

PHYTOCHEMICAL SCREENING AND ANTI-INFLAMMATORY ACTIVITY OF FRACTIONS FROM SAMBUNG RAMBAT (*MICANIA CORDATA*) LEAF

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Received: 20 March 2018, Revised and Accepted: 25 April 2018

ABSTRACT

Objective: The present study is to evaluate the anti-inflammatory activity of various fractions from Sambung Rambat leaf (*Micania cordata*) using ethyl acetate, hexane, and water solvent as the solvent.

Methods: Investigation of phytochemical compound was using standard phytochemical screening method, while the anti-inflammatory was using the carrageenan-induced paw edema method in animal (rats) model.

Results: The phytochemical screening showed that the fractions of *M. cordata* had a lot of phytochemical compounds such as flavonoids, glycosides, steroids, triterpenoids, tannins, and saponins. Anti-inflammatory assessment showed that the strongest activity of anti-inflammatory produced by ethyl acetate fraction with 200 mg/kg BW dose.

Conclusion: This investigation could be concluded that ethyl acetate fraction of *M. cordata* might be a potential for the treatment of inflammatory.

Keywords: Anti-inflammatory, Carrageenan, *Micania cordata*, Phytochemical, Screening.

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INTRODUCTION

Inflammation is defined as the local response of living mammalian tissues to injury due to any agent. It is a body defense reaction to eliminate or limit the spread of injurious agent, followed by removal of the necroses cells and tissues [1]. Inflammation is characterized in acute phase by increased blood flow and vascular permeability along with the accumulation of fluid, leukocytes, and inflammatory mediators such as cytokines [2].

Micania cordata (Compositae) also known as "sambung rambat or heartleaf hempvine" is a plant that grows widely in tropical area, such as Asian, Africa, and South America [3]. In traditional medicine, this plant has been used occasionally for the treatment of ulcers, analgesic, inflammatory, cancer, stressness [4-6] dysentery, itching, cough, headache [7,8], and wounds [9]. In this regard, it makes great sense to evaluate the anti-inflammatory activity of various *M. cordata* leaf fractions in a carrageenan-induced inflammatory using animal (rats) model.

METHODS

Collection and authentication of plant materials

Fresh sambung rambat (*M. cordata*) leaf was collected in March 2017 from local area of Punden Rejo, Deli Serdang (North Sumatera, Indonesia) and authenticated by Indonesian Institute of Sciences: Research Center for Biology.

Preparation of extract and fractions

Extraction was done by a stratified maceration method using ethanolic solvent. 1 kg of powdered *M. cordata* leaf was macerated using 7.5 L ethanol solvent for 5 days, then filtered, do it continuously until the filtrate obtained is clear and colorless. 20 g concentrated ethanolic extract, then fractioned with ethyl acetate, hexane, and water solvent to get the fractions [10,11].

Preliminary phytochemical screening of extract and fractions

Phytochemical screening carried out on *M. cordata* leaf fractions which are ethyl acetate, hexane, and water solvent includes examining the chemical secondary metabolites of alkaloids, flavonoids, glycosides, tannins, triterpenoids, and steroids [12-15].

Preparation of animals

Healthy adult male Wistar rats (175–200 g body weight) from animal house of Faculty of Pharmacy, University of Sumatera Utara, were used for the study. Mice were housed in a polycarbonate cage in a room with 12 hrs day-night circle. They were fed on a standard pellet diet and water *ad libitum* [16]. The study was approved by Animal Research Ethics Committees (AREC) of University of Sumatera Utara (AREC Registration Number: No. 130/KEPH-FMIPA/2017), and the experiments were conducted according to the ethical norms and AREC guidelines.

Inflammation method design

Carrageenan was prepared as a 1% w/v solution in 0.9% saline. Healthy adult male Wistar rats were divided into five groups of six rats each. Edema was induced by injecting 0.05 mL of 1% carrageenan suspension into the subplantar region of the right hind paw of the rats. Control group rats received 0.5% (w/v) Na CMC, and the reference group of rats received 2.25 mg/kg BW sodium diclofenac, orally. The test groups of rats were treated orally with 200 mg/kg BW ethanolic extract, ethyl acetate fraction, and hexane fraction consecutively. The paw volume was measured by plethysmometer before carrageenan injection (V_0) and 30, 60, 90, 120, 150, and 180 min after (V_t). The inflammation was calculated as the increase in volume (mL) of the paw after treatment subtracted of basal volume. Results were expressed as percentage of inhibition of edema, calculated according to the formula [17,18]:

$$\text{Percentage inhibition (\%)} = \frac{\text{Mean paw inflammation of control} - \text{Mean paw inflammation of test}}{\text{Mean paw inflammation of control}} \times 100$$

Table 1: Phytochemical screening result of various *Micania cordata* leaf

No	Screening	Ethyl acetate fraction	Hexane fraction	Water fraction
1	Alkaloids	Negative	Negative	Negative
2	Flavonoids	Positive	Negative	Positive
3	Glycosides	Positive	Negative	Positive
4	Saponins	Positive	Negative	Positive
5	Tannins	Positive	Negative	Positive
6	Triterpenoid/Steroids	Negative	Positive	Positive

Table 2: Effect of various fractions of *Micania cordata* leaf on the paw edema volume

Group	Paw edema volume					
	30'	60'	90'	120'	150'	180'
Group I (control negative)	4.09±0.29 [#]	4.55±0.49 [#]	4.73±0.59 [#]	4.72±0.52 [#]	4.88±0.34 [#]	4.98±0.43 [#]
Group II (control positive)	3.38±0.23 [*]	3.69±0.23 [*]	3.66±0.24 [*]	3.49±0.20 [*]	3.44±0.29 [*]	3.35±0.29 [*]
Group III (ethyl acetate 200 mg/kg)	3.56±0.11 ^{**}	3.86±0.10 ^{**}	3.86±0.13 ^{**}	3.72±0.24 ^{**}	3.64±0.19 ^{**}	3.53±0.22 ^{**}
Group IV (hexane fraction 200 mg/kg)	3.84±0.39 ^{**}	4.35±0.42 ^{**}	4.45±0.40 ^{**}	4.56±0.42 ^{**}	4.88±0.34 ^{**}	4.97±0.36 ^{**}
Group V (water fraction 200 mg/kg)	3.96±0.15 ^{**}	4.38±0.67 ^{**}	4.53±0.24 ^{**}	4.21±0.14 ^{**}	4.33±0.34 ^{**}	4.37±0.36 ^{**}

Values are given as mean±SEM for five mice in each group. ^{*}Differ significantly to control negative; ^{**}Differ significantly to control positive. SEM: Standard error of the mean

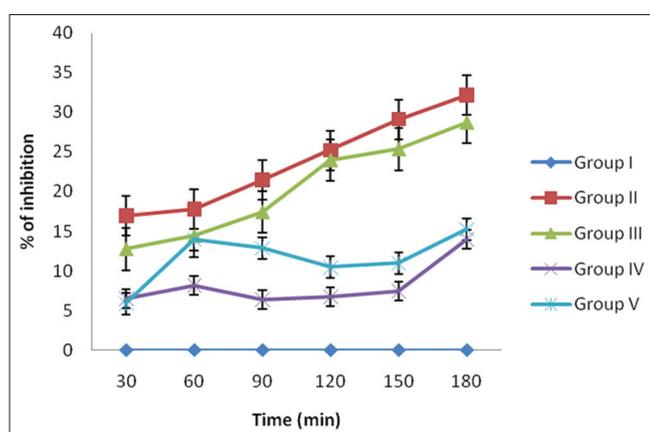


Fig. 1. Effect of various fractions of *Micania cordata* on the percentage of inhibition

Statistical analysis

All the data were expressed as mean ± standard deviation. The significant difference of data between different groups was compared by ANOVA followed by Duncan's test.

RESULTS

Phytochemical screening

Table 1 summarizes the phytochemical screening results of various extract and fractions of *M. cordata* leaf which showed different chemical compound in different extract and fractions.

Effect of *M. cordata* fractions on carrageenan-induced inflammatory

To evaluate the effect of *M. cordata* leaf fractions on the carrageenan induced of inflammatory, the paw volumes and percentage of inhibition of the control, standard, and test groups are shown in Fig. 1 and Table 2. These results showed that the ethyl acetate fraction of *M. cordata* leaf (dose 200 mg/kg BW) has the most potential anti-inflammatory activity.

DISCUSSION

Inflammation is a response triggered when there is a damage on the living tissues. This defense mechanism is a response to protect against infection and injury. The result of this study indicates that the leaf fractions of *M. cordata* process anti-inflammatory activity against carrageenan induction agent. The ethyl acetate fraction showed significant inhibition of edema rather than hexane and water fraction.

This result strongly indicated that the anti-inflammatory activity come from the ethyl acetate fraction with the non steroidal type. The presences of flavonoids in the ethyl acetate fraction may count for its observed pharmacological activities. Many compounds from this class have been found to exhibit anti-inflammatory effects. Previous studies have shown similar relationships between flavonoids and anti-inflammatory effects [19-22]. Therefore, it is possible that the anti-inflammatory action of *M. cordata* may be related to the inhibition of prostaglandin synthesis. The phytochemical profile of *M. cordata* may be explored further to identify the active constituents responsible for its anti-inflammatory activity.

CONCLUSION

It is clearly evident that the ethyl acetate fraction of *M. cordata* leaf possesses significant anti-inflammatory activity in rats. This may be due to the presence of the natural phytochemical constituents. Further studies are needed to determine the exact molecular mechanism involved in the process of anti-inflammatory.

ACKNOWLEDGMENTS

The authors thank Iksen, B.Pharm., M.Sc. of Sekolah Tinggi Ilmu Kesehatan Senior for his support and guidance for this publication.

AUTHOR'S CONTRIBUTIONS

All the authors have contributed equally.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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