

Inhibitory Effects of Dark Tea on Digestive Tract Bacteria

Jingyi Xu^{a,*}, Mengze Du, Xiaomei Li, Qian Tang, Chunlei He, Pinwu Li

Tea Department of College of Horticulture Science, Sichuan Agricultural University, Chengdu 611380, Sichuan, China

^axujytea@126.com

*Corresponding author

Keywords: Dark tea, Staphylococcus aureus, Escherichia coli, antibacterial effect

Abstract: To study the inhibitory effects of dark tea on some intestinal pathogenic bacteria and investigate the healthcare functions of dark tea, the Oxford Cup was employed to determine the antibacterial activities of water extracts from Ya'an tea, Hunan Fu brick and Yunnan Pu'er tea against two bacterial species including Staphylococcus aureus and Escherichia coli and the minimal inhibition concentrations (MICs). The results showed that the extract from three types of dark tea displayed antibacterial activity against Staphylococcus aureus and Escherichia coli. The minimum antimicrobial concentration of all three types of dark tea was 48mg/ml to Escherichia coli. And the minimum antimicrobial concentration of Ya'an tea and Yunnan Pu'er tea was 24mg/ml to Staphylococcus aureus. However, the MIC of Hunan Fu brick was 12mg/ml. It is showed that the extract from three types of dark Tea displayed antibacterial activity on Digestive bacteria, especially Hunan Fu brick have higher antibacterial activity.

1. Introduction

Dark tea is a kind of traditional tea which is unique to China, and it is also an indispensable drink in the daily life of ethnic minorities in China's border areas. According to the place of origin, dark tea in China is divided into Hunan Fu brick tea, Hubei Green brick tea, Ya'an Tibetan tea, Guangxi Liupao tea and Yunnan Pu'er Tea. Due to the complexity of processing technology, various types of dark tea have also formed their own unique flavor. Modern medical research has shown that dark tea has a variety of health benefits. Such as, supplement dietary nutrition, adding to digestion, removing greasy, smoothing the intestinal tract, anti-hyperlipidemia, anti-obesity, softening human blood vessels, preventing cardiovascular disease; anti-oxidation, attenuating aging; anti-cancer, anti-virus; decreasing blood pressure, decreasing blood glucose; antimicrobial, anti-inflammatory, diuresis and detoxification, etc [1-3]. However, there are few reports on the antibacterial function of dark tea and other basic research in-depth.

This paper mainly studied the inhibitory activity and minimum inhibitory concentration of three kinds of dark tea against Staphylococcus aureus and Escherichia coli, and analyzed the antibacterial effect of dark tea, providing theoretical basis for further utilization of tea extract and development of natural food preservatives.

2. Materials and Methods

2.1 Preparation of water extracts contents of tea

The samples of dark tea (Ya'an tea from Ya'an tea Factory, Hunan Fu brick from Hunan Yiyang tea Factory, Yunnan Pu'er tea from Yunnan Dawn Xinghuo Tea Factory) were crushed, and 19.2 g of all kinds of tea samples were taken. They were soaked in boiling 100ml distilled water, and stirred and placed in aseptic state for 1 hour, and then filtrated. Five concentration gradients with doubling dilution method (192mg/ml, 96mg/ml, 48mg/ml, 24mg/ml, and 12mg/ml) were obtained.

2.2 Bacteriostatic test

Using the method of double-layer plate transparent circle [4]. Each tea sample has five concentration gradients, and the urea Agar (lower layer) which was sterilized by 5-7ml was poured into the sterilized petri dish. After coagulation, four Oxford cups (0.6 cm in inner diameter and 0.6 cm in outer diameter 0.8cm) were quickly placed on the plate to cross shape. Under the aseptic condition, 15-20ml of culture medium mixed with the tested bacteria (1ml 1×10^6 cfu/ml suspension was added into the upper 100ml culture medium) was placed on the plate. After the culture medium was solidified, Oxford cup was taken out and then 200 μ L water extract of three kinds of dark tea was added into each hole, and distilled water sterile was used as control and was marked. After the plates were cultured in 37°C illumination incubator for 20h, and the antibacterial effect was observed and the diameter of the inhibition zone was accurately measured with a Vernier caliper. Each inhibition zone was measured 3 times and averaged. Each concentration was repeated 3 times in parallel [5-8].

3. Results and discussions

3.1 Antibacterial activity of three kinds of dark tea

It can be seen from Tables 1 and 2 that all of Ya'an tea, Hunan Fu brick and Yunnan Pu'er tea had obvious antibacterial effects against *Staphylococcus aureus* and *Escherichia coli*, and the inhibition of *Staphylococcus aureus* is stronger. With the decrease of the concentration of tea extracts, the inhibition zone of Ya'an tea and Yunnan Pu'er Tea changed rapidly, while the reduction of Hunan Fu brick was relatively flat. It can be seen from Fig.1 that the inhibitory zone effect of dark tea on *Staphylococcus aureus* is more obvious than *Escherichia coli*. In contrast, the inhibition of *Escherichia coli* is poor, and the inhibition zone is rather vague, as shown in Fig 2.

Table 1. Antibacterial effect of dark tea on *Staphylococcus aureus* (cm)

Species/ concentration	CK	6mg/ml	12mg/ml	24mg/ml	48mg/ml	96mg/ml	192mg/ml
Ya'an tea	-	-	-	1.248 ± 0.13	1.852 ± 0.09	2.207 ± 0.08	2.564 ± 0.05
Hunan Fu brick	-	-	1.393 ± 0.12	1.768 ± 0.10	2.040 ± 0.09	2.224 ± 0.09	2.473 ± 0.06
Yunnan Pu'er tea	-	-	-	1.362 ± 0.14	1.837 ± 0.10	2.246 ± 0.08	2.586 ± 0.05

Table 2. Antibacterial effect of dark tea on *Escherichia coli* (cm)

Species/ concentration	CK	12mg/ml	24mg/ml	48mg/ml	96mg/ml	192mg/ml
Ya'an tea	-	-	-	1.124 ± 0.12	1.658 ± 0.09	2.069 ± 0.05
Hunan Fu brick	-	-	-	1.328 ± 0.11	1.741 ± 0.08	2.092 ± 0.05
Yunnan Pu'er tea	-	-	-	1.269 ± 0.12	1.787 ± 0.08	2.154 ± 0.05

Note: - indicates no bacteriostatic, the control is sterile water.

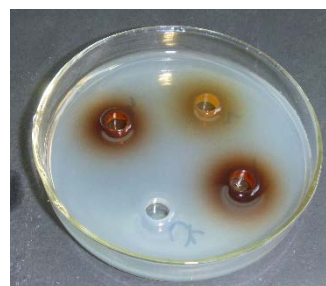


Figure 1. Inhibition effect of dark tea on *Staphylococcus aureus* in 48mg/ml Figure 2. Inhibition effect of dark tea on *Escherichia coli* in 48mg/ml

3.2 Minimum inhibitory concentration (MIC) of three dark teas

According to Table 3 and Table 4, the MIC of three kinds of dark tea against *Escherichia coli* is 48mg/ml. The MIC of Hunan Fu brick was 12 mg / ml against *S. aureus*, while the MIC of Ya'an tea and Yunnan Pu'er tea was 24mg/ml. The difference of antibacterial effects among three kinds of dark tea was supposed to the different processing techniques and origin of the tea.

Table 3. Minimum inhibitory concentration of dark tea against *Staphylococcus aureus*

Species/ concentration	CK	6mg/ml	12mg/ml	24mg/ml	48mg/ml	96mg/ml	192mg/ml
Ya'an tea	-	-	-	+	+	+	+
Hunan Fu brick	-	-	+	+	+	+	+
Yunnan Pu'er tea	-	-	-	+	+	+	+

Table 4. Minimum inhibitory concentration of dark tea against *Escherichia coli*

Species/ concentration	CK	12mg/ml	24mg/ml	48mg/ml	96mg/ml	192mg/ml
Ya'an tea	-	-	-	+	+	+
Hunan Fu brick	-	-	-	+	+	+
Yunnan Pu'er tea	-	-	-	+	+	+

Note: + indicates bacteriostatic, - indicates no bacteriostatic, and the control is sterile water.

4. Conclusion

With the people's life improved, more and more people pay attention to the gastrointestinal diseases caused by intestinal pathogenic bacteria. *Staphylococcus aureus* is a kind of invasive bacteria that produces toxins and is highly destructive to the intestines, causing symptoms such as vomiting, fever, and diarrhea. *Escherichia coli*, is a large group of intestinal bacilli. If the human body is infected with *Escherichia coli*, it will take severe spasmodic abdominal pain and recurrent hemorrhagic diarrhea, accompanied by fever, vomiting and other manifestations [9], which seriously affect the health of the human body. As a daily life drink, tea with rich effective ingredients, has the effect of antivirus and bacteriostatic.

This paper confirmed that Ya'an tea, Hunan Fu brick and Yunnan Pu'er tea all had antibacterial effects against *Staphylococcus aureus* and *Escherichia coli*, especially on *Staphylococcus aureus* and the inhibition zone is obvious. But for *Escherichia coli*, the bacteriostatic effect was slightly worse, and the bacteriostatic zone was vaguer. The minimal inhibitory concentration of the three kinds of dark tea showed that the antibacterial effect of Hunan Fu brick was stronger than other dark tea. According to the research at home and abroad, the polyphenols in tea and its derivatives had strong antibacterial activity, and their antibacterial mechanism was discussed [10]. It has been reported that the active ingredients of tea not only inhibit many kinds of susceptible flora, but also protect and promote the growth of the beneficial bacteria communities along the gastrointestinal tract [11-13].

Acknowledgements

Funding for this research was provided by the Project 063H2300 of Sichuan Agricultural University.

References

- [1] K. B. He, A. L. Jiang, W.Z. Hu, C.H. Liu and G. K. Xu. Processing Technology and Healthcare Functions of Dark Tea [J]. Journal of Anhui Agricultural Sciences, 2012, 40 (27): 13595 - 13597.
- [2] Y. Liang. Research Progress on Chemical constituents and Health Care function of Fu Tea [J]. Newsletter of Sericulture and Tea, 2011 (05): 29 - 31.
- [3] L. L. Wang. Studies on Regulation of Dark Tea on Lipid Metabolism [D]. Hunan Agricultural University, 2012.
- [4] R. Yang, Q. Wang, H. T. Wang, M. J. Xie. Study on bacteriostatic of 10 kinds of Chinese herbal medicines Such as broadleaf holly leaf [J]. Anhui Agricultural Science Bulletin (second half monthly), 2009, 15 (06): 34-36+41.
- [5] D. Zhu, G. C. Niu, X. Y. Sun, X.J. Meng. Study on Antimicrobial Effect of Flavonoids from *Portulaca oleracea* L. [J]. Journal of Anhui Agricultural Sciences, 2006 (01): 7 - 8.
- [6] X. B. Zhang, Y. Zhang, Y. Y. Gao, Y. L. Nie, Y. S. Yu. Bacteriostatic of flavonoids extractive of sweet potato stem and leaf [J]. Food Science and Technology, 2008 (01): 156 - 159.
- [7] S. Ma. Study on Wuyi Rock Tea-polyphenol Research of Wuyi Rock Tea-polyphenol Antibacterial Activity [J]. Journal of Animal Science and Veterinary Medicine, 2012, 31 (01): 24 - 26.
- [8] D. G. Cong, H. Zheng. I Herbivory Effects of Crude Extract of *Heleocharis Tuberosa* Peel [J]. Journal of Henan University of Technology (Natural Science Edition), 2012, 33 (03): 66 - 69.
- [9] Q.Y. Chen. Inhibition function of Tea on harmful bacteria and virus of Human body [J]. Tea Communication, 1998 (04): 31 - 35.
- [10] J. X. Sun, W.J. Wang. Action Mechanism of Antimicrobial Tea Polyphenols on *Pseudomonad* [J]. Meat Research, 2009 (10): 48 - 51.
- [11] H. O.N. Hara, S. Hatano, H. Ichikawa. Effect of tea polyphenols on fecal flora and fecal metabolic products of pigs. J. Vet. Med. Sci [J], 1995, 57 (1): 45 - 49.
- [12] Teerada, H. Hara, S. Nakajyo. Effect of supplements of tea polyphenols on the caecal flora and caecal metabolites of chicks [J]. Microbial Ecol. Health Disease, 1993, (6): 3 - 9.
- [13] T. Okubo, R. Iuneja. Effects of green tea polyphenols on human intestinal microflora [J]. Chemistry and Applications of Green Tea, 1997, (10): 9 - 12.