means in the same concentration that don't bear the same superscript were significantly different at 5% level according to Duncan's multiple range test.

### (V) Superoxide Anion Radical Scavenging Activity

Superoxide anion plays an important role in the formation of other reactive oxygen species (ROS), such as hydrogen peroxide, hydroxyl radical and singlet oxygen, which can induce oxidative damage in lipids, proteins and DNA<sup>(39)</sup>. Furthermore, they have been implicated in several pathophysiological processes due to their transformation into more reactive species such as hydroxyl radical, which is capable of initiating lipid peroxidation. Superoxide has also been observed to initiate lipid peroxidation directly<sup>(40)</sup>.

As illustrated in Figure 5, the superoxide anion radical scavenging activities of  $\alpha$ -tocopherol and BHA at 50  $\mu\text{g/mL}$  concentration was 74% and 68.5% respec-

tively while only 35% for WERJFA. However, there was dosage effect. The scavenging activity of WERJFA could be upgraded by raising its concentration. For example, 200  $\mu\text{g/mL}$  of WERJFA matched 50  $\mu\text{g/mL}$  of the standard antioxidant in performance.

The superoxide anion radical scavenging activity of WERJFA at 200  $\mu\text{g/mL}$  concentration was equal to that of WEBP at 75  $\mu\text{g/mL}$ <sup>(33)</sup>. The superoxide anion radical scavenging activity of WERJFA at 50  $\mu\text{g/mL}$  was higher than that of EEBP at 75  $\mu\text{g/mL}$ .

### (VI) Hydrogen Peroxide Scavenging Activity

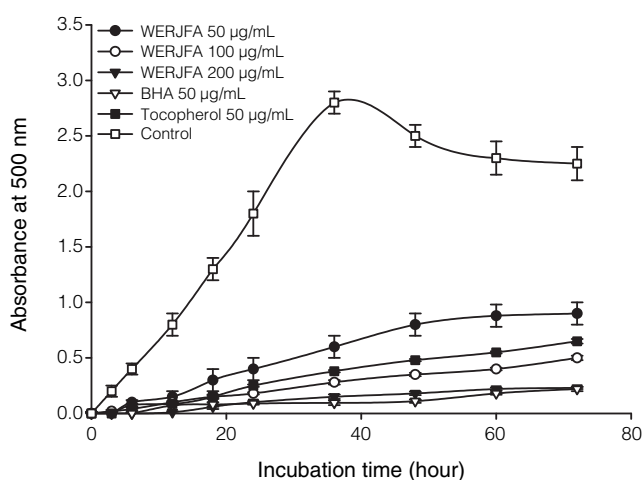
Hydrogen peroxide can be toxic to cells owing to the generation of hydroxyl radicals in cells. Furthermore, hydrogen peroxide in cell culture can lead to the transition of metal ion-dependent OH radical, which is mediated by the oxidative damage of DNA. The scavenging ability for hydroxyl radical by caffeine is relevant to its alleged anticarcinogenic properties<sup>(41)</sup>. The ability to quench the hydroxyl radicals by xylose-lysine Maillard reaction products is directly related to their antimutagenicities<sup>(42)</sup>.

WERJFA exhibited 45% scavenging activity for hydrogen peroxide at the concentration of 50  $\mu\text{g/mL}$ , comparing to 85% and 95% scavenging activities of BHA and  $\alpha$ -tocopherol at the same concentration, respectively (Figure 5). However, dosage effect enabled WERJFA to reach a comparable level (87%) when the concentration was increased to 200  $\mu\text{g/mL}$ . The hydrogen peroxide scavenging activity of WERJFA at 200  $\mu\text{g/mL}$  concentration in the present study was the same as that of WEBP or EEBP at 75  $\mu\text{g/mL}$  reported by Gülçin<sup>(33)</sup>.

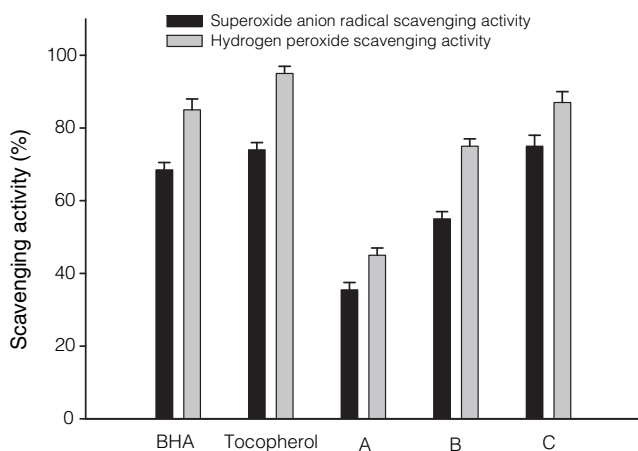
### III. Antibacterial and Antioxidant Components

Numerous herbs, spices and plants have been reported to be potential sources of antimicrobial and antioxidant agents but not many have been studied with respect to levels and ranges of activities<sup>(43)</sup>. The antioxidant effect of plant phenolics has been related to the prevention of coronary diseases, cancer, as well as degenerative brain disorders<sup>(44)</sup>. Phenolic compounds were also reported to be antioxidants that play an important role in delaying lipid peroxidation<sup>(45)</sup>. A highly positive relationship between total phenolics content and antioxidant activity was found in many plant species<sup>(46)</sup>.

The total phenolic content of WERJFA was found to be  $128.2 \pm 1.2$  mg gallic acid equivalent/g. The amounts of phenolics in WERJFA might account for its effective antibacterial and antioxidant properties. WEBP and EEBP contained 54.3 and 42.8 mg gallic acid equivalent/g respectively<sup>(29)</sup>. These extracts from black pepper were recognized with high antioxidant and radical scavenging activities. The present study demonstrated that WERJFA has higher DPPH free radical scavenging activity and reducing capacity, superoxide anion radical scavenging



**Figure 4.** Comparison in antioxidative activity among WERJFA, BHA and  $\alpha$ -tocopherol at various concentrations in 37°C incubation in the ferric thiocyanate test. Antioxidative activity is shown by absorbance at 500 nm.



**Figure 5.** Comparison in scavenging activity for superoxide anion radical and hydrogen peroxide in superoxide generation among  $\alpha$ -tocopherol (50  $\mu\text{g/mL}$ ), BHA (50  $\mu\text{g/mL}$ ), and WERJFA (A: 50  $\mu\text{g/mL}$ , B: 100  $\mu\text{g/mL}$ , and C: 200  $\mu\text{g/mL}$ ).

activity than those of both types of extracts from black pepper, and therefore can be an another good source of natural antioxidant. WERJFA also exerts the inhibitory effect on *E. coli* O157:H7 and *B. cereus*. Its dual role as antioxidant and antibacterial agent grants itself good potential to be applied in the food industry.

## CONCLUSIONS

The results of the present study indicate that WERJFA has antibacterial activity and antioxidant properties and phenolic compounds may be the active components. The residue of jelly fig achenes as an industrial waste has the possibility to be a cheap source of natural food additive. Further research on the isolation and identification of the active components of WERJFA and its application in food systems shall be worthwhile.

## REFERENCES

- Lin, T. P., Liu, C. C., Chen, S. W. and Wang, W. Y. 1989. Purification and characterization of pectinmethylesterase from *Ficus awkeotsang* Makino achenes. *Plant Physiol.* 91: 1445-1453.
- Li, Y. C., Chang, C. T., Hsiao, E. S., Hsu, J. S., Huang, J. W. and Tzen, J. T. 2003. Purification and characterization of an antifungal chitinase in jelly fig (*Ficus awkeotsang*) achenes. *Plant Cell Physiol.* 44: 1162-1167.
- Li, Y. C., Yang, Y. C., Hsu, J. S., Wu, D. J., Wu, H. H. and Tzen, J. T. 2005. Cloning and immunolocalization of an antifungal chitinase in jelly fig (*Ficus awkeotsang*) achenes. *Phytochemistry* 66: 879-886.
- Chua, A. C., Chou, W. M., Chyan, C. L. and Tzen, J. T. 2007. Purification, cloning, and identification of two thaumatin-like protein isoforms in jelly fig (*Ficus awkeotsang*) achenes. *J. Agric. Food Chem.* 55: 7602-7608.
- Jiang, C. M., Li, C. P., Chang, J. C. and Chang, H. M. 2002. Characterization of pectinesterase inhibitor in jelly fig (*Ficus awkeotsang* Makino) achenes. *J. Agric. Food Chem.* 50: 4890-4894.
- Wu, J. S., Wu, M. C., Jiang, C. M., Hwang, Y. P., Shen, S. C. and Chang, H. M. 2005. Pectinesterase inhibitor from jelly-fig (*Ficus awkeotsang* Makino) achenes reduces methanol content in carambola wine. *J. Agric. Food Chem.* 53: 9506-9511.
- Chang, J. H., Wang, Y. T. and Chang, H. M. 2005. Pectinesterase inhibitor from jelly fig (*Ficus awkeotsang* Makino) achene induces apoptosis of human leukemic U937 cells. *Ann. N. Y. Acad. Sci.* 1042: 506-515.
- Ames, B. N., Shigena, M. K. and Hegen, T. M. 1993. Oxidants, antioxidants and the degenerative diseases of aging. *Proc. Nat. Acad. Sci.* 90: 7915-7922.
- Harman, D. 1995. Role of antioxidant nutrients in aging: overview. *Age* 18: 51-62.
- Ames, B. N. 1983. Dietary carcinogens and anticarcinogens: oxygen radicals and degenerative diseases. *Science* 221: 1256-1264.
- Gey, K. F. 1990. The antioxidant hypothesis of cardiovascular disease: epidemiology and mechanisms. *Biochem. Soc. Trans.* 18: 1041-1045.
- Smith, M. A., Perry, G., Richey, P. L., Sayre, L. M., Anderson, V. E., Beal, M. F. and Kowal, N. 1996. Oxidative damage in Alzheimer's. *Nature*. 382: 120-121.
- Diaz, M. N., Frei, B., Vita, J. A. and Keaney, J. F. 1997. Antioxidants and atherosclerotic heart disease. *N. Engl. J. Med.* 337: 408-416.
- Salah, N., Miller, N. J., Paganga, G., Tijburg, L., Biolwell, G. P. and Rice-Evans, C. 1995. Polyphenolic flavonols as scavenger of aqueous phase radicals and as chain breaking antioxidants. *Arch. Biochem. Biophys.* 322: 339-346.
- Hu, C. and Kitts, D. D. 2000. Studies on the antioxidant activity of *Echinaceae* root extract. *J. Agric. Food Chem.* 48: 1466-1472.
- Espin, J. C., Soler-Rivas, C., Wichers, H. J. and Viguera-Garcia, C. 2000. Anthocyanin-based natural colorants: a new source of antiradical activity for foodstuff. *J. Agric. Food Chem.* 48: 1588-1592.
- Rice-Evans, C. A., Miller, N. J. and Paganga, G. 1997. Antioxidant properties of phenolic compounds. *Trends Plant Sci.* 2: 152-159.
- Shimada, K., Fujikawa, K., Yahara, R. and Nakamura, T. 1992. Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. *J. Agric. Food Chem.* 40: 945-948.
- Yen, G. C. and Chen, H. Y. 1995. Antioxidant activity of various tea extracts in relation to their antimutagenicity. *J. Agric. Food Chem.* 43: 27-32.
- Dinis, T. C. P., Madeira, V. M. C. and Almeida, L. M. 1994. Action of phenolic derivatives (acetoaminophen, salicylate and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. *Arch. Biochem. Biophys.* 315: 161-169.
- Mitsuda, H., Yuasumoto, K. and Iwami, K. 1996. Antioxidation action of indole compounds during the autoxidation of linoleic acid. *Eiyo to Shokuryo* 19: 210-214.
- Liu, F., Ooi, V. E. C. and Chang, S. T. 1997. Free radical scavenging activity of mushroom polysaccharide extracts. *Life Sci.* 60: 763-771.
- Ruch, R. J., Cheng, S. J. and Klaunig, J. E. 1989. Prevention of cytotoxicity and inhibition of intracellular communication by antioxidant catechins isolated from Chinese green tea. *Carcinogenesis* 10: 1003-1008.
- Slinkard, K. and Singleton, V. L. 1977. Total phenol analyses: automation and comparison with manual methods. *Am. J. Enol. Viticult.* 28: 49-55.
- Saadoun, I. and Hameed, K. M. 1999. Antibacterial activity of *Orobancha cernua* extract. *J. Basic Microbiol.* 39: 377-380.
- Cruz, J. M., Dominguez, J. M., Dominguez, H. and

- Parajo, J. C. 2001. Antioxidant and antimicrobial effects of extracts from hydrolysates of lignocellulosic materials. *J. Agric. Food Chem.* 49: 2459-2464.
27. Voravuthikunchai, S., Lortheeranuwat, A., Jeeju, W., Sririrak, T., Phongpaichit, S. and Supawita, T. 2004. Effective medicinal plants against enterohaemorrhagic *Escherichia coli* O157: H7. *J. Ethnopharmacology* 94: 49-54.
28. Nasar-Abbas, S. M. and Halkman, A. K. 2004. Antimicrobial effect of water extract of sumac (*Rhus coriaria* L.) on the growth of some food borne bacteria including pathogens. *Int. J. Food Microbiol.* 97: 63-69.
29. Covacci, V., Torsello, A., Palozza, P., Sgambato, A., Romano, G., Boninsegna, A., Cittadini, A. and Wolf, F. I. 2001. DNA oxidative damage during differentiation of HL-60 human promyelocytic leukemia cells. *Chem. Res. Toxicol.* 14: 1492-1497.
30. Cheung, S. C., Szeto, T. Y. and Benzie, I. F. 2007. Lipid oxidation of edible oil. *Plant Foods Hum. Nutr.* 62: 39-42.
31. Elmastaş, M., Gülçin, İ., Beydemir, Ş., Küfrevioğlu, Ö. İ. and Aboul-Enein, H. Y. 2006. A study on the in vitro antioxidant activity of juniper (*Juniperus communis* L.) seeds extracts. *Anal. Lett.* 39: 47-65.
32. Gülçin, I., Mshvildadze, V., Gepdiremen, A. and Elias, R. 2006. Screening of antioxidant and antiradical activity of monodesmosides and crude extract from *Leontice smirnowii* tuber. *Phytomedicine* 13: 343-351.
33. Gülçin, I. 2005. The antioxidant and radical scavenging activities of black pepper (*Piper nigrum*) seeds. *Int. J. Food Sci. Nutr.* 56: 491-499.
34. Hou, W. C., Wu, W. C., Yang, C. Y., Chen, H. J., Liu, S. Y. and Lin, Y. H. 2004. Antioxidant activities of methanolic and hot-water extracts from leaves of three cultivars of Mai-Men-Dong (*Liriope spicata* L.). *Bot. Bull. Acad. Sin.* 45: 285-290.
35. Tanaka, M., Kuie, C. W., Nagahima, Y. and Taguchi, T. 1988. Application of antioxidative Maillard reaction products from histidine and glucose to sadine products. *Nippon Suisan Gakkaishi* 54: 1409-1414.
36. Halliwell, B. 1991. Reactive oxygen species in living systems: source, biochemistry, and role in human disease. *Am. J. Med.* 91: 14-22.
37. Yamauchi, R., Tatsumi, Y., Asano, M., Kato, K. and Ueno, Y. 1988. Effect of metal salts and fructose on the autoxidation of methyl linoleate in emulsions. *Agric. Biol. Chem.* 52: 849-850.
38. Yang, J. H., Tsai, S. Y., Han, C. M., Shih, C. C. and Mau, J. L. 2006. Antioxidant properties of *Glossogyne tenuifolia*. *Am. J. Chin. Med.* 34: 707-726.
39. Pietta, P. G. 2000. Flavonoids as antioxidants. *J. Nat. Prod.* 63: 1035-1042.
40. Wickens, A. P. 2001. Aging and the free radical theory. *Resp. Physiol.* 128: 379-391.
41. Shi, X., Dalal, N. S. and Jain, A. C. 1991. Antioxidant behaviour of caffeine: efficient scavenging of hydroxyl radicals. *Food Chem. Toxic.* 29: 1-6.
42. Yen, G. C. and Hsieh, P. P. 1995. Antioxidative activity and scavenging effects on active oxygen of xylose-lysine Maillard reaction products. *J. Sci. Food Agric.* 67: 415-420.
43. Al-Fatimi, M., Wurster, M., Schröder, G. and Lindequist, U. 2007. Antioxidant, antimicrobial and cytotoxic activities of selected medicinal plants from Yemen. *J. Ethnopharmacol* 13: 515-521.
44. Parr, A. J. and Bolwell, G. P. 2000. Phenols in the plant and in man. The potential for possible nutritional enhancement of the diet by modifying the phenols content or profile. *J. Sci. Food Agric.* 80: 985-1012.
45. Yen, G. C., Duh, P. D. and Tsai, C. L. 1993. Relationship between antioxidant activity and maturity of peanut hulls. *J. Agric. Food Chem.* 48: 67-70.
46. Velioglu, Y. S., Mazza, G., Gao, L. and Oomah, B. D. 1998. Antioxidant activity and total phenolics in selected fruits, vegetables and grain products. *J. Agric. Food Chem.* 46: 4113-4117.