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Acute Antinflammatory Effect of *Marsdenia tinctoria* Leaf Water Extract in Carragenan-induced Rats

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ABSTRACT

Introduction*: Tarum areuy (Marsdenia tinctoria) is a plant whose efficacy has not been widely explored. Traditionally used for infections and inflammation related disease..* ***Aims****: To determine the anti-inflammatory effect of M. tinctoria leaf water extract on animals induced by carrageenan.****Methods****: Extract preparation by boiling in water. Animals were divided into groups, namely control, comparison (diclofenac sodium 4.5 mg/kgBW) and M. tinctoria leaf water extract (20, 40, 80 mg/kgBW). Induction of 0.1 mL of 1% carrageenan was given as much as intraplantar and the test preparation was given orally. Inflammatory and inflammatory inhibition percentages were measured every 30 minutes for 240 minutes. The t-test was utilized in data analysis.* ***Result****: All test doses of M. tinctoria leaf water extract was effective to reduce inflammation in comparison to the control ($p<0.05$). A dose of 20 mg/kgBW was the optimal anti-inflammatory..* ***Conclusion****: M. tinctoria leaf water extract has the potential as an anti-inflammatory.*

KEYWORDS*:* Tarum Areuy, *Marsdenia tinctoria,* Water extract, Anti-inflammatory, Carrageenan

INTRODUCTION

Inflammation is one of the body's homeostatic responses. Inflammation occurs as a response of living tissue to various stimuli from outside or inside the body. The following are symptoms of inflammation: redness (rubor), heat (kalor), enlargement (tumor), pain (dolor), and loss of function (function laesa) (Calhelha et al., 2023).

Inflammation often accompanies several other diseases, such as wounds, burns, pneumonia, asthma, gout, arthritis or other diseases. In Indonesia, the incidence of diseases related to the inflammatory process in the body is quite high. Some of the prevalence of diseases related to inflammation in Indonesia, including asthma 1.6%, upper respiratory tract infections 25.7%, pneumonia 11.28%, and

hepatitis 0.2% (Badan Kebijakan Pembangunan Kesehatan, 2023).

Tarum areuy (*Marsdenia tinctoria*, Asclepiadaceae) is a shrub or small tree with upright branches. The use of M. tinctoria leaf decoction that is often used is as an indigo dye (Mauliza & Putri, 2019). Several *Mardenia* species that have been studied for their anti-inflammatory effects and/or diseases related to inflammation include *M. tencissima, M. brunoniana, M. thyrsiflora*, and *M. latifolia*. To date, there is little scientific information on the use of *M. tinctoria* to treat inflammation. Traditionally *M. tinctoria* is used for diseases related to inflammation. In China, the stem is traditionally used to treat rheumatoid arthritis and hepatomegaly (Gao et al., 2009), where rheumatoid arthritis is characterized by inflammation of the joints (Poznyak et al., 2024) and hepatomegaly can be induced by inflammation of the liver (Wolf & Lavine, 2000). Tribal of Assam in India use a paste of *M. tinctoria* leaves twice a day for 3 days to treat dog bite wounds (Yadav et al., 2022), where the phases of open wound healing are inflammatory, proliferative and remodeling (Gushiken et al., 2021). Another study that has been conducted on *M. tinctoria* leaves is the antifertility effect (Chowdhury et al., 1994), while scientific studies of the potential for other diseases have not been widely conducted. Therefore, the objective of this study is to evaluate the anti-inflammatory effects of *M. tinctoria* leaf water extract in a carrageenan-induced animal model.

MATERIAL AND METHODS

Material and Chemicals

The materials and chemicals used are λ carrageenan (Sigma®), diclofenac sodium (PT Kimia Farma, Bandung, Indonesia), sodium carboxy methyl cellulose, aquadest (Amidis®), toluene (Merck®), amyl alcohol (Merck®), HCl (Merck®), NH4OH (Merck®), FeCl3 (Merck®), ethanol (Merck®), eter (Merck®), KOH (Merck®), CHCl3 (Merck®), gelatine (Merck®), magnesium (Merck®), bismuth subnitrate (Merck®), kalium iodide (Merck®), anhidrida asetat (Merck®), H2SO4 (Merck®), HgCl2 (Merck®)), and formaldehide (Merck®).

Material Collection and Identification

M. tinctoria leaves were obtained from Pataruman-Indigo Experimental Station, South Nagrak Village, Sukabumi, West Java, Indonesia. The Herbarium Laboratory of the School of Life Sciences and Technology, Bandung Institute of Technology, identified the leaves of *M. tinctoria.* The leaves that have been collected are then dried.

Quality Control of Plant Material

Quality control examination of *M. tinctoria* leaves includes macroscopic exa-

mination, determination of water content, determination of ash (total, water soluble, acid insoluble), and determination of extractable matter (in water and ethanol) (Kementerian Kesehatan RI, 2022).

Determination of water content

Determination of water content was done using distillation. Five grams of dry leaves in all were weighed and put into a distillation flask. After adding 200 mL of water-saturated toluene, distillation was continued until a consistent volume of water was achieved. The dried leaf weight was used to calculate the water content (Kementerian Kesehatan RI, 2022). A duplicate test was conducted.

Determination of total ash

As much as 2 g of dry leaves were weighed and put into a silicate crucible that had been incandescent and tared. Then the crucible was incandescent again until the charcoal was charred and incandescent in a furnace until the charcoal was gone. The crucible was then cooled and weighed until the weight was constant. The total ash content was calculated against the weight of the dry leaves (Kementerian Kesehatan RI, 2022). A duplicate test was conducted.

Determination of water soluble ash

The ash obtained in the determination of total ash was boiled for 5 minutes in 25 mL of water. Then it was filtered using ash-free filter paper, washed with hot water and the residue was ignited at a temperature of about 450°C until the weight remained constant. The water-soluble ash content was recorded and calculated against the dry leaf weight when determining the total ash content (Kementerian Kesehatan RI, 2022). A duplicate test was conducted.

Determination of acid insoluble ash

The ash that was obtained during the total ash determination was heated in 25 mL of dilute HCl LP for 5 minutes. After gathering and filtering the acid-insoluble portion using ash-free filter paper, it was cleaned with hot water and heated to 800±25oC in a crucible until its weight stayed constant. The acid-insoluble ash content has been measured and calculated against the dry leaf weight when determining the total ash content (Kementerian Kesehatan RI, 2022). A duplicate test was conducted.

Determination of extractable matter in water

A total of 5 g of dried leaves were weighed and put into a flask. Then 100 mL of chloroform-saturated water was added. After that, it was shaken for the first 6 hours and then left for 18 hours. After filtration, 20.0 mL of the filtrate was heated in an oven at 105oC and evaporated to dryness. Furthermore, the remaining residue was heated at 105oC until the weight remained constant. The calculation of the extractable matter in water was carried out on the weight of the dried leaves used. (Kemente-

ian Kesehatan RI, 2022). A duplicate test was conducted.

Determination of extractable matter in ethanol

A total of 5 g of dried leaves were weighed and put into a flask. Then 100 mL of ethanol P was added. After that, it was shaken for the first 6 hours and then left for 18 hours. After filtration, 20.0 mL of the filtrate was heated in an oven at 105oC and evaporated to dryness. Furthermore, the remaining residue was heated at 105oC until the weight remained constant. The calculation of the ethanol soluble extract content was carried out on the weight of the dried leaves used (Kementerian Kesehatan RI, 2022). A duplicate test was conducted.

Extraction Process

Extraction was done using water as a solvent. A total of 100 g of leaf powder was put into an infusion pan containing 1 L of distilled water. Then it was heated at 95° C for 15 minutes. Then filtering was carried out and the filtrate was dried using freeze drying (Bitwell et al., 2023; Verep et al., 2023).

Phytochemical Screening

Phytochemical screening of the extracts is carried out for the presence or absence of secondary metabolite compounds such as alkaloids, quinones, flavonoids, polyphenols, tannins, saponins, steroids and terpenoids (Rajkumar et al., 2022).

Anti-inflammatory Assay Using Carrageenan-Induced Edema Model

The study was conducted in accordance with the approval of the institutional ethics committee of Universitas Jenderal Achmad Yani with certificate number 10013/KEP-UNJANI/III/2024. The male Wistar rats used were obtained from the Bandung Institute of Technology. The rats were acclimatized for one week before the experiment. A total of 25 animals were divided into five groups (five per group), namely: negative control, positive control (diclofenac sodium 4.5 mg/kgBW), *M. tinctoria* extract (20, 40, 80 mg/kgBW). At the beginning of the test, marking was carried out on the left foot and measuring the volume of the foot to the hand using a manual mercury plethysmometer. After measuring the initial foot volume, each animal was given the preparation according to the group orally. The test preparation was given as much as 1 ml/100 gBW. Thirty minutes later, each test animal on the foot was induced with 0.1 mL of 1% carrageenan intraplantar. After induction, repeated measurements of foot volume were carried out every 30 minutes for 240 minutes. The percentages of inflammation and inhibition of inflammation were the parameters that were determined. Through a comparison of the initial volume of the foot with the volume at a specific time, the percentage of inflammation was ascertained. In the

Table 1. Results of the examination of the characteristics of *M. tinctoria*

Parameter	Result
Organoleptic	 The leaves are oval, light green in color, have a distinctive smell and taste bitter
Water content (%v/w)	4.89
Total ash (%w/w)	12.80
Water soluble ash (%w/w)	5.62
Acid insoluble ash (%w/w)	1.98
Water soluble matter (%w/w)	32.40
Ethanol soluble matter (%w/w)	15.82

meantime, the percentage of inflammation inhibition was computed by contrasting the test group's and the control group's percentages of inflammation (Vikasari et al., 2024).

Data Analysis

Inflammation percentage data were expressed as mean±standard deviation. ANOVA followed by Posthoc was used to compare inflammation percentage in test and control groups, using GraphPad Prism 9 software. A p value <0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Before collecting many samples, determination and identification were carried out to determine the truth of the type and species. The results of identification and determination stated that the sample was indeed a *Marsdenia tinctoria* from the Apocynaceae family (Figure 1).

Quality standards are carried out to support the safe and efficacious medicinal use of herbal medicinal products (Länger et al., 2018). Quality control of medicinal plants in Indonesia is usually written in the form of monographs in the Herbal Pharmacopoeia, but the *M. tinctoria* monograph has not been written in the Indonesian Herbal Pharmacopoeia, so the results of this study are expected to be used as a guide for its quality parameters. The results of the examination of the characteristics of *M. tinctoria* are shown in Table 1.

The water content of *M. tinctoria* leaves meets the requirements of no more than 10% because the presence of water in the leaves can cause the growth of microorganisms such as bacteria and fungi (Pujaningsih et al., 2021). Total ash quality parameters are measured to determine the physiological ash content (derived from the plant itself) and non-physiological ash (derived from air, soil or water pollution). The examination of water-soluble ash content aims to determine the ash content derived from water-soluble salts (sodium and magnesium). The examination of acid-insoluble ash content is carried out to determine the presece of absence of silica (Rao & Xiang, 2009)

Figure 1. *Marsdenia tinctoria* (personal documentation)

Extraction is one of the steps to separate metabolite compounds that are suspected to be potentially or pharmacologically active (Patel et al., 2019). The extraction method chosen in this study is simple and conventional, namely by heating in a water solvent (infusion). The dry extract of *M. tinctoria* leaves was obtained as much as 19.2 g with an extract yield of 2.953% w/w.

The purpose of phytochemical screening is to find secondary metabolites in *M. tinctoria* extract. The results of phytochemical screening can be seen in the Table 2.

In the study, the parameters observed were inflammation of the feet. Tissue inflammation is caused by carrageenan induction. Carrageenan induces inflammation through proteomic and genomic pathways. Carrageenan can induce the formation of the cytokine IL-8, but does not induce TNF-alpha and IL-16 levels (Myers et al., 2019). Swelling caused by carrageenan occurs in three phases, namely the release of inflammatory mediators histamine and serotonin (up to the 90th minute), the release of bradykinin (90-150 minutes), and the release of prostaglandins (180-300 minutes) (Singh et al., 2014). The results of the observation of the anti-inflammatory test of *M. tinctoria* extract can be seen in Figure 2-3.

The test results (Figure 2) showed that the percentage of inflammation of the extract groups with doses of 20, 40 and 80 mg/kg bw were lower compared to the control group ($p<0.05$). The onset of the decrease in the percentage of inflammation in the diclofenac sodium group was at 150 minutes, while the onset of the M. tinctoria extract was at 120 minutes. The results of the total inflammation calculation (Figure 3) showed that all extracts gave lower values compared to the control and when compared to the diclofenac sodium.

Table 2. Results of phytochemical screening of *M. tinctoria* extract

Secondary Metabolites	Results
Alkaloids	+
Quinones	+
Flavonoids	+
Polyphenols	+
Tannins	-
Saponins	+
Steroid-Terpenoid	+

+ = presence, - = absence

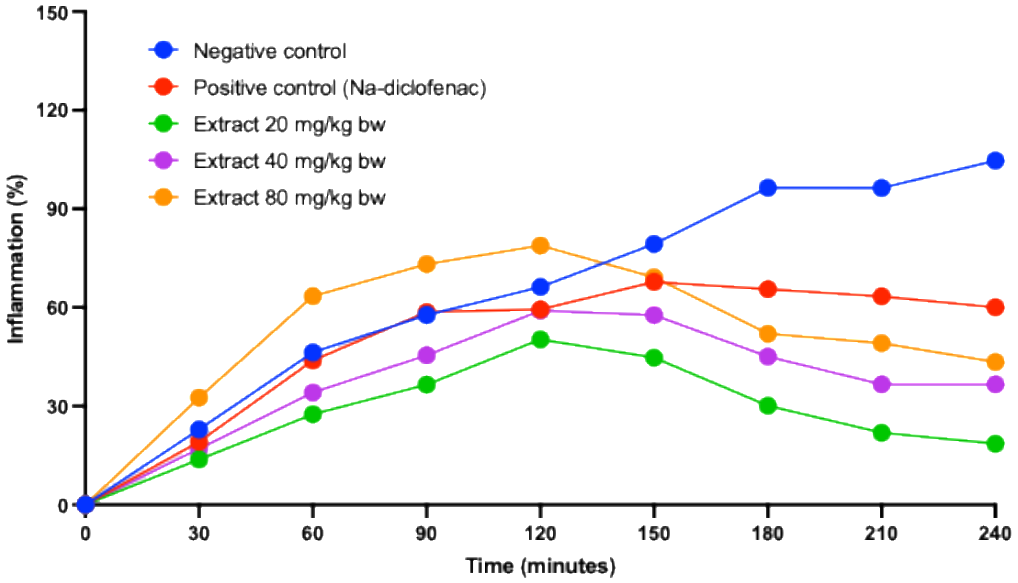

Figure 2. Percentage of inflammation during 240 minutes of observation.

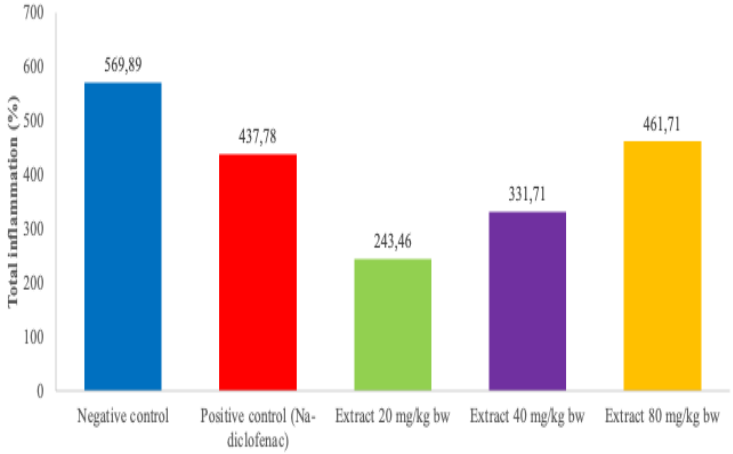

Figure 3. Total percentage of inflammation

The percentage of inflammation inhibition is the ability to inhibit inflammation compared to the control group (Table 3). The greater the inflammation inhibition value, the better the anti-inflammatory effect. Based on the inflammation inhibition data, it shows that *M. tinctoria* extract at all doses can inhibit inflammation (has an anti-inflammatory effect), with the optimal dose being 20 mg/kg bw.

Several secondary metabolite compounds found in *M. tinctoria* that are suspected to have anti-inflammatory effects include phenolic and alkaloid compounds. M. brunoniana is a plant with the same genus and has immunomodulatory effects.

The plant contains compounds phenolic (1,4-benzenediol-(CAS)-hydroquinone, 1,2,3-propanetriol-(CAS)-glycerol), and alkaloid (piperidine, 1-methyl-(CAS)-N-methylpiperidine), and are thought to contribute to influencing the immune system (Savira et al., 2024). The results of qualitative phytochemical screening showed the presence of steroids. One type of steroid that is common in Apocynaceae is

Table 3. Inhibition of inflammation of the anti-inflammatory of *M. tinctoria* extract

Group	Inflammation inhibition (%) at minutes-								**Inflammatory inhibition (%)**
	30	60	90	120	150	180	210	240	
Na-diclofenac	16.47	5.34	-1.56	10.26	14.54	31.99	34.23	42.66	19.24
Extract 20 mg/kg bw	40.10	40.70	36.68	24.09	43.60	68.74	77.22	82.22	51.67
Extract 40 mg/kg bw	25.57	26.32	21.23	10.86	27.23	53.26	61.99	65.02	36.43
Extract 80 mg/kg bw	-41.94	-36.81	-26.75	-19.04	12.82	46.05	48.97	58.50	5.22

pregnane glycosides (Si et al., 2022). Polyoxypregnane glycosides compounds isolated from the root of M. tenacissima have anti-inflammatory effects, as demonstrated by their ability to inhibit nitric oxide formation in lipopolysaccharide-induced RAW 264.7 cells (Na et al., 2023).

M. tinctoria has several flavonoid compounds, including 3,2'-dihydroxyflavone, 1-methylcyclobutene and dimethyl isatoate (Mohd Nasuha & Choo, 2016). Flavonoid compounds contained in *M. tinctoria* extract are thought to contribute to its anti-inflammatory effects. Flavonoids are known to inhibit inflammation in the arachidonic acid pathway and reduce the production of proinflammatory cytokines. (Al-Khayri et al., 2022).

In this study, the anti-inflammatory effect of M. tinctoria extract was better than diclofenac sodium. The anti-inflammatory effect of sodium diclofenac occurs by inhibiting the cyclooxygenase enzyme so that prostaglandin secretion is also inhibited (Al-Otaibi et al., 2023) (the third phase of inflammation due to carrageenan induction), and in this study the onset of the extract was faster than diclofenac sodium, presumably because it can inhibit the early phase of inflammation related to the mediators histamine, serotonin and bradykinin. This assumption needs to be further analyzed with other tests using different inductors. In addition, this study did not determine the levels of secondary metabolite compounds quantitatively, so that the correlation of secondary metabolite content with anti-inflammatory effects could not be carried out.

CONCLUSION

Water extract of *M. tinctoria* leaves can prevent inflammation. The optimal dose of water extract of *M. tinctoria* leaves that has the potential as an anti-inflammatory agent is 20 and 40 mg/kg bw. Several compounds contained in *M. tinctoria* extract are thought to contribute to its anti-inflammatory effects, but further research is needed into their mechanisms of action.

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