CHAPTER 4

RESULTS AND DISCUSSION

The results of the studies were divided into three parts :

4.1 Selection of the 49 Lanna anti-inflammatory medicinal plant recipes

4.1.1 A total of 11,130 translated recipes collected from seven provinces (Chiang Mai, Chiang Rai, Lamphun, Lampang, Phayao, Phrae and Nan) were put in the "MANOSROI II" database. Fortynine anti-inflammatory recipes were selected from the "MANOSROI II" database. Nine recipes were used for treating of acne, 10 recipes for gum abscess, 13 recipes for boiled/bruised/skin edema and 17 for insect/animal sting and bite (Appendix B, Table B.1, B. 2). The results of selection of the recipes were as follows:

4.1.2 The forty-nine recipes were reviewed and selected for the present study based on the criteria were described and shown in Table 4.1.

(A) Frequency of the same plants used in recipes were 7, 4, 3, 2 and 1 recipes, the score 20, 11.4, 8.6, 5.7 and 2.8 respectively as shown in column (1) and (2) in Table 4.1.

(B) Availability of plants in the recipes, the score was 10 for the recipe which had the same plants as in the recipe, as shown in column (3), (4) and (5) in Table 4.1.

(C) The completeness of recipes which composed of the four criteria were; composition of the recipe, dosage form of the recipe, preparation of the recipe and indication uses of the recipe as shown in column (6), (7), (8) and (9) in Table 4.1.

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 which were specified the completeness of the recipes; the composition, dosage form, preparation and indication uses, thus these were selected for Lanna medicinal anti-inflammatory test. These 7 recipes were treated for insect sting and bite, acne, boiled and gum abscess as shown in Table 4.1.

Table 4.1 Survey of the recipes for the study

List	Province	Recipe no.	Lanna use	(A) Frequency of same plants appeared (score=20)		(B) Availability of plants (score=10)			(C) Completeness of recipe (score=20)				Total score =50	(D) Priority ranking
				(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)		
1	Lampang	896	insect	7	20.0	4	4	10	5	5	5	5	50.0	1
2	Chiang Mai	105	insect	7	20.0	9	8	8.9	5	5	5	5	48.9	2
3	Phrae	192	insect	7	20.0	16	14	8.6	5	5	5	5	48.6	3
4	Lampang	25	insect	4	11.4	3	3	10	5	5	5	5	41.4	4
5	Chiang Mai	895	gum ab	4	11.4	6	5	8.3	5	5	5	5	39.7	5

Table 4.1 Survey of the recipes for the study (continued)

List	Province	Recipe no.	Lanna use	(A) Frequency of same plants appeared (score=20)		(B) Availability of plants (score=10)			(C) Completeness of recipe (score=20)				Total score =50	(D) Priority ranking
				(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)		
6	Chiang Mai	717	boiled	3	8.6	6	6	10	5	5	5	5	39.0	6
7	Phrae	346	acne	2	5.7	4	4	10	5	5	5	5	36.0	7
8	Phrae	21	boiled	7	20.0	4	2	5	0	5	0	0	34.3	8
9	Chiang Mai	855	insect	7	20.0	15	5	3.3	5	0	0	5	33.3	9
10	Phayao	119	gum ab	3	8.6	5	4	8	5	0	5	5	31.6	10

Table 4.1 Survey of the recipes for the study (continued)

List	Province	Recipe no.	Lanna use	(A) Frequency of same plants appeared (score=20)		(B) Availability of plants (score=10)			(C) Completeness of recipe (score=20)				Total score =50	(D) Priority ranking
				(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)		
11	Chiang Mai	891	boiled	3	8.6	4	3	7.5	5	5	0	5	31.1	11
12	Chiang Mai	277	gum ab	2	5.7	2	2	10	5	5	0	5	30.7	12
13	Lampang	29	insect	4	11.4	7	3	4.3	5	0	5	5	30.7	12
14	Lampang	605	gum ab	4	11.4	5	2	4.0	5	5	5	0	30.4	13
15	Lamphun	129	gum ab	4	11.4	5	2	4.0	5	0	5	5	30.4	13

Table 4.1 Survey of the recipes for the study (continued)

List	Province	Recipe no.	Lanna use	(A) Frequency of same plants appeared (score=20)		(B) Availability of plants (score=10)			(C)Completeness of recipe (score=20)				Total score =50	(D) Priority ranking
				(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)		
16	Chiang Mai	964	gum ab	3	8.6	3	2	6.7	5	5	0	5	30.3	14
17	Chiang Mai	829	boiled	7	20	2	1	5	0	0	0	5	30.0	15
18	Phrae	3	boiled	7	20	1	1	0	0	5	0	5	30.0	15
19	Phayao	70	gum ab	4	11.4	3	1	3.3	0	5	5	5	29.7	16
20	Lampang	22	insect	4	11.4	10	8	8	5	0	0	5	29.0	17

Table 4.1 Survey of the recipes for the study (continued)

List	Province	Recipe no.	Lanna use	(A) Frequency of same plants appeared (score=20)		(B) Availability of plants (score=10)			(C) Completeness of recipe (score=20)				Total score =50	(D) Priority ranking
				(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)		
21	Chiang Mai	107	boiled	4	11.4	11	8	7.3	5	0	0	5	28.7	18
22	Phrae	488	boiled	3	8.6	2	2	10	5	0	0	5	28.6	19
23	Phrae	310	boiled	3	8.6	4	2	5	5	0	5	5	28.6	19
24	Lampang	123	acne	3	8.6	1	1	0	5	5	5	5	28.6	19
25	Phayao	22	gum ab	4	11.4	3	2	6.7	5	0	0	5	28.1	20

Table 4.1 Survey of the recipes for the study (continued)

List	Province	Recipe no.	Lanna use	(A) Frequency of same plants appeared (score=20)		(B) Availability of plants (score=10)			(C) Completeness of recipe (score=20)				Total score =50	(D) Priority ranking
				(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)		
26	Lamphun	179	insect	4	11.4	8	5	6.25	5	0	5	0	27.7	21
27	Lampang	1	insect	1	2.8	4	3	7.5	5	5	5	0	25.3	22
28	Phrae	11	gum ab	3	8.6	3	2	6.7	5	0	0	5	25.3	22
29	Phrae	22	boiled	2	5.7	12	10	8.3	5	0	0	5	24.0	23
30	Chiang Rai	353	boiled	1	2.8	5	3	6	5	0	5	5	23.8	24

Table 4.1 Survey of the recipes for the study (continued)

List	Province	Recipe no.	Lanna use	(A) Frequency of same plants appeared (score=20)		(B) Availability of plants (score=10)			(C) Completeness of recipe (score=20)				Total score =50	(D) Priority ranking
				(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)		
31	Lampang	119	insect	3	8.6	1	1	0	5	0	5	5	23.6	25
32	Lampang	196	insect	3	8.6	1	1	0	5	5	0	5	23.6	25
33	Chiang Mai	476	gum ab	3	8.6	2	1	5	5	0	0	5	23.6	25
34	Lampang	136	boiled	2	5.7	2	2	2	5	5	0	5	22.7	26
35	Phrae	20	boiled	1	2.8	9	4	4.4	5	5	0	5	22.2	27

Table 4.1 Survey of the recipes for the study (continued)

List	Province	Recipe no.	Lanna use	(A) Frequency of same plants appeared (score=20)		(B) Availability of plants (score=10)			(C) Completeness of recipe (score=20)				Total score =50	(D) Priority ranking
				(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)		
36	Lampang	891	insect	4	11.4	1	1	0	0	5	5	0	21.4	28
37	Chiang Mai	801	insect	1	2.8	1	1	0	5	0	5	5	17.8	29
38	Phrae	264	insect	1	2.8	1	1	0	0	5	5	5	17.8	29
39	Lampang	2	insect	1	2.8	1	1	0	0	5	5	5	17.8	29
40	Phayao	280	acne	1	2.8	1	1	0	0	5	5	5	17.8	29

Table 4.1 Survey of the recipes for the study (continued)

List	Province	Recipe no.	Lanna use	(A) Frequency of same plants appeared (score=20)		(B) Availability of plants (score=10)			(C) Completeness of recipe (score=20)				Total score =50	(D) Priority ranking
				(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)		
41	Phrae	261	acne	1	2.8	1	1	0	5	5	5	0	17.8	29
42	Phrae	263	acne	1	2.8	1	1	0	0	5	5	5	17.8	29
43	Lamphun	395	acne	1	2.8	1	1	0	0	5	5	5	17.8	29
44	Phrae	262	acne	1	2.8	1	1	0	5	5	5	0	17.8	29
45	Phrae	264	acne	1	2.8	1	1	0	5	5	5	0	17.8	29

Table 4.1 Survey of the recipes for the study (continued)

List	Province	Recipe no.	Lanna use	(A) Frequency of same plants appeared (score=20)		(B) Availability of plants (score=10)			(C) Completeness of recipe (score=20)				Total score =50	(D) Priority ranking
				(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)		
46	Phayao	100	boiled	1	2.8	1	1	0	0	0	5	5	13.0	30
47	Lampang	147	insect	1	2.8	1	1	0	0	0	5	5	12.8	31
48	Phrae	260	acne	1	2.8	1	1	0	0	0	5	5	12.8	31
49	Chiang Mai	931	insect	2	5.7	1	1	0	0	0	5	0	10.7	32

The top seven recipes were selected for further study. The seven recipes were 1) recipe nos, 25, 105, 192, and, 896 for insect sting and bite, 2) recipe no.346 for acne abscess, 3) recipe no.895 for gum abscess, and 4) recipe no.717 for boiled or bruised. The preparations of these recipes were prepared in the instruction in the Lanna traditional uses were described and shown in Table 4.2-4.8.

1) The recipe no. 25 has been used to treat insect sting and bite of Lanna traditional uses. The recipe contained of 4 plants and prepared by Lanna traditional instruction. The dosages form of this recipe was boluses and solution. In addition, the recipe also used for the treatment of gum abscess. The drug administer was oral and topical route. The preparation of this recipe was shown in Table 4.2.

Table 4.2 Preparation of recipe no. 25 (Lampang/014-128R. 0025), indication for insect sting and bite

Plants	**Preparation**	**Dosage form**	**Lanna indications of uses**
Cassia alata L. Roxb., *Datura metel* L.var.*fastuosa* (Bernh.) Danert,	**Preparation:** 1. equal amount of the tree part of Thorn Apples and tree and leave of Belly Yaches and soak in water	-Boluses form -Solution form	For insect bite or animal sting and bite (from scorpion and dog). The recipe

Table 4.2 Preparation of recipe no. 25 (Lampang/014-128R. 0025), indication for insect sting and bite (continued)

Plants	Preparation	Dosage form	Lanna indications of uses
Jatropha gossypifolia L.	for 3 nights, then bring it to boil and simmer until the ingradients become puree. 2. Soak Ring Worm fruits in water and changes the soaking water several times, bring them to roast and grind into powder. Then mixed it with the puree from no.1. 3. Mix these products with the liquid of Thorn Apples and Belly Yaches. Compressed the products from no.2 into small pellets/bolus.		is taken orally (mix with water for drinking). Mix the boluses with sesame oil and apply it topically on the injured area caused by falling from tree. For the gum abscess mix the recipe with sesame oil and apply on the affected area or mix with a small ginger tea as a mouth wash.

2) The recipe no.105 has been used to treat insect sting and bite of Lanna traditional uses. The recipe contained the eight plants was prepared as the instruction in the recipe. The dosage form of this recipe was cream and solution. The drug administration was oral and topical route. The preparation of the recipe was shown in Table 4.3.

Table 4.3 Preparation of recipe no. 105 (Chiang Rai 013-100 / R. 0105), indication for insect sting and bite

Plants	**Preparation**	**Dosage form**	**Lanna indications of uses**
Aegle marmelos (L.) Correa ex Roxb., *Azadirachta indica* A. Juss.var.siamensis Valeton ., *Cyperus rotundus* L., *Oryza sativa* L., *Phyllanthus emblica* L., *Piper chaba* Hunt., *Terminalia chebula* Retz., *Zingiber officinale* Roscoe.	Prepare the equal amount of : 1. Bael fruit, Emblic Myrobalan, Myrobalan, Nut Grass, Long Java Peper and Ginger and grind them into powder 2. Grind the same amount of Neem Tree flower 3. Boil Bael fruit and steam rice and strain for the liquid. Mix the liquid with powder from no.1 and 2	-Solution -Cream	- Drinking - Applying a thick coat on the affected area

3) The recipe no.192 has been used to treat insect sting and bite of Lanna traditional uses. The recipe contained of fifteen plants was prepared by Lanna traditional instruction. In addition, the recipe also used for treatment of stomach pain. The dosage form of this recipe was small size of bolus. The drug administer was oral route. The preparation of the recipe was shown in Table 4.4.

Table 4.4 Preparation of recipe no. 192 (Phrae 010-010 / R. 192), indication for insect sting and bite

Plants	**Preparation**	**Dosage form**	**Lanna indications of uses**
Anethum graveolens L.**,** *Baliospermum solanifolium* (Burm) Suresh., *Croton Oblongifolius* Roxb.**,** *Croton tiglium* L.**,** *Dioecrescis erythroclada* (Kurz.) Tirveng., *Foeniculum vulgare* Mill. Var. vulgare (Miller) Thell.,	Composition: Plao Noi, plao Yai, Plao Tong Taek, Makhang Dang, Hassakhun, Rose Colour Lead Wort, White Lead Wort, Ka Bien, Fennel Fruit, Dill, Garden Cress Seed, Black Cumin, ginger, black peper, long Peper Java and Paddy Plant 1.Mixed the equal amount of all ingradients and pound	-Small size bolus form for chewing -Mixed with boiled water for drinking	-Drink the mixed recipe with hot water when feel a stomach pain -Chew the bolus when bitten for

Table 4.4 Preparation of Recipe no. 192 (Phrae 010-010 / R. 192), indication for insect sting and bite (continued)

Plants	**Preparation**	**Dosage form**	**Lanna indications of uses**
Lepidium sativum L.**,** *Nigella sativa* L.**,** *Oryza sativa* L.**,** *Phyllanthus emblica* L., *Piper chaba* Hunt.**,** *Piper nigrum* L.**,** *Plumbago indica* L.**,** *Plumbago zeylanica* L**,** *Terminalia chebula* Retz.	them into powder and compress into small balls/bolus the size of a jujube fruit.	-Small bolus	stinged by the animal or insect

4) The recipe no.346 has been used to treat acne abscess of Lanna traditional uses. The recipe contained of three plants was prepared by Lanna traditional instruction. The dosage form of this recipe was liquid. The drug administration was oral and topical route. The preparation of the recipe was shown in Table 4.5.

Table 4.5 Preparation of recipe no. 346 (Phrae 010-010), indication for acne

Plants	**Preparation**	**Dosage form**	**Lanna indications of uses**
Caesalpinia digyna Rottle. *Lagenaria siceraria* (Molina) Standl.**,** *Passiflora foetida* L.	**Composition:** Terripod Plants, Bottle Guards, Stinking Passion flowers Rub off the root of Terripod Plants and calyx of Bottle Guards and mix them together for oral application. In case the powder mix tastes spicy, add Stinking Passion flowers	- Liquid (condense)	- Drinking - Topical application

5) The recipe no.717 has been used to treat boiled and bruised of Lanna traditional uses. The recipe contained of six plants was prepared by Lanna traditional instruction. The dosage form of this recipe was powder and bolus. The drug administration was oral and topical route. The preparation of the recipe was shown in Table 4.6.

Table 4.6 Preparation of recipe no. 717 (Chiang Mai 017-036), indication for boiled and bruised

Plants	**Preparation**	**Dosage form**	**Lanna indications of uses**
Caryota bacsonensis Magalon. *Cassia occidentalis* L., *Dregea volubilis* (L.F) Hook.f., *Fagraea fragrans* Roxb., *Psophocarpus tetragonolobus*, *Quisqualis indica* L.	**Ingredients:** Root of Wart Fish Tail Palm , Coffee Senna , Kratung-Ma-Ba, Tembusa , Winged Bean, Stinking Passion flower , 1. Grind all ingredients into powder for immediate use 2. Compress the powder from no.1 into small ball /bolus for later use	-Powder for a drink mix -Bolus	- Drink the fresh mix of powder with warm water for abscess, insect sting and bite, snake bite -Topical application on the affected area

6) The recipe no.895 has been used to treat gum abscess of Lanna traditional uses. The recipe contained of five plants was prepared by Lanna traditional instruction. The dosage form of this recipe was liquid. The drug administration was oral route. The preparation of the recipe was shown in Table 4.7.

Table 4.7 Preparation of recipe no. 895 (Chiang Mai 018-048), indication for gum abscess

Plants	**Preparation**	**Dosage form**	**Lanna indication of uses**
Cassia alata L., *Gardenia turgida* Roxb. Terveng., *Sauropus androgynus* L. Merr., *Siphonodon celastrineus* Griff, *Tiliacora triandra* Diels.	**Composition:** Ring Worm Cassia, Ka Bian, Star Gooseberry , Ma Duk , Yanang -Rub off all the ingredients into powder for mixing with rice soaked water, then drink it	- Liquid (condense)	-Drink the mix for gum abscess

7) The recipe no.896 has been used to treat for insect sting and bite of Lanna traditional uses. The recipe contained of four plants were prepared by Lanna traditional instruction. The dosage form of this recipe was oily liquid. The drug administration was oral route. The preparation of the recipe was shown in Table 4.8.

Table 4.8 Preparation of recipe no. 896 (Lampang 007-045), indication for insect sting and bite

Plants	**Preparation**	**Dosage form**	**Lanna indications of uses**
Coccinia grandis L., *Sesamum indicum* L., *Vitex trifolia* L., *Zingiber officinale* Roscoe.	**Composition:** Leaves of Ivy Gourd , liquid of Ginger , Sesame oil , liquid of leaf of Indian Wild Pepper Grind Ivy Gourd leaves and leaves separately and squeeze one cup of juice out of each of them 1.Prepare 3 cups of Ginger tea and 6 cups of Sesame oil 2.Mix the ingredients from no.1 and no.2 and boil the mix until it is reduced to oily liquid	-Oily liquid	-Suck for the pain of root of the tooth -Apply on the area of insect sting and bite

4.2 Preparation of plants and crude extracts of the selected recipes

The literature review was done on the chemical constituents and biological activities of the plants in the selected seven recipes. Also, the name list of the plant species which were authenticated and the dried plant parts were deposited at the herbarium, Faculty of Pharmacy, Chiang Mai University (Appendix D).

Each selected recipe was extracted according to the instruction in the recipes as shown in Table 4.9 :

Table 4.9 Percentage yields of each recipe extracts

Recipe No.	Dry weight of recipe (gm)	Crude extract (gm)	% yield
25	50	11.85	23.70
105	50	15.55	31.10
192	50	7.96	15.92
346	50	9.70	19.40
717	50	12.20	24.40
895	50	10.00	20.00
896	50	11.21	22.43

The doses of crude extracts were calculated the oral feeding and topical application in animal study (Appendix A). The appearance of the crude recipe extracts were shown in Figure 4.1.

Figure 4.1 The crude extracts of the 7 selected recipes

A = crude extract of recipe no. 192, B = crude extract of recipe no. 25

C = crude extract of recipe no. 896, D = crude extract of recipe no. 105

E = crude extract of recipe no. 346, F = crude extract of recipe no. 717

G = crude extract of recipe no. 895

4.3 Phytochemical tests

Preliminary phytochemical tests were used to identify/confirm chemical compounds present in each of the selected seven recipes. A total of 11 chemical compounds were found as a result of the tests. Such chemical compounds included alkaloids, anthraquinone glycocides, carbohydrate, cardiac glycosides, carotenoids, coumarins, flovonoids, lipids, saponin glycosides, tannins, and xanthones.

4.3.1 Test for alkaloids

The four models used for testing of alkaloid substances in the extracts were Dragendorff, Hager, Meyer and Wagner tests. The results were as follows :

Alkaloids were found in all seven recipe extracts. The Dragendorff's test (orange in color) and Wagner's test (orange brown in color) gave strong positive results for all recipes, except recipe no.105 which was responded only for the Hager's test (yellow in color) and Wagner's test (orange brown in color). The test results were shown in Figure 4.2.

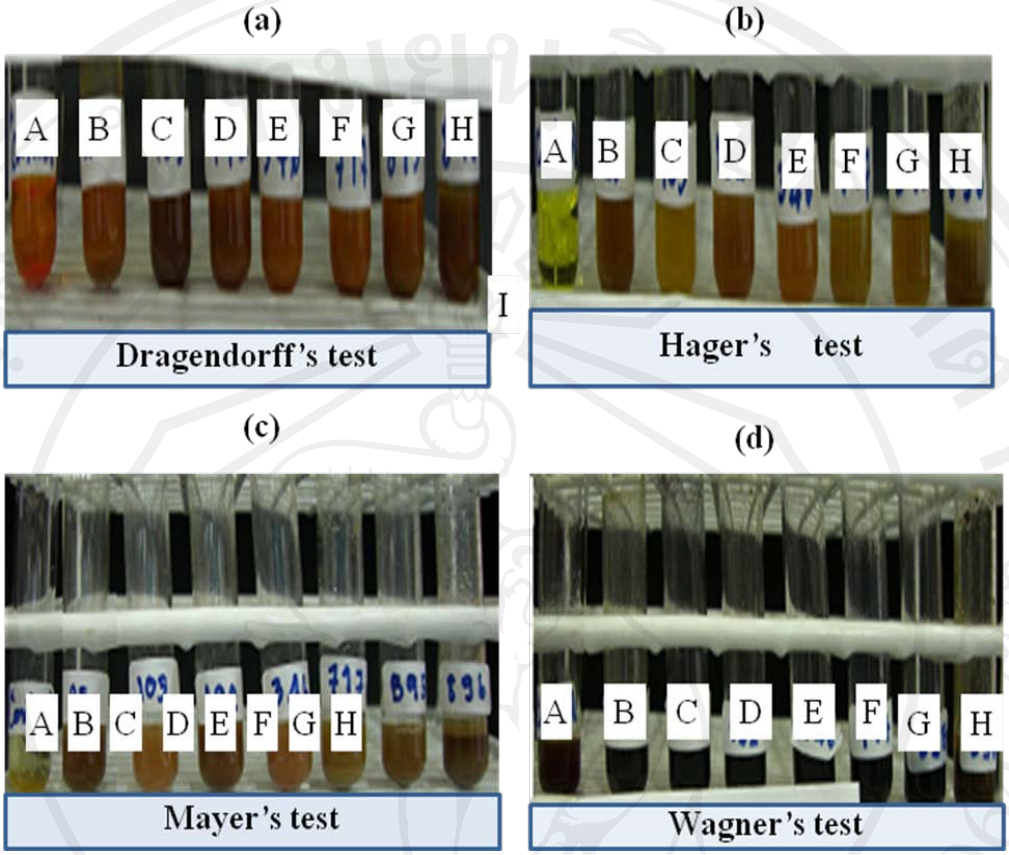

Figure 4.2 Test for alkaloids

Note: (a) = Dragendorf's test, (b) = Hager's test, (c) = Meyer's test, (d) = Wagner's test

A = positive control, B = crude extracts of recipe no.25, C = crude extracts of recipe no.105, D = crude extracts of recipe no.192, E = crude extracts of recipe no.346, F = crude extracts of recipe no.717, G = crude extracts of recipe no.895, H= crude extracts of recipe no.896

As a result, it could be confirmed that all recipes had alkaloid which complied with data from the literature review of plant composition in the recipe. These plants were *Cassia alata*, *Datura metel*, *Jatropha gossypifolia*, *Aegle* marmelos, *Azadirachta indica*, *Cyperus rotundus*, *Oryza sativa*, *Phyllanthus emblica*, *Piper chaba*, *Croton oblongifolius*, *Croton tiglium*, *Nigella sativa*, *Piper nigrum*,

Plumbago zeylanica, Lagenaria siceraria, Dregea volubilis, Fagraea fragrans, Gardenia turgid.

Alkaloids were found in all seven recipe extracts. Results of the Dragendorff's test (orange in color when positive) and Wagner's test (orange-brown in color) showed strong positive outcomes for all recipes, with the exception of recipe no.105 which responded positively only to the Hager's test (yellow in color) and Wagner's test (orange-brown in color). The test results were shown in Figure 3.2 below.

4.3.2 Test for anthraquinone glycosides

Small amount of anthraquinone glycosides of above recipe nos. 25 and 895 from Modified Bontrager's test (the upper aqueous layer become soft pink-red in color) was found. This complied with the literature review that a single plant in these recipes had the anthraquinone glycosides which were *Cassia alata* in recipe nos. 25 and 895.

For the other five recipes, they gave the negative results. Recipe nos. 105 and 717 in this study gave the negative results of anthraquinone glycosides, but the previous report has shown anthraquinone glycosides in the plants containing in these recipes. These plants were *Aegle mamelos, Terminalia chebula* and *Cassia occidentalis*. The results were shown in Figure 4.3.

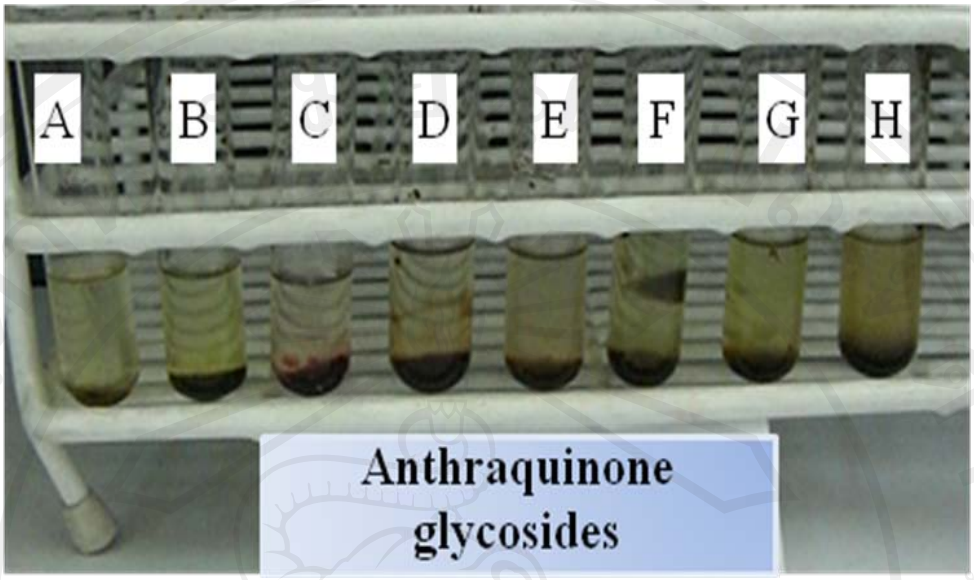

Figure 4.3 Test for anthraquinone glycosides

Note: A = positive control, B = crude extracts of recipe no.25, C = crude extracts of recipe no.105, D = crude extracts of recipe no.192, E = crude extracts of recipe no.346, F = crude extracts of recipe no.717, G = crude extracts of recipe no.895, H = crude extracts of recipe no.896

It could be confirmed that anthraquinone glycosides were present in recipe nos.25 and 895. The anthraquinone glycosides were absent in the extracts of recipe nos. 105 and 717. Anthraquinone glycosides might be reduced during the process of plant preparation.

4.3.3 Test for carbohydrate

The reagent tests for carbohydrate which aimed to reduce sugar. These were Barfoed's, Benedict's, Molisch's, and Seliwanoff's reagents test. The results showed that almost all recipe extracts gave moderate positive results of all tests for carbohydrate with the exception of recipe no.895 which gave the negative result for the Seliwanoff's test. There was no report of the carbohydrate presence in the plants

containing in all recipes except recipe no. 346 which found carbohydrate in the fruit of *Lagenaria siceraria*. The test results were shown in Figure 4.4.

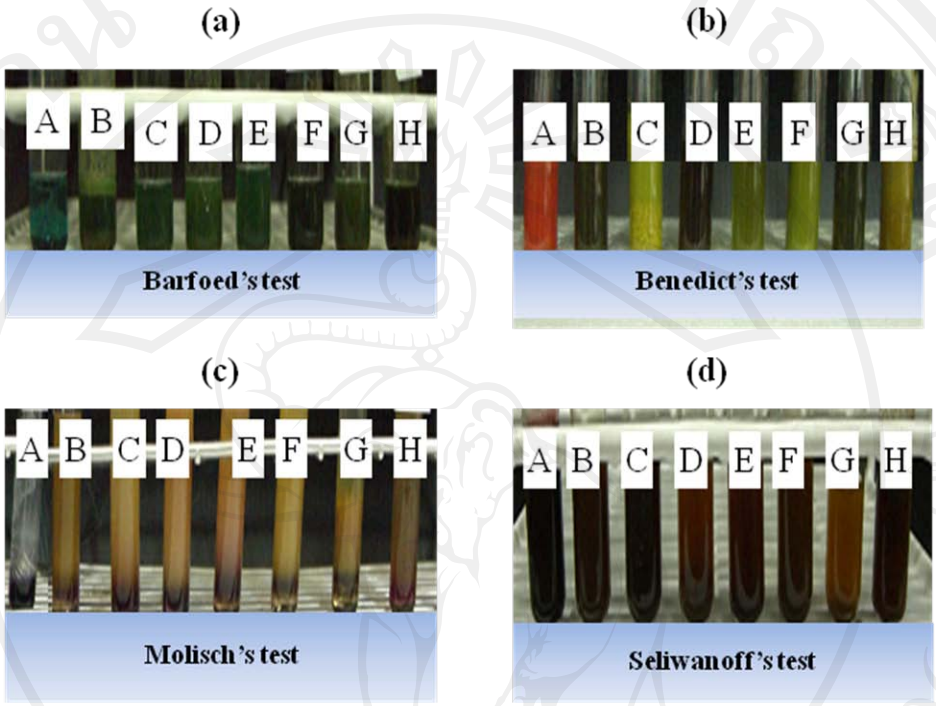

Figure 4.4 Test for carbohydrate

Note: (a) = Barfoed's test, (b) = Benedict's test, (c) = Molisch's test, (d) = Seliwanoff's test

A = positive control, B = crude extracts of recipe no.25, C = crude extracts of recipe no.105, D = crude extracts of recipe no.192, E = crude extracts of recipe no.346, F = crude extracts of recipe no.717, G = crude extracts of recipe no.895, H = crude extracts of recipe no.896

All the recipe extracts have revealed the presence of moderate amount of carbohydrate. When positive, the Barfoed's test gave a brick red precipitate in 5 minutes which indicated a presence of monosaccharide. The Seliwanoff's test was confirmed to have ketose due to a formation of hydroxyl methyl furfural which was condensed with resorcinol to produce an orange red colour. The Benedict's test has

indicated aldehyde group due to a formation of cuprous oxide which produced a brick-red precipitation. The Molisch's test gave the carbohydrate in regarding to the formation of a brownish purple ring between the two layers.

4.3.4 Test for cardiac glycosides

The results showed a positive action with brownish red ring between the layers of the Libermann-Burchard's test (to determine the components of steroid neucleus) and the Keller-Kelliani's test (for de-oxy sugars in cardiac glycosides). But, the Keller-Kelliani's test gave negative result for the recipe no. 105. The results were shown in Figure 4.5.

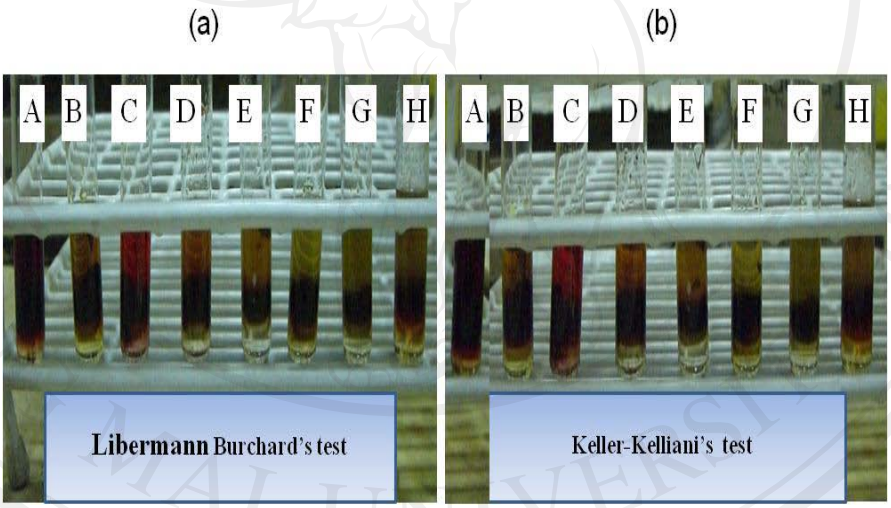

Figure 4.5 Test for cardiac glycosides

Note: (a) = Liberman Burchard's test, (b) = Keller-Kelliani's test:

A = positive control, B = crude extracts of recipe no.25, C = crude extracts of recipe no.105, D = crude extracts of recipe no.192, E = crude extracts of recipe no.346, F = crude extracts of recipe no.717, G = crude extracts of recipe no.895, H = crude extracts of recipe no.896

There was no cardiac glycosides presence in the plant composition of all recipes. In regard to the positive results of the tests, it might be caused by the interaction of other plant components in the recipes.

4.3.5 Test for carotenoids

The two reagents, Conc.H_2SO_4 and Antimony three chloride, were used for the testing of the carotenoids substance. These tests gave negative results (yellow brownish colour) of the extracts from the selected seven recipes under the study. Data from the literature review of the plant composition in these seven recipes gave no evidence on carotenoids. The results were shown in Figure 4.6.

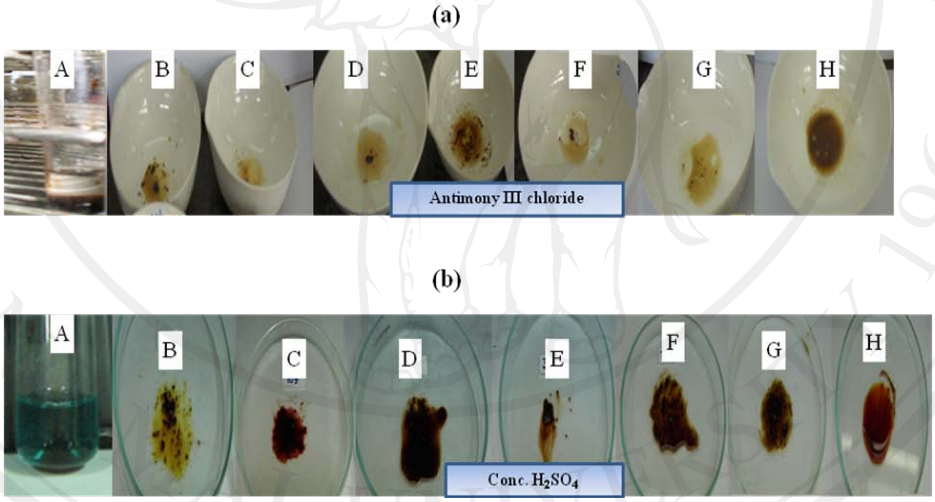

Figure 4.6 Test for carotenoid

Note: (a) = Antimony III chloride, (b) = Conc. H_2SO_4

A = positive control, B = crude extracts of recipe no. 25, C = crude extracts of recipe no.105, D = crude extracts of recipe no. 192, E = crude extracts of recipe no.346, F = crude extracts of recipe no.717, G = crude extracts of recipe no.895, H = crude extracts of recipe no.896

Particularly, carotenoids also give the yellow, orange and red colors of many fruits and flowers. As a result of the study, there were no carotenoids in the plant composition of all tested recipes. Therefore, it might depend on using the leaves, stems or other parts which did not contain carotenoids. Usually, carotenoids were found in fruits and flowers.

4.3.6 Test for coumarins

No coumarins were found in all recipe extracts (no fluorescence on the paper test) which was not compliance with the literature reviews. From the reports, the rhizome of *Cyperus rotundus* and the fruit of *Phyllanthus emblica* which were the plant composition of recipe no.105 and the roots and leaves of *Plumbago zeylanica* which were in recipe no.192 have been reported to have coumarin. The results were shown in Figure 4.7.

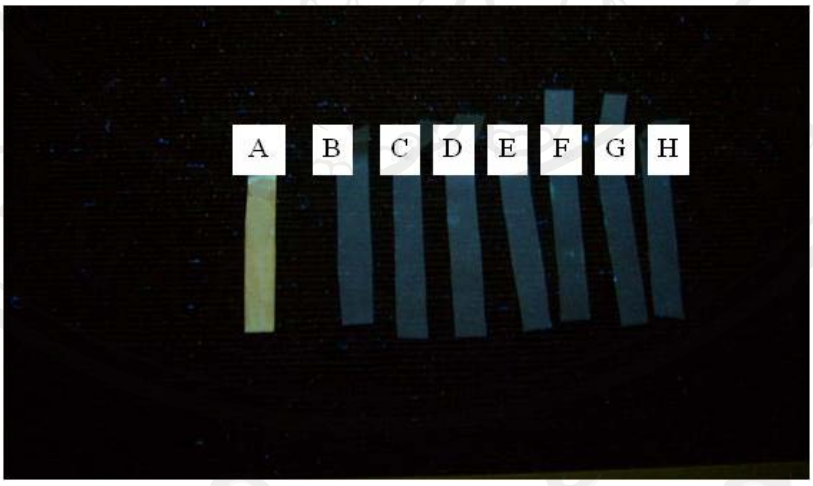

Figure 4.7 Test for coumarins

Note: A = positive control, B = crude extracts of recipe no.25, C = crude extracts of recipe no.105, D = crude extracts of recipe no.192, E = crude extracts of recipe no.346, F = crude extracts of recipe no.717, G = crude extracts of recipe no.895, H = crude extracts of recipe no.896

The negative results of the recipe extracts for test for coumarin may be caused by the interaction among the compounds plant in the composition. According to the coumarin derivatives have contributed the antioxidant and anti-inflammatory activity, therefore, the investigation by using more sophisticated techniques to confirm the accurately results are needed.

4.3.7 Test for flavonoids

This study aimed at the testing of various members of flavonoid classes such as flavone, flavonol, flavanone, flavanonol and flavonoid glycocides. Negative results were found in all recipe extracts through the tests using Magnesium ribbon and Zinc dust reagent. However, literature reviews have indicated the presence of flavonoids in the plant composition in all recipes as follow: the barks and leaves of *Cassia alata*, the leave of *Cassia occidentalis*, the leaves and seeds of *Azadirachta indica*, the leaves and roots of *Oryza sativa*, the leaves and fruits of *Phyllanthus emblica*, the fruit of *Piper chaba*, the rhizome of *Zingiber officinale*, seed of *Anethum graviolens*, the aerial part of the stem bark of *Croton oblongilolius*, the seed of *Croton tiglium*, the leave and branch of *Dioecrescis erythroclada*, the seed of *Foeniculum vulgare*, the seed of *Lepidium sativum*, the seed of *Piper nigrum*, the root of *Plumbago indica*, the root and leaf of *Plumbago zeylanica*, the resin of *Passiflora foetida*, the fruit of *Lagenaria seceraria*, the root of *Caesalpinia digyna*, and the seed of *Sesamum indicum*. The results were shown in Figure 4.8.

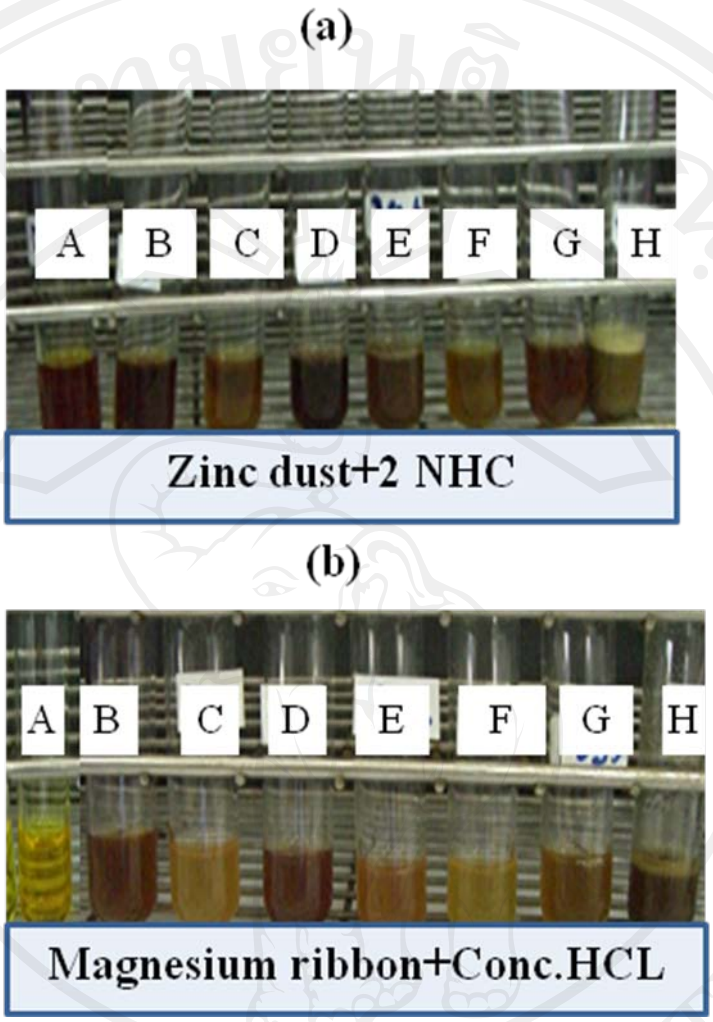

Figure 4.8 Test for flavonoid glycosides

Note: (a) = Zinc dust + HCL, (b) = Magnesium ribbon + Conc. HCL:

A = positive control, B = crude extracts of recipe no.25, C = crude extracts of recipe no.105, D = crude extracts of recipe no.192, E = crude extracts of recipe no.346, F = crude extracts of recipe no.717, G = crude extracts of recipe no.895, H = crude extracts of recipe no.896,

Regard to the flavonoid glycoside tests, the negative results of the recipe extracts disagreed with the previous studies. Therefore, it could be explained that the plant composition in all recipes which contained various kinds of chemical substances

or small amount of flavonoid may cause of the interaction among them and gave completely negative results. Hence, it may be required to find an appropriate technique to test for these compounds in order to evaluate the interaction or synergistic effects of the plants composition.

4.3.8 Test for lipids

Sudan IV test for lipid gave a positive result only for recipe no.896. Most results of the recipe extracts were against the literature review that found lipids in plants which were composition of the six recipes. These plants were seed oil of *Terminsalia chebula*, seeds of *Croton tiglium*, *Nigella sativa* and *Psophocarpus tetragonolobus*, fruits of *Caryota bacsonensis* and fruits of *Vitex trifolia*. The results were shown in Figure 4.9.

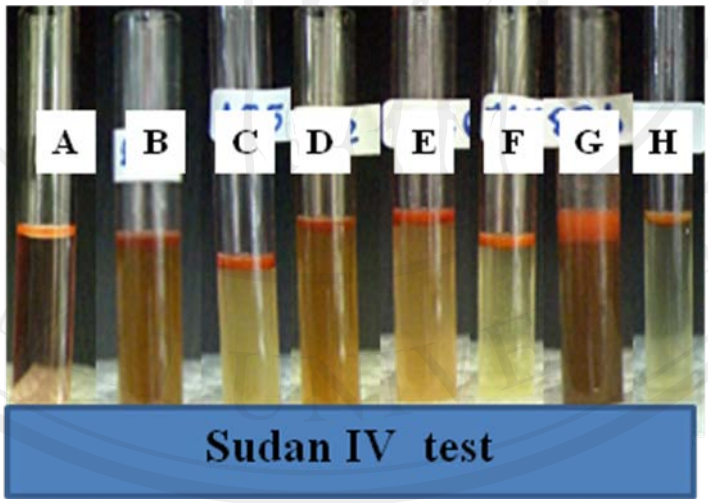

Figure 4.9 Test for lipid

Note: A = positive control, B = crude extracts of recipe no.25, C = crude extracts of recipe no.105, D = crude r extracts of recipe no.192, E = crude extracts of recipe no.346, F = crude extracts of recipe no.717, G = crude extracts of recipe no.895, H = crude extracts of recipe no.896

It could be concluded that lipids were found presence in the crude extracts of plants composition of recipe no. 896. Data from a previous study indicated the presence of fatty acids in the fruits of *Vitex trifolia*. The negative results of tests for lipids in the other six recipe extracts may have caused by the interaction of plant compounds during the process of plant extraction. However, it is required sophisticate technique of extraction for accurately results.

4.3.9 Test for saponin glycosides

The froth test gave a positive result only for recipe no.895 which was against the results of previous studies of plants containing in the selected recipes. In addition, the literature review showed that there was saponin in the dried leaves of *Dregea volubilis*, the fruit of *Lagenaria siceraria*, the leaves of *Terminalia chebula*. The results were shown in Figure 4.10

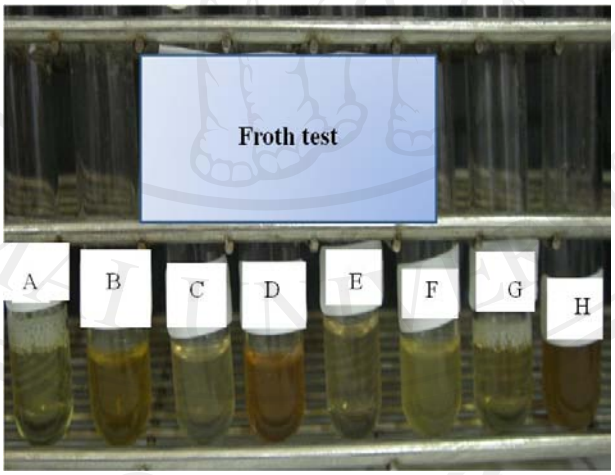

Figure 4.10 Test for Saponin glycosides

Note: A = positive control, B = crude extracts of recipe no.25, C = crude extracts of recipe no.105, D = crude extracts of recipe no.192, E = crude extracts of recipe no.346, F = crude extracts of recipe no.717, G = crude extracts of recipe no.895, H = crude extracts of recipe no.896

For the froth test, it might gave a false positive result for recipe no. 895, since data from many previous studies have indicated that there were the saponins in the plant composition of this recipe.

4.3.10 Test for tannins

The vanillin reagent gave a positive result (crimson colour) for all recipe extracts except the extracts from recipe nos. 717 and 895. The literature reviews have confirmed the presence of tannin in the plant composition of the five recipes which gave positive results. These plants were the leaves of *Jatropha gossypifolia*, the fruits of *Aegle marmelos*, the trunk barks of *Azadirachta indica*, the fruits of *Phyllanthus emblica*, the seeds of *Lepidium sativum*, the barks and seeds of *Caesalpinia digyna* and the leaves of *Coccinia grandis*. The test results were shown in Figure 4.11.

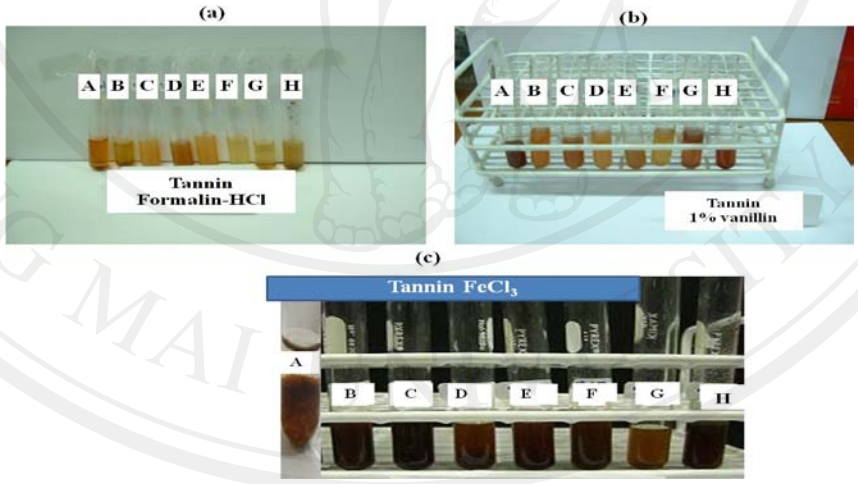

Figure 4.11 Test for tannins

Note: (a) =Tannin Formalin-HCl, (b) Tannin 1% Vanillin, (c) Tannin $FeCl_3$

A = positive control, B = crude extracts of recipe no.25, C = crude extracts of recipe no.105, D = crude extracts of recipe no.192, E = crude extracts of recipe no.346, F = crude extracts of recipe no.717, G = crude extracts of recipe no.895, H = crude extracts of recipe no.896

It could be concluded that there were tannins in the extracts of recipe nos. 25, 105, 192, 346 and 896. These results were confirmed by the preliminary tests and the previous studies.

4.3.11 Test for xanthones

The cyanidin test gave positive result (pink colour) for recipe nos. 105 and 192 which was against the literature reviews of plants containing in the recipes. Infact, xanthone was found in the roots of *Cassia occidentalis* which was the plant in recipe no.717. For the 5% KOH test, it gave positive result in all extracts from the recipes except recipe nos. 192 and 896. The test results were shown in Figure 4.12.

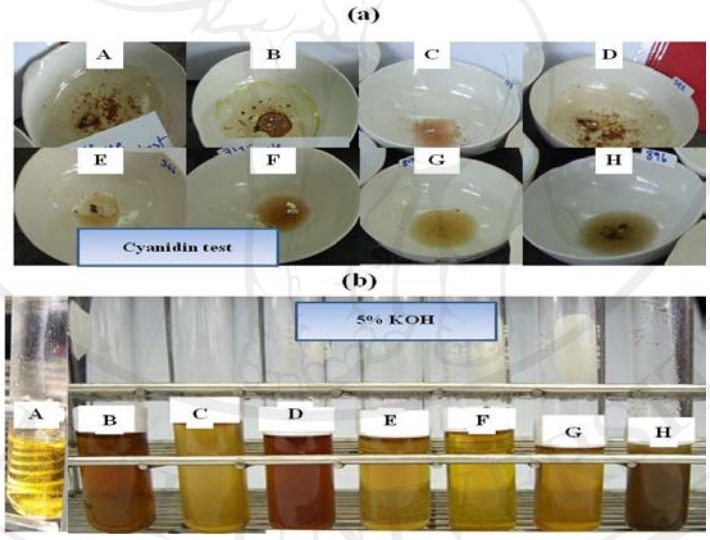

Figure 4.12 Test for xanthones

Note: (a) = Cyanidin test, (b) = 5 % KOH test:

A = positive control, B = crude recipe extracts of recipe no.25, C = crude recipe extracts of recipe no.105, D = crude recipe extracts of recipe no.192, E = crude recipe extracts of recipe no.346, F = crude recipe extracts of recipe no.717, G = crude recipe extracts of recipe no.895, H = crude recipe extracts of recipe no.896

It could be concluded that all recipe extracts gave positive results for xanthone with the exception of recipe no. 896. Recipe no. 25 gave the strongest positive result.

The phytochemical tests were conducted for the presence of chemical compounds with anti-inflammatory activity in the crude extracts of all selected seven recipes in the present. Results of the tests can be summarized as followed:

Tests for alkaloid were performed by using four test models, Dragendorff, Hager, Mayer and Wagner. Through all four test models conducted, alkaloids were found present in all recipe extracts with the strongest positive results on recipe no.896. Confirmation tests for anthraquinone using the Modified Borntrager's test found anthraquinone in recipe nos.25 and 895 with low positive results. Tests for carbohydrate were performed with using four test models, Molisch, Barfoed, Benedict and Seliwanoff tests. All crude extracts of the recipes, except that of recipe no. 895 were found to contain carbohydrate with low positive results. Tests for cardiac glycosides were conducted using Libermann-Burcharsche and Keller-Kelliani test models. The substances were found with strong positive results in recipe nos. 25, 105, 192, 717, and 896 by the Libermann-Burcharsche's test. However, three chemical substances, carotenoids, coumarins, flavonoids were found to be absent from all recipe extracts tested. Tests for lipid were performed using the Sudan IV test. A presence of lipids was found with a strong positive result in recipe no. 896. Tests for a presence of saponin were performed with Froth test. Positive results of saponin were found across the board with the exception of recipe no. 895 which had the low positive result. A presence of tannin was confirmed by three test models, Vanillin reagent, $FeCl_3$ and Formalin HCL. Results of the Vanillin reagent test found tannin with strong positive results in recipe no.25 and low positive result in recipe

nos.105, 192, 346, and 896. Tests performed by $FeCl_3$ test model found tannin with strong positive results in recipe nos. 25 and 105 and low positive results in recipe nos.192, 346 and 717. Formalin HCL tests also found tannin with strong positive results in recipe no.896 and low positive results in recipe nos. 25, 192 and 895. Two test models of KOH 5% and Cyanidin were performed to confirm the presence of xanthone, which yielded strong positive results in recipe no.25 and low positive results in recipe nos. 105, 346, 717 and 895. In addition, the cyanidin test conducted found xanthone with low positive results in recipe nos. 105, 192, 346 and 717.

In conclusion, the preliminary tests suggested that almost all extracts showed positive results. The results were consistent with the previous studies which have indicated the phytochemical in the plants containing in the recipes. These substances showed potent anti-inflammatory activity, for examples, the alkaloids, flavonoids and tannins (Burkill, 1985 and Williamson, 2002; Atal and Kapur, 1982; Thakur *et al*., 1989; EI-Dakhakhny *et al*., 2002). In addition, the recipes, when extracted with distilled water, gave the false negative results. Also the hydrophilic properties of the compounds were known to dissolve better than the hydrophobic compounds.

Table 4.10 Phytochemical tests of the seven recipes

Test	Recipes no.							
	25	**105**	**192**	**346**	**717**	**895**	**896**	**Result**
1. Alkaloids								
Dragendorff's	+	+	+	+	+	+	+++	Reddish-brown
Hager's	+	+	+	+	+	+	+	Yellow
Meyer's	+	+	+	+	+	+	+	Cream
Wagner's	+	+	+	+	+	+	+++	Orange-brown
2. Anthraquinone glycosides								
Modified Borntragers	+	-	-	-	-	+	-	Pink red
3. Carbohydrates								
Molisch's	+	+	+	+	+	+	+	Browish purple ring
Barfoed's	++	+	+	+	+	+	+	Red
Benedict solution	+	+	+	+	+	+	+	Brick red
Seliwanoff	+	+	+	+	+	-	+	Orange red

Table 4.10 Phytochemical tests of the seven recipes (continued)

Test	Recipes no.							
	25	**105**	**192**	**346**	**717**	**895**	**896**	**Result**
4. Cardiac glycosides								
Libermann Burcharsche's	+++	+++	+++	+	+++	+	+++	Brown or brownish-red ring
Keller-Kelliani's	+	-	+	+	+	+	+	Brown red or green ring
5. Carotenoids								
Conc. H_2SO_4	-	-	-	-	-	-	-	Greenish-blue
Antimony III chloride	-	-	-	-	-	-	-	Dark-blue-red
6. Coumarins								
10% Ammonium	-	-	-	-	-	-	-	Fluorescence
7. Flavonoid glycosides								
Flavone	-	-	-	-	-	-	-	Reddish-orange
Flavonol	-	-	-	-	-	-	-	Crimson

Table 4.10 Phytochemical tests of the seven recipes (continued)

Test	Recipes no.							
	25	**105**	**192**	**346**	**717**	**895**	**896**	**Result**
Flavanone	-	-	-	-	-	-	-	Crimson-purplish-red
Flavanonol								Red
8. Lipids								
Sudan IV test	-	-	-	-	-	-	+++	Floating red droplets or red layer
9. Saponins								
Froth test	-	-	-	-	-	+	-	Foam on the mixture
10. Tannins								
Vanillin reagent	+++	+	+	+	-	-	+	Crimson
$FeCl_3$	+++	+++	+	++	++	-	-	Red
Formalin HCL	++	-	++	-	-	++	+++	Red
11. Xanthones								
KOH 5%	+++	+	-	+	+	+	-	Yellow
Cyanidin	-	+	+	+	+	-	-	Pink

Note = + to +++ were low to high color intensity;

= - no color change from negative control

4. 4 *In vivo* anti-inflammatory tests

4.4.1 Rat hind paw edema method

Prednisolone acetate was capable of paw edema inhibition at doses of 1.5 mg/kg b.w. was 36.36 %, 54.24 %, and 48.45 % at time 1 hr, 2 hrs, and 3 hrs respectively; for dose of 2.0 mg/kg b.w. was 56.82 %, 79.10 %, and 59.28 % at time 1 hr, 2 hrs, and 3 hrs respectively; and for dose of 4.0 mg/kg b.w. was 70.45 %, 53.11 % and 34.02 % at time 1 hr, 2 hrs, and 3 hrs respectively. The dose for prednisolone acetate which showed the highest inhibition of the paw edema was 79.10 % of dose of 2.0 mg/kg b.w. at time 3 hrs. The result was shown in Table 4.11.

Table 4.11 Effects of test substance of negative and positive controls on carrageenan-induced hind paw edema in rats

Samples	**Dose in mg/kg/ BW**	**Time after carrageenan injection**						
		EV(ml)				**% EI**		
		1 hr	**2 hrs**	**3 hrs**	***p*-value**	**1hr**	**2hrs**	**3hrs**
Negative control (distilled water)	1 ml	0.23 ± 0.44	0.62 ± 0.22	0.71 ± 0.26	0.021*	0	0	0

Table 4.11 Effects of test substance of negative and positive controls on carrageenan-induced hind paw edema in rats (continued)

Samples	Dose in mg/kg/ BW	Time after carrageenan injection						
		EV(ml)				% EI		
		1 hr	2 hrs	3 hrs	*p*-value	1hr	2hrs	3hrs
Positive control (prednisolone acetate)	1.5 mg/kg b.w.	0.07 ± 0.05	0.17 ± 0.05	0.33 ± 0.04	0.01*	36.36	54.24	48.45
	2.0 mg/kg b.w.	0.05 ± 0.03	0.09 ± 0.02	0.20 ± 0.05	0.01*	56.82	79.10	59.28
	4.0 mg/kg b.w.	0.03 ± .02	0.21 ± 0.07	0.32 ± 0.07	0.01*	70.45	53.11	34.02

Note : EV = Edema volume of the paw (paw volume increase), % EI = percent edema inhibition of Samples at time**;** n = 4 of each group; Values are expressed as mean ± SD**,** * Significantly different in EV for a different time in each dose at $p < 0.05$

Recipe no. 25 showed a significant reduction of the edema formation (*p*-value <0.001) after carrageenan injection only at a dose of 1.97 mg/kg b.w., which had the edema inhibition was 6.25 % at 3 hrs. The maximum edema inhibition of this recipe was 61.36 % of dose 7.88 mg/kg b.w. at time 2 hrs. While the prednisolone acetate exhibited the highest edema inhibition was 79.10 % of dose 2.0 mg/kg b.w. at time 2 hrs. Recipe no. 25 showed no inhibition effect at 1 hr of all doses, however

gave the inhibition effect of dose 7.88 and 31.52 mg/kg b.w..at 2 and 3 hrs. The results were shown in Table 4.12 and Figure 4.13.

Table 4.12 Effects of recipe no.25 crude extracts on carrageenan-induced hind paw edema in rats

Samples	**Dose in mg/kg/ BW**	**Time after carrageenan injection**						
		EV(ml)				**% EI**		
		1 hr	**2hrs**	**3hrs**	***p*-value**	**1hr**	**2hrs**	**3hrs**
Recipe no. 25	1.97 mg/kg b.w.	0.19 ± 0.05	0.44 ± 0.17	0.45 ± 0.15	0.037*	-42.11	0.00	6.25
	3.94 mg/kg b.w.	0.13 ± 0.13	0.37 ± 0.18	0.47 ± 0.22	0.091	-15.38	15.91	2.08
	7.88 mg/kg b.w.	0.15 ± 0.07	0.17 ± 0.05	0.19 ± 0.09	0.745	-26.67	61.36	60.42
	15.76 mg/kg b.w.	0.48 ± 0.11	0.50 ± 0.06	0.47 ± 0.16	0.837	-77.08	-13.64	2.08
	31.52 mg/kg b.w.	0.19 ± 0.13	0.24 ± 0.09	0.19 ± 0.11	0.668	-42.11	45.45	60.42

Note : EV = Edema volume of the paw (paw volume increase), % EI = percent edema inhibition of Sampless at time**;** n = 4 of each group Values are expressed as mean $\pm$ SD**,** * Significantly different in EV for a different time in each dose at $p < 0.05$

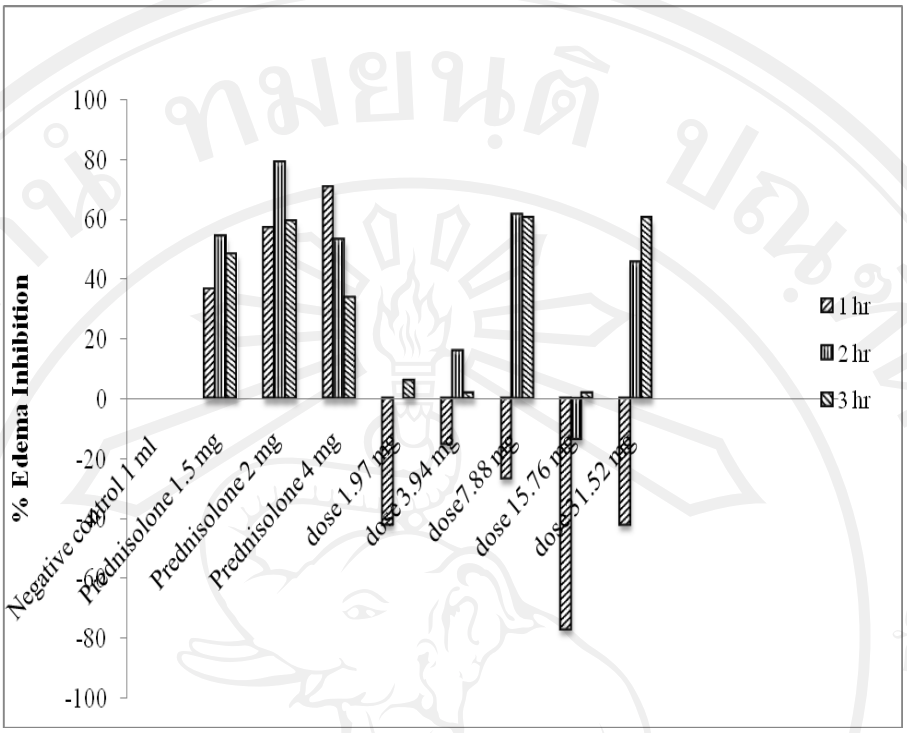

Figure 4.13 Percentages of rat hind paw edema inhibition of recipe no.25 and prednisolone acetate at various doses and time intervals compared with the negative control

The best edema inhibition of recipe no. 105 was 87.50 % at dose of 5.18 mg/kg b.w. at time 3 hrs which was better than the prednisolone acetate of dose 2.0 mg/kg b.w. gave the highest edema inhibition effect of 79.10 % at time 3 hrs. The second best edema inhibition of recipe no. 105 was 75.00 % of dose 41.54 mg/kg b.w. at 3 hrs. Recipe no. 25 showed the edema inhibition effect of all doses at 2 and 3 hrs. The results were shown in Table 4.13 and Figure 4.14.

Table 4.13 Effects of recipe no.105 crude extracts on carrageenan-induced hind paw edema in rats

Samples	**Dose in mg/kg b.w.**	**Time after carrageenan injection**						
		EV(ml)				**% EI**		
		1 hr	**2 hrs**	**3hrs**	***p*-value**	**1 hr**	**2 hrs**	**3hrs**
Recipe no. 105	2.59 mg/kg b.w.	0.16 ± 0.02	0.21 ± 0.11	0.24 ± 0.13	0.644	-45.45	52.27	50.00
	5.18 mg/kg b.w.	0.08 ± 0.05	0.13 ± 0.08	0.06 ± 0.08	0.491	27.27	70.45	87.50
	10.36 mg/kg b.w.	0.14 ± 0.08	0.18 ± 0.08	0.25 ± 0.09	0.234	-27.27	56.82	47.92
	20.72 mg/kg b.w.	0.20 ± 0.05	0.30 ± 0.12	0.34 ± 0.15	0.437	-81.82	31.82	29.17
	41.54 mg/kg b.w.	0.11 ± 0.08	0.14 ± 0.10	0.12 ± 0.09	0.779	0.00	68.18	75.00

Note : EV = Edema volume of the paw (paw volume increase), % EI = percent edema inhibition of Sampless at time**;** n = 4 of each group; Values are expressed as mean ± SD**,** * Significantly different in EV for a different time in each dose at $p < 0.05$

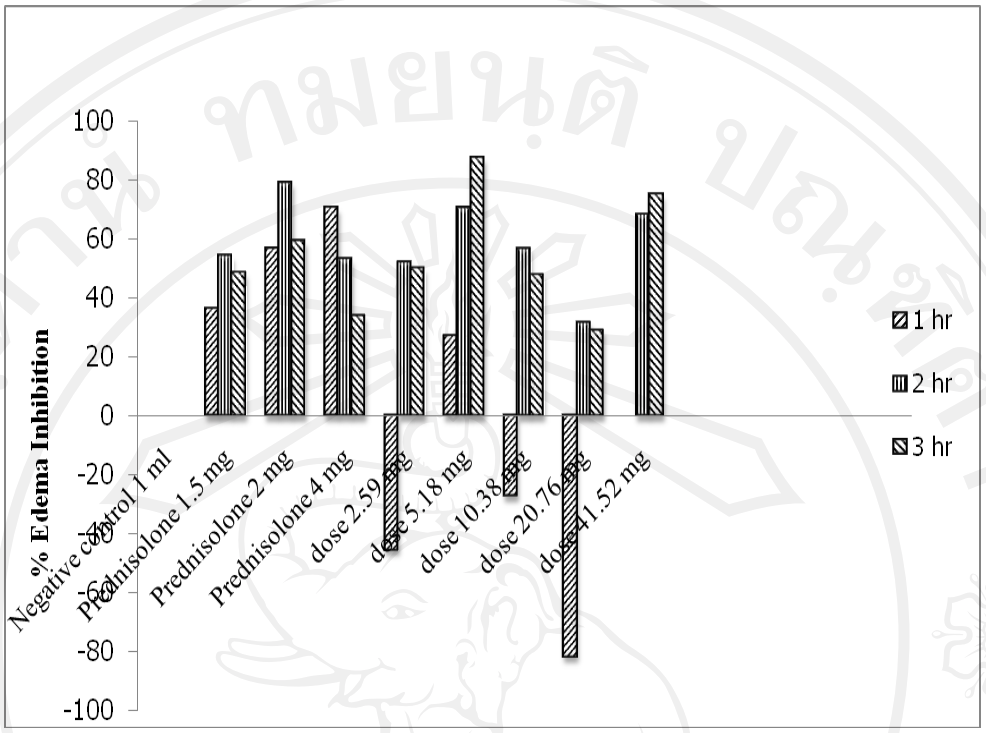

Figure 4.14 Percentages of rat hind paw edema inhibition of recipe no.105 and prednisolone acetate at various doses and time intervals compared with the negative control

The maximum edema inhibition of recipe no. 192 was 61.36 % of dose 5.32 mg/kg b.w. at time 2 hrs. The prednisolone acetate had edema inhibition effect 79.10 % of dose 2.0 mg/kg b.w. at 2 hrs which had better than edema inhibition effect of the recipe no. 192 of all doses and times. The results were shown in Table 4.14 and Figure 4.15

Table 4.14 Effects of recipe no.192 crude extracts on carrageenan-induced hind paw edema in rats

Samples	**Dose in mg/kg/ BW**	**Time after carrageenan injection**						
		EV(ml)				**% EI**		
		1 hr	**2 hrs**	**3hrs**	***p*-value**	**1 hr**	**2 hrs**	**3hrs**
Recipe no. 192	1.33 mg/kg b.w.	0.15 ± 0.08	0.31 ± 0.23	0.36 ± 0.20	0.078	-36.36	29.55	20.83
	2.66 mg/kg b.w.	0.06 ± 0.06	0.10 ± 0.09	0.23 ± 0.17	0.109	0.45	0.77	0.52
	5.32 mg/kg BW	0.14 ± 0.04	0.17 ± 0.086	0.25 ± 0.12	0.456	-27.27	61.36	47.92
	10.64 mg/kg BW	0.37 ± 0.19	0.37 ± 0.20	0.64 ± 0.27	0.170	-236.36	15.91	-33.33
	21.28 mg/kg b.w.	0.12 ± 0.02	0.42 ± 0.46	0.37 ± 0.11	0.077	-9.09	4.55	22.92

Note : EV = Edema volume of the paw (paw volume increase), % EI = percent edema inhibition of Sampless at time**;** n = 4 of each group ; Values are expressed as mean ± SD**,** * Significantly different in EV for a different time in each dose at $p < 0.05$

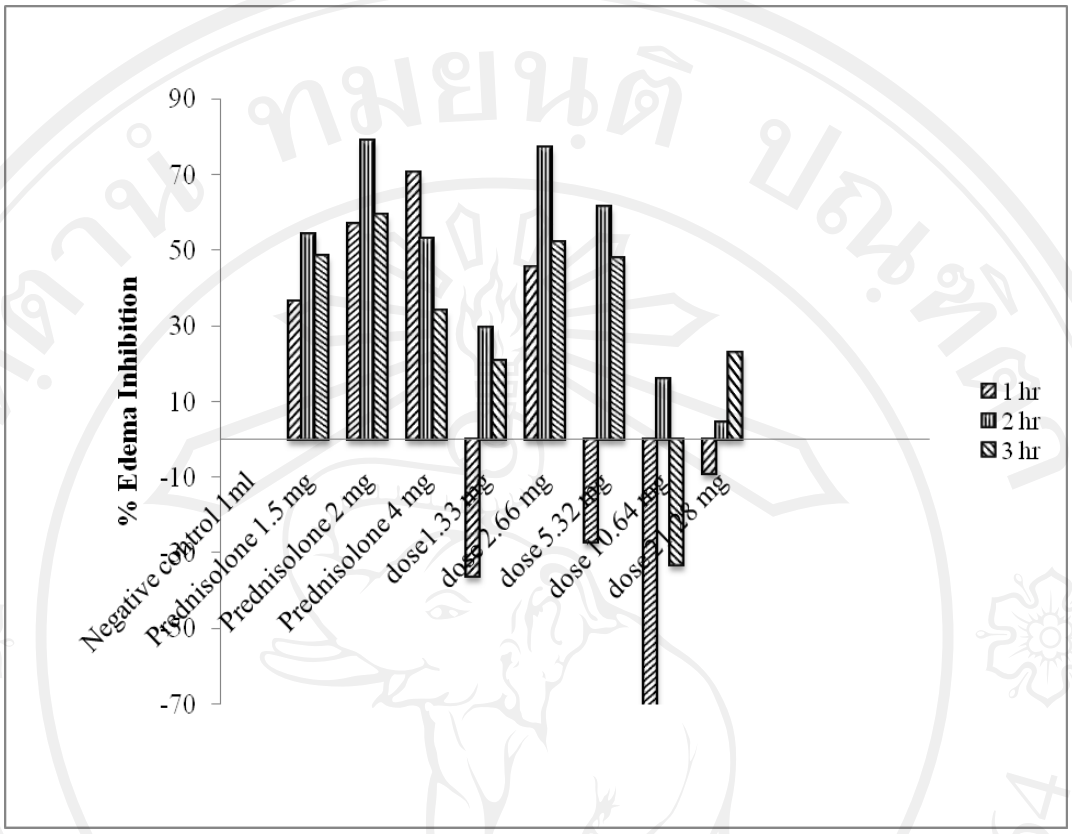

Figure 4.15 Percentages of rat hind paw edema inhibition of recipe no.192 and prednisolone acetate at various doses and time intervalsss compared with the negative control

It was found that the rat hind paw edema of recipe no.346 increased gradually and reached maximum edema at time 3 hrs. It should also be noted that the maximum edema inhibition effect of the crude extracts of the recipe was found to be as high as 75.00 % of dose 3.24 mg/kg b.w. at time 2 hrs but this was not statistically significant. However, the dose of 2.0 mg/kg b.w., prednisolone acetate had the highest edema inhibition effect of 79.10 % at 2 hrs, as shown Table 4.15 and Figure 4.16.

Table 4.15 Effects of recipe no.346 crude extracts on carrageenan-induced hind paw edema in rats

Samples	**Dose in mg/kg b.w.**	**Time after carrageenan injection**						
		EV(ml)				**% EI**		
		1 hr	**2 hrs**	**3hr**	***p*-value**	**1 hr**	**2 hrs**	**3hrs**
Recipe no. 346	1.62 mg/kg b.w.	0.37 ± 0.05	0.34 ± 0.11	0.50 ± 0.29	0.874	0.00	25.00	-4.17
	3.24 mg/kg b.w.	0.16 ± 0.10	0.11 ± 0.07	0.14 ± 0.06	0.500	-236.36	75.00	70.83
	6.48 mg/kg b.w.	0.21 ± 0.12	0.19 ± 0.04	0.31 ± 0.05	0.124	-36.36	56.82	35.42
	12.96 mg/kg b.w.	0.12 ± 0.10	0.17 ± 0.10	0.30 ± 0.05	0.059	-9.09	61.36	37.50
	25.92 mg/kg b.w.	0.15 ± 0.11	0.35 ± 0.14	0.56 ± 0.11	0.010*	-36.36	20.45	-16.67

Note : EV = Edema volume of the paw (paw volume increase), % EI = percent edema inhibition of Sampless at time**;** n = 4 of each group; Values are expressed as mean ± SD**,** * Significantly different in EV for a different time in each dose at $p < 0.05$

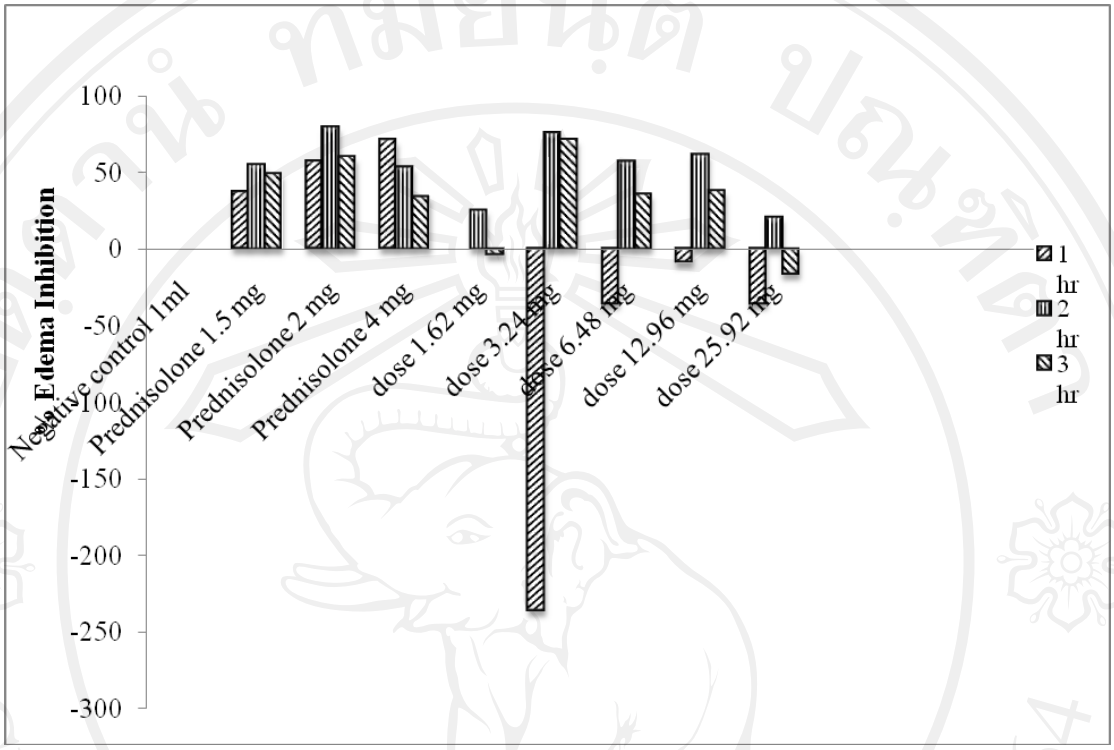

Figure 4.16 Percentages of rat hind paw edema inhibition of recipe no.346 and prednisolone acetate at various doses and time intervalsss compared with the negative control

The maximum edema inhibition effect of recipe no.717 was 70.83 % at dose of 2.03 mg/kg b.w. at time 3 hrs after the carrageenan injection which was not found to be statistically significant. From the observation was found the rat hind paw edema increased gradually and reached the maximum edema at time 3 hrs. The dose of 4.06 mg/kg b.w. and 8.12 mg/kg b.w. showed a significantly reduction of the edema (p-value<0.001) after the carrageenan injection on rat hind paw. Prednisolone acetate at dose of 2.0 mg/kg b.w. had the highest edema inhibition 79.10 %, as shown in Table 4.16 and Figure 4.17.

Table 4.16 Effects of recipe no.717 crude extracts on carrageenan-induced hind paw edema in rats

Samples	**Dose in mg/kg b.w.**	**Time after carrageenan injection**						
		EV(ml)				**% EI**		
		1 hr	**2 hrs**	**3hrs**	***p*-value**	**1 hr**	**2 hrs**	**3hrs**
Recipe no.717	2.03 mg/kg b.w.	0.21 ± 0.04	0.17 ± 0.12	0.14 ± 0.10	0.694	-90.91	61.36	70.83
	4.06 mg/kg b.w.	0.27 ± 0.04	0.25 ± 0.07	0.63 ± 0.10	0.024*	-136.36	43.18	-31.25
	8.12 mg/kg b.w.	0.20 ± 0.08	0.21 ± 0.04	0.44 ± 0.13	0.039*	-72.73	54.55	8.33
	16.24 mg/kg b.w.	0.33 ± 0.12	0.43 ± .13	0.55 ± .22	0.227	-200.00	2.27	-14.58
	32.48 mg/kg b.w.	0.16 ± 0.07	0.20 ± 0.18	0.33 ± 0.24	0.368	-45.45	54.55	31.25

Note : EV = Edema volume of the paw (paw volume increase), % EI = percent edema inhibition of Samples at time**;** n = 4 of each group; Values are

expressed as mean ± SD**,** * Significantly different in EV for a different time in each dose at $p < 0.05$

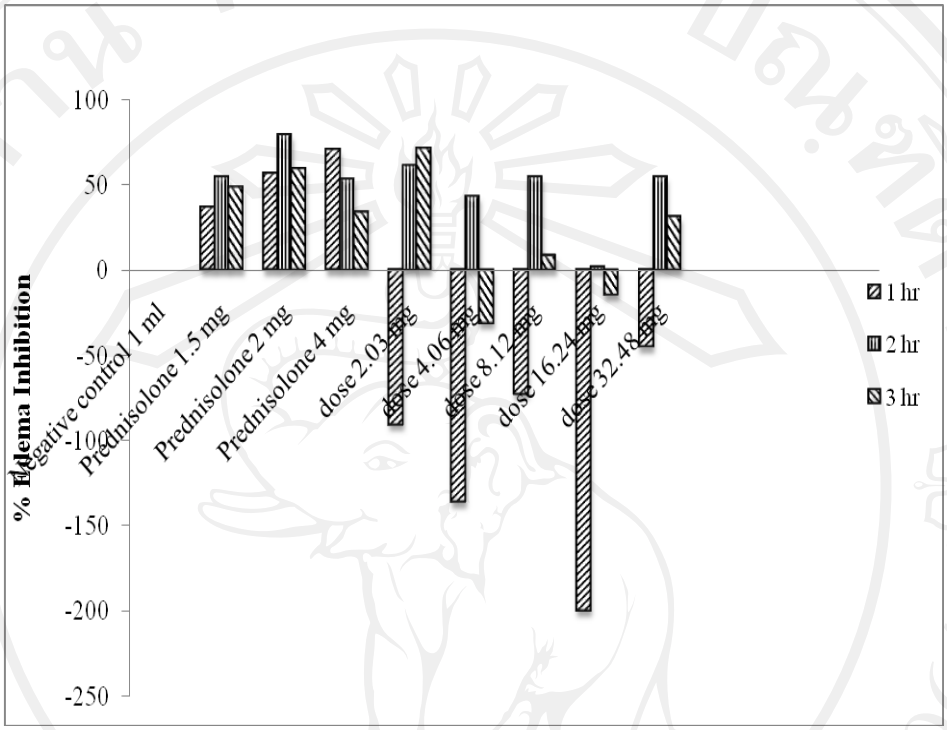

Figure 4.17 Percentages of rat hind paw edema inhibition of recipe no.717 and prednisolone acetate at various doses and time intervals compared with the negative control

Recipe no. 895 showed a significant reduction (p-value<0.001) of the edema after the carrageenan injection on rat hind paw at doses of 1.67 mg/kg b.w., 3.34 mg/kg b.w., and 6.68 mg/kg b.w. which was unable to inhibit the paw edema. There was no edema inhibition effect of recipe no. 895. The results were shown in Table 4.17 and Figure 4.18.

Table 4.17 Effects of recipe no.895 crude extracts on carrageenan-induced hind paw edema in rats

Samples	**Dose in mg/kg b.w.**	**Time after carrageenan injection**						
		EV(ml)				**% EI**		
		1 hr	**2 hrs**	**3hrs**	***p*-value**	**1 hr**	**2 hrs**	**3hrs**
Recipe no.895	1.67 mg/kg b.w.	0.29 ± 0.17	0.66 ± 0.05	0.73 ± 0.08	0.018*	-154.55	-50.00	-52.08
	3.34 mg/kg b.w.	0.36 ± 0.12	0.87 ± 0.45	0.71 ± 0.13	0.025*	-218.18	-97.73	-47.92
	6.68 mg/kg b.w.	0.19 ± 0.10	1.24 ± 0.28	0.66 ± 0.22	0.007*	-72.73	-181.82	-37.50
	13.36 mg/kg b.w.	0.42 ± 0.31	0.62 ± 0.24	0.62 ± 0.21	0.397	-281.82	-40.91	-29.17
	26.72 mg/kg b.w.	0.47 ± 0.16	0.64 ± 0.11	0.56 ± 0.12	0.246	-327.27	-45.45	-16.67

Note : EV = Edema volume of the paw (paw volume increase), % EI = percent edema inhibition of Sampless at time**;** n = 4 of each group; Values are expressed as mean ± SD**,** * Significantly different in EV for a different time in each dose at $p < 0.05$

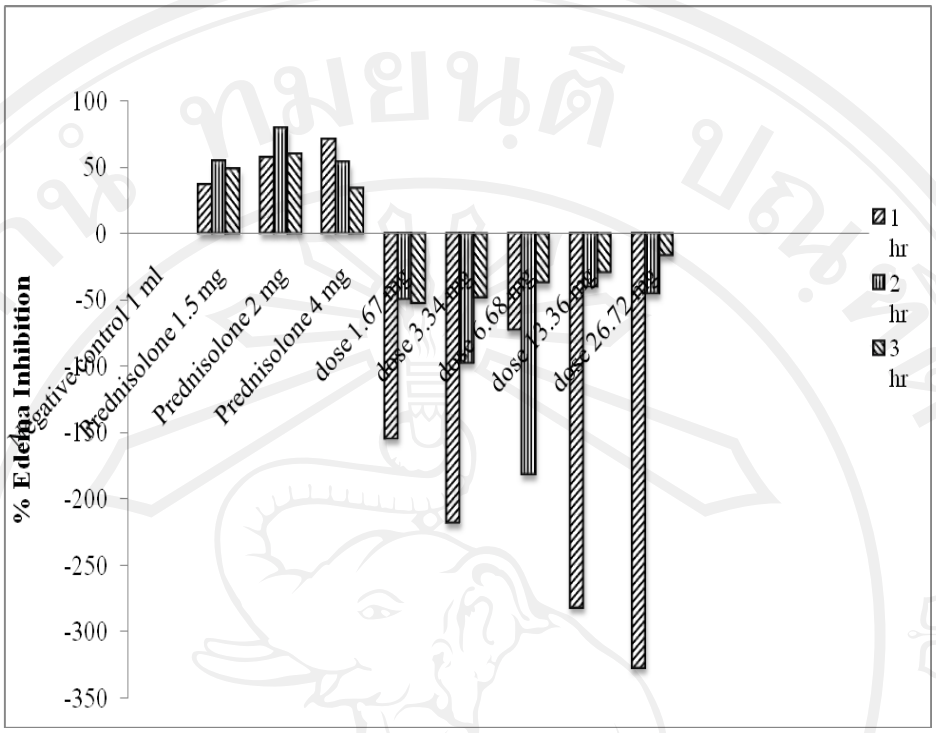

Figure 4.18 Percentages of rat hind paw edema inhibition of recipe no.895 and prednisolone acetate at various doses and time intervalsss compared with the negative control

Recipe no.896 showed a significant reduction of the edema (p=0.025) of dose 29.92 mg/kg b.w. which produced edema inhibition effect of 79.55 % at 2 hrs which was nearly potent edema inhibition to prednisolone acetate rate of 79.10 % at dose of 2.0 mg/kg b.w. and at time 2 hrs as shown in Table 4.18 and Figure 4.19.

Table 4.18 Effects of recipe no.896 crude extracts on carrageenan-induced hind paw edema in rats

Samples	Dose in mg/kg b.w.	Time after carrageenan injection						
		EV(ml)				% EI		
		1 hr	2 hrs	3 hrs	*p*-value	1 hr	2 hrs	3hrs
Recepie no.896	1.87 mg/kg b.w.	0.29 ± 0.17	0.48 ± 0.16	0.56 ± 0.13	0.174	-163.64	-9.09	-16.67
	3.74 mg/kg b.w.	0.12 ± 0.05	0.22 ± 0.12	0.30 ± 0.17	0.154	-9.09	50.00	37.50
	7.48 mg/kg b.w.	0.17 ± 0.07	0.28 ± 0.12	0.34 ± 0.10	0.098	-54.55	36.36	29.17
	14.96 mg/kg b.w.	0.17 ± 0.07	0.28 ± 0.12	0.34 ± 0.10	0.098	-54.55	36.36	29.17
	29.92 mg/kg b.w.	0.04 ± 0.01	0.09 ± 0.05	0.18 ± 0.13	0.025*	63.64	79.55	62.50

Note: EV = Edema volume of the paw (paw volume increase), % EI = percent edema inhibition of Sampless at time**;** n = 4 of each group; Values are expressed as mean $\pm$ SD**,** * Significantly different in EV for a different time in each dose at $p < 0.05$

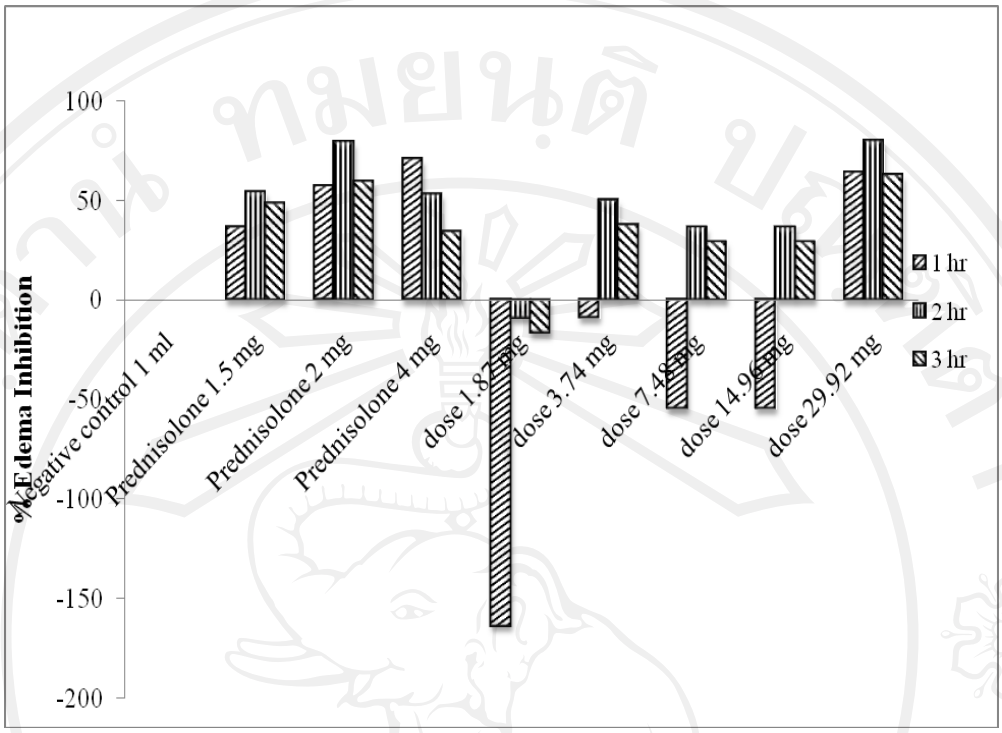

Figure 4.19 Percentages of rat hind paw edema inhibition of recipe no.896 and prednisolone acetate at various doses and time intervals compared with the negative control

Carrageenan induced hind paw edema was considered to be a suitable method for evaluating anti-inflammatory properties for natural drugs because of its sensitivity in detecting orally active anti-inflammatory agents particularly in the acute phase of inflammation (Di Rosa *et al.*, 1971). Development of edema in the rat paw after the injection of carrageenen was a biphasic event (Vinger *et al*., 1969). The initial phase observed during the first hour was attributed to the release of histamine and serotonin. The second phase of edema was due to the release of prostaglandins, protease, and lysosome (Crunkhon and Meacock, 1971). The results of this study demonstrated that the crude extracts of the selected seven recipe extracts exhibited significant anti-inflammatory activities at 2 hrs and 3 hrs after the carrageenan injection. The recipe no.105 was the best recipe which gave the maximum edema

inhibition at 87.50 % of dose 10.1 mg/kg b.w. at time 3 hrs after carrageenan injection. The second rank recipe was recipe no.896 which gave the maximum edema inhibition at 79.55 % of dose 29.92 mg/kg b.w. at 3 hrs after carrageenan injection. While the prednisolone acetate gave maximum edema inhibition at 79.10 % of dose 2.0 mg/kg b.w. at 2 hrs after carrageenan injection. These recipe extracts which contained the active constituents may have shown the anti-inflammatory activity or either inhibition the synthesis, release or action of inflammatory mediators e.g. histamine, serotonin and prostaglandins.

Moreover, the result from phytochemical tests in this study indicated that the chemical constituents in these recipes were of alkaloids, cardiac glycosides, lipids, tannins and xanthones as shown in Table 4.14. Several researchers have reported that these chemical constituents have anti-inflammatory activity (Harborne *et al*, 1999). This is in consistence with the results of this study which was described as follows:

From the literature reviews, recipe no. 105 which has been traditional used in Lanna to treat insect sting and bite, only five out of eight plants showed anti-inflammatory activity. These plants were *Azadirachta indica* (Atal and Kapur, 1982; Thakur *et al*., 1989), *Aegle marmelos* (Burkill, 1985 and Williamson, 2002), *Cyperus rotundus* L. (Jeong *et al*., 2000; Ha *et al*., 2002; Sayed *et al*., 2007), *Phyllanthus emblica* L.(Iyer and Pillay, 1958; Thakur *et al*., 1989; EI-Mekkawy *et al*., 1995; Khanna and Bansal, 1975) and *Zingiber officinale* Roscoe (Young *et al*., 2005 and Suekawa *et al*., 1986) as shown in Table 4.19.

Table 4.19 Compounds in the plants in recipe no. 105 which have been reported to have anti-inflammatory activity

Botanical Name	Part used	Chemical compounds	References
Aegle marmelos	Barks, leaves and fruits	Alkaloid (aeglenine and aegeline), tannin (tannic acid), coumarins (umbelliferone, xanthotoxin, dimethexin, coumarin and scopolein), astringents, aromatic	Burkill, 1985 and Williamson, 2002.
Azadirachta indica	Leaves and barks	Flavoanoid , quercetin, Alkaloid: nimbosterol (β- sitosterol), kaempferol and myricetin, desacetylnimbin, azadirachtin, nimbin, nimbinin , nimbidin,	Atal and Kapur, 1982; Thakur *et al*., 1989

Table 4.19 Compounds in the plants in recipe no. 105 which have been reported to have anti-inflammatory activity (continued)

Botanical Name	Part used	Chemical compounds	References
Azadirachta indica	Young leaves	nimbostero, essential oil, tannins , rhynchosin *3-O-b-D-glucoside* substances, anthraquinone and triterpenes (lupeol	Sithisarn *et al*., 2004
Cyperus rotundus L**.**	Roots	Sesquiterpene alkaloids: rotundines A-C	Jeong *et al*., 2000
	Roots	Triterpenes, oleanolic acid	Ha *et al*., 2002
	Roots	Steroid glycoside, sitosteryl-β-D-galactopyranoside, furochromones, khellin, visnagin, ammiol, coumarin, salicylic acid, caffeic acid, protocatechuic	Sayed *et al*., 2007

Table 4.19 Compounds in the plants in recipe no. 105 which have been reported to have anti-inflammatory activity (continued)

Botanical Name	Part used	Chemical compounds	References
Phyllanthus emblica L.	Fruits and Roots	Gallic acid and flavonoid derivative Ellagic acid, lupeol, quercetin and β-sitosterol	Iyer and Pillay, 1958 Thakur *et al*., 1989
	Roots	astragalin–flavonol	EI-Mekkawy *et al.*, 1995
	Fruits and leaves	Alkaloid : phyllantine and phyllantidine	Khanna and Bansal, 1975
Zingiber officinale Roscoe.	Rhizome (ginger)	(6)-gingerol (6)- shogaol	Young *et al.*, 2005 and Suekawa *et al.*, 1986

From the literature reviews, recipe no. 192 which has been traditional used in Lanna to treat insect sting and bite, only ten out of sixteen plants showed anti-inflammatory activity. These plants were *Croton oblongifolius* Roxb.(Roengsumran *et al*.,1999, 2004,2004; Morikawa *et al*., 2009; Dagli, 2004), *Foeniculum vulgare* Mill. var. vulgare (Miller) Thell. (Aggarwal and Shishodia, 2004; Parejo, 2004; Sebastian, 2006; Nickavar and Abolhasani, 2009; Raaman, 2007), *Lepidium sativum* L.(Al-Yahya *et al.*, 1994), *Nigella sativa* L.(Gilani *et al.*, 2004; EI-Dakhakhny *et al.*,

2002), *Oryza sativa* L. (Punyatong *et al.*, 2008 and Anne, 2000), *Piper chaba* Hunt. (Rukachaisirikul *et al.*, 2002), *Piper nigrum* L. (Duke and Ayensu, 1985; Srinivas and Rao**,** 1999; Khajuria *et al*., 2002; Bang *et al*., 2009), *Plumbago indica* L.((Schmelzer and Gurib-Fakim, 2008; Satyavati *et al*., 1987), *Plumbago zeylanica* L. (Salminsen *et al.*, 2008; Sandur *et al*., 2006), *Zingiber officinale* Roscoe (Young *et al.*, 2005 and Suekawa *et al.*, 1986) as shown in Table 4.20

Table 4.20 Compounds in the plants in recipe no. 192 which have been reported to have anti-inflammatory activity

Botanical Name	**Part used**	**Chemical compounds**	**References**
Croton oblongifolius Roxb.	Stem bark	Alkaloids: the diterpenoids, the cembranoid diterpenes crotocembranoic acid and neocrotocembranal	Roengsumran *et al*.,1999
	Stem bark	the labdane nidorellol, the furoclerodane	Roengsumran *et al*.,2002
		croblongifolin and the clerodane crovatin	
	Stem bark		Roengsumran *et al.* , 2004

Table 4.20 Compounds in the plants containing recipe no. 192 which have been reported to have anti-inflammatory activity (continued)

Botanical Name	Part used	Chemical compounds	References
Croton oblongifolius Roxb.	Fruits	the halimanes crotohalimaneic acid , crotohalimoneic acid, 12-benzoyloxycrotohalimaneic acid	Morikawa *et al*., 2009
	Stem barks	Alkaloid: piperine, pipernonaline, guineensine, and the isobutylamide of 11-(3,4ethylenedioxyphenyl) undeca-2,4,10-trienoic acid	Dagli, 2004
Foeniculum vulgare Mill. var. vulgare (Miller) Thell.	Fruits	Anethol, such as dianethol and photoanethol	Aggarwal and Shishodia, 2004
	Fruits	flavonoids glycoside and flavonoid aglycone	Parejo, 2004; Sebastian, 2006;

Table 4.20 Compounds in the plants in recipe no. 192 which have been reported to have anti-inflammatory activity (continued)

Botanical Name	Part used	Chemical compounds	References
Foeniculum vulgare Mill. var. vulgare (Miller) Thell.			Nickavar and Abolhasani, 2009
	Fruits	Phenolic: gallic acid	Raaman, 2007
Lepidium sativum L.	Seeds	Alkaloids: cyanogenic glycosides (traces), flavonoids, tannins, glucosinolates, sterols and triterpenes	Al-Yahya *et al.*, 1994
Nigella sativa L.	Seeds	Flavonoid: Thimoquinone	Gilani *et al.*, 2004
	Seeds	Flavonoid: Nigellone	EI-Dakhakhny *et al.*, 2002
Oryza sativa L.	Fruits	Anthocyanin: Proanthocyanidin**,** cyanidin 3-glucoside	Punyatong *et al.*, 2008 and Anne, 2000
Piper chaba Hunt.	Stems	Alkaloid: Chabamide	(Rukachaisirikul *et al.*, 2002)
	Fruits	Alkaloid: Piperanine	(Morikawa *et*

Table 4.20 Compounds in the plants in recipe no. 192 which have been reported to have anti-inflammatory activity (continued)

Botanical Name	Part used	Chemical compounds	References
Piper nigrum L.	Fruits	Alkaloid: piperine,	*al.*, 2004) Duke and Ayensu, 1985
	Fruits	Isopiperolein B: an alkamide	Srinivas and Rao, 1999
	Fruits	Alkaolid: iperine (1-Piperoyl piperidine: major alkaloid)	Khajuria *et al.*, 2002
	Fruits	Alkaloid:piperine	Bang *et al.*, 2009
Plumbago indica L.	Roots	Quinone: plumbagin (naphthoquinone)	(Schmelzer and Gurib-Fakim, 2008)
	Roots	Quinone: plumbagin oil	Satyavati *et al.*, 1987
Plumbago zeylanica L.	Roots	Alkaloid: Terpenoids	Salminsen *et al.*, 2008
	Roots	Quinone: Plumbagin	Sandur *et al.*, 2006
Zingiber officinale Roscoe.	Rhizome (ginger)	Phenylpropanoid: (6)-gingerol (6)- shogaol	Young *et al.*, 2005 and Suekawa *et al.*, 1986

From the literature reviews, recipe no. 346 which has been traditional used in Lanna to treat acne abscess, only two out of three plants showed anti-inflammatory activity. These plants were *Lagenaria siceraria* (Molina) Standl. (Ghule *et al*., 2006) and *Caesalpinia digyna* Rottle. (Rastogi and Rawat, 2008) as shown in Table 4.21.

Table 4.21 Compounds in the plants in recipe no. 346 which have been reported to have anti-inflammatory activity

Botanical Name	Part used	Chemical compounds	References
Lagenaria siceraria (Molina) Standl.	Fruit juice	Flavonoids, cucurbitacin (alkaloid), saponins, proteins, and carbohydrates	Ghule *et al*., 2006
Caesalpinia digyna Rottle.	Roots	Flavonoid: bergenin	Rastogi and Rawat, 2008

From the literature reviews, recipe no. 717 which has been traditional used in Lanna to treat skin abscess and insect sting and bite, only two out of six plants showed anti-inflammatory activity. These plants were *Cassia occidentalis* L. (Mazumder *et al*., 2008) and *Dregea volubilis* (L.F) Hook.f. (Biswas *et al*., 2009; Nandi *et al*., 2009) as shown in table 4.22.

Table 4.22 Compounds in the plants in recipe no. 717 which have been reported to have anti-inflammatory activity

Botanical Name	Part used	Chemical compounds	References
Cassia occidentalis L.	Roots	Anthraquinone: chryso-phanol, emodin, Xanthone: pinselin, questin, singueanol-I pinselin and 1,7-dihydroxy-3-methylxanthone, hydroanthracene: germichrysone,methylger mitorosone 1,8-dihydroxyanthraquinone, 2 new bis (tetrahydro) anthracene derivativeoccidentalol-1 and occidentalol-II, glycosidic flavonoids, cassiaoccidentalins A, B and C anthraquinones	Mazumder *et al.*, 2008
Dregea volubilis (L.F) Hook.f.	Fruits ,Dried leaves	Taraxerol, Saponins	Biswas *et al.*, 2009; Nandi *et al.*, 2009

From the literature reviews, recipe no. 896 which has been traditional used in Lanna for treats gum abscess and tooth pain, all four plants showed anti-inflammatory activity. These plants were *Coccinia grandis* L. Voigt., (Nadkami and Nadkami, 1992) *Vitex trifolia* L. (Wen-Xin *et al.*, 2005), *Sesamum indicum* L. (Yun *et al.*, 2008) and *Zingiber officinale* Roscoe**.** (Young *et al.*, 2005 and Suekawa *et al.*, 1986) as shown in Table 4.23.

Table 4.23 Compounds in the plants in recipe no. 896 which have been reported to have anti-inflammatory activity

Botanical Name	**Part used**	**Chemical compounds**	**References**
Coccinia grandis L. Voigt.	Leaves	Triterpenoids, alkaloids and tannins	Nadkami and Nadkami, 1992
Vitex trifolia L.	Leaves	Flavonoids: persicogenin, artemetin, luteolin, penduletin, vitexicarpin, chrysoplenol-D	Wen-Xin *et al.*, 2005
Sesamum indicum L.	Seeds oil	Phenylpropanoid: Sinapic acid	Yun *et al.*, 2008
Zingiber officinale Roscoe**.**	Rhizome (ginger)	Phenolic compound: (6)-gingerol (6)- shogaol	Young *et al.*, 2005 and Suekawa *et al.*, 1986

The best inflammation inhibition effects were demonstrated in the recipe no. 105. The crude extracts was able to significantly reduce edema in the rat hind paw when compared with the positive control, ($p<0.001$). Recipe no.105 indicated the ability to inhibit the edema of rat hind paw was better than the positive control at the level of 87.50 %. at dose of 5.18 mg/kg b.w. at 3 hrs while the positive control could inhibit edema of 79.10 % at a dose of 2.0 mg/kg b.w. at the observed time of 2 hrs. Recipe no. 896 had edema inhibition 79.55 % of dose 29.92 mg/kg b.w. at 2 hrs which was slightly higher than that of the positive control.

The results of this study confirmed a strong anti-inflammatory activity in two of the selected seven recipe extracts of the recipes by showing higher percentages in edema inhibition on rat hind paw in recipe nos.105 and 896 than those in the other five recipes. Previous studies also established the findings that flavonoids and alkaloids which were the main chemical constituents in both recipe nos.105 and 896, played the important role in anti-inflammatory activity and anti-cancer action (Chevallier, 1996).

Recipe no.105 contained the bark of *Aegle marmelos* which has alkaloids and has been reported to possess the anti-inflammatory activity (Veerappan *et al.*, 2005). The study of Burkill and Williamson have indicated that it was found alkaloids in the barks, leaves and fruits of *Aegle marmelos* (Burkill, 1985 and Williamson, 2002). *Azadirachta indica* contained flavonoids in their leaves and barks which have anti-inflammatory activity (Sithisarn *et al.*, 2004). *Cyperus rotundus* L**.** containing alkaloids (rotundines) in the roots (Jeong *et al*., 2000) was also used for the treatment of stomach and bowel disorder and inflammatory diseases (Seo *et al.*,

2001). The fruits and leaves of *Phyllanthus emblica* L**.** contained alkaloids (phyllantine and phyllantidine) (Khanna and Bansal, 1975). Rhizome of *Zingiber officinale* containedthe (6)-gingerol (Young *et al.*, 2005) and (6)-shogaol which have the anti-inflammatory activity (Suekawa *et al.*, 1986).

Recipe no. 896 contained the leaves of *Coccinia grandis* L. Voigt which has triterpenoid, alkaloid and tannin (Nadkami and Nadkami, 1992). The stem has been used for anti-inflammatory activity (Phupattanapong and Wongprasert, 1987). The leaves of *Vitex trifolia* L. contained flavonoids (Wen-Xin *et al.*, 2005). Rhizome of *Zingiber officinale* contains (6)-gingerol (Young *et al.*, 2005) and (6)-shogaol which have the anti-inflammatory activity (Suekawa *et al.*, 1986).

4.4.2 Rat ear edema method

The investigation was conducted on the effects of the treatment of the EPP-induced rat ear edema by the selected three of the seven recipe extracts of the Lanna medicinal plant recipes. The three recipe extracts were selected from the seven recipes used in the present study of rat hind paw edema, based mainly on their topical application by using rat ear edema tests to confirm the inhibitory effects. These three selected recipes were recipe nos. 25, 346 and 717. The negative control study employed distilled water while the positive control was phenylbutazone. Tests conducted on the use of the negative control to treat inflammation of rat ear showed that the rat ear thickness increased and peaked at 15 mins after EPP application. The edema was then slightly reduced at time 360 mins and then began to increase approximately at time 1440 mins. The negative control was found to significantly reduce the edema formation in all assessment times after the EPP application (*p*-value

<0.001). Similarly, a use of positive control showed that the rat ear thickness increased and peaked at time 30 mins after EPP application. The edema was then slightly reduced from time 60 mins. The positive control was found to significantly reduce the edema formation in all assessment times after the EPP application (*p*-value <0.001). The highest edema inhibition effect was 95.41 % of dose 1.0 mg/20 µL/ear and at time 360 mins as shown in Table 4.24.

Table 4.24 Effects of the negative control and positive control on ethyl phenyl propriolate induced ear edema in rats

Samples	**Dose in mg**/20µl/ear	**Time after ethyl phenyl propriolate application**										
		ET(mm)						**% EI**				
		15 mins	**30 mins**	**60 mins**	**360 mins**	**1440 mins**	***p*-value**	**15 mins**	**30 mins**	**60 mins**	**360 mins**	**1440 mins**
Negative control	1mg/ 20µl/ear	0.25 ± 0.05	0.34 ± 0.04	0.33 ± 0.04	0.14 ± 0.02	0.06 ± 0.04	0.001*	0	0	0	0	0
Positive control	1 mg/20µl/ear	0.12 ± 0.04	0.18 ± 0.07	0.16 ± 0.02	0.06 ± 0.02	0.003 ± 0.01	0.001*	55.24	45.37	88.49	95.41	95.0

Note : ET=Edema thickness, % EI = percent edema inhibition of test substance at time; Values are expressed as mean ± SD

*Significantly different from the control at $p < 0.05$

Recipe no. 25 showed a significant reduction in the edema formation (*p*-value <0.001) after the EPP- induced inflammation on rat ear at all doses, 0.5 mg/20 µL/ear, 1.0 mg/20 µL/ear, and 2.0 mg/20 µL/ear at all observed times. At dose of 0.5 mg/20 µL/ear the rat ear edema thickness increased and reached the maximum at 30 mins and reduced gradually throughout the assessment time intervals and reached the maximum edema inhibition rate of 89.09 % at time 360 mins. At dose of 1.0 mg/20 µL/ear, the rat ear thickness increased and peaked at 30 mins and reduced gradually throughout the assessment times and reached the maximum edema inhibition rate of 91.57 % at time 360 mins. At dose of 2.0 mg/20 µL/ear, the rat ear edema thickness increased and peaked at 360 mins and then reduced gradually throughout all the assessment times and reached the maximum edema inhibition rate of 93.60 % were shown in Table 4.28 and Figure 4.20.

Table 4.25 Effects of recipe no.25 crude extracts on ethyl phenylpropriolate induced ear edema in rats

Samples	**Dose in mg**/20µl/ear	**Time after ethyl phenyl propriolate application**										
		ET(µm)						**% EI**				
		15 mins	**30 mins**	**60 mins**	**360 mins**	**1440 mins**	***p*-value**	**15 mins**	**30 mins**	**60 mins**	**360 mins**	**1440 mins**
Recipe no. 25	0.5mg/20µl/ear	0.31± 0.03	0.35± 0.03	0.29± 0.04	0.15± 0.02	0.10± 0.03	< 0.001*	-21.37	-2.69	78.57	89.09	-73.33
	1 mg/20µl/ear	0.26± 0.04	0.31± 0.04	0.21± 0.03	0.12± 0.02	0.08± 0.02	< 0.001*	-3.23	10.15	84.28	91.57	-36.66
	2 mg/20µl/ear	0.28± 0.04	0.41± 0.06	0.50± 0.02	0.09± 0.02	0.05± 0.01	< 0.001*	-12.5	-22.39	62.78	93.6	18.33

Note : ET=Edema thickness, % EI = percent edema inhibition of test substance at time; Values are expressed as mean ± SD

*Significantly different from control at $p < 0.05$

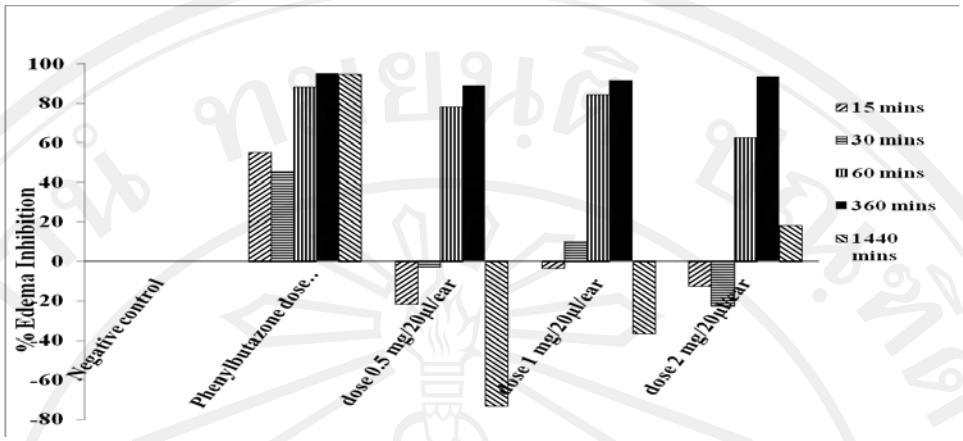

Figure 4.20 Percentages of rat ear edema inhibition of recipe no.25 and phenylbutazone at various doses and time intervals compared with the negative control

Recipe no. 346 showed a significant reduction of the edema formation (*p*-value <0.001) after the EPP- induced inflammation on rat ear at all doses, 0.5 mg/20 µL/ear 1.0 mg/20 µL/ear and 2.0 mg/20 µL/ear and at all the observed time intervals. At dose of 0.5 mg/20 µL/ear, the rat ear thickness exhibited an increase and peaked at 60 mins and then reduced gradually throughout the assessment times. The maximum capacity of edema inhibition was indicated to be 93.90 % at time 360 hrs. At dose of 1.0 mg/20 µL/ear, the rat ear thickness increased and peaked at 30 mins and remained at that level for another 60 mins, after which it then reduced gradually from 360 mins throughout the assessment times. This indicated the maximum edema inhibition rate of 93.08 % at time 350 mins. At dose of 2.0 mg/20 µL/ear, and increase in the rat ear thickness was observed and peaked at 60 mins and then reduced throughout the assessment times. This showed the maximum edema inhibition rate of 96.09% at time 360 mins as shown in and Table 4.26 and Figure 4.21.

Table 4.26 Effects of recipe no.346 crude extracts on ethyl phenyl propriolate induced ear edema in rats

Samples	**Dose in mg**/20µl/ear	**Time after ethyl phenyl propriolate application**										
		ET(µm)						**% EI**				
		15 mins	**30 mins**	**60 mins**	**360 mins**	**1440 mins**	***p*-value**	**15 mins**	**30 mins**	**60 mins**	**360 mins**	**1440 mins**
Recipe no.346	0.5 mg/20µl/ear	0.26± 0.03	0.34 ± 0.078	0.45 ± 0 .02	0.08 ± 0.02	0.03 ± 0.02	< 0.001*	-6.45	-1.19	66.61	93.9	-380
	1 mg/20µl/ear	0.18± 0.02	0.43 ± 0.05	0.43 ± 0 .06	0.09± 0.02	0.04 ± 0.01	< 0.001*	26.61	-26.86	67.81	93.08	31.66
	2 mg/20µl/ear	0.23± 0.04	0.26 ± 0.02	0.28 ± 0.02	0.05 ± 0.02	0.04 ± 0.01	< 0.001*	9.27	23.58	79.54	96.09	36.66

ET = Edema thickness, % EI = percent edema inhibition of test substance at time; Values are expressed as mean ± SD

* Significantly different from control at $p < 0.05$

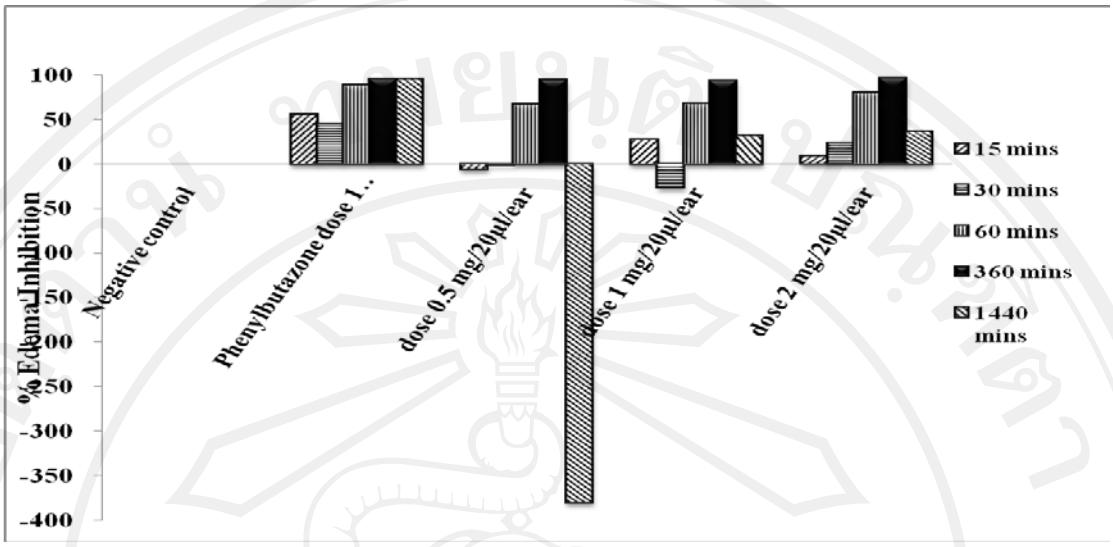

Figure 4.21 Percentages of rat ear edema inhibition of recipe no.346 and phenylbutazone at various doses and time intervals compared with the negative control

Recipe no. 717 showed a significant reduction in the edema formation (*p*-value <0.001) after the EPP-induced inflammation on rat ear at all doses, of 0.5 mg/20 µL/ear 1.0 mg/20 µL/ear and 2.0 mg/20 µL/ear and at all the observed time intervals. At dose of 0.5 mg/20 µL/ear, an increase in the rat ear thickness was exhibited and peaked at 60 mins and then reduced gradually throughout the assessment time intervals. This indicated the maximum edema inhibition rate to be 92.70 % at time 360 hrs. At dose of 1.0 mg/20 µL/ear, a gradual increase in the rat ear thickness was shown and peaked at 60 mins throughout the assessment time intervals. This prduced the maximum edema inhibition rate to be 95.86 % at time 360 mins. At dose of 2.0 mg/20 µL/ear, an increase in the rat ear thickness was seen and peaked at time 60 mins, then reduced throughout the assessment time intervals. This showed the maximum inhibition rate to be 94.58 at time 360 mins, as shown in Table 4.30 and Figure 4.22.

Table 4.27 Effects of recipe no.717 crude extracts on ethyl phenylpropriolate induced ear edema in rats

Samples	**Dose in mg/**20µl/ear	**Average weight in gms**	**Time after ethyl phenyl propriolate application**										
			ET(µm)						**% EI**				
			15 mins	**30 mins**	**60 mins**	**360 mins**	**1440 mins**	***p*-value**	**15 mins**	**30 mins**	**60 mins**	**360 mins**	**1440 mins**
Recipe no.717	0.5 mg/20µl/ear	50	0.30± 0.03	0.45± 0.06	0.48± 0.02	0.10± 0.03	0.05± 0.03	< 0.001*	-22.18	-33.13	64.06	92.7	10.0
	1 mg/20µl/ear	52	0.27± 0.04	0.32± 0.06	0.39± 0.05	0.06± 0.01	0.02± 0.01	< 0.001*	-10.48	4.77	70.52	95.86	76.66
	2 mg/20µl/ear	55	0.25± 0.04	0.34± 0.01	0.33± 0.02	0.07± 0.03	0.02± 0.01	< 0.001*	9.27	0.29	75.03	94.58	71.66

Note : ET = Edema thickness, % EI = percent edema inhibition of test substance at time; Values are expressed as mean ± SD*

Significantly different from control at $p < 0.05$

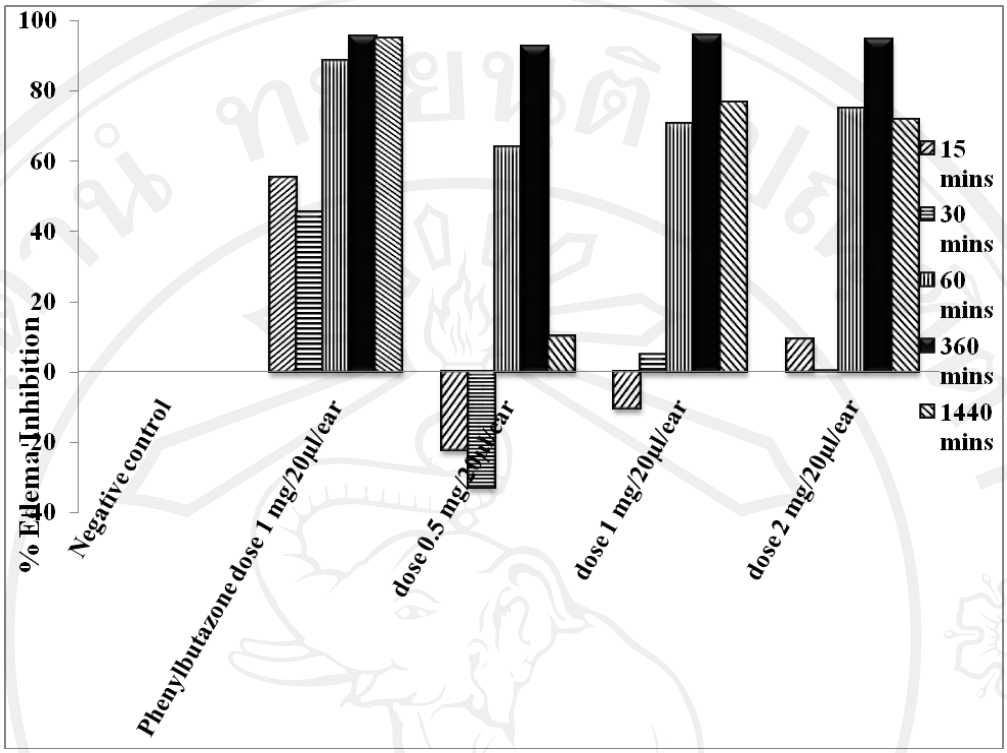

Figure 4.22 Percentages of rat ear edema inhibition of recipe no.717 and phenylbutazone at various doses and time intervals compared with the negative control

The finding suggests correlation of various doses and time intervals of the three selected recipe extracts for their ability to inhibit the rat ear edema. The best dose of recipe no. 25 was 2.0 mg/kg b.w., which gave the edema inhibition was 93.6 % at time 360 mins. The best dose of recipe no.346 was 2.0 mg/kg b.w. which gave the ear edema inhibition was 96.0 % at 360 mins. The best dose of recipe no.717 was 1.0 mg/kg b.w. which gave the ear edema inhibition was 95.86 % at time 360 mins.

Phenylbutazone, a COX-inhibitor at a dose 1 mg/20 µL/ear showed marked reduction of the ear edema. The EPP-induced ear edema tests had a good predictive value for screening anti-inflammatory agents. EPP caused a release of many inflammatory mediators such as, kinin, serotonin and PGs (Brattsand *et al.*,

1982). It is possible that the three selected recipe extracts affect the release and/or synthesis of these mediators. The edema inhibition percentage of recipe no.346 showed its highest effect in inflammatory inhibition on rat ear of 96.09 % at dose of 2 mg/20 µL/ear and at the observed time intervals 360 mins, after which the percentage of edema inhibition reduced at the observed time intervals 1440 mins.

Moreover, the result from the phytochemical tests have confirmed the chemical constituents in these recipes to consist of the alkaloids, cardiac glycosides, tannins and xanthones, all with the anti-inflammatory activity (Table 4.14). The extracts of recipe no.25 which has been used in the Lanna traditional medicines for treating insect sting and bite contained all three plants which possessed anti-inflammatory activity. These three plants included the bark of *Cassia alata* L. Roxb. which possessed alkaloids and flavonoids (Mazumder *et al.*, 2008), all parts of *Datura metel* L. var. *fastuosa* (Bernh.) Danert. that possessed alkaloid megastigmane sesquiterpenes (Kuang *et al.*, 2008) and leaves of *Jatropha gossypifolia* L. that gave flovonoids, vitexin and apigenin (Subramanian *et al.*, 1971). The extracts of recipe no.346 which has been used in the Lanna traditional medicines for treating acne abscess contained two plants which possessed the anti-inflammatory action namely, the fruits juice of *Lagenaria siceraria* (Molina) Standl. that possessed flavonoids, alkaloids (cucurbitacin) (Ghule *et al.*, 2006) and the roots of *Caesalpinia digyna* Rottle that contained flavonoids (bergenin) (Rastogi and Rawat, 2008) (table 4.25). The extracts of recipe no.717 has been used in the Lanna traditional folklore medicines for the treatment of insect sting and bite and skin abscess. Two out of six plants used in this recipe had anti-inflammatory activity (Harborne *et al.*, 1999). These two plants included the leaves of *Cassia occidentalis* L. which had

anthraquinone, xanthone and flavonoid glycosides (Mazumder *et al.*, 2008) and the fruits and dried leaves of *Dregea volubilis* (L.F) Hook. f. which contained Taraxerol (Biswas *et al*., 2009) and saponins (Nandi *et al*., 2009), respectively (Table 4.26).

Effects of recipe no.25 crude extracts on carrageenan-induced hind paw edema in rats