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Research Article

ANTI-INFLAMMATORY STUDY OF *ANREDERA CORDIFOLIA* LEAVES AND *CENTELLA ASIATICA* HERBS AND ITS COMBINATIONS USING HUMAN RED BLOOD CELL-MEMBRANE STABILIZATION METHOD

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ABSTRACT

Objective: Inflammation is body reactions in response to tissue injury and infection. In 2011, non-steroidal anti-inflammatory drug (NSAID) was the highest demand drug in Indonesia. However, long-term treatment using NSAID can cause several side effects to cardiovascular and digestive system. This research aimed to investigate anti-inflammatory properties of binahong leaves (*Anredera cordifolia*) and pegagan herbs (*Centella asiatica*).

Methods: Ethyl alcohol extract of *A. cordifolia* leaves and *C. asiatica* herbs was evaluated for its anti-inflammatory properties using human red blood cell (RBC) – membrane stabilization assay. The extract concentrations used in this study was 100, 200, 400, and 800-ppm, and apigenin and asiaticoside concentration were 1, 2, 3, 4, 5, 6, 10, and 100 ppm. Diclofenac natrium (DN) was used as a standard drug.

Results: The results showed that *A. cordifolia* extract (ACE) alone, *C. asiatica* extract (CAE) alone, and the combination of ACE and CAE could inhibit the hemolysis of RBC in hypotonic solution. The optimum concentration for ACE alone was 100 ppm; for CAE alone was 400 ppm; and for the combination of ACE and CAE was 50 ppm and 50 ppm, respectively. Apigenin and asiaticoside in concentration of 1-10 ppm showed more than 97% inhibition of hemolysis. DN as a standard drug showed optimum inhibition at concentration of 400 ppm.

Conclusion: The ethyl alcohol extract of *A. cordifolia* leaves and *C. asiatica* herbs showed anti-inflammatory activity, both as a single treatment or as combinations, and apigenin and asiaticoside were responsible for anti-inflammatory activity in *C. asiatica*.

Keywords: Anti-inflammation, Human red blood cell – membrane stabilization, *Anredera cordifolia*, *Centella asiatica*.

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INTRODUCTION

Inflammation is body reactions in response to tissue injury and infection. When inflammation occurred, blood element, for example, leukocyte, and chemical messenger accumulated at injured area. There are five characteristics of inflammation: Redness, swelling, the rise of temperature, pain, and loss of function [1]. Non-steroidal anti-inflammatory drug (NSAID) is one of the most common medications for inflammation. This drug acts by stabilizing lysosome membrane and inhibiting hydrolytic enzyme release. In 2011, NSAID was the highest demand drug in Indonesia. However, long-term treatment using NSAID can cause several side effects to the cardiovascular and digestive system [2]. Hence, it is an opportunity to find an alternative medicine for inflammation. Herbal medicine is one of the most potential drug resources, and Indonesia has a very large biodiversity.

Anredera cordifolia and *Centella asiatica* are commonly used as a traditional medicine in Indonesia. *A. cordifolia*, belongs to Basellaceae family [3], known as Binahong in Indonesia, has been evaluated for its analgesic activity [4], anti-hyperlipidemic activity [5], and in improving kidney failure [6]. Formulation for *A. cordifolia* as wound healing gel has been evaluated [7]. Whereas *C. asiatica*, belongs to Apiaceae family, known as pegagan in Indonesia, traditionally used to heal wound and improve appetite [8]; it's also used for its activity as diuretics, hemostatic, and anti-infection [9]. This plant has been reported having activity including antioxidant, antimicrobial, cytotoxic, neuroprotective, anti-inflammatory, anti-diabetic, anti-ulcer, and wound healing. The bioactive constituents of these plants are the triterpenic acid (asiatic acid and madecassic acid), triterpenic saponin (madecassoside and asiaticoside), flavonoids (quercetin, kaemferol, catechin, rutin, apigenin, and naringin), and other phenolic compounds [10,11].

Based on the traditional use and another study, the combination of these two plants was thought to be effective as an anti-inflammatory agent. Moreover, the combination of these two plant extracts in inhibiting inflammation has never been investigated before. Hence, the aim of this study was to evaluate anti-inflammatory activity of *C. asiatica* and *A. cordifolia*, as a single treatment or as combinations.

METHODS

Identification and authentication of plant materials

C. asiatica and *A. cordifolia* plants were obtained from Manoko Botanical Garden, Bandung, West Java, Indonesia. Fresh plants were dried at 50-60°C and then grinded into small pieces. Plant identity's authentication was performed by Herbarium Bandungense, School of Natural Science and Technology, Bandung Institute of Technology.

Preparation of ethanolic extract of *C. asiatica*

About 200 g dried herbs of *C. asiatica* was macerated using 2 L ethanol [12]. The mixture was filtered using a filter paper, and the filtrate was concentrated using rotary vacuum evaporator at 60°C.

Preparation of ethanolic extract of *A. cordifolia*

About 200 g dried leaves of *A. cordifolia* was macerated using 2 L ethanol [7]. The mixture was filtered using a filter paper, and the filtrate was concentrated using rotary vacuum evaporator at 60°C.

Membrane stabilization (MS) assay

Anti-inflammatory activity was evaluated using human red blood cell – MS (HRBC-MS) assay [13]. Blood was collected from healthy volunteer who did not consume any NSAID for 2 weeks before the experiment. The collected blood was mixed with a silver solution in equal amount. The mixture was centrifuged in 3000 rpm and obtained packed cell

was washed with isotonic solution (NaCl 0.9%) in equal volume. The mixture was once again being centrifuged and obtained packed cell was made into 10% suspension in isotonic solution (NaCl 0.9%) [14,15].

The evaluated mixture was made by mixing 2 ml of hypotonic solution (NaCl 0,36%), 1 ml of phosphate buffer solution (pH 7.4), 0.5 ml of HRBC 10% v/v in isotonic solution (NaCl 0.9%), and 1 ml of evaluated sample (*C. asiatica* extract [CAE], *A. cordifolia* extract [ACE], apigenin, asiaticoside, or diclofenac natrium [DN]). The concentration of extract and DN were 100, 200, 400, and 800 µg/ml. Whereas apigenin and asiaticoside concentration were 1, 2, 3, 4, 5, 6, 10, and 100 µg/ml. DN was used as a standard drug. This mixture was incubated at 37°C for 30 minutes, and then being centrifuged. The supernatant was collected and being measured using spectrophotometer ultraviolet-visible at 560 nm [15,16].

The percentage of hemolysis was calculated using following equation:

$$\%\text{Inhibition of hemolysis} = 100\% - \frac{(\text{Sample absorbance})}{(\text{Control absorbance})} \times 100\%$$

RESULTS AND DISCUSSION

The extract's percentage of inhibition of hemolysis was shown in Fig. 1.

From the experiment, the extract showed an ability to inhibit hemolysis of RBC in hypotonic solution. HRBC membrane shows similarity with lysosome membrane. During inflammation, the lysosomic enzyme is released and therefore producing several characteristics of inflammation. The ability to stabilize lysosome membrane is thought can prevent inflammation. This is the NSAID's mechanism of action as anti-inflammatory agent [13].

According to Fig. 1, CAE at concentration 100 ppm showed 78% inhibition of hemolysis. The increasing concentration enhanced the ability to stabilize the RBC membrane from hemolysis. The highest percentage of inhibition was shown at concentration 400 ppm with 91% inhibition. DN, which was used as a standard drug, showed 92.12% inhibition at the same concentration. Based on these data, *C. asiatica* was proved to be having an anti-inflammatory activity. Moreover, this result was comparable with another study which also evaluated its anti-inflammatory properties [17,18].

Asiaticoside and apigenin also showed the ability to inhibit the hemolysis of RBC (Fig. 2).

Apigenin, which was one of the flavonoid compounds in *C. asiatica*, showed more than 90% inhibition of hemolysis in concentration of 1-100 ppm. As for asiaticoside, which was the triterpene compound in *C. asiatica*, showed similar percentage of inhibition to apigenin in concentration of 1-10 ppm. However, at concentration of 100 ppm, asiaticoside showed declining in percentage of hemolysis inhibition to 45%. From these data, it was proved that the active compound which was responsible for anti-inflammatory activity in *C. asiatica* was apigenin and asiaticoside.

As for ACE, the highest percentage of inhibition was achieved at concentration of 100 ppm with 81% inhibition of hemolysis, which was the lowest evaluated concentration. And raising in concentration affected in decreasing the extract's ability to stabilize the RBC membrane from hemolysis. DN as a standard drug showed 12.94% inhibition at the same concentration. According to these data, *A. cordifolia* showed anti-inflammatory activity. From another study, it was known that *A. cordifolia* contain oleanolic acid which has anti-inflammatory activity that can reduce pain in burns [19].

When these two extracts were combined, the result showed that the maximum inhibition of hemolysis activity was shown in combination of 50 ppm CAE and 50 ppm ACE, which was the lowest concentration

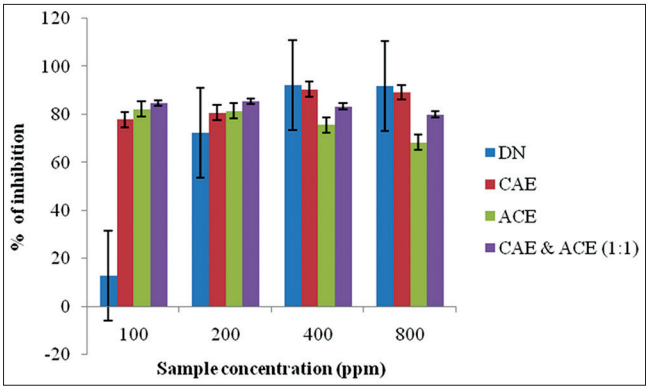

Fig. 1: Percentage of hemolysis inhibition of extracts. DN: Diclofenac natrium, CAE: *Centella asiatica* extract, ACE: *Anredera cordifolia* extract, n=2 per group

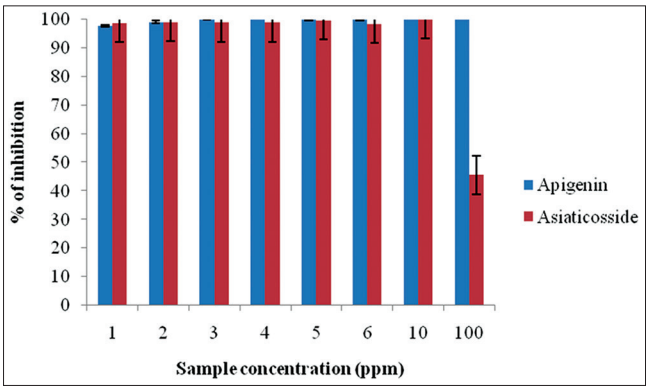

Fig. 2: Percentage of hemolysis inhibition of apigenin and asiaticoside, n=2 per group

of the combinations. And rising concentration in combination did not show a significant elevation in inhibiting hemolysis.

CONCLUSION

It was concluded that CAE and ACE had the anti-inflammatory activity, both as a single treatment or as combinations; and apigenin and asiaticoside were responsible for anti-inflammatory activity in *C. asiatica*.

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