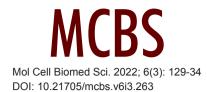
RESEARCH ARTICLE



Methanol Extract of *Katuk* (*Sauropus androgynus*) Leaves as an Anti-inflammatory Agent: Animal Study in Carrageenan-induced Rat Models of Inflammation

Desy Andari¹, Farah Ibnu Khan², Safira Idofia Jakfar²

¹Department of Histology, Faculty of Medicine, University of Muhammadiyah Malang, Malang, Indonesia

Background: Inflammation is a response in the human body to survive during infection, injury and tissue damage. Acute inflammation causes edema and polymorphonuclear neutrophils (PMNs) release. *Sauropus androgynus* leaves which contain flavonoids, tannins, saponins, steroids and triterpenoids may have anti-inflammatory properties. These compounds can be extracted with methanol. This research aimed to determine the anti-inflammatory effect of *S. androgynus* leaves methanol extract (SALME) on carrageenan induced-rats.

Materials and methods: True experimental study was conducted using 12 Wistar rats. Rats were induced with carrageenan subcutaneously on the plantar pedis. Carrageenan-induced rats were treated with/without various doses of SALME. Edema volume was measured with a plethysmometer. The plantar pedis tissues were collected and stained with haematoxylineosin (HE) staining, then PMNs were observed and counted under a light microscope. All data were analyzed by one-way ANOVA, Bonferroni post hoc test, Pearson correlation and linear regression.

Results: SALME had significant effects on the volume of edema (p=0.000) and the number of PMNs (p=0.000). The most effective dose to reduce the edema volume and decrease the PMNs cell number was 37.80 mg/200 g body weight (BW). SALME doses may affect 91.0% of edema volume and 89.2% of PMNs cell number. Edema volume had a significant, robust correlation (92.3%) with PMNs cell number.

Conclusion: SALME is confirmed to have an anti-inflammatory activity by reducing the edema volume and decreasing the PMNs cell number.

Keywords: Sauropus androgynus, methanol extract, anti-inflamamatory, carrageenan, plantar pedis, rats

Introduction

Inflammation is a process that occurs in the body in response to harmful external stimuli, such as microbial infections and tissue damage, as well as other physiological conditions, including tissue healing. The acute inflammatory process is characterized by redness, edema, increase of temperature

Date of submission: March 18, 2022 Last Revised: June 8, 2022 Accepted for publication: June 9, 2022

Corresponding Author:

Desy Andari
Department of Histology
Faculty of Medicine, University of Muhammadiyah Malang
Jl. Bendungan Sutami 188A, Malang 6514, Indonesia
e-mail: desy_andari@umm.ac.id





²Undergraduate Student, Faculty of Medicine, University of Muhammadiyah Malang, Malang, Indonesia

and pain. Acute inflammation causes changes in blood flow and vessel permeability, which are intended to increase the exudation of fluid and plasma proteins, and the emigration of leukocytes, predominantly polymorphonuclear neutrophils (PMNs) to injury site.² Administration of anti-inflammatory drugs both steroid (corticosteroid) and non-steroidal (aspirin and acetaminophen) is one of the methods used to treat inflammation. Unfortunately, these drugs may cause several side effects. The most common side effects caused by shortterm oral corticosteroid therapy in children are vomiting, behavioral changes, sleep disorders and increase the susceptibility to infections.³ In adults, the risk of developing various corticosteroid-related complications, such as infections, diabetes, osteoporosis, and psychiatric disorders increases with long-term exposure to oral corticosteroids.⁴ Aspirin is also known to be involved in acid-induced injury and may increase the risk of cardiovascular and cerebrovascular events.2

Katuk (Sauropus androgynus) leaves are known to have antioxidant compounds. Phytochemical test using thin layer chromatography (TLC) shows that *S. androgynus* leaves contain flavonoids (flavones and flavonols), saponins, tannin gallate, steroids and triterpenoids.⁵ *S. androgynus* leaves extracted with maceration method have been reported to produce more extract yield compared to soxhletation. Methanol as a solvent has the advantage over the more commonly used 70% ethanol since methanol can extract the triterpenoid content.⁶ *S. androgynus* leaf extraction with methanol also results in higher antioxidant activity than other solvents, such as n-hexane and chloroform.⁷

S. androgynus leaves methanol extract (SALME) may have anti-inflammatory properties since it contains flavonoid, tannin, saponin, steroid and triterpenoid.⁵ Higher intakes of flavonoids are associated with lower plasma concentrations of inflammatory biomarkers, such as soluble vascular cell adhesion molecule-1 (sVCAM-1)⁸ and interleukin (IL)-18.^{9,10} Steroids exert their anti-inflammatory effect by switching off several inflammatory genes.¹¹ At low concentrations, triterpenes show an immunomodulatory activity by inhibiting the production of pro-inflammatory mediators, such as nitric oxide (NO), IL-6 and cyclooxygenase-2 (COX-2).¹²

The aim of this research was to determine the effect of SALME as an anti-inflammatory using carrageenan-induced rat models of inflammation. In this study, *S. androgynus* leaves were extracted using methanol as a solvent to yield

more active compounds, particularly triterpenoids, which cannot be extracted with ethanol in previous studies. The parameters measured as indicators of inflammation in this study were edema volume and the number of PMNs. This study also determined the correlation between the volume of edema and the PMNs number.

Materials and methods

Preparation of SALME

S. androgynus leaves were obtained from Materia Medika Batu, Malang. Three hundred g of *S. androgynus* leaf powder was macerated three times using methanol at the ratio of 15% for 3 days at room temperature and concentrated using a rotary evaporator at 60°C. The resulting extract was stored at 4°C for further use.

Experimental Animals and Study Design

This research was a true experimental study using 12 male Wistar rats (180-200 g). Rats were acclimatized under controlled room temperature, fed with standard diet and water *ad libitum* for 7 days prior to the experiment. Rats were induced with 0.1 mL 1% carrageenan suspended in salines subcutaneously on the plantar pedis. Carrageenan-induced rats were then divided into 4 groups, which were control and SALME groups at dose 2.48, 6.15, and 37.80 mg/200 g body weight (BW). Each group contained three rat models of inflammation. SALME was administered orally to rat models 4 hours after fasting. This protocol was approved by Health Research Ethics Committee, University of Muhammadiyah Malang (number: E.5.a/147/KEPK-UMM/V/2018).

Edema Volume Measurement and PMNs Calculation

To measure the edema volume, plantar pedis of rats were inserted into the plethysmometer before and 4 hours after carrageenan injection. The rats were sacrificed under anesthesia and the plantar pedis tissues were collected and stained with haematoxylin-eosin (HE) staining. PMNs in plantar pedis were observed and counted by 2 observers (supervised by a pathologist) under a light microscope in 5 fields of view (averaged) with 400× magnification.

Statistical Analysis

All data were analyzed by one-way ANOVA, Bonferroni *post hoc* test, Pearson correlation and linear regression using SPSS version 24 (IBM, Armonk, NY, USA).

Results

Administration of SALME decreased the average of PMNs cell number in plantar pedis tissue of rats in a dosedependent manner (Figure 1). One-way ANOVA test results showed that SALME had significant effects on the volume of edema (p=0.000) and the number of PMNs (p=0.000). PMNs were the most abundant in the control group (Figure 1A). The groups that received 2.48 and 6.15 mg/200 g BW of SALME had fewer PMNs (Figure 1B, Figure 1C). Meanwhile, the group that received 37.80 mg/200 g BW SALME had the least number of PMNs (Figure 1D). The increase in SALME doses also reduced the volume of edema. Bonferroni post hoc test results showed that 37.80 mg/200 g BW SALME had the most significant effect on the volume of edema and the number of PMNs (Figure 2, Figure 3). R² values suggested that 91.0% edema volume could be affected by SALME doses. Meanwhile, 89.2% PMNs cell number could be affected by SALME doses (Table 1). There was a strong (92.3%), significant (p=0.000) correlation between the PMNs cell number and the volume of edema.

Discussion

According to a previous study, SALME has a half-maximal inhibitory concentration (IC $_{50}$) of 80.81. 14 This value is lower compared to the *S. androgynus* leaves extracted with 80% (IC $_{50}$ =813) and 96% ethanol (IC $_{50}$ =1,024). 15 The IC $_{50}$ value of a compound is inversely proportional to its antioxidant capacity. Lower IC $_{50}$ values indicate stronger antioxidant activity. 20

Extracts obtained from several plant and fungi species have been reported to have anti-inflammatory properties. In this study, SALME had a significant effect on the volume of edema and the number of PMNs. SALME has been reported to contain flavonoids, saponins, tannin gallate, steroids and triterpenoids which have anti-inflammatory properties. Numerous studies show that the high-flavonoid diets can reduce inflammatory markers, such as C-reactive protein (CRP)9,22,23, IL-1 $\beta^{24,25}$, IL- $\beta^{26,27}$, IL- β^{28} and tumor necrosis factor-alpha (TNF- α). In addition, saponin extracts have been reported to reduce the volume of edema in the feet of rats that have been induced by carrageenan. The reduction in edema volume is thought

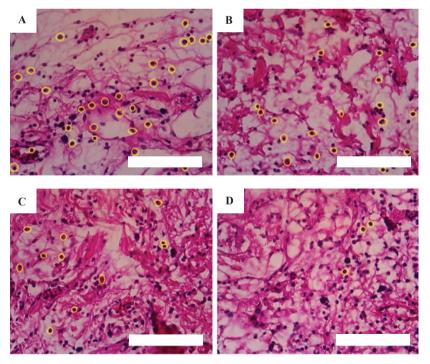


Figure 1. PMNs in plantar pedis tissue of carrageenan-induced rat models of inflammation. A: Control group. B: SALME group at a dose of 2.48 mg/200 g BW. C: SALME group at a dose of 6.15 mg/200 g BW. D: SALME group at a dose of 37.80 mg/200 g BW. White bar: 100 μm.

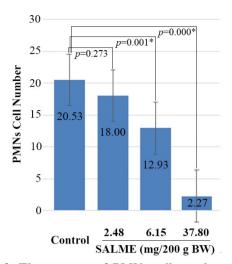


Figure 2. The average of PMNs cell number. *p<0.05, Bonferroni *post hoc* test.

to be the mechanism that inhibits the production and release of inflammatory agents during the inflammatory phase. Steroids are known as anti-inflammatory agents that work through several pathways. The main function of steroids is to inhibit the activation of several inflammatory genes, including nuclear factor kappa B (NF- κ B), a regulator of inflammatory genes. Triterpenoids have been known to have antioxidant and anti-inflammatory properties in both animals and humans. Previous study had shown that triterpenoids inhibit the production of pro-inflammatory mediators, such as TNF- α , NO, IL-6 and COX-2, as well as induce the production of anti-inflammatory cytokines, such as IL-10. In addition, triterpenoids show no potential cytotoxicity to macrophages. The inflammatory is a such as IL-10. In addition, triterpenoids show no potential cytotoxicity to macrophages.

Carrageenan has been known to be a potent inflammatory agent in rodents that induces the production of TNF- α . Inflammation caused by carrageenan upregulates the production of prostaglandins and COX-2, increases vascular permeability, and causes edema. Carrageenan has low toxicity and has not been shown to be teratogenic. Carrageenan-induced rat foot edema is widely used to

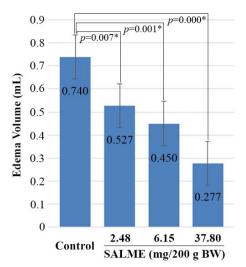


Figure 3. The average of edema volume. *p<0.05, Bonferroni post hoc test.

determine anti-inflammatory activity, a simple and routine way to evaluate pain in the inflamed area without any injury or damage to the inflamed foot.³²

SALME at a dose of 37.80 mg/200 g BW had the most significant effect on the volume of edema and the number of PMNs. This result is slightly different from the result of a previous study which concludes that *S. androgynus* leaves extract at a dose of 48.6 mg/200 g BW is the best in decreasing TNF- α expression, improving histopathological features of duodenum (epithelium and villi), decreasing the number of inflammatory cells (neutrophils) and vasodilation of blood vessels.³³

Lower doses (37.80 mg/200 g BW) of SALME in this study had a significant result in decreasing the number of PMNs. This is consistent with the results of a previous study.³⁴ SALME also decreased the edema volume. This is in accordance with the previous studies that flavonoids, steroids, saponins, tannins and terpenoids have anti-inflammatory properties, including reducing edema.^{11,12,31} Higher intakes of selected subclasses of flavonoids (flavones and flavanones) is associated with a 20% modestly lower

Table 1. Linear regression results between SALME and edema, and SALME and number of PMNs.

	Model	R	R^2	Adjusted R ²	Standard Error of the Estimate
Edema volume	1	0.954^{a}	0.910	0.900	0.05666
Number of PMNs	1	0.945 ^a	0.892	0.882	2.54527

^aPredictors: (Constant), dose.

plasma concentrations of inflammatory biomarkers, such as IL-18° as well as TNF-α and NO.35 Several mechanisms have been described regarding the anti-inflammatory activity of flavonoids. These compounds have free radical scavenging and antioxidative activities. They also regulate the activities of inflammation-related cells and enzymes which are involved in arachidonic acid metabolism (lipoxygenase, cyclooxygenase, phospholipase A2) and nitrogen monoxide synthase. Moreover, flavonoids regulate the expression of pro-inflammatory gene and production of other pro-inflammatory molecules.36 Triterpenoids as antioxidants are characterized by their abilities to remove free radicals and inhibit their enzymatic generation, as well as block the oxidation of cells and extracellular compounds.37

The volume of edema had a strong, significant correlation with the number of PMNs. The cardinal characteristic of the inflammatory reaction is the emigration of leukocytes from small blood vessels into the injured tissues, causing leukocytes accumulation at the injury site. This emigration is due to vascular dilatation, which is followed by an increase in vascular permeability and exudation of PMNs-predominant fluid, leading to edema.³⁸

Conclusion

SALME is confirmed to have an anti-inflammatory activity by reducing the edema volume and decreasing the PMNs cell number in plantar pedis tissue. Edema volume has a strong correlation with PMNs cell number.

References

- Medzhitov R. Origin and physiological roles of inflammation. Nature. 2008; 454 (7203): 428–35.
- Kumar V, Abbas AK, Jon CA. Robbins Basic Pathology. 10th ed. Philadelphia: Elsevier; 2018.
- Aljebab F, Choonara I, Conroy S. Systematic review of the toxicity of short-course oral corticosteroids in children. Arch Dis Child. 2016; 101(4): 365–70.
- Volmer T, Effenberger T, Trautner C, Buhl R. Consequences of long-term oral corticosteroid therapy and its side-effects in severe asthma in adults: A focused review of the impact data in the literature. Eur Respir J. 2018; 52(4): 1800703. doi: 10.1183/13993003.00703-2018
- Djamil R, Zaidan S. Isolasi senyawa flavonoid dari ekstrak metanol daun katuk (Sauropus androgynus (L.) Merr), Euphorbiaceae. J Ilmu Kefarmasian Indones. 2017; 14(1): 57-61.
- 6. Hikmawanti NPE, Fatmawati S, Arifin Z, Vindianita V. Pengaruh variasi metode ekstraksi terhadap perolehan senyawa antioksidan pada daun katuk (Sauropus androgynus (L.) Merr). J Farm Udayana. 2021; 10(1): 1-12.
- 7. Andari A. Uji Aktivitas Ekstrak Daun Katu (Sauropus androgynus

- L. Merr.) Sebagai Antioksidan pada Minyak Kelapa [Thesis]. Yogyakarta: Universitas Islam Negeri Sunan Kalijaga; 2010.
- Febriza A, Ridwan R, As'ad S, Kasim VN, Idrus HH. Adiponectin and its role in inflammatory process of obesity. Mol Cell Biomed Sci. 2019; 3(2): 60-6.
- Landberg R, Sun Q, Rimm EB, Cassidy A, Scalbert A, Mantzoros CS, et al. Selected dietary flavonoids are associated with markers of inflammation and endothelial dysfunction in U.S. women. J Nutr. 2011; 141(4): 618–25.
- Purnama CA, Meiliana A, Barliana MI, Dandan KL, Wijaya A. Apoptosis and efferocytosis in inflammatory diseases. Indones Biomed J. 2021; 13(3): 242-55.
- Barnes PJ. How corticosteroids control inflammation: Quintiles Prize Lecture 2005. Br J Pharmacol. 2006; 148(3): 245–54.
- de Almeida PD, Boleti AP, Rüdiger AL, Lourenço GA, da Veiga Junior VF, Lima ES. Anti-inflammatory activity of triterpenes isolated from Protium paniculatum oil-resins. Evid Based Complement Alternat Med. 2015; 2015: 293768. doi: 10.1155/2015/293768.
- Mansouri MT, Hemmati AA, Naghizadeh B, Mard SA, Rezaie A, Ghorbanzadeh B. A study of the mechanisms underlying the antiinflammatory effect of ellagic acid in carrageenan-induced paw edema in rats. Indian J Pharmacol. 2015; 47(3): 292–8.
- Zuhra CF, Tarigan JB, Sihotang H. Aktivitas antioksidan senyawa flavonoid dari daun katuk (Sauropus androgunus (L) Merr.). J Biol Sumat. 2008; 3(1): 7-10.
- Arista M. Aktivitas antioksidan ekstrak etanol 80% dan 96% daun katuk (Sauropus andrognus (L.) Merr.). Calyptra J Ilm Mhs Univ Surabaya. 2013; 2(2): 1–16.
- Sovia E, Anggraeny D. Sugar palm fruits (Arenga pinnata) as potential analgesics and anti-inflammatory agent. Mol Cell Biomed Sci. 2019; 3(2): 107-14.
- Sudiono J, Hardina M. The effect of Myrmecodia pendans ethanol extract on inflamed pulp: Study on Sprague Dawley rats. Mol Cell Biomed Sci. 2019; 3(2): 115-21.
- Rizal A, Sandra F, Fadlan MR, Sargowo D. Ganoderma lucidum polysaccharide peptide reduce inflammation and oxidative stress in patient with atrial fibrillation. Indones Biomed J. 2020; 12(4): 384-
- Abdelkader RS, El-Beih NM, Zaahkouk SA, El-Hussieny EA. Ameliorative effect of Eruca sativa seeds and its rutin on Gentamicin-induced nephrotoxicity in male rats via targeting inflammatory status, oxidative stress and kidney injury molecule-1 (KIM-1)/cystatin C expression. Indones Biomed J. 2022; 14(1): 74-83.
- Elisha IL, Dzoyem JP, McGaw LJ, Botha FS, Eloff JN. The antiarthritic, anti-inflammatory, antioxidant activity and relationships with total phenolics and total flavonoids of nine South African plants used traditionally to treat arthritis. BMC Complement Altern Med. 2016; 16(1): 307. doi: 10.1186/s12906-016-1301-z.
- Djati MS, Dwijayanti DR, Nurmamulyosari LD, Fuadah Y, Basyarudin M, Jannah N. Elephantopus scaber and Sauropus androgynus regulate macrophages and B lymphocyte cells during Salmonella typhi infection. UNEJ e-Proceeding. 2016; [n.v]: 42–4.
- Ham D, Jun S, Kang M, Paik HY, Joung H, Shin S. Consumption of Korean foods with high flavonoid contents reduces the likelihood of having elevated C-reactive protein levels: Data from the 2015-2017 Korea National Health and Nutrition Examination Survey. Nutrients. 2019; 11(10): 2370. doi: 10.3390/nu11102370.
- Lukito AA, Bakri S, Kabo P, Wijaya A. The mechanism of coronary artery calcification in centrally obese non-diabetic men: Study on

- the interaction of leptin, free leptin index, adiponectin, hs-C reactive protein, bone morphogenetic protein-2 and matrix Gla protein. Mol Cell Biomed Sci. 2020; 4(2): 88-93.
- Chalons P, Amor S, Courtaut F, Cantos-Villar E, Richard T, Auger C, et al. Study of potential anti-inflammatory effects of red wine extract and resveratrol through a modulation of interleukin-1-beta in macrophages. Nutrients. 2018; 10(12): 1856. doi: 10.3390/nu10121856
- Domiciano TP, Wakita D, Jones HD, Crother TR, Verri WA Jr, Arditi M, et al. Quercetin inhibits inflammasome activation by interfering with ASC oligomerization and prevents interleukin-1 mediated mouse vasculitis. Sci Rep. 2017; 7: 41539. doi: 10.1038/srep41539.
- Lombardo Bedran TB, Morin MP, Palomari Spolidorio D, Grenier D.
 Black tea extract and its theaflavin derivatives inhibit the growth
 of periodontopathogens and modulate interleukin-8 and β-defensin
 secretion in oral epithelial cells. PLoS One. 2015; 10(11): e0143158.
 doi: 10.1371/journal.pone.0143158.
- Chen PC, Wheeler DS, Malhotra V, Odoms K, Denenberg AG, Wong HR. A green tea-derived polyphenol, epigallocatechin-3gallate, inhibits IκB kinase activation and IL-8 gene expression in respiratory epithelium. Inflammation. 2002; 26(5): 233–41.
- 28. Bobe G, Albert PS, Sansbury LB, Lanza E, Schatzkin A, Colburn NH, *et al.* Interleukin-6 as a potential indicator for prevention of high-risk adenoma recurrence by dietary flavonols in the polyp prevention trial. Cancer Prev Res. 2010; 3(6): 764–75.
- Oz HS, Chen T, de Villiers WJ. Green tea polyphenols and sulfasalazine have parallel anti-inflammatory properties in colitis models. Front Immunol. 2013; 4: 132. doi: 10.3389/fimmu.2013.00132.
- Castilla P, Dávalos A, Teruel JL, Cerrato F, Fernández-Lucas M, Merino JL, et al. Comparative effects of dietary supplementation with red grape juice and vitamin E on production of superoxide by

- circulating neutrophil NADPH oxidase in hemodialysis patients. Am J Clin Nutr. 2008; 87(4): 1053–61.
- Hassan HS, Sule IM, Musa MA, Musa YK, Abubakar SM, Hassan SA. Anti-inflammatory activity of crude saponin extracts from five Nigerian medicinal plants. Afr J Tradit Complement Altern Med. 2011: 9(2): 250–5.
- Necas J, Bartosikova L. Carrageenan: A review. Vet Med. 2013; 58(4): 187–205.
- Roosdiana A, Yudandi SA, Erika A. The potency of ethanolic extract of Sauropus androgynus (L.) Merr leaves as therapeutic herbal of rats (Rattus norvegicus) peptic ulcer model induced by Aspirin. IOP Conf Ser: Mater Sci Eng. 2018; 299: 012018. doi: 10.1088/1757-899X/299/1/012018.
- Perera HDSM, Samarasekera JKRR, Handunnetti SM, Weerasena OVDSJ, Weeratunga HD, Jabeen A, et al. In vitro pro-inflammatory enzyme inhibition and anti-oxidant potential of selected Sri Lankan medicinal plants. BMC Complement Altern Med. 2018; 18(1): 271. doi: 10.1186/s12906-018-2335-1.
- Ramos-Romero S, Pérez-Cano FJ, Ramírez-Santana C, Castellote C, Franch A, Castell M. Modulation of oxidative stress and TNFα secretion by peritoneal macrophages of arthritic rats fed with a flavonoid-enriched diet. J Transl Med. 2010; 8(Suppl 1): P24. doi: 10.1186/1479-5876-8-S1-P24.
- García-Lafuente A, Guillamón E, Villares A, Rostagno MA, Martínez
 JA. Flavonoids as anti-inflammatory agents: Implications in cancer
 and cardiovascular disease. Inflamm Res. 2009; 58(9): 537–52.
- Han N, Bakovic M. Biologically active triterpenoids and their cardioprotective and anti-inflammatory effects. J Bioanal Biomed. 2015; S12: 005. doi: 10.4172/1948-593X.S12-005.
- Shen G, Sarembock IJ. The inflammatory response. J Vasc Res. 1999; 36(5): 431–2.