CHAPTER 4

RESULTS AND DISCUSSIONS

Part 1 is the finding of optimal extraction condition of *B. pandurata* by solvent extraction and hydrodistillation processes. This part evaluated the optimal condition by Disc Diffusion Method, and Broth Microdilution Method. Two methods were usually used for evaluation of inhibition efficiency of microorganism such as *Streptococcus mutans*.

Part 2 was the testing of developed mouth care production mixing with *B. pandurata* extraction from part 1. This part evaluated inhibition efficiency of microorganism in two products by Time kill analysis. Time kill analysis was used to determine the bacterial activity of antimicrobials. This technique was guaranteed the product quality and quantity.

Part 3 was the results from questionnaire in technology transfer workshop. It included the results from general information and technical information.

4.1 Optimal extraction condition of *B. pandurata*

4.1.1 Extraction of *B. pandurata* by solvent extraction

These extracts by solvent extraction process were strong yellow and odor of *B. pandurata*. The inhibition of *S. mutans* ATCC 25175 was applied for decisions of optimal condition including percent of ethanol in solvent, immersing time, and solvent
dilution ratio. The inhibition of *S. mutans* ATCC 25175 was analyzed by Disc Diffusion Method and Broth Microdilution Method.

First, the study was finding of percentage of Ethyl Alcohol and dilution ration (g/ml) between *B. pandurata* and volume of solvent. These findings were fixed immersing time at 1 day. Table 4.1 and Figure 4.1 was the results of inhibition of *S. mutans* ATCC 25175 with *B. pandurata* extract from solvent extraction at 1 day of immersing time, and the amount of *S. mutans* was calculated by Broth Microdilution Method and showed in Table 4.2.

Inhibition of *S. mutans* ATCC 25175 with *B. pandurata* extract from solvent extraction at 1 day of immersing time by Disc Diffusion Method were reported by Minimum inhibitory concentration (MIC). The high values of MIC defined as the high inhibition efficiency of microorganism. The MIC at 40% of Ethyl Alcohol was stability at 0.65 cm for all dilution ratios. The MIC at 60% of Ethyl Alcohol was increased at 1:1 of dilution ratios and decreased when the dilution ratios was high. The pattern of MIC at 80% was similar to 60%.

The best condition for *B. pandurata* extract by solvent extraction was 80% of Ethyl Alcohol and 1:1 solvent dilution ratio. The value of MIC at 80% of Ethyl Alcohol and 1:1 solvent dilution ratio was 0.80 cm. But, the commercial condition concerned on the highest of solvent dilution ratio, the suitable commercial condition was 95% of Ethyl Alcohol, and 1:7 solvent dilution ratios. The value of MIC at 95% of Ethyl Alcohol and 1:7 solvent dilution ratios was 0.70 cm.
Table 4.1  Inhibition of *Streptococcus mutans* ATCC 25175 with *B. pandurata* extract from solvent extraction at 1 day of immersing time by Disc Diffusion Method

<table>
<thead>
<tr>
<th>Percentage of Ethanol in extraction (% v/v)</th>
<th>Minimum inhibitory concentration (MIC) (cm.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dilution Ratio (Extract: Deionized water)</td>
</tr>
<tr>
<td></td>
<td>1:1</td>
</tr>
<tr>
<td>40</td>
<td>0.65±0.05</td>
</tr>
<tr>
<td>60</td>
<td>0.775±0.225</td>
</tr>
<tr>
<td>80</td>
<td>0.8±0.10</td>
</tr>
<tr>
<td>95</td>
<td>0.6±0.00</td>
</tr>
</tbody>
</table>

*Paper Diameter is 0.53 cm*

Figure 4.1 Minimum inhibitory concentrations (MIC) of *S. mutans* ATCC 25175 with *B. pandurata* extract from Solvent extraction at 1 day of immersing time
The increasing of percentage of ethanol in solvent extraction inhibited on the growth of S. mutans ATCC 25175 in Disc diffusion method and Broth Microdilution Method. The results of inhabitation of S. mutans ATCC 25175 with B. pandurata extract at 40%, 60%, 80% and 95% was not significant. The optimal dilution ratio was interested in the high volume and high bacteria inhabitable.

According to the suitable commercial condition of B. pandurata extract by solvent extraction, Table 4.2 was evaluation of the amount of S. mutans ATCC 25175 (Log CFU/ml) at 1:7 of dilution ratio. The amount of S. mutans ATCC 25175 was compare with the control sample without extract. The best condition at 95% of Ethyl Alcohol and 1:7 solvent dilution ratio was 2.60±0.00 Log CFU/ml of S. mutans ATCC 25175. The inhibition standard condition concern at the amount of microorganism less than 4.00 Log CFU/ml and the value must be less than the control sample. The worst condition was still at 40% of Ethyl Alcohol. The occurrence condition at least 4.00 Log CFU/ml was shown at 60% of Ethyl Alcohol. The high percentage of Ethanol in extraction can decreases the growth of S. mutans ATCC 25175.

After the percentage of ethanol extraction and dilution ration was fit at 95% 95% of ethanol extraction and dilution ratio was 1:7 (Extract: Deionized water, v/v), the immerging time was the important parameter must be controlled. Table 4.3 and Figure 4.2 were shown the MIC of 95% of ethanol extraction at several of immerging time. The increasing of immerging time and percentage of ethanol can improve the inhibition of microorganisms. Table 4.3 indicated that the optimal immerging time was 7days and the MIC at 7 days with 1:7 (extract: water) is the least value (1.20 cm.).
Table 4.2  Inhibition of *Streptococcus mutans* ATCC 25175 with *B. pandurata* extract from solvent extraction at 1 day of immersing time and dilution ratio at 1:7 by Broth Microdilution Method

<table>
<thead>
<tr>
<th>Percentage of Ethanol in extraction (% ,v/v)</th>
<th>Amount of <em>Streptococcus mutans</em> ATCC 25175 dilution ratio at 1:7 (Log CFU/ml)</th>
<th>Average (mean ± SD; n=2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No extract (control)</td>
<td>No.1 7.10 No.2 7.13</td>
<td>7.12±0.01</td>
</tr>
<tr>
<td>40</td>
<td>3.38 No.1 3.56 No.2 3.56</td>
<td>3.47±0.13</td>
</tr>
<tr>
<td>60</td>
<td>2.60 No.1 2.70 No.2 2.70</td>
<td>2.65±0.07</td>
</tr>
<tr>
<td>80</td>
<td>2.70 No.1 2.60 No.2 2.60</td>
<td>2.65±0.07</td>
</tr>
<tr>
<td>95</td>
<td>2.60 No.1 2.60 No.2 2.60</td>
<td>2.60±0.00</td>
</tr>
</tbody>
</table>

Table 4.3  Inhibition of *Streptococcus mutans* ATCC 25175 with *B. pandurata* extract from 95% of ethanol extraction at several of immersing time by Disc diffusion method

<table>
<thead>
<tr>
<th>Immerging time (day)</th>
<th>MIC diameter (cm.)</th>
<th>Dilution Ratio (Extract: Deionized water)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1:1</td>
</tr>
<tr>
<td>1</td>
<td>0.6±0.00</td>
<td>0.7±0.00</td>
</tr>
<tr>
<td>3</td>
<td>0.9±0.10</td>
<td>1±0.00</td>
</tr>
<tr>
<td>5</td>
<td>1±0.00</td>
<td>1.1±0.00</td>
</tr>
<tr>
<td>7</td>
<td>1.175±0.075</td>
<td>1.1±0.00</td>
</tr>
</tbody>
</table>
Figure 4.2 Minimum inhibitory concentration of *S. mutans* ATCC 25175 with *B. pandurata* extract from solvent extraction at 95% with 1:7 dilution ratio, and several immersing day

Table 4.4 showed inhibition of *S. mutans* ATCC 25175 with *B. pandurata* extract from 95% of ethanol extraction at several of immersing time. The increasing of immersing time can produced the high efficacy of inhibition in extraction. The best immersing day was 7 days. The extraction at that condition can reduce the amount of *S. mutans* ATCC 25175 from $10^7$CFU/ml to $10^4$CFU/ml. The critical value of inhibition of *S. mutans* ATCC 25175 was $10^4$CFU/ml. Finally, it concluded that the optimal condition of solvent extraction was 95% of ethanol, immersing time at 7 days, and dilution ratio at 1:7 (v/v).
Table 4.4 Inhibition of *S. mutans* ATCC 25175 with *B. pandurata* extract from 95% of ethanol extraction with dilution ratio at 1:7 and several of immersing time by Broth Microdilution Method

<table>
<thead>
<tr>
<th>Immersing time (day)</th>
<th>Amount of <em>Streptococcus mutans</em> ATCC 25175 (log CFU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.1</td>
</tr>
<tr>
<td>No extraction (control)</td>
<td>7.18</td>
</tr>
<tr>
<td>1</td>
<td>2.59</td>
</tr>
<tr>
<td>3</td>
<td>2.67</td>
</tr>
<tr>
<td>5</td>
<td>2.97</td>
</tr>
<tr>
<td>7</td>
<td>2.00</td>
</tr>
</tbody>
</table>

Table 4.5 Inhibition of *Streptococcus mutans* ATCC 25175 with *B. pandurata* Hydrodistillation extract by Disc Diffusion Method

<table>
<thead>
<tr>
<th><em>B. pandurata</em> Hydrodistillation Product</th>
<th>MIC diameter (cm.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dilution Ratio (extraction: ionized water)</td>
</tr>
<tr>
<td>Hydrodistillation temperature (°C)</td>
<td>Product</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>30°C</td>
<td>Oil</td>
</tr>
<tr>
<td></td>
<td>Distillate</td>
</tr>
<tr>
<td></td>
<td>Bottom</td>
</tr>
<tr>
<td>60°C</td>
<td>Oil</td>
</tr>
<tr>
<td></td>
<td>Distillate</td>
</tr>
<tr>
<td></td>
<td>Bottom</td>
</tr>
<tr>
<td>90°C</td>
<td>Oil</td>
</tr>
<tr>
<td></td>
<td>Distillate</td>
</tr>
<tr>
<td></td>
<td>Bottom</td>
</tr>
</tbody>
</table>

NA: It cannot be extracted by hydrodistillation. ND: It cannot detect the amount of bacteria.
4.1.2 Extraction of Boesenbergia pandurata by hydrodistillation

The hydrodistillation product generally received oil, distillate, and bottom produce or residue. Three products were analyzed on inhabitation of S. mutans ATCC 25175 by disc diffusion method. All of laboratory testing results was explained on Table 4.5. The hydrodistillation temperature related to the occurrence of hydrodistillation products. The hydrodistillation temperatures of B. pandurata at 90 °C easily produced oil and distillate. It differed on 30 °C and 60 °C that cannot receive those products. The bottom product of 30 °C and 90 °C cannot inhibit the growth of S. mutans ATCC 25175 but it was different at 60°C. The oil and distillate only receive from the hydrodistillation temperatures at 90 °C and they can inhibit the growth of S. mutans ATCC 25175. The results of this experiments concluded that the optimal extraction temperature for B. pandurata hydrodistillation was 90 °C.

4.2 Testing of developed mouth care with B. pandurata extraction with Time Kill Analysis

Time kill analysis related with time factor that impacted on the inhibition of S. mutans ATCC 25175 in developed tooth care products. This section concerned on the efficiency of inhibition of S. mutans ATCC 25175 in mouthwash and powder toothpaste. The results were shown in Figure 4.3. The inhibition of powder toothpaste A1 and A2 product can inhibit of S. mutans ATCC 25175. The efficiency of inhibition of A1 product was less than A2. The amount of bacteria of A1 product decreased from $10^7$ CFU/ml to $10^5$ CFU/ml while A2 product reduced from $10^7$ CFU/ml to $10^6$ CFU/ml. However, both of the inhibition of mouthwash B1 and B2 product declined from $10^7$ CFU/ml to $10^5$ CFU/ml. The comparison between developed toothpaste and
mouthwash product indicated that the inhibition of *S. mutans* ATCC 25175 of mouthwash product *S. Mutans* ATCC 25175 was better than the powder toothpaste product. The product mixing with *B. pandurata* extract by solvent extraction process was more inhibition than hydrodistillation process because of hydrophobia in oil product.

![Time kill curve analysis](image)

**Figure 4.3** Time kill curve analysis of mouth care products

4.3 Results of technology transfer workshop

These results received from local people in Phisanulok who live near industrial area. The mouth care technology was transfer by workshop. The 60 people joined in this workshop and answer the questionnaire after finish the workshop (Figure
4.4). The answer was analyzed by mathematical program and the results were displayed in Table 4.6 and Table 4.7.

Table 4.6 General Information

<table>
<thead>
<tr>
<th>General Information</th>
<th>Number (person)</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>14</td>
<td>23.3</td>
</tr>
<tr>
<td>Female</td>
<td>46</td>
<td>76.7</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20-29 years</td>
<td>9</td>
<td>15.0</td>
</tr>
<tr>
<td>30-39 years</td>
<td>15</td>
<td>25.0</td>
</tr>
<tr>
<td>40-49 years</td>
<td>14</td>
<td>23.3</td>
</tr>
<tr>
<td>Over 50 years</td>
<td>22</td>
<td>36.7</td>
</tr>
<tr>
<td>Marital Status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>8</td>
<td>13.3</td>
</tr>
<tr>
<td>Married</td>
<td>46</td>
<td>76.7</td>
</tr>
<tr>
<td>Widow</td>
<td>5</td>
<td>8.3</td>
</tr>
<tr>
<td>Divorce</td>
<td>1</td>
<td>1.7</td>
</tr>
<tr>
<td>Career</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Farmers</td>
<td>13</td>
<td>21.7</td>
</tr>
<tr>
<td>Government officer</td>
<td>3</td>
<td>5.0</td>
</tr>
<tr>
<td>Employees</td>
<td>44</td>
<td>73.3</td>
</tr>
<tr>
<td>Income</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lower 2,000 bath</td>
<td>15</td>
<td>25.0</td>
</tr>
<tr>
<td>2,001-5,000 bath</td>
<td>20</td>
<td>33.3</td>
</tr>
<tr>
<td>5,001-10,000 bath</td>
<td>19</td>
<td>31.7</td>
</tr>
<tr>
<td>10,001-15,000 bath</td>
<td>1</td>
<td>1.7</td>
</tr>
<tr>
<td>15,001-20,000 bath</td>
<td>4</td>
<td>6.7</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>1.7</td>
</tr>
<tr>
<td>Total</td>
<td>60</td>
<td>100</td>
</tr>
<tr>
<td>Questions</td>
<td>Level Evaluation</td>
<td>Average</td>
</tr>
<tr>
<td>--------------------------------------------------------------------------</td>
<td>------------------</td>
<td>---------</td>
</tr>
<tr>
<td>The dental disease is the problems in Thailand.</td>
<td>Most (%)</td>
<td>15 (25.0)</td>
</tr>
<tr>
<td></td>
<td>More (%)</td>
<td>33 (55.0)</td>
</tr>
<tr>
<td></td>
<td>Medium (%)</td>
<td>12 (20.0)</td>
</tr>
<tr>
<td></td>
<td>Little (%)</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td>Least (%)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>The oral disease is the chronic problem.</td>
<td>13 (21.7)</td>
<td>34 (56.7)</td>
</tr>
<tr>
<td></td>
<td>3 (5.0)</td>
<td>5 (8.3)</td>
</tr>
<tr>
<td></td>
<td>10 (16.7)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>The application of fluoride can prevent tooth decay.</td>
<td>16 (26.7)</td>
<td>35 (58.3)</td>
</tr>
<tr>
<td></td>
<td>8 (13.3)</td>
<td>1 (1.7)</td>
</tr>
<tr>
<td></td>
<td>1 (1.7)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Inadequate of Thai dentists.</td>
<td>3 (5.0)</td>
<td>26 (43.3)</td>
</tr>
<tr>
<td></td>
<td>26 (43.3)</td>
<td>4 (6.7)</td>
</tr>
<tr>
<td></td>
<td>1 (1.7)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>The high price of dental treatment.</td>
<td>11 (18.3)</td>
<td>36 (60.0)</td>
</tr>
<tr>
<td></td>
<td>8 (13.3)</td>
<td>4 (6.7)</td>
</tr>
<tr>
<td></td>
<td>1 (1.7)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>The mouthwash products are mostly imported.</td>
<td>3 (5.0)</td>
<td>22 (36.7)</td>
</tr>
<tr>
<td></td>
<td>12 (20.0)</td>
<td>22 (36.7)</td>
</tr>
<tr>
<td></td>
<td>1 (1.7)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Are you interested in the herbal mouthwash product?</td>
<td>20 (33.3)</td>
<td>36 (60.0)</td>
</tr>
<tr>
<td></td>
<td>3 (5.0)</td>
<td>1 (1.7)</td>
</tr>
<tr>
<td></td>
<td>1 (1.7)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Herbal mouthwash is cheaper than conventional products.</td>
<td>9 (15.0)</td>
<td>49 (81.7)</td>
</tr>
<tr>
<td></td>
<td>2 (3.3)</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>B. pandurata can be used to produce mouthwash.</td>
<td>6 (10.0)</td>
<td>53 (88.3)</td>
</tr>
<tr>
<td></td>
<td>1 (1.7)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>B. pandurata can reduce bacteria in the mouth.</td>
<td>6 (10.0)</td>
<td>51 (85.0)</td>
</tr>
<tr>
<td></td>
<td>3 (5.0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>If there is a transfer technology in the manufacture B. pandurata mouthwash. Will you be interested in this transfer technology workshop?</td>
<td>4 (6.7)</td>
<td>48 (80.0)</td>
</tr>
<tr>
<td></td>
<td>8 (13.3)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>The production of mouthwash and toothpaste products are promote to local commercial product. Will you be interested to join in technical workshop?</td>
<td>6 (10.0)</td>
<td>36 (60.0)</td>
</tr>
</tbody>
</table>
Table 4.7  Knowledge and Technology Transfer (Continue)

<table>
<thead>
<tr>
<th>Questions</th>
<th>Level Evaluation</th>
<th>Average</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Both of mouthwash and toothpaste products from B. pandurata can increase the household income of local community revenue. Will you be interested to join in technical workshop?</td>
<td>Most (%)</td>
<td>7</td>
<td>11.7</td>
</tr>
<tr>
<td></td>
<td>More (%)</td>
<td>35</td>
<td>58.3</td>
</tr>
<tr>
<td></td>
<td>Medium (%)</td>
<td>10</td>
<td>16.7</td>
</tr>
<tr>
<td></td>
<td>Little (%)</td>
<td>8</td>
<td>13.3</td>
</tr>
<tr>
<td></td>
<td>Least (%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Will you be interested to join in transfer workshop of production of mouthwash and toothpaste products from B. pandurata?</td>
<td>Most (%)</td>
<td>5</td>
<td>8.3</td>
</tr>
<tr>
<td></td>
<td>More (%)</td>
<td>52</td>
<td>86.7</td>
</tr>
<tr>
<td></td>
<td>Medium (%)</td>
<td>3</td>
<td>5.0</td>
</tr>
<tr>
<td></td>
<td>Little (%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Least (%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Will you promote both of mouthwash and toothpaste products from B. pandurata to OTOP?</td>
<td>Most (%)</td>
<td>12</td>
<td>20.0</td>
</tr>
<tr>
<td></td>
<td>More (%)</td>
<td>45</td>
<td>75.0</td>
</tr>
<tr>
<td></td>
<td>Medium (%)</td>
<td>3</td>
<td>5.0</td>
</tr>
<tr>
<td></td>
<td>Little (%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Least (%)</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Most of local people was 23.3% of male and 76.7% of female, generally had got married. The occupation is plant employees, farmer and government officer, sequentially. The age of people was over than 50 years old. The lowest income for local people is lower 2,000 baht per month (25%), most of people receive the income between 2,000 to 10,000 baht per month.

The range of many fundamental questions about dental disease and treatment by dentist received average value around 3.40 to 4.50. It explained that people understood on the problem and basic solutions between moderate and high level. The next question group related with the mouth care and technology transferring. The answers on these questions were average value around 3.00 to 4.25. It showed that the
people in this workshop can understand the techniques for mouth care production technology at moderate and high level.