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Research Article

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Pharmacological evaluation of ethanol extract of *Sida rhombifolia* L. roots (Malvaceae)

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ABSTRACT

Many herbs are usually practiced by Indonesian people to treat tooth pain, one of them is the roots of S. rhombifolia. However there are lack studies in dentistry to evaluate them either pharmacological activities. The aim of the present study was to investigate anti-inflammation and analgesic activities ethanol extract of S. rhombifolia roots. Anti-inflammatory using carrageenan-induced acute inflammation on rat and analgesic activities using hot plate method on mice, in vivo. Highest anti-inflammatory activities was found at dose 2.4 g/kg/BW with 20.78% of anti-inflammatory index compared to negative control ($p<0.05$). While the longest duration was for analgesic activities was found at concentration 2.4 g/kg/BW in 13.02 min. These results suggest that ethanol extract of S. rhombifolia roots have potential to be developed as dentistry formula.

Keywords: *S. rhombifolia*, antibacterial, anti-inflammation, analgesic.

INTRODUCTION

Indonesia is the largest archipelago in the world and rich in flora including many unique varieties of tropical plant life in various forms[1]. Many of the plants is used by local residents as sources of medicine or drug. They had been using the plants with their traditional knowledge, one of them is Sidaguri[2].

Sidaguri, also named *Sida rhombifolia* a genus of flowering plants in the mallow family, Malvaceae. It is used in Indonesia folklore medicine for the treatment of gout [3]. In India it is used against hypertension and diabetes [4], in Europe used as anti-tuberculosis agent [5] and Australian Aborigines use the roots to treat diarrhea and indigestion [6].

Various classes of chemical constituents have been characterized from the *S. rhombifolia*. They belong to sterol: sitosterol, stigmasterol, sitosterol-3-O-b-D-glucopyranoside, stigmasterol-3-O-b-D-glucopyranoside, phaeophytin A, 17^3-ethoxypheophorbide A, 13^2-hydroxy phaeophytin B, 17^3-ethoxypheophorbide B, 5,7-dihydroxy-4'-methoxyflavone, cryptolepinone and a salt of cryptolepine have vasorelaxant activity in ratsmesenteric artery ring model[7]; ecdysteroids; 20-hydroxyecdysone, 2-deoxy-20-hydroxyecdysone-3-O-beta-D-glucopyranoside and 20-hydroxyecdysone-3-O-beta-D-glucopyranoside [8]; n-hexacos-11-enoic acid, stigmasterol and β-sitosterol have antimicrobial activities [8].

Since this plant has important medicinal properties, the present study to evaluate selected pharmacological activities of ethanol extract of *S.rhombifolia* L. roots: antimicrobial, anti-inflammation and analgesic activities.

EXPERIMENTAL SECTION

Material
All the chemicals used are of analytical reagent grade. Ethanol 96%, nutrient agar, DMSO and carrageenan were obtained from Merck - Indonesia. Indomethacin and paracetamol are obtained from drugstores – Indonesia.

Plant collection and extraction
The roots were collected from Watampone, South Sulawesi, Indonesia. The roots were separated from undesirable materials, plants or plant parts and sun-dried. The dried roots were ground into a coarse powder and extracted. Extract was made by maceration method using ethanol 96%. The extract filtered through Whatman filter paper and evaporated under the vacuum at 40^{O}C (Buchi) and then dried to a powder using a freeze dryer at -80^{O}C (Scanvac).

Carrageenan-Induced Acute Inflammatory Model
Male Wistar rats (200-225 g) were housed in a controlled environment and provided with standard rodent chow and water, *at libitum*. To test inhibitory effects on acute inflammation in an animal model, paw oedema was induced by subcutaneous injection of 100 μL of freshly prepared solution of carrageenan (1%) into the right hind paw, as was previously described with modification[9, 10]. Five animals per group were tested. The Animal and Ethics Review Committee at the Hasanuddin University evaluated and approved the protocol used in this study.

Procedure: Animals of group A, B, C, D and E were treated with the single dose of ethanol extract of *S. rhombifolia* root at 0.15; 0.30; 0.60; 1.20 and 2.40 g/kgBB; group F and G were treated with indomethacin 0.01 g/kgBB and vehicle, respectively, 30 min prior to carrageenan injection. Paw oedema volume were measured using plethysmometer just before the carrageenan injection, that is, at 0 min and then at 15, 30, 60, 90, 120, 150, 180, 210, 240, 270 and 300 min after carrageenan injection. Increase in paw oedema volume was measured as the difference in paw oedema volume at 0 min and paw oedema volume at respective min.

Hot-plate Analgesic test
Male BALB/c mice (20-22 g) were housed in a controlled environment and provided with standard rodent chow and water, at libitum. The method used was described with modification[11, 12]. Briefly, a metal hot-plate was heated to a constant temperature 50 ± 0.5^{O}C. The temperature of the plate was monitored at all times. A colorless acrylic cylinder of 20 cm diameter was placed on the hot-plate to confine the mice to a certain observation area. Five animals per group were tested. The Animal and Ethics Review Committee at the Hasanuddin University evaluated and approved the protocol used in this study.

Procedure: Group A, B, C, D and E were treated with the single dose of ethanol extract of *S. rhombifolia* root at 0.21; 0.42; 0.84; 1.68 and 3.36 g/kgBB; group F and G were treated with paracetamol 0.014 g/kgBB and vehicle, respectively. The mice were placed on the hot-plate and response to the thermal stimulus. Responses were characterized as licking or flinching of one of the paws, jumping or vocalization was recorded.

STATISTICAL ANALYSIS
Statistical analyses were performed by One-Way ANOVA supplemented with Tukey multiple comparisons test ($p<0.05$ was considered to indicate statistical significance).

RESULTS AND DISCUSSION

Acute Inflammatory
The ethanol extract of *S. rhombifolia* root significantly inhibited carrageenan-induced paw oedema. The extract produced a dose-dependent inhibition of carrageenan (1%) which was comparable with indomethacin anti-inflammatory drugs. The extract produced significant anti-inflammatory activity. Significant reduction of paw oedema was observed at 0.6 g/kgBB till 2.4 g/kgBB (Figure 1).

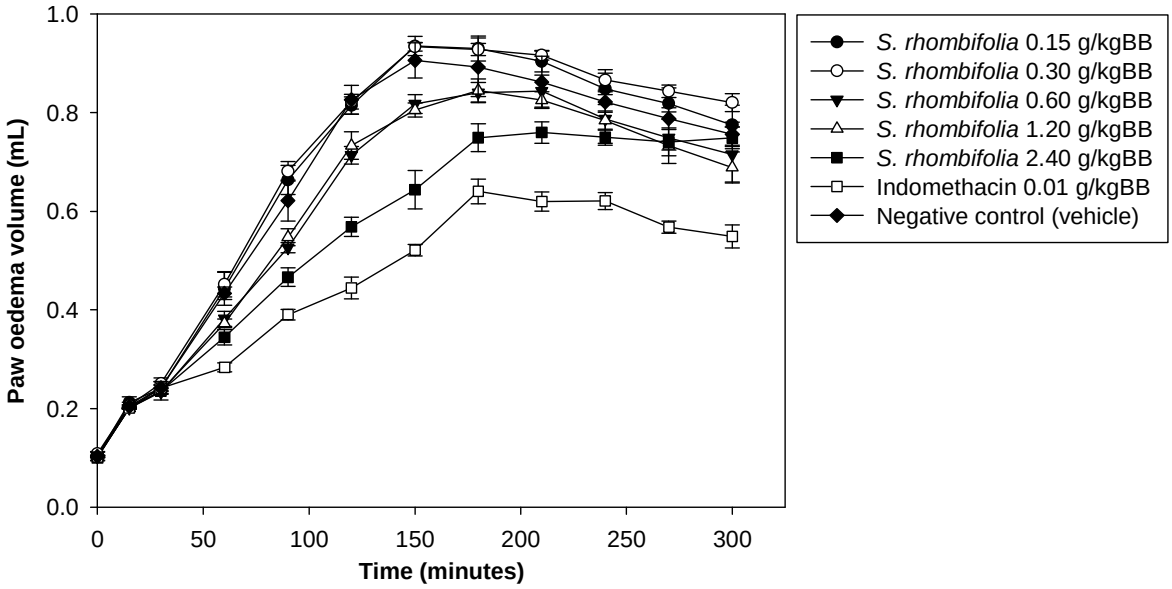

Figure 1. Change in paw edema volume (mL) of ethanol extract of *S. rhombifolia*root at 0.15; 0.30; 0.60; 1.20 and 2.40 g/kgBB compared with indomethacin 0.01 g/kgBB and vehicle (Na-CMC). Edema was induced bysubcutaneous injection of 100 µL of freshly prepared solution of carrageenan (1%) into the right hind paw
Data are expressed as mean ± SD of five rats per group

Carrageenan-induced paw oedema is a biphasic response mediated through the release of cytokines pro-inflammation: histamine, serotonin and kinins[13]. Cytokines pro-inflammation induced to release prostaglandin or prostaglandin-like substances in 2–3 h [14, 15]. The extract can inhibit carrageenan-induced paw oedema that may act through inhibition of cytokines pro-inflammation so that inhibited release of prostaglandin or prostaglandin-like [16, 17]. In the present study, maximum paw oedema was observed at 180 min after carrageenan injection, i.e., after the release of all these mediators of inflammation. The probable cause of anti-inflammatory action against acute inflammation might be due to the inhibition of some or all of the mediators released within 180 min of carrageenan injection.

Anti-inflammatory index

Percentage inhibition of paw oedema volume was measured by SPSS 16 using multivariant analysis.

Table 2. Percent inhibition in paw oedema volume
Percentage of oedema reduction was measured using multivarian analysis by using software (Table 2)

No.	Sample	Dose	Oedema Reduction (Percentage)
1	Ethanol extract of *S. rhombifolia* root	0.15 g/kgBB	-3.62
		0.30 g/kgBB	-5.25
		0.60 g/kgBB	9.49
		1.20 g/kgBB	9.95
		2.40 g/kgBB	20.78
2	Indomethacin	0.01 g/kgBB	38.13
3	Negative control	vehicle	1.00

As shown in Table 2, the reduction of oedema showed dose-dependent inhibition. The highest inhibition 20.78% was showed by extract 2.4 g/kgBB, although not equally effective to that produced by the standard (indomethacin) 38.13%, indicating an anti-inflammatory effect.

Carrageenan can induce cytokines pro-inflammation: histamine, serotonin and kinins that can be inhibited by antioxidant and anti-inflammation. *S. rhombifolia* stems and roots have antioxidant and anti-inflammation activities

on adjuvant induced arthritis in experimental rats[18]. The roots have good scavenging activity on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radically the same on this research [19]. Various studies reported that *S. rhombifolia* has anti-inflammatory on NF-Kb cell line inflammation model [20].

Analgesic test

The result of the hot plat test revealed that latency time was significant ($p<0.05$). The extract showed dose-dependent inhibition and the effect of extract at the dose of 3.36 mg/kgBB was better than that of extract at the below-dose and negative control when given orally on mice.

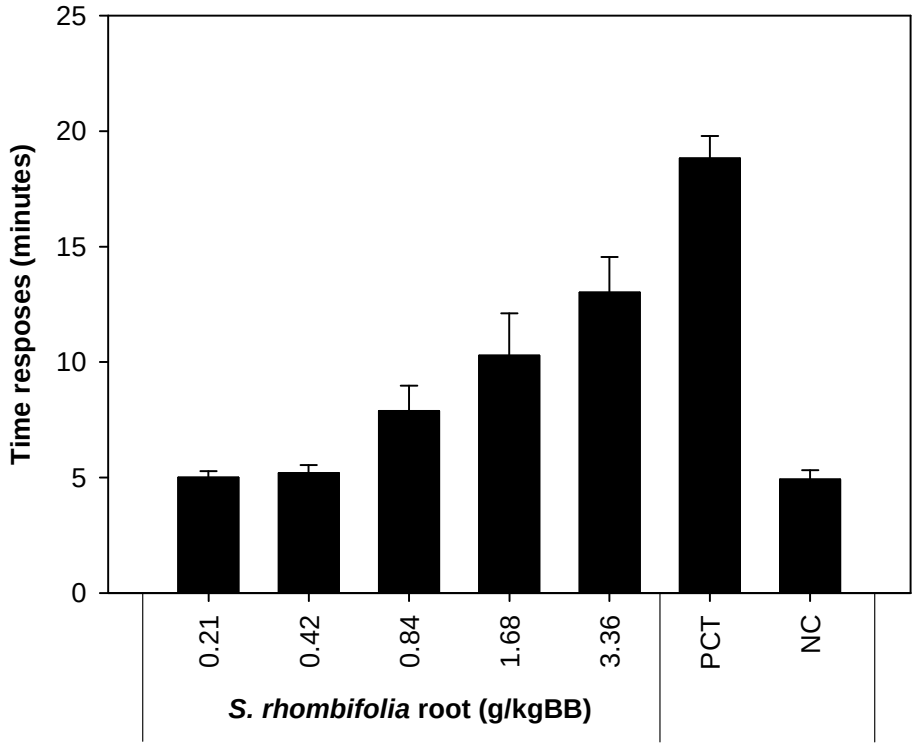

Figure 2.Effects of ethanol extract of *S. rhombifolia* root at 0.21; 0.42; 0.84; 1.68 and 3.36g/kgBB were compared with paracetamol 0.014 g/kgBB and vehicle (Na-CMC)

The mice were placed on the hot-plate and responded to the thermal stimulus. Data are expressed as mean ±SD of five rats per group.

Hot-plate model aims to study the spinal anti-nociceptive action. This method measures animal nociceptive response latencies to thermal stimulus predominantly on supraspinal[21-23]. Treating the mice with extract of *S. rhombifolia* root, at dose 0.21 g/kgBB till 3.36 g/kgBB alters mice latency to painful thermal stimulus in hot plate tests. From the results, though the extract showed analgesic actions in hot plate models, may not be fully mediated through central mechanism or peripheral mechanism. The analgesic effect produced may be via central mechanisms involving these receptor systems or via peripheral mechanisms or inhibition of cytokines pro-inflammation [24, 25]. From the results, though the extract showed analgesic actions in hot plate models, the results may not be fully mediated through central mechanism or peripheral mechanism.

Phytochemical analysis and HPTLC fingerprint of *S. rhombifolia* confirmed the presence of phenolic, especially flavonoids. The antioxidant activity of root may be attributed to its flavonoid content. *S. rhombifolia,* such capacity of a compound may serve as a significant indicator of its potential antioxidant activity[19].

CONCLUSION

The results obtained in this study indicate that the extract of *S. rhombifolia* root has antimicrobial activity on *E. faecalis* but not antimicrobial activity on *Actinomycetes* spp. The extract possesses anti-inflammation and analgesic

activities, which are mediated although may not be fully mediated through central mechanism or peripheral mechanism. Further studies should be performed for the isolation of new chemical constituents of the plant as well as for a better understanding of the mechanism of pharmacological activity presented by the extract.

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