Full Length Research Paper

Study on bactericidal effect on fresh *Zanthoxylum bungeanum* juice

1Guo Hu and 2Shangqin Hu

1College of Fine Arts Chongqing Normal University, Chongqing, China
2College of Life Science Chongqing Normal University, Chongqing, China

Corresponding Author’s E-mail: hushangqin@163.com

Accepted 20th March, 2014

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 *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 south, 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 of bacteria 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 as, the green Zanthoxylum bungeanum was used as experimental material.

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, Aspergillus and 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 (Xinzi and Junhong, 2010).

Main instruments and equipment

Asepsis room, ultra clean table (SW-CJ-1F), 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 (G80W23YSY-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 X10^2/ mL, 4.3 X10^2/mL, 3.5 X10^2/mL, 2.8 X10^2/mL concentration gradient. Take 4.3 X10^2/mL, 3.5 X10^2/mL, 2.8 X10^2/mL 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 X10^2/ mL, 3.5 X10^2/ mL, 2.8 X10^2/ mL 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 X10^2/mL sterilization bacteria liquid 60min

<table>
<thead>
<tr>
<th>Strain Name</th>
<th>2%</th>
<th>4%</th>
<th>8%</th>
<th>contrast</th>
<th>2%</th>
<th>4%</th>
<th>8%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli</td>
<td>28.6</td>
<td>22.6</td>
<td>0</td>
<td>42.6</td>
<td>33%</td>
<td>47%</td>
<td>100%</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>29.4</td>
<td>20.5</td>
<td>0</td>
<td>42.6</td>
<td>31%</td>
<td>52%</td>
<td>100%</td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>30.7</td>
<td>20.5</td>
<td>4</td>
<td>42.6</td>
<td>28%</td>
<td>52%</td>
<td>99.1%</td>
</tr>
<tr>
<td>yeast</td>
<td>29.5</td>
<td>24.0</td>
<td>3</td>
<td>42.6</td>
<td>30%</td>
<td>43%</td>
<td>99.9%</td>
</tr>
<tr>
<td>Aspergillus niger</td>
<td>30.0</td>
<td>23.9</td>
<td>3</td>
<td>42.6</td>
<td>29%</td>
<td>44%</td>
<td>99.3%</td>
</tr>
</tbody>
</table>

Table 2. Effect of different concentration of Zanthoxylum bungeanum(Z. bungeanum) juice processing 3.5 X10^2/mL sterilization bacteria liquid 60min

<table>
<thead>
<tr>
<th>Strain Name</th>
<th>2%</th>
<th>4%</th>
<th>8%</th>
<th>contrast</th>
<th>2%</th>
<th>4%</th>
<th>8%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli</td>
<td>196</td>
<td>134</td>
<td>0</td>
<td>343</td>
<td>43%</td>
<td>61%</td>
<td>100%</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>209</td>
<td>147</td>
<td>0</td>
<td>343</td>
<td>39%</td>
<td>57%</td>
<td>100%</td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>216</td>
<td>152</td>
<td>3</td>
<td>343</td>
<td>37%</td>
<td>55%</td>
<td>99.1%</td>
</tr>
<tr>
<td>yeast</td>
<td>210</td>
<td>141</td>
<td>2</td>
<td>343</td>
<td>38%</td>
<td>58%</td>
<td>99.4%</td>
</tr>
<tr>
<td>Aspergillus niger</td>
<td>213</td>
<td>165</td>
<td>2</td>
<td>343</td>
<td>37%</td>
<td>52%</td>
<td>99.4%</td>
</tr>
</tbody>
</table>

Table 3. Effect of different concentration of Zanthoxylum bungeanum(Z. bungeanum) juice processing 2.8 X10^2/mL sterilization bacteria liquid 60min

<table>
<thead>
<tr>
<th>Strain Name</th>
<th>2%</th>
<th>4%</th>
<th>8%</th>
<th>contrast</th>
<th>2%</th>
<th>4%</th>
<th>8%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli</td>
<td>148</td>
<td>86</td>
<td>0</td>
<td>274</td>
<td>46%</td>
<td>68%</td>
<td>100%</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>140</td>
<td>90</td>
<td>0</td>
<td>274</td>
<td>49%</td>
<td>67%</td>
<td>100%</td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>159</td>
<td>95</td>
<td>2</td>
<td>274</td>
<td>42%</td>
<td>65%</td>
<td>99.3%</td>
</tr>
<tr>
<td>yeast</td>
<td>167</td>
<td>89</td>
<td>1</td>
<td>274</td>
<td>39%</td>
<td>67%</td>
<td>99.6%</td>
</tr>
<tr>
<td>Aspergillus niger</td>
<td>135</td>
<td>84</td>
<td>1</td>
<td>274</td>
<td>50%</td>
<td>68%</td>
<td>99.6%</td>
</tr>
</tbody>
</table>

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
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 sterilization rate. Cases As in the treatment for 60min, 4.3 $\times 10^5$/mL-1, 3.5 $\times 10^5$/mL-1, 2.8 $\times 10^5$/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 90 min.

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.

REFERENCE


