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# In Vitro Study of *Kaempferia galanga L*. Compound, δ- 3-Carene, Against 5-Lipoxygenase

Lusi Putri Dwita<sup>1,\*</sup> Supandi<sup>2</sup> Yeni<sup>3</sup>

<sup>1,2,3</sup> Universitas Muhammadiyah Prof. DR. HAMKA, Delima II/IV Street, East Jakarta 13460, Indonesia \*Corresponding author. Email: <u>lusi\_putridwita@uhamka.ac.id</u>

#### ABSTRACT

*Kaempferia galanga* has anti-inflammatory activity and worth developing as lipoxygenase inhibitors. The aim of this study was to determined  $\delta$ -3-carene, one of the potential compounds of *K. galanga*, as a lipoxygenase inhibitor. The LOX-inhibitor activity was tested in-vitro. The result showed that the IC50 of  $\delta$ -3-carene was 23.10  $\mu$ M, which was less potential compare to zileuton (7.54  $\mu$ M). The study showed that *K. galanga* contains possible active components that could be developed as 5-LOX-inhibitor.

*Keywords:* Kaempferia galanga, Anti-inflammatory, lipoxygenase,  $\delta$ -3-carene

#### 1. INTRODUCTION

Kaempferia galanga rhizome has been known for its various activities, such as anti-inflammatory[1]. The main components are ethyl p-methoxycinamic (30%), ethyl cinnamate (25%), p-methoxycinamic acid, monoterpenes ketone, and 3-carene-5-one [2]. It has shown potential in inhibiting cyclooxygenase (COX) and perhaps also binds to the lipoxygenase (LOX) enzyme. In previous study, the docking and molecular dynamics stimulation study showed that, among the compounds of *K. galanga*, the highest affinity towards 5 –LOX was shown by  $\delta$ -3-carene. Therefore, in this study, we further examined the activity of one of the components of *K. galanga* as LOX inhibitor by in-vitro study.

#### 2. METHOD

The Lipoxygenase (LOX) inhibitor Screening assay kit (Abnova, Taiwan) was used in this study. Zileuton (sigma, US) used as comparator. Lipoxygenase enzyme was used as a standard and linoleic acid as the substrate. The kit detects and measures the hydroperoxides produced in the lipoxygenase reaction. The zileuton and  $\delta$ -3-carene (Sigma, US) were dissolve in ethanol. The procedure was done based on the kit instruction. The result was read at 490 nm.

#### 3. RESULT AND DISCUSSION

*K. galanga* has been studied as anti-inflammatory [3–8]. Despite many studies of the rhizome on inflammation, there has not been research of the compounds of *K. galanga* as lipoxygenase inhibitor. Lipoxygenase (LOX) plays a role in the metabolic process of arachidonic acid (AA) that could produce leukotriene (LT), which mediates inflammation. LOX inhibition can reduce LT, resulting in anti-inflammatory effects [9]. Inflammatory mediators produced

by cyclooxygenase (COX) and lipoxygenase (LOX) pathways are responsible for many diseases in humans, such as cancer, arthritis, autoimmune, cardiovascular, and neurological disorders. Studies showed mediators produced by 5-LOX, 12-LOX, and 15-LOX associated with allergic reactions, and the pathogenesis of atherosclerosis [10]. The research was started by in-silico study of 21 potential compounds of K. galanga, : ethyl cinnamate, ethyl pmethoxycinamic acid, p-methoxycinamic acid, 3-carene-5one, camphene,  $\delta$ -3- carene, p-methoxy styrene,  $\gamma$ -pinene,  $\beta$ -myrcene, p- cymene, 1,8-cineole, iso-myrcene, camphor,  $\alpha$ -terpineol, p-cymene- 8-ol, eucarvone,  $\delta$ -cadinene, kaempferol, quercetin, cyanidin and delphinidin. The highest affinity towards LOX was shown by  $\delta$ -3-carene, then this compound was continued to be tested in in-vitro study. The result of the study showed that the IC50 of  $\delta$ -3carene was 23.10 µM, which was less potential compare to zileuton (7.54 µM) (Figure 1 and 2). This result indicated that carene has the activity towards 5-LOX. Therefore, further study is crucial to confirm the 5-LOX inhibitor activity of  $\delta$ -3-carene and other compounds of K. galanga.



Figure 1. Zileuton Lipoxygenase Inhibition





Figure 2. δ-3-carene Lipoxygenase Inhibition

#### 4. CONCLUSION

Kaempferia galanga has anti-inflammatory activity and worth developing as lipoxygenase inhibitors. The aim of this study was to determined  $\delta$ -3-carene, one of the potential compounds of K. galanga, as a lipoxygenase inhibitor. The LOX-inhibitor activity was tested in-vitro. The result showed that the IC50 of  $\delta$ -3-carene was 23.10  $\mu$ M, which was less potential compare to zileuton (7.54  $\mu$ M). The study showed that K. galanga contains possible active components that could be developed as 5-LOX-inhibitor.

#### **AUTHORS' CONTRIBUTIONS**

Lusi Putri Dwita (Concepts, experimental studies, data analysis, manuscript preparation), Supandi (Concepts, design), Yeni (Literature search).

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