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EFFECTIVENESS TEST OF THE N-HEXANE FRACTION OF ASOKA FLOWERS (*Saraca asoca*) AS AN ANTIINFLAMMATORY AGAINST FOOT EDEMA CARAGENAN-INDUCED RATS

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Abstract

This study aimed to evaluate the anti-inflammatory effectiveness of n-hexane fraction of Ashoka flower (Saraca). This study aimed to test the effectiveness of Ashoka flower (FNBA) against carrageenan-induced paw edema in rats. Inflammation is a protective response of the body against injury, but synthetic treatments often cause side effects. Ashoka flower, traditionally used for various conditions, is known to contain secondary metabolites such as flavonoids, saponins, alkaloids, triterpenoids, and tannins, which have anti-inflammatory potential. Extraction was obtained by maceration using 96% ethanol, followed by n-hexane fractionation. Anti-inflammatory testing was carried out on 25 male white rats divided into 5 treatment groups: negative control (Na CMC 0.5%), positive control (Na Diclofenac 2.25 mg/kg BW), and n-hexane fraction of Ashoka flower (FNBA) at concentrations of 20%, 40%, and 60%. Edema measurements were carried out using a plethysmometer every 30 minutes for 180 minutes. The results of the study showed that FNBA can reduce inflammation, with a concentration of 60% being the most effective and comparable to Diclofenac Sodium in reducing edema in rat paws.

1. Introduction

Inflammation is a fundamental defense response that remove pathogens and invading foreign objects to restore homeostasis. However, excessive activation of inflammatory response is definitely harmful to the host and causes diseases, such as cancer, sepsis, and autoimmunity. These complex biological processes cause clinical symptoms, including heat, pain, redness, and swelling, is widely triggered by infection and tissue damage . This condition is a basic pathological process, inflammation associated with with most human diseases. Studies have shown that excessive ROS are highly reactive and can kills cells in the body by oxidizing cellular components, including proteins, lipids, and nucleic acids, which cause inflammation. In addition, ROS function as signaling molecules and regulate various types of kinases and transcription factors in the initiation and progression of inflammation. It is widely believed that ROS participate in the inflammatory response and increases inflammation in various diseases, including diabetes, inflammatory bowel disease (IBD), chronic obstructive pulmonary disease (COPD), and osteoarthritis (OA). (Liu et al, 2023).

Anti-inflammatory effects associated with the inhibition of proinflammatory cytokines and the activation of anti-

inflammatory cytokines. (Santa Maria, 2023).

Treatment for inflammation generally involves synthetic anti-inflammatory drugs, both steroidal (NSAID) and nonsteroidal (NSAID). While effective, long-term use of NSAIDs, especially in elderly patients, can cause serious side effects on the digestive system, kidneys, and liver (Alfanda et al., 2021) .

In several clinical trials, it has been shown that the combination of NSAID and chemotherapy drugs (CTD) is able to obtain appropriate results. (Thiruchenthooran, 2023). However, its use is often associated with gastrointestinal (GIT) side effects due to inhibition of cyclooxygenase (COX)-1 and COX-2 enzymes, which leads to a decrease in gastric-protective prostaglandins (PGs). (Sohail, 2023).

The risk of gastric or intestinal ulceration, anemia due to gastrointestinal bleeding, urticaria, nausea, vomiting, and decreased renal blood flow, potentially leading to acute renal vasoconstriction and impaired renal function, are major concerns. Therefore, developing alternative anti-inflammatory therapies with minimal side effects is urgently needed . Traditional medicine using medicinal plants has long been a part of Indonesian society, offering abundant availability and minimal side effects (Fitriyanti et al., 2020) .

One plant that has attracted attention is the asoka (Saraca asoca), which is popular as an ornamental plant in Indonesia. Besides its aesthetic value, various parts of the asoka plant have health benefits. The asoka flower, for example, is used to relieve menstrual pain, dysentery, hemorrhoids, diabetes, and diarrhea. The roots can be brewed as an herbal tea, while the leaves and stems are used to treat diarrhea, fever, headaches, wounds, and stomach ulcers (Tinting et al., 2024) .

The HRMS profile of the methanol extracts of leaf and callus samples showed a predominance of flavonoids, phenols, saponins, and alkaloids. (Srivastava, 2025)

Flavonoids, for example can be extracted with non-polar solvents such as n-hexane and is known to inhibit enzymes involved in the inflammatory process (Fitriyanti et al., 2020) . Given this potential, this study aims to test the effectiveness of the n-hexane fraction of asoka flowers (*Saraca asoca) as an anti-inflammatory agent in reducing carrageenan-induced rat paw edema, as well as comparing its effectiveness with* diclofenac sodium .

2. RESEARCH METHOD

Type, Location, and Time of Research

This research was conducted using an experimental method to evaluate the effectiveness of the n-hexane fraction of Asoka flowers as an anti-inflammatory. All stages of the research, from sample collection to anti-inflammatory activity testing, were conducted in the Pharmacognosy Laboratory and Quantitative Chemistry Laboratory of the Deli Husada Deli Tua Health Institute.

Collection and Processing of Plant Samples

Asoka flower (Saraca asoca) samples were collected purposively from Aek Korsik Village, Bilah Hulu District, Labuhan Batu Regency, North Sumatra Province, with a total weight of 5 kg. After collection, the asoka flowers were washed thoroughly with running water to remove dirt, then drained and weighed as wet weight. The drying process was carried out in a drying cabinet using an incandescent lamp, avoiding direct sunlight exposure to maintain the quality of the active substance. Drying was continued until the simplicia was completely dry and brittle, facilitating the process of grinding into powder using a blender. The resulting simplicia powder was then weighed and stored in a tightly closed plastic container. Botanical identification of the plants was carried out at the Medanese Herbarium Laboratory, University of North Sumatra, to ensure species accuracy.

Examination of Simplicia Characterization and Phytochemical

Screening

Characterization examination of Asoka flowers includes examination (shape color, flavor taste) and microscopic (observation of cells using microscope) ensure the identity and of the simplicia. In addition determination of extract content , ethanol- total acid content, and insoluble acid content

such as tannins saponins , steroids /triterpenoids to have anti-inflammatory activity.

Preparation Of Test Preparations and Reagent Solutions

The process of making preparation began the extraction of the simplicia of flower maceration method with 96% ethanol solvent, producing a thick extract. This extract was with n-hexane using funnel to the non-polar components, and the n-hexane fraction obtained was until thick. A 0.5% Na-CMC colloidal solution prepared as a carrier for the test preparation The n-hexane fraction extract suspension of flower was concentrations of 20%, 40%, and 60%. In addition a 1% carrageenan was prepared as an indicator edema, and a Na-Diclofenac was prepared as a positive control. Various solutions reagents such as Mayer, Dragendorf, Bo uchardat, Molish, FeCl3 1%, Lead (II) Acetate, NaOH 2N, HCl 2N, and Al um inium (III) Chloride 5% were also prepared for phytochemical screening (Fauzi et al. 2024) .

Anti-inflammatory Effectiveness Testing Procedure

A total of 25 male white ticks 2-3 with weight of 150-300 grams were test animals The ticks acclimatized for a week acclimatized hours before . They were divided into 5 treatment groups), positive control (2.25 mg/kg BW of NaDiclofenac), and three test groups n-hexane fraction of Ashoka at concentrations of 20%, 40%, and 60%. Edema was by intraplantar injection of 1% carrageenan. The foot volume the ticks measured a before treatment) and every 1 hour until 5 treatment . The percentage of inflammation and the percentage of were the values .

3. RESULTS

The results of the extraction of B Asoka flowers 500 mg were obtained yield as much as 57% (Table 1) and the results inspection Macroscopic and Microscopic can seen in tables 2 and 3.

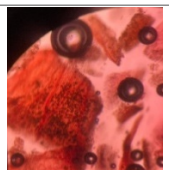
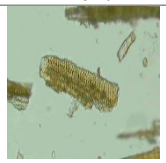
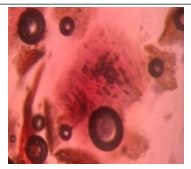
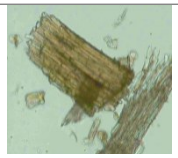
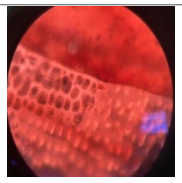
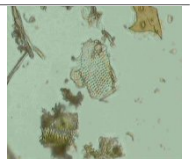
Table 1. Yield Results Asoka Flower simple

Heavy Simple ingredients	Heavy Extract	Yield (%)	FHI 2017
500 gr	285 gr	57%	>10%

Table 2. Examination Macroscopic N-hexane fraction of Asoka Flower

Components checked	Fresh herbs	Simple ingredients
Bent uk	Compound flowers, bisexual, funnel-shaped petals, four stamens, 0.5 cm long, anthers attached to the crown.	Powder
Color	Red	Red
Ba u	Typical	Typical
Flavor	Sweet	Bitter

Table 3. Examination Microscopic N- hexane fraction of Ashoka Flower

No.	Sample	Fragment Name	Pustaka Pharmacopoeia 2017
1.		Xylem	
2.		Scleren chyma	
3.		carrier file	

The results of the characteristics of Asoka flowers can seen in table 4.

Table 4. Characteristics Simplisia B Asoka flower

No.	Description	Results	Condition (FHI, 2010)
1.	Water content	5.79%	<10%
2.	Water soluble content	15.02%	>12.3%
3.	Ethanol soluble content	20.71%	>5.4%
4.	Total ash content	8.23%	<13.2%
5.	The ash content is in soluble in acid	1.77%	<2.7%

Screening Results Phytochemicals of B Asoka flowers can seen in table 5.

Table 5. Screening Phytochemicals of B Asoka flowers

No.	Metabolites Sek under	Reagent	Results
1.	Alkaloid	Mayer Bo uchart Dragendorff	(+) (+) (+)
2.	Flavonoid	Powder Mg + Amyl Alcohol + HCL	(+)
3.	Saponin	Hot water , shaken + 2N HCL	(+)
4.	Tannin	FeCL3	(+)
5.	Steroid	Lieberman-Bouchart	(+)

The results of the Asoka Flower fraction can seen in table 6.

Table 6. Results of N- hexane Fraction Asoka Flower

Heavy Simple ingredients	Heavy Extract	Heavy Faction	Fraction Results Thick
500 gr	285 gr	100 gr	10

grams

Anti- inflammatory test results extract ethanol flower Ashoka can seen in table 7.

Table 7. Anti- inflammatory Test Results Extract Ashoka Flower Ethanol

Group Test	Tick us	Inflammation Before Ind uksi	Inflammation After Ind uksi	Observation Period After Inspection					
				1	2	3	4	5	6
CMC Na 0.5%	1.	0.56	0.75	0.71	0.69	0.67	0.65	0.63	0.61
	2.	0.52	0.87	0.68	0.65	0.63	0.61	0.59	0.57
	3.	0.64	0.67	1.03	0.99	0.95	0.92	0.90	0.89
	4.	0.50	0.92	0.89	0.87	0.85	0.82	0.70	0.69
	5.	0.34	0.59	0.56	0.54	0.51	0.49	0.47	0.45
Na diclofenac 2.25 mg/kg BW	1.	0.41	0.80	0.61	0.71	0.62	0.54	0.43	0.39
	2.	0.50	1.02	0.98	0.81	0.72	0.64	0.58	0.49
	3.	0.50	0.89	0.87	0.81	0.73	0.65	0.54	0.47
	4.	0.50	0.98	0.95	0.83	0.74	0.67	0.53	0.47
	5.	0.64	1.16	1.14	0.96	0.88	0.74	0.65	0.58
FNBA 20%	1.	0.50	0.87	0.82	0.78	0.74	0.70	0.68	0.63
	2.	0.56	0.98	0.74	0.70	0.68	0.65	0.61	0.59
	3.	0.62	1.29	0.89	0.85	0.81	0.78	0.74	0.70
	4.	0.43	0.85	0.67	0.63	0.59	0.57	0.54	0.51
	5	0.66	0.89	0.87	0.84	0.81	0.77	0.72	0.69
FNBA 40%	1.	0.50	0.89	0.75	0.67	0.61	0.57	0.55	0.52
	2.	0.50	1.03	0.72	0.68	0.63	0.60	0.58	0.53
	3.	0.56	0.87	0.85	0.78	0.72	0.67	0.61	0.53
	4.	0.62	1.30	1.29	1.00	0.96	0.87	0.83	0.77
	5.	0.34	0.55	0.50	0.48	0.45	0.40	0.38	0.36
FNBA 60%	1.	0.52	0.70	0.68	0.62	0.61	0.58	0.55	0.50
	2.	0.34	0.72	0.56	0.51	0.47	0.40	0.34	0.30
	3.	0.50	0.93	0.87	0.79	0.72	0.65	0.58	0.52
	4.	0.43	0.70	0.67	0.62	0.57	0.52	0.48	0.42
	5.	0.56	0.89	0.85	0.79	0.73	0.66	0.59	0.55

Table 8. Percentage results average inflammation

Time u (Hour)	Percent average inflammation				
	CMC Na 0.5% mg/kg BW	Na Diclofenac 2.25 mg	FNBA 20% mg/kg BW	FNBA 40% mg/kg BW	FNBA 60% mg/kg BW
0	52.34%	77.38%	45.46%	60.17%	55.41%
1	47.14%	62.63%	38.37%	42.34%	42.49%
2	41.84%	45.34%	31.99%	26.23%	30.48%
3	37.04%	27.86%	26.21%	22.32%	19.59%
4	28.96%	7.28%	19.78%	16.11%	17.46%
5	25.58%	6.24%	13.47%	9.08%	8.85%
Average	38.82%	37.79%	29.21%	29.38%	29.05%

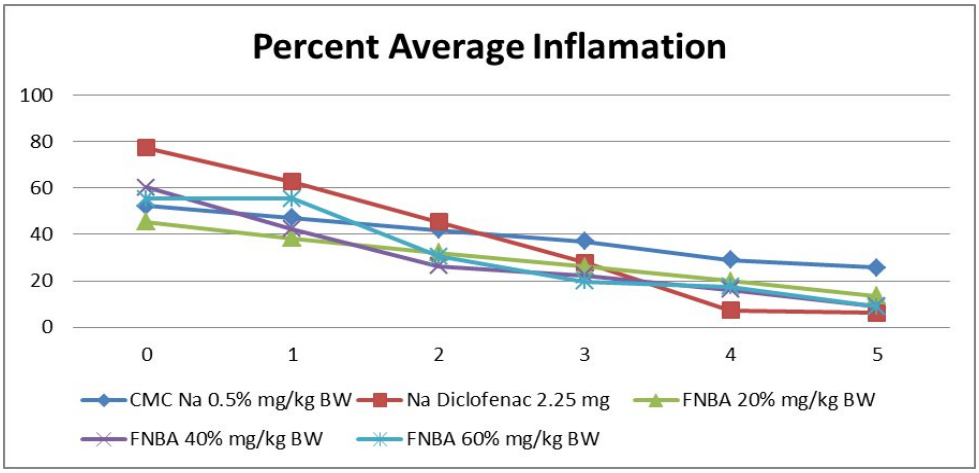


Figure 1. Average Inflammation Percentage Graph

4. DISCUSSION

Identification and Characterization of Simplicia Asoka Flower

This study b asoka flower (Saraca asoca) extraction of active compounds with maceration method using 96% ethanol resulting in a thick blackish red extract with a yield of 57%, meeting standards of the Indonesian Herbal Pharmacopoeia. Macroscopic examination showed simplicia was red, had a distinctive smell and tasted sweet, according the characteristics of asoka flower. Microscopically, xylem fragments, sclerenchyma, and vascular bundles were found which the the sample and function in reducing loss (Findriyani et al. 2022) .

Quality of Simple Drugs and Phytochemical Content

Characterization of Ashoka flower simplicia shows good quality according to standards.The water content of 5.79% is below the maximum limit of %, men unj yukkan k x good shelf life. The content of soluble extract out of water (15.02%) and the content of soluble extract out of ethanol (20.71%) both meet pharmacopoeial requirements, indicating that the chemical compounds are more readily soluble in ethanol. The total ash content (8.23%) and acid-insoluble ash content (1.77%) also met standards, indicating a reasonable mineral content and minimal soil impurities. Phytochemical screening confirmed the presence of alkaloids, flavonoids, saponins, tannins, and steroids in ashoka flowers (Kavitha 2021). The presence of flavonoids, indicated by a red-orange color change, is particularly relevant as these compounds are known to have anti-inflammatory activity (Indriaty et al. 2023).

n-Hexane Fractionation and Anti-Inflammatory Mechanism

Fractionation of the ethanol extract with n-hexane produced a thick, reddish-black fraction weighing 10 grams. The non-polar n-hexane solvent is effective in attracting lipid and oil contents

metabolite compounds such as flavonoids, tannins, and terpenoids. Flavonoids act as anti-inflammatories by inhibiting the enzymes cyclooxygenase and lipoxygenase, which play a role in the production of

genes have antioxidant properties and inhibit pro-inflammatory . The presence of these compounds in the n-hexane fraction of asoka flower anti -inflammatory potential (Hannoodee and Nasuruddin 2020) .

Anti-inflammatory Effect

Anti-inflammatory effect testing in carrageenan-induced rats showed that the n-hexane fraction of Ashoka flower (FNBA) had a positive effect in reducing edema volume. The negative control group (CMC Na 0.5%) showed the highest percentage of inflammation, while the positive control (Na Diclofenac 2.25 mg/kg BW) showed a significant decrease in inflammation. FNBA at concentrations of 20%, 40%, and 60% gradually showed better edema reduction as the concentration increased. FNBA 60% showed almost equivalent effectiveness to Na Diclofenac, indicating that high doses of this fraction have strong anti-inflammatory abilities.

5. CONCLUSION AND SUGGESTION

Conclusion

The n-hexane fraction of asoka flowers (Saraca asoca) can reduce carrageenan-induced inflammation of rat paws with an effective concentration of 60% which is almost equivalent to diclofenac sodium as a comparative control.

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