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ANTI-INFLAMMATORY ACTIVITY OF ETHANOLIC EXTRACT OF TARO LEAVES (*Colocasia esculenta* (L.) Schott) AND ITS FRACTIONS IN RATS USING EGG WHITE-INDUCED MODEL

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ABSTRACT

Taro plant (*Colocasia esculenta* (L.) Schott) is empirically known to have anti-inflammatory activity. Taro leaves contain secondary metabolites such as alkaloids, saponins, tannins, flavonoids, and polyphenols. This study aimed to prove the anti-inflammatory effectiveness of ethanol extract of taro leaves (EETL), ethyl acetate fraction (EAFTL), and residual fraction (RFTL) in rats induced by 1% egg white solution, and to identify the chemical compound groups contained in EETL and its fractions. Thirty-two rats were divided into eight groups, namely group I as a negative control (CMC-Na 0.5%), group II as a positive control (Diclofenac sodium 4.5 mg/kgBW), groups III-IV were administered EETL 100 and 200 mg/kgBW, groups V-VI were administered EAFTL 100 and 200 mg/kgBW, and groups VII-VIII were administered RFTL 100 and 200 mg/kgBW. The animal models were induced with 0.1 mL of 1% egg white solution subplantar 1 hour after oral treatment. The volume of rat leg edema was measured every 1 hour for six consecutive hours. This research shows that EETL 100 mg/kgBW and RFTL doses of 100 and 200 mg/kgBW have an anti-inflammatory effect with a percent inhibitory power of >50%. Phytochemical screening showed that EETL contains alkaloids, saponins, tannins, and phenolics. EAFTL contains tannins and phenolics, whereas RFTL contains saponins, tannins, and phenolics.

Keywords: Taro leaves, antiinflammatory, egg white solution, fractionation

INTRODUCTION

Inflammation is a body response to tissue injury caused by trauma, chemical damage, or microbiology. Inflammation is characterized by classic signs, such as heat, pain, swelling, redness, and loss of function in the acute form (Hasanah & Hidayah, 2019). Inflammation can increase vasodilation and blood vessel permeability (Bahrudin, 2018). Inflammation can be caused by several pathogenic events that damage tissues, such as infection, myocardial infarction, and tissue damage (Chen et al. 2018). This shows that many processes involve inflammation; therefore, anti-inflammatory drugs are required. Inflammation can be treated with various drugs, such as steroids and non-steroidal anti-inflammatory drugs (NSAID). The long-term side effects of NSAIDs include kidney, cardiovascular, gastrointestinal, cerebrovascular, and nervous system disorders (Cabassi et al., 2020). Therefore, finding an alternative herbal treatment that is safe is necessary. Herbal treatments are believed to have relatively few side effects.

One plant that has the potential to be studied as an anti-inflammatory drug is Taro (*Colocasia esculenta* (L.) Schott). The general public mainly uses Taro plants as food, which have been used as ingredients in traditional medicine for a long time (Ladeska et al., 2021). Administration of Taro leaf water extract at 800 mg/kgBW showed several signs of toxicity. This indicates that a dose of less than 800 mg/kgBW has been proven safe and non-toxic within the therapeutic dose range (Camille et al., 2018). Taro plants have been confirmed in

vivo or in cell line tests to test their efficacy, and no toxicity has been found (Pereira et al., 2021). Taro leaf ethanol extract contains secondary metabolites, such as flavonoids, alkaloids, saponins, tannins, and polyphenols (Nurhayati et al., 2023). Secondary metabolites of flavonoids and saponins have the potential to exhibit anti-inflammatory activity (Audina et al., 2018). Tannins act as anti-inflammatory agents by inhibiting NO and prostaglandin-E2 (Wijesinghe et al., 2013). Anti-inflammatory research has been carried out, namely the stalk (Cahyani et al., 2023), Taro leaf (Biren et al., 2006; Sjamsudin et al., 2021), and roots (Baro et al., 2023) of Taro plants in mice that were induced carrageenan

To date, no research has tested the activity of the ethanol extract of Taro leaves and its fractions using an egg white solution as an inducer. In addition to fresh egg white, various irritants or phlogistics such as formaldehyde, brewer's yeast powder, dextran solution, aerosil, kaolin, sulfated polysaccharides like carrageenin, and naphthoylheparamine solution are used to induce paw edema in mice or rats. Some irritants cause paw edema for more than 24 hours, whereas others cause only brief inflammation (Vogel, 2008). Egg white, as an inducing agent, has high availability, bioactivity, easy handling, and low cost (Jalili-Firoozinezhad et al., 2020). Inflammation inducers can use egg whites because they contain proteins that act as edema inductors, namely, protein fractions (Barung et al., 2021). It is hoped that extracts from natural ingredients can be used as an alternative treatment, one of which is anti-inflammatory. This study aimed to prove the effectiveness of an ethanol extract from Taro leaves and its non-polar, semi-polar, and polar fractions as anti-inflammatory agents in rats induced by egg white, along with their phytochemical screening.

RESEARCH METHODS

Equipment and Materials

Analytical balance (Ohaus), 1 mL and 5 mL injection syringes (OneMed), oral probe, mercury pletysmometer (Pyrex Iwaki Glass), glassware (Pyrex), water bath (Memmert), Buchner vacuum, sonicator (Branson), and separating funnel. Samples of Taro leaf extract, 70% ethanol (technical), n-hexane (technical), ethyl acetate (technical), distilled water, aqua pro injection, diclofenac sodium powder (pharmaceutical grade), CMC-Na, 1% chicken egg white, distilled water, NaOH, Mayer's reagent (p.a), Dragendorf's reagent (p.a), HCl 2N (p.a), sulfuric acid (p.a), anhydrous acetic acid (p.a), ethanol (technical grade), and $FeCl_3$ (p.a).

Research Procedure

1. Preparation of Determination and Ethical Clearance

   Taro leaves were collected at the Biology Laboratory of FKIP Universitas Muhammadiyah Surakarta to ensure the authenticity and accuracy of the plants studied. Based on the statement letter No. 023/A.E-1/LAB.BIO/VIII/2023, states that the key to plant determination and morphology for research is the species *Colocasia esculenta* (L.) Schott, with the synonym *Colocasia antiquorum* Schott. The test design using experimental animals has been declared to have passed, based on the Ethical Eligibility Letter No. 5034/A.1/KEPK-FKUMS/IX/2023 from the Faculty of Medicine, Universitas Muhammadiyah Surakarta.
2. Sample Preparation

   Taro leaves were obtained from Sambongwangan, Randublatung, Blora Regency, Central Java in September 2023. The samples were cleaned, washed, and dried in a drying cupboard at 60 °C for 2 days.
3. Extraction and Fractionations

   The extraction was performed using the maceration method. Simplicia were macerated in an organic solvent (70% ethanol) at a powder: solvent (1:10) (Amelinda et al., 2018). Taro leaf powder was soaked in 70% ethanol and covered tightly for 3 × 24 hours in a place not exposed to sunlight with occasional stirring. Filtering was performed using a Buchner vacuum to obtain a filtrate and concentrates using an evaporator at a temperature of ± 60 °C, followed by evaporation in a water bath until the extract thickened.

Fractionation was carried out every 10 g of ethanolic extract of Taro leaves (EETL) was dissolved in 100 mL of hot distilled water, and a liquid-liquid partition was carried out using 100 mL of n-hexane as a non-polar solvent until the hexane was clear (5x partition). The dissolved n-hexane phase was then evaporated using a rotary evaporator and concentrated in a water bath for ± 2 hours until the non-polar hexane fraction of taro leaves (HFTL) was obtained. The hexane-insoluble phase was partitioned into liquid liquid using 100 mL ethyl acetate until transparent (5x partition). The phase dissolved in ethyl acetate was separated, evaporated on a rotary evaporator, and then concentrated in a water bath for ± 6 hours to obtain the semi-polar ethyl acetate fraction of taro leaves (EAFTL). The remaining fraction (insoluble ethyl acetate) was evaporated and concentrated in a water bath for ± 1 day until the residual fraction of taro leaves (RFTL) was obtained as a polar fraction. A schematic of the extraction and fractionation processes for taro leaves is shown in **Figure 1**.

Calculation of yield percentage

$$\%\ yield = \frac{Weight\ of\ extract\ obtained\ (g)}{Weight\ of\ simplicia\ (g)}\ x\ 100 \quad \text{..... (Eq.1)}$$

$$\%\ yield\ of\ dry\ powder = \frac{Simplicia\ (g)}{Fresh\ Taro\ Leaves\ (g)}\ x\ 100$$

$$\%\ yield\ of\ EETL = \frac{EETL\ (g)}{Dry\ powder\ (g)}\ x\ 100$$

$$\%\ yield\ of\ HFTL = \frac{HFTL\ (g)}{EETL\ (g)}\ x\ 100$$

$$\%\ yield\ of\ EAFTL = \frac{EAFTL\ (g)}{EETL\ (g)}\ x\ 100$$

$$\%\ yield\ of\ RFTL = \frac{RFTL\ (g)}{EETL\ (g)}\ x\ 100$$

See **Table II** for the abbreviation information

4. Phytochemical Screening

The phytochemical screening method used to determine the class of secondary metabolite compounds present in taro leaves requires a detection reagent for each compound. Phytochemical testing included flavonoids, alkaloids, saponins, steroids/triterpenoids, tannins, and phenolics. Qualitative tests using the tube method were as follows:

a. Flavonoids

A total of 0.5 g of extract or fraction was dissolved in distilled water and a few drops of NaOH reagent were added, and the positive reaction of the flavonoids changed color to orange (Ikalinus et al., 2015).

b. Alkaloids

The total extract or fraction (0.5 g) was weighed and dissolved in 2 mL of distilled water, placed in a porcelain cup, and evaporated in a water bath. The obtained residue was dissolved in 5 mL of 2N HCL the residue obtained. The solutions were then divided into 3 test tubes. First, a few drops of 2N HCl were added to the blank tube. A few drops of Dragendorf reagent were added to the second tube, which was positive for alkaloids if an orange precipitate was present. A few drops of Mayer's reagent were added to the third tube, positive for alkaloids if there was a yellow precipitate (Farnsworth, 1966) and modified by Kusumo et al. (2022).

c. Steroids/Triterpenoids

A total of 0.5 g of extract or fraction was dissolved in 2 mL of distilled water, and then acetic anhydride, concentrated sulfuric acid, and acetic anhydride were added. positive steroids if the color changed to green-blue and positive triterpenoids if the color changed to red-purple (Ikalinus et al., 2015).

d. Saponin

An amount of extract or fraction (0.5 g) was added to sufficient distilled water into a test tube heated for ± 15 minutes until it boiled, allowed to cool, and then filtered using filter paper. A few drops of 2 N HCL were added to the filtrate. If the foam persisted and did not disappear for at least 10 minutes, it indicated the presence of saponin. Foams that form at least 1 cm in height indicate the presence of saponins (Harborne, 1996;Kusumo et al., 2022).

e. Tannin
A total of 0.5 g of extract or fraction was boiled with 4 mL of distilled water (filtered), then a few drops of $FeCl_3$ were added and the reaction was positive if the color changed to blackish blue or greenish brown (Farnsworth, 1966;Ikalinus et al., 2015).

f. Phenolic
A total of 0.5 g of extract or fraction was added to 1 mL of 70% ethanol and a few drops of $FeCl_3$ reagent were added. The reaction was positive if the color changed to black or blue (Farnsworth, 1966) and was modified by Rismawati et al. (2018).

5. Anti-inflammatory Testing

Test animals were acclimatized or adapted for approximately 7 days Rats were fasted for ± 18 hours before treatment and received water ad libitum. All the experimental animals were treated under the same conditions. This test used 32 rats that were randomly divided into 8 groups. Each group was given oral treatment **Tabel I**.

Table I. Groups of Treatment of Extract and Fraction of Taro Leaves for Anti-inflammatory Test (n=4)

Groups	Treatment
I	CMC-Na 0.5% (negative control)
II	Diclofenac sodium 4.5 mg/kgBW (positive control)
III	EETL dose 100 mg/kgBW
IV	EETL dose 200 mg/kgBW
V	EAFTL dose 100 mg/kgBW
VI	EAFTL dose 200 mg/kgBW
VII	RFTL dose 100 mg/kgBW
VIII	RFTL dose 200 mg/kgBW

Each rat was marked with a marker on the rat's right ankle so that it remained the same when the paw was entered into the plestysmometer each time it was tried. The initial volume (V_0) of the rat legs was measured immediately before treatment. Induction was carried out using 0.1 mL of 1% egg white solution subplantar after 1 hour of oral treatment. The right foot of each test animal was cleaned using ethanol. A plestysmometer was used to measure the volume of leg edema in rats every 1 hour for 6 consecutive hours as the final volume (V_t) (Bodhi et al., 2021).

Data analysis

The results were analyzed based on the volume of the rat paws before and after induction of 1% egg white solution in the anti-inflammatory test. Swelling volume (edema) is the change in volume after induction with the initial volume of the rat paw (V_0).

The edema volume was calculated using the following formula:

$$(V_u) = V_t - V_0 \quad \text{.....(Eq. 2)}$$

V_t: Volume of rat paws after induction with 1% egg white solution at a certain time.
V_0: Rat leg volume before being induced by 1% egg white solution
V_u: Volume of swelling (edema) in a rat's paw at a certain time

The area under the curve was calculated based on edema volume data. The AUC value is the average value of the area under the curve, which describes the relationship

between the average volume of edema and time (Sujono et al., 2012). The formula used to calculate this is as follows:

$$AUC_{t_{n-1}}^{t_n} = \frac{Vu_{n-1} + Vu_n}{2} (t_n - t_{n-1}) \text{ ..... (Eq. 3)}$$

Vu_n: Average edema volume at t_n

Vu_{n-1}: Average edema volume at t_{n-1}

The percentage of *Anti-Inflammatory Power* (% AIP) was calculated from the AUC data using the following formula:

$$\% \text{ AIP} = \frac{AUC_k - AUC_p}{AUC_k} \times 100\% \text{ ..... (Eq. 4)}$$

AUC_p : AUC curve of edema volume against time in the treatment group for each individual (rats)

AUC_k : AUC of time-averaged edema volume curve for negative control

RESULTS AND DISCUSSION

The maceration method is one of the extraction methods chosen because it has several advantages, such as simplicity, ease of processing, and no use of heating, so it can prevent damage to the active substances contained in the sample due to the influence of temperature (Sa'adah & Nurhasnawati, 2017). The difference in pressure inside and outside the cell causes the cell membrane and cell wall to lyse (break), so that compounds also lyse (break) in the cytoplasm and dissolve in organic solvents. The weakness of the maceration method is that it uses room temperature, resulting in less soluble compounds (Chairunnisa et al., 2019). The extract was then fractionated to obtain non-polar, semi-polar, and polar fractions. The fractionation principle involves the extraction of compounds by separating two types of solvents with different polarities that do not mix (Putri et al., 2023). 70% ethanol was chosen because it is more selective towards the compounds drawn, namely polar compounds in Taro leaves. Ethanol is also a selective and volatile solvent for organic compounds (Sulistiani & Isworo, 2022). The extraction and fractionation processes for taro leaves are shown in **Figure 1**. The percentage yields of the ethanol and taro leaf fractions are presented in **Table II**.

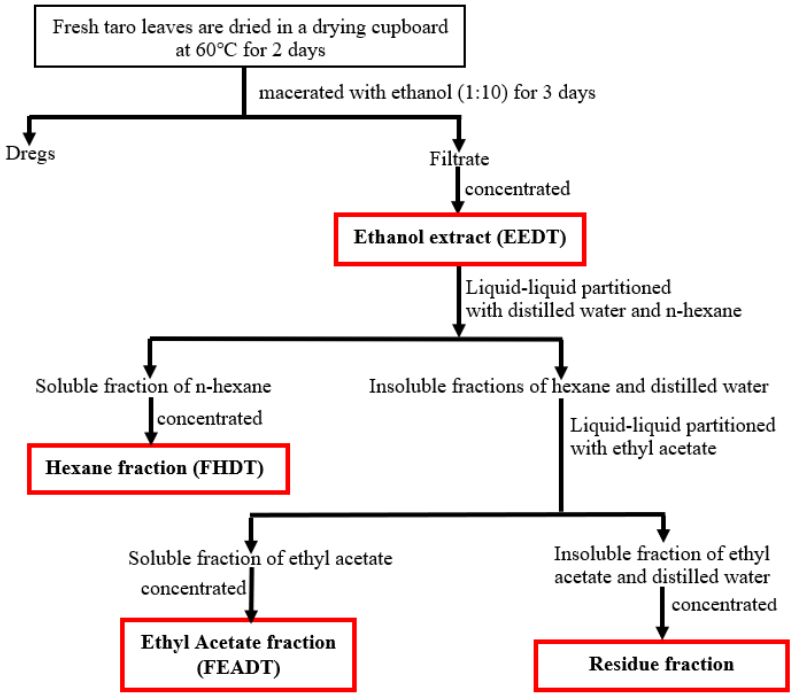

Figure 1. Scheme of Extraction and Fractionation of Taro Leaves Using The Liquid-liquid Partition Method

Table II. Yield (%) of Ethanol Extract and its Fractions in Taro Leaves

Extract or fraction	Weight (g)	Yield (%)
Taro leaves (fresh)	3000	-
Simplicia (dry powder)	585	19.50
Ethanol extract of Taro leaves (EETL)	107.88	18.44
n-hexane fraction (HFTL)	0.73	0.68
Ethyl acetate fraction (EAFTL)	6.97	6.46
Residue fraction (RFTL)	74.16	68.74

In this study, the n-hexane fraction was minimal, yielding a yield of only 0.68%. This is possible because the content of non-polar compounds in Taro leaves is low, so the resulting fraction is also small, and it is not possible to use it as a treatment material. The yield was calculated by comparing the weight of the thick extract with the initial weight of the simplicial weight. The higher the % yield, the more bioactive compound components it contains (Hidayati et al., 2022). The yield can be influenced by the polarity differences and boiling points of each solvent (Kanifah et al., 2015).

Phytochemical Screening Results

Taro plants contain primary metabolites, including proteins and carbohydrates, as well as secondary metabolites such as tannins, steroids, saponins, and flavonoids (Nurainun et al., 2021). Flavonoids and saponins exhibit anti-inflammatory activities (Audina et al., 2018). This pharmacological activity is influenced by the number of active substances in each part of the plant, which can have different pharmacological effects. The results of the tube method for phytochemical screening of the ethanol extract and taro leaf fractions are presented in **Table III**.

Table III. Results of Phytochemical Screening of Taro Leaf Extracts and Fractions

Group of secondary metabolites	Reagent	Phytochemical screening results	Ethanol extract	Ethyl acetate fraction	Residue fraction
Flavonoids	NaOH 10%	Positive if an orange/orange color forms.	-	-	-
Alkaloids	Mayer	Positive if a yellow precipitate forms	+	-	-
	Dragendorf	Positive if an orange color forms	+	-	-
Steroids	Acetic anhydrous + H_2SO_4	Positive for steroids if a green-blue color forms.	-	-	-
Triterpenoids	Acetic anhydrous + H_2SO_4	Positive for triterpenoids if a red-purple color forms	-	-	-
Saponin	Aquadest + HCl 2 N	Positive if foam forms >1 cm in at least 10 min	++	-	++
Tannin	$FeCl_3$	Positive if a green-brown/blue-black color forms	+	+	+
Phenolics	Ethanol 70 % + $FeCl_3$	Positive if a blue/black color forms	+	++	+

Notes: + : intensive color
++ : more intensive color

As shown in **Table III**, the results of phytochemical screening of taro leaf extracts and fractions did not reveal the presence of flavonoids, possibly because when concentrating with an evaporator or water bath, the temperature was high/not constant, making it challenging to determine the flavonoid content. Flavonoid compounds are not resistant to heating; therefore, they are easily damaged at high temperatures (Widayanti et al., 2023). The flavonoids in Taro leaves are vitexin, isovitexin, orientin, and isoorientin, which can be used as anti-inflammatory agents and painkillers (Rubiono et al., 2020). Although no flavonoids were found in the phytochemical screening in vivo, the test results showed that taro leaves showed anti-inflammatory activity. This is possible because, apart from flavonoids, several secondary metabolites, such as alkaloids, saponins, steroids, tannins, and phenolics, can potentially have anti-inflammatory effects. The mechanism of action of alkaloids as potential anti-inflammatory agents is to prevent histamine release by mast cells (Wasiaturrahmah & Amalia, 2023). The mechanism of action of saponins is to prevent exudate formation and inhibit vascular permeability. Steroids can also prevent prostaglandins and arachidonic acid from forming inflammatory mediators by inhibiting phospholipase enzymes. These compounds can reduce the inflammatory response at specific concentrations, because they are thought to have a synergistic effect on each other (Astika et al., 2022). Tannins have an antioxidant effect, acting as anti-inflammatory agents, preventing the production of oxidants by monocytes, macrophages, and neutrophils, and directly preventing the occurrence of reactive oxidants such as hypochlorous acid and hydroxyl radicals (Anisa et al., 2019). Phenolics act as anti-inflammatory agents to capture free radicals that trigger arachidonic biosynthesis by inhibiting cyclooxygenase and prostaglandin enzymes to prevent the formation of inflammatory mediators (Khotimah, 2016).

Anti-Inflammatory Effect Test Results

This study was conducted to test the inflammation-inhibiting effect of the ethanol extract of Taro leaves and its fractions in rats induced by 1% egg white solution. Inflammation is caused by the egg white solution, which contains proteins such as lysozyme (3.2%), ovomucin (3.5%), ovomucoid (11%), ovotransferrin (12%), and ovalbumin (54%). The inflammatory response is thought to originate from a protein injected into the soles of the rat's feet, causing swelling, and this protein has been identified as the primary allergen. The swelling mechanism during acute inflammation induced by the egg white solution is mediated by the release of histamine and serotonin, which causes increased vascular permeability and edema formation (Barung et al., 2021). Egg white was chosen as the inflammation-inducing agent because of its high availability and bioactivity, easy handling, and low cost (Jalili-Firoozinezhad et al., 2020).

As shown in **Table IV**, the average edema volume was the highest at the 3rd hour, and all groups experienced a decrease in edema volume after the 3rd hour. The largest average edema volume was observed in the CMC-Na 0.5% group as a negative control group. This shows that the negative control group treated with CMC-Na had no effect on reducing the swelling caused by the 1% egg white solution.

Table IV. Edema Volume ± SD in Each Group with n=4

Groups of treatment	Average edema volume at hour (Vt) (mL)					
	V1	V2	V3	V4	V5	V6
Negative control	0.33 ± 0.12	0.45 ± 0.10	0.61 ± 0.07	0.59 ± 0.11	0.57 ± 0.09	0.52 ± 0.09
Diclofenac sodium	0.23 ± 0.06	0.25 ± 0.08	0.28 ± 0.04	0.20 ± 0.07	0.15 ± 0.07	0.10 ± 0.07
EETL 100 mg/kgBW	0.16 ± 0.04	0.25 ± 0.04	0.36 ± 0.06	0.27 ± 0,02	0.21 ± 0.04	0.16 ± 0.04
EETL 200 mg/kgBW	0.20 ± 0.10	0.28 ± 0.08	0.40 ± 0.00	0.31 ± 0.04	0.21 ± 0,08	0.16 ± 0.04

EAFTL mg/kgBW	100	0.22 ± 0.06	0.30 ± 0.05	0.42 ± 0.10	0.35 ± 0.07	0.30 ± 0.07	0.21 ± 0.06
EAFTL mg/kgBW	200	0.27 ± 0.09	0.36 ± 0.07	0.42 ± 0.08	0.36 ± 0.10	0.28 ± 0.07	0.22 ± 0.08
RFTL mg/kgBW	100	0.23 ± 0.04	0.28 ± 0.02	0.36 ± 0.04	0.28 ± 0.04	0.20 ± 0.04	0.13 ± 0.02
RFTL mg/kgBW	200	0.21 ± 0.08	0.27 ± 0.08	0.38 ± 0.09	0.28 ± 0.11	0.20 ± 0.12	0.06 ± 0.09

Notes:
EETL (ethanol extract of Taro leaves); EAFTL (ethyl acetate fraction of Taro leaves); dan RFTL (residue fraction of Taro leaves)

Based on **Table IV**, the average edema volume of the positive control (Diclofenac sodium) was the highest at 0.28 mL at the 3rd hour and the lowest at 0.10 mL at the 6th hour. Diclofenac sodium reduced swelling caused by a 1% egg white solution on the rats' right feet. Diclofenac sodium is a phenylacetate derivative of the NSAID group that has anti-inflammatory, analgesic, and antipyretic activities. Diclofenac sodium has strong anti-inflammatory properties and relatively mild side effects compared with other NSAID. In a study by Suwandi et al. (2021), diclofenac sodium was used to treat swelling or inflammation by inhibiting cyclooxygenase, an enzyme that synthesizes prostaglandins as a mediator of inflammation. The positive control validates the method and shows the potential efficacy of the extract or fraction compared with drugs already available on the market.

The AUC calculation was obtained from the volume of edema used to obtain the % anti-inflammatory power value. The AUC value describes the extent of inflammation in the soles of the rat's feet, while %DAI is the percentage of anti-inflammatory power. The lower the AUC value, the greater its ability to inhibit swelling, resulting in greater anti-inflammatory activity (Safitri et al., 2023). The highest percentage of anti-inflammatory power was the diclofenac sodium group at 79.29 ± 1.80%, followed by the EETL 100 mg/kgBW, RFTL 200 and 100 mg/kgBW, EETL 200 mg/kgBW, EAFTL 100 and 200 mg/kgBW groups with the percentage value of anti-inflammatory power which can be seen below in **Table V**.

Table V. AUC and % Anti-inflammatory Power (PAI) in Each Treatment Group

Groups of treatment	Average AUC± SEM (mL.h)	Average PAI± SEM (%)
Negative control	2.65 ± 0.22	-
Diclofenac sodium	1.05 ± 0.13*	79.29 ± 1.80
EETL 100 mg/kgBW	1.26 ± 0.09*	52.47 ± 3.44
EETL 200 mg/kgBW	1.39 ± 0.08*	47.52 ± 3.17
EAFTL 100 mg/kgBW	1.59 ± 0.16*	40.00 ± 6.03
EAFTL 200 mg/kgBB	1.68 ± 0.18*	36.47 ± 6.96
RFTL 100 mg/kgBB	1.32 ± 0.06*	50.11 ± 2.33
RFTL 200 mg/kgBB	1.28 ± 0.23*	51.52 ± 8.79

Notes : *significantly different from the negative control ($p<0.05$), each group consisted of 4 rats); SEM (*standard error mean*); AUC (*area under the curve*); PAI (anti-inflammatory power). EETL (ethanol extract of Taro leaves); EAFTL (ethyl acetate fraction of Taro leaves); and RFTL (residue fraction of Taro leaves)

Statistical analysis was performed based on the volume of edema obtained, and AUC and %PAI values were calculated. AUC data were analyzed using SPSS, which carried out a normality test using the Shapiro-Wilk test because the population was <50 samples. The data were usually and homogeneously distributed, followed by one-way Analysis of Variance (ANOVA) because they were significantly different ($p<0.05$), followed by post hoc least significant difference (LSD) with a confidence level of 95%.

The results of the LSD test showed that there was an insignificant difference between the AUC values of the EETL group at doses of 100 and 200 mg/kgBW and RFTL at doses of 100 and 200 mg/kgBW (not significantly different) compared with the positive control (diclofenac sodium). This shows that EETL and RFTL had equivalent effects on diclofenac sodium. The EAFTL groups at doses of 100 and 200 mg/kgBW showed a significantly different effect than the positive control; this is possible because the ethyl acetate fraction of the Taro leaf extract was not optimal.

Generally, a drug has a good safety profile if its therapeutic index is greater than 10 (Tamargo et al., 2015). Previous research stated that Taro leaf water extract at a dose of 800mg/kgBW showed several signs of toxicity (Camille et al., 2018). Based on the safety therapy index in animals, namely by comparing LD50/ED50, it is stated that a dose of 200 mg/kgBW is considered too large because the therapeutic index value is less than 10, so in future research, it is recommended to use a maximum dose of 100 mg/kgBW or lower. Therefore, further research is needed to determine the optimal dose that can inhibit inflammation in the proper soles of rats.

Testing the anti-inflammatory effect of Taro leaf extract and its fractions at a dose of 100 mg/kgBW was able to reduce swelling in the soles of the right feet of mice; this is in line with research by (Biren et al., 2006), which stated that the ethanol extract of Taro leaves (*Colocasia esculenta* (L.) Schott) at a dose of 100 mg/kgBW orally can inhibit carrageenin-induced swelling of rat's feet. A material is said to have anti-inflammatory power if the percentage of anti-inflammatory power is 50% or more (Maifitrianti et al., 2019). The results of this study prove that EETL at a dose of 100 mg/kgBW and RFTL at a dose of 100 and 200 mg/kgBW have anti-inflammatory activity because they have a percentage of anti-inflammatory power of more than 50% **Table V**.

The effectiveness of a drug is determined by the dose given; if the dose given is too small, then the effect will not be as maximal as desired, and conversely, if the dose given is too large, it will cause toxic effects (Basir et al., 2022). The increase in effect is proportional to the increase in dose. However, the higher the dose, the more likely the effect will be constant when it reaches a dose that can no longer increase the response.

CONCLUSION

EETL at a dose of 100 mg/kgBW and RFTL at a dose of 100 and 200 mg/kgBW have an anti-inflammatory effect with a percentage of anti-inflammatory power of more than 50%. EETL contains alkaloids, saponins, tannins, and phenolics. EAFTL contains tannins and phenolics. Meanwhile, RFTL contains saponins, tannins, and phenolics.

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