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Anti-inflammatory Effect of Ethyl Acetate Fraction Of Ethanol Extract Of *Guazuma ulmifolia* Lamk

Emsutrisna*, Sri Wahyuni**, Tanti Azizah S**, Maryati**

*Medical faculty of UniversitasMuhammadiyah Surakarta
**Pharmacy Faculty of UniversitasMuhammadiyah Surakarta
*Corresponding author : es233@ums.ac.id

Abstract

The aim of this research is to investigate the anti-inflammatory effect of ethyl acetate fraction of ethanol extract of *Guazuma ulmifolia* Lamk. The research used pre and post test with control group design. A number 25 white rats wistar strain were devided into 5 groups. Group 1 was treated by Diclofenac sodium at dose of 50mg/kgbw; group 2 was treated by Aqueous 2 mL/rats; group 3, 4 and 5 were treated by ethyl acetate fraction of ethanol extract of *Guazuma ulmifolia* Lamk. (EAGU) at dosage of 250; 500 and 1000 mg/kgbw respectively. After treated, volume of rats's right paw was measured by plethysmometer for 330minutes. The compound of ethyl acetate fraction of ethanol extract of *Guazuma ulmifolia* Lamk was analyzed by phytochemicals analysis. The EAGU at dosage of 1000 mg/kgbw can reduce edema volume at minutes 60 and minutes 90 significantly (by anova P. <0.05). The phytochemical compounds of EAGU are alkaloid, flavonoid, tannin, saponin and triterpen.

Keywords: anti-inflammatory, *Guazuma ulmifolia* Lamk, pre and post test with control group design

INTRODUCTION

In Indonesia, *Guazuma ulmifolia* Lamk.plant (GUL.) was studied by scientists relating to antioxidants effect. Essential oils from (GUL.) leaves have antioxidant effects with DPPH method with IC_{50} 7.61 ± 0.09 µg / mL (Boligon et al., 2013). Ethanol extracts 95% of (GUL.) leaves at a concentration of 800 ug/mL has an antioxidant effect (Hidayat et al., 2014).

Guazuma ulmifolia Lamk.stem ethyl acetate fraction has antioxidant effect with IC_{50} 8.09 ± 0.14 µg/ mL by DPPH method; while butanol fraction, showed IC_{50} 12, 61 ± 0.25 µg/ mL (Feltrin et al., 2012).

Excessive production of ROS will attack tissues and then trigger inflammation through the production of pro-inflammatory mediators and chemotactic factors. This factors can cause chronic inflammation (Mittal et al.,2014). The inhibition of the formation of ROS or enzymes involved in the formation of free radicals can reduce inflammation. Thus plants that have anti-oxidant effects are also thought to have anti-inflammatory effects.

The aim of this study is to investigate anti-inflammatory effect of ethyl acetate fraction of *Guazuma ulmifolia* Lamk. (EAGU) and its phytochemical contains.

METHOD

This research has been approved by Health Research Ethics Committee of Medical Faculty of Universitas Muhammadiyah Surakarta, (2064/A.2/ KEPK-FKUMS/III/2019).

Materials

Dry simplisia was found from Gede market, Surakarta, Central of Java in March 2019. The rats wistar strain that used in this research were found from Pharmacology laboratory of Medical

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Drug and chemical : Ethanol, Ethyl acetate, Diclofenac sodium (Kimia Farma) and Carrageenan (Sigma Aldrich)

Preparation of extracts

A total of 500 g of plant samples were macerated in 8 liters of ethanol 96% for 5 days, and re maceration was carried out in 3 days. The results of maceration were then fractionated with ethyl acetate.

Anti- inflammatory activity

A total 25 rats were divided into 5 groups. Group 1 was treated by diclofenac sodium at dose 50 mg/kgbw, group II was treated by aqueous 2 ml/ rat, group III, IV and V were treated by EAGU at dosage of 250, 500 and 1000 mg/kgbw respectively Thirty minutes later, all rats were injected by carrageenan 0.1 mL 1% w/v sub cutaneous into back rat's left paw. Paw volume were measured on minutes 0: 60; 90; 150; 210; 330 by Plethysmometer. Area under the curve (AUC) were calculated on 0-30; AUC 0-90; AUC 0-150; AUC 0-210 and AUC 0-330 minutes.

RESULT

In this research, carrageenan is used to induce inflammation. Diclofenac sodium (50mg/kg bw per oral) was used as synthetic drug during the activity inflammatory evaluation of EAGU. The result of the volumes of rat paw edema in the five groups are presented in Table 1.

Table 1. The rat's paw edema on minutes 30; 60; 90;150; 210; 330

Groups	Edema volume (ml±SD)					
	Minute 30	Minute 60	Minute 90	Minute 150	Minute 210	Minute 330
Diclofenac sodium at dose of 50mg/kgbw	0.018±0.018	0.044±0.005	0.046±0.015	0.038±0.023	0.026±0.015	0.004±0.005
Aquaous 2mL/ rats	0.020±0.018	0.066±0.011	0.068±0.018	0.060±0.010	0.034±0.025	0.034±0.025
EAGU at dose 250mg/kgbw	0.026±0.016	0.037±0.011	0.034±0.008	0.330±0.019	0.060±0.013	0.060±0.017
EAGU at dose 500mg/kgbw	0.018±0.014	0.054±0.013	0.056±0.015	0.064±0.028	0.042±0.013	0.006±0.013
EAGU at dose 1000mg/kgbw	0.006±0.005	0.046±0.015	0.048±0.017	0.050±0.023	0.018±0.014	0.010±0.014

From the rat's paw edema, the AUC were calculated and describe in Table 2.

Table 2. The AUC of edema volume on minutes 0-60; AUC 0-90; AUC 0-150; AUC 0-210 and AUC 0-330.

		Groups				
		Diclofenac sodium at dose of 50mg/kgbw	Aquaous 2mL/rats	EAGU at dose 250mg/ kgbw	EAGU at dose 500mg/ kgbw	EAGU at dose 1000mg/ kgbw
AUC(ml±SD)	AUC 0-60	0.930±0.32*	1.29±0.43	1.50±0.28	1.08±0.36	0.78±0.16*
	AUC 0-90	3.21± 0.87*	4.59±1.22	5.13±0.66	3.81±1.06	2.97±0.81*
	AUC0-150	4.80±1.49*	7.14±1.51	7.65±1.08	6.33±1.87	5.13±1.83
	AUC0-210	6.72±2.42*	9.96±2.15	9.99±2.02	9.51±2.99	7.17±2.30
	AUC0-330	8.52±3.34*	14.04±4.71	11.43±3.51	12.39±4.26	8.85±2.79

Note: *: Significantly different with negative control on Anova (p.0.05)

From this table, it can be concluded that EAGU at dosage of 1000 mg / kgbw can decrease edema volume on minute 0-90. The compound active of ethyl acetate fraction of ethanol extract of *Guazuma ulmifolia* Lamk were performed by the phytochemicals analysis. The results of phytochemical analysis were expressed in Table 3.

Table 3. The chemical constituent of EAGU by pytochemical analysis

The chemical constituent	**Results**
Alkaloid	+
Flavonoid	+
Tannin	+
Saponin	+
Triterpen	+
Steroid	-

DISCUSSION

In this research , inflammation rat's model was induced by injecting 0.1 ml carrageenan (1% w/v) into the left hind paw. Ethyl acetate fraction of *Guazoma ulmifolia* L. (EAGU) at dose of 1000 mg/ kg bw showed a reduction of edema volume in minutes 0 – 90. The pytochemical analysis show that the fraction contains alkaloid, flavonoid, tannin, saponin and triterpen.

This Phytochemical results inline with previous research by Choi and Wang ,2005. They found that the extract of *Guazoma ulmifolia* leaves contains some phytocemical, among others : tannins and other phenolic compounds (Choi and Hwang, 2005). Another active constituen of the leaves is the alkaloid, Beta-sitosterol and phytosterol (Delporte et al., 2007).The leaf also contain essential oil wich rich in beta-caryophyllene (Arriaga et., al., 1997).

The mechanism of anti-edema effect of this extract is not clear. Alkaloids are one of the largest single classes of plant secondary metabolites. Several alkaloids have antiinflammatory action, among others: Ailanthamide, Sinomenine, Scytonemin, Scholaricine and Riparin II. Ailanthamide is one of alkaloid that inhibit activity on superoxide generation by human neutrophils with $IC_{50} \leq 5.34$ µg/mL and inhibit activity on elastase release by human neutrophils with $IC_{50} \leq 5.53$ µg/mL (Chen

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et al., 2009). Sinomenine (alkaloid class) at dose of 3,036mg/kgbw have antiinflammatory effect in rat's arthritis induced by collagen II (Garcia-Argaez et al., 2000). Scytonemin at dose of 5–100 µg/ear can reduce edema induced by phorbol of the mouse ear (Stevenson et al., 2002). Scholaricine (100uM) can inhibit Cox-1 and COX -2 in mice and reduced ear edema induced by xylene at dose of 5mg/kgbw (Shang et al., 2010). Riparin II (from *Aniba riparia*) at dose of 25mg/kgbw reduced rat's pedal edema induced by carrageenan (Leite et al., 2011).

Research by Tambe et al., 1996, found that sesquiterpene act as anti-inflamatory by inhibition gastric mucosal lesions in rats when administered orally, without affecting gastric acid secretion(Tambe et al., 1996). The ethanolic extract from leaves and flowers of *Guazuma ulmifolia* at doses of 500, 250 and 125mg/kg can protect grastric mucosa rats injuries induced by diclofenac (Bereguer et al., 2007).

CONCLUSION

The ethyl acetate fraction of ethanol extract of *Guazuma ulmifolia* Lamk at dosage of 1000mg/kgbw can decrease edema volume of rat's paw induced by carrageenan.

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