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An Effectiveness Test Combination of Ethanol Extracts of Buas-buas Leaves (*Premna serratifolia* L.) and Sappan Wood (*Caesalpinia sappan* L.) as Topical Antiinflammatory Agent in Male White Rats (*Rattus novergicus*)

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Abstract

Background*: Inflammation is the reaction of the immune system to tissue damage resulting from physical injury, harmful chemicals, or microbial agents. Steroid and non-steroid drugs are commonly used to treat inflammation, but long-term use can result in side effects, such as hormonal disorders and gastric ulcers. Buas-buas leaves and sappan wood has potential as traditional medicines, and based on empirical evidence, they utilize both plants as anti-inflammatory, antibacterial, antioxidant, antiallergic, and antiarthritic agents, as well as for the treatment of cardiovascular disorders, cough, and leprosy.* ***Objective****: This study aimed to evaluate the effect of combining 96% ethanol extracts from buas-buas leaves and sappan wood to deliver topical anti-inflammatory benefits.* ***Methods****: This research was experimental, using thirty male rats divided into six treatment groups: a negative control group with 2% carrageenan, Biocream® control group, a positive control group with Hydrocortisone Acetate, and three treatment groups receiving a combination of ethanol extracts from buas-buas leaves and sappan wood at concentrations of 1.67%, 2.5%, and 3.75%. The test compounds were administered after carrageenan injection. Measurement of skinfold thickness on the backs of the rats was conducted every hour for 6 h using digital calipers, and the difference in skinfold thickness of each rat, AUC value, and percentage of inflammation inhibition was calculated. Data analysis involved the Kolmogorov-Smirnov test and Levene's test to assess normality and homogeneity, respectively, and was subsequently followed by One-way ANOVA and a post-hoc test.* ***Conclusion****: The combination of ethanol extracts of Buas-Buas leaves and sappan wood can provide a topical anti-inflammatory effect, with the most effective concentration being 3.75% and an inflammation inhibition percentage of 32.77%.*

Keywords*: anti-inflammatory, topical, buas-buas leaves, sappan wood, ethanol extract*

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INTRODUCTION

Inflammation is the response of the immune system to tissue injury caused by physical trauma, harmful chemical substances, or microbiological agents (Marbun, 2015). The inflammatory response is characterized by *calor* (heat), *dolor* (pain), *rubor* (redness), and *tumor* (swelling) (Anggraini, 2018). Inflammation begins with the presence of a stimulus that causes cell damage, leading to the release of several phospholipids, including arachidonic acid. After arachidonic acid is released, it is activated by several enzymes, including cyclooxygenase and lipoxygenase (Fitriyanyi, 2020). To treat inflammation, drugs from steroidal and non-steroidal anti-inflammatory groups are usually used, but long-term use can result in side effects such as hormonal disturbances and gastric ulcers (Setia, A. I. D., 2016).

Traditional plants are safer than synthetic chemicals (Parawansah, 2017). (Parawansah, 2017). Traditional treatments with plants have long been used by the Indonesian community for the treatment of various diseases. Additionally, owing to their abundant ingredients, plants are also claimed to have minimal side effects (Fitriyanyi, 2020). Buas-buas (Premna serratifolia L.) and sappan (Caesalpinia sappan L.) are planted with the potential to be used in traditional medicine. Empirically, the community uses buas-buas and sappan plants as anti-inflammatory, antimicrobial, antioxidant, antiallergic, and antiarthritic agents, as well as to treat heart disorders, cough, and leprosy (Agusti., 2022; Meilina., 2019, Nomer, N. M. G. R., 2019). The main secondary metabolites found in buas-buas and sappan plants include flavonoids and brazilin, which are known to have anti-inflammatory effects (Puspita, W., 2020; Nifinluri, C. M. B., 2019).

Research has explored the potential of Buas-Buas plants as anti-inflammatory agents, including studying the impact of ethanol extract fromBuas-Buass leaves (*Premna serratifolia L.*) on paw edema in white rats (*Rattus norvegicus*). This investigation utilized ethanol extract concentrations of 100 mg, 200 mg, and 300 mg. The results indicated that the ethanol extract of buas-buas leaves effectively alleviated inflammation symptoms, with higher extract concentrations showing greater efficacy in reducing inflammation in rat paws (Marbun, E. M. A., 2015). Another study on the topical ethanol extract of sappan wood (*Caesalpinia sappan* L.) showed its effectiveness on collagen density during the wound healing process of white rats (*Rattus novergicus*) at sample concentrations of 6.5, 15, and 30%. The results showed that a 6.5% sample concentration had a good and effective effect on the wound-healing process and increased collagen density. Meanwhile, the 15% and 30% samples also had positive effects, but not as much as the 6.5% sample. This is because oxygenation and moisture content are important factors that influence the wound-healing process. The 6.5% sample concentration is known to have a higher moisture content than the 15% and 30% sample concentrations (Sucita et al. 2019).

MATERIALS AND METHODS

Materials

Buas-buas Leaves, Sappan Wood, 96% Ethanol, Carrageenan, 2.5% Hydrocortisone Acetate, Biocream®, 0.95% NaCl Physiological Solution, veet cream.

The study utilized 33 Wistar male white rats (Rattus norvegicus), aged 2-3 months and weighing–100-200 grams, sourced from rat breeders in the Pontianak region. This research was approved by the Ethics Review Division of the Faculty of Medicine at Tanjungpura University (No. 2322/UN22.9/PG/2023)3.

Instrument

This study used Rotary Evaporator (*Buchi*), Maceration Vessel, Oven (*Reveriberi*), Vacuum Pump, dried plants material Crusher, Blender, Analytical Balance (*Ohauss*), Animal Scale (*Ohauss*), Digital Caliper (*Mitutoyo digital sigma*), Mortar and Pestle, Scissors, Surgical Tools, 3 ml Injection Syringe, Glassware (*Pyrex Iwaki*).

Method

Determination of buas-buas leaves and sappan wood

Plant determination was carried out at the Biology Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences (FMIPA), Tanjungpura University, Pontianak, and it was confirmed that the plant species used were correctly identified as buas-buas (*Premna serratifolia* L and sappan wood (*Caesalpinia sappan* L.) (Isnindar et al., 2016).

Extraction of buas-buas leaves and sappan wood

The dried plant material buas-buas leaves (203.3 g), and sappan wood (413 g) were extracted separately. Each material was macerated in 96% ethanol using separate maceration vessels for 3 × 24 h, protected from sunlight, and stirred occasionally. After standing for 24 h, the macerate was filtered using a vacuum pump and the residue was replaced with fresh solvent three times. The resulting filtrate was concentrated using a rotary evaporator and an oven to produce a concentrated extract (Nomer, 2019). The extract yield was calculated using the following formula (Wijay, 2018):

P-ISSN: 2406-9388
E-ISSN: 2580-8303

$$\%\text{Yield} = \frac{\text{Total weight of the extract obtained (gram)}}{\text{Total weight of dried plant material before extraction (gram)}} \times 100\%$$

Determination of cream concentrations of the combination of ethanol extract of buas-buas leaves and sappan wood

The positive control used was 2.5% hydrocortisone acetate as the basis for determining the concentration of the combination of ethanol extracts of buas-buas leaves and sappan wood. This middle concentration was increased to obtain the third concentration and decreased to obtain the first concentration, thus obtaining three concentration series: 1.67, 2.5, and 3.75%.

Preparation of cream from the combination of ethanol extract of buas-buas leaves and sappan wood

A cream formulation combining the ethanol extracts of buas-buas leaves and sappan wood at concentrations of 1.67%, 2.5%, and 3.75% in a 1:1 ratio was prepared by weighing each ethanolic extract of buas-buas leaves and sappan wood at 0.0835 g, 0.125 g, and 0.1875 g, respectively. They were then mixed with 5 g of Biocream® base using a mortar and pestle to obtain a homogeneous mixture. The formulation details for the cream combining the ethanol extracts of the buas-buas leaves and sappan wood are presented in Table 1.

Table 1. The formulation of the cream combining ethanolic extracts of buas-buas leaves and sappan wood

No	Ingredient Name	Formula		
		F1	F2	F3
1	Ethanol Extract of buas-buas Leaves	0.0835 g	0.1250 g	0.1875 g
2	Ethanol Extract of Sappan Wood	0.0835 g	0.1250 g	0.1875 g
3	Biocream® (Base)	5.0000g	5.0000 g	5.0000 g

Preparation of carrageenan suspension

Carrageenan was prepared with three concentration series: 1, 2, and 3% by dissolving 0.2, 0.5, and 0.75 g of carrageenan in a 0.9% physiological solution up to 20 ml.

Preliminary test

Three experimental animals were used and divided into three treatment groups based on the concentrations of the carrageenan prepared, namely administration of 1, 2, and 3% with each administration volume of 0.2 ml subcutaneously. Measurements were taken for 6 h, and every 1 hour, the thickness of the back skin fold was gauged with a digital caliper. The concentration series of carrageenan that resulted in an increase in the thickness of the rat back skin fold by 2-3 times from the initial fold thickness was selected as the subsequent inducer.

Preparation of experimental animals

The study employed male Wistar strain rats, aged 2–3 months and weighing 100-200 grams, all in good health and acclimated to the laboratory environment for one week. Then, the hair on the rat's back was shaved to a size of 3 × 3 cm^2 using scissors, and vein *cream* was applied to remove the remaining hair and left for 1 × 24 h to prevent post-shaving inflammation. Each part of the rat tail was numbered to facilitate the administration of treatment.

Grouping of experimental animals

A total of 30 rats were divided into six treatment groups, with each group consisting of 5 rats. These groups were as follows: Group I, negative control carrageenan; Group II, Biocream® control; Group III, positive control 2.5% Hydrocortisone Acetate; Groups IV, V, and VI, cream with a combination of ethanol extracts of buas-buas leaves and sappan wood at concentrations of 1.67%, 2.5%, and 3.75%, respectively. Subsequently, they were subcutaneously induced with 2% carrageenan subcutaneously at 0.2 ml in each treatment group.

The cream was tested using a combination of ethanol extracts of buas-buas leaves and sappan wood.

Normal skin on the back of the rat was gauged with a digital caliper before the administration of 2% carrageenan. Edema was observed after administration of 2% carrageenan. The increase in edema was observed and measured with a digital caliper 1 h after injection for 6 h every hour. In Groups II, III, IV, V, and VI, after induction with carrageenan, the backs of the test animals that developed edema were treated with Biocream®, 2.5% hydrocortisone acetate, and cream prepared using a combination of ethanol extracts of buas-buas leaves and sappan wood at concentrations of 1.67, 2.5, and 3.75%. The inhibition and reduction of edema were observed using a digital caliper every 1 h for 6 h.

Data analysis

Measurement of edema thickness in dorsal rat skin

The analysis was conducted by measuring the thickness of the dorsal skin edema in rats using a digital caliper.

P-ISSN: 2406-9388
E-ISSN: 2580-8303

Calculation of AUC (Area Under the Curve) of the difference in thickness of dorsal rat skin fold

The difference in edema thickness was measured every hour, and the total AUC value for each treatment was calculated using the following formula :

$$AUC_{0-6} = \sum_{0}^{6} [\frac{(y_{n-1}y_n)(x_n - x_{n-1})}{2}]$$

Where :

AUC_{0-6} = area under the curve from hour 0 to hour 6 (mm.hours)

y_{n-1} = skin fold thickness at hour (n-1) (mm)

y_n = skin fold thickness at hour n (mm)

x_{n-1} = hour (n-1) (hours)

Calculation of percentage (%) suppression of inflammation

The percentage of inflammatory suppression was determined using the following formula:

$$\text{Inflammation inhibition (\%)} = \frac{(AUC_{0-x)0}\,(AUC_{0-n})n}{(AUC_{0-\times})0} \times 100\%$$

Where :

$(AUC_{0-x})0$ = Mean total AUC of the negative control group (mm.hr)

$(AUC_{0-n})n$ = AUC value of each rat in the group treated with the test compound at a concentration of n (mm.hr)

Analysis of results

Observational data were collected and presented in the form of tables, graphs, and statistical analyses using *ANOVA (Analysis of Variance)*. AUC data for edema volume over time were subjected to the *Shapiro-Wilk* test to check for normal distribution, followed by a homogeneity test to assess the homogeneity of variances. Since the data were normally distributed and homogenous, further analysis was conducted using One-way ANOVA at a 95% confidence level. Post-hoc tests, including *Scheffé* and *Duncan*, were then performed to determine if the observed differences were statistically significant. Data analysis was conducted using the *Statistical Product and Service Solutions* (SPSS) version 25 .

RESULTS AND DISCUSSION

Microscopic and morphological data of sappan wood indicated that the sample used was sappan wood. The extraction process employed was maceration, which involved soaking the powdered herbal materials in a solvent. The maceration method was used because of the practicality and simplicity of the tools used. This results in a long processing time. Soaking the herbal materials allows the solvent to penetrate the cell walls and enter the cell cavities containing active substances, thereby dissolving the active substances. The difference in the concentration between the active substance solution inside and outside the cell causes the most concentrated solution to be pushed out. This event recurs, leading to an equilibrium in the concentration between the solution outside the cell and inside the cell (Indrato et al., 2019). Buas-buas leaves, and sappan wood were extracted separately using 96% ethanol. Extraction was performed for 3 × 24 h with occasional stirring using a spatula to ensure that the solvent solution could draw more compounds contained in the plants, and re-maceration was performed with the same solvent every 24 h. The filtrate obtained from the maceration and subsequent re-maceration processes was evaporated using a rotary evaporator at 50°C. The evaporation process was stopped when all the solvents evaporated, as indicated by the absence of solvent vapor droplets. The evaporated product was then heated in an oven at 50°C until a thick extract was formed. The yield obtained was then calculated and is presented in Table 2.

The purpose of determining the yield is to quantify the quantity of secondary metabolites dissolved in the solvent without specifying the particular types of compounds (Kumalasari, K, 2023). Yields correlate with the active compounds present in the extract; thus, an increase in yield corresponds to a higher concentration of active compounds in the extract. The presence of active compounds in a sample is indicated by a high yield (Safitri, S., 2022).

Organoleptic observations included the aroma, appearance, and color of the tested extracts through sensory perception. The purpose of organoleptic observation is initial objective recognition (Rinduana, 2021). The results of the organoleptic observation of the ethanol extract of buas-buas leaves showed a thick appearance, distinct aromatic, and dark green colour, while that of the ethanol extract of sappan tree showed a thick appearance, distinct aromatic, and reddish colour. The organoleptic results are shown in Table 3. Images of the ethanol extracts of buas-buas leaves and sappan wood are shown in Figure 1.

P-ISSN: 2406-9388
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Table 2. Yield of Buas-buas Leaves and Sappan Wood Extracts

	Dried Plants Material (gram)	Extract (gram)	Total yield (%)
Buas-buas Leaves	203.3 gram	44 gram	21.64 %
Sappan Wood	413 gram	26.1 gram	6.319%

Table 3. The organoleptic results of the ethanol extracts of buas-buas leaves and sappan wood

Organoleptic Characteristics	Buas-buas Lead Ethanol Extract	Sappan Wood Ethanol Extract
Appearance	Thick	Thick
Colour	Dark Brown	Reddish
Aroma	Distinct Aroma	Distinct Aroma

Figure 1. Ethanol extracts of buas-buas leaves and sappan wood

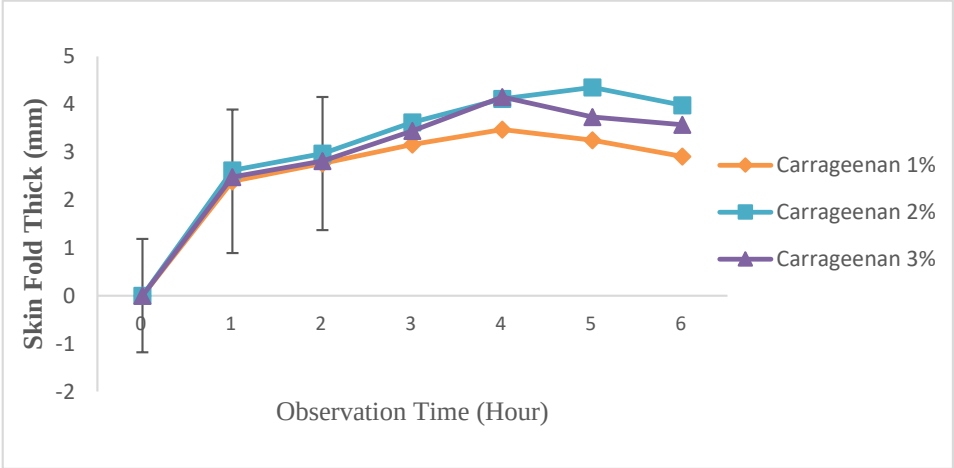

Figure 2. Measurement of edema every 1 hour up to 6 hours from various subcutaneous carrageenan concentrations

Preliminary tests showed the optimal concentration of carrageenan for use as an inducer. To observe the difference in the thickness of the rat's back skinfold, the fold thickness was two to three times greater than the initial or normal thickness, and this increase was maintained until the 6 h (Santoso, Y. I. K., 2014). Preliminary tests used 1, 2, and 3% carrageenan. Carrageenan administration to the test animals resulted in edema of the rat's back skin for 6 h, as shown in Figure 2.

As shown in Figure 2, each subcutaneously injected carrageenan concentration caused an increase in edema of up to 2-3 times the initial skinfold thickness until the 4th hour. However, at concentrations of 1% and 3%, skinfold thickness was maintained until the 4th hour, with a decrease observed at the 5th and 6th hours, indicating that concentrations of 1% and 3% could not maintain the increase in skinfold thickness of the rat back until the 6th hour. A concentration of 2% carrageenan led to an increase in edemaof up to 3-4 times the initial skinfold thickness. Therefore, the 2% carrageenan concentration was used in this study because it showed an increase in the skinfold thickness of the rat back by 3-4 times from the initial skinfold thickness, and it was able to maintain its thickness until the 5th hour.

P-ISSN: 2406-9388
E-ISSN: 2580-8303

Figure 3. Measurement of edema formed

Following the selection of carrageenan concentration, the topical anti-inflammatory effect of the combination of ethanol extracts from buas-buas leaves and sappan wood on edema on the rat's back was evaluated. The concentrated extracts from the buas-buas leaves and sappan wood were mixed into a cream preparation for ease of application to the rat back. The anti-inflammatory efficacy of this combination was observed in terms of the reduction in skinfold thickness on the backs of rats after topical application of the cream at concentrations of 1.67, 2.5, and 3.75%.

In this study, the rats were divided into six groups. Group I was the negative control (NC), with 2% carrageenan as the edema-inducing agent. Group II represented the Biocream® group (BC), which served as the base for formulating the cream containing ethanol extracts from buas-buas leaves and sappan wood. Group III was positive control (PC), receiving 2.5% Hydrocortisone Acetate. Groups IV, V, and VI received a combination of the ethanol extracts from buas-buas leaves and sappan wood at concentrations of 1.67, 2.5, and 3.75%, respectively. Each of these concentrations, with doses of 0.835, 1.25, and 1 g of Biocream® base, was then administered to rats' back skin, which white rats previously injected with 2% carrageenan. Following the application of the combination of ethanol extracts from buas-buas leaves and sappan wood at different concentrations, comparisons were made with the control groups in Category I (NC), II (BC), and III (PC).

The measurement of the thickness of the white rat's back skin fold was conducted every hour for 6 h, starting from hour 0, as the measurement of normal skin from the white rat's back before being injected with carrageenan. The data in the form of a graph of the average difference in the thickness of the white rat back skinfold induced with 2% carrageenan are shown in Figure 4.

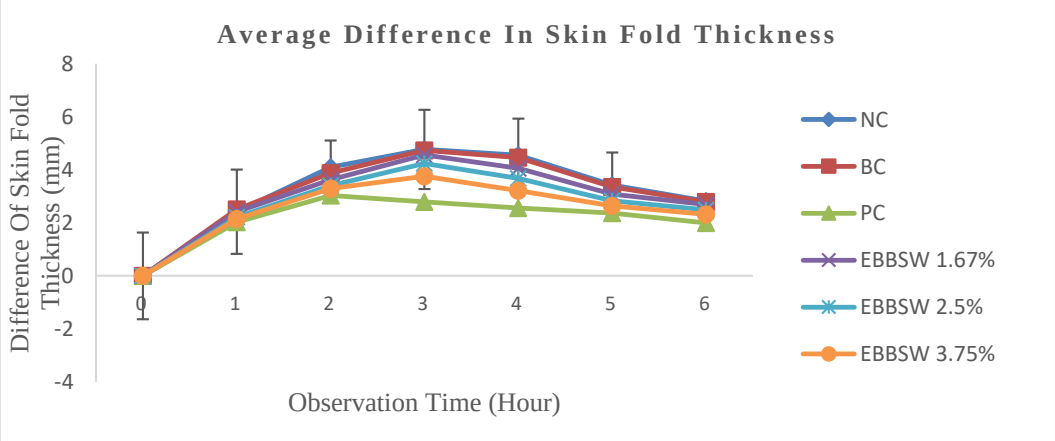

Figure 4. Graph of the average difference in the thickness of the white rat back skin fold from the measurement time of 1 h to 6 h.

Where :

NC	: Negative Control (2% Carrageenan)
BC	: Biocream® Control
PC	: Positive Control (2.5% Hydrocortisone Acetate)
EBBSW 1.67%	: 1.67% Ethanol Extract of Buas-buas Leaves and Sappan Wood
EBBSW 2.5%	: 2.5% Ethanol Extract of Buas-buas Leaves and Sappan Wood
EBBSW 3.75%	: 3.75% Ethanol Extract of Buas-buas Leaves and Sappan Wood

P-ISSN: 2406-9388
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Figure 4 illustrates that following the subcutaneous injection of 2% carrageenan, the rats in all treatment groups exhibited an increase in back skin-fold thickness. Carrageenan-induced edema formation occurs in three phases. The initial phase included the release of histamine and serotonin, lasting up to 90 min. The second phase involves the release of bradykinin, which occurs between 1.5 and 2.5 hours following induction. In the third phase, prostaglandin release occurs 3 h post-induction, followed by rapid edema development, reaching its maximum volume at approximately 6 h post-induction.

The curve demonstrates that both the negative control and Biocream® groups experienced significant edema formation, with a threefold increase in skinfold thickness compared with the initial thickness. Conversely, the positive control group, which received 2.5% hydrocortisone acetate, and the three concentrations of the combination of the ethanol extracts from buas-buas foliage and sappan tree exhibited an opposite trend compared to the Biocream® and negative control groups, showing a decrease in back skin-fold thickness in rats.

In this study, 2.5% hydrocortisone acetate was selected as a positive control because of its corticosteroid properties. Corticosteroids function by inhibiting phospholipase A2 activity, thus preventing the formation of arachidonic acid, which triggers inflammation. Consequently, the positive control group displayed a decrease in the difference in skin fold thickness approaching normal skin over the 6 hours.

After 6 h of measurement, the skin fold thickness of the back in the Biocream® group did not return to its initial thickness. Biocream® was utilized as the base for preparing the combination cream to investigate its influence on the anti-inflammatory effect. This was evident from the Biocream® control curve, demonstrating an increase in skinfold thickness similar to that of the negative control. In the treatment group that received the combination cream, the 1.67% concentration exhibited the greatest edema thickness compared to the 2.5% and 3.75% concentrations. The 3.75% concentration group tended to display smaller edema than the 1.67% and 2.5% concentrations.

Data analysis was continued by calculating the total average AUC for each treatment group compared with the control. The total average AUC is the area under the curve representing the average difference in back skinfold thickness in rats showing edema from hour 0 to hour 6.

The effectiveness of the topical anti-inflammatory treatment using the combination of the ethanol extracts from buas-buas leaves and sappan wood is evident from the significant decrease in skinfold thickness compared to both the negative control and Biocream® control groups. This reduction can be seen in the decline in total average AUC values. Conversely, larger total average AUC values corresponded to smaller reductions in the skin-like fold thickness difference. The total average AUC values for each treatment group are presented in Table 4 below.

Table 4. Total Average AUC Values for Each Treatment Group

Group	Total average AUC ± SD (mm.hour)
NC	4.531 ± 0.184
BC	4.544 ± 0.183
PC	2.059 ± 0.25
EBBSW 1.67%	4.149 ± 0.314
EBBSW 2.5%	3.917 ± 0.254
EBBSW 3.75%	3.046 ± 0.153

Where :
NC : Negative Control (2% Carrageenan)
BC : Biocream® Control
PC : Positive Control (2.5% Hydrocortisone Acetate)
EBBSW 1.67% : 1.67% Ethanol Extract of Buas-buas Leaves and Sappan Wood
EBBSW 2.5% : 2.5% Ethanol Extract of Buas-buas Leaves and Sappan Wood
EBBSW 3.75% : 3.75% Ethanol Extract of Buas-buas Leaves and Sappan Wood
SD : Standard Deviation
AUC : Area Under the Curve (mm.hours)

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Table 5. %Inflamantion Inhibition of each Group

Group	%Inflammantion Inhibition±SD
NC	-±0.185
BC	-0.2869±0.184
PC	8.43±0.257
EBBSW 1.67%	13.55±0.315
EBBSW 2.5%	13.55±0.255
EBBSW 3.75%	32.77±0.153

Where :
NC : Negative Control (2% Carrageenan)
BC : Biocream® Control
PC : Positive Control (2.5% Hydrocortisone Acetate)
EBBSW 1.67% : 1.67% Ethanol Extract of Buas-buas Leaves and Sappan Wood
EBBSW 2.5% : 2.5% Ethanol Extract of Buas-buas Leaves and Sappan Wood
EBBSW 3.75% : 3.75% Ethanol Extract of Buas-buas Leaves and Sappan Wood

The negative control and Biocream® groups exhibited notably higher mean values than the other groups, indicating the inflammatory process induced by the subcutaneous injection of 2% carrageenan. Additionally, Biocream®, as the base for making the combination cream of the ethanol extracts from buas-buas leaves and sappan wood, did not demonstrate the ability to inhibit inflammation or reduce carrageenan-induced edema. Conversely, the treatment groups receiving the combinations of the ethanol extracts from buas-buas leaves and sappan wood at concentrations of 1.67, 2.5, and 3.75% displayed lower mean total AUC values compared to the carrageenan control and Biocream® control groups. This suggests that each treatment effectively reduced the edema on the rat's back skin induced by the injection of 2% carrageenan. The obtained AUC data were used to calculate the percentage of inflammation suppression for each treatment group.

The mean AUC data were analyzed using Statistical Product and Service (SPSS) 25 at a confidence level of 95%. Before conducting this analysis, the Kolmogorov-Smirnov and Levene's tests were performed to ensure the normality and homogeneity of the data, which are prerequisites for conducting the one-way ANOVA test. The results indicated that the data were normally distributed ($p > 0.05$) and homogeneous ($p > 0.05$), meeting the requirements for conducting a one-way ANOVA test.

The One-way ANOVA yielded a significant p-value of 0.000 ($p<0.05$), indicating significant differences between the test groups. Subsequently, post-hoc Duncan and Scheffé tests were conducted to identify the group with the best anti-inflammatory effect and to determine the statistical significance of the observed differences. According to the post-hoc Duncan test, the positive control group demonstrated the most effective anti-inflammatory efficacy. The treatment group with a concentration of 3.75% showed a significantly different anti-inflammatory effect compared to the positive control. Meanwhile, the concentrations of 2.5% and 1.67% exhibited statistically similar anti-inflammatory effects. Conversely, the negative control and Biocream® control groups demonstrated progressively smaller anti-inflammatory effects.

The results of the Scheffe post-hoc test indicated a significant difference between the negative and positive control groups ($p<0.05$). This suggests that the negative control group lacked wound-healing effects, validating the methodology employed. In the 1.67% and 2.5% groups, there was no significant difference compared to the negative control group ($p>0.05$), indicating that these concentrations did not exhibit a robust anti-inflammatory effect. However, the group with a concentration of 3.75% displayed a significant difference compared with the negative control group ($p<0.05$) and the positive control group ($p<0.05$). This suggests that the 3.75% concentration group had a notably different anti-inflammatory effect from the negative and positive control groups, indicating the most effective anti-inflammatory compared to the concentrations of 2.5% and 1.67%.

The compounds present in the ethanol extracts of Buas-Buas leaves and sappan wood include flavonoids, phenolics, saponins, tannins, terpenoids, and brazilin. Flavonoids and brazilin are responsible for these anti-inflammatory effects. The combination of buas-buas leaves and sappan wood exhibits synergistic effects because the compounds in both plants interact and collaborate to enhance the anti-inflammatory effects, thus reducing the thickness of the ras back skin folds.

This research aligns with a study conducted by Marbun, E. M. A. & Restuati, M (2015), which explored the potential of buas-buas plants as anti-inflammatory

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agents in edema of white rat legs. They concluded that ethanol extracts of buas-buas leaves could effectively reduce inflammation symptoms, with higher treatment concentrations providing better and more effective results in reducing inflammation symptoms. Additionally, Choi and Hwang (2019) stated in their research that brazilin can improve the skin barrier through its anti-inflammatory activity and has the potential to treat psoriasis.

In this study, the combination of ethanol extracts from buas-buas leaves and sappan wood demonstrated topical anti-inflammatory effects, as evidenced by the reduction in the thickness of rat back skin folds induced by 2% carrageenan. This indicates that Buas-Buas leaves and sappan wood can be utilized as topical anti-inflammatory treatments. Other studies on the antioxidant and anti-inflammatory activities of sappan flowers have also shown significant anti-inflammatory activity at the highest concentration in terms of HbRc stability. The methanolic extract of Buas-Buas leaves has been found to exhibit inhibitory activity against rat paw edema, with the highest concentration showing the most significant effect. Anti-inflammatory activity is also demonstrated by compounds isolated from sappan wood, particularly brazilin, which has inhibitory effects on the production of inflammatory mediators such as NO, PGE2, and TNF-α, as well as transcription factors produced by RAW 264.7 cells. Another study also reported that ethanol extracts of sappan wood exhibited anti-inflammatory activity in macrophages and osteoarthritic chondrocytes.

However, the limitation of this study lies in the fact that the effect produced by the combination of ethanol extracts from buas-buas leaves and sappan wood in cream form still did not compare with the effect of the positive control in terms of the duration of reduction in thickness of rat back skin folds. This may be attributed to the samples used as crude extracts, the active compounds of which are unknown, compared to the positive control, which comprises a single active compound. Nonetheless, both are equally effective at exerting anti-inflammatory effects. Further research is warranted to explore the purification of the active extract.

CONCLUSION

Based on the findings of this study, it is evident that the combination of the ethanol extracts from buas-buas leaves and sappan wood exhibits topical anti-inflammatory properties, with a concentration of 3.75% proving to be the most effective, resulting in a 32.77% inhibition of inflammation. Further investigation is warranted to enhance the purification of the active extract.

AUTHOR CONTRIBUTIONS

Conceptualization, I.; Methodology, I., S.L.; Software, I.; Validation, A.P.P, I., S.L.; Formal analysis, A.P.P.; Investigationon, A.P.P., I.; Resources, A.P.P, I.; Data Curration: A.P.P, I., S.L.; Writing - Original Draft, A.P.P, I.; Writing - Review & Editing, A.P.P., I.; Visualization: A.P.P., I.; Supervision, I.; Project Administration, I.; Funding Acquisition, I.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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