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INTRODUCTION

Sapindus rarak DC. or "Ma-Kum-Dee-Kwao" in Thai is a herbal plant which is prevalent in many parts of Thailand. It has been known for its treating skin diseases properties[1]. Triterpenoid saponins[2,3] and flavonoids[4] have been reported from this plant species. Previous studies showed antidermatophytic[5] and pancrease lipase inhibitory[3] activities of extracts from *S. rarak* pericarps. However, there is no scientific report concerning the anti-inflammatory activity of this plant, especially the effect of *S. rarak* on skin inflammation. In this study, the topical anti-inflammatory effect of saponin rich extract from *S. rarax* pericarps was investigated using croton oil induced rat ear edema model.

MATERIALS AND METHODS

Plant material : Mature fruits of *S. rarax* were purchased from Doi Moo Sur Market, Tak Province, Thailand in June, 2010 and identified by Mr. Winai Supatanakul, the botanist of Agriculture Department , Thailand Institute of Scientific and Technological Research (TISTR). Voucher specimen (SR 001) was deposited in the Department of Pharmaceutical and Natural Products, TISTR.

Extraction and Fractionation: Pericarps (50 g) were cut into small pieces and then extracted with distilled water (1:10 for 2 h) on water bath. The aqueous solution was filtered and put on through 500 ml macroporous resin (Diaion HP-20, Mitsubishi Chemical Industry, Japan) then eluting with water-ethanol 1:0,1:1,0:1 (500x3 ml/each solvent propotion). Fractions were monitored by TLC using silica gel GF_{254} (Alufolien , Merck, Germany) as stationary phase , mixture of chloroform : methanol: water 20:5:0.25 as mobile phase and anisaldehyde sulfuric acid as detection reagent. Similar fractions of saponin mixture were combined and lyophilized to give 3.32 g of dried powder (SRE).

Anti-inflammatory Test [6]

Animals: Male Wistar rats were purchased from Laboratory Animal Centre, Mahidol University, Salaya, Nakornprathom, Thailand. The animals were housed in the animal facility of Thailand Institute of Scientific and Technological Research under standard conditions (25±2°C), 50-60% of humidity and 12 h/12 h light/dark cycles. The animals were kept under laboratory conditions for one week prior to the start of the experiments. Food and water were allowed *ad libitum*.

Croton oil- induced ear edema : Rats weighing 80-100 g were divided in groups of six. Each rat received 3.6 µl of croton oil (Sigma , USA) dissolved in 20 µl of acetone on the right ear. This was applied by an autopipette in 20 µl volume to both anterior and posterior surfaces of the right ear. The left ear control received the same volume of vehicle. The plant extract, SRE at 3 doses i.e. 1.25, 2.5, 5 mg/20 µl or the reference drug, diclofinac (Sigma , USA) 5 mg/20µl separatedly dissolved in 70 % aqueous ethanol were administered topically 120 min after treatment with croton oil. The ear thickness was measured by pocket thickness gage at 1, 2, 3 and 4 h after test samples application. The percentage of edema inhibition was calculated with reference to vehicle control group.

Analysis of Data: The results are expressed as means ± S.E.M. Differences in mean values between groups were analyzed by a one-way analysis of variance (ANOVA)

RESULTS

Saponin rich extract (3.32 g/50 g pericarps) was obtained from the water:ethanol 0:1 eluate. It appeared as hygroscopic light brown powder. This fraction obviously showed the foam forming property indicating the presence of saponin constituent. TLC analysis of the fraction using anisaldehyde sulfuric acid spray reagent showed the characteristic bluish band color of saponins. Six bluish bands were obviously observed (Fig. 1).

Topical anti-inflammatory effect of SRE is demonstrated in Table 1. SRE at 1.25, 2.5 mg/ear at 4 h after application showed significant inhibition of croton oil induced ear edema with percentage inhibition of 43.67 and 39.40 %, respectively while the reference drug, diclofinac at 5 mg/ear showed 50% inhibition. SRE at 5 mg/ear did not show significant edema inhibitory effect.

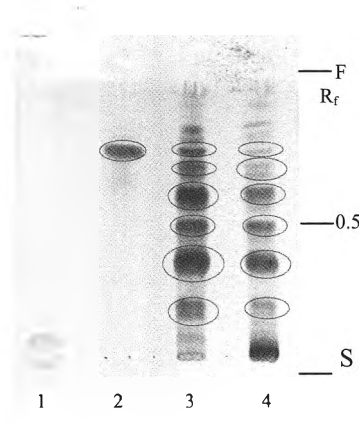

Figure 1 TLC chromatogram of saponin rich extract from *S. rarax* pericarps

Stationary Phase : Silica gel GF_{254}
Mobile phase : Chloroform : Methanol : Distilled water : 20:5:0.25
Detection : Anisaldehyde sulfuric acid reagent

Track 1 asiaticoside
Tract 2 asiatic acid
Track 3 Saponin rich extract (SRE)
Track 4 *S. rarax* water extract
= bluish band

Table 1 Edema inhibitory effect of saponin rich extract (SRE) from *S. rarax* pericarps tested by croton oil induced ear edema in rat.

Samples	% Swelling				Edema inhibition (%)			
	1 h	2 h	3 h	4 h	1 h	2 h	3 h	4 h
Control	51.67±2.43	38.14±1.25	38.15±0.84	36.32±2.24	-	-	-	-
Diclofenac 5 mg/ear	39.88±5.88*	20.92±3.60*	19.53±3.36*	18.16±2.76*	22.82*	45.15*	48.80*	50.00*
SRE 1.25 mg/ear	38.66±2.24*	26.41±1.64*	22.75±2.20*	20.46±3.37*	25.18*	30.75*	40.36*	43.67*
SRE 2.5 mg/ear	40.48±3.87*	25.67±2.79*	24.71±2.55*	22.01±2.84*	21.66*	32.69*	35.23*	39.40*
SRE 5 mg/ear	46.17±3.77	37.26±3.35	33.14±4.06	29.33±3.63	10.64	2.31	13.13	19.24

n = 6, * significant from control, $P < 0.05$

$$\% \text{ Swelling} = \frac{(T_t - T_0) \times 100}{T_0}$$

T_0 = ear thickness before induced edema
T_t = ear thickness after sample application

DISCUSSION

The present study demonstrated the topical anti-inflammatory effect of saponin rich extract (SRE) from *S. rarak* pericarps. SRE was prepared from the aqueous extract using adsorption-desorption column chromatography on macroporous resin (Diaion HP-20), this technique is convenient and commonly practised for industrial separation of saponins. In this experiment mixture of saponins was obtained from the ethanolic eluate. The result confirms the previous report on the presence of saponin mixtures in *S. rarak* pericarps[3]. Based on the mobile phase polarity and R*f* values compared to asiaticoside and its aglycone, asiatic acid, saponin components in SRE could be classified into the medium polar group.

SRE from *S. rarak* pericarps has a relevant topical anti-inflammatory effect in a model of cutaneous inflammation in rat ear. Croton oil contains 12-*O*-tetradecanoylphorbol-13-acetate (TPA) as the main phorbol ester. Topical application of croton oil or TPA promotes an acute inflammatory reaction characterized by vasodilatation, polymorphonuclear leukocyte infiltration to the tissue and edema formation[7]. SRE at 1.25, 2.5 mg/ear exhibited significant edema inhibitory effect in every monitoring period (1,2, 3, and 4 h) in the same manner as that of diclofinac at 5 mg/ear. It is noteworthy that the edema inhibitory effect of SRE was not in a dose dependent manner. SRE at 5 mg/ear did not show significant edema inhibitory effect. SRE at 5 mg/20µl of 70 % aqueous ethanol appeared as viscous solution. Consequently, this might interfere with the delivery of saponin component to the target area. SRE at 1.25 mg/ear seemed to be the most active dose in our study. Dose response of the edema inhibitory effect of SRE probably could be observed when lower concentrations than 1.25 mg/ear were administered.

CONCLUSION

The present study provides pharmacological evidence for the topical anti-inflammatory activity of saponin rich extract obtained from *S. rarak* pericarps. In croton oil -induced rat ear edema, saponin rich extract at 1.25 -2.5 mg/ear showed significant edema inhibitory activity. The result confirms ethnomedical uses of *S. rarak* for the treatment of skin diseases.

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