Anti-inflammatory effectiveness of combination of basil leaf extract (Ocimum americanum L) and turmeric rhizome (Curcuma domestica Val) in rats with carrageenan induction

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ABSTRACT

Background : Inflammation is the body's response to tissue injury, usually caused by physical trauma, damaging chemicals or microbiological agents. Basil leaves and turmeric rhizomes have been shown to be effective as anti-inflammatories. The combination of these plants aims to determine the effective dose as an anti-inflammatory.

Objective: To determine the anti-inflammatory effectiveness of a combination of basil leaf extract *(Ocimum americanum L)* and turmeric rhizome *(Curcuma domestica Val)* in rats with carrageenan induction.

Method: This study is an experimental laboratory study using 28 male white rats of the Wistar strain induced by 1% carrageenan. KN using CMC Na, KP sodium diclofenac, EK dose of 10 mg/KgBW, RK extract dose of 600 mg/KgBW and EKRK dose composition (2.5: 450 mg/KgBW, 5: 300 mg/KgBW, and 7.5: 150 mg/KgB B) . Continued with anti-inflammatory power. then data analysis using SPSS version 26 began with a normality test and a homogeneity test as a requirement for the one-way ANOVA test.

Results: The percentage results of anti-inflammatory power of KP 28.71%, EK 10 mg/KgBB 33.00%, RK 600 mg/KgBB 33.23%, and EKRK 2.5: 450 mg/KgBB, 5: 300 mg/KgBB, dose 7.5: 150 mg/KgBB respectively, namely 31.21%, 28.65%, and 28.35% . The data obtained were continued with statistical tests using SPSS version 26. The results of statistical tests using normality and homogeneity tests were P> 0.05 and the one-way ANOVA test value was P> 0.05.

Conclusion: It can be concluded that the results of this study showed that in all treatment groups it was proven effective as an anti-inflammatory. So it is hoped that this study can be used as a reference for further research.

Keywords: anti-inflammatory; basil leaves; turmeric rhizome

INTRODUCTION

Inflammation is the body's response to tissue injury usually caused by physical trauma, damaging chemicals or microbiological substances. Symptoms of inflammation are pain (*dolor*), heat (*kalor*), redness (*rubor*), swelling (*tumor*), and changes in function (*fungsiolaesa*) (Sukmawati, Wati, and Muflihunna,2022) . Chronic inflammation is recognized as the most significant cause of death in the world today with more than 50% of deaths. Inflammation underlies various diseases including asthma, GERD, rheumatoid arthritis, autoimmune diseases, diabetes mellitus and stroke. Inflammation is a normal condition of the body that functions to protect damaged body tissue and accelerate healing (Hidayati et al,2022) . Treatment of inflammation is divided into two groups, namely steroids and non-steroids. Anti-inflammatory drugs that are often used are the steroid group (NSAIDs) where the working mechanism of this non-steroid drug group is to inhibit the synthesis of prostaglandins and affect the enzymes that play a role such as cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) enzymes (Meltyza, Indriyanti, and Rahimah,2015).

Indonesia is one of the countries known for its abundant natural wealth, in which there are various plants that can be used as medicine, people believe that medicines derived from nature rarely cause side effects that can be detrimental. One of the plants that has the potential as an anti-inflammatory is basil leaves *(Ocimum americanum L.)* and turmeric rhizomes *(Curcuma domestid Val.).*

Basil leaves *(Ocimum americanum L.)* have active flavonoid compounds where the anti-inflammatory activity of flavonoids is to inhibit cyclooxygenase and lipoxygenase so that there is a limitation of the number of inflammatory cells that migrate to the injured tissue, so that the inflammatory reaction will last shorter (Sa'adah et al, 2020) . Basil leaves have flavonoid compounds that have been proven effective as anti-inflammatories by reducing the number of leukocytes and reducing complement activity, thereby reducing leukocyte adhesion to the endothelium and resulting in a decrease in the anti-inflammatory response (Istiqomah, Nofita, and Hidayaturahmah,2023) . Based on previous research on basil herbs with a dose of 10 mg / kgBW, it is effective in having the largest percentage of edema inhibition, which is 78.17% (Sukaina,2013) . The results of the study showed that a dose of 160 mg / 200 g BW showed the best inhibition of inflammation, which was 44.83% (Saputri and Zahara,2016) .

Turmeric *(Curcuma domestid Val.)* has an active compound curcumin where the anti-inflammatory mechanism of curcumin is to inhibit prostaglandins, but is also associated with inhibition of pro-inflammatory cytokines, and COX-2 (Nugraha,

Harfiani, and Pramesyanti,2022) . In a study, the suspension of ethanol extract of turmeric rhizomes at a dose of 600 mg/kgBW showed a significant anti-inflammatory effect compared to indomethacin suspension at a dose of 100 mg/kgBW (Meilina and Mukhtar,2019) . In the ethanol extract of white turmeric at a dose of 900 mg/KgBW, it was proven to have the same effectiveness as 25 mg of sodium diclofenac (Meilina and Mukhtar 2019) . Turmeric rhizomes at doses of 20 and 25 mg/KgBW have anti-inflammatory activity with an average protection of 64.79% (Viera Valencia and Garcia Giraldo,2019) .

The combination of anti-inflammatory is 2 types of anti-inflammatory used simultaneously and mutually affect the work of each anti-inflammatory. The combination is often used to maximize the effectiveness of anti-inflammatory. Basil leaves and turmeric rhizomes have been proven effective as anti-inflammatory. The combination of these plants aims to determine the effective dose as an anti-inflammatory.

METHOD

Material

The materials used in this study were: a combination of ethanol extract of basil leaves and turmeric rhizomes, 1% carrageenan, Sodium diclofenac with the trade name novel, 28 male white Wistar rats as test animals.

Tool

The tools used in this study are as follows: rat cage, rat food and drink containers, analytical balance, 3 cc syringe, 1 cc syringe, sonde needle, stirring rod, rotary evaporator, water bath, pyrex glassware.

Determination and collection of basil leaves and turmeric rhizomes

Before conducting research on basil leaves *(Ocimum americanum L.)* and turmeric rhizomes *(Curcuma domestica Val.),* plant determination was carried out to identify the type and ensure the truth of the simplicia. Plant determination was carried out at the UAD Yogyakarta Biology Laboratory.

Extraction of basil leaves and turmeric rhizomes

Basil leaves and turmeric rhizomes were extracted using the maceration method with 96% ethanol solvent for 3 days and stirred occasionally and re-maceration was carried out for 1 day on the 4th day. Then concentrated using *a Vacuum Rotary evaporator* at a temperature of 50ºC and evaporated again until a thick extract was obtained (Fitriyanti, Hikmah, and Astuti 2020; Pratiwi et al,2023) .

Phytochemical screening of basil leaf and turmeric rhizome extracts

Phytochemical screening was carried out with 5 tests, namely flavonoids, saponins, tannins, steroids/terpenoids and alkaloids. Flavonoid testing by adding Mg powder and concentrated HCl, the presence of flavonoids is indicated by the formation of a red, yellow or orange color. Saponin testing by adding hot water, then shaking for 10 seconds. Saponin content is indicated by the presence of foam. Tannin testing by adding FeCl3 , if it contains tannins a blackish blue color will form. Steroid/terpenoid testing by adding 3 drops of anhydrous acetic acid and concentrated sulfuric acid The formation of a reddish orange ring indicates the presence of terpenoids, while if there is a change in the red color in the first solution then turns blue indicates the presence of steroids. Alkaloid testing by adding dragendrof reagent, the formation of a yellow to light brown color indicates the presence of alkaloids (Astika, Sani K, and Elisma,2022)

Test of the anti-inflammatory effectiveness of a combination of basil leaf extract and turmeric rhizome

Preparation of test animals

The test animals used were male white rats of the Wistar strain that were adapted for 1 week (acclimatization) before being given treatment. The test animals were grouped into 7 treatment groups, each group consisting of 4 rats. The anti-inflammatory effectiveness test was carried out by preparing a CMC Na solution for KN, Diclofenac Sodium solution for KP, EK 10 mg/KgBB, RK 600 mg/KgBB, and EKRK (2.5: 450 mg/KgBB, 5: 300 mg/KgBB, and 7.5: 150 mg/KgBB), and making 1% carrageenan for the formation of edema in the rat's feet.

Testing the anti-inflammatory effectiveness of basil leaves and turmeric rhizomes

Weigh the rat first and mark one of the rat's feet then measure the volume of the rat's feet using a plethysmometer then record the results of V_0 and continue with the administration of 1% carrageenan inraplantarly to all treatment groups and observed for 1 hour, re-measurement and record the results with the results of V_t. Then the rats were given oral treatment. Measurements were carried out for 6 hours at each hour the volume of edema was measured using a plethysmometer. evaluation of the decrease in edema on the soles of the rat's feet every hour.

Data analysis

The data collected is the decrease in edema volume calculated using the following formula: $\% \ edema = \frac{Vt - V0}{V0} \times 100\%$

Description: Vt: volume of edema after administration of carrageenan

Vo: initial volume before carrageenan injection

Then continue with the calculation of % edema inhibition with the following formula: $\%\ in\ h\ ibisi\ edema = \frac{a-b}{a} \times 100\%$

Information :

a : average percentage of edema in the negative control group after carrageenan injection

b : average percentage of edema in the treatment group after carrageenan injection

Continue by calculating the relationship between % edema and time and calculating AUC $_{0\text{-}6}$ using the formula:

$$AUC\ {}^{tn}_{tn-1} = \frac{Vt_{n-1}+Vt_n}{2}\ (t_n\text{-}t_{n-1})$$

$$\%\ daya\ antiinflamasi\ \frac{AUC_k - AUC_p}{AUC_k} x100\%$$

Information :

Vt_{n-1} = Average edema volume at t_{n-1}

Vt_n = Average edema volume at t_n

AUC_k = Average edema volume curve versus time for negative control

AUC_p = Average edema volume curve versus time for treatment

The data analysis used was the One Way ANOVA (Analysis Of Variance) test in the SPSS application. The steps of the *One Way* ANOVA test are started with the *Spahiro-Wilk normality test* and the *Levene homogeneity test,* with the requirement to have normal data distribution and homogeneous data variance, namely with a p value> 0.05. After the normality and homogeneity test requirements are met, *the One Way* ANOVA test is continued. If the p value <0.05 then all treatment groups have different anti-inflammatory effectiveness, if the p value> 0.05 then all treatment groups have the same anti-inflammatory effectiveness.

RESULTS AND DISCUSSION

Plant Determination

Basil leaves and turmeric rhizomes were taken from the Bondowoso area. The Determination Certificate of basil leaves with number 014/Lab.Bio/B/I/2024 and

turmeric rhizome with number 020/Lab.Bio/B/I/2024 were issued by the UAD Yogyakarta Biology Learning Laboratory.

Results of basil leaf and turmeric rhizome extraction

Extraction of basil leaves and turmeric rhizomes using the maceration method for 3 x 24 hours and remaceration was carried out for 1 x 24 hours with the aim of attracting compounds left over in the first maceration. The percentage yield was calculated using the thick extract obtained . To calculate the yield, the weight of the extract produced was compared with the weight of the weighed simplicia. A high yield value indicates that there are many bioactive ingredients in it. The percentage yield value produced is proportional to the amount of compound content (Hidayati et al,2022) . In this study, the yield value obtained can be seen in Table 1. The percentage yield results show the number of metabolite compounds that are attracted but cannot determine the type of compound .

The yield value obtained from the extraction of basil leaves using 96% ethanol was 19.90 % . In the extraction of turmeric rhizomes, a yield value of 14.89 % was obtained. In previous studies, the extraction of basil leaves using 96% ethanol obtained a yield value of 5.71% (Hamka, Arief NNoena, and Arsya Putri Azmin,2022) . Previous studies obtained the results of turmeric rhizome extraction using 96% ethanol of 23.33% (Ningsih, Nurrosyidah, and Hisbiyah,2018) . Looking at the results obtained in this study, the difference in the yield% results with previous studies can be caused by whether or not the yield results obtained are good.

Phytochemical screening results

Phytochemical screening was conducted to determine the chemical compounds contained in basil leaf extract *(Ocimum americanum L)* and turmeric rhizome *(Curcuma domestica Val)* . The screening results of basil leaf extract *(Ocimum americanum L) and* turmeric rhizome *(Curcuma domestica val)* can be seen in table 2.

The results of phytochemical screening that have been carried out show that basil leaf extract contains flavonoid, tannin, steroid and alkaloid compounds. This is in accordance with other studies where basil leaves have secondary metabolites of flavonoids, tannins, steroids, and alkaloids (Pratiwi et al,2023) .

The results of phytochemical screening of turmeric rhizome extract showed that it has flavonoid, tannin and alkaloid compounds. Previous studies have shown that it has flavonoid, tannin, alkaloid, and steroid compounds (Putri Rahayu et al,2024) . The difference between the results of this study and previous studies is that in this

study no steroids were found in previous studies steroids were found. The sensitivity of different test methods to the amount of chemical content of the natural ingredients tested can cause different phytochemical screening results.

Data on decreased edema in the soles of the feet of mice

Anti-inflammatory activity testing in this study was conducted for 4 days with 7 treatment groups in each group induced by carrageenan. Anti-inflammatory activity can be seen from the magnitude of the decrease in edema in the rat's feet. Data on the volume of edema in the rat's feet obtained from the measurement results for 6 hours after being induced by carrageenan can be seen in Figure 1.

Figure 1 shows an increase in the volume of edema in the soles of the rat's feet after carrageenan induction. Carrageenan can be used as an edema inducer because it does not leave scars, does not cause tissue damage, and increases the response to anti-inflammatory drugs (Istiqomah et al,2023) . The image above shows a significant decrease in edema volume in the positive control group and in all treatment groups.

Data on the results of percent edema and percent edema inhibition

Calculating the percentage of edema and the percentage of edema inhibition to determine how much inflammation inhibition is provided by each treatment tested. If the test animals induced by carrageenan experience a reduction in swelling (inhibition) of up to 50% or more, the material is considered to have an anti-inflammatory effect. The higher the level of inflammation inhibition, the greater the anti-inflammatory effect of the extract (Istiqomah et al,2023) . The results of this study can be seen in table 3 showing a decrease in edema in all treatment groups and positive controls and the percentage value of edema inhibition in the EK treatment group was 66.88%, RK 73.56%, EKRK composition 2.5: 450 is 68.72%, EKRK composition 5: 300 is 72.02% and EKRK composition 7.5: 150 is 71.34%. This shows that the RK sample group and the EKRK sample composition 5:300 have the ability to inhibit inflammation equivalent to the positive control, which is 72.10 % . In the EK and EKRK samples, the composition 2.5 :450 has the smallest percentage of inflammation compared to other compositions. The combination of EKRK composition 5:300 in this study is still greater than the previous study, namely the combination of turmeric rhizome and betel lime of 43.08% (Susanto et al,2023) .

The results of the area under the curve (AUC) data and the results of the percentage value of anti-inflammatory power (DAI)

The area under the curve between the average edema volume and time is called the area under the curve (AUC) (Fitriyanti et al,2020) . A drug has less anti-inflammatory activity in reducing edema volume if its AUC value is higher. Conversely, a lower AUC value indicates that the drug's anti-inflammatory activity is greater (Pramitaningastuti and Anggraeny,2017) . In this study, the AUC value and the percentage value of anti-inflammatory power can be seen in Table 4. It can be seen that the smallest AUC value is at a dose of RK 600 mg/KgBB of 1.40 while at the smallest combination dose, namely EKRK composition 2.5: 450 mg/kgBB of 1.44 and the largest AUC value is in the negative control of 2.10. In previous studies, the combination of Lombok basil *(Ocimum bacilicum)* and bay leaves *(Syzygium polyanthum)* had the smallest AUC value, namely (32.46) at a dose of 250:75 mg/KgBW (Putri et al,2022) Anti-inflammatory power (DAI) is the presentation of inhibition of inflammation symptoms. DAI can be represented as a percentage, and the percentage of anti-inflammatory power indicates the percentage of a compound's ability to carry out anti-inflammatory activity (Fitriyanti et al,2020) . The results of the percentage data on anti-inflammatory power in table 4 show that the highest DAI value in RK 600 mg/KgBB is 33.23. In the combination group in EKRK composition 2.5 : 450 mg/KgBB of 31.21. The results of the% DAI value obtained in all treatment groups have almost the same value . It can be concluded that all treatment groups have anti-inflammatory effectiveness.

The results of phytochemical screening showed that basil has flavonoid compounds where these flavonoids have strong anti-inflammatory activity. It can be seen in table 4 the % DAI value of EK is 33.00 % . The value obtained is greater than KP which is 28.71 % . Turmeric has curcumin compounds. Curcumin has strong anti-inflammatory activity, the same as basil, it can be seen in table 4 the % DAI value in RK is 33.23%, this value is almost the same as the EK value of 33.00%. Phytochemical screening shows that basil has steroid compounds while turmeric does not have steroid compounds. It can be seen from the results of phytochemical screening obtained without steroids, its anti-inflammatory activity is better.

Statistical test results using SPSS Version 26

The results of the percentage data of anti-inflammatory power obtained were continued with statistical tests using SPSS version 26 which began with the normality test and homogeneity test. The results of the normality and homogeneity tests can be seen in table 5. In the normality test using *Spahiro-Wilk* and the results of the data

distribution were normally distributed, namely P> 0.05 , then continued with the homogeneity test using *Levene,* the results were P> 0.05, which means that the data varies homogeneously. Then continued with the one-way anova test to determine the significant differences from all samples. The results of the anova test showed a value of P> 0.05 , which means that all treatment groups were not significantly different. This shows that all treatment groups had anti-inflammatory effectiveness. In previous studies on the toxicity of basil leaves, the results of the apparent LD50 value were obtained as much as> 2000 mg / kgBW (Syahroni 2023) . While in the turmeric rhizome extract, the results of the apparent LD50 were> 2000 mg / kgBW with practically non-toxic criteria (Health,2021) .

In another study, the results showed that basil leaves with a dose of 10 mg/kgBW inhibited edema better than basil leaf extract with a dose of 5 mg/kgBW (Sukaina,2013) . In another study, turmeric rhizome ethanol extract suspension with a dose of 600 mg/kgBW given orally had better anti-inflammatory performance than turmeric rhizome ethanol extract suspension with a dose of 400 mg/kgBW and 500 mg/kgBW. Turmeric rhizome ethanol extract suspension with a dose of 600 mg/kgBW is almost the same as indomethacin suspension with a dose of 100 mg/kgBW (Meilina and Mukhtar,2019) .

The anti-inflammatory effect found in basil leaf extract is due to flavonoids that function as anti-inflammatories where Flavonoids also have the ability to stop the secretion of arachidonic acid and lysosomal enzymes and stop the cyclooxygenase and lipoxygenase pathways, which are part of the anti-inflammatory properties (Hidayati et al,2022) . While in turmeric rhizomes there are curcumin compounds where the anti-inflammatory mechanism produced by curcumin is to inhibit the activity of the cyclooxygenase-2 (COX-2) protein. COX-2 acts as an enzyme that mediates the production of prostaglandins. Indirectly, inhibition of COX-2 by curcumin can inhibit the production of prostaglandins (Fahryl and Novita,2019) .

CONCLUSION

This study concluded that the yield of basil leaf extract was 19.90 % and turmeric rhizome was 14.89%. Basil leaves contain flavonoids, tannins, steroids, and alkaloids. While turmeric rhizomes contain flavonoids, tannins, and alkaloids. In the EK sample of 10 mg/KgBB, RK 600 mg/KgBB, EKRK 2.5:450 mg/KgBB, EKRK 5:300 mg/KgBB, and EKRK 7.5:150 mg/KgBB can reduce the volume of edema in the soles of the feet of mice.

From the statistical test, the results of the One Way Anova test were obtained P = 0.162, which means that all treatment groups were not significantly different. This

study concluded that all treatment groups had anti-inflammatory effectiveness as indicated by the results of the statistical test.

Hopefully this research can be used as a reference for further research in the field of health, especially alternative anti-inflammatory treatments. And this research can add insight for readers and provide a contribution to scientific knowledge related to herbal medicine.

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Table 1 Extraction of basil leaves and turmeric rhizomes

Weight of simple substances	Extract weight (g)	Yield (%)
500 grams of basil leaves	99,479	19.90
500 grams of turmeric rhizome	76,459	14.89

Table 2 Results of phytochemical screening of basil leaf extract *(Ocimum americanum L.)* and turmeric rhizome *(Curcuma domestica val)*

Compound	Reagent	Screening results	
		Basil	Turmeric
Flavonoid	Mg + HC l concentrated	+	+
Saponins	Hot water	-	-
Tannin	$FeCl3_{10}$ %	+	+
Steroid	Liberman	+	-
Alkaloid	Dragendorff	+	+

Figure 1 shows data graph of decreased edema volume in the soles of the feet mice

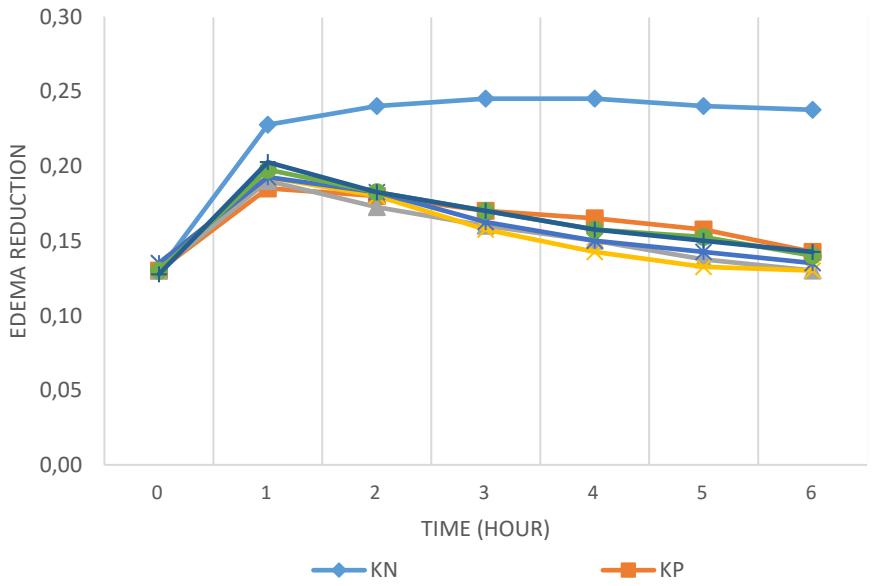

Table 3 Data on Percentage of Edema and Percentage of Edema Inhibition in the Soles of Mice's Feet after being induced with carrageenan and given treatment

Treatment	$\bar{X}$ % Edema ± SE	$\bar{X}$ % Edema Inhibition ± SE
KN	84.19 ± 2.690	-
KP	23.49 ± 2.264	72.10 ± 2.689
EK 10 mg/KgBW	27.88 ± 2.474	66.88 ± 2.939
RK 600 mg/KgBW	22.26 ± 1.050	73.56 ± 1.203
EKRK 2.5 : 450 mg/KgBW	26.34 ± 4.045	68.72 ± 4.805
EKRK 5: 300 mg/KgBW	23.56 ± 1.612	72.02 ± 1.916
EKRK 7.5 : 150 mg/KgBW	24.13 ± 2.043	71.34 ± 2.424

Table 4 Calculation of the average AUC0-6 and percentage value of anti-inflammatory power in each treatment group

Treatment	$\bar{X}$ AUC ± SE	$\bar{X}$ %DAI ± SE
KN	2.10 ± 0.45	-
KP	1.50 ± 0.37	28.71 ± 1.82
EK 10 mg/KgBW	1.41 ± 0.38	33.00 ±1.85
RK 600 mg/KgBW	1.40 ± 0.51	33.23 ± 2.40
EKRK 2.5:450 mg/kgBW	1.44 ± 0.27	31.21 ± 1.32
EKRK 5:300 mg/KgBW	1.50 ± 0.14	28.65 ± 0.65
EKRK 7.5:150 mg/KgBW	1.50 ± 0.31	28.35 ± 1.48

Table 5 Data on normality and homogeneity results

Group	Normality test (P>0.05)	Homogeneity test (P>0.05)	One Way ANOVA test (p<0.05)
KP	0.524		
EK 10 mg/KgBW	0.465		
RK 600 mg/KgBW	0.556		
EKRK 2.5:450 mg/KgBW	0.345	0.182	0.162
EKRK 5:300 mg/KgBW	0.646		
EKRK 7.5:150 mg/KgBW	0.615		