

The Relationship of Secondary Metabolites: A Study of Indonesian Traditional Herbal Medicine (Jamu) for Post Partum Maternal Care Use

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The Relationship of Secondary Metabolites: A Study of Indonesian Traditional Herbal Medicine (Jamu) for Post Partum Maternal Care Use

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Abstract. Indonesian traditional herbal medicine for post partum contains many plants such as Kunyit (*Curcuma longa*), meniran (*Phyllanthus urinaria*), akar manis (*Glycyrrhiza glabra*), lempuyang (*Zingiber zerumbet*), temulawak (*Curcuma zanthorrhiza*), jungrahap (*Baeckea frutescens* L.), bangle (*Zingiber montanum*), binahong (*Anredera cordifolia*), asem (*Tamarindus Indica*). These plants are important sources of phenolic compounds such as flavonoid, quercetin, kaempferol that potentially act as antioxidant. Antioxidants are important substances which possess the ability to protect the body from damage caused by free radical oxidative stress. The post partum herbal medicine were extracted using aquades. The antioxidant activity of extracts was evaluated using 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity assay and an improved (2,2'-azino-bis-[3-ethylbenzothiazoline sulphonate]) ABTS radical cation decolorization assay using *in vitro*. Trolox was used as the standard with IC_{50} 5.89 $\mu\text{g/mL}$ in DPPH assay and 3.06 $\mu\text{g/mL}$ in ABTS assay. The variant B1 showed the highest free radical scavenging activity (IC_{50} = 28.37 $\mu\text{g/mL}$ in DPPH assay and IC_{50} = 27.17 $\mu\text{g/mL}$ in ABTS assay). The variant E2 also showed the highest free radical scavenging activity (IC_{50} = 29.56 $\mu\text{g/mL}$ in DPPH assay and IC_{50} = 26.16 $\mu\text{g/mL}$ in ABTS assay). This study indicated that Indonesian traditional herbal medicine possess antioxidant activity and it needs to be investigated more for other bioactivity such as antimicrobial and anticholesterol.

Keywords: post partum herbal medicine, Indonesian traditional herbal medicine, phenolic compounds, DPPH, ABTS

INTRODUCTION

Jamu is a traditional herbal medicine in Indonesia. Most of the Indonesian people especially in Java use *jamu* as traditional medicines derived from plants. The academics, industry and Indonesian government all recognize that the further development of *jamu* production is needed. The Indonesian government divide the preparation of *jamu* into three steps, *jamu*, standardized herbal medicines and fitomarka [1]. There are many researches to be done to establish the safety and efficacy of *jamu* productions; the raw material consists of simplicia or galenic preparations that have been fulfilled in applicable terms.

The post partum herbal medicine is a herb of some medicinal plants used for postpartum care after delivery. The puerperium is a period that begins after the birth of the placenta and ends when the uterus returns as it was before pregnancy and lasted for about 6 weeks. During the postpartum period many changes occur in women either anatomically, physiologically, or psychologically. There is correlation between the changes that occur and the women who take post partum herbal frequently during the first month after delivery [2]. The change is in fact not uncommon to cause complaints and problems for post partum mothers. Therefore, care and monitoring of the condition of the mother is needed so that the mother could avoid the possibility of problems or complications that follow during childbirth. Common complications of postpartum women with Pre Eclamps include: high blood pressure, excessive weight gain, swollen legs, headache and visual impairment [3].

Javanese people usually consume special maternity herbal medicine passed down from generation to generation by the ancestors. This herbal medicine consists of several types of herbs that are drunk in sequence; starting from herbal papeja, laxative, charcoal aring, bangkes, sambedan, selokarang, white pomegranate. The efficacies of this birth herbal medicine are, removing the blood of childbirth until clean, launching milk, overcoming stiffness, feeding disorders, irregular menstruation, helping the body to reduce fat, and eliminating unpleasant body odor, preventing eating disorders, infections, sick and cough [4].

This herbal medicine consists of several medicinal plants such as majakani, turmeric, dinglo bengle, red and white pomegranate. Each of these plants has different phytochemical content. Manjakani fruit contains tannins, vitamins A

and C, iron, and carbohydrates that can increase antioxidant activity. The white pomegranate contains flavonoids, saponins, tannins, and triterpenoids. The Madurese use this ingredient in a mixture of post partum herbs medicine because it is useful to overcome leucorrhoea. Other benefits of white pomegranate are that it is antibacterial, antihypertensive, antidiabetes, anticancer prostate; and it also acts as a cough medicine [5]. The content of phytochemicals in this herbal medicine makes it rich in efficacy. High quality herbal ingredients and good phytochemical content will lead to the synergism of some extracts of this post partum herb material.

7 The use of herbal medicine has not been standardized and there has been no scientific research. The results showed that it is still difficult to determine its accuracy in compounding the composition of herbal medicine. The community uses a handful of dossiers, a sheet and aberration based on the experience of the ancestors [6]. The composition of this herb has an effect on the effectiveness of the activity and toxicity of post partum herbal medicine. Based on Health Ministerial Decree no. 381 of 2007 on National Traditional Medicines Policy, a scientific research is needed to ensure a safe and beneficial traditional medicine and to protect it from inappropriate use. Therefore, research and development on the effectiveness of the composition of this herbal medicine is one of the efforts to preserve the culture of the nation.

The purpose of this research is to screen secondary metabolites and the activity of antioxidants from the composition variation of post partum herbal medicine based on scientific evidence.

MATERIALS AND METHODS

Materials

Plant materials were collected from East Java. The composition of post partum traditional herbal medicine 1 consists of *meniran*, *kunyit* (turmeric), *jungrahap*, *akar manis* (licorice), *binahong*. The composition of post partum traditional herbal medicine 2 consists of Bangle, *lempuyang*, *asem* (tamarind), majakani, *kunyit* (turmeric). The solvent aquades was purchased from Bratachem, DMSO and the methanol and ethanol were purchased from Wako Pure Chemical Industries Japan. The DPPH and ABTS reagent were purchased from Wako (Japan). Trolox (6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid; Sigma Aldrich) was used as the antioxidant standard. All other reagents were of analytical grade.

General Experimental Procedures

The preparation of post partum traditional herbal medicine has to adhere to safety criteria. We used both online and offline literature sources in selecting the raw materials and information about the productions of post partum traditional herbal medicine. Interviews on plant composition used for post partum traditional herbal medicine were conducted on traditional herbal medicine sellers in Jombang, Madura and Surabaya. Based on the results of the survey and literature study, there are 9 plants that could potentially be used as materials for post partum traditional herbal medicine. The process of post partum traditional herbal medicine production consists of sorting, grating, scraping, mixing and boiling the plants. All of the plants were dried in room temperature and ground into powder. Each plant was weighed according to the variation of the composition.

TABLE 1. Composition of post partum traditional herbal medicine 1 (total weight 5g)

Name of Plant	A1 (g)	B1 (g)	C1 (g)	D1 (g)	E1 (g)
<i>Meniran</i>	1.0	0.9	1.4	1.2	1.0
<i>Kunyit</i> (turmeric)	1.0	1.4	0.9	1.1	0.5
<i>Jungrahap</i>	1.0	0.7	1	0.5	0.9
<i>Akar manis</i> (licorice)	1.0	0.5	1.2	1.2	1.2
<i>binahong</i>	1.0	1.5	0.5	1.0	1.4

TABLE 2. Composition of post partum traditional herbal medicine 2 (total weight 5g)

Name of Plant	A2 (g)	B2 (g)	C2 (g)	D2 (g)	E2 (g)
<i>Bangle</i>	1.0	0.9	1.4	1.2	1.0
<i>Lempuyang</i>	1.0	1.4	0.9	1.1	0.5
<i>Asem</i> (tamarind)	1.0	0.7	1	0.5	0.9
<i>Majakani</i>	1.0	0.5	1.2	1.2	1.2
<i>Kunyit</i> (turmeric)	1.0	1.5	0.5	1.0	1.4

Post partum traditional herbal medicine are divided into two types. Post partum traditional herbal medicine 1 is the herbal medicine taken for the first 40 days after giving birth. Composition on TABLE 1. are plants used in post partum traditional herbal medicine 1 with five variations of composition, namely A1, B1, C1, D1 and E1. Post partum traditional herbal medicine 2 is an advanced herbal medicine taken in the next 40 days after that. Composition on TABLE 2. are plants used in post partum traditional herbal medicine 2 with five variations of composition, namely A2, B2, C2, D2 and E2. The total weight of herbs used was 5 grams [7].

Five grams of the variation were extracted using 100 mL of Aquades. The liquid extracts were filtered with filter paper. The filtrates were frozen and dried to remove the solvent and get crude extracts. Each variation was tested for DPPH and ABTS antioxidant activity.

DPPH Radical Scavenging Assay

The free radical scavenging activity of post partum traditional herbal medicine was measured in terms of hydrogen donating or radical scavenging ability against the DPPH radical [8]. Post partum extracts were dissolved in methanol 33.33 μ L (maximum dissolved concentration). One milliliter of DPPH 6×10^{-5} M radical solution which was prepared daily, was mixed with 33.33 μ L methanolic solutions of *M. oleifera* extracts. The contents were mixed vigorously and incubated at 37°C for 20 minutes. The absorbance decrease of the mixture was read with spectrophotometer UV at 515 nm (*As*). The colour of the solution changed from violet to pale yellow during the reduction process by the antioxidant. Blank samples with 33.33 μ L of methanol in the above DPPH radical solution were prepared and measured daily at the same wavelength (*Ab*). Trolox was used as the positive control. The experiment was carried out in triplicate. IC₅₀ value was the concentration required to scavenge 50% of DPPH radical free, it was calculated using the following formula.

$$\text{DPPH scavenging effect (\%)} = \frac{[Ab - As]}{Ab} \times 100 \quad (1)$$

Where *Ab* was the absorbance of the blank sample and *As* was the absorbance in the presence of the sample. This analysis was done in triplicate.

Radical Scavenging by ABTS Radical

ABTS assay was in accordance with the methods described previously [9]. The ABTS [2,2' azino - bis (3-ethylbenzothiazoline-6-sulfonic acid)] radical scavenging based on the ability of antioxidant molecules to quench the long living ABTS^{•+}. The ABTS^{•+}, which is the oxidant, was generated by peroxydisulfate oxidation. ABTS stock solution was prepared with 5 mL of 7 mM ABTS in ammonium aqueous solution. The ABTS radical cation (ABTS^{•+}) was the product of reaction between the ABTS stock solution with 88 μ L of 140 mM potassium persulfate (Na₂S₂O₈). The ABTS^{•+} solution was allowed to be kept in the refrigerator storage for 12-16 hours at 4°C in darkness to yield a dark blue solution. This solution was subsequently diluted with ethanol 99.5% before use which gave an absorbance of 0.7 ± 0.02 at 734 nm as the working solution. One milliliter of working solution was mixed with 10 μ L of *M. oleifera* extract (maximum dissolved concentration) and shaken well for 10 seconds; after 4 minutes of incubation at 30°C, the absorbance of the reaction mixture was measured at 734 nm to give *As* values. Trolox was used as the positive control. Ethanol 99.5% was used as a blank (absorbance was *Ab*). The antioxidative activity of the *M. oleifera* extracts was calculated by determining the decrease in absorbance at different concentrations by using equation 1. This analysis was done in triplicate.

RESULTS AND DISCUSSIONS

The synergy between plant components in post partum traditional herbal medication increases boactivity. Based on the phytochemical screening of each compound variation of the post partum traditional herbal medication, the composition showed a higher amount of secondary metabolite compounds. There are two types of post partum traditional herbal medication based on the function and composition of the plant. Based on the survey results, post partum traditional herbal medication 1 was taken a month after childbirth. This herb served to maintain the stamina of the mother after childbirth, accelerate wound healing, increase antibodies, and is anti-inflammatory. While the post partum traditional herbal medication 2 was taken in the second month after childbirth. The function of the post partum traditional herbal medication 2 is to speed up the process of returning the size of the uterus, to provide tranquility for the mother, and to tighten the female organs.

DPPH Radical Scavenging Assay

The DPPH inhibition of post partum traditional herbal medicine 1 was measured with trolox as the positive control. The activities are reported in **FIGURE 1**.

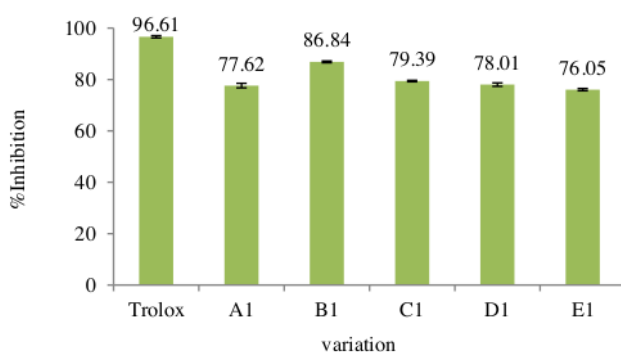


FIGURE 1. DPPH inhibition of post partum traditional herbal medicine 1 at a concentration of 399.32 $\mu\text{g/mL}$; values are the mean \pm SD of three independent experiments in triplicate at each concentration.

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The *in vitro* antioxidant activity assay of the extracts of post partum traditional herbal medication1 had been done as shown in **FIGURE 1**. Variation B1 had the highest antioxidant activity of DPPH at 86.84%. The value of IC_{50} for variation B1 is shown in **FIGURE 2**.

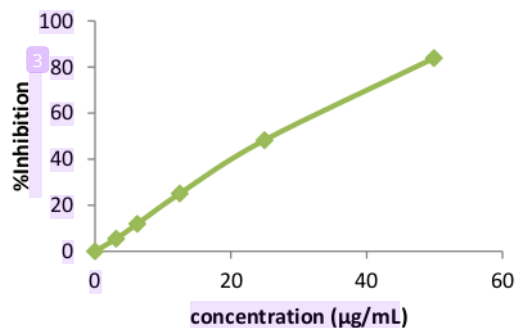


FIGURE 2. DPPH inhibition of Variation B1

The DPPH inhibition of post partum traditional herbal medication 2 was measured with trolox as the positive control. The activities are reported in **FIGURE 3**.

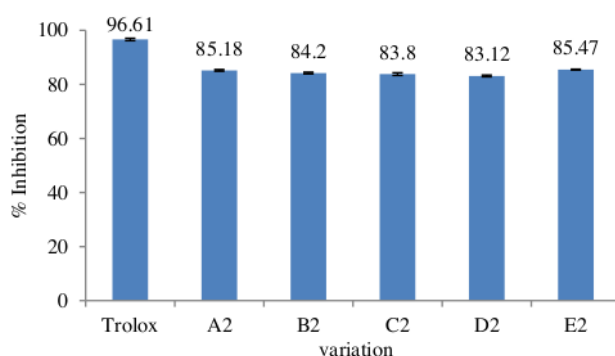


FIGURE 3. DPPH inhibition of post partum traditional herbal medication 2 at a concentration of 399.32 $\mu\text{g/mL}$; values are the mean \pm SD of three independent experiments in triplicate at each concentration.

While in post partum traditional herbal medication 2, E2 variation had the highest antioxidant activity of DPPH at 85.47%. The value of IC_{50} for variation E2 is shown in **FIGURE 4**.

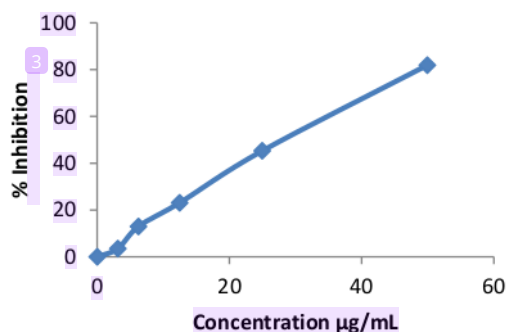


FIGURE 4. DPPH inhibition of Variation E2

Antioxidant activity is influenced by the donation of hydrogen atom that is able to stabilize free radical of DPPH. DPPH radicals are relatively stable so it is often used to measure the ability of a secondary metabolite compound as a hydrogen donor [10]. The ability of secondary metabolite compounds in binding free electrons in radical DPPH is characterized by the color purple to pale yellow. The absorbance result from the color change was measured (its absorbance value) by using UV-vis spectrophotometer at 515 wavelength. Then, the absorbance result was compared to the absorbance value of positive control (Trolox) and negative control (Aquades).

ABTS Radical Scavenging Assay

In this study, ABTS test was conducted because this method is often used to analyze antioxidants in the food and agricultural products of industry. Post partum herbal medicine is a drink consumed by the people of Indonesia. Making ABTS cation radical solution can be done by adding strong oxidizers such as potassium persulfate ($\text{K}_2\text{S}_2\text{O}_8$) to ABTS

solution. The resulting solution is concentrated blue with a high absorbance value when measured at a wavelength of 734 nm. Therefore, before use, it must be diluted first with 99.5% ethanol until the produced radical ABTS cation solution has an absorbance value of 0.7 ± 0.02 . During the reaction process, the ABTS cation solution (blue) will change to a colorless ABTS solution, because the electron donation from the test compound can stabilize the ABTS cation radical [11]. This decrease in absorbance is measured by a UV spectrophotometer and can be used to determine the antioxidant activity of a compound.

The ABTS inhibition percentage of post partum traditional herbal medicine 1 was measured with trolox as the the positive control. The activities are reported in **FIGURE 5**.

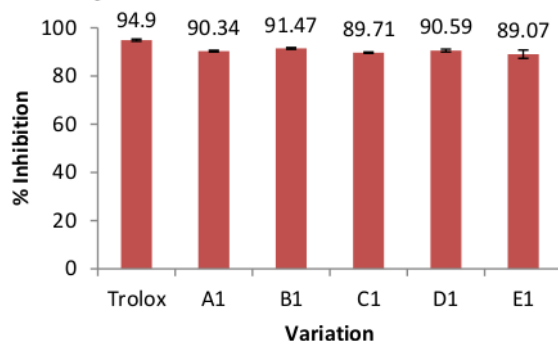


FIGURE 5. DPPH inhibition of post partum traditional herbal medicine 1 at a concentration of 399.32 $\mu\text{g/mL}$; values are the mean \pm SD of three independent experiments in triplicate at each concentration.

The value of IC_{50} for variation B1 is shown in **FIGURE 6** .:

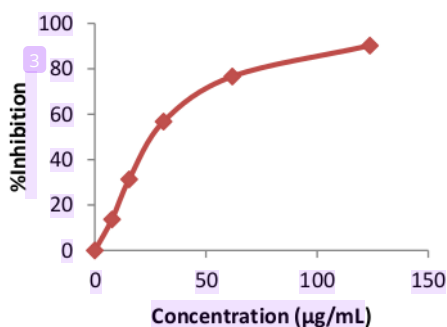


FIGURE 6. ABTS inhibition of Variation B1

The ABTS inhibition percentage of post partum traditional herbal medicine 2 was measured with trolox as the the positive control. The activities are reported in **FIGURE 7**.

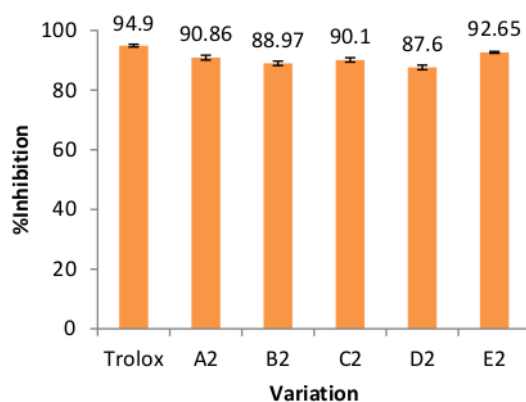


FIGURE 7. ABTS inhibition of post partum traditional herbal medicine 2 at a concentration of 123.76 $\mu\text{g}/\text{mL}$; values are the mean \pm SD of three independent experiments in triplicate at each concentration.

The value of IC_{50} for variation E2 is shown in **FIGURE 8.** :

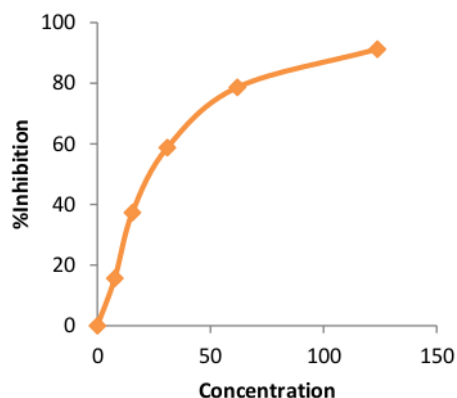


FIGURE 8. ABTS inhibition of Variation E2

Variation B1 and E2 exhibited fine scavenging abilities against DPPH and ABTS radicals with concentration dependent manner. Thus, the Variation B1 was exhibited as a high ABTS radical scavenging efficiency rather than the DPPH radicals, which could be indicated to a different mechanism. In the DPPH assay, the scavenging action may be due to the hydrogen donating ability, whereas scavenging of ABTS radical is due to scavenging of proton radicals induced through donation of electrons.

The ability of compounds to act as antioxidants can be seen from the value of IC_{50} . The value of IC_{50} is the concentration of antioxidant compounds required to lower the DPPH radicals by 50%. IC_{50} values are derived from linear line equations which express the relationship between concentrations (X axis) and the percentage of inhibition (Y axis) [12]. Smaller IC_{50} value means better antioxidant activity. Variations B1 and E2 showed good radical DPPH inhibitory activity, this was seen from IC_{50} B1 value of 28.37 $\mu\text{g} / \text{mL}$ and IC_{50} E229.56 $\mu\text{g} / \text{mL}$ as compared to IC_{50} trolox.

Antioxidant activity can also be measured using ABTS test. The principle of ABTS test is that it is a compound that stabilizes the radical cation of ABTS [13]. It is characterized by the radical cation's color change of ABTS (light blue) to be less colorful. Variations of B1 and E2 indicated good inhibitory activity of ABTS radical cation, this was seen from the IC_{50} B1 value of 27.17 $\mu\text{g} / \text{mL}$ and IC_{50} E2 of 26.16 $\mu\text{g} / \text{mL}$ when compared to IC_{50} trolox 8.75 $\mu\text{g} / \text{mL}$.

CONCLUSIONS

The effectiveness of post partum traditional herbal medication composition has been researched in terms of antioxidant activity. In vitro antioxidant of all compounds was evaluated by using DPPH and ABTS method.

Variations B1 and E2 showed the best value of antioxidant activity. This was indicated by the value of IC_{50} to DPPH variation of B1 28.37 μ g / mL and E2 29.56 μ g / mL compared to trolox μ g / mL. While for IC_{50} to ABTS the variation of B1 27.17 μ g / mL and E2 26.16 μ g / mL was compared to trolox μ g / mL. This result indicated that the variation composition of B1 and E2 could be used as a good potential source of antioxidant.

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