

## Phytochemical, antioxidant and antibacterial activities of medicinal plants used in Northern Thailand as postpartum herbal bath recipes by the Mien (Yao) community

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### Abstract

The Mien (Yao) community in northern Thailand uses selected medicinal plants as traditional recipes for postpartum bath to avoid puerperal sepsis and related conditions. Current study was designed to investigate pharmacological studies to validate folk use of such herbal recipes in northern Thailand. Phytochemical tests revealed the presence of phenolic compounds, flavonoids, triperpene and lactone glycosides. All plants were then tested for DPPH, FRAP, and total phenolic content. It was found that *Phlogacanthus curviflorus* (Wall.) Nees var. *curviflorus* (Hung Teaw Yam) has the highest DPPH anti-oxidant activity ( $EC_{50} = 0.219$  mcg), its FRAP value, expressed as  $FeSO_4$  equivalents (mg/g extract), is  $949.143 \pm 0.074$  and its total phenolic content, expressed as gallic acid equivalents (mg/g extract), equals  $17,368.421 \pm 0.009$ . Results of the antimicrobial showed *Schefflera* sp. cf *S. bengalensis* Gamb. and *Plumbago indica* L. to be the most active plants against *Staphylococcus aureus* with MIC and MBC of 0.726; 1.453 mg/ml and 0.782; 0.782 mg/ml, respectively. TLC finger print of *P. indica* L. showed the band having  $R_f = 2.3$  was equivalent to morin with antibacterial property against *S. aureus*. Present investigation showed significant therapeutic effect of folk medicinal recipes used as traditional postpartum herbal bath by the Mien community in northern Thailand.

**Keywords:** Mien (Yao), Thailand, Postpartum herbal bath

### Introduction

Since people have the advantage of medical technology, they live longer and are faced with diseases that come along with age and illnesses such as cancer and diabetes mellitus. Diseases caused by stress and the pollution of an industrial lifestyle, which may not be cured by modern medicine, force many people to seek out complementary and alternative

medicine and/or in form of new drugs or functional foods. The use of medicinal plants in a traditional way also is becoming revitalized over the world.

Moreover, since modern medicine is becoming more widespread but at a high cost. In rural area, it is useful in certain cases, like surgery. Traditional medicine still plays an important role and commonly used as primary form of health care for people especially in the critical events such as in postpartum women. The rapidly deterioration of the mother's health is due to the loss of a large amount of blood, the tearing of the organs, and the physiological and hormonal changes of mother's body. Medicinal plants that are used in postpartum herbal bath and food supplement may be responsible for antioxidation action, anti-inflammatory and antimicrobial activity on target organs of mother.

Over the past few decades there has been a dramatic increase in the number of puerperal infected patients. Puerperal sepsis is responsible for 5% to 23% of all maternal deaths worldwide, and causes the loss of more than one third of woman's healthy years of life because of resulting illness (Salama *et al.*, 2008; Onah *et al.*, 2007). In developing country, puerperal sepsis causes at least 75,000 maternal deaths every year. In Africa, it was estimated that 9.7% (95% CI 6.3-12.6) of maternal deaths were due to puerperal sepsis (Khan *et al.*, 2006). It has not only found puerperal sepsis in developing countries, but also found in western countries, USA, and Japan (Mikamo *et al.*, 1998; Janice *et al.*, 2009; Areschoug *et al.*, 2004; Chen *et al.*, 2008). Gram-positive cocci aerobic bacteria such as *Staphylococcus aureus* and *Staphylococcus epidermidis*, (Group A, B streptococcus) were the most commonly species found in puerperal infections in Japanese report, infection with the fungi, *Candida albicans*, was also found (in percentage of 51, and 2.6, respectively) (Fortney and Hussein, 2004). By traditional way, confinement and steam bath are the common way to primary health care of postpartum in many ethnic groups in the world.

The Mien people are of Chinese origin and are well known for their knowledge of ethnomedicinal plants for primary health care. Since the Mien migrated from southern China to northern Thailand in 1854, knowledge of medicinal plants was brought with them, and some of this knowledge has been revitalized in their society. Especially the knowledge of the use of medicinal plants for bathing and health tonics in postpartum women has been used for more than thousands years. These recipes have potential plants for use in primary health care for postpartum women.

However, their use needs more support by scientific knowledge regarding their active ingredients and the bioactivity of the plants in the preparations to answer the question why herbal bathing can promote health, heal the wound and made the mother recover in as short time, and which plants in these preparations that are responsible to against the pathogens that common found in a puerperium vagina.

This study was then designed to determine phytochemicals, and tests antioxidation and antibacterial activities on selected medicinal plants, which lack of their bioactivity information. Thin Layer Chromatography (TLC) was used to determine the chemical constituents and also to measure antibacterial activity, in preparations that are the most popular in the Mien's community in northern Thailand.

## Plant material

### Collection and identification

The indices used in ethnobotany (Treyvaud *et al.*, 2005; Gazzaneo *et al.*, 2005; Phillips *et al.*, 1994; Friedman *et al.*, 1986; Hoffman and Gallaher, 2007; Trotter and Logan, 1986) were used to determine potential value of the recipes and plants. Ten of forty-four plants, which are used in postpartum herbal bath recipes, were collected from forests near Sancharurn Village in November 2010. Identification was done by a taxonomist from the Chiang Mai University Herbarium, Thailand. All voucher specimens are deposited there with specimen numbers KPHB 201-210.

### Preparation of extracts

In order to perform phytochemical analysis and antioxidant activities, one hundred grams of dry ground plant material was macerated in 70% ethyl alcohol, shaken for 5 hours then kept at room temperature for 24 hrs. in closed containers. The extraction process was repeated 3 times (extraction for 3 days). Then the extracts were filtered under vacuum and concentrated at reduced pressure using a rotary evaporator. The dried extracts were kept in the refrigerator at 4 °C until use. Similarly for antibacterial activity, twenty-five grams of dry ground plant material was boiled in distilled water for 1 hr. and allowed to cool at room temperature, filtered and concentrated at reduced pressure using a rotary evaporator. The dried extracts were kept in the refrigerator at 4 °C until use.

### Phytochemical analysis

Identification of the chemical constituents: polyphenolic compounds, flavonoids, glycosides, saponins, tannins, alkaloids, and anthraquinones was carried out using ethanolic extracts according to the methods of Farnsworth (1962) and Farnsworth *et al.* (1962; 1966).

### Determination of anti-oxidant activity

#### *1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging activity*

The modified method of Hatano *et al.* (1989) was used to measure the free radical scavenging activity of each of the ethanol extracts (with different dilutions) by DPPH (2,2-diphenyl-1-picrylhydrazyl, Sigma, Germany). One hundred microliters of extract was added to each well containing 100 µl of DPPH in ethanol, mixed well, and then the absorbance was measured at 520 nm after exactly 30 minutes by a microplate reader (DTX880 multimode detector, Beckman Coulter). All samples were run in triplicate. Determination of % scavenging of test samples is as follows:

$$\% \text{ Scavenging} = \frac{[C-(A-B)]}{C} \times 100$$

Where A, B, and C are the absorbance of DPPH in the resulting reaction mixture, the blank, and the control respectively. Percent of scavenging was plotted via the log of the

concentration. Substituting  $y = 50$  in the resulting linear equation obtains the  $x$  value. The antilog  $x$  calculated the EC50 (conc. of 50 % scavenging) value (Ballantyne *et al.*, 1995). Butylated hydroxyl toluene (BHT) was used as reference standards ( $Y = 29.649 + 144.513 (\log X)$ ;  $R^2 = 0.999$ ).

#### *Ferric-reducing power (FRAP) assay*

One hundred microliters of extract (with different dilutions) was added to wells containing 100  $\mu$ l of FRAP reagent, mixed well, and absorbance was then measured at 595 nm at 5 minutes by a microplate reader (DTX880 multimode detector, Beckman Coulter). All samples were run in triplicate. The FRAP was expressed as FeSO<sub>4</sub> and trolox equivalents in mg per gram extract ( $Y = 5.803X + 0.034$ ;  $R^2 = 0.999$ ).

#### *Total phenolic content (TPC)*

Total phenolic content of the extracts was determined using the Folin-Ciocalteu assay adapted from method of Kähkönen *et al.* (1999) and Waterhouse (1999). Five hundred microliters of samples were introduced into test tubes followed by 5 ml. of Folin-Ciocalteu's reagent (10 x dilution) and 4 ml. of sodium carbonate solution. The tubes were kept in the dark for 2 hours then poured into cuvettes before absorbance was measured at 765 nm. TPC was expressed as gallic acid equivalents in mg per gram of extract ( $Y = 5.115X - 0.008$ ,  $R^2 = 0.999$ ).

#### *Determination of antimicrobial activities*

##### *Disc diffusion method*

Antimicrobial activity was determined by disc diffusion method of NCCLS (1997). Briefly, 0.1 ml of  $10^8$  cells per ml of a suspension of the tested microorganism (*Staphylococcus aureus* ATCC 25923) was spread on the solid media plates. Filter paper discs (6 mm in diameter) were impregnated with 15  $\mu$ l of the aqueous extracts and placed on the inoculated plates. These plates, after staying at 4 °C for 2 hours, were then incubated at 37 °C for 24 hours. The diameters of the inhibition zones were measured in millimeters. Vancomycin was used as positive control.

##### *Minimum inhibition concentration (MICs)*

Microdilution broth susceptibility assay was used as recommended by NCCLS for the determination of MIC (1999). All tests were performed in Mueller Hinton broth (MHB; BBL) supplemented with Tween 80 detergent (final concentration of 0.5 % (v/v)). Bacterial strain was cultured overnight at 37 °C in MHA. Test strain was then suspended in MHB to give a final density of  $5 \times 10^5$  cfu/ml and these were confirmed by viable counts. Geometric dilutions ranging from 0.036 – 72.00 mg/ml of the extracts were prepared in a 96-well microtiter plate, including one growth control (MHB+Tween 80) and one sterile control (MHB+Tween 80+test extracts). Plates were incubated under normal atmospheric conditions at 37 °C for 24 hrs. The MIC of vancomycin was determined in order to control the sensitivity.

ty of the test organism. The bacterial growth was indicated by the presence of a white “pellet” on the well bottom.

### ***Determination of chemical constituents and their activity against S. aureus by Thin Layer Chromatography (TLC)***

The analysis was performed on precoated 20x20 cm TLC plates of silica gel 60F<sub>254</sub> by Whatman. Ten µl of each extract was applied as spots onto TLC sheets. Six different mobile phases were selected from Marica *et al.* (2004) to establish the optimization of chromatographic conditions in thin layer chromatography of flavonoids and phenolic acids. The plates were developed at room temperature in a vertical separating chamber to the height of approximately 17 cm from the start. The chamber was previously saturated with the appropriate mobile phase (saturation time was 1 hour). After drying, visualization was performed in two ways: in short UV light (254 nm), and spraying with sulfuric acid and then chromatograms were interpreted in long wave UV light (366 nm). The *R<sub>f</sub>* value was identified by comparison with *R<sub>f</sub>* of standards in the similar conditions.

Antibacterial assay against *S. aureus* of Chomnawang *et al.* (2005) was adapted for this experiment. The dried developed TLC plates were placed onto smeared agar plates then kept in an incubator at 37 °C for 24 hours. The inhibition zone was then measured and analyzed.

## **Results**

### ***Phytochemical analysis***

Ten species of medicinal plants commonly used in postpartum herbal bath of the Mien were selected for testing of their chemical compounds by phytochemical screening. All of them showed positive results with 1% ferric chloride of polyphenolic compounds, and also showed negative results for cyanotic and cardiac glycoside, anthraquinones, saponins, and alkaloids

Although all of plants showed positive results with 1% ferric chloride, none of them showed positive results with another test for tannins. Three of the plants, *Olex imbricata* Roxb., *Gouania leptostachya* DC., *Plumbago indica* L., showed positive results for Shinoda's test of flavonoids. Of these, *Olex imbricata* Roxb. displayed the darkest shade of pink color. Eight of plants, *Adenia penangiana* (G. Don) W.J. de Wilde., *Olex imbricata* Roxb., *Trevesia palmate* (Roxb. ex Lindl.) Vis., *Poikilospermum suaveolens* Merr., *Schefflera* sp. cf. *S. bengalensis* Gamb., *Phlogacanthus curviflorus* (Wall.) Nees var. *curviflorus*, *Plumbago indica* L., also showed positive results for lactone glycosides.

Preparation 1 consisted of *Poikilospermum suaveolens* Merr., *Gouania leptostachya* DC., *Schefflera* sp. cf. *S. bengalensis* Gamb., *Phlogacanthus curviflorus* (Wall.) Nees var. *curviflorus*, *Ricinus communis* L., *Chromolaena odoratum* L. King et Robin, *Blumea balsamifera* (L.) DC., *Cymbopogon citrates* (DC.) Stapf., *Crinum asiaticum* L., *Leea indica* (Burm. f.) Merr.. Preparation 2 contained of 10 selected plants, that each of plants of this preparation are virgin from any tests, was designed to test for antioxidant activities and antibacterial activity in this study. Plants in each preparation were boiled together in water for 1 hour then the extracts were tested by phytochemical screening. We found that both of

Table 1. Phytochemical Screening of Ten Used Species of Selected Plants in Postpartum Herbal Bath of the Mien community in Northern Thailand

| Plant   | Polyphenolic compounds and Tannin |            |              |              |            |           | Triterpene |           | Cardiac glycosides |                   |                | Flavonoids         |                     | Lactone glycosides | Cyanotic glycosides | Saponins       | Anthraquinones | Alkaloids |       |       |             |        |               |
|---|-----------------------------------|------------|--------------|--------------|------------|-----------|------------|-----------|--------------------|-------------------|----------------|--------------------|---------------------|--------------------|---------------------|----------------|----------------|-----------|-------|-------|-------------|--------|---------------|
|   | 1% ferric chloride                | 1% gelatin | Gelatin salt | Formalin-HCl | Lime-water | L.B. test | L.B. test  | L.B. test | Kedde's            | Keller-Killiani's | Shimoda's test | Lactone glycosides | Cyanotic glycosides | Frost test         | Saponins            | Anthraquinones | Dragendorff    | Hager     | Marme | Mayer | Tannic acid | Wagner | Dragen. spray |
| <i>Adenia penangiana</i> (G.Don) W.J. de Wilde.                       | ++                                | -          | -            | -            | -          | ++        | ++         | ++        | +                  | -                 | #              | +                  | -                   | -                  | -                   | -              | +              | +         | -     | -     | -           | -      | -             |
| <i>Olax imbricate</i> Roxb.   | ++                                | -          | -            | -            | -          | ++        | ++         | ++        | -                  | +                 | +++            | +                  | -                   | -                  | -                   | -              | -              | +         | +     | -     | -           | -      | -             |
| <i>Trevesia palmata</i> (Roxb. ex Lindl.) Vis.                        | +                                 | -          | -            | -            | -          | ++        | ++         | ++        | #                  | ++                | #              | +                  | -                   | -                  | -                   | -              | -              | -         | -     | -     | -           | -      | -             |
| <i>Poikilospermum suaveolens</i> Merr.                                | +                                 | -          | -            | -            | -          | ++        | ++         | +         | -                  | +                 | #              | +                  | -                   | -                  | -                   | -              | -              | +         | -     | -     | -           | -      | -             |
| <i>Gouania leptostachya</i> DC. var. <i>leptostachya</i>              | ++                                | -          | -            | -            | -          | ++        | ++         | +         | -                  | ++                | +              | -                  | -                   | -                  | -                   | -              | -              | +         | +     | -     | -           | -      | -             |
| <i>Tetrastigma</i> sp.  | ++                                | -          | -            | -            | -          | ++        | ++         | ++        | -                  | +                 | #              | -                  | -                   | -                  | -                   | -              | -              | +         | +     | -     | -           | -      | -             |
| <i>Porhios chinensis</i> (Raf.) Merr.                                 | ++                                | -          | -            | -            | -          | ++        | ++         | +         | -                  | ++                | #              | -                  | -                   | -                  | -                   | -              | -              | +         | -     | -     | -           | -      | -             |
| <i>Schefflera</i> sp. cf <i>S. bengalensis</i> Gamb.                  | ++                                | -          | -            | -            | -          | ++        | ++         | +         | -                  | ++                | #              | +                  | -                   | -                  | -                   | -              | -              | -         | +     | -     | -           | -      | -             |
| <i>Phlogacanthus curviflorus</i> (Wall.) Nees var. <i>curviflorus</i> | ++                                | -          | -            | -            | -          | ++        | ++         | ++        | -                  | -                 | -              | +                  | -                   | -                  | -                   | -              | -              | +         | -     | -     | -           | -      | -             |
| <i>Plumbago indica</i> L.   | ++                                | -          | -            | -            | -          | ++        | ++         | ++        | -                  | +                 | +              | +                  | -                   | -                  | -                   | -              | +              | +         | -     | -     | -           | -      | -             |
| Herbal Bath Preparation 1   | ++                                | -          | -            | -            | -          | +         | +          | -         | -                  | -                 | +              | +                  | -                   | -                  | +                   | -              | -              | -         | -     | -     | -           | -      | -             |
| Herbal Bath Preparation 2   | ++                                | -          | -            | -            | -          | +         | +          | -         | +                  | -                 | +              | +                  | -                   | -                  | -                   | -              | -              | -         | -     | -     | -           | -      | -             |

the preparations showed positive results for 1% ferric chloride, Liebermann-Burchard test, Shinoda's test, and the lactone glycosides test. Moreover, preparation 1 also showed positive results for the froth test of saponins. Details of the phytochemicals of the plants are shown in Table 1.

### **Total phenolic content (TPC)**

Total phenolic content was determined by the Folin-Ciocalteu method. We found that the extract of *Phlogacanthus curviflorus* (Wall.) Nees var. *curviflorus* showed the highest amount of phenolic compounds as expressed as gallic acid equivalents ( $17,368.421 \pm 0.009$  mg/g extract), it was followed by extracts of *Gouania leptostachya* DC. var. *leptostachya* ( $1,624.294 \pm 0.006$  mg/g extract). Preparations 1 and 2 also showed a high quantity of phenolic compounds. These are also expressed as gallic acid equivalents of  $1,960.696 \pm 0.017$  and  $2,328.313 \pm 0.014$  mg/g extract, respectively. Details of the total phenolic content of plants are shown in Table 2.

### **Antioxidant activity**

According to results of antioxidant activity by DPPH methods, extract of *Phlogacanthus curviflorus* (Wall.) Nees var. *curviflorus* showed the highest amount of free radical scavenging activity as expressed as BHT equivalents ( $6,588.367$  mcg/mg extract). It was followed by extracts of *Tetrastigma* sp. ( $146.253$  mcg/mg extract).

In case of antioxidant activity by the FRAP assay, it was found that extract of *Phlogacanthus curviflorus* (Wall.) Nees var. *curviflorus* showed the highest amount of ferric reducing power expressed as FeSO<sub>4</sub> and trolox equivalents ( $949.143 \pm 0.074$  and  $12.914 \pm 0.714$  mg/g extract, respectively). It was followed by extracts of *Olex imbricata* Roxb. ( $179.229 \pm 0.013$  and  $9.936 \pm 0.033$  mg/g extract, respectively). Preparations 1 and 2 also showed high amount of ferric reducing power, these are  $501.429 \pm 0.073$ ,  $25.794 \pm 0.226$  and  $425.829 \pm 0.094$ ,  $22.708 \pm 0.021$  mg/g extract, respectively. Details of antioxidant activities of plants are shown in Table 2.

### **Antibacterial activity**

Results of the antibacterial activity by Disk Diffusion with Minimal Inhibiting Concentration (MIC) method revealed that *Schefflera* sp. Cf *S. bengalensis* Gamb. and *Plumbago indica* L. have considerable antibacterial activity against *Staphylococcus aureus* with MIC and MBC of 0.726; 1.453 mg/ml and 0.782; 0.782 mg/ml, respectively.

### **Chemical constituents and their antibacterial activity against *S. aureus* measured by TLC method**

The chromatograms of chemical constituents studied via TLC plates (based on silica gel 60F<sub>254</sub>) were measured using 6 different mobile phases. TLC-fingerprints showed that there are more than 14 separate spots in the *Plumbago indica* L. extract and more than 12 separate spots in the *Schefflera bengalensis* Gamb. extract. In comparison with standards in similar conditions, the five spots in the chromatogram of *P. indica* L. extract and the 11 spots of *S. bengalensis* Gamb. were equal with the standards (*R<sub>f</sub>* values = 0.77, 0.80, 0.23, 0.60, 0.56

Table 2 Anti-oxidation Activities of Ten Commonly Used Species of Medicinal Plants in Postpartum Herbal Bath Preparations.

| Name of medicinal plants<br>(Mien name)   | DPPH                     |                                     | FRAP  |   | Total phenolic<br>content<br>(Gallic acid<br>equivalents<br>(mg/g extract) |
|---|--------------------------|-------------------------------------|---|---|--|
|   | EC <sub>50</sub><br>(mg) | BHT equivalents<br>(mcg/mg extract) | FeSO <sub>4</sub> equivalents<br>(mg/g extract) | Trolox<br>equivalents<br>(mg/g extract) |  |
| <i>Phlogacanthus curviflorus</i> (Wall.) Nees var. <i>curviflorus</i> (Hung teaw yam) | 0.219 (mcg)              | 6588.367                            | 949.143 ± 0.074                                 | 12.914 ± 0.714                          | 17,368.421 ± 0.009   |
| <i>Adenia penangiana</i> (G.Don) W.J. de Wilde. (Sung bung ma lui)                    | 0.946 (mcg)              | 15.0879                             | 97.12 ± 0.072                                   | 5.943 ± 0.072                           | 70.647 ± 0.013   |
| <i>Pothos chinensis</i> (Raf.) Merr. (Ha dia ngaeng)                                  | 8.950 (mcg)              | 15.4587                             | 115.11 ± 0.007                                  | 6.576 ± 0.007                           | 108.713 ± 0.008  |
| <i>Tetrasigma</i> sp. (Faan til your)   | 9.170 (mcg)              | 146.2534                            | 79.233 ± 0.033                                  | 2.433 ± 0.005                           | 74.255 ± 0.018   |
| <i>Schefflera</i> sp. cf <i>S. bengalensis</i> Gamb. (Au cha pee)                     | 9.34 (mcg)               | 14.8132                             | 58.514 ± 0.085                                  | 3.150 ± 0.085                           | 46.444 ± 0.011   |
| <i>Trevesia palmata</i> (Roxb. ex Lindl.) Vis. (Thoe fim)                             | 9.375 (mcg)              | 14.7579                             | 47.524 ± 0.043                                  | 2.625 ± 0.033                           | 10.863 ± 0.019   |
| <i>Poikilospermum suaveolens</i> Merr. (Pwang dia tom)                                | 0.0455                   | 3.0408                              | 40.659 ± 0.040                                  | 2.727 ± 0.040                           | 31.982 ± 0.017   |
| <i>Plumbago indica</i> L. (Hong lin)  | 0.0630                   | 2.1961                              | 50.286 ± 0.009                                  | 2.580 ± 0.009                           | 67.284 ± 0.003   |
| <i>Olax imbricate</i> Roxb. (Dia djan)  | 0.0688                   | 2.0110                              | 179.229 ± 0.013                                 | 9.936 ± 0.033                           | 559.089 ± 0.021  |
| <i>Gouania leptostachya</i> DC. var. <i>leptostachya</i> (Pwang dia yao)              | 0.1810                   | 0.7644                              | 76.564 ± 0.076                                  | 4.798 ± 0.176                           | 1,624.294 ± 0.006  |
| Herbal Bath Preparation 1   | 0.0182                   | 7.6020                              | 501.429 ± 0.073                                 | 25.794 ± 0.226                          | 1,960.696 ± 0.017  |
| Herbal Bath Preparation 2   | 0.2388                   | 5.7940                              | 425.829 ± 0.094                                 | 22.708 ± 0.021                          | 2,328.313 ± 0.014  |



Table 3 Rf Value of Flavonoids, Phenolic Acids (standards) and 2 Sample Extracts

| Standard / TLC System          | 1                   | 2                   | 3                   | 4                   | 5                   | 6                   |
|--------------------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
| Flavanone                      | 0.67                | 0.62                | 0.38                | 0.62                | 0.75                | 0.76                |
| Naringenin                     | 0.54                | (0.58) <sub>1</sub> | 0.24                | 0.44                | 0.52                | (0.73) <sub>1</sub> |
| Flavone                        | (0.88) <sub>1</sub> | 0.92                | 0.66                | 0.85                | 0.91                | 0.92                |
| 3-Hydroxyflavone               | (0.77) <sub>2</sub> | 0.80                | 0.56                | 0.66                | 0.82                | 0.83                |
| 6-Hydroxyflavone               | 0.67                | 0.61                | 0.36                | 0.56                | 0.75                | (0.80) <sub>2</sub> |
| 6'-Hydroxyflavone              | 0.52                | 0.46                | 0.28                | 0.48                | 0.56                | 0.73                |
| 7-Hydroxyflavone               | 0.46                | 0.42                | 0.26                | 0.46                | 0.47                | 0.70                |
| 3,6-Dihydroxyflavone           | 0.54                | 0.51                | 0.34                | 0.46                | 0.56                | 0.72                |
| 3,7-Dihydroxyflavone           | 0.54                | 0.50                | 0.33                | 0.47                | 0.54                | 0.70                |
| Morin                          | (0.23) <sub>1</sub> | 0.14                | 0.13                | (0.23) <sub>2</sub> | (0.13) <sub>1</sub> | 0.32                |
| Chrysin                        | (0.62) <sub>1</sub> | (0.60) <sub>2</sub> | 0.36                | (0.56) <sub>2</sub> | (0.68) <sub>1</sub> | 0.74                |
| Quercetin                      | 0.39                | 0.27                | 0.22                | 0.35                | 0.30                | 0.60                |
| Galangin                       | 0.65                | 0.64                | 0.37                | 0.60                | (0.72) <sub>1</sub> | 0.85                |
| Apigenin                       | 0.44                | 0.47                | (0.21) <sub>1</sub> | 0.37                | 0.39                | 0.67                |
| Kaempferol                     | 0.51                | 0.50                | 0.23                | 0.40                | 0.47                | 0.77                |
| O-Coumaric acid                | 0.55                | 0.51                | 0.37                | 0.48                | (0.73) <sub>1</sub> | 0.75                |
| p-Coumaric acid                | 0.55                | 0.51                | 0.34                | 0.47                | (0.69) <sub>1</sub> | 0.75                |
| Caffeic acid                   | 0.38                | 0.30                | 0.22                | 0.34                | 0.43                | 0.62                |
| Ferulic acid                   | 0.56                | 0.49                | 0.28                | 0.45                | (0.63) <sub>1</sub> | 0.70                |
| <i>Plumbago indica</i> L.      | 0.89                | 0.03                |                     | 0.61                | 0.87                | 0.96                |
|                                | 0.10                | 0.87                |                     | 0.43                | 0.61                | 0.57                |
|                                | 0.03                |                     |                     |                     | 0.44                | 0.29                |
|                                |                     |                     |                     |                     | 0.145               |                     |
|                                |                     |                     |                     |                     | 0.97                |                     |
|                                |                     |                     |                     |                     | 0.77                |                     |
|                                |                     |                     |                     |                     | 0.067               |                     |
|                                |                     |                     |                     |                     | 0.57                |                     |
|                                |                     |                     |                     |                     | 0.53                |                     |
|                                |                     |                     |                     |                     | 0.49                |                     |
|                                |                     |                     |                     |                     | 0.45                |                     |
|                                |                     |                     |                     |                     | 0.40                |                     |
|                                |                     |                     |                     |                     | 0.35                |                     |
|                                |                     |                     |                     |                     | 0.27                |                     |
| <i>Scheffera bengalensis</i> G | 0.29                |                     |                     | 0.96                | 0.77                | 0.27                |
|                                | 0.13                |                     |                     | 0.89                | 0.32                | 0.06                |
|                                | 0.04                |                     |                     | 0.17                | 0.96                |                     |
|                                |                     |                     |                     |                     | 0.69                |                     |
|                                |                     |                     |                     |                     | 0.40                |                     |
|                                |                     |                     |                     |                     | 0.95                |                     |
|                                |                     |                     |                     |                     | 0.36                |                     |

\*This table was adapted from Maricaet *al.*, 2004

Solvents used in chromatographic system

No. 1 = toluene:ethylacetate:formic acid, 36:12:5

No. 2 = toluene:ethylacetate:acetic acid, 36:12:5

No. 3 = n-hexane:ethyl acetate: formic acid, 31:14:5

No. 4 = toluene:acetone:formic acid, 38:10:5

No. 5 = toluene:acetone:acetic acid, 31:14:5

No. 6 = petroleum ether: ethyl acetate:formic acid, 30:15:5

and 0.58, 0.73, 0.88, 0.23, 0.13, 0.62, 0.68, 0.21, 0.73, 0.69, 0.63, respectively). These results indicated that *Plumbago indica* L. may contain 3-Hydroxyflavone, 6-Hydroxyflavone, Morin, and Chrysin while *S. bengalensis* Gamb may contain Naringenin, Flavone, Morin, Chrysin, Galangin, Apigenin, *O*-Coumaric acid, *p*-Coumaric acid, Ferulic acid. However, there are unknowns that need to be identified in further research. The details are shown in Table 3.

Determination of antibacterial activity against *S. aureus* was determined by placing TLC plates onto smeared agar plates. An inhibition zone of the band at  $R_f = 0.23$  (equivalent to morin in TLC system 4) of *P. indica* L. extract was found, while the ethanolic extract of *S. bengalensis* Gamb. did not show antibacterial activity like its aqueous extract.

## Discussion

The results from the antioxidant assays show that there is a close correlation between the results of the DPPH and total phenolic content (TPC) when tested by a Pearson correlation ( $r = 0.984$ ,  $p < 0.000$ ). The correlation between FRAP and total phenolic content (TPC) results and the correlation between DPPH and FRAP results are also high ( $r = 0.901$ ,  $p < 0.000$  and  $r = 0.832$ ,  $p < 0.001$ , respectively). These results confirm the anti-oxidant activity of each method. The very high amount of antioxidant activity of each plant in these recipes can play an important role in the postpartum recovering by prevent the formation of reactive oxidant species that could damage DNA, RNA, modify proteins, and cause lipid peroxidation of cellular targets. Antioxidants also may inhibit the initiation or propagation of oxidation (Wilfred and Ralph, 2006).

Phytochemical data showed a relationship between the antioxidant and antibacterial results. Plants which had positive results in the 1% ferric chloride test of polyphenolic compounds and flavonoids also showed positive results in the antioxidant and antibacterial activities. In this study, there is antibacterial activity against *S. aureus* of the two selected plants. This positive effect against *S. aureus*, which is the commonly strain of microorganisms that can cause of infected wound in the postpartum. Chromatograms on Thin Layer Chromatography plates confirmed their chemical constituents, especially flavonoids from *Plumbago indica* L. which are responsible for antibacterial against *S. aureus* in this study. The results support that flavonoids are known as phytochemical compounds that provide protection against ultraviolet radiation, pathogens, and herbivores (Marica et al., 2004).

Some other plants commonly used in postpartum herbal baths of the Mien such as *Gouania leptostachya* DC., *Cymbopogon citrates* (DC.) Stapf., *Leea indica* (Burm. f.) Merr, and *Chromolaena odoratum* (L.) King et Robin have shown antimicrobial activities in previous reports (Ke et al., 1999; Srinivasan et al., 2009; Mahabir et al., 1997; Ajaiyeoba et al., 2003). However some plants such as *Ricinus communis* L., *Chromolaena odoratum* (L.) King et Robin, *Cymbopogon citrates* (DC.) Stapf., *Leea indica* (Burm. f.) Merr, and *Dianella ensifolia* (L.) DC. also showed anti-inflammatory, and analgesic activities in previous studies (Ke et al., 1999; Ilavarasan et al., 2006; Silva et al., 2010; Irobi, 1997; Srinivasan et al., 2009). These studies supported the folkloric uses of medicinal plant of the Mien community. Other recipes of herbal bath of other ethnic group such as in Thai, Lao PDR, Vietnam, southern China, Malaysia, Indonesia, Philippines, and Myanmar (Bour et al., 1992; Wang et

*al.*, 2003; Lamxay *et al.*, 2011; Zumsteg and Weckerle, 2007; Mathews and Manderson, 1981; Manderson, 1997: 2003; Huai and Pei, 2004; Hart, 1965; Nash, 1965), which these recipes have influent to their lifestyle, should be more investigated to promote local knowledge of the use of medicinal plants for the primary health care of the postpartum women in this region.

All tests in this study and in the reviewed literature showed that there are beneficial effects from postpartum herbal baths and the Mien's postpartum herbal bath recipes. Phytochemicals like phenolic compounds and flavonoids, when combined with the release of good smelling volatile oils in hot water can boost immunity, increase blood circulation, clear airways, make one feel relaxed, and may help recovery in postpartum woman by their antioxidation activities. Antibacterial and other activities such as analgesic and anti-inflammatory activity of these phytochemicals are also good for postpartum care.

The scientific knowledge found in this study and in the reviewed literature has been provided to the entire village to raise their awareness of the value of plants and their uses and for conservation of knowledge of the use of medicinal plants and their habitats. Information regarding good agriculture practices and monitoring by the World Health Organization has been provided to villagers for good practice in cultivation and production of herbal remedies. The Mien's recipes should be further researched by pharmacognosy to study about the active phytochemicals, by pharmacology to provide appropriate dosage information and toxicity, and also for pharmaceuticals to provide good formulations and wider range uses in the future.

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