

Study on bactericidal effect of fresh *Zanthoxylum bungeanum* juice

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Abstract. 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 *Zanthoxylum bungeanum* 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, The longer 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.

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 south western ,China ,(Not including Inner Mongo and northeastern China ,and xinjiang).But due to the different [1] 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[2]. 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[3].

In order to better the development and utilization of Chinese prickly as, the green *Zanthoxylum bungeanum* was used as experimental 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.

Materials and methods

Material. Zanthoxylum bungeanum. Zanthoxylum bungeanum (Abbreviation: Z.bungeanum),

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; agar18g; water1000mL ; PH 7 to 7.2; 121 °C for 30min; Potato Dextrose Medium: potato 200g; agar 20g; glucose20g; water1000mL; 121 °C for 30min; Malt extract medium:malt extract 20g ; agar 20g; water1000mL; 121 °C for 20min[4].

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 bungeanum16g 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. Using 10 times dilution method[5-6],all the strains were diluted to respectively for the amount of bacteria : 5.0×10^2 / mL-1 , 4.3×10^2 / mL-1 , 3.5×10^2 / mL-1 , 2.8×10^2 / mL-1 concentration gradient. Take 4.3×10^2 / mL-1 , 3.5×10^2 / mL-1, 2.8×10^2 / 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 1mL 4.3×10^2 / mL-1 , 3.5×10^2 / mL-1 , 2.8×10^2 / mL-1 joined the A group number corresponding to the tube, and mixing evenly, and see its bactericidal effect.

bactericidal effect of Zanthoxylum bungenum 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

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.

Table 1 Effect of different concentration of Zanthoxylum bungeanum(Z.bungeanum) juice processing 4.3 X 10²/ mL-1 sterilization bacteria liquid 60min

Strain Name	Effect of different concentration of <i>Z.bungeanum</i> juice 60min No.of live bacteria (X10 ¹ CFU/mL ⁻¹)				different <i>Z.bungeanum</i> juice concentrated sterilization ratio(%)		
	2%	4%	8%	contrast	2%	4%	8%
<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 X10²/ mL-1 sterilization bacteria liquid 60min

Strain name	Effect of different concentration of <i>Z.bungeanum</i> juice 60min No.of live bacteria (X10 ¹ CFU/mL ⁻¹)				different <i>Z.bungeanum</i> juice concentrated sterilization ratio(%)		
	2%	4%	8%	Contrast	2%	4%	8%
<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 X10²/ mL-1 sterilization bacteria liquid 60min

Strain name	Effect of different concentration of <i>Z.bungeanum</i> juice 60min No.of live bacteria (X10 ¹ CFU/mL ⁻¹)				different <i>Z.bungeanum</i> juice concentrated sterilization ratio(%)		
	2%	4%	8%	contrast	2%	4%	8%
<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%

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 bungenum juice concentration after treatment, the sterilization rate; with different

concentration, sterilization 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.

Table 4. 4% Zanthoxylum bungeanum sauce sterilization solution with 4.3×10^2 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 tim 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 3.5×10^2 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%

From table 4, 5 data, seen from the table for different concentrations of Zanthoxylum bungenum 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 82%, 84%. (In the table 6 94% data have been omitted)

Conclusion

Zanthoxylum bungeanum sauce has significant bactericidal effect for the tested bacteria;

different concentration of bacteria has different its 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.

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.

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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