## CHAPTER 5

## CONCLUSIONS

anti-inflammatory Forty-nine recipes were selected from the "MANOSROI II" database. Seven recipes were selected from the forty-nine recipes. This study conducted in *in vivo* tests in rats to investigate the anti-inflammatory activity of the recipes. Data from the literature review have also confirmed the antiinflammatory activity of each plant which made up the composition in the selected recipes. The phytochemical tests were conducted to prove the chemical constituents of the recipe extracts. The rat hind paw edema and rat ear edema were the two methods to explore the potential anti-inflammatory activity of the crude plant extracts on the selected recipes through the oral and topical administrations respectively. The male rats Sprague dawley were used in the animal study. Carrageenan was used to induce inflammation for the rat hind paw method and ethyl phenylpropiolate (EPP) was similarly used in the rat ear method. Prednisolone acetate was used as the positive control substance in the rat hind paw edema method while phenylbutazone was used for the same purpose in the rat ear edema method. This study aimed to test the anti-inflammatory effects of the crude extracts of the selected recipes of drugs made from medicinal plants traditionally prepared according to the Lanna traditional medicinal recipes. The results from this study can be concluded as follows:

1) The forty-nine recipes were selected from a total of 11,130 translated recipes from Lanna Medicinal Plant Textbooks Database which collected from seven provinces (Chiang Mai, Chiang Rai, Lamphun, Lampang, Phayao, Phrae and Nan

were put in the "MANOSROI II" database. Forty-nine anti-inflammatory recipes were found in the "MANOSROI II" database. Nine recipes were used to treat acne, 10 recipes for gum abscess, 13 recipes for boiled/bruised/skin edema and 17 for insect/animal sting and bite. Forty-nine recipes were selected by using the criteria of that 1) frequency of the same plants used in recipes, 2) the ease availability of plants in the recipes 3) completeness of recipes which composed of the four criterian were; a) composition of the recipe, b) dosage form of the recipe, c) preparation of the recipe and d) indication uses of the recipe. The forty-nine recipes were ranked for 50 scores. It was found the recipe nos. 896, 105, 192, 25, 895, 717 and 346 were in the top seven of the priority ranking respectively. These recipes were treated for insect sting and bite, acne, boiled and gum abscess which matched all the criteria as mentioned above, thus they were selected for the study. The seven selected recipes were from various provinces in the northern part of Thailand, namely, recipe nos. 25 and 896from Lampang, recipe no.105 from Chiang Rai, recipe nos.192 and 346 from Phrae, recipe nos.717 and 895 from Chiang Mai. Plants in the recipe were available in the local district markets or in scrub forests where they were collected. According to the recipes, some plants appeared as ingredients in more than one recipe.

2) The phytochemical tests confirmed the presence of alkaloids, anthraquinone glycosides, carbohydrate cardiac glycosides, lipids, saponins, tannins and xanthones in the crude extracts of all the selected seven recipes. Alkaloid was found high color intensity in the crude extracts from recipe no.896 and low color intensity in the crude extracts from recipe nos. 25, 105, 192, 346, 717 and 895. Anthraquinone glycosides were found low color intensity in the crude extracts from recipe nos. 25 and 895. Carbohydrate was found low color intensity in the crude

extracts from all seven selected recipes. Cardiac glycosides were found high color intensity in the crude extracts from recipes nos. 25, 105, 192, 717 and 896 but were found low color intensity in the crude extracts from recipe nos. 346 and 895. Lipids were found high color intensity in the crude extracts from recipe no. 896. Saponins were found low color intensity in the crude extracts from recipe no.895. Tannins were found high color intensity in the crude extracts from recipe nos. 25 and 105. Xanthones were found in the crude extracts from recipe nos. 105, 346, 717 and 895, moreover the high color intensity of xanthones was found in the crude extract of recipe no. 25. It was confirmed that almost all extracts of the recipes did not contain flavonoid, coumarin and carotenoid.

3) The results from rat hind paw edema assay of the extracts for oral administration for anti-inflammatory activity are summarized as follows:

3.1 The crude extracts from 6 recipes were found to have antiinflammation effects, compared with the positive control (prednisolone acetate). The recipe nos. 105 and 896 was better edema inhibition than positive control. The detailed results were as follows:

3.1.1 The extracts of recipe no.25 indicated the maximum inhibitory effects on the rat paw edema of 61.36 % of dose 7.88 and 31.52 mg/kg b.w. at time 3 hrs which was less inhibitory effects than the positive control at maximum inhibitory effects 79.10 % of dose 2 mg/kg b.w. at 2 hrs.

3.1.2 The extracts of recipe no.105 showed the highest inhibitory effects on rat paw edema of 87.50 % of dose of 5.18 mg/kg b.w. at time 3 hrs which was better inhibitory effects than the prednisolone acetate at maximum inhibitory effects 79.10 % of dose 2 mg/kg b.w. at 2 hrs. 3.1.3 The extracts form recipe no.192 showed maximum inhibitory effects on rat paw edema of 77.27 % of dose 2.66 mg/kg b.w. at time 2 hrs whereas, the positive control's best inhibitory effects on rat paw edema was 79.10 % of dose 2.0 mg/kg b.w. at time 2 hrs.

3.1.4 The extracts of recipe no.346 exhibited maximum inhibitory effects on rat paw edema 75.0 % of dose 3.24 mg/kg b.w. at 2 hrs whereas, the positive control's best inhibitory effects on rat paw edema was 79.10 % of dose 2.0 mg/kg b.w. at time 2 hrs of observation.

3.1.5 The extracts of recipe no.717 showed the highest inhibitory effects on rat paw edema 70.83 % at dose of 2.03 mg/kg b.w. at time 3 hrs whereas, the positive control's best inhibitory effects on rat paw edema was 79.10 % of dose 2.0 mg/kg b.w. at time 2 hrs of observation.

3.1.6 The extracts of recipe no.895, it was ineffective to inhibit the rat paw edema.

3.1.7 The extracts of recipe no.896 showed the highest inhibitory effects on rat paw edema 79.55 % of dose of 29.92 mg/kg b.w. at time 2 hrs whereas, the positive control's best inhibitory effects on rat paw edema was 79.10 % of dose 2.0 mg/kg b.w. at time 3 hrs of observation.

3.2 The reduction of edema by the seven selected recipe extracts and prednisolone acetate at 2 hrs or at longer time interval after carrageenan injection suggested that these treatments may produce anti-inflammatory effects in the second phase of edema, indicating the inhibition of prostaglandin synthesis.

3.3 Data from the literature reviews have confirmed the presence of chemical compounds anti-inflammation action in each plants used as the ingredients

in recipes nos. 105 and 896 which gave the best inhibitory effects were shown as follows:

3.3.1 The extracts of recipe no.105 which has been traditional used for the treatment of insect sting and bite was found to have alkaloids from barks, leaves and fruits of *Aegle marmelos*; flavonoids, alkaloids, quercitins, anthraquinones and tannins from leaves and barks of *Azadirachta indica*; alkaloids, steroid glycosides and coumarins from the roots of *Cyperus rotundus*; alkaloids and gallic acid from the fruits of *Phyllanthus emblica* and phenolic compound (6-shogoal and 6-gingerol) from the rhizomes of *Zingiber officinale*.

3.3.2 The extracts of recipe no. 896 which has been traditional used to treat insect sting and bite was found to have alkaloid triterpenoids and tannins from leaves of *Coccinia grandis*; phenylpropanoid (sinapic acid) from seed oils of *Sesamum indicum*; flavonoids from leaves of *Vitex trifolia* and phenolic compound (6-shogoal and 6-gingerol) from the rhizomes of *Zingiber officinale*.

4) The results from rat ear edema tests whereby the extracts from the selected three (recipe nos. 25, 346 and 717) for the anti-inflammatory activity were topically administered. These three recipes were selected from the rat hind paw tests which notified the indication uses by topical administration were summarized as follows:

4.1 Extracts from three selected recipes were tested statistically significant in their anti-inflammation effects of reducing the rat ear edema (p<0.05) when compared with the positive control (phenylbutazone). The positive control exhibited better inhibition effect than the extracts of only recipes no.25. It was, therefore, possible to claim that the three selected recipe extracts and phenylbutazone

(positive control) at time 360 mins of the observation became effect when release and/or synthesis of kinin, serotonin and prostaglandins occurred. More detailed results were shown as follows:

4.1.1 The extracts of recipe no.25 indicated the significantly reduced the rats ear edema thickness (*p*-value<0.001) when compared to the positive control. The best dose of recipe no.25 was 1.0 mg/20µL/ear at time 360 mins when the highest edema inhibition on rat ear edema was shown to be 91.57 %, whereas, phenylbutazone showed the highest inhibitory effects on rat ear edema of 95.41 % of dose 1 mg/20µL/ear at time 360 mins and significantly reduced the edema formation at time 360 mins. The minimum inhibitory effect on rat ear edema of phenylbutazone was 45.37 % of dose 1 mg/20µL/ear at time 30 mins better than the extracts of recipe no.25 of all doses at time 15 mins which were unable to inhibit the rat paw edema.

4.1.2 The extracts of recipe no.346 exhibited maximum inhibitory of the rat ear edema was 96.09 % of dose 2.0 mg/20 $\mu$ L/ear at time 360 mins, whereas, the positive control showed the highest inhibitory effects on the rat ear edema was 95.41 % of dose 1 mg/20 $\mu$ L/ear at time 360 mins. The minimum inhibitory effect on rat ear edema of phenylbutazone was 45.37 % of dose 1 mg/20 $\mu$ L/ear at time 30 mins better than the extracts of recipe no.346 of -380 % of dose 0.5 mg/20 $\mu$ L/ear at time 1440 mins which was unable to inhibit the rat paw edema.

4.1.3 The extracts of recipe no.717 showed the highest inhibitory effects on the rats ear edema 95.86 % of dose 2.0 mg/20 $\mu$ L/ear at time 360 mins, whereas, the positive control showed the highest inhibitory effects of rat ear edema 95.41 % of dose 1 mg/20 $\mu$ L/ear at time 360 mins. The lowest inhibitory

effects on rat ear edema of phenylbutazone was 45.37 % of dose 1 mg/20 $\mu$ L/ear at time 30 mins better than the extracts of recipe no.717 of -33.13 % of dose 0.5 mg/20 $\mu$ L/ear at time 60 mins which was unable to inhibit the rat paw edema.

4.2 The study indicated that the extracts from recipe no.346 was the best recipe extracts and produced the maximum inhibitory activity on the rats ear edema of 96.09 % at dose of 2.0 mg/20 $\mu$ L/ear at time 360 mins. The extracts from recipe no.346 produced the maximum inhibitory activity on the rats ear edema was 96.09 % of dose 2.0 mg/20 $\mu$ L/ear at time 360 mins. The positive control exhibited the highest inhibitory rate on the rats ear edema was 95.41 % of dose 1.0 mg/mg/20 $\mu$ L/ear at time 360 mins. While the positive control exhibited better inhibition effect on rat ear edema than the extracts of recipe no.25, the extracts of recipe no.346 showed better inhibition effect than that of the positive control.

4.3 Data from the literature reviews were consistent with the findings of the present study and thus confirm the anti-inflammatory activity and chemical compounds found in the plants composition in recipe nos. 25, 346 and 717 tested on the rat ear model. These recipes which have been used in the Lanna traditional medicine for treating insect sting and bite and acne abscess respectively contained many kinds of plants which have been proved to possess the anti-inflammatory activity from two main chemical compounds, flavonoids and alkaloids.

4.3.1 The extracts of recipe no.25 which has been used in the Lanna traditional medicines for treating insect sting and bite was found to have flavonoids and alkaloids from the bark of *Cassia alata* L.Roxb; and all parts of *Datura metel* L.var.*fastuosa* (Bernh.) Danert was found to have alkaloid

megastigmane sesquiterpenes and leaves of *Jatropha gossypifolia* L. gave flovonoids, vitexin and apigenin.

4.3.2 The extracts of recipe no.346 which has been used in Lanna traditional medicines for treating acne abscess was found to have flavonoids and alkaloids (cucurbitacin) from the fruits juice of *Lagenaria siceraria* (Molina) Standl and flavonoids (bergenin) from the roots of *Caesalpinia digyna* Rottle.

4.3.3 The extracts of recipe no.717 which has been used in the Lanna traditional medicines for the treatment of insect sting and bite and skin abscess was found to have anthraquinone, xanthone and flavonoid glycosides from the leaves of *Cassia occidentalis* L.; as well as Taraxerol and saponins from the fruits and dried leaves of *Dregea volubilis* (L.F) Hook. f.

## **Suggestions for further study:**

Results from this study have suggested the potential of the selected recipes for further research novel anti-inflammatory drugs and developments. These recipes, however, need further in-depth investigation to reconfirm their chemical compounds and establish their potency, interaction among these compounds, doses and effectiveness as follows:

1. The present study attempted to closely follow the preparation of the traditional recipes, particularly in the plants selection and preparation instructions. Of much interest was the use of extraction method. Extraction with organic solvent and test of the extracts for the anti-inflammatory activity should be performed.

2. The isolation of compounds with active principles in the medicinal plants in the recipe extracts and its mechanism of anti-inflammatory action should be considered for further studies to understand the way the recipes work and their better selection for the treatment purpose.

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