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ANTIBACTERIAL AND ANTIOXIDATIVE ACTIVITIES OF MULBERRY RED PIGMENT

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ABSTRACT
In this article, the antibacterial and antioxidative effects of mulberry red pigment were studied in order to contribute to edible and medicinal values of mulberry. Antibacterial actions of mulberry red pigment against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas stutzeri*, *Asparagillus niger* and *Saccharomyces cerevisiae* were studied by inhibitory zone with filter paper and analysis of minimum inhibitory concentration, and the antioxidant effects of mulberry red pigment were investigated by scavenging DPPH free radical and scavenging superoxide radical. As a result, antibacterial and antioxidative abilities increased gradually with increase of concentration of mulberry red pigment. The mulberry red pigment showed antibacterial effects against six bacterial stains: *E. coli* > *S. aureus* > *B. subtilis* > *P. stutzeri* > *A. niger* > *S. cerevisiae*, and had the most strongly antibacterial activity on *E. coli* with IC_{50} value of 16.27 mg/mL and the most weakly antibacterial action on *S. cerevisiae* with IC_{50} value of 195.7 mg/mL. And mulberry red pigment could scavenge DPPH free radical and superoxide radical, and EC_{50} were 3.91 g/L and 1.93 g/L, respectively, but the antioxidant properties were lower than that of Vc. In general, mulberry red pigment had antibacterial and antioxidative effects, antibacterial ability against bacteria was higher than agaist fungi, and could scavenge DPPH free radical and superoxide radical.

1. Introduction

The mulberry belongs to the *Morus* genus of the Moraceae family which is widely distributed around the world. There are 24 species in the world, and there are 15 species and 4 variations in China (Muhammad et. al., 2012). Mulberry leaves are used for sericulture. Mulberry fruit is a kind of a purple fruit, and has a long history in China as one of the traditional fruit. And the mulberry fruit has been widely used in Chinese health foods and folk medicines for several thousands years. The mulberry fruit containes rich protein, polysaccharides, flavonoids, anthocyanins, amino acids and vitamins, etc, including antimicrobial, antioxidation and antitumor properties (Muhammad et. al., 2012; Bae and Suh, 2007; Chang et al. 2007). Muhammad Arfan reported the sugar-free extracts of *Morus* nigra had much oxidation resistance though

determining ABTS and DPPH (Muhammad et. al., 2012). Mulberry red pigment contained anthocyanins and carotene as a natural pigment, including antimicrobial and antioxidation properties. Duan Honglian reported the mulberry red pigment could strongly inhibit on *E. coli*, weakly inhibit on *S. aureus* and *B. subtilis*, and had no effect on fungi and yeast (Duan et al., 2007). Lu Yinghua reported mulberry pigment had excellent natural antioxidant and free radical scavenger (Lu et al., 2007). Niu Tianyu reported that mulberry had the highest anthocyanin content and had the strongest free radical scavenging capacity (Niu et al., 2016). Some studies had been done on antimicrobial and antioxidative activities of mulberry fruits. However, few studies had been done on researching mulberry red pigment both to inhibit food microorganism and to have antioxidant activity. As a good natural pigment, mulberry red pigment can be widely used in beverages, cold drinks, baked products, chewing gum, jelly and wine, etc. Therefore, mulberry red pigment has extensive effect on the food industry. In this paper, antibacterial and antioxidant activities of mulberry red pigment were studied in order to study food preservatives effect of mulberry fruits and mulberry red pigment. In this study, the alcohol abstraction method was used to gain mulberry red pigment, and inhibitory zone with filter paper and analysis of minimum inhibitory concentration were used to investigate antimicrobial effects of mulberry red pigment against *B. subtilis*, *S. aureus*, *E. coli*, *P. stutzeri*, *A. niger* and *S. cerevisiae* which were isolated from food. Antioxidant activities were studied by scavenging superoxide anion and DPPH free radical, and compared with Vc. The objective of this study was to evaluate antibacterial and antioxidant activities of mulberry red pigment in order to promote the comprehensive utilization of mulberry.

2. Materials and methods

2.1. The Mulberry samples collection

The mulberry fruits samples were collected from Yanbian county of Panzhihua city in Sichuan province of China. And the collected samples were freezed and preserved.

2.2. The mulberry red pigment extracts

The mulberry red pigment was extracted by the alcohol extraction. Fifty gram mulberry fruits were weighed, and were broken by mechanical method and soaked in 50% ethanol for 48 hours. The samples were filtered and centrifuged, and the supernatant was mulberry red pigment.

2.3. Antimicrobial sensitivity assay

Bacillus subtilis, *S. aureus*, *E. coli*, *P. stutzeri*, *A. niger* and *S. cerevisiae* were separated and purified from food. The antimicrobial effects of mulberry red pigment against *B. subtilis*, *S. aureus*, *E. coli*, *P. stutzeri*, *A. niger* and *S. cerevisiae* were tested by inhibitory zone with filter paper. The diameter of inhibition zones was measured and the average was calculated. And the inhibition rate was assayed by the inhibition zone diameters (Diao and Yang, 2014).

The inhibition rate (%) = (the inhibition zone diameters - filter diameter)/ the inhibition zone diameters × 100% (1)

Toxicity regression equations and 50% inhibiting concentration(IC_{50}) were got in order to determine antibacterial property of mulberry red pigment. The minimal inhibitory concentration (MIC) was scaled by agar dilution method (Wiegand et al., 2008; Eloff, 1999; Andrews, 2001)

2.4. Antioxidant effect analysis

Scavenging DPPH free radical and scavenging superoxide radical were determined and compared with Vc (Muhammad et. al., 2012; Bae and Suh, 2007; Chang et al. 2007).

3. Results and discussions

3.1. Antibacterial assay of mulberry red pigment

3.1.1. The antibacterial action

Figure 1 showed the inhibition rate of mulberry red pigment against *B. subtilis*, *S. aureus*, *E. coli*, *P. stutzeri*, *A. niger* and *S. cerevisiae* increased with the increase of concentration of mulberry red pigment. When concentration of mulberry red pigment increased from 0.2 mg/mL to 125 mg/mL, the suppression ratio against *E. coli* was the highest, with 92.89% ± 3.88%; it was followed by *S. aureus*, *B. subtilis*, *P. stutzeri*, and *A. niger*; and it showed the lowest inhibiting rates against *S. cerevisiae*, with 34.36% ± 2.96%. Wholly, mulberry red pigment could inhibit *E. coli*, *B. subtilis*, *S. aureus*, *P. stutzer*i, *A. niger* and *S. cerevisiae*, it had the strongest antimicrobial effect on *E. coli* and the weakest antimicrobial effects on *S. cerevisiae*, and antibacterial ability against bacteria was higher than against fungi.

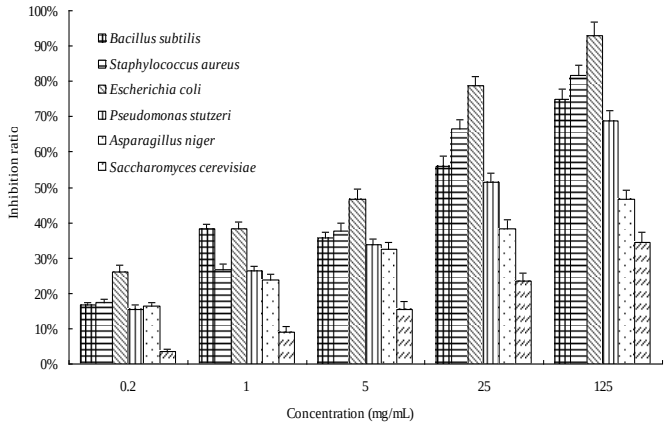

Figure 1. The antibacterial action of mulberry red pigment

3.1.2. Regression analysis of antimicrobial activity

As shown in Table 1, that regression equations and IC_{50} were obtained from the inhibition rates when *E. coli*, *B. subtilis*, *S. aureus*, *P. stutzeri*, *A. niger* and *S. cerevisiae* were inhibited by mulberry red pigment. IC_{50} against *E. coli* was the lowest, with 16.27 mg/mL; it was followed by *S. aureus*, *B. subtilis*, *P. stutzeri* and *A. niger*; and it showed the highest IC_{50} against *S. cerevisiae*, with 195.70 mg/mL. There was significant difference between IC_{50}. Wholly, IC_{50} value descended orderly: *S. cerevisiae* > *A. niger* > *P. stutzeri* > *B. subtilis* > *S. aureus* > *E. coli*. Therefore, mulberry red pigment had the strongest antibacterial activity on *E. coli* and the weakest antibacterial action on *S. cerevisia*.

3.1.3. Analysis of minimum inhibitory concentration (MIC)

Table 2 showed that *E. coli*, *B. subtilis*, *S. aureus*, *P. stutzeri*, *A. niger* and *S. cerevisiae* were inhibited by mulberry red pigment, and minimum inhibitory concentration (MIC) were attained. MIC against *E. coli* was the lowest, with 45 mg/mL, it was followed by *S. aureus*, *B. subtilis*, *P. stutzeri* and *A. niger*; and it showed the highest MIC against *S. cerevisiae*, with 540 mg/mL. And there were significant difference between MIC. The MIC descended orderly: *S. cerevisiae* > *A. niger* > *P. stutzeri* > *B. subtilis* > *S. aureus* > *E. coli*. Therefore, mulberry red pigment had the most strongly antibacterial activity on *E. coli* and the most weakly antibacterial action on *S. cerevisiae*.

3.2. Antioxidative assay of mulberry red pigment

3.2.1. Scavenging effects of mulberry red pigment on DPPH free radical

It could be seen from Figure 2 that the scavenging effects on DPPH free radical increased with the increase of concentration of mulberry red pigment. When the concentration of Vc was in the 1 g/L, scavenging rate of Vc reached its maximum. When the concentration of mulberry red pigment was in the 8 g/L, scavenging effects on DPPH free radical reached its maximum, And the scavenging rate of mulberry red pigment was lower than that of Vc, and scavenging rates were 89.95% ± 2.86% and 97.38% ± 2.1%, respectively.

Regression equations of scavenging DPPH free radical was $y = 0.1128x + 0.059$ ($R^2 = 0.9815$), and EC_{50} of scavenging DPPH free

radical was 3.91 g/L. So mulberry red pigment had antioxidant abilities against DPPH free radical.

Table 1. Regression equations and IC_{50}

Strains	Regression Equation	R^2	IC_{50} (mg/mL)	T0.01
Bacillus subtilis	y = 0.0036x + 0.3312	0.9568	46.89	D
Staphylococcus aureus	y = 0.0043x + 0.3251	0.985	40.67	D
Escherichia coli	y = 0.0044x + 0.4284	0.9583	16.27	E
Pseudomonas stutzeri	y = 0.0035x + 0.283	0.9282	62.00	C
Asparagillus niger	y = 0.0018x + 0.2586	0.966	134.11	B
Saccharomyces cerevisiae	y = 0.002x + 0.1086	0.9429	195.70	A

Table 2. Minimum inhibitory concentration (MIC)

Strains	MIC (mg/mL)	T0.01
Bacillus subtilis	88	D
Staphylococcus aureus	80	D
Escherichia coli	45	E
Pseudomonas stutzeri	125	C
Asparagillus niger	450	B
Saccharomyces cerevisiae	540	A

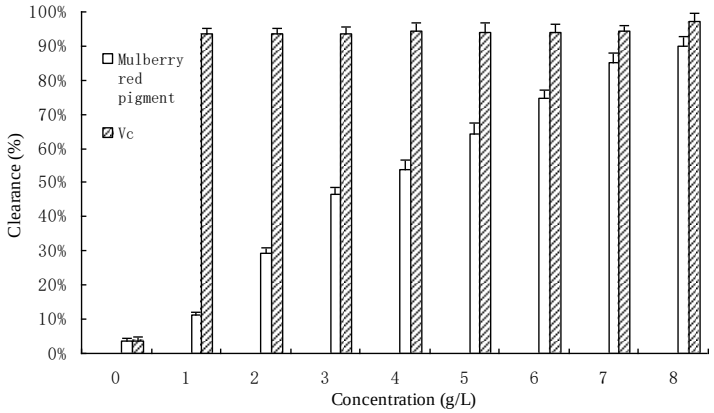

Figure 2. Scavenging effects of mulberry red pigment on DPPH free radical

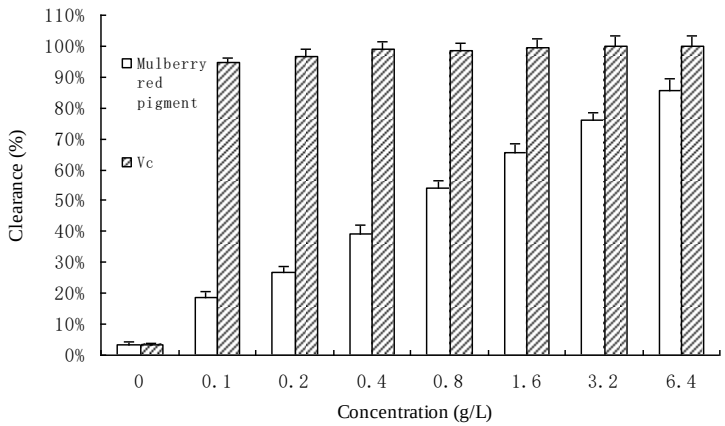

Figure 3. Scavenging effects of mulberry red pigment on superoxide radical

3.2.2. Scavenging effects of mulberry red pigment on superoxide radical

Figure 3 showed that the scavenging effect on superoxide radical increased with the increase of concentration of mulberry red pigment. When the concentration of Vc was in the 0.1 g/L, scavenging ability of Vc reached its maximum. When the concentration of mulberry red pigment was in the 6.4 g/L, scavenging effects on superoxide radical reached its maximum. But the scavenging rate of mulberry red pigment was lower than that of Vc, and scavenging rates were 85.78% ± 3.66% and 100% ± 3.46%, respectively. Regression equations of scavenging superoxide radical was $y = 0.1092x + 0.2889$ ($R^2 = 0.9594$), and EC_{50} of scavenging superoxide radical was 1.93 g/L. So mulberry red pigment had antioxidant abilities against superoxide radical.

4. Conclusions

Mulberry is widely distributed in China. Mulberry leaves are used as forage plant for silkworms, and they are also used as a herbal medicine. Mulberry fruit with rich nutrient is a natural fruit, but its storage period is short in natural condition, which can affect sales and prices of mulberry fruits. So extraction, antibacterial and antioxidative activities of mulberry red pigment were studied in order to promote further processing of mulberry fruits and to improve the comprehensive utilization of mulberry red pigment.

In this study, antibacterial and antioxidative effects of mulberry red pigment were studied. Mulberry red pigment could inhibit *E. coli*, *B. subtilis*, *S. aureus*, *P. stutzeri*, *A. niger* and *S. cerevisiae*, antibacterial ability against bacteria was higher than against fungi, and mulberry red pigment had the strongly antibacterial activity on *E. coli* and the weakly antibacterial action on *S. Cerevisiae*, the research result was are different from Duan Jianglian's reports (Duan and Xu, 2007). Mulberry red pigment could scavenge DPPH free radical and superoxide radical, and EC_{50} were 3.91 g/L and 1.93 g/L, respectively. The antioxidant properties were lower than that of Vc, but EC_{50} was lower than that of Lu Yinghua's reports (Lu et al., 2007)

The present study suggested mulberry red pigment had certain bacteriostatic and antioxidative action, and had nutrient hygienical function. So mulberry red pigment could be used in food additives, which contributed to food safety.

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