

## RESEARCH ARTICLE

### ED<sub>50</sub> from anti-inflammatory properties of *Vitex trifolia* L. ethanol extract

Resmi Mustarichie<sup>1</sup>, Sri Adi Sumiwi<sup>2</sup>, Annisa Trisfalia Hanifah<sup>1</sup>

<sup>1</sup>Department of Pharmaceutical Analysis and Medicinal Chemistry, Faculty of Pharmacy, Universitas Padjadjaran, Jawa Barat, Indonesia,

<sup>2</sup>Department of Pharmacology and Clinic, Faculty of Pharmacy, Universitas Padjadjaran, Jawa Barat, Indonesia

Correspondence to: Resmi Mustarichie, E-mail: resmi.mustarichie@unpad.ac.id

Received: November 22, 2017; Accepted: January 13, 2018

#### ABSTRACT

**Background:** In Indonesia, *Vitex trifolia* grown as a crop or bush is even used as a guardrail. Conventionally, *V. trifolia* is used as a swelling drug caused by breast cancer by drinking the decoction of its leaves. Our group of researchers in previous articles has been able to prove scientifically that *V. trifolia* has anti-inflammatory activity using the carrageenan-induced method in five groups of rats. Because in the previous study found a large dose of 21.44%, 30.78%, and 41.46% for the dose of extract 500 mg / KgBW, 1000 mg / kg, and 2000 mg / KgBW for efficacious as anti-inflammatory, it is necessary to study the magnitude of the effective dose of the extract. **Objective:** This study aims to determine the median effective dosage of anti-inflammatory of *V. trifolia* L. ethanol extract. **Materials and Methods:** The dried *V. trifolia* leaves were finely chopped and then macerated with 96% ethanol in the macerator for 3 × 24 h, thickened with a rotary evaporator, and then evaporated over the water bath. Phytochemical screening was performed on the Farnsworth method. Against the extract, examination of extract parameters and TLC examination were performed based on Indonesian pharmacopoeia IV. Tests on ED<sub>50</sub> were based on the induction procedure for inflammation of rats using carrageenan. The extract was administered orally to five test groups containing five mice at doses 1000, 1500, 2000, 2500, and 3000 mg/kg BW. The rat foot volume was measured and calculated percent inflammation inhibition after it was made a curve on probit log paper to determine ED<sub>50</sub> extract. **Results:** From the result of substitution on plot equation of probit, log curve obtained effective dose median of Legundi leaf extract of 1428,8 mg/kg. Dose 1428.8 mg/kg BW was then defined as the ED<sub>50</sub> anti-inflammatory activity of Legundi leaves extract. Through the calculation of one-way ANOVA and least significance different advanced test ( $P < 0.05$ ), it could be proved that all the doses of the extract gave a significant difference to the negative control. The anti-inflammatory activity was shown by the dose of extract 2000 m/kg BW, 2500 mg/kg BW, and 3000 mg/kg BW statistically did not differ significantly with positive control of 10 mg/kg BW. **Conclusion:** The effective dose of median (ED<sub>50</sub>) extract ethanol leaves *V. trifolia* Linn. which was efficacious anti-inflammatory was found 1428.8 mg/kg body weight. So far, no other researcher reported the ED<sub>50</sub> of *V. trifolia*. These results suggest if the leaves legundi will be made anti-inflammatory herbal medicine, the preparation should be a drug to drink not a tablet considering the required dose big enough.

**KEY WORDS:** *Vitex Trifolia* Linn.; Anti-inflammation; ED<sub>50</sub>; Extraction; ANOVA

#### Access this article online

Website: [www.njppp.com](http://www.njppp.com)

Quick Response code

DOI: 10.5455/njppp.2018.8.1145113012018



#### INTRODUCTION

Legundi (Indonesian) or *Vitex trifolia* is an important medicinal plant in many traditional medicine methods in the world, among them Ayurveda (India) and Unani (Greece). [1-4] In Indonesia, legundi grown as a crop or bush is even used as a guardrail.[5] In other words, these plants are easy

National Journal of Physiology, Pharmacy and Pharmacology Online 2018. © 2018 Mustarichie et al. This is an Open Access article distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), allowing third parties to copy and redistribute the material in any medium or format and to remix, transform, and build upon the material for any purpose, even commercially, provided the original work is properly cited and states its license.

to develop and are available in large quantities. Utilization of Legundi has been reported as medicinal plants include leaves, stems, fruit, seeds, and until the roots.<sup>[6]</sup> Over time, the use of medicinal plants *Vitex trifolia* empirically by the community is now tested its activity through scientific research. Conventionally, in Krawang district, West Java, *V. trifolia* is used as a swelling/inflammation drug caused by breast cancer by drinking the decoction of its leaves. Legundi plant extract has been scientifically proven to have many activities, among them as anti-inflammatory and anticancer.<sup>[1,7,8]</sup>

Inflammation is a form of immune response of the body. Inflammation indicates that the body is protecting itself from attacks of foreign substances. This process causes pain, redness of the skin, and even swelling. Inflammation aims to eliminate harmful stimuli such as irritation, cell damage, and pathogens from the body and initiate wound healing.<sup>[9]</sup> There are times when inflammatory pain is so unbearable that it is necessary to take painkillers. The common medicine used to treat pain is nonsteroidal anti-inflammatory drugs (NSAIDs). NSAIDs are proven to be clinically effective but may have side effects such as gastric or intestinal mucosal injury.<sup>[10]</sup> Inflammation and cancer are two inseparable things. Inflammation is caused by cancer. The presence of cancer cells causes inflammation.<sup>[11]</sup> Previous studies have shown that both the ethanol extract and the water extract of *V. trifolia* Linn. leaf produce anti-inflammatory activity against carrageen-induced rat-induced rat-rodents without significant adverse effects.<sup>[8,12]</sup> However, until now, there has been no research that leads to the search for an effective dose of extracts that effectively lead to anti-inflammatory properties (ED<sub>50</sub>). This paper reports the ED<sub>50</sub> extract of *V. trifolia* leading to the developmental steps toward the formulation of its anti-inflammation drugs.

## MATERIALS AND METHODS

### Animal Test

The experimental animals used were female white rats weighing between 180 and 200 g, obtained from the School of Pharmacy, Bandung Institute of Technology. Before the use, mice were acclimatized for 1 week.

### Plant Materials

The leaves of the plant *V. trifolia* Linn. or Legundi obtained from Tanjung Pakis Beach in Karawang, West Java, and had been through the process of determination by the Department of Biology FMIPA, Universitas Padjadjaran.

### Extraction

The extraction method was performed according to the Indonesian Herbal Pharmacopoeia.<sup>[13]</sup> The dried Legundi leaves were finely chopped and then soaked with 96%

ethanol solvent in the macerator for 3 × 24 h. The macerate was thickened with a rotary evaporator and evaporated over a water bath to remove the residual solvent to obtain a thick viscous extract with a constant weight.

### Phytochemical Screening

Phytochemical screening based on the Farnsworth method<sup>[14]</sup> was performed to determine the secondary metabolite content of Legundi plant leaves. These tests included the detection of alkaloids (reagent Dragendorff, Mayer, and Bouchard), flavonoids (Shinoda test), tannins and polyphenols (Harborne method), saponins (foam test), triterpenoids (Lieberman-Bouchard reagents), sesquiterpenoids (vanillin reagents in H<sub>2</sub>SO<sub>4</sub>), and quinone (KOH reagent).

### Extract Parameter Examination

This procedure included the determination of water content, ash content, and the determination of the yield of the extract, based on Indonesian Herbal Pharmacopoeia.<sup>[13]</sup>

### Thin-Layer Chromatography

Examination of thin-layer chromatography on extracts was performed by comparison of 2:1 hexane and ethyl acetate phases and silica gel 254 as the stationary phase.

### ED<sub>50</sub> Anti-inflammatory Extract

The method of determining ED<sub>50</sub> is based on modified Weil method.<sup>[15]</sup> The ethanol extract of Legundi leaves was tested for inflammatory activity using induction method of edema in rat's leg. There were five dose variations tested, i.e., 1000 mg/kg BW, 1500 mg/kg BW, 2000 mg/kg BW, 2500 mg/kg BW, and 3000 mg/kg BW which were administered 1 h after the injection of carrageenan in the subplanar area to the test animals. The amount of edema was measured every 1 h for 5 h with a plethysmometer. The activity of ethanol extract of Legundi fruit was shown by the percent of production given to the formation of edema calculated by the formula:

$$\% \text{ inflammation} = \frac{V_t - V_o \times 100\%}{V_t}$$

where V<sub>t</sub> = Average volume of rat foot after induced carrageenan

V<sub>o</sub> = volume of rat foot before induced carrageenan.

After that calculated the percentage of inflammatory inhibition. This value indicates the anti-inflammatory activity of the test material.

$$\% \text{ inflammatory inhibition} = \frac{\% \text{ inflammatory test} - \% \text{ inflammatory control}}{\% \text{ inflammatory control}} \times 100\%$$

To the animal, the negative control group was given 2% pulvis gummi arabicum (PGA) solution while the positive control was acetosal 10 mg/kg BW. The results of percent inflammation inhibition calculation in each group were then plotted in the probit log chart after which it could be observed a dose that gave effect to 50% of test animals. The ANOVA method followed by the least significant different advanced test (LSD) was then used to verify the data obtained.<sup>[16]</sup>

## RESULTS

### Extraction

Extraction of 250 g of Legundi leaves with ethanol yielded a yield of 12.05 %W/W.

## PHYTOCHEMICAL SCREENING RESULTS

### Water Content

The ethanol extract of Legundi leaves used in this study contained the moisture content of 9.51% v/w.

### Ash Content

It was found that the ash content of the Legundi leaves was 9.46% w/w.

## THIN LAYER CHROMATOGRAPHY

### ED<sub>50</sub> of Legundi Extracts

Figure 2: The relationship between percentage inhibition inflammation with test dose. Red = Rat test 1, Green = Rat test 2, Purple = Rat test 3, Blue = Rat test 4, Orange = Rat test 5, Yellow = Mean percent inflammatory inhibition

## DISCUSSIONS

### Extraction

Extraction of 250 g of Legundi leaves with ethanol yielded a yield of 12.05 %W/W.

### Phytochemical Screening Results

Secondary metabolite compounds contained in Legundi leaves can be seen in Table 1. Flavonoids are known to be efficacious anti-inflammatory positive detected in ethanol extract of the Legundi leaf.

Murugan and Mohan<sup>[17]</sup> and Rajakan *et al.*,<sup>[18]</sup> in their articles, revealed the presence of alkaloids, flavonoids, saponins, phenolics, tannins, and terpenoids in their *V. trifolia* from Tamil Nadu, India. We had tried several times in our samples but could not find the alkaloids. It was not clear as to whether this was caused by location. Thenmozhi *et al.*,<sup>[19]</sup> however, did not find alkaloid in their research on *V. trifolia* from Tamil Nadu, India.

### Water Content

The ethanol extract of Legundi leaves used in this study contained the moisture content of 9.51%, so it was stated still eligible. As stated in Farmakope Herbal Indonesia,<sup>[13]</sup> a water content of the extract should be no more than 10% v/w as it may increase the risk of mold and mold growth.

### Ash Content

It was found that the ash content of the legundi leaves was 9.46% w/w. Pharmacopoeia Herbal Indonesia<sup>[13]</sup> states that good simplicia have the maximum ash content of 11% w/w. This meant that the Legundi leaves of simplicial ash content used in this study were eligible. Basically, ash contains inorganic radicals such as phosphates, carbonates, and silicates of sodium, potassium, magnesium, or calcium. Calculation of ash content is useful in determining the quality and purity of the raw material to be used as medicine.<sup>[19]</sup>

### Thin-Layer Chromatography

Examination of thin-layer chromatography on extract with the comparison of hexane and ethyl acetate phases of 2:1 and silica gel 254 as stationary phase yielded six dots at 254 nm and 366 nm [Figure 1]. There was no further effort to determine the type of spot. Jangwani *et al.*<sup>[20]</sup> reported that they had isolated β-sitosterol and two triterpenoids: Ursolic acid acetate and platanic acid from ethanolic extract of *V. trifolia* leaves.

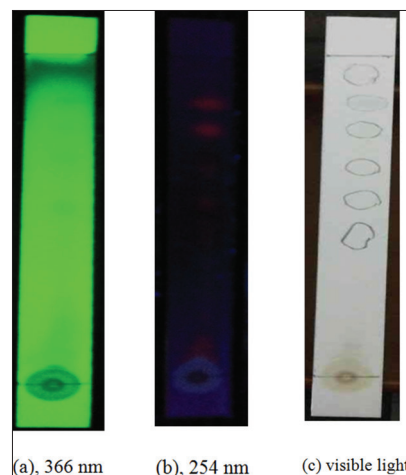


Figure 1: TLC spots of Legundi ethanol extract

### ED<sub>50</sub> of Legundi Extracts

The procedure of determination of ED<sub>50</sub> extract was based on induction method of rat's inflammation of rats using carrageenan. This method was simple, quick, and gave constant results on testing of anti-inflammatory drugs without causing tissue damage.<sup>[21,22]</sup> Edema of carrageenan injection resulted in a gradual inflammatory response. Histamine, serotonin, and bradykinin were detected in the early phase of carrageenan induction, while prostaglandins that increased vascular permeability were detected in the final phase of inflammation.<sup>[23]</sup> Infiltration and activation of local neutrophils also increased the inflammatory response by producing free radical compounds of oxygen derivatives.<sup>[24]</sup> Another mediator that plays an important role in acute inflammation was the nitrous oxide (NO). Carrageenan stimulated the production and release of NO in tissues.<sup>[23]</sup> Legundi leaves were known to contain casticin.<sup>[25]</sup> Flavonoid compounds are efficacious anti-inflammatory. Liquid extract was made by soaking the simplicia leaves in macerator with ethanol 96% for 3 days at room temperature. Ethanol was known as the preferred solvent for crop extraction because it has good extractive power, in the sense of being able to selectively dissolve the flavonoid component of the plant. The liquid extract of the Legundi leaves was further evaporated using a rotary evaporator and dried over the water bath to obtain a viscous extract with a constant weight because it no longer contains ethanol in significant amounts. From this procedure, the yield of extract was 12.05%. The extract was then applied for ED<sub>50</sub> determination.

Examination of ED<sub>50</sub> was performed by preparing a 1% carrageenan solution in 0.9% saline for injection with a dose of 0.1 mL. According to Estakhr *et al.*,<sup>[26]</sup> a fresh solution of 1–3% carrageenan in saline was commonly used as much as 50–150 µL for intraplantar injection. Carrageenan was known to have no nutritional value but contains high levels of cellulose, so it was widely used by the food and medicine industry for making gels and emulsifiers.<sup>[23,27,28]</sup> The other use was as a material testing of experimental drugs that are suspected anti-inflammatory efficacious.<sup>[29]</sup>

In this study, there were seven groups of mice who received five different treatment types. To the negative control group, 2% PGA solution was administered per oral while for the positive control group was given an assay of acetosal 10 mg/kg BB per oral. The test group was given a solution of *V. trifolia* leaves in 2% PGA with doses of 1000 mg/kg BW, 1500 mg/kg BW, 2000 mg/kg BW, 2500 mg/kg BW, and 3000 mg/kg BW as shown in Table 2. Based on the percentage data of rat leg inflammation inhibition in each fraction of the test group, the effective dose of Legundi leaves extract could be determined by finding the dose variable which had at least 50% of the sample population with the percent of rat foot inflammation inhibition which reached 50%.

The relationship between the extract dose and percent of inflammatory inhibition in the test group was shown in Figure 1 and Table 2.

The results of anti-inflammatory activity of extract can be seen in Figure 1 and Table 2. Based on the percentage data of rat foot inflammation inhibition in each fraction of the test group, the effective dose of Legundi leaves extract could be determined, i.e., by finding dose variables that

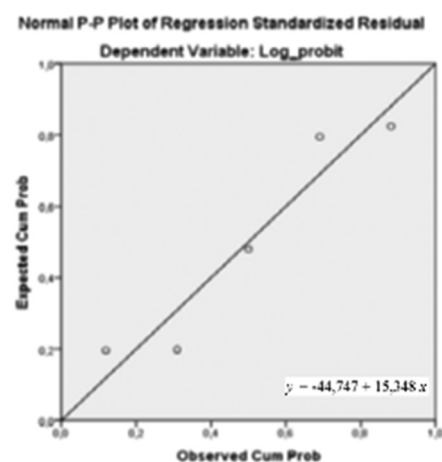
**Table 1: Phytochemical screening of Legundi leaves**

Chemical compounds	Results
Alkaloids	-
Polyphenols	+
Tannin	-
Flavonoids	+
Steroids	+
Triterpenoids	-
Monoterpene and sesquiterpene	+
Quinone	+
Saponins	-

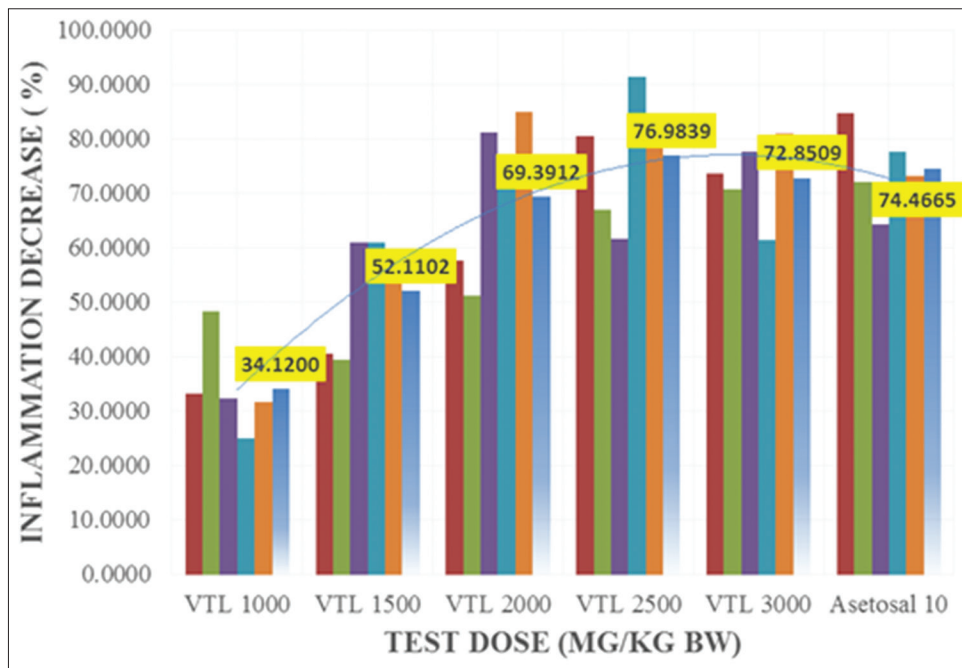
**Table 2: Percent of sample inflammatory inhibition of rats in each test group**

Treatments	Percent populations that have inflammatory inhibition minimum 50(%)
PGA 2%	-
VTL 1.0 g/kg BW	0
VTL 1.5 g/kg BW	60
VTL 2.0 g/kg BW	100
VTL 2.5 g/kg BW	100
VTL 3.0 g/kg BW	100
Acetosal 0.010 g/kg BW	100

PGA: Pulvis gummi arabicum, VTL: *Vitex trifolia* leaf extract



**Figure 3: SPSS calculation results**



**Figure 2:** The relationship between percentage inhibition inflammation with test dose. Red = Rat test 1, Green = Rat test 2, Purple = Rat test 3, Blue = Rat test 4, Orange= Rat test 5, Yellow = Mean percent inflammatory inhibition

		Coefficients				
Model B		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		Std. Error	Beta			
1	(Constant)	-44,747	13,193	-	-3,392	0.043
	Log_doses	15,348	4,029	0.910	3,810	0.032

a. Dependent Variable: Log<sub>e</sub>probit

have at least 50% of the sample population with a percent inhibition of leg inflammation of rats that reached 50%. It was shown that an increase in the mean percentage of inflammatory inhibition as increasing doses of the extract was administered. Exceptions occurred in the dose variables of 2500 mg/kg BW and 3000 mg/kg BW which showed a decrease in the percentage of inhibition, even doses of 2500 mg/kg BW reached a greater percentage of inflammatory inflammation than the positive control of the acetosal.

With the help of SPSS software, graph of the relationship between probit log and dosage log, the equation of the line as  $y = -44,747 + 15,348 x$ , as shown in Figure 2, with the standard error 13,193 and 4,029 ( $P < 0.05$ ;  $n = 5$ ). This results in the search for ED<sub>50</sub> values requiring adjustment of calculations when substituted into the equations manually. Substitution y with probit value 5 (log probit 50) in the line equation yielded the antilogarithm value  $x = 1428.8$  [Figure 3]. Dose 1428.8 mg/kg BW was then defined as the ED<sub>50</sub> anti-inflammatory activity of Legundi leaves extract. ED<sub>50</sub> was understood as median effective dose, the dose required to achieve 50% of the desired response in 50% of

the population, whereas TD<sub>50</sub> is median toxic dose of 50% for 50%, the dose required to get 50% of the population reporting this specific toxic effect; LD<sub>50</sub> (median lethal dose), the dose required to achieve 50% mortality from toxicity; EC<sub>50</sub> (Half maximum effective concentration), the concentration of a drug at which 50% of its maximum response was observed; and LC<sub>50</sub> (half lethal concentration), the concentration of a drug at which 50% mortality from toxicity was observed.<sup>[30,31]</sup> Our previous project reported that scientifically *V. trifolia* had anti-inflammatory activity of 21.44%, 30.78%, and 41.46% for doses of 500 mg extract/kgBW, 1000 mg/kg, and 2000 mg/KgBW.<sup>[32]</sup> It was understood that research on anti-inflammatory of *V. trifolia* had been done by other researchers<sup>[8,12,19,33,34]</sup> as well, but none of them mentioning about ED<sub>50</sub> of their results.

The analysis of the results of the research in the form of ANOVA ( $P < 0.05$ ) showed that there was a significant difference between the increase of dose and the percentage of inflammatory inhibition in rat's leg. This procedure was followed by Advanced LSD Test which gave the result that the doses of 1000 mg and 1500 mg were from non-identical populations, whereas larger doses of extracts show identical

results with positive controls of 10 mg/kg BW. Matsui *et al.*<sup>[33]</sup> mentioned that *V. trifolia* Linn. gave inflammatory inhibitory activity by regulating the expression of iNOS in macrophage cells.

## CONCLUSIONS

The effective dose of median (ED<sub>50</sub>) extract ethanol leaves *V. trifolia* Linn. which was efficacious anti-inflammatory was found 1428.8 mg/kg body weight. These results suggest that, if the leaves of Legundi will be made anti-inflammatory herbal medicine, the preparation should be a drug to drink not a tablet considering the required dose big enough.

## REFERENCES

- Laxmikant K. *Vitex trifolia* (Verbenaceae): A review on pharmacological and biological effects, isolated and known potential phytoconstituent of therapeutic important. *Int J Res Pharm Sci* 2012;3:441-5.
- Singh U, Meena AK, Yadav AK, Singh B, Rao MM. Pharmacological and phytochemical evidences for the extracts from plants of the genus *Vitex*. *Int J Pharm Clin Res* 2010;2:1-9.
- Mary RN, Banu N. Screening of antibiofilm and anti-quorum sensing potential of *Vitex trifolia* in *Pseudomonas aeruginosa*. *Int J Pharm Pharm Sci* 2015;7:242-5.
- Sahib HB, Al-Zubaidy AA, Hussain SM, Jassim GA. The anti-angiogenic activity of *Vitex Agnus astus* leaves extracts. *Int J Pharm Pharm Sci* 2014;6 Suppl 2:863-9.
- Dalimartha S. Atlas of Indonesian Medicinal Plants Volume 5 (Atlas Tanaman Obat Indonesia Jilid 5); 2008. p. 93-4. Available from: [http://lms.aau.ac.id/library/ebook/U\\_14822\\_09/#/0](http://lms.aau.ac.id/library/ebook/U_14822_09/#/0).
- Departemen Kesehatan Republik Indonesia. *Materia Medika Indonesia* 5<sup>th</sup> ed. Jakarta: Departemen Kesehatan Republik Indonesia; 2008. p. 510-3.
- Garbi MI, Osman EE, Kabbashi AS, Saleh MS, Yuosof YS, Mahmoud SA, *et al.* Cytotoxicity of *Vitex trifolia* leaf extracts on MCF-7 and Vero cell lines. *J Sci Innov Res* 2015;4:89-93.
- Kulkarni LA. Anti-inflammatory activity of *Vitex trifolia* L. (*Verbenaceae*) leaves extracts. *Int J Pharm Sci Res* 2011;2(8):2037-40.
- Nordqvist C. What Is Inflammation? What Causes Inflammation?; 2017. Available from: <http://www.medicalnewstoday.com/articles/248423.php>. [Last accessed on 2017 Aug 01].
- Mitchell RN. Acute and chronic inflammation. In: Cotran RS, editor. *Robinsons Basic Pathology*. 7<sup>th</sup> ed. New Delhi: Harcourt Pvt. Ltd; 2002.
- Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation, the seventh hallmark of cancer: Links to genetic instability. *Nature* 2008;454:436-44.
- Pfuzia A, Devi RK, Sharatchandra KH, Debashree BN, Banylla SN, Monica KS, *et al.* Studies on the anti-inflammatory effect of the aqueous extract of the leaves of *Vitex trifolia* L. In albino rats. *Int J Pharm Bio Sci* 2013;4:588-93.
- Departemen Kesehatan RI. *Farmakope Herbal Indonesia*. Jakarta: Departemen Kesehatan RI; 2008. p. 110-5, 134-144.
- Farnsworth NR. Biological and phytochemical screening of plants. *J Pharm Sci* 1966;55:225-76.
- Weill CS. Tables of convenient calculations of median effective dose ED<sub>50</sub> or LD50 and instruction on their use. *Biometric* 1952;8:249-63.
- Dodge Y. *The Concise Encyclopedia of Statistics*. New York: Springer; 2008. p. 302-4.
- Murugan M, Mohan VR. Efficacy of different solvent extracts of *Vitex trifolia* L. and *Aristolochia indica* L. for potential antibacterial activity. *Sci Res Rep* 2012;2:110-4.
- Rajakani NI, Balakrishnan M, Karthikeyan R, Jagadheesan V. Screening of antibacterial activity, qualitative and quantitative analysis of phytochemicals in *Vitex trifolia*. *Int J Res Dev Pharm Life Sci* 2014;3:1085-8.
- Thenmozhi S, Sundaram RS, Kumar JP, Bihari CG. Pharmacognostical and phytochemical investigation on leaves of *Vitex trifolia* Linn. *J Pharm Res* 2011;4:1259-62.
- Jangwani JS, Aquino RP, Mencherini T, Picerno P, Singh R. Chemical constituents of ethanol extract of leaves and Molluscicidal activity of crude extracts from *Vitex trifolia* Linn. *Herba Pol* 2012;59:19-32.
- Naik SR, Sheth UK. Inflammatory process and screening methods for anti-inflammatory agents (a review). *J Postgrad Med* 1976;22:5-21.
- Petersson M, Wiberg U, Lundeberg T, Uvnäs-Moberg K. Oxytocin decreases carrageenan induced inflammation in rats. *Peptides* 2001;22:1479-84.
- Necas J, Bartosikova L. Carrageenan: A review. *Vet Med* 2013;58:187-205.
- Salvemini D, Wang ZQ, Wyatt PS, Bourdon DM, Marino MH, Manning PT, *et al.* Nitric oxide: A key mediator in the early and late phase of carrageenan-induced rat paw inflammation. *Br J Pharmacol* 1996;118:829-38.
- Kulkarni LA. Analgesic potential of *Vitex trifolia* L. (*Verbenaceae*). *Asian J Pharm Clin Res* 2014;7:157-9.
- Estakhr J, Sanchooli N, Najafi SH, Javdan N. Antiinflammatory activity of ethanolic extract of *Physalis alkekengi*. *Res J Pharm Biol Chem Sci* 2011;2:421-5.
- Takamatsu S, Tosa T. Production of L-alanine and D-aspartic acid. *Bioprocess Technol* 1993;16:25-35.
- Van de Velde F, Lourenco ND, Pinheiro HM, Bakkerd M. Carrageenan: A food-grade and biocompatible support for immobilization techniques. *Adv Synth Catal* 2002;344:815-35.
- Zacharopoulos VR, Phillips DM. Vaginal formulations of carrageenan protect mice from herpes simplex virus infection. *Clin Diagn Lab Immunol* 1997;4:465-8.
- Brunton LL, Laso JS, Parker KL. *Goodman and Gilman's the Pharmacological Basis of Therapeutics*. 11<sup>th</sup>ed. New York: McGraw-Hill Medical Pub. Division; 2006.
- Chen M, Chen C, Ke Q. The effect of age on the median effective dose (ED<sub>50</sub>) of intrathecally administered plain bupivacaine for motor block. *Anesth Analg* 2014;118:863-8.
- Mustarichie R, Indriyati W, Wardhana YW, Wilar G, Levita J, Muhtadi A. Comparison of chemical and pharmacological research data between *Vitex trifolia* L. and *Vitex rotundifolia* L. *World J Pharm Res* 2013;2:1942-57.
- Matsui M, Kumar-Roine S, Darius HT, Chinain M, Laurent D,

- Pauillac S, *et al.* Characterisation of the anti-inflammatory potential of *Vitex trifolia* L. (*Labiatae*), a multipurpose plant of the pacific traditional medicine. *J Ethnopharmacol* 2009;126:427-33.
34. Ankalikar A, Viswanathswamy AH. Effect of leaves of *Vitex. Trifolia* linn on different stages of inflammation. *Indian J Pharm Educ Res* 2017;51:461-71.

**How to cite this article:** Mustarichie R, Sumiwi SA, Hanifah AT. ED<sub>50</sub> from anti-inflammatory properties of *Vitex trifolia* L. ethanol extract. *Natl J Physiol Pharm Pharmacol* 2018;8(5):719-725.

**Source of Support:** Nil, **Conflict of Interest:** None declared.