The Potency of *Legetan warak* (*Adenostemma lavenia*) and *Kersen* Leaf (*Muntingia calabura*) Extract as a Candidate for Chronic Obstructive Pulmonary Disease (COPD) Herbal Medicine

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***Abstract*—Lung inflammation is a normal response in COPD patients that are usually treated using anti-inflammatory drugs. Most anti-inflammatory drugs work to inhibit the activity of cyclooxygenase which produces inflammatory mediators. This study aims to examine the anti-inflammatory potency of *legetan warak (Adenostemma lavenia)* and kersen leaf *(Muntingia calabura)* extract for treating COPD through inhibition of Cyclooxygenase-2 (COX-2) and to predict the chemical compounds that play a role in it through laboratory tests and literature studies. Phytochemical assays show that both *legetan warak* and *kersen* leaf contain flavonoid compounds. Based on some literature studies, it is concluded that the extract of *legetan warak* and *kersen* leaf have the potency as an anti-inflammatory by inhibiting COX-2 activity. The compounds that assumed to be COX-2 inhibit are certain compounds from flavonoid and phenolic compounds groups.**

Keywords—Anti-inflammatory, Chronic Obstructive Pulmonary Disease (COPD), Cyclooxygenase-2 (COX-2), Adenostemma lavenia, Muntingia calabura

I. INTRODUCTION

According to Global Initiative for Chronic Obstructive Lung Disease (GOLD), COPD is a disease with respiratory system constrain characteristic which is not completely reversible. The respiratory constrain is typically progressive and also related to inflammation due to harmful substance or gas contact [1].

Chronic Obstructive Pulmonary Disease is one of the global leading causes of death. WHO stated COPD is the 4th global cause of death. It is assumed COPD has been the cause of 2.75 million death or equals to 4.8%. Based on the epidemiology study of The Burden of Obstructive Lung Disease (BOLD) program, COPD cases increased to 384 million in 2010 with 11.7% global prevalence [2].

COPD treatment without medication involves a better life style, regular exercise, stop smoking, and healthy diet. On the other hand, COPD treatment with medication could be done with consuming lozenges or oral steroids. However, steroids consumed in long period of time would impact on other diseases such as diabetes, osteoporosis, cataract, higher risk of infection, hyperglycemia, and high blood pressure [3]. In China, as study of COPD medication and treatment has been conducted. It resulted to a combination of medical method and Traditional Chinese Medicine (TCM) as the most effective attempt for COPD patients [4]. The latest study showed that there was endothelial nitric oxide synthase (eNOS) dysfunction on COPD patients which caused respiratory inflammation. One of the therapies as the treatment was anti-inflammatory therapy [5].

As mentioned before, certain COPD drugs have several side effects. Thus, the use of herbal medication is preferable to minimize the side effects. Treatment using natural herbal medicine compounds is frequently done for it is expected to generate harmless and more effective medication compounds for COPD treatment. One of herbal medication is *legetan warak* (*Adenostemma lavenia* (L) [6]. Also nowadays, a lot of researches to find potential COPD medicine from herbal medicinal plants have been conducted. According to Jiang et al. [7], milkvetch root (*Astragalus monholicus*) has the ability to recover COPD patients' immune system with acute exacerbation.

According to Reference Arpini [8], *legetan warak* herb ethanol 70%` extract had 19.09% anti-inflammation effect in inhibiting cyclooxygenase-2 (COX-2) enzyme. *Muntingia calabura* methanol extract had 76% anti-inflammation effect. Thus, it could be used as COPD drugs [9]. Reference Sarimanah et al. [10] summarized that *kersen* leaf 90% ethanol extract of 50 mg/kgBW and 100 mg/kgBW presented anti-inflammation effect with 58.33% and 52.78% inflammation inhibition percentage. The active material in *legetan warak* root

was 11-*Hydroxylated kauranic acids* compounds group [11] which was beneficial as anti-inflammation, escalating the lungs and lever function, pain killer, influenza and measles preventers and hepatitis transmission preventer. Research had also defined the compound characteristic 11-*Hydroxylated kauranic acids* was found in *legetan warak* as anti-inflammation and highly potential as COPD medication, this compound was also very difficult to be synthesized [12]. In accordance to the potencies above, this study aims to scrutinize the anti-inflammation potency of *legetan warak* (*A. lavenia*) and *kersen* leaf (*M. calabura*) extract for COPD medication through inhibition of COX-2. In addition to that, this study also aims to discover the chemical compound that actively functions in the extract through literature studies. The study hypothesis was *legetan warak* and *kersen* leaf extract could inhibit COX-2 in the inflammation process by *in vitro*. The result study was expected to provide scientific reference of *legetan warak* and *kersen* leaf mixture benefits as anti-inflammation along with facilitating it as COPD herbal alternative medication.

II. Experimental Section

A. Sample Preparation

The applied sample in this study was *legetan warak* and *kersen* leaf herbs. *Legetan warak* herbs sampling was taken from Pekalongan District, Central Java, while *kersen* leaf sampling was taken from Bogor District, West Java. Both samplings were being cleaned and dried in room temperature, which then were grinded into 80 mesh powders.

B. Water Content Calculation [13]

An empty petri dish was dried in 105 °C oven for 30 minutes, then was cooled off in desiccator for 30 minutes and weighed. As much as 3 gram simplicia was weighed and added into an already weighed dish. Simplicia filled dish was dried in 105 °C oven for 3 hours. The dish was then cooled off in desiccator for 30 minutes and weighed. The drying procedure was completed until constant weighed. The water content test was performed in three replicates. Water content formula was calculated as follow:

$$Water\ Content\ (\%) = \frac{A - B}{A} \times 100\% \quad (1)$$

Notes:

A = sample wet weight (g)

B = sample weight after drying (g)

C. Sample Extraction

Extraction was carried out by maceration using water and 70% ethanol solvent in room temperature. Sample powder was weighed, *legetan warak* and *kersen* leaf herbs in 1:9 sample and solvent ratio. Sample and solvent were shaken and set aside for 24 hours before being filtered. Filtrate was separated and repeated three times. Filtrate was then being concentrated using rotary evaporator in 40 °C. The generated extract was weighed and measured for its yield value.

$$\text{Yield Extract } (\%) = \frac{a}{b(1 - \frac{c}{100})} \times 100\% \quad (2)$$

Notes:

a = extract weight (g)

b = sample weight (g)

c = water content (%)

D. Phytochemical Assay

- *Test for Alkaloids:* As much as 0.05 g sample extract was diluted with several drops NH_3 and 5 mL $CHCl_3$ before being filtered and put into a test tube. To filtrate filled test tube was added with 1 mL H_2SO_4 2 M and shaken into forming two separated layers. The upper layer or acid layer was separated into dropping plates. To acid layer was dropped by Meyer, Wagner and Dragendorff reagents which would generate colored sediments accordingly in white, brown and orange-ish red as it contained alkaloid.
- *Test for Triterpenoids and Steroids:* As much as 0.05 g sample extract was diluted with 5 mL hot ethanol and then filtered into a test tube. The filtrate was then evaporated to dry and added with 1 mL diethyl ether before being relocated into evaporating dish. A drop of concentrate H_2SO_4 and one drop of CH_3COOH anhydrate was added into the solution. Red or purple color showed triterpenoid content and green or blue color showed steroid content.
- *Test for Flavonoids:* As much as 0.05 g sample extract was diluted with 5 mL distilled water and then boiled for 5 minutes before being filtered. To generated filtrate then was added with Mg powder, 1 mL HCl, 1 mL ethanol, and 1 mL amyl alcohol. The mixture was then shaken strongly for couple of minutes. Positive result signified in red, yellow or orange on amyl alcohol layer.
- *Test for Saponins:* As much as 0.05 g sample extract was diluted with 5 mL distilled water and then boiled for 5 minutes before being filtered. The generated filtrate was shaken strongly into forming some foam. Saponin content was signified in 15 minutes firmed foam.
- *Test for Tannins:* As much as 0.05 g sample extract was diluted with 5 mL distilled water and then boiled for 5 minutes before being filtered. To the filtrate was added several drops $FeCl_3$ 10%. Tannin compound content signified as greenish black color was appeared.

E. Literature Study

Literature study was implemented to obtain information about shrimp larvae toxicity test and inhibition potency test of

COX-2. The perceived literature in the study was referred from the following database such as *Google Scholar, Medicine, The Royal Society of Chemistry, PubMed, Science Direct, Scopus, Scifinder, Portal Garuda*, and *IPB Repository*.

III. Results and Discussion

A. Water Content

The sample used in the study was *legetan warak* herbs and *kersen* leaf powder. The generated average water content from *legetan warak* and *kersen* leaf simplicia were 2.05% and 9.57%. The sample water content value was considered low since it was less than 10%. The water content within a substance was less than 10 %, its optimum stability would be achieved as well as reduced the microbe growth. Fig. 1 shows *legetan warak* and *kersen* plants.

(a)

(b)

Fig. 1. (a) legetan warak, (b) kersen.

B. Yield Extraction

The result showed that *kersen* leaf ethanol 70% extract yield (16.42 %) had bigger yield value compared to its water extract (13.76 %), while *legetan warak* water extract yield had the smallest value compared to *kersen* leaf extract (12.73%). It showed that both samplings had different secondary metabolites contents.

C. Secondary Metabolites Components

Phytochemical assay was the preliminary process on a phytochemistry study to provide some depiction of the compounds group within the observed plants. *Legetan warak* and *kersen* leaf herbs phytochemical assay was on Table I and II.

TABLE I. *Legetan Warak* Herbs Secondary Metabolites Components

Compounds	Result	
	Water Extract	***Ethanol 70% Extract***
Alkaloid	-	-
Flavonoid	+	+
Saponin	+	-
Tannin	+	+
Terpenoid	-	-
Steroid	-	+

Notes: (+) secondary metabolites detected on sample tes, (-) secondary metabolites undetected on sample test.

TABLE II. *Kersen* leaf extract secondary metabolites components

Compounds	Result	
	Water Extract	***Ethanol 70% Extract***
Alkaloid	-	-
Flavonoid	+	+
Saponin	+	+
Tannin	-	+
Terpenoid	+	+
Steroid	+	+

Notes: (+) secondary metabolites detected on sample tes, (-) secondary metabolites undetected on sample test.

Referred to Table I and II, it was shown there was no alkaloids within *legetan warak* herbs and *kersen* leaf extract. Test of flavonoids positive was marked by the color orange on alcohol amyl layer. Test of flavonoids gave positive result on all the samples. Test of saponins was negative result on *legetan warak* herbs ethanol 70% extract, while on another test sample was positive. Test of tannins was positive result on *legetan warak* extract and *kersen* leaf ethanol 70 % extract. Positive steroids result showed in 70% ethanol extract and *kersen* leaf water.

D. Various Extracts Inhibition Capacity Potency to COX-2 Activity

Prostaglandin is one of the mediators often associated with pain, fever, and inflammation. Inflammatory process is commonly contributed by histamine, prostaglandin, eicosanoids, leukotrienes, cytokine, nitric oxide, and many more. COX-1 and COX-2 functioned differently. COX-2 took part in initiation process mainly when there were some stimuli such as; growth factor, cytokine, lipopolysaccharide, interleukin-1, tumour necrosis factor, and also endotoxin. COX-2 induction was temporary and would gradually subside as the inflammation overcome. COX-1 took its part in platelets aggregation by generating thromboxane A_2. The initial generated product from both COX-1 and COX-2 enzymatic reactions was Prostaglandin G_2 (PGG_2) which would then be metabolized into Prostaglandin H_2 (PGH_2). Furthermore, PGH_2 was the precursor for prostanoid compounds such as prostaglandin D (PGD_2), prostaglandin E (PGE_2), prostaglandin F (PGF_2), prostacyclin (PGI_2) dan thromboxane (TX_2). *Legetan warak* (*A. lavenia*) and *kenikir* (*Cosmos*

caudatus) extracts cyclooxygenase-2 inhibition as anti-inflammatory were extracted [8]. Both plants were from Asteraceae family. The research was performed by *in vitro* with ELISA method using COX Inhibitor Screening Assay Kit and following Cayman Chemical Catalog No. 701080 protocol. The applied *legetan warak* concentrations were 10, 50, dan 100 µg/mL. Fig. 2 shows the research result of Arpini study [8].

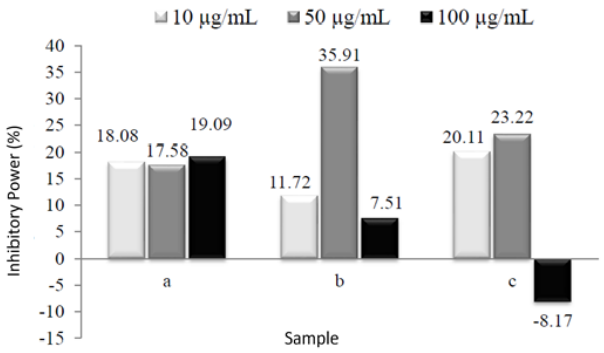

Fig. 2. Inhibition sample to COX-2 activity (a) legetan warak herbs ethanol 70% extract, (b) kenikir water extract, (c) kenikir ethanol 70% extract [8].

Fig. 2 showed *legetan warak* and *kenikir* herbs ethanol 70% extract inhibition capacity. *Legetan warak* was potential as COX-2 activity inhibitory since it could hinder COX-2 activity. Based on the inhibition value generated from all concentrations, *legetan warak* ethanol extract 100 µg/mL concentration owned the highest inhibition value as much as 19.09%. Another research by Chen et al. [14] about anti-inflammatory activity from *legetan warak* extract ethyl acetate fraction resulted on inhibition power to COX-2 existence.

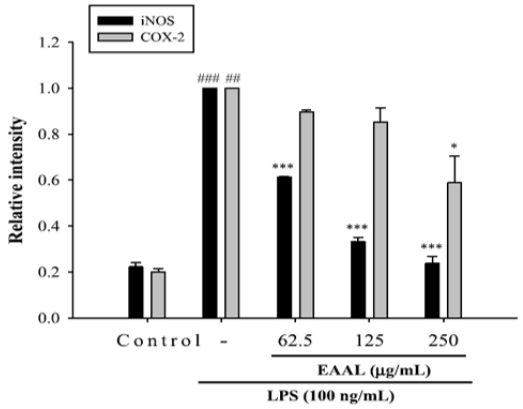

Fig. 3. Legetan warak ethanol extract ethyl acetate inhibitory fraction to COX-2 and iNos [14].

Referred to Fig. 3 from Chen et al. [14] research of inhibition activity to COX-2 and iNOS, iNOS was an isoenzyme responsible in forming nitric oxide (NO) which mainly functioned in pathology process as well as blood flow and blood pressure regulations. Blood vessel dilatation commonly occurred during inflammation. [14] result study mentioned *legetan warak* ethanol extract was able to inhibit COX-2 activity. The applied solvent sample was *legetan warak* ethanol extract in three concentration values, which were 62.5, 125, dan 250 µg/mL.

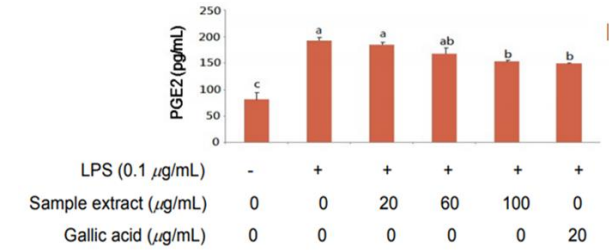

Fig. 4. The influence of Muntingia calabura fruit ethanol extract to PGE2 production that stimulated by LPS [15].

Fig. 4 showed the influence of *Muntingia calabura* fruit ethanol extract to PGE_2 production. The research by Lin *et al.* [15] used *kersen* fruit extract as COX-2 inhibitory agent with the following concentrations: 20, 60, and 100 µg / mL. The applied positive control was gallic acid as it was the main phenolics within the extract with three hydroxyl groups as cathecol B-ring structure in flavonoids. Thus, gallic acid was expected to play main function in anti-inflammatory activity. Khozuni *et al.* [16] stated that gallic acid had the ability assisting the inflammation inhibition process by reducing TNF-α production. TNF-α was a pro-inflammatory cytokine crucially functioned in pathogenesis mechanism of several chronic inflammation disease. PGE_2 production could be reduced with *Muntingia calabura* fruit extract addition. It showed in Fig. 4 explained as the concentration of *Muntingia calabura* fruit extract got higher, it reduced PGE_2 amount. 100 µg/mL extract concentration had almost as much amount of PGE_2 concentration as the applied positive control.

Based on the phytochemical assay, it obtained that all fourth tested extracts contained flavonoids and seemed dominating as its color intensity compared to the other secondary metabolite. Flavonoids functioned in inhibiting the important phase of prostaglandin biosynthesis, cyclooxygenase pathway.

E. Several Plants in Asteraceae Family Inhibition on the COX-2 Activity

The study by Alberto *et al.* [17] was reported that *B. incarum* and *C. Atacamensis* ethanol extract with a total of 8 µg/mL phenolics concentration owned the highest COX-2 inhibition percentage as much as 84% compared to other extracts. Other compounds assumed to have vital function as COX inhibitory in this study were phenolics and flavonols. Almeida *et al.* [18] did research of *Arctium lappa L* plant anti-inflammatory activity with large intestine inflammation model *2,4,6-trinitrobenzenesulfonic acid* (TNBS) induced. The plant was collected from Mogi Mirim, Brazil, and owned COX-2 inhibition activity. The result showed that onopordopicrin within *A. Lappa L* extract was not reacted to COX-1. However,

it has inhibitory activity to COX-2 which vitally functioned in inflammatory process after being induced with TNBS.

Sesquiterpene lactones commonly found in *Asteraceae* family plants had inhibition activity to COX-2 [19]. COX-2 inhibition activity assay was done by *in vitro* method The result was the docked sesquiterpene lactones into *(-)-a-Santonin (2,2,2-trifluoroethyl) oxime ether* had the highest COX-2 inhibition activity value as much as 74.3% in 1 μM concentration. *Arnica montana* and *Calendula officinalis* were the *Asteraceae* family plants assumed to have COX-2 inhibition activity. A literature study result of several *Asteraceae* family plants potency was on the following Table III.

F. Certain Malvales Order Plants Inhibition Capacity on the COX-2 Activity

Research of anti-inflammatory activity from *Hibiscus rosa-sinensis* and *Hibiscus rosa-sinensis* var. Alba ethanol extract have been performed [20]. Both plats were collected from Selangor and Serawak, Malaysia. *H. rosa-sinensis* var alba and *H. rosa-sinensis* L. flower and leaf ethanol extract anti-inflammatory activity was determined using carrageenan method by *in vivo*. Rats as the testing animal were injected with carrageenan 30 minutes before giving the extract in 5, 50, and 100 mg/kg dosages. 50 and 100 mg/kg dosages of *H. rosa-sinensis* flower and leaf extract led to significant edema inhibition ($P<0.05$). Based on the result, it was concluded that all extracts had the ability to handle acute inflammation by *polymorphonuclear infiltration* (PNL) inhibition caused by carrageenan.

Research reported that *Malva neglecta* water extract could reduce TNF-α, IL-1β, iNOS, IL-18, and COX-2 expression in sinoviocytes [21]. The plant, taken from Iran genetics research center, was induced with LPS and calculated using *Enzyme Linked Immunosorbent Assay* (ELISA). It resulted on significantly prostaglandin reduction as much as 28% as *M. Neglecta* extract was added. Maryam [22] also reported on *M. Neglecta* water extract was effectively suppressed pro-inflammatory cytokines expression. TNF-α cytokines gene expression reduced into 95.04%, IL-1B cytokines gene expression reduced into 73.81% and COX-2 gene expression reduced into 93.79%.

Malva sylvestris and *Sida cordifolia* plants anti-inflammatory effect have been reported [23]. Both plants were under the same order as *kersen* plant, *Malvales*, and collected from Onta Grossa Parana and Mato Grosso Do Soul, Brazil. The anti-inflammatory activity measurement using lipopolysaccharide (LPS) induced cell. *M. Sylvestris* flower crude extract could inhibit PGE_2 production as much as 50.8%. Oenin, quercetin, and scopoletin were the chemical compounds assumed to facilitate anti-inflammatory activity by inhibiting COX-2 function *S. cordifolia* ethanol extract residue fraction provided the highest prostaglandin production inhibition percentage as much as 69.08% in 50 μg/mL concentration. The inhibition power was a lot higher compared to the applied positive control.

Reference Cinthura et al. [24] reported COX-2 inhibitor activity from *Abutilon indicum*. The applied *Abutilon indicum* extract was collected from Green Chem Herbal Extracts, India. The COX-2 inhibition assay was *enzyme immunoassay* by *in vitro*. The applied extract concentrations were 15.625, 31.25, 62.5, 125, 250, 500, and 1000 μg/ml. The highest inhibition percentage generated in 1000 μg/ml extract concentration as much as 95.33%.

Hibiscus sabdariffa ethanol extract anti-inflammatory effect have been studied [25]. The used plant sample was taken from Cairo University. *H. sabdariffa* leaf was extracted with ethanol 70% solvent before being partitioned with various solvents such as water, methylene chloride, ethyl acetate, and *n*-butanol. The anti-inflammatory activity test was done by *in vitro* with *COX-2 inhibitory assay*. COX-2 test result generated IC_{50} value on *H. sabdariffa* ethanol extract concentration of 0.27 μg/ml. To confirm the *in vitro* result, *in vivo* assay using carrageenan-induced rat hind paw edema method have been conducted. The result was *H. sabdariffa* ethanol extract significantly could inhibit leg edema with 65.71% maximum inhibition on 400 mg/kg after six days.

IV. CONCLUSION

This experimental study tested the effectiveness of *legetan warak* and *kersen leaf* extracts to inhibit COX-2 in the inflammation process of COPD patients. The results showed that *legetan warak* and *kersen leaf* mixture was proven as anti-inflammation agent and could be alternative medication for COPD patients.

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