Antibacterial and Toxicity Activities of Indonesian Herbal Medicine Extracts Used for Postpartum Treatment

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1. Introduction

Bacterial infections are one of the most serious postpartum clinical conditions. According to a WHO report, postpartum bacterial infections lead to over 75,000 maternal deaths worldwide (WHO 2015). These infections are experienced after both vaginal and caesarean section births. Certainly, many factors associated with postpartum infections arise from other complications, such as malnutrition, obesity, diabetes, bacterial vaginosis, and Streptococcus infections. There are many types of bacteria known to cause postpartum infections. For instance, Escherichia coli and Streptococcus equi, subspecies of zooepidemicus, have been reported to cause equine uterine disease in the early postpartum period (Dollen et al. 2019). The same postpartum uterine disease also occurred in dairy cattle (Sheldon and Owens 2017) (Sheldon et al. 2009). These studies link postpartum uterine disease to E. coli, Trueperella pyogenes, Arcanobacterium pyogenes, as well as anaerobic pathogenic bacteria such as Prevotella species, Fusobacterium necrophorum, and Fusobacterium nucleatum (Sheldon and Owens 2017). Additionally, Mycoplasma hominis was responsible for an infection in a 27-year-old female patient following caesarean section (Yamaguchi et al. 2009). Staphylococcus aureus is known to be the bacterium responsible for infections resulting in acute mastitis throughout postpartum lactation periods (Rodriguez and Fernandez 2017). From these reasons, antibiotics are urgently needed to treat pathogenic bacteria during the postpartum period. Interestingly, women in Indonesia are known to consume traditional herbal medicines as postpartum antibiotic treatments.

An herbal medicine called “Jamu bersalin” is one of the folk medicines commonly used for postpartum treatment by women in Asia, especially in Indonesia (Fitriana et al. 2018) and Thailand (Panyaphu et al. 2019).
known for their bioactivity. For example, exploration of Indonesia traditional herbal medicines scientifically based on our previous reports about an diabetic bioactive substances. Evidence of this is in which to find antioxidant, antibacterial, and anti-
countries. Indonesia has abundant natural resources

For the first time, this research aims to investigate
supplement in postpartum treatment. To this end,

2. Materials and Methods

2.1. Material

All of the herbal plants were collected in Jombang, East Java, in Indonesia. The composition of PHM-1 was made up of Phyllanthus urinaria (18%), Curcuma longa (28%), Baeckea frutescens L. (14%), Glycyrrhiza glabra (10%), and Anredera cordifolia (30%). For PHM-

2.2. Preparation of Extracts

PHM-1 (150 g) and PHM-2 (150 g) were both re-extracted using methanol (300 ml) for 24 hours at room temperature. After the 24 hour period, the extracts were filtered using Whatman 41 filter paper. The filtrates then were evaporated using a rotary evaporator to obtain the PHM-1 and PHM-2 crude extracts.

2.3. Antibacterial Assay

The antibacterial activity was assayed in accordance with our previous method (Putri et al. 2018). A working solution was made from mixing 5 μl of a sample solution at a concentration of 100 μg/ml, NB media of 445 μl, and a bacterial suspension of 104 CFU/ml, and then homogenized using a vortex. 150 μl of working solution of was then put
into 96-micro-well plates and incubated using an incubator shaker at room temperature for 18 hours. The inhibitory activity was measured as a percentage using a microplate reader at a wavelength of 630 nm (OD$_{630}$). The experiment was replicated three times, using Chloramphenicol as a positive control and DMSO as a blank.

### 2.4. Evaluation of In Vivo Toxicity in Mice

The in vivo acute toxicity test was performed using parameters described in the guide book of PERKA BPOM RI No. 7/2014 on male and female mice weighing 18 to 25 g purchased from the Faculty of Pharmacy, at the University of Indonesia. The mice were divided into five groups; four were used for testing and one group served as a normal control. Each group of five male and female mice were given four doses dissolved with CMC 0.5 %. Dose amounts were 0.88, 1.75, 3.50, and 7.01 g/kg BW. Before testing, the mice fasted for 10–12 hours. After 24 hours, LD$_{50}$ values were determined using the Weil formula as follows:

$$\text{Log } m = \text{log } D + d \times (f+1)$$

(1)

Where:
- $m$ = LD$_{50}$ value
- $D$ = the smallest dose value
- $d$ = log of dose
- $f$ = Weil table factor

### 2.5. Statistical Analysis

The data obtained consisted of differences between the two groups and were analysed using a one-way analysis of variance (ANOVA). The significant value was set as $\alpha = 0.05$ and the graphical representation was designed by Microsoft Excel 2016.

### 3. Results

#### 3.1. Extraction of Postpartum Herbal Medicines (PHM)

Two yields of crude extracts were determined, as shown in Table 1. The yield of PHM-2 was higher than that of PHM-1. For each of the 150 g samples in 300 ml of methanol, PHM-2 yielded 23.27% extract, while PHM-1 yielded 8.82%. In the present study, PHM-1 and PHM-2 made up postpartum herbal medicines with differing compositions. The exact composition was determined based on previous studies. Based on the present studies, PHM-2 had a higher yield than PHM-1.

#### 3.2. Antibacterial Activity of Postpartum Herbal Medicines (PHM)

The two crude extracts’ antibacterial activity was determined using a broth dilution method. The antibacterial activities are presented in Figure 1 and 2. The results showed that both PHM-1 and PHM-2 at concentrations of 100 μg/ml had positive inhibitory activity against *E. coli*, *S. aureus*, *B. subtilis*, *P. aeruginosa*, *R. palustris*, *R. pickettii*, and *S. epidermidis*, as compared with the positive control, chloramphenicol. PHM-1 was shown to be antibacterial against *E. coli*, *S. aureus*, *B. subtilis*, *P. aeruginosa*, *R. palustris*, *R. pickettii*, and *S. epidermidis* with IC$_{50}$ values of 22.70, 25.51, 25.58, 17.37, 16.30, 11.16, and 91.74 μg/ml, respectively. In addition, PHM-2 showed antibacterial activity against *E. coli*, *S. aureus*, *B. subtilis*, *P. aeruginosa*, *R. palustris*, *R. pickettii*, and *S. epidermidis* with IC$_{50}$ values of 20.51, 23.70, 20.21, 19.11, 11.46, 6.26, and 14.88 μg/ml, respectively (Table 2.). Among these antibacterial activities, the antibacterial activity of both PHM-1 and PHM-2 against *R. pickettii* performed the highest in terms of inhibitory activity, as evidenced by the lowest IC$_{50}$ value, indicating good antibacterial activity against *R. pickettii* for both.

#### 3.3. Acute Toxicity in Mice Exposed to Postpartum Herbal Medicines (PHM) In Vivo

The in vivo acute toxicity test was performed using five groups of mice in the methodology described above. The results showed a several of deaths. After introducing the smallest to largest dose, deaths after 24 hours for male mice were 0, 0, 1, and 2, and 0, 1, 1, and 2 for female mice. LD$_{50}$ value was then
Figure 1. Antibacterial activities of PHM-1 against (a) *E. coli*, (b) *S. aureus*, (c) *B. subtilis*, (d) *P. aeruginosa*, (e) *R. palustris*, (f) *R. pickettii*, and (g) *S. epidermidis*
Figure 2. Antibacterial activities of PHM-2 against (a) *E. coli*, (b) *S. aureus*, (c) *B. subtilis*, (d) *P. aeruginosa*, (e) *R. palustris*, (f) *R. picketti*, and (g) *S. epidermidis*
determined using the Weil formula. The LD$_{50}$ value for both male and female mice was 3.28 g/kg BW. Thus, there is a slight potential for acute toxicity in male and female mice.

4. Discussion

Dried powder samples of 150 g of PHM-1 and PHM-2 were re-extracted using methanol, as it has shown to be an effective solvent to extract all of a substance from herbal plants. In accordance with our previous studies, methanol shows the best biological activity of all extractors used, including n-hexane, dichloromethane, ethyl acetate, and water (Putri and Fatmawati 2019) (Hidayati et al. 2017) (Fitriana et al. 2016) (Khumaidah et al. 2018). Differences in extract yielded are known to be a result of their unique compositions. For example, PHM-2 might have a large number of chemical substances as well as secondary metabolites. Methanol is also understood to be a solvent that can extract polar compounds well. Therefore, PHM-1 and PHM-2 extract may contain polar compounds, the potential of which ought to be investigated in the next study.

Based on these results, PHM-1 and PHM-2 both strongly inhibit Gram-positive and Gram-negative bacteria. To reiterate, E. coli and S. aureus are the Gram-negative and Gram-positive bacteria, respectively, that commonly cause postpartum infections (Dollen et al. 2019) (Rodriguez and Fernandez 2017). Generally speaking, these bacteria have different morphological structures. Gram-negative bacteria have a three-layer plasma membrane making them more complex than Gram-positive bacteria. This thicker membrane does not make it easy for chemical substance to enter the cell wall. However, PHM-1 and PHM-2 showed high percentages of inhibition against both E. coli and P. aeruginosa, compared with the positive control. Therefore, PHM-1 and PHM-2 contain bioactive chemical substances.

Interestingly, this is the first known report investigating the antibacterial effectiveness of PHM-1 and PHM-2. Our present study showed that both had high levels of inhibitory activity against R. pickettii, a pathogenic Gram-negative bacteria that can grow in various water sources (Ryan and Adley 2013). R. pickettii was reported to be found in several drinking and hospital water supplies, as well as in industrial-grade purity water. Furthermore, both hot and cold water is used as the main component of pharmaceutical preparation for people to consume herbal medicines. As we know, water is a vital necessity for human life. Therefore, PHM-1 and PHM-2 have great potential for use as an antibacterial against R. pickettii. However, no sufficient data exist about the antibiotic resistance of R. pickettii over the course of a few decades.

Based on previously reported studies, ten plants in northern Thailand were selected for use as postpartum herbal bath medicines (Panyaphu et al. 2012). Among these plants, only two plants showed antibacterial activity against S. aureus strains, namely Schefflera bengalensis and Plumbago indica. However, the effect was weak, with minimum inhibitory concentration (MIC) values of 0.73 and 1.45 mg/ml, respectively. In another example, E. coli, pathogenic bacteria widely known to cause postpartum uterine infections in equine (Dollen et al. 2019) and dairy cattle (Sheldon and Owens 2017), was not inhibited by ceftiofur. This common antibiotic is used for the early postpartum treatment of mares, and was not shown to be effective in decreasing bacterial growth of E. coli at a concentration of 0.5 μg/ml (Dollen et al. 2019). In contrast, this study successfully revealed that Indonesian herbal medicines exhibit excellent antibacterial activity.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Chloramphenicol (positive control)</th>
<th>PHM-1 Inhibition (%)</th>
<th>IC$_{50}$ (μg/ml)</th>
<th>PHM-2 Inhibition (%)</th>
<th>IC$_{50}$ (μg/ml)</th>
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</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>100.00</td>
<td>99.69</td>
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<td>S. aureus</td>
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<td>97.41</td>
<td>25.51</td>
<td>97.89</td>
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<td>B. subtilis</td>
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<td>101.25</td>
<td>25.58</td>
<td>92.33</td>
<td>20.21</td>
</tr>
<tr>
<td>P. aeruginosa</td>
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<td>98.49</td>
<td>17.37</td>
<td>98.72</td>
<td>19.11</td>
</tr>
<tr>
<td>R. palustris</td>
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<td>94.63</td>
<td>16.30</td>
<td>94.48</td>
<td>11.46</td>
</tr>
<tr>
<td>R. pickettii</td>
<td>99.81</td>
<td>100.39</td>
<td>11.16</td>
<td>96.80</td>
<td>6.26</td>
</tr>
<tr>
<td>S. epidermidis</td>
<td>100.70</td>
<td>62.47</td>
<td>91.74</td>
<td>97.18</td>
<td>14.88</td>
</tr>
</tbody>
</table>

*Concentration samples were 100 μg/ml

Table 2. Antibacterial activities of Indonesia postpartum herbal medicines
We also found a structure-activity relationship between the PHM substances and antibacterial activity. PHM-1 and PHM-2 both contain more *C. longa* powder than others tested. This suggests that its chemical substances might play a primary role in pathogenic bacteria inhibition. Previously, *C. longa* has been reported as an antibacterial agent working against *S. aureus* (Gupta et al. 2015) (Niamsa and Sittiwet 2009) and *E. coli* (Niamsa and Sittiwet 2009) with significant inhibitory effects. Data from this present study also support this and furthermore, indicate that the Indonesian herbal medicines PHM-1 and PHM-2 should be considered as viable antibacterial postpartum treatments for infection.

In addition, we also reported on the acute toxicity effect of PHM-1. Acute toxicity refers to the investigation of the toxic effect present from a natural or synthetic product consumed by people. This test is a crucial part of herbal medicine discovery and development related to finding safe drugs. Currently, very little information about the toxicity of some herbal medicines exists (Sharwan et al. 2015). For this reason, toxicity testing of postpartum herbal medicines is urgently needed. The toxicity test, one of several pre-clinical studies, is expressed using LD₅₀ values. LD₅₀ is the median lethal dose required to kill half of the mice tested. In this present study, an acute toxicity test was done by Weil method. As mentioned above, the effect of PHM-1 on both male and female mice was slightly toxic. In addition, the results showed mortality occurring after a test period of 24 hours. Consequently, autopsies were performed and showed that the size, shape, and colour of vital organs compared to the normal control group were not differently affected. Therefore, it was not necessary to continue with histological organ tests. Based on these results, herbal medicine from Indonesia can be safely consumed by women in the first 40 days of the postpartum period.

5. Conclusion

This was the first report on antibacterial activities of PHM-1 and PHM-2. Excellent antibacterial activity against pathogenic bacteria including *S. aureus*, *B. subtilis*, *E. coli*, *P. aeruginosa*, *R. palustris*, *R. pickettii*, and *S. epidermidis* was shown. In addition, acute toxicity testing indicated low risk for human consumption. In conclusion, the postpartum herbal medicines from Indonesia called PHM-1 and PHM-2 are recommended for postpartum treatment. Further research is needed to reveal the bioactive chemical substances at work and should be pursued for future study.

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References


