CHAPTER 3
RESULTS

3.1 GC/MS analysis of the extract

The analysis of the chromatogram indicated the presence of 19 compounds which 17 of them could be identified as shown in Table 1. The three most abundance were Benzyl benzoate, Ethane isothiocyanate, and 2-Phenanthrenol, 7-ethenyl-1,2,3,4, 4a,4b,5,6,7,9,10,lOa-dodecahydro-1,1,4a,7-tetramethyl-, [2S-(2.alpha.,4a.alpha., 4b.beta.,7.beta.,lOa.beta.)] which constituted 22.71%, 17.28%, and 11.18%, respectively. The other 16 compounds constituted only 48.84% of the extract.

3.2 Anti-inflammatory activity

3.2.1 Effects of SI extract and diclofenac on EPP-induced ear edema

The ear edema thickness of both control groups were gradually increased and peaked at 1 h after the EPP application, and slightly decreased after that. Both of SI extract and diclofenac at the dose of 5 mg/ear could significantly reduce ear edema at all time-points. The percentages of edema inhibition of SI extract were comparable to those of diclofenac (Table 2).

3.2.2 Effects of SI extract and diclofenac on carrageenin-induced hind paw edema

In the control group, the paw edema was produced and reached the peak level at 3 h after the carrageenin injection and maintained until the 5^{th} h. SI extract at all doses (75, 150, and 300 mg/kg) and diclofenac at the dose of 10 mg/kg could significantly inhibit paw edema at all time-points. The percentages of edema inhibition of all doses of SI extract and diclofenac were peaked at 3 h after the carrageenin injection (Table 3).

3.2.3 Effects of SI extract, diclofenac, and prednisolone on AA-induced hind paw edema

The paw edema volume of control group was increased after AA injection. Both reference drugs, diclofenac at the dose of 10 mg/kg and prednisolone at the dose of 5 mg/kg significantly inhibited paw edema, whereas SI extract showed significantly inhibitory effect only at doses of 150 and 300 mg/kg. SI extract at the dose of 300 mg/kg and prednisolone exerted equal effect on edema inhibition with percentage of 58% (Table 4).

3.2.4 Effects of SI extract, diclofenac, and prednisolone on transudative and granuloma components, and ulcerogenic effect on cotton pellet-induced granuloma formation

At the 8^{th} d after cotton pellet implantation, transudative and granuloma weights were intensively increased. All test drugs (diclofenac and prednisolone at the same dose of 5 mg/kg/d, and SI extract at the dose of 300 mg/kg/d) could significantly reduce both parameters (Table 5). However, the effects of SI extract on these parameters were less pronounced when compared to those of diclofenac and prednisolone. SI extract at this dose showed only 24% of granuloma inhibition when compared to 50% of diclofenac and 72% of prednisolone. SI extract did not elicit any ulcerogenic effect (data not shown).

3.2.5 Effects of SI extract, diclofenac, and prednisolone on body weight and thymus weight on cotton pellet-induced granuloma formation

The body weight and dry thymus weight were not significant difference among all groups (normal, control, diclofenac, and SI extract groups), except in the prednisolone group which revealed significant decrease from those of both normal and control groups (Table 6).

3.2.6 Effects of SI extract, diclofenac, and prednisolone on ALP activity on cotton pellet-induced granuloma formation

As shown in Table 7, ALP activity was significantly elevated in the control group (50.02 ± 3.26 U of enz/mg of total protein $\times 10^{-4}$) when compared with that of

the normal group (33.77 ± 4.28 U of enz/mg of total protein × 10^{-4}). However, all test drugs could normalize serum ALP activity to nearly normal level.

3.3 Analgesic activity

3.3.1 Effects of SI extract and diclofenac on acetic acid-induced writhing response

The writhing response was intensively produced in the control group. Diclofenac at the dose of 10 mg/kg and SI extract at all doses (18.75, 37.5, and 75 mg/kg) possessed inhibitory effect on writhing response. The percentage of writhing response inhibition of SI extract at the dose of 75 mg/kg was comparable to that of diclofenac (76% and 81%, respectively) (Table 8).

3.3.2 Effects of SI extract, diclofenac, and codeine on tail-flick test

The test reaction time at all time-points in the control, 10 mg/kg of diclofenac, and 18.75 mg/kg of SI extract groups as well as the test reaction time at the 3rd h of SI extract at the dose of 37.5 mg/kg were not different from their baseline values. Codeine at the dose of 200 mg/kg and SI extract at doses of 37.5, and 75 mg/kg could significantly increase test reaction time at all time-points (Table 9).

3.4 Antipyretic activity

At 18 h after yeast injection, the hyperthermia was generated and maintained at all time-points of the control group. Diclofenac at the dose of 10 mg/kg could significantly reduce hyperthermia at all time-points, whereas SI extract, at all doses (75, 150, 300 mg/kg), could not show this effect (Table 10).

Table 1 Chemical components of *S. involucratus* ethanolic extract as analyzed by GC/MS.

Peak	RT	% of Total	Compound
1	13.702	6.95	Benzenecarboxylic acid
2	15.562	1.64	Resorcinol
3	19.980	17.28	Ethane isothiocyanate
4	20.038	9.11	Heptanoic acid
5	22.082	22.71	Benzyl benzoate
6	22.787	0.71	l-Methoxy-3-(2-hydroxyethyl) nonane
7	24.404	2.42	n-Hexadecanoic acid
8	24.935	6.36	Hexadecanoic acid, ethyl ester
9	26.656	4.39	Phytol
10	26.922	1.71	9,12-Octadecadienoic acid (Z,Z)
11	27.014	2.02	cis,cis,cis-7,10,13-Hexadecatriena
12	27.418	3.73	Linoleic acid ethyl ester
13	27.505	2.96	7,10,13-Hexadecatrienoic acid, methyl ester
14	27.898	0.68	D-Gluconic acid, 2,3,4,5-tetra-O-m ethyl-6-O-*(2*, 3,4,6-tetra-O-methyl-.alpha.-D-glucopyranosyl)-, methyl ester
15	28.729	11.18	2-Phenanthrenol, 7-ethenyl-1,2,3,4,4a,4b,5,6,7,9, 10,lOa-dodecahydro-1,1,4a,7-tetramethyl-, [2S-(2.alpha.,4a.alpha.,4b.beta.,7.beta.,lOa.beta.)]
16	29.884	2.52	2-Isopropylidenehydrazono-3-methyl -6-chloro-2,3-dihydrobenzothiazole
17	31.554	0.87	No match found
18	33.621	2.03	1,2-Benzenedicarboxylic acid, di-isooctyl ester
19	34.620	0.74	No match found

RT = retention time (min)

Table 2 Percentage of edema inhibition of SI extract and diclofenac on EPP-induced ear edema.

Group	Dose (mg/ear)	Edema thickness (μm)				% Edema inhibition			
		15 min	30 min	1 h	2 h	15 min	30 min	1 h	2 h
Control of diclofenac	-	70 ± 25	115 ± 18	137 ± 10	110 ± 26	-	-	-	-
Diclofenac	5	18 ± 8*	38 ± 12*	50 ± 6*	42 ± 13*	74	67	63	62
Control of SI extract	-	75 ± 28	123 ± 16	143 ± 14	122 ± 17	-	-	-	-
SI extract	5	27 ± 8*	45 ± 6*	52 ± 8*	43 ± 5*	64	63	64	65

Values are expressed as mean ± S.D. (n = 6)

* Significantly different from the control group, $p < 0.05$

Control of diclofenac = 5% DMSO in acetone

Control of SI extract = Acetone

Table 3 Percentage of edema inhibition of SI extract and diclofenac on carrageenin-induced hind paw edema.

Group	Dose (mg/kg)	Edema volume (mL)			% Edema inhibition		
		1 h	3 h	5 h	1 h	3 h	5 h
Control	-	0.27 ± 0.13	0.75 ± 0.12	0.74 ± 0.20	-	-	-
Diclofenac	10	0.07 ± 0.02*	0.15 ± 0.03*	0.24 ± 0.06*	74	81	68
SI extract	75	0.19 ± 0.04*	0.42 ± 0.05*	0.58 ± 0.05*	30	44	22
SI extract	150	0.15 ± 0.02*	0.38 ± 0.05*	0.57 ± 0.05*	44	49	23
SI extract	300	0.10 ± 0.04*	0.17 ± 0.06*	0.30 ± 0.12*	63	77	60

Values are expressed as mean ± S.D. (n = 6)

* Significantly different from the control group, $p < 0.05$

Table 4 Percentage of edema inhibition of SI extract, diclofenac, and prednisolone on AA-induced hind paw edema.

Group	Dose (mg/kg)	Edema volume at 1 h (mL)	% Edema inhibition
Control	-	0.26 ± 0.03	-
Diclofenac	10	0.17 ± 0.08*	35
Prednisolone	5	0.11 ± 0.09*	58
SI extract	75	0.25 ± 0.10	4
SI extract	150	0.16 ± 0.06*	39
SI extract	300	0.11 ± 0.06*	58

Values are expressed as mean ± S.D. (n = 6)

* Significantly different from the control group, $p < 0.05$

Table 5 Transudative weight and percentage of granuloma inhibition of SI extract, diclofenac, and prednisolone on cotton pellet-induced granuloma formation.

Group	Dose (mg/kg/d)	Granuloma wet weight (mg)	Granuloma dry weight (mg)	Transudative weight (mg)	Granuloma weight (mg/mg cotton)	% Granuloma inhibition
Control	-	565.88 ± 133.92	89.47 ± 16.42	476.42 ± 119.18	3.47 ± 0.82	-
Diclofenac	5	310.43 ± 34.86*	54.56 ± 4.12*	255.88 ± 32.48*	1.73 ± 0.21*	50
Prednisolone	5	225.83 ± 28.48*	39.29 ± 3.48*	186.54 ± 26.88*	0.96 ± 0.17*	72
SI extract	300	415.93 ± 34.28*	72.52 ± 7.01*	343.41 ± 29.03*	2.63 ± 0.35*	24

Values are expressed as mean ± S.D. (n = 6)

* Significantly different from the control group, $p < 0.05$

Table 6 The changes of body weight and dry thymus weight of SI extract, diclofenac, and prednisolone on cotton pellet-induced granuloma formation.

Group	Dose (mg/kg/d)	Body weight (g)			Dry thymus weight (mg/100 g BW)
		Initial	Final	Gain	
Normal	-	187.67 ± 7.31	245.00 ± 10.18	57.33 ± 6.65	57.46 ± 1.73
Control	-	185.00 ± 5.48	244.67 ± 11.43	59.67 ± 8.04	61.20 ± 3.34
Diclofenac	5	183.33 ± 8.16	239.00 ± 12.12	55.67 ± 7.84	61.36 ± 6.41
Prednisolone	5	182.00 ± 4.00	188.00 ± 14.59$^{\#, *}$	6.00 ± 14.09$^{\#, *}$	23.60 ± 2.26$^{\#, *}$
SI extract	300	185.67 ± 4.97	248.33 ± 9.91	62.67 ± 5.61	59.08 ± 7.33

Values are expressed as mean ± S.D. (n = 6)

Significantly different from the normal group, $p < 0.05$

* Significantly different from the control group, $p < 0.05$

Table 7 ALP activity of SI extract, diclofenac, and prednisolone on cotton pellet-induced granuloma formation.

Group	Dose (mg/kg/d)	Alkaline phosphatase (U/L)	Total protein (g/dL)	Alkaline phosphatase activity (U of enz/mg of total protein × 10^{-4})
Normal	-	192.17 ± 28.91	5.68 ± 0.30	33.77 ± 4.28
Control	-	246.17 ± 21.45#	4.92 ± 0.16#	50.02 ± 3.26#
Diclofenac	5	174.33 ± 21.83*	4.95 ± 0.47#	35.62 ± 6.63*
Prednisolone	5	178.33 ± 37.80*	5.60 ± 0.21*	31.89 ± 6.90*
SI extract	300	204.00 ± 23.66*	5.12 ± 0.15#	39.96 ± 5.24*

Values are expressed as mean ± S.D. (n = 6)

Significantly different from the normal group, $p < 0.05$

* Significantly different from the control group, $p < 0.05$

Table 8 Percentage of writhing response inhibition of SI extract and diclofenac on acetic acid-induced writhing response.

Group	Dose (mg/kg)	Number of writhes	% Writhing response inhibition
Control	-	21 ± 2	-
Diclofenac	10.00	4 ± 2*	81
SI extract	18.75	17 ± 1*	19
SI extract	37.50	11 ± 1*	48
SI extract	75.00	5 ± 2*	76

Values are expressed as mean ± S.D. (n = 6)

* Significantly different from the control group, $p < 0.05$

Table 9 Effects of SI extract, diclofenac, and codeine on tail-flick test.

Group	Dose (mg/kg)	Baseline reaction time (sec)	Test reaction time (sec)			% Maximum possible response		
			1 h	2 h	3 h	1 h	2 h	3 h
Control	-	2.25 ± 0.26	2.50 ± 0.21	2.40 ± 0.17	2.50 ± 0.26	-	-	-
Diclofenac	10.00	3.63 ± 0.31	3.77 ± 0.41	3.68 ± 0.35	3.63 ± 0.33	2	1	0
Codeine	200.00	3.12 ± 0.19	8.33 ± 1.36*	9.70 ± 0.74*	8.77 ± 1.47*	76	96	82
SI extract	18.75	2.70 ± 0.28	2.82 ± 0.41	2.78 ± 0.37	2.73 ± 0.43	2	1	0
SI extract	37.50	2.02 ± 0.04	3.05 ± 0.10*	2.18 ± 0.08*	2.05 ± 0.06	13	2	0
SI extract	75.00	2.82 ± 0.37	4.62 ± 0.22*	3.53 ± 0.19*	3.25 ± 0.19*	25	10	6

Values are expressed as mean ± S.D. (n = 6)

* Significantly different from the baseline reaction time, $p < 0.05$

Table 10 Antipyretic activity of SI extract and diclofenac on yeast-induced hyperthermia.

Group	Dose (mg/kg)	Baseline rectal temperature (°C)	18 h after yeast injection rectal temperature (°C)	Test rectal temperature (°C)				
				30 min	60 min	90 min	120 min	180 min
Control	-	37.5 ± 0.2	39.0 ± 0.2	39.2 ± 0.3	39.2 ± 0.3	39.1 ± 0.3	39.0 ± 0.3	39.2 ± 0.4
Diclofenac	10	37.4 ± 0.2	38.9 ± 0.3	38.2 ± 0.3*	37.6 ± 0.3*	37.2 ± 0.1*	36.9 ± 0.2*	37.4 ± 0.3*
SI extract	75	37.6 ± 0.2	39.2 ± 0.3	39.3 ± 0.3	39.1 ± 0.4	39.1 ± 0.3	39.1 ± 0.3	39.2 ± 0.3
SI extract	150	37.6 ± 0.1	39.1 ± 0.2	39.4 ± 0.2	39.2 ± 0.2	39.1 ± 0.3	39.1 ± 0.3	38.9 ± 0.3
SI extract	300	37.6 ± 0.3	39.2 ± 0.5	39.3 ± 0.4	39.2 ± 0.3	39.2 ± 0.2	39.2 ± 0.2	39.2 ± 0.2

Values are expressed as mean ± S.D. (n = 6)

* Significantly different from the 18 h after yeast injection rectal temperature, $p < 0.05$