

Full Length Research Paper

Study on bactericidal effecton fresh *Zanthoxylum bungeanum* juice

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In order to make clear the pepper bactericidal effect of *Zanthoxylum bungeanum*, this study used different concentration of *Zanthoxylum bungeanum* sauce for sterilization test on the tested strains in different treatment time. Ten fold dilution of the tested fungi and fresh Bitter Melon Juice by gradual concentration dilution method was used .They were diluted into different concentrations. The balsam pear juice was mixed respectively with different processing test bacteria. Plate dilution culture method was used to calculate the number of live bacteria and its sterilization rate. The results show that: *Zanthoxylum bungeanum* juice has bactericidal effect on *Aspergillus niger*, yeast, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis* and other bacteria, But with the increase of *Zanthoxylum bungeanum* juice concentration, bactericidal effect was strengthening. With 4% juice p*Zanthoxylum bungeanum* treatment for 60min, the bactericidal effect on *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Aspergillus niger*, and *Candida* is respectively 61%, 57%, 55%, 58%, 52%. The same treatment with 8% *Zanthoxylum bungeanum* juice concentration for 60min resulted in the sterilization rate of 100%; 100%; 99.1%; 99.4%; 99.4%. The sterilization rate varies with the different rates of concentration and action time on different strains of higher concentration, Thelonger the better sterilization effect. This study is significant in the application of *Zanthoxylum bungeanum* and is especially important for treatment of diseases caused by bacteria in agriculture and medicine sterilization.

Keywords: *Zanthoxylum bungeanum* juice; bacteria; bactericidal effect, application

INTRODUCTION

Zanthoxylum bungeanum, belonging to the Rutaceae *Zanthoxylum bungeanum* genus, They bring warmth to minimize pain and has sterilization and antipruritic effect. China is also the biggest producer for *Zanthoxylum bungeanum* cultivation and production .production is , mainly distributed in northern China and tsouth western ,China ,(Not including Inner Mongo and northeastern China ,and xinjiang).But due to the different (Limin et al.,2008) influence of climate rainfall and soil structure and other natural factors ,the chemical composition and content of *Zanthoxylum bungeanum* may be different from place to place.*Zanthoxylum bungeanum* has very high economic value,it not only can be used as medicine ,food additives and insecticide. It

has a wide range of applications .Especially in the field of agriculture, in recent years the research and development of Botanical Fungicide against plant diseases catches many scholars attention and become a hot research field (Sixiao et al., 2006). The development of bio-pesticide with high efficiency, low toxicity,low residue and environmentally friendliness ,become the development direction of new pesticides .According to reports ,*Zanthoxylum* extracts have excellent effect (Sixiao et al., 2006) and are significant in killing virus, bacteria and microorganisms.

Zanthoxylum bungeanum extract has strong secticidal and bactericidal effect in significant inhibition and killing ofbacteria in maize,weevil ,*Tribolium castaneum*,

Aspergillus and Penicillium, This has long been reported (Xiaolong and Yi, 1993). It has significant effect especially in killing virus, bacteria and microorganisms. On the other hand, compared with the antibacterial effect of chemical agent, natural product is more secure. In order to better the development and material. Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Aspergillus and yeast were used as testing strains, to explore its bactericidal effect and to provide the theory basis for further research and development of *Zanthoxylum bungeanum* resources for the benefit of mankind. Utilization of Chinese prickly ash, the green *Zanthoxylum bungeanum* was used as experimental

MATERIALS AND METHODS

Material

Zanthoxylum bungeanum

Zanthoxylum bungeanum (Abbreviation *Z. bungeanum*), purchased from Shapingba District of Chongqing City, Chen Yonghui supermarket.

Test strains

Escherichia coli, *Staphylococcus aureus*, *Bacillus subtilis*, yeast, *Aspergillus niger*, were provided by the Microbial Laboratory of Chongqing Normal University.

Medium

Beef extract peptone medium: beef extract 3g; peptone 10g; NaCl 5g; agar 18g; water 1000mL; PH 7 to 7.2; 121 °C for 30min.

Potato Dextrose Medium: potato 200g; agar 20g; glucose 20g; water 1000mL; 121 °C for 30min. Malt extract medium: malt extract 20g; agar 20g; water 1000mL; 121 °C for 20min (Xinzhi and Junhong, 2010)

Main instruments and equipment

Asepsis room, ultra clean table (SW-CJ-1FD), vertical pressure steam sterilizer (YXQ-LS-100), mold incubator (SHH-250JS), biochemical incubator (SHH-250L), electronic balance (JA5003A), a flask of various types (500mL/250mL/100mL), a Petri dish (90mm/75mm), electromagnetic oven (IC-TW2104), Galanz microwave oven (G80W23YSL-V90), JJ-2 Waring Blender (DS-200) etc..

Methods

Zanthoxylum bungeanum juice preparation

The *Zanthoxylum bungeanum* 16g, 37 °C and use cold boiling water rinse several times, wash and dry the water

reserve, the mortar inside and outside with 75% alcohol wipe disinfection, and sterile water purification, and dried *Zanthoxylum bungeanum* to be ground to powder, and *Zanthoxylum bungeanum* powder in 250mL flask, add 200mL of sterile water to soak about 24h; the soak was conducted with sterile gauze filter and 8% *Zanthoxylum bungeanum* sauce was produced. Then 8% of *Zanthoxylum bungeanum* juice, *Zanthoxylum bungeanum* juice was diluted to obtained solution containing 2%, 4%, 8%. effective components.

Bacterial suspension preparation

Strain activation: will the inoculation in fresh medium for culture of bacteria activation for 24h in 37 °C, and 48h in 28 °C for yeast culture, and 72-96h in 28 °C for mold culture. The various bacteria activated were taken respectively with inoculating loop to a triangular bottle of 100mL by using sterile water and glass beads to wash down, concussion after 10min made 10^{-2} concentration of bacterial suspension, and then used the solution to get concentration using 10 times dilution method[5], all the strains were diluted to respectively for the amount of bacteria: $5.0 \times 10^2 / \text{mL}^{-1}$, $4.3 \times 10^2 / \text{mL}^{-1}$, $3.5 \times 10^2 / \text{mL}^{-1}$), $2.8 \times 10^2 / \text{mL}^{-1}$ concentration gradient. Take $4.3 \times 10^2 / \text{mL}^{-1}$, $3.5 \times 10^2 / \text{mL}^{-1}$, $2.8 \times 10^2 / \text{mL}^{-1}$ three concentration gradients and do plate counts of bacteria, and conduct germicidal test, each concentration was repeated three times test, finally take the average.

Bactericidal effect of different concentrations of *Zanthoxylum bungeanum* juice

The sterilization tube number, divided into two groups of A and B groups. Group A was the experimental group, group B as control group respectively, and number each strain in A and B groups of different concentration. *Zanthoxylum bungeanum* juice solution of 5mL in the A group each tube were added 2%, 4%, 8% concentration, and distilled water equivalent to join in group B as control. Then the concentration of each bacterium liquid with $1 \text{mL } 4.3 \times 10^2 / \text{mL}^{-1}$, $3.5 \times 10^2 / \text{mL}^{-1}$, $2.8 \times 10^2 / \text{mL}^{-1}$ joined the A group number corresponding to the tube, and mixing evenly, and see its bactericidal effect.

Bactericidal effect of *Zanthoxylum bungeanum* in different time

Using plate culture method, respectively, in every 30min, 60min, 90min uses liquid transfer pipe for the mixed bacteria 0.2mL plate culture, culture of bacteria for 24h at 37 °C, culture of yeast for 48h at 28 °C, cultured of mold for 72 ~ 96h at 28°C, afterward, do colony to colony counting, colony number (CFU) in experimental group of A as the colony growth after sterilization, the control group B colony number as not sterilization itself. And calculation of their bactericidal rate

Table 1. Effect of different concentration of *Zanthoxylum bungeanum*(Z.bungeanum) juice processing $4.3 \times 10^2 / \text{mL}^{-1}$ sterilization bacteria liquid 60min

Strain Name	Effect of different concentration of Z.bungeanum juice 60min				different Z.bungeanum juice concentrated sterilization ratio(%)		
	No. of live bacteria ($\times 10^1 \text{ CFU mL}^{-1}$)	2%	4%	8%	contrast	2%	4%
<i>Escherichia coli</i>	28.6	22.6	0	42.6	33%	47%	100%
<i>Staphylococcus aureus</i>	29.4	20.5	0	42.6	31%	52%	100%
<i>Bacillus subtilis</i>	30.7	20.5	4	42.6	28%	52%	99.1%
yeast	29.5	24.0	3	42.6	30%	43%	99.3%
<i>Aspergillus niger</i>	30.0	23.9	3	42.6	29%	44%	99.3%

Table 2. Effect of different concentration of *Zanthoxylum bungeanum*(Z.bungeanum) juice processing $3.5 \times 10^2 / \text{mL}^{-1}$ sterilization bacteria liquid 60min

Strain name	Effect of different concentration of Z.bungeanum juice 60min				different Z.bungeanum juice concentrated sterilization ratio(%)		
	No. of live bacteria ($\times 10^1 \text{ CFU mL}^{-1}$)	2%	4%	8%	Contrast	2%	4%
<i>Escherichia coli</i>	196	134	0	343	43%	61%	100%
<i>Staphylococcus aureus</i>	209	147	0	343	39%	57%	100%
<i>Bacillus subtilis</i>	216	152	3	343	37%	55%	99.1%
yeast	210	141	2	343	38%	58%	99.4%
<i>Aspergillus niger</i>	213	165	2	343	37%	52%	99.4%

Table 3. Effect of different concentration of *Zanthoxylum bungeanum*(z.bungeanum) juice processing $2.8 \times 10^2 / \text{mL}^{-1}$ sterilization bacteria liquid 60min

Strain name	Effect of different concentration of Z.bungeanum juice 60min				different Z.bungeanum juice concentrated sterilization ratio(%)		
	No. of live bacteria ($\times 10^1 \text{ CFU mL}^{-1}$)	2%	4%	8%	contrast	2%	4%
<i>Escherichia coli</i>	148	86	0	274	46%	68%	100%
<i>Staphylococcus aureus</i>	140	90	0	274	49%	67%	100%
<i>Bacillus subtilis</i>	159	95	2	274	42%	65%	99.3%
yeast	167	89	1	274	39%	67%	99.6%
<i>Aspergillus niger</i>	135	84	1	274	50%	68%	99.6%

RESULTS AND ANALYSIS

Bactericidal effect of different concentrations of

Zanthoxylum bungeanum juice

Bactericidal effect of different concentrations of *Zanthoxylum bungeanum* juice at the same time under

the conditions of various strains, each concentration was repeated three test, finally take the average. The results are shown in table 1,2, 3.

From table 1,2,3 data, seen from the table in *Zanthoxylum bungeanum* juice has marked bactericidal effect for various bacteria; when the processing time is 60min, different concentrations of *Zanthoxylum bungeanum* juice concentration after treatment, the sterilization rate; with different concentration, sterilization

Table 4. 4% *Zanthoxylum bungeanum* sauce sterilization solution with $4.3 \times 10^2/\text{mL}^{-1}$ bacteria liquid at different time

Strain name	Live bacteria at different time after th <i>Z.bungeanum</i> Juice ($\times 10^1$ CFU mL^{-1})				<i>Z.bungeanum</i> Juice at different time sterilization rate (%)		
	30min	60min	90min	contrast	30min	60min	90min
<i>Escherichia coli</i>	30.7	20.4	8.6	42.6	28%	52%	80%
<i>Staphylococcus aureus</i>	28.5	20.4	6.8	42.6	33%	52%	84%
<i>Bacillus subtilis</i>	32.4	22.6	11.1	42.6	24%	47%	74%
yeast	29.4	22.6	8.9	42.6	31%	47%	79%
<i>Aspergillus niger</i>	30.7	21.7	9.0	42.6	28%	49%	78%

Table 5. 4% *Zanthoxylum bungeanum* sauce sterilization solution and $3.5 \times 10^2/\text{mL}^{-1}$ bacteria liquid at different time

Strain name	Live bacteria at different time after th <i>Z.bungeanum</i> Juice ($\times 10^1$ CFU mL^{-1})				<i>Z.bungeanum</i> Juice at different time sterilization rate (%)		
	30min	60min	90min	contrast	30min	60min	90min
<i>Escherichia coli</i>	22.0	12.7	6.2	34.3	36%	63%	82%
<i>Staphylococcus aureus</i>	19.9	13.4	6.2	34.3	42%	61%	82%
<i>Bacillus subtilis</i>	22.3	14.7	7.2	34.3	35%	57%	79%
yeast	22.3	14.1	6.4	34.3	35%	59%	81%
<i>Aspergillus niger</i>	21.3	14.7	6.4	34.3	38%	57%	81%

Table 6. 4% *Zanthoxylum bungeanum* sauce sterilization solution and $2.8 \times 10^2/\text{mL}^{-1}$ bacteria liquid at different time

Strain name	Live bacteria at different time after th <i>Z.bungeanum</i> Juice ($\times 10^1$ CFU mL^{-1})				<i>Z.bungeanum</i> Juice at different time sterilization rate (%)		
	30min	60min	90min	contrast	30min	60min	90min
<i>Escherichia coli</i>	13.2	7.4	2.2	27.4	52%	73%	92%
<i>Staphylococcus aureus</i>	14.0	6.6	1.6	27.4	49%	76%	94%
<i>Bacillus subtilis</i>	15.6	9.0	4.9	27.4	43%	67%	82%
yeast	14.2	8.8	4.4	27.4	48%	68%	84%
<i>Aspergillus niger</i>	14.5	8.7	4.4	27.4	47%	68%	84%

rate gets higher. When the concentration was 8%, the sterilization rate can reach more than 99.1%. As the strain is different, because the bacillus and fungi have a certain resistance, therefore bactericidal effect decreases. The less containing bacteria liquid, the better sterilization effect.

Bactericidal effect of different *Zanthoxylum bungeanum* juice with different time processing

Bactericidal effect of the same concentration of *Zanthoxylum bungeanum* juice in processing time under different conditions, each time to do three repeat tests, finally take the average. The results are shown in table 4,5,6.

From table 4, 5, 6 data, seen from the table for different concentrations of *Zanthoxylum bungeanum* juice, the processing time is not the same time, the test on bacteria had obvious bactericidal effect, in the treatment of 30min, the sterilization of *Escherichia coli*,

Staphylococcus aureus, *Bacillus subtilis*, *Candida*, *Aspergillus niger* rates were 52%, 49%, 43%, 48%, 47%. Respectively With the time prolonging the sterilization effect will be better, in 90min treatment, sterilization of *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Candida*, *Aspergillus niger* rates were 92%, 94%, 82%, 84%, 84%. For the sterilization effect on yeast, *Aspergillus niger*, it was inferior to that in non bacillus; Bacillus and fungi (*Candida*, *Aspergillus niger*) due to the structure of the cell wall is different, so the bactericidal effect on *Zanthoxylum bungeanum* juice decreased.

CONCLUSION

i. *Zanthoxylum bungeanum* sauce has significant bactericidal effect for the tested bacteria; different concentration of bacteria has different it sterilization rate. Cases As in the treatment for 60min, $4.3 \times 10^2/\text{mL}^{-1}$, $3.5 \times 10^2/\text{mL}^{-1}$, $2.8 \times 10^2/\text{mL}^{-1}$ concentration of

Escherichia coli have sterilization rates of the concentration of rates 52%, 63%, 73%. But on *Aspergillus niger*, bactericidal effect of yeast is inferior to other bacteria.

ii. With different *Zanthoxylum bungeanum* juice concentration, in dealing with the same time its bactericidal effect is more obvious, The sterilization rate is as higher and higher Up to 99% sterilization rate at the treatment of 90min.

iii. With the same concentration of *Zanthoxylum bungeanum* juice to process bacteria, if the treatment time is different, its antibacterial rate is also different, When the processing time is longer, the sterilization rate is bigger, and the sterilization effect is more obvious.

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