Antibacterial and Toxicity Activities of Indonesia herbal medicine extracts used for a postpartum treatment

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Abstract

A postpartum treatment has been recognized by local women in Indonesia since a long time ago. This treatment was commonly used with consuming of traditional postpartum herbal medicines during the first 40 days after childbirth called PHM-1 and the next 40 days after that namely PHM-2 as well. However, there is no sufficient evidence about this postpartum herbal medicine scientifically. In this study, antibacterial activities of the PHM-1 and PHM-2 was evaluated for the scientific evidence. The results showed that both of PHM-1 and PHM-2 were potential to be antibacterial with very low IC_{50} values against pathogenic bacteria such as *Escherichia coli*, *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Rhodopseudomonas palustris, Ralstonia pickettii,* and *Staphylococcus epidermidis* compared with chloramphenicol as a positive control. Among these antibacterial activities, the antibacterial activity of both PHM-1 and PHM-2 against *R. pickettii* has the highest inhibitory activity with the lowest IC_{50} values of 11.16 and 6.26 µg/mL, respectively. Furthermore, an *in vivo* toxicity acute test showed that PHM-1 has low LD_{50} for both of male and female mice with LD_{50} value of 3.28 g/kg BW. These results implied that both of PHM-1 and PHM-2 are recommended as antibacterial for women postpartum treatment.

Keywords: antibacterial, toxicity, IC_{50}, LD_{50}, postpartum treatment, herbal medicines.

Introduction

A postpartum infection is one of postpartum clinical conditions caused by bacterial infections. According to WHO's report, these postpartum bacterial infections are to be serious disease leading of over 75,000 maternal deaths worldwide [1]. Either by vaginal birth or caesarean section, the bacterial infections could be experienced for during childbirth and postpartum condition. Certainly, many factors have been associated with postpartum infections because of other complications such as malnutrition, obesity, diabetic, bacterial vaginosis, and group *Streptococcus* infections. There are many types of bacteria related to postpartum infections. For instance, *Escherichia coli* and *Streptococcus equi* which are subspecies *zooepidemicus* had been reported as causing of equine uterine disease in early postpartum period [2]. The postpartum uterine disease also happened in dairy cattle [3] [4]. These studies reported that postpartum uterine disease is related to *E. coli*, *Trueperella pyogenes*,

Arcanobacterium pyogenes, and anaerobic pathogenic bacteria such as *Prevotella* species, *Fusobacterium necrophorum*, and *Fusobacterium nucleatum* [3]. Moreover, *Mycoplasma hominis* infection affected on a 27-year-old female patient after caesarean section [5]. In addition, *Staphylococcus aureus* is the main bacterium of acute mastitis throughout a lactation period related with postpartum condition as well [6]. From these reasons, antibiotic is urgently needed against pathogenic bacteria during on postpartum period. Indeed, women in Indonesia have consumed traditional herbal medicines as antibiotic for their postpartum treatment.

The postpartum herbal medicine called as "Jamu bersalin" in Indonesia, is one of the folk medicines commonly used for the postpartum treatment. These herbal medicines are widely used by women during postpartum period in Asia area especially in Indonesia [7] and Thailand [8]. Definitely, the herbal medicine is the best choice as an alternative medicine for local people in the developing countries. On the other hand, Indonesia has a huge of natural resources as bioactive substances; antioxidant, antibacterial, and antidiabetic. These evidences are scientifically based on our previous reports about an exploration of Indonesia traditional herbal medicines through their bioactivities. Currently, *Chromolaena odorata* known as a folk medicine in Ambon-Indonesia had been revealed as a potent antioxidant [9] and other natural antioxidants including *Syzygium Polyanthum* [10], *Moringa oleifera* [11] as well as *Cajanus cajan* [12]. In addition, *S. polyanthum* [13], and *Sonneratia ovata* [14] was reported as antibacterial and *Garcinia mangostana* as antidiabetic agent [15]. These evidences implied that Indonesia herbal medicine might be potential as a good health supplement with a low side effect for women in postpartum treatment.

Based on our previous studies, the composition of various herbal components was evaluated based on a preliminary radical scavenging assay. There were two postpartum herbal composition types. Each of types consist of five composition groups. The first type was postpartum herbal medicine 1 (PHM-1) which was used to consuming by women for the first 40 days after childbirth. The second type was postpartum herbal medicine 2 (PHM-2) for the next 40 days. The results showed that both the PHM-1 and the PHM-2 had the lowest an inhibitory activity concentration of 50% (IC_{50}) against both DPPH and ABTS of other group compositions [7]. Hence, these results implied that the PHM-1 and PHM-2 compositions are recommendation for the best composition of Indonesia postpartum herbal medicines. Furthermore, both the PHM-1 and PHM-2 composition, *Curcuma longa* is the most substance of others. These evidences inferred that the best postpartum herbal medicine should contain a lot of *C. longa*. Moreover, many published report presented that *C. longa* showed significant pharmacological effects such as antimicrobial [16] [17], antifungal [18], antidiabetic [19], antimalarial [20], and anti-inflammatory [21] as well. Definitely, *C. longa* is recommended as a health supplement for the postpartum treatment.

For the first time, we have investigated antibacterial activities of Indonesia postpartum herbal medicines namely the PHM-1 and PHM-2 samples for more their scientific evidences. The antibacterial activity assay had been done against pathogenic bacteria of Gram-positive bacteria

such as *S. aureus*, *Bacillus subtilis*, and *Staphylococcus epidermidis* as well as Gram-negative bacteria such as *E. coli*, *Pseudomonas aeruginosa*, *Rhodopseudomonas palustris,* and *Ralstonia pickettii*.

Experimental

Materials

All of the herbal plants were collected in Jombang, East Java, Indonesia. The composition of PHM-1 contains *Phyllanthus urinaria* (18%), *Curcuma longa* (28%), *Baeckea frutescens L.* (14%), *Glycyrrhiza glabra* (10%), and *Anredera cordifolia* (30%). For the PHM-2, the compositions are *Zingiber montamun* (20%), *Zingiber zerumbet* (10%), *Tamarindus indica* (18%), *Quercus infectoria* (24%), and *Curcuma longa* (28%). Methanol and dimethyl sulfoxide (DMSO) were used as organic solvents. The antibacterial assay materials were supported with nutrient agar (NA), nutrient broth (NB), chloramphenicol as a positive control, and stock of bacteria *S. aureus*, *B. subtilis* (NBRC 3009), *E. coli* (NBRC 3301), *P. aeruginosa* (NBRC 3080), *R. palustris* (NBRC 16661), *R. pickettii* (NBRC 102503), and *S. epidermidis* (NBRC 12993). These bacteria were collected from Laboratory of Microbial Chemistry, ITS. For *in vivo* non-clinical toxicity test, it used male and female mice with body weight range of 18 – 25 g. These mice were obtained from Laboratory of pharmacy, UI.

Methods

Preparation of Extracts

The PHM-1 (150 g) and PHM-2 (150 g) were respectively re-extracted by using methanol (300 mL) for 24 hours at room temperature. After the 24 hours, the extracts were filtered by filter paper Whatman 41. Then, the filtrates were evaporated by rotary evaporator to obtain the PHM-1 and PHM-2 crude extracts.

Antibacterial Assay

The antibacterial activity was assayed by our previous method [22]. Working solution was made from mixture of sample solution of 5 µL at concentration of 100 µg/mL, NB media of 445 µL, and bacteria suspension at 10^4 CFU/mL, which homogenized by using a vortex. The working solution of 150 µL was put into 96-micro-well plate and incubated by using incubator shaker at room temperature for 18 hours. The inhibitory activity (%) was measured by using microplate reader at a wavelength of 630 nm (OD_{630}). Chloramphenicol was used as positive control and DMSO as a blank, which experiment was done in triplicate.

Evaluation of *in vivo* Toxicity in Mice

The *in vivo* toxicity acute test was performed based on guide book of PERKA BPOM RI No. 7/2014. Experiments were implemented on male and female mice weighing from 18 to 25 g purchased from Faculty of Pharmacy, Universitas Indonesia. The mice were divided into five groups. The four groups were used as test and one group as a normal control. Each group including five of male and female mice respectively was induced with four doses which were dissolved in CMC 0.5 %. Those doses are 0.88, 1.75, 3.50, and 7.01 g/kg BW. Before testing, the mice were fasted about 10 – 12 hours. After that, all the tested mice were induced the sample as the four doses. After 24 hours, LD_{50} value was determined with Weil formula as follow:

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

Where, m : LD_{50} value, D : the smallest dose value, d : log of dose, and f : Weil table factor.

Statistical Analysis

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

Results and Discussion

Extraction of Postpartum Herbal Medicines (PHM)

The two yields of crude extracts had been determined as shown in Table 1. The yield of PHM-2 is higher than that of the PHM-1. From each of samples (150 g) in 300 mL methanol, the PHM-2 had the yield of 23.27 %, while the PHM-1 had 8.82 %. In present study, the PHM-1 and PHM-2 are the postpartum herbal medicines with different composition. The determination of composition had been determined based on our previous studies [7]. Based on our present studies, the PHM-2 yield has the higher yield than that of the PHM-1.

Antibacterial Activity of Postpartum Herbal Medicines (PHM)

The antibacterial activity of two crude extracts was determined by broth dilution method [22]. The antibacterial activities are presented in Figure 1 and 2. The results showed that both of the PHM-1 and PHM-2 at concentration of 100 µg/mL had good inhibitory activity against *E. coli*, *S. aureus*, *B. subtilis*, *P. aeruginosa*, *R. palustris*, *R. pickettii*, and *S. epidermidis* compared with chloramphenicol as positive control. The results indicated that the PHM-1 is potential

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. On the other hand, the PHM-2 had the antibacterial activities 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 inhibitory activity. It was shown with the lowest IC_{50} value. Hence, both of them have a good antibacterial activity against *R. pickettii*.

In vivo Toxicity Acute in Mice of Postpartum Herbal Medicines (PHM)

The *in vivo* toxicity acute test was performed for five groups of mice. The four groups were used as test and one group as a normal control. Each group including five of male and female mice weighing from 18 to 25 g. Each of mice was induced with four doses (0.88, 1.75, 3.50, and 7.01 g/kg BW) which were dissolved in CMC 0.5 %. The results showed that a number of deaths based on the inducing from the smallest to largest dose after 24 hours was for male mice 0,0,1,2 and for female mice 0,1,1,2. Then, LD_{50} value was determined by Weil formula. The LD_{50} value for both of male and female mice was 3.28 g/kg BW. Thus, based on this result, the potential for acute toxicity in male and female mice is slightly toxic [23].

Discussions

The dried powder of PHM-1 and PHM-2 samples of 150 g was re-extracted by using methanol. Methanol is an effective solvent to extract all of substance from the herbal plants. According to our previous studies, the methanol extract showed the best biological activity of all used extracts; *n*-hexane, dichloromethane, ethyl acetate, and water [9] [14] [10] [11]. Definitely, the differences of yield are caused by the compositions. Hence, the postpartum herbal medicine (PHM-2) might have a lot of chemical substances and secondary metabolite as well. Furthermore, methanol is a polar solvent which can extract polar compounds also very well. Therefore, the PHM-1 and PHM-2 extract might have the polar compounds. These compounds are potent to reveal for the next study.

Based on these results, both of the PHM-1 and PHM-2 are very strong to inhibit Gram-positive and Gram-negative bacteria. *E. coli* and *S. aureus* are the Gram-negative and Gram-positive bacteria, respectively, that commonly related to infection after childbirth [2] [6]. Generally, Gram-positive and Gram-negative bacteria have different morphological structures. The Gram-negative bacteria have more complex morphological structure rather than of the Gram-positive bacteria. There are three layers of plasma membrane in the Gram-negative bacteria. It does not make easy for the chemical substance to enter the cell wall. However, the PHM-1 and PHM-2 could inhibit both of *E. coli* and *P. aeruginosa* very well. They showed high

percentage of inhibition compared with the positive control. Therefore, the PHM-1 and PHM-2 have bioactive chemical substances.

Interestingly, this is the first report about antibacterial activity of the PHM-1 and PHM-2. Based on our present studies, both of them showed the highest inhibitory activity against *R. pickettii*. *R. pickettii*, known as Gram-negative bacteria, is one pathogenic bacteria that can grow in various water sources [24]. However, there is no sufficient data about the antibiotic resistance of *R. pickettii* for few decades. As we know, water is a vital necessary for human life. Besides, *R. pickettii* was reported that they have been found in several drinking water supplies, hospital water supplies, and industrial purity water. Furthermore, either hot or cold water are the main component of pharmaceutical preparation for people to consume the herbal medicine. Therefore, PHM-1 and PHM-2 are potential to be the new antibacterial against *R. pickettii*.

Based on previous reported studies, ten selected plants in northern Thailand were used as postpartum herbal bath medicines [8]. Among ten selected plants, only two plants showed antibacterial activity against *S. aureus* namely *Schefflera bengalensis* and *Plumbago indica*. However, both of them did not significantly exhibit it with minimum inhibitory concentration (MIC) values of 0.73 and 1.45 mg/mL, respectively. Furthermore, *E. coli* was reported as pathogenic bacteria which are widely found in some animal postpartum uterine infection cases in equine [2] and dairy cattle [3] for example. Moreover, ceftiofur, a common antibiotic used for early postpartum treatment of mares, was not effective to decreasing bacterial growth of *E. coli* at concentration 0.5 µg/mL [2]. Hence, this present study successfully revealed that Indonesia herbal medicines have a good antibacterial activity.

In this present study, we also revealed that there is a structure-activity relationship between the PHM substances and antibacterial activity. Both of the PHM-1 and PHM-2 consist of much more *C. longa* powder than others. It means *C. longa* chemical substances might be a main role to inhibit the pathogenic bacteria. Scientifically, *C. longa* has been reported as an antibacterial against S*. aureus* [17] [25] and *E. coli* [25] with significant effect. Hence, this present study successfully revealed that Indonesia herbal medicines named PHM-1 and PHM-2 should be serve as antibacterial for postpartum infection treatment.

In addition, we also reported toxicity acute effect of PHM-1. The toxicity acute study means the investigation of the toxic effect from the natural or synthetic products consumed by people. This study is an important part of herbal medicine discovery related to finding safe drugs. Currently, there was very little information about the toxicity of some herbal medicines [26]. For this reason, the toxicity test of postpartum herbal medicine is also urgently determined. The toxicity test, one of pre-clinical studies, is expressed with LD_{50} value. LD_{50} is the median lethal dose which means the amount of the extract required to kill half of the test mice. In this present study, the toxicity acute test was done by Weil method. As mentioned before, the toxicity acute effect of PHM-1 for the male and female mice is slightly toxic. In addition, the results showed that there was mortality after test time of 24 hours. So, an autopsy was performed. The autopsy results presented that there were no difference effects in size, shape, and colour of vital organs

compared to the normal control group. Therefore, the histological organ test is not continued. Based on these results, the first 40 days of postpartum herbal medicine from Indonesia is safe for consumed by women after childbirth.

Conclusion

In conclusion, the postpartum herbal medicines from Indonesia called PHM-1 and PHM-2 were recommended for the postpartum treatment. This was the first report on antibacterial activities of the PHM-1 and PHM-2. They showed very good antibacterial activities against pathogenic bacteria including *S. aureus*, B. subtilis, *E. coli*, *P. aeruginosa, R. palustris, R. pickettii,* and *S. epidermidis*. Thus, the determination of bioactive chemical substances should be revealed for the future study.

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