Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2014, 6(11):156-160

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Evaluation of the antifungal activity of the acetone extract of Japanese apricot fruit

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ABSTRACT

The aim of this study is to evaluate the antifungal activity of the acetone extract of Japanese apricot fruit against plant phytopathogenic fungi. The results revealed that the acetone extract of Japanese apricot fruit could inhibit the mycelial growth of phytopathogenic fungi, including Phytophthora capsici Leonian, Fusarium solani (Mart.) Sacc., Fusarium graminearum Schwl, Botryosphaeria dothidea (Moug.) Ces. et De Not, Colletotrichum fragariae Brooks with different antifungal activities. The mycelial growth of phytopathogenic fungi was affected by the concentration of acetone extract of Japanese apricot fruit. We also evaluated the effect of the organic acids including oxalic, tartaric, malic, ascorbic, acetic, citric, maleic, fumaric and succinic acid isolated through the reverse HPLC inhibitions on the mycelial growth of Botryosphaeria dothidea (Moug.) Ces. et De Not. The results showed that the inhibitions were significant differences among the nine kinds of organic acids. In summary, we can infer that the inhibition of the extract of Japanese apricot fruit against phytopathogenic fungi is related to the kinds of organic acid presented in the fruit of Japanese apricot.

Key words: Japanese apricot, acetone extract, mycelial growth, inhibition.

INTRODUCTION

The use of synthetic pesticides in plant protection had made a great contribution to protect plant from suffering diseases, but many of them were no longer used because of economic, environmental or healthy concerns, as well as the resistant pathogen population of production emerges due to the indiscriminate and excessive use of a wide range of fungicides. Therefore, the demand for organic agricultural products cultivated without using any agricultural chemicals or chemical fertilizers is increasing and it caused more and more scientists to concentrate researches on the bactericidal activity of plant extracts. It was reported that 1389 kinds of plants including different kinds of compounds used as antiseptic, and the volatile compounds from plants, especially essential oils, had antifungal activity [1,2]. Oxenham et al. [3] found that two chemotypes of basil (*Ocimum basilicum*) oil inhibited the mycelial growth of pathogenic fungus *Botrytis fabae* in plant. Chaetoviridins A and B which were isolated from barnyard grass, showed antifungal activities on rice blast (*Magnaporthe grisea*) and wheat leaf rust (*Puccinia recondite*) [4]. Melaleuca alternifolia essential oil could reduce in vitro growth of fungal mycelium and single purified components were more active [5].

Japanese apricot (*Prunus mume* Sieb. et Zucc.), a deciduous tree of the genus Rosaceae, originates in China and widely cultivates in the regions of Zhejiang, Guangdong, Jiangsu, Fujian, Yunnan and Taiwan of China, and also in Japan and Korea [6,7]. The fruits of Japanese apricot are consumed as preserved fruits and drink in China and various parts of which have been used as herbal medicines for alleviating some common disorders in China for a long time [8]. In recent years, scientists begin to pay more attention on the function of Japanese apricot. Yoshikawa et al. [9] found that the concentrated fruit juice have been found to improve human blood fluidity in vitro and Shi et al. [10] discovered the methanol extract from Japanese apricot flowers exhibits inhibitory activity on aldose reductase and platelet aggregation in vitro. The extract of Japanese apricot fruit shows the inhibition of the some cancer cell lines growth, such as breast, lung, liver, colon and pancreatic cancer [11, 12, 13, 14, 15]. There were seven kinds of organic acids in the presence of Japanese apricot fruit [16], nevertheless, there have been no researches on the antifungal activity of Japanese apricot fruit and the organic acids extracted from the fruit.

The function of the extract of Japanese apricot fruit on anti-cancer agents, antibacterial has been studied, and up to our knowledge there are no reports on the antifungal activity of Japanese apricot against plant pathogenic fungi. In the research, we investigated antifungal activity of the acetone extract of Japanese apricot fruit against pathogenic fungi of *Phytophthora capsici* Leonian, *Fusarium solani* (Mart.) Sacc., *Fusarium graminearum* Schwl, *Botryosphaeria dothidea* (Moug.) Ces. et De Not and *Colletotrichum fragariae* Brooks to come to herbaceous and woody pathogenic fungi. This is the first report on the inhibition effect of the extract of Japanese apricot on the pathogenic fungi, which would provide evidence to produce organic fungicides.

EXPERIMENTAL SECTION

2.1 Plant materials
The fruit samples of Japanese apricot fruit were collected from the National Field Genbank for Japanese apricot located in Nanjing Agricultural University, Nanjing, P. R. China during the harvest time (80 days after bloom) and stored at -20 °C for further studies.

2.2 Fungi for test
Phytophthora capsici Leonian, *Fusarium solani* (Mart.) Sacc. and *Fusarium graminearum* Schwl were provided by the Laboratory of Phytopathology of Nanjing Agricultural University in Nanjing, P. R. China, *Botryosphaeria dothidea* (Moug.) Ces. et De Not was isolated and purified from apple tree and *Colletotrichum fragariae* Brooks was isolated and purified from strawberries. The growth of these fungi were maintained on potato dextrose agar medium at 4 °C.

2.3 Chemicals
The reagents used including analytical-grade metaphosphoric acid for oxalic, tartaric, malic, ascorbic, acetic, citric, maleic, fumaric and succinic acid were purchased from SIGMA company.

2.4 Extraction of Japanese apricot fruit
The pulp of the Japanese apricot fruit was extracted with the same amount of extraction agent at room temperature for 24 h, and then heated for 10 min at 90 °C, centrifuged at 10,000 rpm for 30 min. The supernatant was filtered through a 0.22 μm membrane filter. The extraction concentration of Japanese apricot is 1 g/mL and stored at 4 °C until use.

2.5 Mycelial growth inhibition test
Potato dextrose agar (PDA) medium was used for the culture maintenance and the bioassays. The isolated compounds dissolved in distilled water were tested for antifungal activity in vitro, and the extract of Japanese apricot fruit was in acetone.

The medium incorporating the extract of Japanese apricot fruit at series of concentrations of 100 mg/mL, 200 mg/mL, 300 mg/mL, 400 mg/mL, 0 mg/mL (CK), actone contrast, and were inoculated at the center with agar discs of the test fungi (6 mm diameter). And the organic acid concentrations were 5 μg/mL, 10 μg/mL, 15 μg/mL, 20 μg/mL, respectively. The Petri dishes were then incubated at 28 °C for 72 h, followed by the measurement of the diameters of zones of inhibition. All experiments were carried out in triplicate and the report data represents average values. Acetone was used as a positive control. The mycelial growth of fungi in both test (T) and control (C) Petri dishes was measured diameter (mm) in three different directions and the percentage growth inhibition (I) was calculated using the formula [17,18]:

$$I (\%) = [(C - T) / C] \times 100$$

2.6 Statistical analysis
Statistics were analyzed using SPSS 17.0 software. All the measurements were replicated three times for each treatment and the data are reported as means ± standard error. The statistical analysis was performed by one-way analysis of variance (ANOVA) using statistical software. The correlation coefficient was calculated between the content of mycelial biomass and the extract concentration.

RESULTS AND DISCUSSION

3.1 Results interpretations

Among all the five kinds of fungi, the extract of the fruit of Japanese apricot exhibited different antifungal activities, and the results of the percentages of inhibition against plant pathogenic fungi after inoculated 72 h were shown in Table 1. There were significant differences between the mycelial growth at the concentration of 100 mg/mL and CK, 100 mg/mL and 200 mg/mL. And there were highly significant difference between 200 mg/mL, 300 mg/mL, and CK.

The concentration of the extract at 100 mg/mL, the mycelial growth of *Fusarium graminearum* Schwl (10.67 mm) was the lowest and the inhibition percentage was 76.75%, followed by *Botryosphaeria dothidea* (Moug.) Ces. et De Not (70.22%), the same result showed in the extract of Japanese apricot fruit at the concentration of 200 mg/mL, and the inhibition percentages of *Botryosphaeria dothidea* (Moug.) Ces. et De Not, *Fusarium graminearum* Schwl and *Phytophthora capsici* Leonian were more than 90%. When the concentration of the extract was 300 mg/mL and 400 mg/mL, there were no signs of mycelial growth of the five kinds of plant pathogenic fungi.

Table-1: The zone of the mycelial growth and the percentage of growth inhibition against plant pathogenic fungi

Plant pathogenic fungi	CK	actone contrast	control one	control two	control three	control four
Botryosphaeria dothidea	81.17	55.67(31.41%)a	24.17(70.22%)b	3.92(95.18%)c	0(100%)c	0(100%)c
Fusarium graminearum schwl	46.08	35.00(23.89%)a	10.67(76.75%)b	0.75(98.20%)c	0(100%)c	0(100%)c
Colletotrichum fragariae Brooks	37.33	31.17(16.73%)a	16.92(54.56%)b	6.00(83.92%)c	0(100%)c	0(100%)c
Fusarium solani	38.08	35.50(6.82%)a	14.00(65.10%)b	4.33(88.60%)c	0(100%)c	0(100%)c
Phytophthora capsici Leonian	40.00	33.58(15.92%)a	13.58(66.26%)b	2.08(94.80%)c	0(100%)c	0(100%)c

Notation：Processing one, the extract of the fruit of Japanese apricot was 100 mg/ml in the medium, treatment two was 200 mg/ml, and the fllowing were 300 mg/ml and 400 mg/ml respectively. In the brackets were bacteriostatic rate, with a lower case of a, b, c showing significant difference.

Table-2: The virulence of the extract of Japanese apricot fruit against plant pathogenic fungi

Plant pathogenic fungi	virulence curve	The correlation coefficient	EC50	confidence interval, CI
Botryosphaeria dothidea	y=45.969+0.167x	0.891	24.14	33.51-58.43
Fusarium graminearum schwl	y=44.670+0.175x	0.845	30.46	28.36-60.98
Colletotrichum fragariae Brooks	y=28.650+0.212x	0.920	100.71	15.43-41.87
Fusarium solani	y=27.852+0.221x	0.884	100.22	10.70-45.01
Phytophthora capsici Leonian	y=35.018+0.202x	0.873	74.17	18.48-51.55

Table-3: Zone of the mycelial growth and the percentage growth inhibition against *Botryosphaeria dothidea* (Moug.) Ces. et De Not

Organic acids	CK	one	two	three	four
Citric acid	79.17a	66.50(16.00%)b	30.6(61.26%)b	17.83(77.48%)c	17.67(77.68%)c
Oxalic acid	79.17a	0(100%)b	0(100%)b	0(100%)b	0(100%)b
Tartaric acid	79.17a	57.00(28.00%)ab	28.33(64.22%)bc	14.33(81.90%)abc	2.33(97.06%)c
Acetic acid	79.17a	0(100%)b	0(100%)b	0(100%)b	0(100%)b
Fumaric acid	79.17a	39.00(50.74%)b	22.00(72.21%)c	17.50(77.90%)d	2.00(97.47%)e
Maleic acid	79.17a	11.8(85.06%)b	0(100%)c	0(100%)c	0(100%)c
Malic acid	79.17a	65.17(17.68%)ab	45.67(42.03%)bc	34.50(56.42%)cd	20.33(74.32%)d
Succinic acid	79.17a	70.33(11.17%)a	52.83(33.27%)b	34.83(56.84%) c	37.67(52.42%)c
Ascorbic acid	79.17a	63.50(19.79%)b	51.50(34.95%)c	43.17(45.17%)d	32.00(59.58%)e

Notation: Different lowercase letters standed for LSD test significant difference at 5% level. One, two, three, four showed adding organic acid concentrations into PDA medium were 5 μg/mL, 10 μg/mL, 15 μg/mL and 20 μg/mL, respectively.

The resistance of the extract of Japanese apricot fruit against five kinds of plant pathogenic fungi depended on obtaining EC50 values as presented in Table 2. And the inhibition of the extract of Japanese apricot fruit against *Botryosphaeria dothidea* (Moug.) Ces. et De Not was the highest, and the EC50 (24.14 mg/mL) was the lowest, followed by *Fusarium graminearum* Schwl (30.46 mg/mL). Among the five kinds of plant pathogenic fungi, the inhibition effect of the extract on *Colletotrichum fragariae* Brooks and *Fusarium solani* (Mart.) Sacc. is worse than the others, and the EC50s were 100.71 mg/mL and 100.22 mg/mL, respectively.

The extract of Japanese apricot was analyzed for the compositions and contents of organic acid in the fruit of Japanese apricot, and there were seven kinds of organic acids in the presence of the fruit of Japanese apricot. In the research, we isolated nine kinds of organic acids from Japanese apricot fruit, and they are citric, oxalic, tartatic, acetic, fumatic, maleic, malic, succinic, and ascorbic acids. The inhibition of organic acids from the fruit of Japanese apricot was determined against the mycelial growth of *Botryosphaeria dothidea* (Moug.) Ces. et De Not. Table 3 shows that the oxalic and acetic acids could inhibit the mycelial growth of *Botryosphaeria dothidea* (Moug.) Ces. et De Not, the mycelial was not grown on the PDA with oxalic and acetic acid. And the rest organic acids caused a significant dose-dependent inhibition against *Botryosphaeria dothidea* (Moug.) Ces. et De Not.

3.2 Summary of the research work performed

In this study, the extract of Japanese apricot fruit has been studied againsts *Phytophthora capsici* Leonian, *Fusarium solani* (Mart.) Sacc. and *Fusarium graminearum* Schwl, *Botryosphaeria dothidea* (Moug.) Ces. et De Not, *Colletotrichum fragariae* Brooks for the first time. All these kinds of fungi are plant pathogenic and are responsible for diseases at field levels. As a result, considering the importance of the extract as ecofriendly agents, it was studied against these fungi. It was interesting to note that the extract of Japanese apricot fruit exhibited different antifungal activities against all the five tested fungi. The extract exhibited higher antifungal activities against *Botryosphaeria dothidea* (Moug.) Ces. et De Not, *Fusarium graminearum* Schwl and *Phytophthora capsici* Leonian than that of *Colletotrichum fragariae* Brooks and *Fusarium solani* (Mart.) Sacc.. Considering this fact, the extract was subjected to HPLC analysis to isolate from the components present in it and it was found that the antifungal activity of the extract of Japanese apricot fruit was partly related to the organic acids presented in the fruit by detecting the antifungal activity against the plant pathogenic fungi.

During the experiment, we found that the mycelial of plant pathogenic fungi on the PDA medium with the extract of Japanese apricot fruit was different from that on the control medium, the mycelial of the former was intensive, and the mycelial was growing upwards around the agar discs and not suitable to spread around. While the control showed the contrast, the mycelial growth continuously extended to the surrounding in the horizontal covered the entire petri dish, and then the vertical.

3.3 The specific potential of Japanese apricot fruit

The potential of effective substances as antifungal agents were reported in recent years [19]. The acetone was used as a universal solvent because it was less toxic to the growth of plant pathogens and the medium was polarity, both water-soluble and fat-soluble [20]. Japanese apricot was used as herbal medicines for alleviating fever, cough, and intestinal disorders since ancient times [21], and modern researches have found that the fruits of Japanese apricot were important sources of antioxidants, anti-cancer, and antibacterial agents [22,23]. Japanese apricot was found to be antibacterial against *S. mutans*, *S. mitis*, *S. sanguis*, *P. gingivalis*, *B. bronchi* and *H. pylori* [24, 25, 26, 27]. Xia et al. [28] found that the extract of Japanese apricot seeds could inhibit the growth of this study including *Staphylococcus aureus*, *Escherichia coli*, *Salmonella enterica*, *Vibrio para-haemolyticus*, *Candida albicans*, *Saccharomyces cerevisiae* and Aspergillus niger, and the antibacterial activity was related to the phenolic compounds.

Recently, the use of fungicidal chemical has fallen into discourage because of the detrimental effects on environmental pollution and nontarget organisms [29]. If the natural plant productions can inhibit the plant pathogens and control disease development, and these plant extracts which have potential as environmentally safe alternatives may be exploited for their fungi-toxic potency because of the synergistic activity of their different compounds [30, 31]. There were more and more reports on antifungal activities of the extracts of plant against the plant pathogenic fungi. Yu et al. [32] tested 62 species of plant extracts with isolate hypha of *Botrytis cirerea Pers.*, *Fusarium graminerum* Schwl, *Phytophthora capsici* Leonian, *Colletotrichum gloeosporioides* Penz, and *Exserohilum turcicum* Pass and found the antifungal rate of 39 species of the plant extracts was more than 60% to one or more tested fungi at a concentration of 100 mg/mL. That of 23 species (for example, *Salix polyadenia*, *Juglans cathayonsis* etc.) was up to 80% to one or more tested fungi. While the antifungal activity of the extract of Japanese apricot fruit against *Colletotrichum fragariae* Brooks was more than 50% at the concentration, that against *Botryosphaeria dothidea* (Moug.) Ces. et De Not, *Fusarium graminearum* Schwl, *Fusarium solani* (Mart.) Sacc., and *Phytophthora capsici* Leonian was up to 60%.

CONCLUSION

In conclusion, the extract of Japanese apricot, a rich source of organic acids, included oxalic, maleic, and acetic acid, could effect the mycelial growth of plant pathogenic fungi and showed strong antifungal activity against plant pathogenic fungi due to the presence of oxalic and acetic acid. The results suggest that the extract of Japanese apricot fruit may have the potential to be used in agriculture as a natural plant pathogenic fungi inhibitor. The organic acids contribute significantly to the antifungal activity, and further studies should be carried out to probe into the specific components responsible for higher antifungal activity.

Acknowledgments
We gratefully acknowledged the Fundamental Research Funds for the Central University (KYZ201208) and the Jiangsu Province Science Technology Independent Innovation Fund [CX (12) 2011] for providing financial support.

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