AJARCDE
ASIAN JOURNAL OF APPLIED RESEARCH FOR COMMUNITY DEVELOPMENT AND EMPOWERMENT

Journal home page: http://ajarcde-safe-network.org ISSN 2581-0405

Utilization of Suji Leaves Extract (*Pleomele Angustifolia* N.E Brown) in Inhibiting Carrageenan-Induced Inflammation on Rats

Reni Koja[1], Endang Prangdimurti [2], Puspo Edi Giriwono[2]

[1]*Department of Food Technology and Agricultural Products, Faculty of Agricultural Technology, Andalas University, West Sumatra, Indonesia*
[2]*Department of Food Science and Technology, Faculty of Agricultural Technology, IPB University, Darmaga Bogor Campus, West Java, Indonesia*

ARTICLE INFO

Article History:
Received: 24 December 2023
Final Revision: 07 February 2024
Accepted: 08 February 2024
Online Publication: 09 February 2024

KEYWORDS

antioxidant, anti-inflammatory, bioactive compounds, total chlorophyll, suji

CORRESPONDING AUTHOR

*E-mail: *reni.koja@gmail.com*

A B S T R A C T

Suji leaf (*Pleomele angustifolia* N.E. Brown) has long been used as an ingredient for traditional medicines. This study seeks to evaluate the anti-inflammatory activity of suji leaf extract in terms of its ability to reduce oedema in the hind paws of rats. Six groups were treated: negative control, positive control given diclofenac sodium as anti-inflammatory medication, two groups of suji leaf extract powder (SEP) with doses of 300 and 800 mg/kg, acetone extract of suji leaf (AES), and a group of acetone extract of SEP (EA-SEP) at a dose of 500 mg/kg with oral administration. Injections of 1% carrageenan suspension into the right hind paw of rats induced inflammation. The results demonstrated that SEP administered at a dose of 800 mg/kg has an inflammatory capacity (80.56%), AES (56.94%), and AE-SEP (75.7%). Chlorophyll, total phenol, and antioxidant capacity in SEP (9.0809 mg /g sample; 3.7354 mg GAE/g sample; 3.04 mg AAE/g sample), AES (4.6471 mg /g sample; 0.9994 mg GAE/g sample; 3.26 mg AAE/g sample), and AE-SEP (6.4912 mg /g sample; 2.1703 mg GAE/g sample; 2.55 mg AAE/g sample). According to qualitative test results, Suji extract contains bioactive compounds of the flavonoid group, saponins, steroids, and triterpenoids. It is believed that phenolic compounds function as anti-inflammatory agents.

1. INTRODUCTION

1.1. Research Background

Inflammation is a response to the body's protective mechanism. Inflammation is a local reaction to damage to body cells and tissues caused by various factors such as infection, chemical, thermal, and mechanical. Tissue inflammation is characterised by fever, heat, redness, swelling, and pain [1]. The continuing process is followed by changes in tissue structure that can cause loss of function. A commonly used anti-inflammatory drug is a non-steroidal anti-inflammatory (AINS). However, using AINS has side effects that can cause damage to the digestive process [2].

The use of productive plants as an anti-inflammatory has been widely used as an alternative treatment with relatively smaller side effects. Traditional plants with bioactive components and antioxidant content show potential as anti-inflammatory agents, such as piper beetle leaf [3], *Mimusops elengi* L leaf [4] and *Andrographis paniculata* leaf [5]. Research on several plants says that the bioactive components of the phenolic group [6], antioxidants [7,8], act as anti-inflammatories.

One of the green plants that is an indigenous plant as a source of antioxidants with a fairly high chlorophyll content and widely used by the community is suji leaves. Suji leaves (*Pleomele angustifolia* N.E. Brown) have long been used in traditional medicine. Suji leaves have a high content of chlorophyll and antioxidant activity [9]. Chlorophyll from green leaves is reported to have anti-inflammatory activity [10]. Various studies have also shown that chlorophyll derivatives have the ability to

act as antioxidants, have antimutagen activity, perform xenobiotic detoxification, and induce apoptosis in cancer cells [11].

1.2. Literature Review

Inflammation is a local reaction to cell and tissue damage caused by various factors such as infection, chemical, thermal, and mechanical. Inflammation is a body's protective response involving immune cells, blood vessels, and various molecular mediators that work by eliminating the causes of cell damage and repairing body tissues [12]. Inflammation is a pathological condition that underlies various diseases. Inflammation plays a role in various health conditions, including various degenerative diseases such as cancer, diabetes, Alzheimer's, arthritis and heart disease. There are indications that cancer begins with chronic inflammation. Chronic inflammation caused by a continuous inflammatory response and tissue destruction is associated with increased disease risk. Chronic inflammation occurs due to pathological conditions caused by tissue damage and a continuous or long-term inflammatory response. In general, treating inflammation and pain uses a non-steroidal anti-inflammatory drug (NSAID), which effectively controls pain as a treatment for pain, inflammation, rheumatoid arthritis and osteoarthritis. However, NSAIDs have side effects that can cause damage to the digestive process, so it is necessary to explore natural ingredients.

Consuming vegetables and fruits containing bioactive components can help reduce disease risk. The utilization of natural materials has contributed to the development of modern medicine. Phytochemicals acting as anti-inflammatory agents continue to be developed in food, pharmaceutical and nutraceutical products to reduce the risk of diseases that protect health [13]. A diet that contains nutrients and bioactive chemical compounds can help modulate immunomodulators in the inflammatory process. Epidemiological studies demonstrate that dietary patterns significantly impact immunological and inflammatory processes [14]. Bioactive compounds or phytochemicals have the potential to effectively prevent and treat disease risks through interrelated biological processes and mechanisms. The mechanism of bioactive components as anti-inflammatories can be through various ways, such as by having antioxidant activity, scavenging free radicals, modulating cellular activities related to inflammation and modulating the activity of pro-inflammatory enzymes.

Prevention and repair of damage caused by free radicals can be neutralized by antioxidants. Superoxide dismutases (SODs) enzymes convert superoxide radicals to hydrogen peroxide and molecular oxygen, while catalase and peroxidase convert hydrogen peroxide to water products. The toxic superoxide radicals and hydrogen peroxide are converted to harmless aqueous products. SODs enzyme grouping is divided into three types, namely manganese superoxide dismutase (MnSOD), which is present in mitochondria; Cu and Zn superoxide dismutase (CuZnSOD), which is found in the cytoplasm and nucleus. Then extracellular SOD (ECSOD) which is expressed in several tissues [15]. Other antioxidant enzymes, such as catalase, can be found in peroxisomes and cytoplasm, while Glutathione peroxidase (GPx) is found mainly in mitochondria and nuclei.

Dietary components, as well as phytochemicals, can modulate the inflammatory process. The bioactive components modulate various chemical mediators during the inflammatory process. This modulation controls the inflammatory process, which can inhibit and reduce the risk of disease that occurs as a result. Bioactive compounds can modulate at various points of the inflammatory process. Dysregulation of inflammatory mechanisms can lead to chronic disease. Arachidonic acid metabolites are mediators of inflammation. Arachidonic acid metabolism takes two different pathways, producing many prostaglandins and thromboxane, namely the cyclooxygenase (COX) pathway, and producing leukotrienes, namely the lipooxygenase (LOX) pathway. Prostaglandins play a role in creating an inflammatory response. Prostaglandin biosynthesis is significantly increased in inflamed tissues and contributes to the hallmarks of acute inflammation [16]. Other mediators of inflammation, such as reactive oxygen species, hydrolytic enzymes, cytokines (TNF-α, IL-1, IL-6) IFN-γ, and other growth factors trigger chronic disease. The inflammatory response is initiated due to damage caused by viruses, chemicals, and reactive oxygen/nitrogen species, which then increase the synthesis and secretion of proinflammatory cytokines such as tumour necrosis factor-α (TNF-α), interleukin (IL)-1β, IL-6, IL -12, and IFN-γ in macrophages [17]

The explosion of natural ingredients has long been carried out as a prevention against various diseases. Bioactive compounds found in plants are secondary metabolites with pharmacological (specific doses) and toxicological (high doses) effects for humans and animals [18]. Consumption of fruits and vegetables is significant for the health of the body. Besides fulfilling nutritional intake, such as containing vitamins and minerals needed by the body, fruit and vegetable consumption is also associated with a reduced risk of cardiovascular disease, stroke, mouth cancer, pharynx, oesophagus, lung, stomach, and colon. This is associated with the bioactive components or phytochemicals contained in these materials. Plants with high polyphenol content are reported to have physiological benefits as a health protector. Regularly consuming plants containing polyphenolic compounds is associated with a decrease in several chronic diseases, such as cancer, cardiovascular disease, and neurodegenerative disorders [19]. Bioactive compounds of the phenolic group, which are secondary metabolites from plants in abundant quantities, have high antioxidant activity and play a role in preventing oxidative stress and cancer in vitro and in vivo research studies with experimental models using test animals and interventions in humans [20]. Another phytochemical compound that is reported to have health effects is chlorophyll. Chlorophyll, a green dye found in natural ingredients in fruits and green vegetables, has many known benefits, especially for health. Studies show that chlorophyll and its degradation products act as widely available anti-inflammatory agents and provide opportunities for developing phytomedicine or conventional medicine to treat inflammation and related diseases [21].

Chlorophyll is a natural dye that is widely found in nature. Natural chlorophyll can reduce the cytotoxicity of heme compounds that trigger colon cancer in rats. However, chlorophyllin (derivatives) of chlorophyll, which no longer has a phytyl group, does not show this ability [22]. Chlorophyll a and pheophytin a (chlorophyll a, which has lost the magnesium group) from green leaves have anti-inflammatory activity in swelling experiments caused by carrageenan induction in mice and formalin induction in rat feet. Chlorophyll a can inhibit TNF-α gene expression but does not affect the expression of nitric acid

synthase and cyclooxygenase-2 [23]. A chlorophyll derivative, chlorophyllin has antitumor activity [24].

1.3. Research Objective

This study aimed to determine the anti-inflammatory activity of suji leaf extract *(Pleomela angustifolia* N.E. Brown) by measuring rat hind paw oedema induced with carrageenan.

2. MATERIALS AND METHODS

2.1. Material and Tools

There are 3 types of samples used, namely Suji leaf Extract Powder (SEP), Acetone Extract of Suji leaf (AES) and Acetone Extract of SEP (EA-SEP). The suji leaf extract solution is made by cleaning the suji, then cut into pieces and crushed with 0.5% NaHCO3 solution (1:10, w/v) [25]; after filtering and centrifuging the supernatant is dried with *a freeze dryer* so that SEP (suji leaf extract powder) is obtained. Acetone Extract of Suji leaf (AES) is prepared by crushing suji leaves using 85% acetone solvent (1: 2.5 b / v). After filtering, the acetone solvent is removed using a rotavapor at a temperature of 45+50C for 90 minutes, and then nitrogen gas is exhaled so that an AES viscous liquid is obtained. EA-SEP is obtained by extracting SEP using 100% acetone solvent, concentrating it using a rotavapor and exhaling nitrogen gas to get EA-SEP viscous liquid. Analysis for chemical and biological activity included analysis of the presence of phytochemical compounds (alkaloids, flavonoids, tannins, saponins, steroids, triterpenoids, and hydroquinones), analysis of total chlorophyll, total phenols, antioxidant activity, and testing of the anti-inflammatory properties of suji leaf extract in vivo.

The tools used in this research include blenders, water baths, centrifuges, freeze dryers, refrigerators, rotary evaporators, spectrophotometers, analytical scales, vortexes, micro pipers, mortars, syringes, plethysmometers, and other glass equipment.

2.2. Design Experiment and Analysis

2.2.1. Total Chlorophyll Analysis

Determination of chlorophyll levels using spectrophotometric method [14]. 0.5 grams of sample and 20 ml of 80% acetone vortexed and filtered were added. The extract was taken 1 ml, mixed with 9 ml of 80% acetone, and then left for one night in the refrigerator. It is then centrifuged at 3000 rpm for 10 minutes. Direct measurements of supernatant absorbance at 645 and 663 nm wavelengths were performed to analyze total chlorophyll levels. Calculation of total chlorophyll levels using the formula

Total chlorophyll (mg/L) = 20.2 (Abs $_{645}$ nm) + , (,(Abs $_{663\,nm}$)

2.2.2. Total Phenol Analysis

Analysis of total phenol content using Folin-Ciocalteu reagent and gallic acid as standard[15]. The test procedure was carried out by weighing samples as much as 0.5 mg of material and extracted with 95% ethanol (1:10, w/v) vortexed and filtered, then mixed with 5 ml of Folin-Ciocalteu reagent (1:10, v/v) then added 4 ml of Na 2 CO_3 vortexed and incubated for 15 minutes at a temperature of 45ºC. As a standard used gallic acid. Total phenol was analyzed by measuring the absorbance of the sample at a wavelength of 765 nm using a spectrophotometer. The total phenol content is expressed as *Gallic Acid Equivalent* (GAE) mg/g of the sample.

2.2.3. Antioxidant Capacity Testing

Antioxidant capacity testing using DPPH[16] free radicals. Extract samples of 50mg/ml concentration in equates, then dilute up to 10 times. 1 ml of diluted sample is added, 1 ml of 500 μM DPPH, and 1 ml of ethanol. The homogenized solution was then kept in a dark room for 20 minutes, and its absorbance was measured at 517 nm. Blank solutions are used aquades instead of sample solutions. Ascorbic acid is used as standard. The fading of the color of the reaction mixture indicates the more neutralised DPPH radicals. The antioxidant content of the ingredient is expressed in *Ascorbic Acid Equivalent* (AAE) mg/g . Results of antioxidant capacity with the formula :

Antioxidant capacity = [1-Abs sample/Abs blank] x 100.

2.2.4. In Vivo Anti-Inflammatory Testing

Testing anti-inflammatory activity *in vivo* with the *rat hind paw edema* method, which measures the volume of swelling in the legs of experimental rats[17]. Experimental animals used male white rats of *the Sprague Dawley* strain, healthy, 2 months old, weighing 150-250 grams. Rats were first adapted for 7 days before being treated. White rats were given standard rations and drinking water ad libitum during the adaptation period. The test animals were divided into 6 groups of 5 mice. The negative control group was given a 0.5% Na-CMC solution; the positive control group was given diclofenac sodium 2.25 mg/kg body weight. The test group consists of 4 groups. The sample solution of the Suji Extract Powder (SEP) test group was given at doses of 3 00 mg /kg and 800 mg /kg. The sample test group of Suji Acetone Extract (AES) and Suji Extract Powder Acetone Extract (EA-SEP) was given a 500 mg/kg dose.

Testing of anti-inflammatory activity is based on the large inflammation volume the test solution can inhibit. The test was carried out using rats that had been satisfied overnight were marked with a dip limit on their feet, then a test or control solution was given through a single strangulation and then injected 0.1ml of carrageenan 1% w/v. and measured the initial volume of the rat's legs (V0) using a pletismometer. Volume measurement is done by means of rat feet inserted until the mark into the pletismometer device. The magnitude of edema volume can be measured from the increase in mercury volume based on the law of capillarity. Measurements are taken every 30 minutes for 5 hours, starting from the 0th minute. The edema volume measurement data was then used to calculate the Percent Increase in Edema Volume (% KVE), *Area Under Curve* (AUC) value, and Percent Anti-inflammatory Inhibition (% IP). The calculation of Percent Increase in Edema is calculated according to the following formula:

$$\%\,\boldsymbol{KVE} = \frac{\boldsymbol{V_t - V_0}}{\boldsymbol{V_0}}\boldsymbol{x}\ \mathbf{100}\%$$

Information:
%KVE : Percent Increase in Volume of Edema
Vt : Volume rat's foot after carrageenan injection in the t-minute
V_0 : The initial volume of rat legs shortly after carrageenan injection

https://doi.org/10.29165/ajarcde.v8i1.371

The Under Curve Area (AUC) is calculated based on the area under the curve between t=0 and t=300 minutes (AUC 0-300) with the x-axis as time (t) and the y-axis as % KVE. The amount of anti-inflammatory activity is expressed by the Percent Anti Inflammatory Inhibition (% IP), which is calculated based on the following formula:

$$\%\ IP = \frac{AUCk - AUCp}{AUCk} x\ 100\%$$

Information:
% IP: Percent Anti-inflammatory Inhibitor
AUCk: *Area Under Curve* (AUC) of the average negative control group
AUCp: *Area Under Curve* (AUC) of the treatment group average

Data were analyzed using variance analysis (ANOVA). If the real difference is significant, proceed with the Smallest Real Difference Test (BNT) at the significant level of 95% (α = 0.05). Data is processed using SPSS 22.0 software. Pearson's analysis examined the correlation between anti-inflammatory activity and total chlorophyll, phenols and antioxidants.

3. RESULT AND DISCUSSION

3.1. Qualitative analysis of phytochemical compounds

A qualitative analysis of phytochemical compounds was conducted to determine the presence of bioactive compounds in suji leaf extract. The qualitative test results of suji leaf extract in each treatment are presented in Table 1.

Table 1. Phytochemical Test Results

Bioactive compounds	Sample		
	SEP	AES	AE-SEP
Flavonoids	++	+	+
Tannin	-	-	-
Saponin	++	+	+
Hydroquinone	-	-	-
Steroids	+	+	+
Triterpenoids	+	+	+
Alkaloids	-	-	-

Information :
- : negative result; + : exists in low levels; ++ : exists in quite high levels
SEP: Suji leaf extract powder; AES: Acetone Extract of Suji leaf; and AE-SEP: Acetone Extract of SEP

3.2. Chlorophyll contents, total phenolic contents, and antioxidant activities

Differences in the content of bioactive compound components in an extract solution affect the compound's biological and chemical activity content. Physical and chemical activity testing of samples was carried out by analysing total chlorophyll content, total phenol, and antioxidant capacity. SEP samples have higher total chlorophyll and total phenol contents than AES and EA-SEP samples, as shown in Table 2.

Table 2. Chlorophyll contents, total phenolic contents, and antioxidant activities

Sample	Chlorophyll contents (mg klorofil/g sampel)	Total phenolic contents (mg GAE/g sampel)	Antioxidant activities (mg AAE/g sampel)
SEP	9.0809±0.06	3.7354±0.05	3.0434±0.87
AES	4.6471±0.17	0.9994±0.02	3.2568±0.97
EA-SEP	6.4912±0.04	2.1703±0.08	2.5500±0.65

Note: SEP (suji leaf extract powder), AES (acetone extract of suji leaf), and EA-SEP(acetone extract of SEP)

3.3. Anti-Inflammatory Activity of Suji Leaf Extract In Vivo

The acute inflammation model used was to screen anti-inflammatory compounds using carrageenan-induced rat paw edema. The percentage increase in edema volume in each treatment is shown in Figure 1. The percentage increase in edema volume in the negative control group showed the most significant level of edema, while the positive control group showed the lowest level of edema. The four suji extract test solution samples showed the ability to suppress edema volume, which was quite large compared to the negative control group. It was seen from the beginning of the first 30 minutes of observation until the end of the observation that the increase in edema volume in each test solution was lower than in the negative control.

The AUC (Area Under Curve) value was then calculated from the data on the increase in edema volume. AUC value to see the percentage of anti-inflammatory activity of each treatment group. Table 3 contains information on the AUC values in the SEP, AES, EA-SEP, negative control, positive control, and SEP groups.

The results of the analysis indicate a significant difference in anti-inflammatory efficacy between the positive control group and each test solution (SEP, AES, and EA-SEP) used ($p < 0.05$). Specifically, SEP at 300 mg/kg exhibited anti-inflammatory potency that differed significantly from SEP at 800 mg/kg but not significantly from the EA-SEP test solution at a dose of 500

mg/kg BW. The SEP, AES, and EA-SEP test solutions demonstrate anti-inflammatory properties.

Pearson correlation [31] coefficients were calculated to estimate the bioactive components acting as anti-inflammatory agents, as shown in Table 4. The correlation levels between anti-inflammatory activity and total chlorophyll and total phenol were 0.529 (moderately strong) and 0.629 (strong), respectively. These values indicate a proportional relationship, suggesting that higher levels of total chlorophyll and total phenol correspond to increased anti-inflammatory activity.

The content of phytochemical components is related to its anti-inflammatory ability. Flavonoid and saponin compounds are present at high levels in SEP. Flavonoids have been reported to play a role in anti-inflammatory activity through antioxidants, modulating gene expression (cytokines, adhesion molecules), and enzyme activity [32]. The bioactive saponin compound also has pharmacological activity as an anti-inflammatory using the method of testing inflammation through carrageenan induction in experimental mice [33-34]. Steroids have also been reported as effective as anti-inflammatories through modern clinical and preclinical studies [35]. Steroids and triterpenoids show anti-inflammatory activity by inhibiting nitric oxide (NO) production [36]. Chlorophyll is sensitive to light, heat, oxygen, and acid degradation [11]. Suji extract in liquid form is limited because it is less stable.

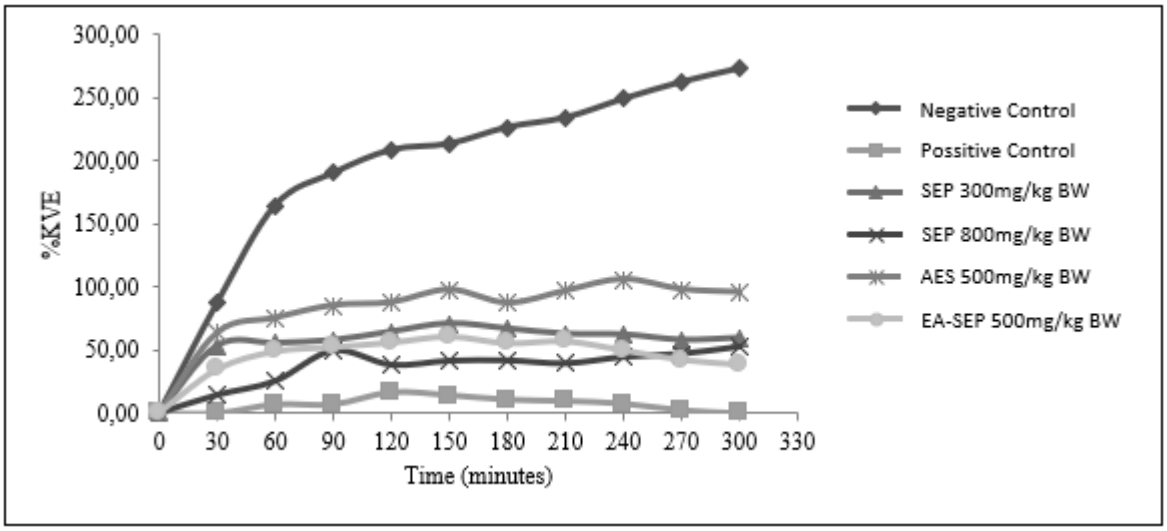

Figure 1. Percentage Increase in Edema Volume in Each Treatment Group

Table 3. Anti-inflammatory Inhibitor, Total Dosage of Chlorophyll, Total Phenol, and Antioxidant Capacity

Samples	Total AUC	IP (%)	Total Dosage of Chlorophyll (mg Chlorophyll/kg)	Total Phenol (mg GAE/kg)	Antioxidant Capacity (mg AAE/kg)
Negative control	59228	-	-	-	-
Positive control	2321	$96.08^{d}\pm5.35$	-	-	-
SEP 300mg/kg BW	17004	$71.39^{b}\pm5.48$	2.7243	1.1206	0.9130
SEP 800mg/kg BW	11513	$80.56^{c}\pm8.59$	7.2647	2.9883	2.4347
AES 500mg/kg BW	25504	$56.94^{a}\pm6.67$	2.3235	0.4997	1.6284
EA-SEP 500mg/kg BW	14356	$75.76^{bc}\pm0.83$	3.2456	1.0852	1.2750

Note: the same letters in the table indicate values that are not significantly different at $p<0.05$
SEP (suji leaf extract powder), AES (acetone extract of suji leaf), and EA-SEP (acetone extract of SEP)

Table 4. Pearson Coefficient Value Between Anti-Inflammatory Activity and Analysis of Total Chlorophyll, Total Phenol and Antioxidant Capacity

Correlation	Pearson Coefficient Value (r)	Level of Correlation
Anti-inflammatory activity - Total chlorophyll	0.529	Strong enough
Anti-inflammatory activity - Total phenols	0.629	Strong
Anti-inflammatory activity - Antioxidant capacity	0.086	Weak

The SEP sample was made with an alkaline $NaHCO_3$ solution as a stabilizer and was dried at low temperatures so that this treatment could maintain the chlorophyll content of the material. This differs from the AES and EA-SEP samples using a rotary evaporator. The heating temperature during the extraction process resulted in a color change in the solution sample from green to brownish green. The process of chlorophyll degradation occurs when chlorophyll turns into pheophytin, resulting in a decrease in the value of the total chlorophyll content of the extract. Apart from being influenced by heating, the formation of pheophytin is also influenced by the presence of acid [37]. Total phenol analysis showed that SEP had the highest total phenol content compared to AES and EA-SEP. The heating process can significantly reduce total phenol levels in vegetables [38]. Reducing the heating temperature can maintain the 80-100% phenol content in

 https://doi.org/10.29165/ajarcde.v8i1.371

some vegetables [39]. Heat reduction in total phenol by heat can occur due to oxidation.

The results of the analysis of antioxidant capacity in AES samples were greater than those of SEP and EA-SEP. It is suspected that during the heating process in AES, chlorophyll compounds change into pheophytin, which also has a high antioxidant content. Pheophytin has higher antioxidant properties than chlorophyll using the DPPH [40] testing method. Even though the chlorophyll content of AES is the lowest, the antioxidant content is the highest. Acetone is used to extract chlorophyll, but the heat received during drying using a rotary evaporator causes chlorophyll degradation. Observing the anti-inflammatory activity of the test solution, the positive control using diclofenac sodium had the most potent anti-inflammatory effect compared to the suji leaf sample extract given. Diclofenac sodium is a non-steroidal anti-inflammatory drug (NSAID) belonging to the phenylacetic acid class, which has anti-inflammatory, analgesic, and antipyretic properties. However, diclofenac sodium has side effects on the gastrointestinal, cardiovascular, and kidney [41].

The anti-inflammatory testing method of creating artificial inflammation in experimental animals is one of the methods for acute inflammation. In acute inflammation, there are changes in the circulation in the micro-blood vessels and fluid exudation. Carrageenan is an inflammatory mediator because it can cause swelling in rats' feet. Carrageenan may cause vascular changes associated with acute inflammation [42]. Carrageenan can cause an increase in the activity of inflammatory marker enzymes such as lipoxygenase (LOX), cyclooxygenase (COX) in peripheral blood mononuclear cells, increasing serum concentrations of ceruloplasmin (proinflammatory) and myeloperoxidase (MPO) [43].

Phytochemical compounds act as anti-inflammatory mechanisms through (1) antioxidant activity and free radical scavengers; (2) modulation of inflammatory cell activity (mastocytes, macrophages, lymphocytes and neutrophils); (3) modulation of the activity of pro-inflammatory enzymes such as phospholipase A2 (PLA2), cyclooxygenase (COX), lipoxygenase (LOX) and enzymes that produce nitric oxide (NO), nitric oxide synthase (NOS); (4) modulating the production of pro-inflammatory compounds and (5) modulating the expression of pro-inflammatory genes [44].

Table 3 shows the total dose of chlorophyll, total phenol, and antioxidant capacity given to each rat for each treatment based on data from Table 2. The anti-inflammatory activity of the SEP 800mg/kg sample is not significantly different from that of EA-SEP 500mg/kg, even though the total dose of chlorophyll contained in the samples given to mice was very different. Other compounds, such as organic acids extracted by acetone solvent, are suspected of anti-inflammatory activity.

Anti-inflammatory activity correlated with chlorophyll and total phenol content. Several other phytochemical compounds in suji leaves also act as anti-inflammatories, such as alkaloids, saponins, steroids, and triterpenoids. Chlorophyll a and pheophytin a (chlorophyll a, which has released the magnesium group) from green leaves have anti-inflammatory activity in experimental swelling caused by carrageenan induction in mice and formalin induction in rat feet [10]. Chlorophyll a can inhibit the expression of the TNF-α gene but does not affect the expression of nitric acid synthase and cyclooxygenase-2.

The amount of antioxidant capacity does not strongly correlate with anti-inflammatory activity. The anti-inflammatory activity is suspected to be modulated by other bioactive compound components, which play a role in suppressing the formation of pro-inflammatory compounds. The presence of ligand binding between bioactive compounds and cell receptors results in changes in gene expression for pro-inflammatory compounds. This study tested antioxidant activity using the DPPH method; the ability to donate H was not closely related to the ligand binding. Bioactive compounds have anti-inflammatory activity by inhibiting the NF-kB signalling pathway and mitogen-activated protein kinases (MAPKs), which produce various pro-inflammatory mediators [45]. Phenolic and flavonoid bioactive compounds have anti-inflammatory activity through the mechanism of inhibiting nitric oxide (NO) activity in RAW 264.7 cells, which is induced by lipopolysaccharide (LPS) and gamma interferon (IFN-γ) as well as through inhibiting the activity of prooxidant enzymes by inhibiting tyrosinase and xantioxidase [46]. The anti-inflammatory mechanisms of bioactive flavonoids include inhibition of pro-inflammatory enzymes such as cyclooxygenase-2, lipoxygenase, NO synthase, NF-kB, AP-1, phase II antioxidant detox activation, MAPK, and protein kinase C and nuclear factor-erythroid 2-related factor 2 (Nrf2) [47].

4. CONCLUSION

Qualitative phytochemical test results show that suji leaf extract contains flavonoids, saponins, steroids and triterpenoid components. The total chlorophyll content and total phenol in SEP samples (9.0809 mg chlorophyll/g samples; 3.7354 mg GAE/g) was greater than AES samples (4.6471 mg chlorophyll/g samples; 0.9994 mg GAE/g samples) and EA-SEP (6.4912 mg chlorophyll/g samples; 2.1703 mg GAE/g samples). The antioxidant content of AES (3.26mg AAE/g sample) was higher than that of the SEP sample (3.04mg AAE/g sample) and EA-SEP (2.5526mg AAE/g sample). Suji leaves act as anti-inflammatory agents, showing the highest anti-inflammatory activity in SEP samples at 80.57%. Components of the phenolic compound group act more as anti-inflammatory agents than chlorophyll. Quantitative testing parameters for chemical characteristics of bioactive components such as flavonoids, saponins, steroids and triterpenoids can be added to see the correlation in anti-inflammatory models of rat hind paw edema with suji leaf extract test solution.

ACKNOWLEDGMENTS

Acknowledgements were conveyed to Mrs Dra. Diana Serlahwaty, M.Si Apt, a lecturer at the Faculty of Pharmacy, Pancasila University, has facilitated anti-inflammatory testing in the QLab laboratory of the Faculty of Pharmacy, Pancasila University.

REFERENCE

[1] Freire MO, Van Dyke TE. Natural resolution of inflammation. *Periodontol 2000*. 2013; 63(1):149-164. doi: 10.1111/prd.12034.

[2] Sasso O, Migliore M, Habrant D, Armirotti A, Albani C, Summa M, *et al*. Multitarget fatty acid amide hydrolase / cyclooxygenase blockade suppresses intestinal inflammation and protects against nonsteroidal anti-inflammatory drug-dependent gastrointestinal damage. *FASEB J*. 2015;29(6):2616-2627. doi:10.1096/fj.15-270637.

[3] Alam B, Akter F, Parvin N, Pia RS, Akter S, Chowdhury J, *et al*. Antioxidant, analgesic and anti-inflammatory activities of the methanolic extract of *Piper betle* leaves. *Avicenna J phytomedicine*. 2013; 3(2):112-125.

[4] Gadamsetty G, Maru S, Sarada NC. Antioxidant and anti-inflammatory activities of the methanolic leaf extract of traditionally used medicinal plant *Mimusops elengi* L. *J Pharm Sci Res*. 2013;5(6):125-130.

[5] Adedapo AA, Adeoye BO, Sofidiya MO, Oyagbemi AA. Antioxidant, antinociceptive and anti-inflammatory properties of the aqueous and ethanolic leaf extracts of *Andrographis paniculata* in some laboratory animals. *J Basic Clin Physiol Pharmacol*. 2015; 26(4):327-334. doi:10.1515/jbcpp 2014-0051.

[6] Dai J, Mumper RJ. Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties. *Molecules*. 2010;15(10):7313-7352. doi:10.3390/molecules15107313.

[7] Zhang C-R, Aldosari SA, Vidyasagar PSP V., Nair KM, Nair MG. Antioxidant and Anti-inflammatory assays confirm bioactive compounds in ajwa date fruit. *J Agric Food Chem*. 2013; 61(24) : 5834 - 5840. doi:10. 1021/jf401371v.

[8] Liu ZH, Wang DM, Fan SF, Li DW, Luo ZW. Synergistic effects and related bioactive mechanism of *Potentilla fruticosa* L. leaves combined with *Ginkgo biloba* extracts studied with microbial test system (MTS). *BMC Complement Altern Med*. 2016;16(1). doi:10.1186/s12906-016-1485-2

[9] Prangdimurti E, Muchtadi D, Astawan M, Zakaria FR. Aktifitas antioksidan ekstrak daun suji (*Pleomele angustifolia* N.E. Brown). *J Teknol dan Ind Pangan*. 2006;XVII(2):79-86.

[10] l A, Velikkakathu V, Asha, Nair SA, Sasidharan SP, Parameswaran K. Sureshkumar, Rajendran KN, *et al.* Chlorophyll revisited: Anti-inflammatory activities of chlorophyll a and inhibition of expression of TNF-α gene by the same. *Inflammation*. 2012; 35(3) : 959 - 966. doi:10.1007/s10753-011-9399-0

[11] Ferruzzi MG, Blakeslee J. Digestion, absorption, and cancer preventative activity of dietary chlorophyll derivatives. *Nutr Res*. 2007;27(1):1-12. doi:10.1016/j.nutres.2006. 12.003.

[12] Liehn EA. Hector A C-F. 2015. Inflammation between defense and disease: impact on tissue repair and chronic sickness. Discoveries 3(1):e42. doi: 10.15190/d.2015.34

[13] Kalpna R, Mital K, Sumitra C. 2011. Vegetable and fruit peels as a novel source of antioxidants. J Med Plants Res. 5(1): 63-71

[14] Watzl B. 2013. Anti-inflammatory effects of plant-based foods and of their constituents. Int J Vitam Nutr Res. 78:293-298. doi: 10.1024/0300-9831.78.6.293.

[15] Weydert CJ, Cullen JJ. 2010. Measurement of superoxide dismutase, catalase, and glutathione peroxidase in cultured cells and tissue. Nat Protoc. 5(1): 51–66. doi:10.1038/nprot.2009.197

[16] Ricciotti E dan FitzGerald GA. 2011. Prostaglandins and Inflammation. Arterioscler Thromb Vasc Biol. doi:10.1161/ATVBAHA.110.207449

[17] Kim YS, Young MR, Bobe G, Colburn NH, Milner JA. 2009. Bioactive food components, inflammatory targets, and cancer prevention (review). AACR. doi: 10.1158/1940-6207.CAPR-08-0141

[18] Bernhoft A. 2010. A brief review on bioactive compounds in plants.Bioactive Compounds In Plants Benefits and Risks for Man and Animals [Online]. http://www.dnva.no/binfil/download. php?tid=48677

[19] Vauzour D, Rodriguez-Mateos A, Corona G, Oruna-Concha, Spencer JPE. Polyphenols and Human Health: Prevention of Disease and Mechanisms of Action. doi: 10.3390/nu2111106

[20] Dai J, Mumper RJ. 2010. Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. Molecules 15. doi:10.3390/molecules 15107313

[21] Appian S., Asha VV., Nair A., Sasidharan SP. 2011.Chlorophyll Revisited: Anti-inflammatory Activities of Chlorophyll a and Inhibition of Expression of TNF-α Gene by the Same. PubMed. doi:10.1007/s10753-011-9399-0

[22] De Vogel J, Denise SML, Jonker-Termont, Martijn B. Katan, R van der Meer. 2005. Natural chlorophyll but not chlorophyllin prevents heme-induced cytotoxic and hyperproliferative effects in rat colon. J Nutr. 2005 135(8):1995-2000.

[23] Subramoniam A, Velikkakathu V, Asha, Nair SA, Sasidharan SP, Parameswaran K. Sureshkumar, Rajendran KN, Karunagaran D, Ramalingam K. 2012. Chlorophyll Revisited: Anti-inflammatory Activities of Chlorophyll a and Inhibition of Expression of TNF-α Gene by the Same. Inflammation. 35. 3. 2012. doi: 10.1007/s10753-011-9399-0

[24] Lihuan D, Jingcun Z, Ning J, Guozeng W, Yiwei C, Wei L, Jing Q, Yuanfang Z, Gang C, 2014. Photodynamic therapy with the novel photosensitiser chlorophyllin f induces apoptosis and autophagy in human bladder cancer cells. Lasers in Surgery and Medicine 46:319–334.

[25] Prangdimurti, E. Kapasitas antioksidan dan daya hipokolesterolemik ekstrak daun suji (*Pleomele angustifolia* N.E. Brown). *Disertasi*. Bogor: Program Studi Ilmu Pangan, Fakultas Teknologi Pertanian, Institut Pertanian Bogor, 2007.

[26] Mujeeb F, Bajpai P, Pathak N. Phytochemical evaluation, antimicrobial activity, and determination of bioactive components from leaves of *Aegle marmelos*. *Biomed Res Int*. 2014;2014. doi:10.1155/2014/497606.

[27] Vimala T, Poonghuzhali T V. Estimation of seaweed pigments using acetone and DMSO. *Int J Sci Res*. 2013;14611(10):2319-7064.

[28] Pourmorad F, Hosseinimehr SJ, Shahabimajd N. Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. *African J Biotechnol*. 2006;5:1142-1145. doi:10.1055/ s-2007-987042.

[29] Narsih, Kumalaingsih S, Wijana S, Wignyanto. Microencapsulation of natural antioxidant powder from *Aloe vera* (L.) skin using foam mat drying method. *Int Food Res J*. 2013;20(2):681-685.

[30] Kaushik D, Kumar A, Kaushik P, Rana AC. Analgesic and anti-inflammatory activity of *Pinus roxburghii* Sarg. 2012;2012. doi:10.1155/2012/245431.

[31] Evans JD. *Straightforward statistics for the behavioural sciences.* Pacific Grove, CA: Brooks/Cole Publishing. 1996.

[32] Pérez-Cano FJ, Castell M. Flavonoids, inflammation and immune system. *Nutrients.* 2016;8(10):8-11. doi:10.3390/nu8100659.

[33] Hassan HS, Sule IM, Musa MA, Musa YK, Abubakar SM, Hassan SA. Anti-inflammatory activity of crude saponin extracts from five Nigerian medicinal plants. *African J Tradit Complement Altern Med*. 2012; 9(2):250-255. doi:10.4314/ajtcam. v9i2.10

[34] Sarkhel S. Evaluation of the anti-inflammatory activities of *Quillaja saponaria* Mol. Saponin extract in mice. *Toxicol Reports.* 2016;3:1-3. doi:10.1016/j.toxrep. 2015.11.006

[35] Patel SS, Savjani JK. Systematic review of plant steroids as potential anti-inflammatory agents: Current status and future perspectives. *J Phytopharm JPHYTO*. 2015; 4(42):121-125.

 https://doi.org/10.29165/ajarcde.v8i1.371

[36] Ko HH, Hung CF, Wang JP, Lin CN. Antiinflammatory triterpenoids and steroids from *Ganoderma lucidum* and *G. tsugae*. *Phytochemistry*. 2008; 69(1):234-239. doi:10.1016/j.phytochem.2007.06.008

[37] Gross J. *Pigments in vegetables, chlorophylls and carotenoids.* New York(US): AVI Book, Van Nostrand Reinhold. 1991.

[38] Lima GPP, Lopes TDVC, Rossetto MRM, Vianello F. Nutritional composition, phenolic compounds, nitrate content in eatable vegetables obtained by conventional and certified organic grown culture subject to thermal treatment. *Int J Food Sci Technol*. 2009;44(6):1118-1124. doi:10.1111/j.1365-2621.2009.01928.x

[39] Roy MK, Juneja LR, Isobe S, Tsushida T. Steam-processed broccoli *(Brassica oleracea*) has higher antioxidant activity in chemical and cellular assay systems. *Food Chem*. 2009;114(1):263-269. doi:10.1016/j. foodchem.2008.09.050

[40] Hsu C, Chao P, Hu S, Yang C. The antioxidant and free radical scavenging activities of chlorophylls and pheophytins. *Food Nutr Sci*. 2013;4(August):1-8. doi:10.4236/fns.2013.48A001

[41] Altman R, Bosch B, Brune K, Patrignani P, Young C. Advances in NSAID development: Evolution of diclofenac products using pharmaceutical technology. *Drugs*. 2015;75(8):859-877. doi:10.1007/s 40265-015-0392-z

[42] Corsini E, Di Paola R, Viviani B, Genovese T, Mazzon E, Lucchi L, *et al.* Increased carrageenan-induced acute lung inflammation in old rats. *Immunology*. 2005;115(2):253-261. doi:10.1111/j.1365-2567.2005.02148.x

[43] Mohsin S, Muraleedhara Kurup G. Mechanism underlying the anti-inflammatory effect of sulphated polysaccharide from Padina tetrastromatica against carrageenan induced paw edema in rats. *Biomed Prev Nutr*. 2011;1(4):294-301. doi:10.1016/j.bionut.2011.09.004

[44] Bellik Y, Boukraâ L, Alzahrani HA, Bakhotmah BA, Abdellah F, Hammoudi, *et al.* Molecular mechanism underlying anti-inflammatory and anti-allergic activities of phytochemicals: An update. *Molecules*. 2013;18(1):322-353. doi:10.3390/molecules 18010322.

[45] Arulselvan P, Fard MT, Tan WS, Gothai S, Fakurazi S, Norhaizan ME *et al.* Role of antioxidants and natural products in inflammation. *Oxid Med Cell Longev*. 2016;2016. doi:10.1155/2016/5276130.

[46] Oskoueian E, Abdullah N, Hendra R, Karimi E. Bioactive compounds, antioxidant, xanthine oxidase inhibitory, tyrosinase inhibitory and anti-inflammatory activities of selected agro-industrial by-products. *Int J Mol Sci*. 2011;12(12):8610-8625. doi:10.3390/ijms12128610.

[47] Serafini M, Peluso I, Raguzzini A. Flavonoids as anti-inflammatory agents. *Proc Nutr Soc*. 2010;69(3):273-278. doi:10.1017/S002966511000162X.