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Anti-inflammatory effects of *Eucheuma denticulatum* and *Padina minor* crude extracts in an egg albumin-induced paw edema of ICR mice

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Abstract. *Labiaga AS, Ples MB, Montaño MNE. 2024. Anti-inflammatory effects* of Eucheuma denticulatum *and* Padina minor *crude extracts in an egg albumin-induced paw edema of ICR mice. Nusantara Bioscience 16: 245-250.* Inflammation indicates that the body is unhealthy and exposed to harmful stimuli. Hence, it is important to study natural compounds with anti-inflammatory activities. This study assessed the anti-inflammatory effects of the aqueous and ethanolic crude extracts of *Eucheuma denticulatum* (N.L. Burman) F.S. Collins & Hervey and *Padina minor* (Yamada, 1925) in an egg albumin-induced paw edema of male Institute for Cancer Research (ICR) mice. This in vivo study investigated the anti-inflammatory activity of the aqueous and ethanolic crude extracts from *E. denticulatum* and *P. minor* at a dose of 100 mg/kg and compared it with a commercial anti-inflammatory drug (mefenamic). The study showed a significant decrease in the paw size, body temperature, neutrophil count, and white blood cell count in mice treated with seaweed crude extracts. The results of the first hour showed that the *Eucheuma* ethanolic extract treatment had the highest decrease in paw size, and the highest temperature reduction was found in the treatment of *Padina* ethanolic extract. There are significant differences in paw size, temperature, and leukocytes between the control and treatment groups, as well as between the aqueous and ethanolic extracts. The extracts from *E. denticulatum* significantly decrease the temperature, paw size, and leukocytes, which are the inflammation markers of egg albumin-induced paw edema. The diverse seaweed in the country has various pharmacological potential that should be studied further to maximize utilization.

Keywords: Bioactive compounds, brown algae, ethanolic extracts, red algae, seaweeds

Abbreviations: CBC: Complete Blood Count, EAE: *Eucheuma* Aqueous Extract, EAIPE: egg-albumin-induced paw edema, EDTA: Ethylenediaminetetraacetic Acid, EEE: *Eucheuma* Ethanolic Extract, IACUC: Institutional Animal Care and Use Committee, IBMC: Institutional Biorisk Management Committee, ICR: Institute for Cancer Research, PAE: *Padina* Aqueous Extract, PC: Positive Control, PEE: *Padina* Ethanolic Extract, NC: Negative Control, SC: Sham control, WBC: White Blood Cells

INTRODUCTION

Health and safety are important and concerning matters for everyone. Utilizing natural resources to develop pharmacological and biomedical products still plays an important role in overcoming various diseases. Traditional medicines have low side effects, and natural products remain valuable in the age of traditional and modern medicines.

Marine algae are abundant in the Philippines, and it is famous for its good seaweed cultivation. There are various seaweed species in the country, but most of them remain underexplored. People benefit from abundant seaweeds as food, for export, and as growth promoters or fertilizers, but there are very few studies on the benefits of bioactive products and organic compounds. The red seaweed *Eucheuma denticulatum* (N.L. Burman) F.S. Collins & Hervey is one of the Philippines' four main farmed red seaweed species (Dumilag et al. 2023). However, there are more important benefits of seaweed, namely drug development. Many people consider seaweeds for food; therefore, it is essential to explore information on their utilization for nutraceuticals and pharmaceuticals (Masoumi et al. 2021). Abundant marine algae can also source seaweed-derived bioactive products with high potential for different bioactivities. Only less than half of the species in the country have an economic value, with less than 5% of these species having significant economic value (Magdugo 2020) because many seaweeds are underutilized, and their pharmacological potentials remain unexplored. Ordinary people on the coast have even less notice for the brown seaweed *Padina minor* (Yamada, 1925). Resort owners and swimmers want these brown seaweeds removed because they can add dirt to the beach with the tides.

The phytochemical content of seaweed provides a wealth of potential health benefits, offering a promising avenue for natural medicine against diseases. Various brown, red, and green seaweeds provide sulfated polysaccharides and other bio-active constituents, namely, fucoidan, laminarin, carrageenan, agar, ulvan, tannins, phlorotannin, polyphenols, folic and folinic acids, tocopherols, terpenoids, fucoxanthin, lipids, fatty acids, proteins, and their derivatives (Saraswati et al. 2019; Lomartire and Gonçalves 2022). Seaweeds are

a reservoir of essential bioactivities with the potential as anti-inflammatory, antibacterial, anticancer, antiviral, anti-thrombocytopenia, analgesic, antioxidant, anticoagulant, and antihyperglycemic, cardio- and neuroprotective, and have anti-obesity properties.

Inflammation indicates the body is unhealthy and exposed to harmful stimuli like infections, injuries, and toxins. Acute inflammation displays fever, redness, warmth, swelling, and pain around tissues and joints as a response to injury or disease. It may also progress to chronic degenerative diseases like rheumatoid arthritis, multiple sclerosis, cancer, arthritis, atherosclerosis, heart disease, obesity, diabetes, asthma, dermatitis, migraine, irritable bowel disease, insulin resistance, autoimmune, and other diseases (Barbu et al. 2022). The anti-inflammatory activity of natural compounds is vital in treating the health conditions mentioned above. Some of the seaweed species in the country that are included in the summary of their utilization with an emerging interest in their anti-inflammatory bioactivity are *Asparagopsis taxiformis*, *Porphyra yezoensis*, and *Kappaphycus alvarezii* (Hurtado et al. 2020). The majority of the anti-inflammatory activities of *Eucheuma* species are used in food and industry. Despite the tremendous biodiversity of seaweeds in the Philippines, to the best of the author's knowledge, there are still limited published studies on seaweed utilization for biomedical and pharmacological potential in the country. This study on the anti-inflammatory activities of *E. denticulatum* and *P. minor* in an egg albumin-induced paw edema was aimed at assessing the pharmacological potential of red and brown seaweed extracts and utilizing seaweed bioactive compounds to impact public health and to prevent and overcome diseases.

MATERIALS AND METHODS

Ethical consideration

IACUC certification

The Cebu Technological University-Institutional Animal Care and Use Committee (IACUC) approved laboratory animal safety and proper handling as a protocol under certificate IACUC2020-MA-A03. The study followed the IACUC guidelines, the principles of animal welfare, the Animal Welfare Act of the Philippines (RA 8485), and AO 45 of the Bureau of the Animal Industry.

Biosafety/biorisk clearance

The Institutional Biorisk Management Committee of Cebu Technological University provided the ethical certificate IBMC-2020-MA-004-LAB. The proper procedure for the safety and security of any valuable biological materials was followed throughout the experimentation. Approved safety protocols with necessary precautionary measures to avoid harming humans handling the experiment and providing animal biosafety were observed when dealing with chemicals (e.g., Zoletil).

Research procedure

Seaweed collection and extract preparation

Fresh samples of red seaweed *E. denticulatum* and brown seaweed *P. minor* (25 kg of each) were collected from the shallow coast of Olango Island, Visayas, Philippines, with geographical coordinates at 10°21' 37" North, 124°04'58" East Pangan-an, Lapu-Lapu City, Cebu, Philippines. The samples were naturally air-dried and cut into smaller sizes. The study carried out the phenolic extraction and seaweed bioactive compound preparation techniques of Cotas et al. (2020) with minor modifications. Some seaweeds were preserved as representative samples and submitted for identification to the herbarium curator at the University of San Carlos. The seaweed samples were identified as *P. minor* Yamada (1925) and *E. denticulatum* (N.L. Burman) F.S. Collins & Hervey. The accession number of the herbarium specimen is USCBM2678.

The study used the modified decoction aqueous-extraction method of Godlewska et al. (2016). The red seaweed crude extracts were obtained using a 1:3 seaweed: water ratio. For every 0.5 kg of seaweed powder, 1.5 L of water was used. The extract was heated at 333.15 Kelvin (60°C) and filtered to collect the supernatant. The *Eucheuma* Aqueous Extract (EAE) was stored at 4°C until used. The same procedure was performed to obtain the *Padina* Aqueous Extract (PAE). Air-dried and powdered seaweed samples were used to generate the ethanolic extracts of *E. denticulatum*. The samples were macerated in 95% ethanol (at a 1:3 sample: solvent ratio) for at least 72 hours and then filtered. The *Eucheuma* Ethanolic Extract (EEE) was then collected. The excess alcohol was removed using a rotary evaporator at 100 rpm at 40 to 60°C, with an extract yield of 20-30%. The same method was used to conduct the *P. minor* Ethanolic Extract (PEE). All collected samples were stored in 10-50 mL amber bottles at 4°C until used.

Test animal preparation

The study used 49 male albino Institute for Cancer Research (ICR) mice, 6-8 weeks old, weighing 25-35 g, obtained from the Animal Laboratory in Fil-Scientia Research and Consultancy Services in Cebu City, Philippines. The mice were grouped into seven groups (with seven individuals each) before the 7 days of acclimatization. Three control groups were sham, negative, and positive (SC, NC, and PC), and four treatment groups, PAE, EAE, PEE, and EEE, were closely monitored and observed for their behavior, body score, and attitude. The mice were provided with a regular pellet diet and reverse osmosis water ad libitum from acclimatization until the end of the experiment. The animals were housed in a room with a 12-hour cycle of light and dark at 18-26°C. During the experiment, the animal's paw size was measured using a Vernier caliper (mm), and the temperature (°C) was taken using a digital ear scanner for mice. The temperatures and the paw sizes after induction and post-treatment (first hour, 3 h, 6 h, and 24 h) were then compared. The extracts were given to the treatment groups via oral gavage, 1 h after the egg albumin induction and every 6 h afterward. The animals were weighed before treatment.

Egg albumin inflammation induction and monitoring

After acclimatization, egg albumin was injected into the sub-plantar region of the right hind paw to induce paw edema. The egg albumin-induced paw edema is an animal model for developing rheumatoid arthritis. Next, 0.1 mL of egg albumin in 20% normal saline was induced in all groups except the sham control. This induction procedure followed the method of Akinloye et al. (2020). A digital Vernier caliper and a digital ear scanner thermometer were used to measure the paw size and the temperature, respectively, during pre- and post-induction and -treatments (after the first hour, 3 h, 6 h, and 24 h). The blood samples were collected post-induction and -treatment to compare blood parameters and analyzed for White Blood Cell count (WBC), especially the neutrophil count.

Experimental design and administration of treatments

The study used a randomized experimental design with 49 male albino ICR mice (seven mice for each of the seven groups). The mice had 7 days of acclimatization and 2 days of treatment. Baseline data of uninduced, untreated sham control groups and the groups with egg albumin-induced paw edema were taken. The anti-inflammatory activity of mice treated with seaweed extract was compared to the Sham Control (SC) and the negative and positive control groups. The Negative Control (NC) group was only given after induction, and the Positive Control group (PC) was assigned a dose of 250 mg/kg of mefenamic acid in 0.9% saline (Feng and Wang 2018). Through oral gavage, the treatment groups were given four different seaweed extracts, i.e., EAE, PAE, EEE, and PEE, at a dose of 100 mg/kg (Tumang and Ples 2017; Lomartire and Gonçalves 2022). The experimental extracts were administered an hour after induction and every 6 hours afterward.

Blood collection and analysis

A total of 49 tubes of blood samples were collected using the tail–vein method an hour after induction and 24 hours after treatment. Blood was collected in purple-top ethylenediaminetetraacetic acid (EDTA) vacutainers between 0.25 and 0.5 mL. The WBC and neutrophil counts were compared across the control and the remaining treatment groups. The anesthetic, Zoletil, was used before extraction at 0.2 mL per 0.30 g bodyweight via the intraperitoneal route. The second blood extraction was performed via the orbital sinus method to obtain a blood sample 24 hours after the first treatment, followed by termination. During the terminal blood collection, the capillary tube was placed into the orbital sinus of the mice, and 0.25–0.5 mL of blood was collected and sent to a laboratory for complete blood count, which includes WBC and neutrophil counts. The data of six induced groups (i.e., NC, PC, EAE, PAE, EEE, and PEE) were compared to the uninduced group SC and the data before induction. The anti-inflammatory activity of all treatments was compared to the positive control using a commercial anti-inflammatory drug.

Data analysis

The mean and the standard deviation of the paw size, temperature, WBC, and neutrophil count were presented and compared. The measured parameters were analyzed using one-way Analysis of Variance (ANOVA) with a statistical significance level of 0.05α.

RESULTS AND DISCUSSION

Evaluating the anti-inflammatory effects of bioactive seaweed compounds included markers of inflammation and indicators: paw size, temperature, WBC, and neutrophil count, pre- and post-induction, and post-treatment. Table 1 shows the relative difference between the pre-induction and every succeeding hour until the end of the treatment period. One hour after applying 0.1 mL egg albumin for paw edema induction, a significant increase in the paw sizes of the induced group of mice (NC, PC, EAE, PAE, EEE, and PEE) was recorded as 1.16, 1.19, 1.27, 1.26, 1.67, and 1.38 cm, respectively. The induced groups' temperature increases (in degrees Celsius) were 0.9, 0.8, 1.0, 1.0, 1.4, and 1.5, respectively. It indicates the success of egg albumin-induced paw edema in mice. The paw size significantly decreased 3 h after the animals were treated with seaweed extracts, and the extracts were continuously provided every 6 h for 24 h.

After the EAIPA induction, the results showed an increase in paw size in all of the induced groups, from the negative control to the experimental groups (compared to the baseline data). However, decreased paw size was evident after treatment from the paw size in 1 hour to the 3rd hour or 2 hours after treatment, except for NC. The negative control group (treated with water only) did not decrease after 1 h (instead, it increased from 1.68 to 1.71) and showed the lowest decrease in the following hours, with the decrease range from 0.3 to 0.11 mm. The highest decrease in the paw size was obtained in the EEE between 1 and 3 h post-treatment with a 0.76 mm decrease (2.18 to 1.42), while the PEE ranked second with a 0.69 mm decrease (from 1.88 to 1.19). In all the induced groups, the recorded range of decrease in paw size after treatment was from 0.01 to 0.76 mm. After treatment, the experimental groups showed a significant decrease in paw size 24 hours after the increase in post-induction. The negative control has the least decrease of 0.13 (from 1.68 to 1.55), PC has 0.37 difference, both aqueous extracts (EAE decreased from 1.73 to 1.07 and PAE from 1.76 to 1.10) have 0.66 difference, 1.08 difference from 1.88 to 1.08 in PEE, and the EEE has the highest difference of 1.23 mm for the size dropped from 2.18 to 0.98. The effectiveness of the extracts on mice paw size was higher in the two ethanolic extracts, which might be due to polyphenolic compounds, such as phenols and tannins.

Changes in body temperature are a sign of inflammation and the effectiveness of anti-inflammatory treatments. In this study, the body temperature of the mice control group, housed at 18–26°C, ranged from 35.1°C to 35.4°C. The highest increase in baseline body temperature before induction was recorded as 1.5°C. It is important to note that a significant difference of approximately 1°C can significantly affect the mice (Guo et al. 2012). The normal surface temperature of mice may vary. The temperature induced by diseases may also fall within the normal limits

but show a slight increase or decrease.

Table 2 presented a noticeable increase in mice's body temperature at 1st hour in induced groups (not in Sham) and decreased temperature after treatment (3, 6, and 24 hours) except for the little increase on 3rd hour of NC and 6th hour of PC. The highest temperature difference from the pre-and post-treatments was obtained in the treatment group PEE with a 2.1°C difference, from 37.5°C post-induction temperature to 35.4°C body temperature after 24 hours or after treatment. However, the temperature of mice after treatment ranged from 35.1 °C to 36.2 °C in all groups. The PEE treatment potentially reduced the temperature faster and showed an anti-inflammatory effect. Both aqueous extracts (EAE and PAE) decreased the temperature 3 h after treatment, while the standard drug had a temperature difference of 0.5 at most. There is a significant difference in the temperature of the three control and treatment groups. The temperature change in *P. minor* significantly differed from that in *E. denticulatum*. The temperature of the aqueous extracts also statistically varied in the ethanolic extracts.

The WBC range was within the typical WBC count in mice, which ranged from 2,000 to 10,000 (Table 3). The decreased WBC count in the treatment of seaweed extract indicated the positive effects of the seaweed extracts. In the results of the leukocyte level of the induced groups, the PAE group has the lowest decrease of 1.3, while the decrease in EEE (2.6 difference) is almost the same as the decrease in PC, which is 2.7. The EEE in the treatment group, which is the group treated with ethanolic extract of seaweed, has almost the same effect as the positive control group, which used the commercial anti-inflammatory drug. Polyphenols, such as flavonoids, are bioactive compounds that can inhibit regulatory enzymes or transcription factors to control mediators (Maleki et al. 2019) and reduce the WBC and neutrophil count, important indicators of inflammation. Neutrophil counts are approximately 20 to 30% of the WBC count of laboratory animals with lymphocytic hemograms, like rodents (Tizard 2017). The average neutrophil count decreased post-induction and post-treatment, except in SC (Table 3). The neutrophil count showed no statistical difference between the control and treatment groups an hour after induction and before treatment. Table 3 shows a significant decrease from pre- to post-treatments.

Table 1. The paw size in egg albumin-induced mouse

Group baseline		Paw size (mm)				
		1st hour	3rd hour	4th hour	6th hour	24 hours
SC	0.54±0.04	0.54±0.04	0.55±0.03	0.56±0.03	0.57±0.03	0.57±0.02
NC	0.52±0.09	1.68±0.25	1.71±0.17	1.62±0.17	1.58±0.16	1.55±0.17
PC	0.50±0.06	1.69±0.12	1.65±0.09	1.48±0.16	1.58±0.16	1.32±0.06
EAE	0.46±0.13	1.73±0.16	1.42±0.11	1.27±0.12	1.26±0.08	1.07±0.10
PAE	0.50±0.07	1.76±0.08	1.41±0.07	1.30±0.11	1.26±0.08	1.10±0.06
EEE	0.51±0.09	2.18±0.30	1.42±0.05	1.24±0.10	1.15±0.09	0.98±0.13
PEE	0.50±0.09	1.88±0.18	1.19±0.19	0.94±0.10	0.86±0.06	0.80±0.08
F-computed value	0.293	30.738	43.860	34.219	45.457	40.803
P-value	0.9367667080 39595	0.00000000000007 389494 (**)	0.00000000000000 015629 (**)	0.00000000000001 200810 (**)	0.000000000000 00008242 (**)	0.0000000000 0000056337 (**)

Note: p>0.05: No significant difference, *p<0.05: Significant difference, **p<0.01: Highly significant difference. SC: Sham Control, NC: Negative Control, PC: Positive Control, EAE: *Eucheuma* Aqueous Extract, PAE: *Padina* Aqueous Extract, EEE: *Eucheuma* Ethanolic Extract, PEE: *Padina* Ethanolic Extract (PEE)

Table 2. The temperature and the results of ANOVA of mice after paw edema induction

	Pre-induction (Baseline Data) in °C	1st-hour post-induction (°C)	*3rd hour* Post-treatment (°C)	4th hour Post-treatment (°C)	6th Hours Post-treatment (°C)	24 hours Post-treatment (°C)
SC	35.4	35.4	35.3	35.2	35.2	35.1
NC	35.5	36.4	36.5	36.2	36.2	36.2
PC	35.5	36.3	36.8	36.5	36.3	36.0
EAE	35.2	36.2	36.7	36.2	35.8	35.4
PAE	35.5	36.5	36.5	36.2	35.8	35.6
EEE	35.5	36.9	36.5	36.1	36.0	35.5
PEE	36.0	37.5	35.4	34.8	35.0	35.4
F-computed value	1.902	9.622	16.659	22.499	23.645	10.205
P-value	0.1030810 9061643	0.0000011 74328656 (**)	0.000000001 018497 (**)	0.00000000 0011515 (**)	0.00000000 0005286 (**)	0.0000 00595550103 (**)

Note: p>0.05: No significant difference, *p<0.05: Significant difference, **p<0.01: Highly significant difference. SC: Sham Control, NC: Negative Control, PC: Positive Control, EAE: *Eucheuma* Aqueous Extract, PAE: *Padina* Aqueous Extract, EEE: *Eucheuma* Ethanolic Extract, PEE: *Padina* Ethanolic Extract (PEE)

Table 3. The leukocyte and neutrophil count and results of ANOVA in mice pre- and post-treatment

	Leukocyte count		Neutrophil Count	
	One hour pre-treatment	**24-hour pre-treatment**	**One hour pre-treatment**	**24-hour pre-treatment**
SC	2.7	3.0	2.7	3.0
NC	5.1	2.7	5.1	2.7
PC	4.6	1.9	4.6	1.9
EAE	3.8	2.4	3.8	2.4
PAE	3.6	2.3	3.6	2.3
EEE	5.1	2.5	5.1	2.5
PEE	3.9	2.2	3.9	2.2
F-computed value	7.04	2.47	0.70	2.33
P-value	0.0000314901075156512 (**)	0.04 (**)	0.66	0.05 (*)

Note: p>0.05: No significant difference, *p<0.05: Significant difference, **p<0.01: Highly significant difference. SC: Sham Control, NC: Negative Control, PC: Positive Control, EAE: *Eucheuma* Aqueous Extract, PAE: *Padina* Aqueous Extract, EEE: *Eucheuma* Ethanolic Extract, PEE: *Padina* Ethanolic Extract (PEE)

The extracts and the commercial drug significantly reduced the neutrophil count. There was a considerable decrease in neutrophils between pre- and post-treatments, but no significant difference existed between the control and treatment groups. Several studies on inflammation mediators supported these findings. Bioactive compounds normalize the body's WBC and neutrophil counts. These compounds are associated with anti-inflammatory activity, such as carotenoids, phytosterols, alkaloids, and sulfated polysaccharides from *Caulerpa cupressoides* (Khursheed et al. 2023). Labiaga et al. (2021) showed that the phytochemical analysis results on *E. denticulatum* and *P. minor* contained high alkaloids and carbohydrates. The *E. denticulatum* had a low phenol and sterols, but *P. minor* had paw sizes that were much higher than the critical value of 2.32. Both the aqueous and ethanolic extracts showed a potential anti-inflammatory effect, decreasing neutrophil count and reducing paw edema.

The egg albumin significantly induced paw edema in mice as the paw size increased from pre- to post-induction. The paw size from post-induction treatment was much higher than the critical value of 2.32. The seaweed extracts decreased the paw size in the post-treatment and showed considerable difference in the paw edema of mice in all treatment groups. The highest increase after induction and the highest decrease in paw size post-treatment was evident in the EEE group. The fastest decrease in body temperature was in PEE. The highest reduction of WBC and neutrophil was traced in the positive control group, with only 0.1 difference from the EEE treatment group. A significant difference was also observed between the control group and the groups with seaweed extract treatment and between the aqueous and ethanolic extracts. Extracts of *E. denticulatum* and *P. minor* positively reduced mice's paw size and body temperature.

The brown seaweed *P. minor* significantly decreased the paw size, temperature, WBC, and neutrophil count among the induced mice. The bioactive constituents of these species, which are not well known but are abundant in temperate countries like the Philippines, have a high potential for anti-inflammatory. Previous studies showed that a phlorotannin-rich fraction of brown seaweed, *Cystoseira sedoides*, had great anti-inflammatory effectivity (Lomartire and Gonçalves 2022). Its lectin promotes the reduction of inflammatory hypernociception and inhibits plasma extravasation, cytokine levels, cyclooxygenase-2, and intercellular adhesion molecules (Rivanor et al. 2018). A study in Thailand showed that the aqueous extract of *P. minor* lessened rat ear edema (Peerapornpisal et al. 2010). The strong suppression of edema in the methanolic extract of *Ulva linza* and *Undaria pinnatifida* indicated its anti-inflammatory significance (Lomartire et al. 2021).

Seaweeds, such as *Eucheuma* and *Kappaphycus*, have the potential as anti-inflammatory in solid edema suppression, decreased neutrophil migration, and are potential sources of effective functional metabolites that are potential in inhibiting α-amylase, leukocyte recruitment, and reduced leukocyte influx in the peritoneal cavity (Balasubramaniam et al. 2016). The compounds associated with the anti-inflammatory activity are phenolics, carotenoids, phytosterols, alkaloids, flavonoids, tannins, and sulfated polysaccharides like fucans, flavonoids, tannins, fucoidan, and fucoxanthin (Cotas et al. 2020). Flavonoid is a polyphenol-based bioactive compound that can inhibit regulatory enzymes or transcription factors to control mediators involved in inflammation (Maleki et al. 2019).

Other inflammation mediators are nitric oxide, prostaglandins, and cytokines. Polysaccharides from *Sargassum horneri* showed the highest nitric oxide inhibition and effectively reduced the production of inflammatory cytokines (Jayawardena et al. 2020). *Padina australis* was also assessed to suppress nitric oxide production in lps-induced macrophages and potential as an anti-inflammatory agent (Pechroj et al. 2020). The seaweeds used in previous studies were *C. sedoides*, *Cladostephus spongiosis*, *Padina pavonica*, *Padina gymnospora*, *P. australis*, methanol extracts of *U. linza*, *Ulva pinnatifida*, *Ulva prolifera*, *Chaetomorpha linum*, and *C. cupressoides* (Marques et al. 2012; Ripol et al. 2018; Lomartire et al. 2021; Khursheed et al. 2023). These are just a few, and vast seaweed potentials remain for biomedical and pharmaceutical utilization.

In conclusion, as egg albumin successfully induced paw edema in male ICR mice, the aqueous and ethanolic seaweed extracts from *E. denticulatum* and *P. minor* showed significant anti-inflammatory effects. The seaweed extracts substantially reduced the induced mice's paw size, temperature, WBC, and neutrophil count. The decrease in the paw size and temperature from post-induction to post-treatment indicated the anti-inflammatory effects of the crude extracts. The difference between the WBC and the neutrophil count from post-induction to post-treatment supports the claim that the extracts from the two seaweed species effectively treat inflammation. The bioactive compounds of *E. denticulatum* and *P. minor* potentially affect the inflammation after seaweed extract treatment. This result showed that seaweed extracts have health benefits; therefore, the abundance of seaweeds in the Philippines can have high pharmacological and biomedical potential. Besides being a food and functional food ingredient, seaweed may also be used to fight diseases.

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