

The Combination of *Colocasia esculenta* L. and *Zingiber officinale* Potentially Inhibits Inflammation and Pain

(Kombinasi *Colocasia esculenta* L. dan *Zingiber officinale* Potensial Menghambat Inflamasi dan Nyeri)

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Abstract: The active metabolites of *Colocasia esculenta* L. and *Zingiber officinale* L. have been reported to reduce pain and exert anti-inflammatory effects. This study investigated the anti-inflammatory and painkilling properties of a mixture of *C. esculenta* and *Z. officinale* extracts. Thirty rats and mice were each divided into 6 groups (n=5), namely the normal group, negative control, positive control (Na-diclofenac), and 3 test groups were given a extract combination (dose 1.3 mg/20 gBW, 2.6 mg/20 gBW, and 5.2 mg/20 gBW). Carrageenan was induced in rat paws to conduct anti-inflammatory experiments using Winter's approach, while acetic acid was induced in mice's intraperitoneum to conduct analgesic testing using Sigmund's method. The percentage of inhibition of leg edema in rats was 11.33%, 18.90%, and 19.10% for the three doses of the combined extracts of *C. esculenta* and *Z. officinale*, and 22.72% for Na-diclofenac (p<0.05). The percentage inhibition in the analgesic test in the positive control group and the three test groups was 61.17%, 41.19%, 51.79%, and 52.35%, respectively (p<0.05). The combination of the *C. esculenta* and *Z. officinale* extracts exhibited anti-inflammatory and analgesic effects. Dose of 2.6 mg/20 gBW and 5.2 mg/20 gBW as effective as Na-diclofenac.

Keywords: Analgesic and anti-inflammatory effect, *Colocasia esculenta* L., *Zingiber officinale* L.

Abstrak: Kandungan metabolit aktif dari tanaman *Colocasia esculenta* L. dan *Zingiber officinale* L. dilaporkan dapat mengurangi rasa nyeri dan antiinflamasi. Penelitian ini bertujuan untuk menguji efek anti-inflamasi dan analgesik dari kombinasi ekstrak *C. esculenta* dan *Z. officinale*. Tigapuluh ekor tikus dan mencit masing-masing dibagi menjadi 6 kelompok (n=5) yaitu kelompok normal, kontrol negatif, kontrol positif (Na-diklofenak), dan 3 kelompok uji diberi kombinasi ekstrak (dosis 1,3 mg/20 gBB, 2,6 mg/20 gBB, dan 5,2 mg/20 gBB). Metode Winter digunakan untuk uji antiinflamasi dengan menginduksi karagenan pada kaki tikus, dan metode Sigmund untuk uji analgesik dengan induksi asam asetat secara intraperitoneal pada mencit. Pembengkakan kaki mencapai tingkat maksimum pada jam ke-5. Persentase penghambatan edema kaki pada tikus berturut-turut adalah 11,33%; 18,90%; 19,10% untuk 3 dosis kombinasi ekstrak *C. esculenta* dan *Z. officinale* dan 22,72% untuk Na-diklofenak (p<0,05). Persentase penghambatan uji analgesik pada mencit kelompok kontrol positif dan 3 kelompok uji berturut-turut adalah 61,17%; 41,19%; 51,79% dan 52,35% (p<0,05). Kombinasi ekstrak *C. esculenta* dan *Z. officinale* memberikan efek anti-inflamasi dan analgesik. Dosis 2,6 mg/20 gBB dan 5,2 mg/20 gBB memiliki efek setara dengan Na-diklofenak.

Kata kunci: *Colocasia esculenta* L., efek analgesik dan anti-inflamasi, *Zingiber officinale* L.

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INTRODUCTION

PAIN can be initiated following inflammation and/or peripheral nerve injury. It is a consequence of the pathological functioning of the nervous system, rather than only a symptom. Pathogen-associated and damage-associated molecular patterns are released as an outcome of the inflammatory response carried on by microbes or tissue damage. Pain and inflammation are significant social, health, and economic burdens worldwide. Pain management is a worldwide challenge owing to the side effects of classical treatments⁽¹⁾. Treatment to reduce pain and inflammation aims to enable the patient to perform normal activities and improve the patient's quality of life by reducing limitations in carrying out daily physical activities. Drugs that are usually used in pharmacological therapy are nonsteroidal anti-inflammatory drugs (NSAIDs), which, if used in the long term or at high doses, can cause other complications, especially in the upper digestive tract. The complications mentioned are examples of ulcers, bleeding in the digestive tract, and gastroesophageal reflux disease (GERD)⁽²⁾. Therefore, we need a safer alternative with minimal side effects that is also effective. A source of Indonesia's wealth is its diverse flora. In addition to having been trusted for years, herbal medicines have fewer side effects than chemical drugs. This is also supported by the Indonesian government regulations, which also set standards for herbal medicine regulations in Indonesia. In addition, with increasing public awareness of the back-to-nature movement and the side effects of chemical drugs, other options that are equally effective in reducing pain and inflammation sufferers are sought.

One of the plants that has antinociceptive or analgesic and anti-inflammatory effects is *Colocasia esculenta* L.⁽³⁾. The leaves are used to treat bee stings, ulcers, boils, inflammation with purulent skin, diarrhea, and night sweats. Juice from the leaves is useful as a stimulant, expectorant, astringent, appetite enhancer, and for the treatment of ear pain (otalgia). *C. esculenta* can be useful for aches or pain in the body. The tuber juice can be used as alopecia, laxative, demulcent, analgesic, galactagogue, antidote to bee and wasp stings⁽⁴⁾. *C. esculenta* bulbs contain alkaloid and flavanoid compounds, in which alkaloids have analgesic properties that can reduce pain and flavanoids which are phenolic compounds that act as anti-inflammatory agents⁽⁵⁾. *Zingiberis officinale* is useful for pain management. This is due to the content of bioactive substances in the rhizome, which can be useful as anti-inflammatory agents by inhibiting the expression of pro-inflammatory mediators. Thus, the pain felt by sufferers due to osteoarthritis can be

reduced⁽⁶⁾. *Z. officinale* rhizome which also contains flavanoids as well as several other compounds such as gingerols, shogaol, and zingerone provide analgesic, anti-inflammatory, antioxidant, cardiotoxic and anti-carcinogenic effects⁽⁷⁾.

Many herbal medicines are based on *C. esculenta* and *Z. officinale*. However, there is a very complex process for commercializing herbal medicine, especially at the phase of standardizing the quality of raw materials and during the production process, which consists of extraction, drying, and packaging. In order to turn a herbal medicine into a nutraceutical that is halal, safe, and proven to be effective for treating pain and inflammation that commonly occur in osteoarthritis, a preclinical test was carried out on a combination of *C. esculenta* and *Z. officinale* extracts. Several methods are often used to scientifically prove the efficacy of herbs, namely anti-inflammatory tests using the method of forming edema on the paw of rats by injecting a small amount of irritant intraplantar⁽⁸⁾. Sigmund's method is commonly used to test the analgesic effect which is carried out on mice by giving acetic acid orally and observing the writhing experienced by mice⁽⁸⁾. This study was conducted to determine the effect of the combination of *C. esculenta* and *Z. officinale* based on edema inhibition in the paws of rats induced by 1% carrageenan solution and to determine the analgesic effect based on the inhibition of writhing in mice induced by 3% acetic acid.

MATERIALS AND METHODS

MATERIALS. Experimental animals were obtained from Laboratorium Puslitbang Biomedis and Teknologi Dasar Kesehatan, Balitbangkes, Kemenkes RI. The experimental animals in this study were male Wistar rats aged 2-3 months with a body weight of 150-200 g for the anti-inflammatory test, male white Swiss Webster mice aged 2-3 months with a body weight of 25-30 g for the analgesic test, combination of 350 mg *Colocasia esculenta* L. extract and 150 mg *Zingiber officinale* extract in 500 mg capsules (PT. Titan Pilar Utama Niaga, Indonesia), sodium diclofenac (Novell), CMC (Brataco Chemical), carrageenan (Sigma-Aldrich), acetic acid (Brataco Chemical), and Aquadest (Brataco Chemical).

Tools. Stopwatch, analytical balance, oral gavage, Plethysmometer (Panlab) glassware (Pyrex).

METHODS. Experimental Design. This research was conducted at the Pharmacology Laboratory Faculty of Pharmacy, Universitas Pancasila, Srengseng Sawah Jagakarsa, South Jakarta. The test animals were acclimatized for one week. Test animals were placed in a room with a temperature

of $25 \pm 2^\circ\text{C}$, constant air humidity of $65 \pm 10\%$, adequate lighting (12-hour cycle of light and dark), pelleted food, and water ad libitum. The experimental assays were approved by the Health Research Ethics Committee of the Faculty of Medicine, Universitas Indonesia (number: KET-1355/UN2).F1/ETIK/PPM.00.02/2022.

Anti-Inflammatory Test for the Combination of *C. esculenta* and *Z. officinale* Extracts in Carrageenan-Induced Rat Paw. The anti-inflammatory test was carried out using the Winter method with sub-plantar injection of carrageenan until edema formed on the paw of the rats⁽⁸⁾. At the time of testing, the rats were weighed and as many as 30 rats were randomly divided into six groups with each group consisting of 5 rats. The six groups were the normal control group, the negative control group were given CMC-Na, the positive control group which were given Na diclofenac 2.7 mg/200 g BW, the test group were given combination of *C. esculenta* and *Z. officinale* extracts suspended with CMC-Na at a dose of 1.3 mg/20 g BW, dose of 2.6 mg/20 g BW, and a dose of 5.2 mg/20 g BW. Before treatment, the initial volume of the rat paw was measured by dipping the paw into a Plethysmometer. In each anti-inflammatory test group, rats were orally administered the test substance according to the treatment dose of each group. Rats were given the test drug orally in each anti-inflammatory test group at the appropriate treatment dose. After administration of carrageenan, measured the volume of edema on the paws of the rats every 1 hour for 5 hours (at 1st, 2nd, 3rd, 4th, 5th hour). The data obtained from each group for the anti-inflammatory test are presented in a graph, and the Area Under Curve (AUC) was calculated, the percentage of anti-inflammatory activity in the test group, and the percentage of anti-inflammatory activity when compared to the positive control of Na diclofenac.

Analgesic Test for the Combination of *C. esculenta* and *Z. officinale* Extracts by Acetic Acid-induced Writhing in Mice. The analgesic test effect was carried out on experimental mice using the Siegmund method, by counting at the writhing response in mice given 3% acetic acid⁽⁸⁾. In this test, mice were fasted for ± 18 h while still being given a drink. A total of 30 mice were taken randomly and divided into six groups, with 5 mice in each group, as follows: normal control group, negative control group, positive control group given Na diclofenac 0.39 mg/20 g BW, combination of *C. esculenta* and *Z. officinale* extracts at a dose of 1.3 mg/20 g BW, the test group at a dose of 2.6 mg/20 g BW, the dose 5.2 mg/20 g BW. In each analgesic test group, rats were orally administered the test substance according to the

treatment dose of each group. Thirty minutes later the mice were induced intraperitoneally with 3% acetic acid solution as much as 0.2 mL/20 g BW. The mice were placed in cages. After administration of acetic acid, the mice exhibited a writhing response, which was indicated by rubbing their abdomen against the bottom of the cage and moving the front pair of legs pulled forward and a pair of hind legs pulled back. Recorded the amount of writhing response shown by the mice within 5 minutes for 1 hour (at 5, 10, 15, 20, 25, 30, 35, 40, 45, 55, 60 minutes). The data obtained from each group for the analgesic activity test are presented in a graph and the Area Under Curve (AUC) was calculated, the percentage of analgesia that occurred in the test group, and the percentage of effectiveness of analgesia when compared to the positive control of Na diclofenac.

Data Analysis. The Statistical Package for the Social Sciences application was used to examine the data on the Area Under Curve that were received from each test group. A One-way Analysis of Variance (ANOVA) was carried out if the data had a normal distribution and were homogeneous. If the results of the ANOVA test showed statistically significant differences in each test group, then the analysis was continued using the Least Significant Difference (LSD) test with a significance level of 5% (0.05) to determine whether there was a difference between each individual test. However, if the Area Under Curve did not meet the normal distribution and homogeneity requirements, the Kruskal–Wallis test was used.

RESULTS AND DISCUSSION

Anti-inflammatory Activity of *C. esculenta* and *Z. officinale* Extracts. There was a decrease in the average volume of rat paw edema in the positive control group and the three test groups showed edema 2 hours after being induced by carrageenan. This demonstrates the ability of Na diclofenac and the combination of *C. esculenta* and *Z. officinale* extracts to inhibit edema volume. The data is presented in graphical form, which can be seen in Figure 1. The graph shows a significant difference between the normal and negative control groups ($p < 0.05$), indicating that carrageenan can induce acute edema in rat paws. Between the test group and the negative control group, there was a discernible difference ($p < 0.05$). Between the 2.6 mg/20 gBW and 5.2 mg/20 gBW dosing groups, as well as when compared to the positive group that received Na diclofenac, there was no discernible change.

Assessing the effectiveness of ingredients that can inhibit inflammation is by seeing a decrease in edema volume on the rats' paw, and calculating the

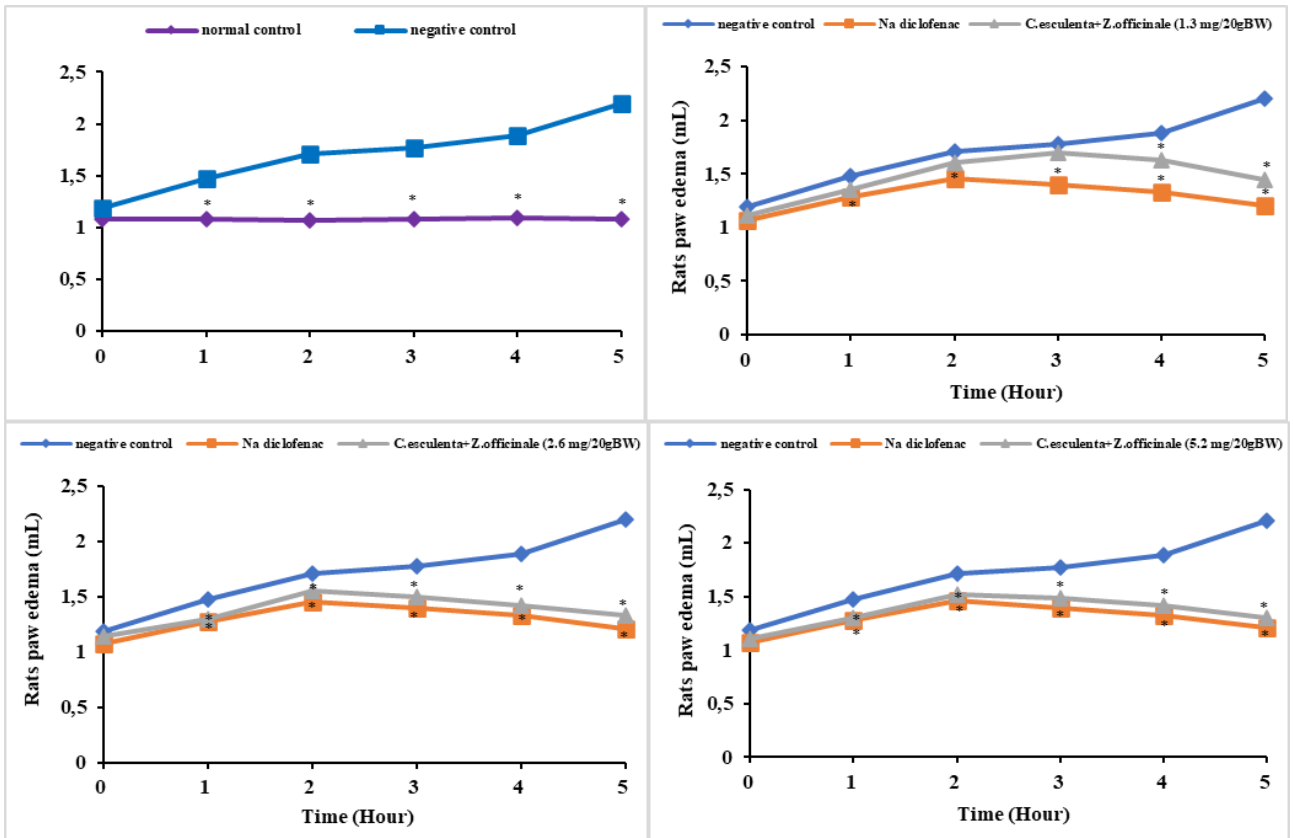


Figure 1. Reduction of edema formation in rat paws. Animals (n=5) were treated with three doses of *C. esculenta* and *Z. officinale* extracts. Sodium diclofenac was used as the positive control (0.39 mg/20 gBW). Data obtained from animal experiments are expressed as the mean. * indicates significant differences ($p < 0.05$) from the negative control (carrageenan-induced group).

AUC (Area Under Curve) from graph on the test for 5 hours. The greater the AUC value, the smaller the effectiveness of the anti-inflammatory drug. The average AUC value of the negative control group was higher than that of other test preparation groups. This shows that carrageenan can induce the formation of edema in the paw of rats ($p < 0.05$) compared to the normal paw of rats. The AUC values of the test preparation and positive control groups were lower than the AUC value of the negative control group ($p < 0.05$). Of the three test doses, it was found that the dose of 5.2 mg/20g BW was better at inhibiting the formation of edema on paw of the rats as indicated by the lowest average AUC value (Figure 2.)

Carrageenan-induced paw edema assays in rats have been widely used to determine anti-inflammatory effects. Carrageenan-induced paw edema in rats is a sensitive and reproducible test used to screen new molecules with anti-inflammatory activities. Therefore, carrageenan-induced inflammation has great predictive value for anti-inflammatory drugs functioning as mediators of acute inflammation because it induces an acute and local inflammatory response that is useful for detecting orally active anti-inflammatory medicines^(9,10). First step of acute inflammatory response is characterized by edema often formed because of

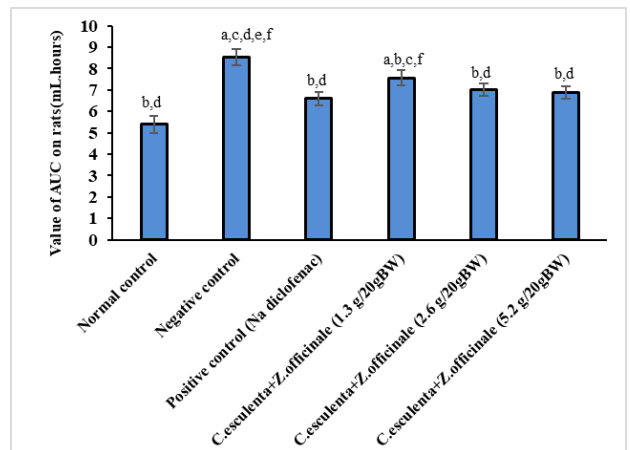


Figure 2. Decreased value of AUC in anti-inflammatory test on rats (mL.hours) after treatment with na diclofenac and combination of *C. esculenta* and *Z. officinale* extracts. (a) p value < 0.05 vs. Normal group; (b) p value < 0.05 vs. negative control group; (c) p value < 0.05 vs. positive control group (Na diclofenac) (d) p value < 0.05 vs. combination of *C. esculenta* and *Z. officinale* extracts (1.3 g/20 gBW) (e) p value < 0.05 vs. combination of *C. esculenta* and *Z. officinale* extracts (2.6 g/20 gBW) (f) p value < 0.05 vs. combination of *C. esculenta* and *Z. officinale* extracts (5.2 g/20 gBW)

exudation of fluid and plasma proteins^(11,12). The early stage (0–1 h) is characterized with the secretion of histamine, serotonin, bradykinin, and the overproduction of prostaglandins in surrounding damaged tissue. The later stage (1–6 h) is the target of the most clinically

effective anti-inflammatory drugs because of the overproduction of pro-inflammatory mediators such as bradykinin, leukotrienes, prostaglandins, platelet-activating factor, nitric oxide, and proteolytic enzymes by neutrophils in inflamed tissues^(13,14). In our work we found that edema formation was reduced significantly at 5-h post-treatment.

Anti-inflammatory test studies were conducted on negative controls, positive controls, and combinations of *C. esculenta* and *Z. officinale* extract doses of 1.3 mg/20 g BW, 2.6 mg/20 g BW and 5.2 mg/20 g BW. Before edema induction, measurements were performed at 0 h (before induction of carrageenan). This was performed to determine and ensure that the rat paws were not edematous and were in normal condition.

The effect of combinations of *C. esculenta* and *Z. officinale* extracts on carrageenan-induced swelling of rat paws was monitored (Figure 1). For 3 dose tested extracts, paw swelling reaches the maximum level on the 5th hour and ranged from 11.33% for dose of 1.3 mg/20 gBW to 19.10 % for dose of 5.2 mg/20 gBW (p value<0.05). In the treated rats, *C. esculenta* and *Z. officinale* extract combination, given at a dose of 2.6 mg/20 gBW and 5.2 mg/20 gBW, caused a significant diminution in paw swelling levels at 5 h of treatment and varied significantly (p<0.05) as compared to dose of 1.3 mg/20 g BW (Figure 1). Na diclofenac was more effective than the combination of *C. esculenta* L. and *Z. officinale* extract (p<0.05), as it caused a 22.72% reduction in paw swelling levels. The decrease in edema volume after the administration of diclofenac Na and the three combination doses of *C. esculenta* and *Z. officinale* extract occurred 2 h after carrageenan induction. This showed that Na diclofenac and a combination of *C. esculenta* and *Z. officinale* extracts reduced edema in paw rats. In the negative control group, there was no decrease in the paw edema volume. Na diclofenac works as an anti-inflammatory by inhibiting COX and prostaglandin synthesis⁽¹⁵⁾. The low anti-edematogenic effect observed for Na diclofenac during the early stage looks to be normal since the non-steroidal anti-inflammatory remedies are unable to inhibit the early stage of swelling as reported by Hmidani A⁽¹⁶⁾. The fact that the oral administration combination of *C. esculenta* L. and *Z. officinale* extracts inhibited paw edema formation significantly (p<0.05) compare to negative control during all phases of inflammation (Figure 1), suggests that combination of *C. esculenta* and *Z. officinale* extracts compounds inhibit diverse facets and chemical mediators of inflammation. Therefore, it can be hypothesized that these compounds might be acting through the inhibition of histamine release, cyclooxygenase enzymes

that produced prostaglandin, lysosomal enzymes as well as scavenging ability the free radical produced by polymorphonuclear leucocytes that would lead to tissue damage in the site of inflammation⁽¹⁶⁾.

According to previous study, the tubers and petioles of *C. esculenta* contain anthocyanins, polyphenols and saponins⁽¹⁷⁾. The anthocyanins contained in *C. esculenta* include pelargonidin 3-glucoside, cyanidin 3-rhamnoside, and cyanidin 3-glucoside which can inhibit the expression of proinflammatory mediators such as TNF- α , IL-6, and nitrogen monoxide (NO)⁽¹⁸⁾. Cyanidin 3-rhamnoside reduced NO, PGE-2, COX-2, and inducible nitric oxide synthase (iNOS) levels by decreasing NF- κ B expression and increasing I κ B α expression⁽¹⁹⁾. Cyanidin 3-glucoside reduces levels of TNF- α , IL-1 β , and IL-6 by inhibiting NF- κ B activation and MAPK phosphorylation⁽²⁰⁾. *C. esculenta* also contains flavonoids *orientin*, *isoorientin*, *isovitexin*, *luteolin 7-O-glucoside*, and *vicenin-2*. *Orientin* can decrease the production of proinflammatory mediators such as IL-1 β , TNF- α , IL-6, IL-18, COX-2, and inducible nitric oxide synthase (iNOS)⁽²¹⁾. *Z. officinale* contains the flavonoids shagaol, gingerol, 6-paradol which can be useful as anti-inflammatories by inhibiting the expression of pro-inflammatory mediators⁽⁶⁾. Therefore, the synergistic effect of these compounds identified in the combination of *C. esculenta* and *Z. officinale* extracts may be responsible for their potent anti-inflammatory activity. These variations in the reduction in paw swelling levels among the combinations of *C. esculenta* and *Z. officinale* extract dose varieties may be related to the quantity and chemical diversity at each dose.

Analgesic Activity of The Combination of *C. Esculenta* and *Z. Officinale* Extracts. In this study, the decreased number of writhes in mice in the positive control group and the three test groups occurred in the 20th minute after induction with acetic acid. This shows the ability of the test substance to inhibit the increase in the number of ticklings in mice. The data is displayed in graphical form, which can be seen in Figure 3. The statistic showed a significant difference between the normal and negative control groups (p value <0.05), this shows that acetic acid can induce pain in mice. A significant difference was observed between the negative control and test groups (p<0.05). There was no significant difference between the positive control groups, doses of 2.6 mg/20 gBW and 5.2 mg/20 gBW. These results indicate that an extract dose of 2.6 mg/20 gBW and 5.2 mg/20 gBW is equally potent as Na diclofenac.

Assessment of the effectiveness of analgesic drugs was performed by observing an increase or decrease in the number of writhing in mice and the AUC value,

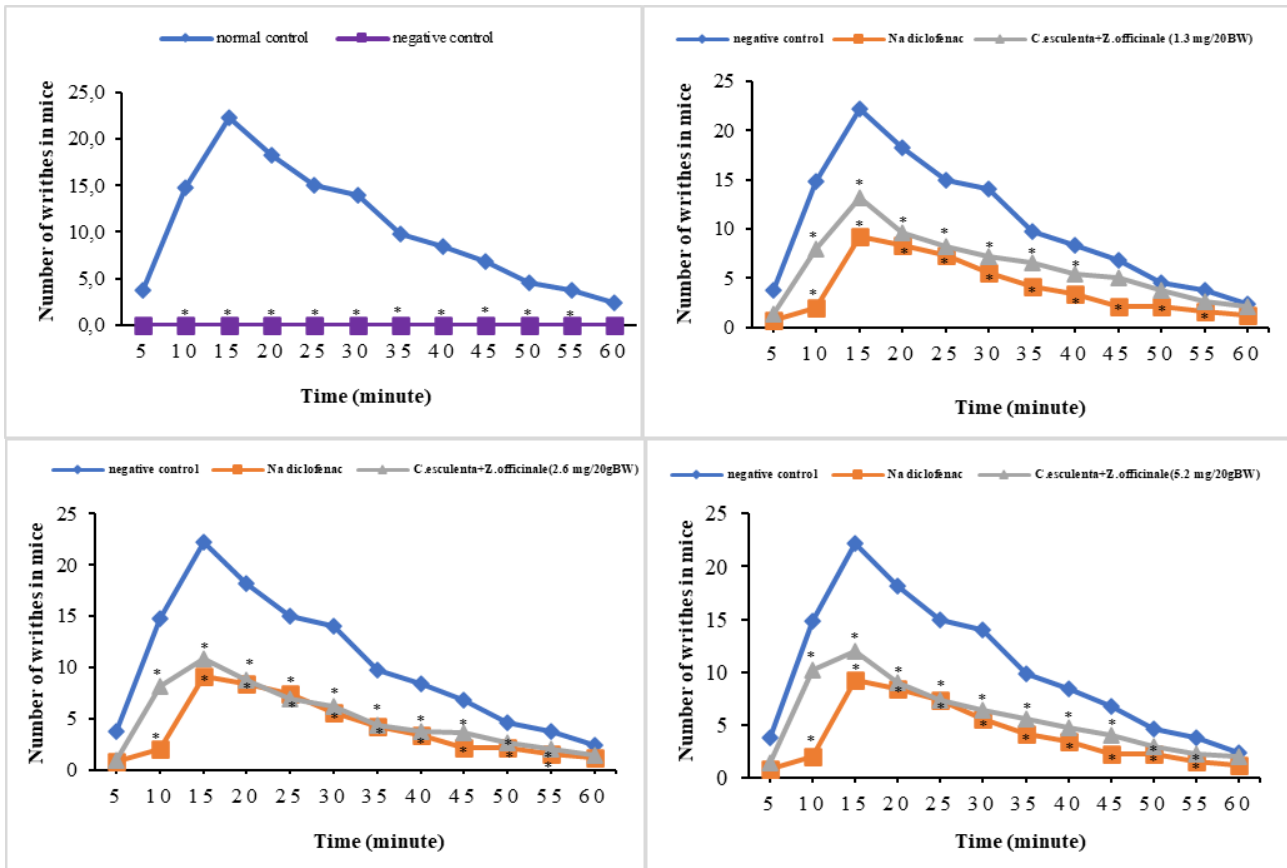


Figure 3. Decreased number of writhes in mice. Animals (n=5) were treated with three doses of *C. esculenta* and *Z. officinale* extracts. Sodium diclofenac was used as a positive control (0.39 mg/20 gBW). Data obtained from animal experiments were expressed as mean. *: points out the significant differences ($p < 0.005$) from the negative control: acetic acid-induced group

as well as the effectiveness of the inflammation test. In this study, it can be seen that Na diclofenac and the three test doses had an analgesic effect because the AUC of the negative control was higher than the AUC of the positive control and the other doses ($p < 0.05$). The AUC value of the 2.6 mg/20 gBW dose had the lowest AUC value compared to the other dose groups and was equivalent to the AUC of Na diclofenac. Based on statistical tests, the results showed that there were significant differences between the negative and positive control groups, with doses of 1.3 mg/20 gBW, 2.6 mg/20 gBW and 5.2 mg/20 gBW. This showed that there was a decrease in the amount of writhing in the group administered sodium diclofenac and the three test doses compared to the negative group. A comparison of the AUC values is shown in Figure 4.

In testing the analgesic effect of the combined extracts of *C. esculenta* and *Z. officinale*, the acetic acid-induced nociception model involves the non-selective cationic channels in the peripheral nociceptive neurons (C fiber), causing the release of pain mediators such as prostaglandins (PGs), cytokines (IL-8, TNF- α , and IL-1 β), cyclooxygenase (COX), lipoxygenase (LOX), serotonin, bradykinin, and histamine in the peripheral tissue fluid⁽²²⁾. This method is also specific

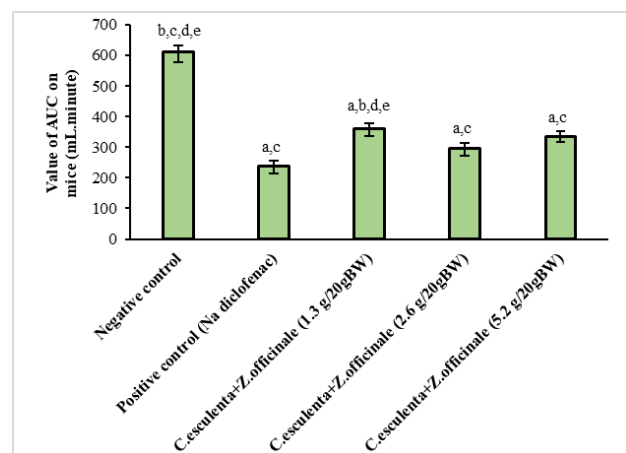


Figure 4. Decreased value of AUC in analgesic test on mice (mL.minute) after treatment with na diclofenac and combination of *C. esculenta* and *Z. officinale* extracts (a) p value < 0.05 vs. negative control group; (b) p value < 0.05 vs. positive control group (Na diclofenac) (c) p value < 0.05 vs. combination of *C. esculenta* L.and *Z. officinale* extracts (1.3 g/20 gBW) (d) p value < 0.05 vs. combination of *C. esculenta* and *Z. officinale* extracts (e) p value < 0.05 vs. combination of *C. esculenta* and *Z. officinale* extracts (5.2 g/20 gBW).

for drugs suspected to have prostaglandin inhibitory activity. The pain caused by glacial acetic acid also only lasts for an hour and then slowly fades away⁽²³⁾.

Therefore, acetic acid-induced writhing was used

as a stimulus to evaluate the anti-nociceptive activity mediated by the peripheral nervous system⁽²⁴⁾ In this model of nociception, behavioral responses, namely abdominal constrictions, the extension of the forelimbs, and elongation of the body^(25,26) were evaluated. These responses are integrated by dorsal horn, and sensitive to peripherally acting analgesics such as NSAID derivatives because increased levels of the pain mediators provoke peripheral nociceptive neurons entering into the dorsal horn of the central nervous system⁽²⁷⁾. The oral single dose of combined extracts of *C. esculenta* and *Z. officinale* showed a significant effect ($p < 0.01$) on acetic acid-induced (nociception model) behavioral responses when compared with the negative control, which was administered with 0.5% CMC Na. We demonstrated that the combined extracts of *C. esculenta* and *Z. officinale* significantly prolonged the latency response time to acetic acid stimuli and reduced nociceptive behavioral responses. These results suggested that the anti-nociceptive effect of the combined extracts of *C. esculenta* and *Z. officinale* in the acetic acid model could be driven by antinociceptive action involving peripheral mechanisms. The antinociceptive effect of combined extracts of *C. esculenta* and *Z. officinale* might be attributed to the blockade of the release of endogenous inflammatory mediators or direct inhibitory activity at nerve endings of the primary afferent neurons and/or inhibition of the transmission pathway entering the dorsal horn⁽²⁷⁾. In addition to this, flavonoid, anthocyanin, shogaol has been shown to downregulate the tumor necrosis factor and suppress of the nuclear factor kappa B (NF- κ B) activation in the brain and spinal cord⁽²⁸⁾. Moreover, the peripheral antinociceptive effect of combined extracts of *C. esculenta* and *Z. officinale* is directly linked to its flavonoid orientin, which has an anti-inflammatory effect via TNF- α , IL-6, COX-2, and may control nociception at dorsal horn through GABA receptors⁽²⁹⁾. The GABA-inhibitory neurons were found abundance in the spinal cord and have strongly been suggested involve in pain inhibition⁽³⁰⁾.

In the analgesic test, it was found that the average number of writhes mice in the negative control was higher than the average number of tickling positive controls and the combination of *C. esculenta* L. and *Z. officinale* extracts doses of 1.3 mg/20 g BW, 2.6 mg/20 g BW, and 5.2 mg/20 g BW. This was because the negative control was only given CMC Na, so there was no inhibition of prostaglandin synthesis. In contrast, the positive control and the combination of *C. esculenta* L. and *Z. officinale* extracts decreased writhes ($p < 0.05$) compared with the negative control. This demonstrates the analgesic actions of Na diclofenac and the three combined doses of *C. esculenta* L. and

Z. officinale extracts. Additionally, the statistical test reveals a significant difference between the three combined doses of *C. esculenta* L. and *Z. officinale* extracts: the positive control, the negative control, and the negative control. The combination of *C. esculenta* L., and *Z. officinale* extracts at doses of 1.3 mg/20 g BW, 2.6 mg/20 g BW and 5.2 mg/20 g BW also had an analgesic effect with the percentage of mice writhing inhibition successively 41.19%, 51.79% and 52.35%. Increasing the combined dose of *C. esculenta* L. and *Z. officinale* extract from 2.6 mg/20 g BW to 5.2 mg/20 g BW did not show a significant difference so a dose of 2.6 mg/20 g BW was effective as an anti-inflammatory and analgesic.

Percentage of Edema and Writhing Inhibition.

The percentage of edema inhibition and writhing inhibition by diclofenac Na and the three combinations of *C. esculenta* L. and *Z. officinale* extracts are shown in Figure 5. It shows that the positive control has edema inhibition, and the amount of stretching inhibition is better than that of the test preparation group. The 2.6 mg/20 gBW and 5.2 mg/20 gBW test groups had comparable amounts of swelling inhibition.

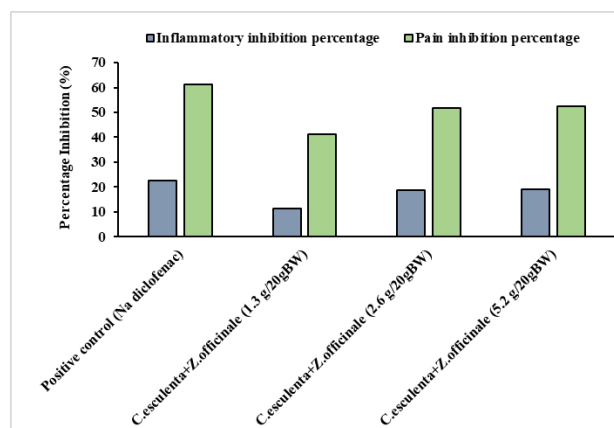


Figure 5. Percentage inhibition of edema and writhing.

Percentage of Anti-inflammatory and Analgesic Effectiveness. The percentage of effectiveness was calculated by comparing the AUC of the test group with the positive control of Diclofenac Na (Table 1.)

In the calculation results (Table 1.) it was found that the analgesic test preparation group at doses of 2.6 mg/20 gBW and 5.2 mg/20 gBW had anti-inflammatory and analgesic effectiveness equivalent to the positive control of Na diclofenac (p -value < 0.05)

Non-steroidal anti-inflammatory drugs (NSAIDs) are the first-line therapy for mild-to-moderate pain. However, the many side effects, especially in the gastrointestinal tract, and cardiovascular risks make NSAIDs unusable in the long term, especially in elderly patients⁽⁹⁾. The search for natural compounds with fewer adverse effects on patients has been growing, and over the past few years, more attention has been

Table 1. Percentage anti-inflammatory and analgesic effectiveness.

Groups	Percentage of Effectiveness	
	anti-inflammatory effectiveness (%)	analgesic effectiveness (%)
<i>C. esculenta</i> L.+ <i>Z. officinale</i> (1.3 g/20 gBW)	49.87	67.33
<i>C. esculenta</i> L.+ <i>Z. officinale</i> (2.6 g/20 gBW)	83.18	84.67
<i>C. esculenta</i> L.+ <i>Z. officinale</i> (5.2 g/20 gBW)	84.01	85.58

given to herbal medicines. Natural ingredients have been used empirically by the people of Indonesia as an alternative treatment for various diseases, such as pain and inflammation because they are believed to be safer and have minimal side effects. In this study, the anti-inflammatory and analgesic effects of the combination of *Colocasia esculenta* L. and *Zingiber officinale* extract were carried out so that it could potentially be given to reduce pain and inflammation. The compounds contained in the tubers of *C. esculenta* L. can be useful for aches and pain in the body. The tuber juice can be used as alopecia, laxative, demulcent, analgesic, galactagogue, antidote to bee and wasp stings⁽⁴⁾. *Zingiber officinale* is also useful for the treatment of pain. This is due to the content of bioactive substances in the rhizome, which can be useful as anti-inflammatory agents by inhibiting the expression of pro-inflammatory mediators. Thus, the pain felt by sufferers can be reduced⁽⁶⁾.

CONCLUSION

This is the first study to investigate the anti-inflammatory and analgesic properties of a combination of *C. esculenta* and *Z. officinale* extract. Our findings suggest that these extracts mediate anti-inflammatory and analgesic activities. These findings may relate to considerable quantity of anthocyanins, polyphenols and saponins in *C. esculenta* and shogaol, gingerol, 6-paradol in *Z. officinale*. The combination of *C. esculenta* and *Z. officinale* extract dose of 2.6 mg/20 gBW and 5.2 mg/20 gBW was as effective as that of sodium diclofenac.

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