

b. Antioxidant activities and total phenolic content

Antioxidants of medicinal plants are also necessary to cope with reactive oxidant species that could damage DNA, RNA, modify proteins, and cause lipid peroxidation of cellular targets. They may inhibit the initiation or propagation of oxidation which can prevent any cells and organs being damaged. So, they may play the role of prophylactic and therapeutic agents [284].

After delivery, the rapid 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. There are medicinal plants that are used for health promotion of the Mien's postpartum women, some of them were claimed about their antioxidant and anti-inflammatory activity [180, 222, 238, 256, 260] while some plants lacked of scientific data. So, two types of antioxidant activity tests, which can be easily performed, were then used for screening in this study. The results are shown as follows:

Determination of antioxidant activity by DPPH assay

Standard curve of butylated hydroxyl toluene (BHT) solution showed linear equation of $Y = 29.649 + 144.513 (\log X)$ where $R^2 = 0.999$ while the activity of ten extracts were found that the extract of *P. curviflorus* (Wall.) Nees var. *curviflorus* showed the highest amount of free radical scavenging activity as expressed as BHT (6,588.367 mcg/mg extract). It was followed by extracts of *Tetrastigma* sp. (146.2534 mcg/mg extract). Linear equation of BHT reference standard, and details of antioxidant activities of plants are shown in figure 14 and Table 9, respectively.

% Absorbance

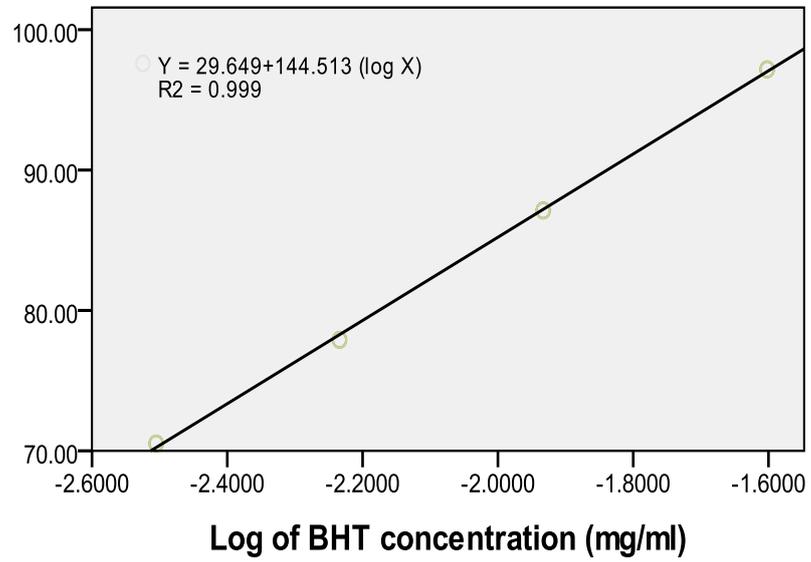


Figure 14 Standard curve of butylated hydroxyl toluene (BHT) solution

Table 9 DPPH radical scavenging activity of crude extracts of medicinal plants used in postpartum herbal bath preparations compared with aqueous extracts of combined ten plants of preparation 1 and 2

Name of medicinal plants	DPPH	
	EC 50	BHT equivalents (mcg/mg extract)
1. <i>Phlogacanthus curviflorus</i> (Wall.) Nees var. <i>curviflorus</i>	0.210 mcg	6588.367
2. <i>Tetrastigma</i> sp.	0.946 mcg	146.2534
3. <i>Adenia penangiana</i> (G.Don) Wilde	9.170Mcg	15.0879
4. <i>Pothos chinensis</i> (Raf.) Merr.	8.950 mcg	15.4587
5. <i>Schefflera</i> sp. aff <i>S. bengalensis</i> Gamb.	9.34 mcg	14.8132
6. <i>Trevesia palmata</i> (Roxb. ex Lindl.) Vis.	9.375 mcg	14.7579
7. <i>Poikilospermum suaveolens</i> (Bl.) Merr.	0.0455 mg	3.0408
8. <i>Plumbago indica</i> L.	0.0630 mg	2.1961
9. <i>Olox imbricata</i> Roxb.	0.0688 mg	2.0110
10. <i>Gouania leptostachya</i> DC. var. <i>leptostachya</i>	0.1810 mg	0.7644
11. Preparation 1 (P1)	0.0182 mg	7.6020
12. Preparation 2 (P2)	0.2388 mg	5.7940

The determination of antioxidant activity by Ferric-reducing power (FRAP) assay

Linear equation of FeSO_4 reference standard showed $Y = 5.803X + 0.034$ where $R^2 = 0.999$ while linear equation of trolox reference standard presented $Y = 0.125X - 0.027$ where $R^2 = 0.999$. The linear equations of standards: FeSO_4 and trolox are shown as follows:

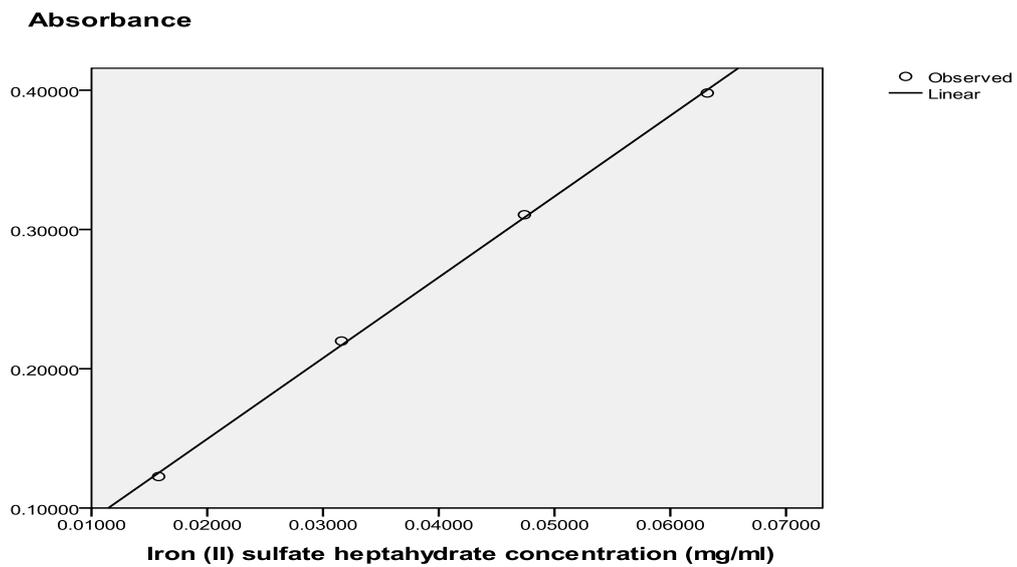


Figure 15 Standard curve of FeSO_4 solution

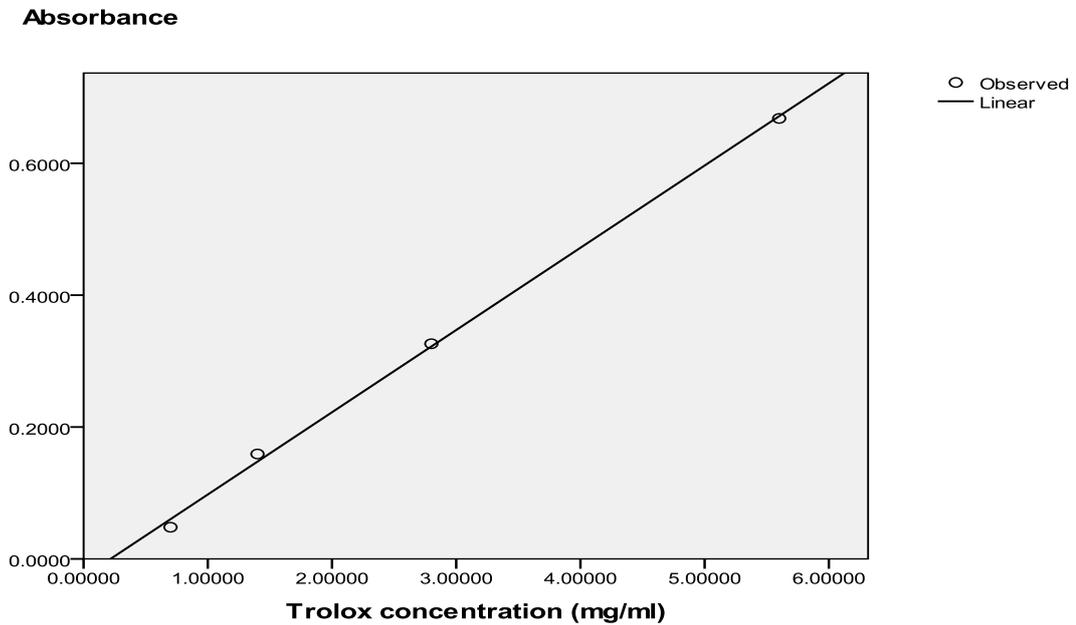


Figure 16 Standard curve of trolox solution

The results found that the extract of *P. curviflorus* (Wall.) Nees var. *curviflorus* showed the highest amount of ferric reducing power expressed as FeSO_4 and trolox equivalents (949.143 ± 0.074 , 12.914 ± 0.714 mg/g extract, respectively). P1 and P2 also showed a high amount of ferric reducing power, these are 501.429 ± 0.073 , 25.794 ± 0.0226 ; 425.829 ± 0.093 , 22.708 ± 0.021 mg/g extract, respectively. Details of antioxidant activities of plants are shown in Table 10

Table 10 Ferric-reducing power of crude extracts of medicinal plants used in postpartum herbal bath preparations compared with aqueous extracts of combined ten plants of preparation 1 and 2

Name of medicinal plants	FRAP	
	FeSO ₄ equivalents (mg/g extract)	Trolox equivalents (mg/g extract)
1. <i>Phlogacanthus curviflorus</i> (Wall.) Nees var. <i>curviflorus</i>	949.143 ± 0.074	12.914 ± 0.714
2. <i>Olax imbricata</i> Roxb.	179.229 ± 0.013	9.936 ± 0.033
3. <i>Pothos chinensis</i> (Raf.) Merr.	115.11 ± 0.007	6.576 ± 0.007
4. <i>Adenia penangiana</i> (G.Don) Wilde	97.120 ± 0.072	5.943 ± 0.072
5. <i>Tetragium</i> sp.	79.233 ± 0.033	2.433 ± 0.005
6. <i>Gouania leptostachya</i> DC. var. <i>leptostachya</i>	76.564 ± 0.076	4.798 ± 0.176
7. <i>Schefflera</i> sp. aff <i>S. bengalensis</i> Gamb.	58.514 ± 0.085	3.150 ± 0.085
8. <i>Plumbago indica</i> L.	50.286 ± 0.009	2.580 ± 0.009
9. <i>Trevesia palmata</i> (Roxb. ex Lindl.) Vis.	47.524 ± 0.043	2.625 ± 0.033
10. <i>Poikilospermum suaveolens</i> (Bl.) Merr.	40.659 ± 0.040	2.727 ± 0.040
11. P1	501.429 ± 0.073	25.794 ± 0.226
12. P2	425.829 ± 0.094	22.708 ± 0.021

Total Phenolic Content (TPC)

The phenolic compounds are compounds that have one or more hydroxyl attached directly to an aromatic ring. There is a huge body of evidence that phenolic compounds are the main compounds responsible for the antioxidant activity of plants

and they also have effects on human health [284]. So, results on the measurement of phenolic contents can confirm the quantity of the antioxidant compound.

Total phenolic content in this study was determined by the Folin Ciocalteu method. Standard curve of gallic acid showed $Y = 5.115x - 0.008$ where $R^2 = 0.999$. Total phenolic content of ten extract were found that the extract of *P. 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 *G. leptostachya* DC. var. *leptostachya* ($1,624.294 \pm 0.006$ mg/g extract). P1 and P2 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. The linear equation of gallic acid, and details of the total phenolic content of plants are shown in figure 17 and in table 11, respectively.

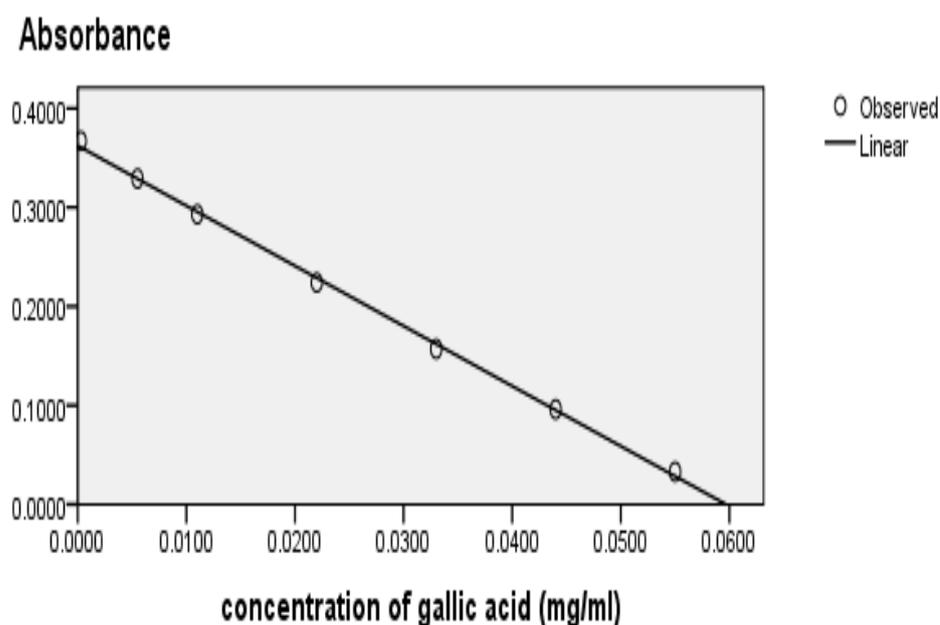


Figure 17 Standard curve of gallic acid solution

Table 11 Total phenolic content of crude extracts of medicinal plants used in postpartum herbal bath preparations compared with aqueous extracts of combined plants of preparation 1 and 2

Name of medicinal plants	Total phenolic content (Gallic acid equivalents) (mg/g extract)
1. <i>Phlogacanthus curviflorus</i> (Wall.) Nees var. <i>curviflorus</i>	17,368.421± 0.009
2. <i>Gouania leptostachya</i> DC. var. <i>leptostachya</i>	1,624.294±0.006
3. <i>Olox imbricata</i> Roxb.	559.089±0.021
4. <i>Pothos chinensis</i> (Raf.) Merr.	108.713± 0.008
5. <i>Tetrastigma</i> sp.	74.255± 0.018
6. <i>Adenia penangiana</i> (G.Don) Wilde.	70.647± 0.013
7. <i>Plumbago indica</i> L.	67.284± 0.003
8. <i>Schefflera</i> sp. cf <i>S. bengalensis</i> Gamb.	46.444± 0.011
9. <i>Poikilospermum suaveolens</i> Merr.	31.982± 0.017
10. <i>Trevesia palmata</i> (Roxb. ex Lindl.) Vis.	10.863± 0.019
11. P1	1,960.696± 0.017
12. P2	2,328.313± 0.014

The prior results showed that there was a close relationship between DPPH and total phenolic content (TPC) results when tested by Pearson correlation ($r = 0.984$, $p < 0.000$) while correlation between FRAP and total phenolic content (TPC) results and the correlation between DPPH and FRAP results also showed the high correlation ($r = 0.901$, $p < 0.000$; $r = 0.832$, $p < 0.001$, respectively), these results can confirm antioxidant activity of each method. The very high amount of antioxidant

activity of recipes of P1 and P2 may come from the combination of phenolic compounds such as flavonoids and others of each plant which were selected and combined to use for treating many conditions involving the postpartum women symptom and/or there are no antagonist effect among them. This antioxidant activity may play an important role in the postpartum recovering by preventing the formation of reactive oxidant species that could damage DNA, RNA, modify proteins, and cause lipid peroxidation of cellular targets. Antioxidants may also inhibit the initiation or propagation of oxidation [285].

c. Antimicrobial tests

Since humans have been suffering from infectious diseases in the native delivery of indigenous people, they have tried to use the various species of medicinal plants to treat puerperal infectious diseases. In the Mien community, it is believed that some plants used in the postpartum herbal bath recipes can treat infectious diseases and heal wounds in a short time. Plants that were mentioned were *P. indica* L., and *L. indica* (Burm. f.) Merr. Other plants like *C. citratus* (DC.) Stapf and *C. asiaticum* L. are used for anti-inflammatory and analgesic activities, and *Schefflera* sp. aff *S. bengalensis* Gamb., *O. imbricata* Roxb. are used to eliminate waste products from the vagina and also make the uterus firm. However, some of these plants still lack of bioactivities that can confirm the usefulness of the traditional use in the literature. In this study, it was then decided to determine the antibacterial activities of the rest of the ten species of medicinal plants that have never been reported. The antibacterial activity results of selected plants are shown as follows:

Disk diffusion method and minimal inhibition concentration (MIC)

The results found that *Schefflera* sp. aff *S. bengalensis* Gamb. and *P. indica* L. have antimicrobial activities against *S. aureus* with MIC of 0.726 mg/ml and 0.782 mg/ml, respectively. The figures of antibacterial activity by disk diffusion method are shown as figure 18.

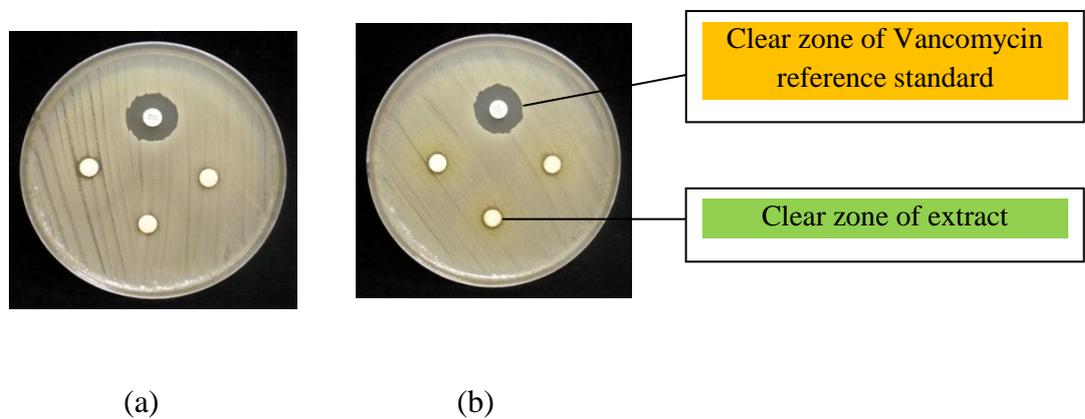


Figure 18 Antibacterial activity against *Staphylococcus aureus* of *Schefflera* sp. aff *S. bengalensis* Gamb. (a) and *Plumbago indica* L. (b) tested by disk diffusion method

d. Analysis of phytochemicals and their bioactivities of medicinal plants that were used in postpartum herbal bath recipes on health of postpartum women

There are more than 33 species of medicinal plant that were traditionally used in the Mien postpartum herbal bath recipes. From these recipes, names of species used and their part used are shown in table 12:

Table 12 Medicinal plants that commonly used in the Mien's herbal bath recipes and their part used

Botanical name	Part used	Botanical name	Part used
1. <i>Blumea balsamifera</i> (L.) DC.*	Leaves	13. <i>Tetrastigma</i> sp.**	Aerial parts
2. <i>Eupatorium odoratum</i> L.*	Leaves, stem	14. <i>Adenia penangiana</i> (G.Don) Wilde**	Aerial parts
3. <i>Crinum asiaticum</i> L.*	Leaves	15. <i>Pothos chinensis</i> (Raf.) Merr.**	Leaves, stem
4. <i>Phlogacanthus curviflorus</i> (Wall.) Nees*,**	Leaves	16. <i>Trevesia palmata</i> (Roxb. ex Lindl.) Vis.**	Leaves
5. <i>Olax imbricata</i> Roxb.*,**	Leaves	17. <i>Acorus gramineus</i> Sol.	leaves
6. <i>Schefflera</i> sp. <i>S. bengalensis</i> Gamb.*,**	Leaves, stem	18. <i>Dianella ensifolia</i> (L.) Red.	Leaves
7. <i>Cymbopogon citratus</i> (DC.) Stapf*	Leaves	19. <i>Crotalaria assamica</i> Bth.	Leaves, stem
8. <i>Ricinus communis</i> L.*	Leaves, stem	20. <i>Kalanchoe</i> sp.	Leaves
9. <i>Poikilospermum suaveolens</i> (Bl.) Merr.*,**	Leaves, stem	21. <i>Pothos scandens</i> L.	Leaves, stem
10. <i>Gouania leptostachya</i> DC. var. <i>leptostachya</i> *,**	Aerial parts	22. <i>Pegia nitida</i> Colebr.	Leaves, stem
11. <i>Leea indica</i> (Burm. f.) Merr.*	Aerial parts	23. <i>Macropanax dispermus</i> Kuntze	Leaves
12. <i>Plumbago indica</i> L.**	Aerial parts	24. <i>Caesalpinia sappan</i> L.	Stem

Table 12 Medicinal plants that commonly used in the Mien's herbal bath recipes and their part used (continued)

Botanical name	Part used	Botanical name	Part used
25. <i>Caesalpinia</i> sp.	Stem	30. <i>Illigera rhodantha</i> Hence	Leaves, stem
26. <i>Commelina diffusa</i> Burm. F.	Aerial parts	31. <i>Eurysolen gracilis</i> Prain	Leaves
27. <i>Pollia secundiflora</i> (Bl.) Bakh.f.	Aerial parts	32. <i>Hyptis rhomboidea</i> . M.	Leaves
28. <i>Cnestis palala</i> (Lour.) Merr.	Leaves, stem	33. <i>Cyclea barbata</i> Miers.	Leaves, stem
29. <i>Cleidion spiciflorum</i> Merr.	Leaves		

* = composed in preparation 1, ** composed in preparation 2

From Table 12, 20 species which high frequency use by villager, showed that there were 10 species that lacked of scientific data, they were then tested for the phytochemicals, antioxidant, and antibacterial activities of these medicinal plant species. The positive effect of laboratory tests when combined to information on phytochemicals and bioactivities both from literature can be used to explain the positive effect that the postpartum women receive from their herbal bath. The pulled information details can be analyzed and then concluded as following:

Ten main plants used in recipes that contain in P1 may provide benefit to postpartum women health from plants' bioactivities as following:

1. Antioxidant activity, there are at least 4 species of plants that were claimed about their antioxidant activity in literature, there are *R. communis* L., *B. balsamifera* (L.) DC., *L. indica* (Burm. f.) Merr., *C. citratus* (DC.) Stapf, [180, 239, 242, 259, 266, 274], and this activity also was found in *P. curviflorus* (Wall.) Nees var. *curviflorus*, *P. suaveolens* Merr., *G. leptostachya* DC., *Schefflera* sp. aff.

S. bengalensis Gamb in this study.

2. Antimicrobial activity there are at least 6 species of plants that their antimicrobial were recorded, there are *G. leptostachya* DC., *Schefflera* sp. aff. *S. bengalensis* Gamb., *R. communis* L., *E. odoratum* L., *C. citratus* (DC.) Stapf, *C. asiaticum* L., and *L. indica* (Burm. f.) Merr. [137, 187, 202, 203, 222, 228, 234, 235, 238, 254, 255, 256, 260].

3. Anti-inflammatory and analgesic activity these activities were found in at least 6 species of plants, there are *R. communis* L., *E. odoratum* L., *B. balsamifera* (L.) DC., *C. citratus* (DC.) Stapf, *C. asiaticum* L., *L. indica* (Burm. f.) Merr. [80, 197, 198, 201, 204, 205, 222, 234, 245, 277, 279].

4. Other activities such as sedative, against effect on mast cell mediators, effect on tumor cell apoptosis, decrease expression of proliferating cell nuclear antigen (PCNA) (in rats), anticonvulsant activity from *C. asiaticum* L. were reported [188, 196, 200, 210, 230, 269-270, 282], antimutagenic activity in mammary cells, inhibition of the liver and intestinal mucous membrane cancer (in mice), also were reported from *C. citratus* (DC.) Stapf [211, 222, 229, 230-233, 236-237], anti-fertility activity and is a potential male contraceptive agent (in mice), hepatoprotective from *R. communis* L. also were claimed [246, 247, 249, 253, 257, 261, 262].

While others plants containing in P2 may be used to supplement for the specific symptoms such for treating common cold, flu, to relieve symptoms of flatulence, congestion, itching. Medicinal plants which contain the similar indication may be used for replacement or may be used to increase their potency of the specific activities.

From this section, it can be concluded that using of many species of medicinal plants especially more than ten of main plants that containing in postpartum herbal bath recipes may be used to improve postpartum women health. In the same time, there are no antagonist effect of each plants which were used in the recipes.

Flavonoids and phenolic compounds that presented in many plants in the Mien's postpartum herbal bath can confirm the mention of Vermerris and Nicholson that is flavonoids are known as phytochemical compounds that provide protection against ultraviolet radiation, pathogens, and herbivores [284]. The above scientific evidence supports the Mien's folklore about using herbs in postpartum bath recipes and answer the question of why are these recipes have been trusted and used for long time in Mien society.

The health promotion of Mien's postpartum baths relates to health promotion in the Thai tradition. In Thai society, there is a belief and cultural use medicinal plants in confinement and the postpartum herbal steam bath. It is believed that confinement and herbal steam bathing can help postpartum women to heal completely, receive the fire element that helps postpartum women keep warm and maintain temperature balance, help to increase the elimination of waste product from the vagina, and make the uterus firm. Moreover, it helps wound dryness, which helps in postpartum recovering.

Many medicinal plants were chosen to use for Thai's bathing. Aromatic plants which have volatile oils were used for muscle relaxants, skin diseases, to relieve nose congestion like the volatile oil from "Phlai" (*Zingiber cassumunar* Roxb.), "Makrud" (*Citrus hystrix* DC.), and Turmeric (*Curcuma longa* L.). Some "sour" plants are used for cleaning the skin like tamarind leaves and soap pods. There were some plants used for specific diseases such as Sea holly (*Acanthus ilicifolius* L.) for skin diseases and cardamom (*Amomum krervanh* Pierre) for eye pain [286].

Previous literature also found that, there are this practices of traditional medicine in South East Asia, their care mostly intend to care the health of vulnerable people such as child, elderly people and women especially the pregnancy and postpartum women. Mother roasting and steam herbal bath also were found in the postpartum women healthcare of ethnic groups in this region such as the confinement of Brou, Saek and Kry in LAO PDR, Minahasa in Indonesia, Vietnamese, Lahu in Jinping Miao, Yao, Dai, Shuhi, and Bai in southwest China. The other ethnic groups in Thailand, Malaysia, Philippines, Indonesia also were found [46-52, 287-289]. These herbal recipes have influenced to their lifestyle in term of holistic care in oriental medicine belief. These recipes should be conserved by documentation and investigation further to promote and integrate to modern medicine to fulfill a weakness point of each other. To promote these medicinal plants use knowledge, it should be intensively performed especially in the primary health care level of the postpartum women in this region. Medicinal plant used in postpartum women of many ethnic groups should be the first priorities to conserve for the sustainable use of these local people. An example of medicinal plants which have been commonly used in postpartum herbal steam baths recipes of the Mien, Yunanese, Lisu and Lahu, four

ethnic groups who come from the southern China and currently live in northern Thailand, and also are used by Thai people that should be promoted are shown in Table 13.

Table 13 Medicinal plants commonly used in herbal bath preparations by four ethnic groups in northern Thailand and also used by Thai people

Scientific Name	Mien*	Yunnanese**	Lisu**	Thai**	Lahu***
<i>Ageratum conyzoides</i> L.	-	X	X	-	-
<i>Blumea balsamifera</i> (L.) DC.	X	X	-	X	X
<i>Clerodendrum fragrans</i> (Vent.) Willd.	-	X	-	-	-
<i>Clerodendrum inerme</i> (L.) Gaertn.	-	-	-	X	-
<i>Cleodendrum paniculatum</i> L.	-	X	-	-	-
<i>Clerodendrum serratum</i> (L.) Moon	-	-	X	-	-
<i>Clerodendrum urticaefolium</i> (Roxb.) Wall. ex Schauer	-	X		-	-
<i>Cymbopogon citratus</i> (DC.) Stapf	X	-	X	X	-
<i>Cymbopogon nardus</i> Rendle	-	X	-	-	-
<i>Elsholtzia</i> sp.	X	X	-	-	X
<i>Morus alba</i> L.	-	X	X	-	-
<i>Rhinacanthus nasutus</i> (L.) Kurz	-	X	-	X	-
<i>Ricinus communis</i> L.	X	X	-	X	-
<i>Vitex</i> spp.	X	X	X	-	-

Sources : *[11], [42-43], **[287-288], ***[289]