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Full Length Research Paper

Evaluation of different kinds of organic acids and their antibacterial activity in Japanese Apricot fruits

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The fruit of Japanese apricot is rich in organic acids, which have strong antibacterial activities. The types and contents of organic acids in six different cultivars of Japanese apricot fruit were evaluated by reverse-phase high performance liquid chromatography (HPLC). The antibacterial activity against *Escherichia coli*, *Bacillus subtilis* and *Streptococcus suis* was also determined. The results revealed that there are nine types of organic acids in the presence of the extracts of Japanese apricot fruit, including oxalic, tartaric, malic, ascorbic, acetic, citric, maleic, fumaric and succinic acids. The total organic acid content of 'Zhonghong' was the highest among the studied cultivars, and the main organic acids present were citric and malic acids. The antibacterial activity on the growth of *E. coli* and *B. subtilis* was higher than on the growth of *S. suis*. The antibacterial effect of acetic acid against bacteria was the best, and the minimum antibacterial concentration (MIC) against *E. coli* and *B. subtilis* was 0.417 mg/mL. The citric and maleic acids were also against these three strains of bacteria. The results suggest that the antibacterial activity is related to the organic acid composition and content of Japanese apricot fruit.

Key words: Japanese apricot, organic acids, high performance liquid chromatography (HPLC), antibacterial activity.

INTRODUCTION

Japanese apricot (*Prunus mume* Sieb. *et* Zucc.), a deciduous tree of the genus *Rosaceae*, originates in China and is widely cultivated in the regions of Zhejiang, Guangdong, Jiangsu, Fujian, Yunnan and Taiwan of China, as well as in Japan and Korea (Chuda et al., 1999; Chu, 1999). Japanese apricots are rich in nutrients and contain varieties of basic minerals and organic acids (Chu, 1999) which are commonly processed into different kinds of food that have beneficial effects (Terada and Sakabe, 1988; Ohtsubo and Ikeda, 1994). The consumption of Japanese apricot as a health food is popular in Japan (Yamaguchi et al., 2004).

Organic acids widely exist in fruits and vegetables, and the content is an important indicator of the nutritional and flavour quality of fruits (Shui and Leong, 2002; Sha et al., 2011). In recent years, researchers have found that organic acids can inhibit the growth of some bacteria and fungi. Kim et al. (2010) reported that red muscadine juice, which is rich in organic acids, showed strong antimicrobial action against *Cronobacter sakazakii*, and the main organic acids present were malic and tartaric acids. Raybaudi-Massilia et al. (2009) discovered that malic acid could inhibit the growth of *Listeria monocytogenes*, *Salmonella gaminara*, and *Escherichia coli* O157: H. Eswaranandam et al. (2004) also found that organic acids such as malic, citric, lactic, and tartaric acid had antibacterial activity with specific pH conditions. The flowers, branches, leaves and seeds of Japanese apricot could be used as medicine for the treatment of many diseases recorded in ancient Chinese medicine (Shi et al., 2009).

Modern medical studies have found that the production of Japanese apricot fruit with broad-spectrum bacterial and antibacterial ability could inhibit the growth of *Streptococcus mutans*, *Streptococcus mitis*, *Streptococcus sanguis, Porphyromonas gingivalis*,

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Bordetella bronchi and *Helicobacter pylori* (Wong et al., 2010; Jung et al., 2010; Miyazawa et al., 2006; Enomoto et al., 2010). Later studies found that the extract of Japanese apricot fruit also highly suppressed the growth of influenza virus (Yingsakmongkon et al., 2008; Sriwilaijaroen et al., 2011). Xia et al. (2011) found that the methanol extract of Japanese apricot seeds could inhibit the growth of certain strains of bacteria. Until now, there have been no reports on the antibacterial activities of Japanese apricot fruit and organic acids on *E. coli*, *Bacillus subtilis* and *S. suis* and the organic acid contents of the different cultivars of Japanese apricot fruits and at the same time, the relationship between the contents of organic acids and the antibacterial activity of Japanese apricot fruit has not been reported.

The objective of the present study was to evaluate the types of organic acids and their antibacterial activity in Japanese apricot fruits. The antibacterial activities of the extracts of six cultivars of Japanese apricot fruit were determined against *E. coli*, *B. subtilis* and *S. suis*. After establishment of the bacteriostatic activities of the six cultivars, their active components were investigated, and the contents of organic acids were in the presence of six cultivars of Japanese apricot fruits.

MATERIALS AND METHODS

The fruit samples (100 g) of six cultivars of Japanese apricot fruit were collected from the National Field Genbank for Japanese apricot of Nanjing Agricultural University in Nanjing, P. R. China during harvest time (80 days after flowering) and stored at −20°C for further studies.

Extraction of organic acids

The pulp of Japanese apricot fruit (10 g) was extracted with 10 mL of water at room temperature for 24 h; then, it was heated for 10 min at 90°C and centrifuged at 10000 rpm for 30 min. The supernatant was filtered through a 0.22 μm membrane filter. The extraction concentration of Japanese apricot was 1 g/mL and stored at 4°C until use for HPLC analysis and antibacterial activity determination.

Chemicals

The reagents used included analytical-grade metaphosphoric acid and chromatography-grade standards for oxalic, tartaric, malic, ascorbic, acetic, citric, maleic, fumaric and succinic acid were purchased from SIGMA (USA).

Reverse-phase high performance liquid chromatography (HPLC) condition

HPLC analysis was performed on a HITACHI apparatus, which consisted of a L-2310 pump (HITACHI, Japan), a L-2400 UV- vis detector (HITACHI, Japan) and a Agilent ZORBAX SB-Aq C_{18} (250 × 4.6 mm, particle size of 5 μm, USA), and its temperature was maintained at 27°C. The flow rate was 0.8 mL/min. The mobile phase used was 0.01 mol/L potassium dihydrogen phosphate (pH: 2.4) (A) and methyl alcohol (B) for a total running time of 20 min. The sample injection volume was 20 μL. The detection wavelength was 215 nm. All solutions and the mobile phase solvents were filtered through a 0.22 μm membrane filter before HPLC analysis.

Bacterial strains

Type cultures of *E. coli* ATCC35218, *B. subtilis* ATCC6633 and *S. suis* were collected from the Laboratory of Animal Pharmacology of Nanjing Agricultural University in Nanjing, P. R. China and stored in glycerine at −20°C.

Suspension preparation

The bacteria used in this study included *E. coli*, *B. subtilis* and *S. suis*. The *E. coli* and *B. subtilis* strain were inoculated onto LB solid media, and the *S. suis* strain was inoculated onto THB at 37°C for 24 h. Then, a single colony was moved with an inoculation loop from the culture media to the MH media and cultured at 37°C until the desired concentration was reached. The suspension of bacteria must be cultured to 0.5 Maxwell turbidity units.

Determination of minimum inhibitory concentration (MIC)

The micro-dilution twice method recommended by CLSI was employed to test the MIC. Cells from cultures grown on Iso-Sensitest slopes were inoculated using a sterile loop into fresh Iso-Sensitest broth and incubated overnight at 37°C until the concentration of the bacteria reached 0.5 Mcfarland turbidity. 96-well plates were put in the ultra-clean work bench to open the UV irradiation for 2 to 3 h before test. 180 μL of medium with bacteria was added into the first well, while 100 μL was added into the rest wells. 20 μL of the sample was added into the first well, mixed, and 100 μL of the mixture of medium of bacteria and the sample of the extract from the first well was pipetted into the second well, mixed. The same method was used for all the other wells up till the 11^{th}; 100 μL of the sample was discarded. The 12^{th} hole without the sample was used as a control, with a three-time repeat. The plates were then incubated at 37°C for 12 h and the MICs were calculated.

Statistical analysis

Statistics were analyzed using SPSS 17.0 software. Comparisons were based on one-way ANOVA followed by Duncan's test. A p-value <0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Screening for antimicrobial activity of different cultivars

The antimicrobial activity of the extract from the fruit of the Japanese apricot cultivar 'Zhonghong', 'Weishanzhong', 'Jietianmei', 'Dayezhugan', 'Huangxiaoda' and 'Hangbaimei' on *E. coli*, *B. subitilis* and *S. suis* were investigated in this study. There were several differences in the antibacterial activities of six cultivars of Japanese apricot fruit on the three strains of experimental bacteria (Table 1). Comparisons demonstrated that the extracts of 'Weishanzhong' and 'Dayezhugan'

Table 1. The MIC of the fruit extracts from 6 Japanese apricot cultivars against three bacteria.

Cultivars	MI Cs(mg/mL)		
	E. coli	***B. subtilis***	***S. suis***
Dayezhugan	12.50[a]	16.67[ab]	25.00[ab]
Hangbaimei	16.67[ab]	16.67[ab]	33.0[ab]
Weishanzhong	12.50[a]	33.33[b]	33.3[ab]
Jietianmei	20.83[ab]	20.83[ab]	58.33[b]
Zhonghong	14.58[ab]	14.58[a]	20.83[a]
Huangxiaoda	25.00[b]	25.00[ab]	25.00[ab]

Different letters (a-b) in each column indicated significant differences at $P < 0.05$ level. The antibacterial activities of the extracts of 6 cultivars of Japanese apricot on *E. coli*, *B. subtilis* and *S. suis.*

Table 2. Linearity, repeat ability and recoveries of organic acids determination.

Organic acids	Regression equation	t_R/min	R^2
Oxalic acid	y=795096x	3.77	0.9965
Tartaric acid	y=543365x	4.21	0.9915
Malic acid	y=211024x	5.28	0.9910
Ascorbic acid	y=814764x	5.65	0.9954
Acetic acid	y=93221x	5.90	0.9994
Citric acid	y=523115x	7.53	0.9930
Maleic acid	y=3E+06x	9.18	0.9950
Fumaric acid	y=3E+06x	11.77	0.9989
Succinic acid	y=5E+06x	16.69	0.9980

Nine types of organic acids were identified; the linearity, repeat ability and recoveries of organic acid determination.

had the highest antibacterial activities on *E. coli*, of which the MIC was 12.5 mg/mL. 'Zhonghong' had the highest antibacterial activity on *B. subtilis* and *S. suis*. The MICs were 14.58 and 20.8 mg/mL, respectively. Comparison by Duncan test demonstrated that the antibacterial activities of 'Dayezhugan', 'Weishanzhong', 'Hangbaimei', 'Jietianmei', and 'Zhonghongmei' on *E. coli* did not differ significantly and that of 'Huangxiaoda' was significantly lower than that of 'Dayezhugan' and 'Weishanzhong', but there were no significant differences between 'Huangxiaoda' and 'Hangbaimei', 'Jietianmei' or 'Zhonghong'.

The antibacterial activity of 'Zhonghong' and 'Weishanzhong' showed significant differences on *B. subtilis*, but neither differed significantly on *E. coli* or *S. suis*. There were significant differences between the antibacterial activities of 'Zhonghong' and 'Dayezhugan', 'Hangbaimei', 'Weishanzhong', 'Huangxiaoda' against *S. suis*, as the same as that between 'Jietianmei' and 'Dayezhugan', 'Hangbaimei', 'Weishanzhong', 'Huangxiaoda'. This result contradicts the report of Chen et al*.* (1989) who showed that juice of Japanese apricot had a significantly positive inhibitory activity on *Staphylococcus aureus*, *E. coli*, *B. subtilis* and *S. mutans* (Chen et al., 1989).

The composition and content of organic acids in Japanese apricot fruit and their antibacterial activity assay

Determination of organic acids in fruit of Japanese apricot by reverse-phase high–performance liquid chromatography (reverse-phase HPLC)

The contents of organic acids in Japanese apricot were determined by reverse-phase HPLC. There are many reports on the contents of organic acids. There are seven types of organic acids in the fruit of Japanese apricot. In the present study, nine types of organic acids were identified; the linearity, repeat ability and recoveries of organic acid determination are listed in Table 2, and the distribution of the nine kinds of organic acids in different cultivars of Japanese apricot are presented in Figure 2. As shown in the figure, there were significant differences in the contents of organic acid among different cultivars of Japanese apricot. The organic acid content of 'Zhonghong' was the highest among the six, followed by 'Weishanzhong', 'Dayezhugan' and 'Jietianmei' without any significant differences among the four. The contents of 'Zhonghong' and 'Weishanzhong' were significantly higher than that of 'Hangbaimei', whereas there were no

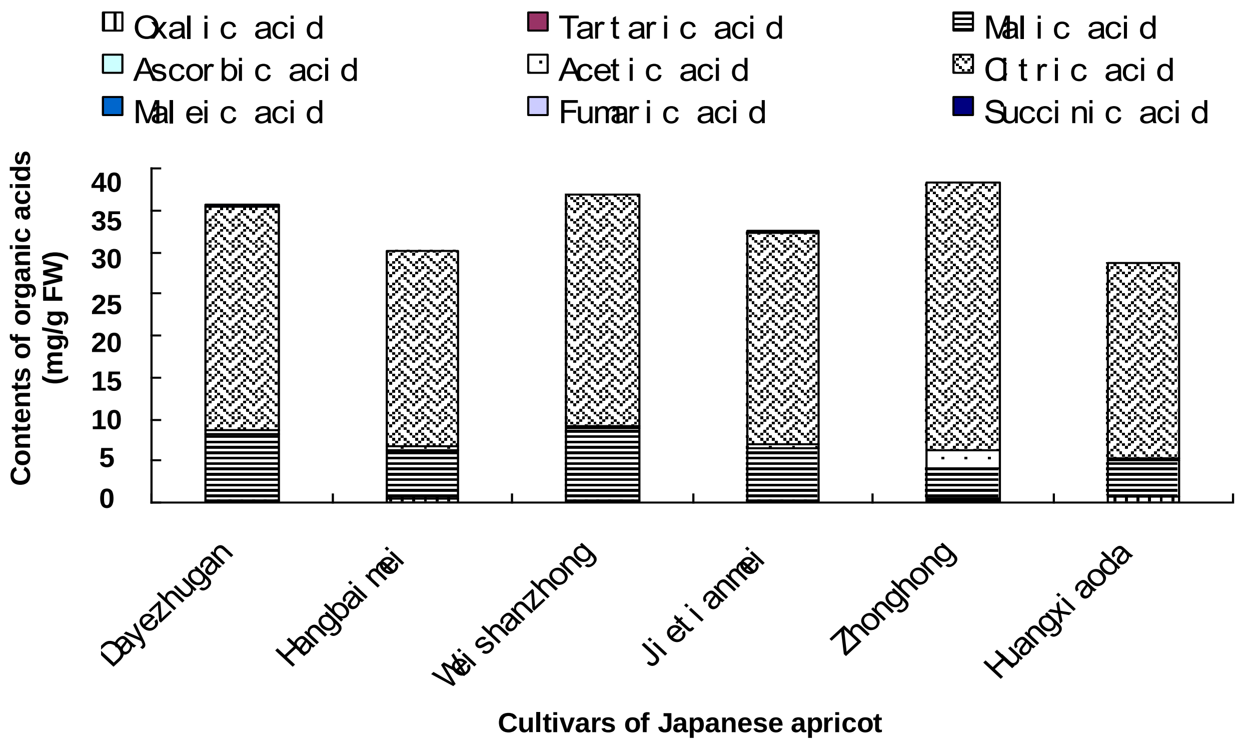

Figure 1. The content of organic acid in fruit of six Japanese apricot cultivars.

significant differences between 'Dayezhugan', 'Jietianmei' and 'Hangbaimei'. The organic acid contents of 'Hangbaimei' and 'Jietianmei' did not differ from 'Huangxiaoda', and that of 'Dayezhugan' was significantly higher than 'Huangxiaoda', whereas there were great significant differences between 'Zhonghong', 'Weishanzhong' and 'Huangxiaoda'. The main organic acid in the fruit of Japanese apricot was citric acid (about 70%), followed by malic acid. The content of citric acid in 'Zhonghong' (32.12 mg/g FW) was the highest of the six cultivars of Japanese apricot fruit, followed by 'Weishanzhong'. The lowest was 'Huangxiaoda', of which the citric acid content was 23.36 mg/g FW. Chen and Lu (2002) identified types of organic acids in *Fructus mume* and the results demonstrated that the main organic acids in Japanese apricot fruit were citric and malic acids, but the content had several differences that were related to the different extraction methods. 10 kinds of organic acids were separated and identified from the flower, branch and leaf extracts of Japanese apricot. Previous results showed that not all 10 were present in flowers, branches and leaves; some kinds of organic acid existed in flowers but not in branches while some were present in leaves but not in flowers and branches (Lin et al., 2011; Pan et al., 2008).

Using reverse-phase HPLC analysis as shown in Figures 3 and 4, we found that the nine types of tested organic acids are present in all the six Japanese apricot cultivars, but the contents of which differed. The contents of the nine kinds of organic acids in the Japanese apricot fruit are shown in Figure 1. The content of the total organic acid in 'Zhonghong' was 38.36 mg/g FW, which was the highest among the six studied cultivars, followed by 'Dayezhugan' while the organic acid content of 'Huangxiaoda' (28.79 mg/g FW) was the lowest. The results showed that the main organic acid in Japanese apricot fruit was citric acid, followed by malic acid, but ascorbic, maleic and fumaric acids were minor. Bureau et al. (2009) reported that the content of citric (15.8 to 16.0 meq g/ 100 gFW) in the fruit of Japanese apricot was higher than that of malic acid (10.4 to 10.6 meq g/ 100 gFW) (Bureau et al., 2009). The result was similar to ours.

Screening of antimicrobial activity of different organic acids

The antibacterial activities of nine kinds of organic acids present in Japanese apricot on the *E. coli*, *B. subtilis* and *S. suis* are shown in Table 3. The organic acid with the highest antibacterial activity on *E. coli* was found to be acetic acid (0.42 mg/mL), followed by oxalic, citric, maleic and succinic acid, with no significant differences between them. In addition, significant differences were observed between fumaric and ascorbic acid (Table 3). The antibacterial activity of organic acid on *B. subtilis* was different from that of *E. coli*. The MIC of acetic acid was

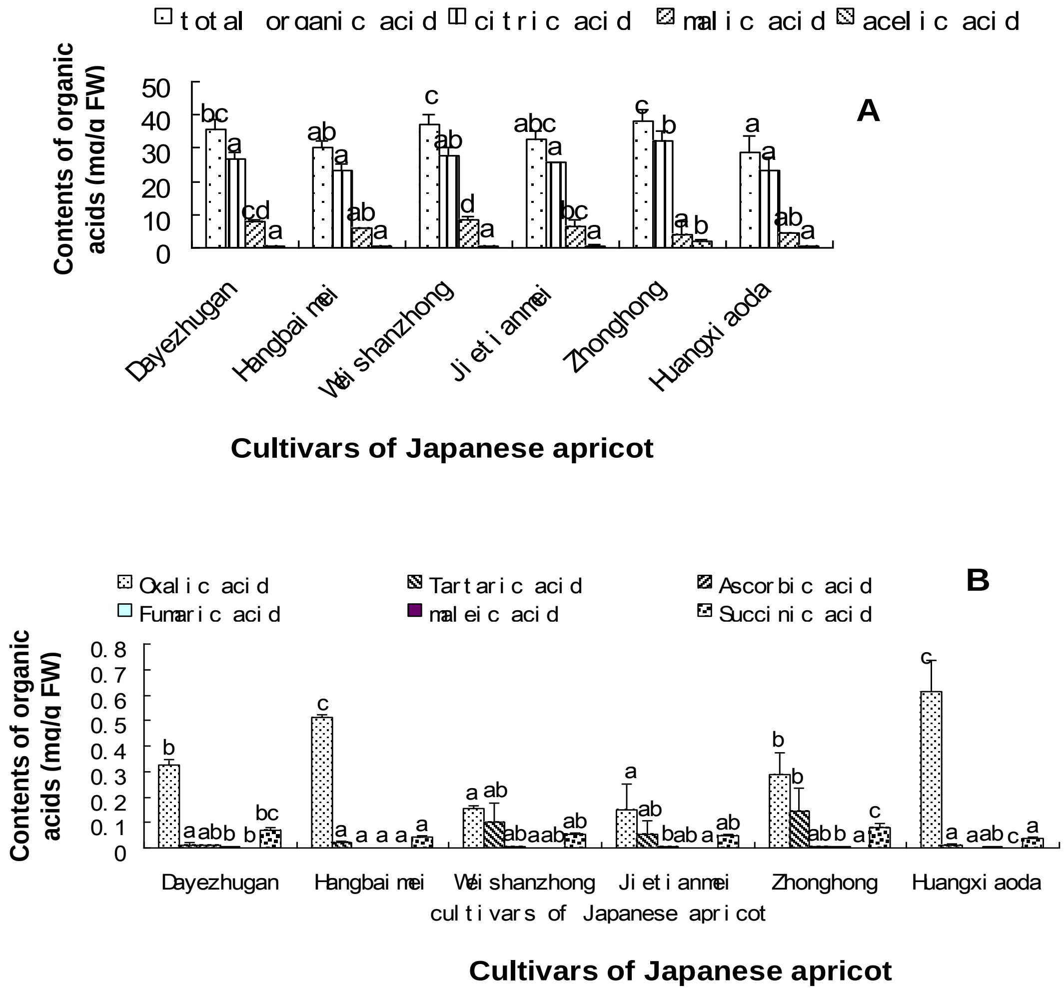

Figure 2. Distribution of organic acids in different cultivars of Japanese apricot.

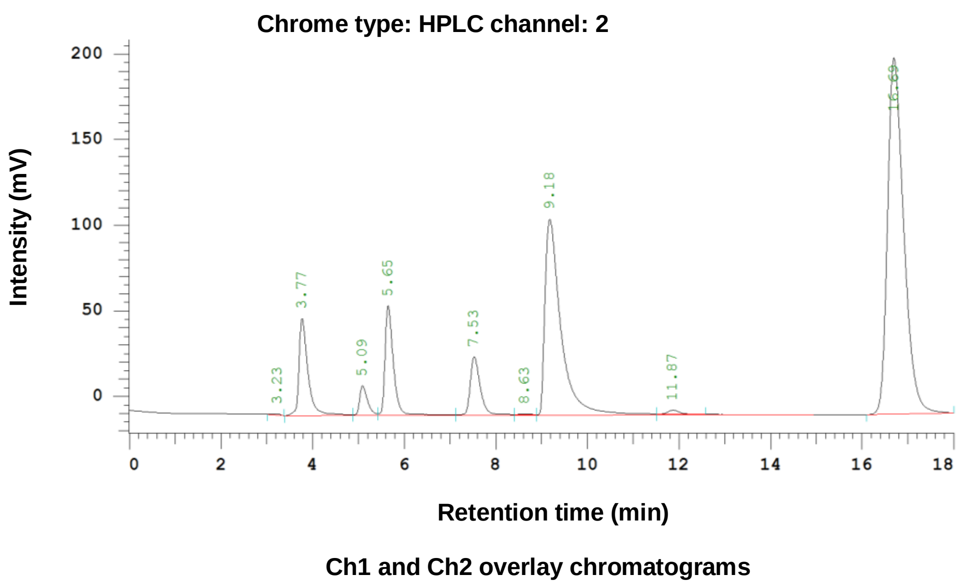

Figure 3. The peak patterns of oxalic, ascorbic, citric, maleic, succinic acid with intensity at 3.77, 5.65, 7.53, 9.18 and 16.69 min, respectively.

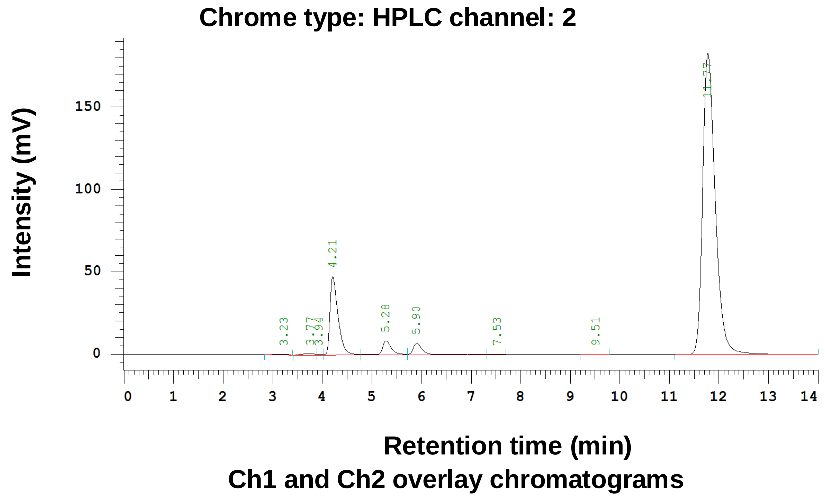

Figure 4. The peak patterns of tartaric, malic, acetic fumaric acid with intensity at 4.21, 5.28 min, 5.90, and 11.77 min, respectively.

Table 3. The MICs of different organic acids against three bacteria.

Organic acids	MIC (mg/mL)		
	E. coli	***B. subtilis***	***S. suis***
Oxalic acid	1.500[a]	1.333[ab]	32.000[d]
Tartaric acid	2.000[a]	2.000[ab]	8.000[b]
Malic acid	2.000[a]	2.000[ab]	6.667[b]
Ascorbic acid	8.000[b]	6.667[b]	32.000[d]
Acetic acid	0.417[a]	0.417[a]	4.000[a]
Citric acid	1.667[a]	2.000[ab]	8.000[b]
Maleic acid	1.667[a]	1.667[ab]	16.000[c]
Fumaric acid	13.333[c]	3.333[b]	8.000[b]
Succinic acid	1.667[a]	6.667[b]	32.000[d]

Different letters (a-d) in each column indicated significant differences at $P < 0.05$ level. The antibacterial activities of nine kinds of organic acids present in Japanese apricot on the *E. coli*, *B. subtilis* and *S. suis* and also the differences between different types of organic acids were shown.

the lowest, followed by oxalic, maleic, tartaric, malic and citric acids without any significant differences between them, while the latter five were also not significantly different from ascorbic, fumaric and succinic acids. There were significant differences among the antibacterial activities of the nine organic acids against *S. suis*, and acetic acid (4.00 mg/mL) had the highest antibacterial activity, followed by malic acid (6.67 mg/mL), tartaric acid (8.00 mg/mL), citric acid (8.00 mg/mL) and fumaric acid (8.00 mg/mL), as presented in Table 3.

We found that the organic acids present in Japanese apricot fruit had high antibacterial activities. Maria et al. (2007) studied the antibacterial activity of red and white wine and found that succinic, malic, lactic, and acetic acids were responsible for these activities. Eswaranandan et al. (2004) reported that citric, lactic, malic and tartaric acids inhibit the growth of *E. coli* O157:H7. Prashanth et al. (2001) found that the extract of *Punica granatum* showed significant antibacterial activities against *B. subtilis*.

The effect of the organic acid on the antibacterial activity of Japanese apricot

We found that the organic acids present in the fruit of Japanese apricot could inhibit the growth of three strains of bacteria, so it could be inferred that there was a relationship between the organic acid content and the antibacterial activity of Japanese apricot.

In the study, we found that 'Zhonghong', which had the highest antibacterial activity, had greater organic acid contents than other varieties of Japanese apricot, whereas, the content of organic acid in 'Huangxiada' was

Table 4. The correlation analysis of the contents of organic acids in Japanese apricot and its antibacterial activity.

Bacterial strains	Correlation
E. coli	$y=20.83+34.55x_1+101.57x_2-3.93x_3+1076.98x_4-21.59x_5-0.082x_6-7250.22x_7+800.93x_8+709.72x_9$
B. subtilis	$y=28.15+2.99x_1+49x_2-2.62x_3+525.67x_4-9.74x_5-0.23x_6-1537.10x_7+6659.55x_8+171.99x_9$
S. suis	$y=77.67-51.31x_1-199.33x_2+4.87x_3-1354.99x_4+32.97x_5-1.91x_6-1948.94x_7+6909.12x_8-634.59x_9$

The correlations between oxalic, tartaric, malic, ascorbic, acetic, citric, maleic, fumaric and succinic acids existed in Japanese apricot, and the antibacterial activity of three detected bacterial strains.

lower and the inhibitory effect on bacteria was also lower. Therefore, it could be inferred that the amount of organic acid influences the antibacterial activity of Japanese apricot. Correlations between oxalic, tartaric, malic, ascorbic, acetic, citric, maleic, fumaric and succinic acids existed in Japanese apricot, and the antibacterial activity of three detected bacterial strains are shown in Table 4.

The fruit of Japanese apricot was rich in organic acids, which likely resulted in the antibacterial activity of the extract of the Japanese apricot fruit. The antibacterial activity of acetic acid was the highest, and the MICs of *E. coli*, *B. sublitis* and *S. suis* were 0.417, 0.417 and 4.00 mg/mL, respectively. We found that the antibacterial activities of nine types of organic acids towards *E. coli* and *B. subtilis* were higher than that of the extract of Japanese apricot fruit. However, the antibacterial activity of *S. suis* was different because the antibacterial activities of some organic acids are higher than that of the extract, while some were lower than that of the extract. The reason for the differences in the inhibitory effect was that the antibacterial activities of different substances against different strains of bacteria were different. For example, formic acid more highly inhibited the growth of *E. coli* than that of succinic acid, but the opposite is true for *S. suis*. Another reason for this was that there may be other compounds in the presence of the fruit of Japanese apricot with bacteriostatic action.

The antibacterial activity of the extract of Japanese apricot fruit on the three tested bacterial strains not only depends on the type and amount of organic acid, but also on several other compounds present in Japanese apricot fruit. Xia et al. (2011) reported that phenolic compounds have antioxidant and antibacterial activities, and scholars like Okada et al. (2007), Nakagawa et al. (2007) and Okada et al. (2008) found that MK615, which is an extract from Japanese apricot fruit, could inhibit the growth of many kinds of cancer cells. Chuda et al. (1999) synthesized mumefural, which is a citric acid derivative, and found that it could improve the flow of blood. In summaries, there are types of ingredients that play important roles in health and antibacterial activity in the fruit of Japanese apricot, and further research is needed.

Conclusions

We found that different cultivars of Japanese apricot fruit were remarkably different in antibacterial activity and that antibacterial activities are related to the antibacterial activity of organic acids present in Japanese apricot fruit. There were nine kinds of organic acids present in the extracts of Japanese apricot fruit, including oxalic, tartaric, malic, ascorbic, acetic, citric, maleic, fumaric and succinic acid. The total organic acid content of ‘Zhonghong’ was the highest among the studied cultivars and the mainly organic acids in it were citric and malic acid. The antibacterial activity of which was higher than that of *S. suis* on the growth of *E. coli* and *B. subtilis*. The antibacterial effect of acetic against bacterial was the highest, and the minimum antibacterial concentration against *E. coli* and *B. subtilis* was 0.417 mg/mL. Citric and maleic acid were also effective against these three strains of bacteria.

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