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ANTIOXIDANT AND ANTI-INFLAMMATORY INSIGHTS OF *PSEUDOPTEROGORGIA AUSTRALIENSIS* COLLECTED FROM WEST COAST OF MUMBAI

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ABSTRACT: The study investigates to find the antioxidant and anti-inflammatory properties of the crude extract of coral *Pseudopterogorgia australiensis.* Various assays were used to assess the biological activities of the crude extract. The antioxidant capacity was evaluated using DPPH, ABTS, and FRAP tests. The DPPH assay indicated a dose-dependent radical scavenging effect whereas the ABTS assay showed a significant inhibition of which is comparable to ascorbic acid. In the case of FRAP assay it confirmed that the antioxidant activity revealing a strong reducing power which was equivalent to Trolox. These results demonstrated that the crude extract of *Pseudopterogorgia australiensis* have strong potential to neutralize ROS and mitigate oxidative damage. The Anti-inflammatory activity was assessed through albumin denaturation, proteinase inhibitory activity, and heat-induced heamolysis. The albumin denaturation assay demonstrated moderate inhibition where as the proteinase inhibitory assay showed stronger inhibition which is comparable to diclofenac sodium. In the case of heat-induced heamolysis it showed notable dose-dependent inhibition of protein denaturation which is comparable to the standard drug diclofenac sodium. From the above findings it underscores that crude extract of *Pseudopterogorgia australiensis* have strong potential to stabilize proteins and prevent their denaturation, positioning it as a promising candidate for anti-inflammatory and cytotoxic activity thus it confirms that the crude extract of *Pseudopterogorgia australiensis* may be useful for therapeutic use in future.

INTRODUCTION: Marine life, especially gorgonian corals, has become a critical focus in the search for new bioactive compounds. These corals, often called sea fans, sea whips, or sea plumes, are integral components of tropical and subtropical marine habitats, thriving in environments ranging from tidal areas to depths of around 4000 meters.

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There are over 6100 species within 13 families of gorgonian corals worldwide. These corals are known to produce a variety of secondary metabolites with distinct chemical characteristics [1].

Research indicates that these metabolites hold considerable promise for medicinal uses, particularly as antioxidants and anti-inflammatory agents [2]. Their ability to neutralize free radicals and influence inflammatory pathways has made them of great interest in therapeutic development [3]. Gorgonian species are especially noted for their production of terpenoids and diterpenes, which have demonstrated potential in blocking crucial

inflammatory mediators, such as cytokines and COX enzymes, offering a promising avenue for pharmaceutical exploration [4]. Oxidative stress, primarily driven by reactive oxygen species (ROS) like superoxide anion, hydroxyl radicals, and hydrogen peroxide, plays a key role in cellular injury and the development of various diseases. Hydrogen peroxide, a byproduct of metabolic activities, can produce hydroxyl radicals, which are among the most damaging agents responsible for oxidative harm [5]. This damage affects essential cellular structures, including lipids, proteins, and DNA, contributing to the onset and progression of diseases such as cancer, diabetes, heart disease, and neurodegenerative disorders. Additionally, oxidative stress is linked to protein denaturation, a pivotal factor in triggering inflammation, which worsens these conditions [6].

Inflammation is a crucial bodily response to protect against tissue damage caused by factors like physical injury, chemical exposure, heat, immune system reactions, or microbial infections. While it serves a protective role, persistent or excessive inflammation can result in tissue harm and contribute to the development of various diseases. Common treatments, including non-steroidal anti-inflammatory drugs (NSAIDs), help alleviate inflammation, pain, and fever but may also lead to side effects such as gastrointestinal ulcers and bleeding. These adverse effects are linked to the inhibition of cyclooxygenase (COX)-1, which plays a role in prostaglandin production, and COX-2, which is activated during inflammatory responses [7, 8].

This study investigates the antioxidant and anti-inflammatory properties of *Pseudopterogorgia australiensis*, with an emphasis on its potential as a source of innovative therapeutic agents for combating oxidative stress and inflammation. In response to the increasing demand for safer and more effective alternatives to NSAIDs, this research aims to advancing our knowledge of bioactive compounds from marine sources and their role in human health.

MATERIALS AND METHODS:

Sample Collection: The gorgonian species *Pseudopterogorgia australiensis* was obtained from the nets of local fishermen at Madh (19° 8’ 47652” N, 72° 47’ 17.8116” E), located in Malad, Mumbai, Maharashtra. The sample was promptly transferred in an icebox to the Biology laboratory at Patkar Varde College, Goregaon West, Mumbai to maintain its freshness and integrity.

FIG. 1: PHOTOGRAPH-1 MAP SHOWING THE COLLECTION SITE

Identification of Sample: The initial analysis of the species was carried out by examining the size and shape of the sclerites and additionally, confirmation was obtained from the Department of Fisheries Biology at the College of Fisheries in Shirgaon, Maharashtra.

Ethical Approval and Deposition of Sample in Repository: Ethical clearance was obtained from the Principal Chief Conservator of Forests, Nagpur (Reference No: Desk-22(8)/Research/CR-10(23-24)/819/2023-24), and the Maharashtra State Biodiversity Board, Nagpur (Reference No: MSBB/Desk-5/Research/885/2023-24) for the collection of species. The voucher specimen was deposited in the repository at the Zoological Survey

of India, Western Regional Office, Pune, India (Reference No: ZSINilRC/MISC./20).

Preparation of Crude Extract: The specimen was thoroughly rinsed with double-distilled water to eliminate any impurities, such as salts and debris, after being brought into the laboratory. It was then left to air-dry in the shade for 24 hours. After drying, the coral was finely ground to ensure consistency for extraction. The powdered material was immersed in a solvent mixture of ethanol and dichloromethane (1:1) for a period of 8 hours. This extraction process was repeated three times to maximize the recovery of bioactive compounds. The collected extracts were processed in a rotary evaporator under reduced pressure to eliminate the solvents, resulting in a concentrated crude extract. The final extract was stored in an airtight container at 4°C until it was ready for further analysis. This method was standardized to preserve bioactive components for subsequent antioxidant and anti-inflammatory assessments.

Assessment of Antioxidant Activity:

DPPH Assay for Antioxidant Activity: The antioxidant activity of the *Pseudopterogorgia australiensis* extract was assessed using the DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay method [9]. Various concentrations of the extract, ranging from 100 µg/mL to 500 µg/mL, were prepared. To each test tube, 1 mL of a 0.1 mM DPPH solution in methanol was added, followed by 1 mL of the extract solution at the appropriate concentration. The mixtures were gently shaken to ensure proper interaction between the DPPH and the extract. The reaction was left in the dark at room temperature for 30 minutes. Absorbance was measured at 517 nm using a spectrophotometer. The scavenging activity was calculated using the formula:

DPPH Scavenging Activity (%) = Absorbance of Blank- Absorbance of Sample / Absorbance of Blank × 100

A standard curve was plotted based on the absorbance values obtained, and the equation of the curve (y = mx + c) was used to determine the ascorbic acid equivalent antioxidant activity of the crude extract.

ABTS Assay for Antioxidant Activity: The antioxidant activity of the bioactive compound was evaluated using the ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) radical cation decolorization assay [10]. ABTS radical cations ($ABTS^+$) were generated by combining a 7 mM ABTS solution with 2.45 mM ammonium persulfate, followed by incubation in the dark for 12–16 hours. On the day of the assay, the $ABTS^+$ solution was diluted 10 times with distilled water to achieve an absorbance of approximately 0.7 ± 0.02 at 734 nm. Various concentrations of the crude extract (20–100 µL/mL) were prepared in methanol. To each concentration of the crude extract, 1 mL of the diluted $ABTS^+$ solution was added, and absorbance was measured at 734 nm using a spectrophotometer. Ascorbic acid at concentrations ranging from 5 to 50 µg/mL served as the standard. A standard curve was constructed using the absorbance values of ascorbic acid, and the antioxidant activity of the bioactive compound was calculated as ascorbic acid equivalent antioxidant activity (AEAC). All experiments were conducted in triplicate to ensure reliability, and results were presented as mean ± standard deviation.

Ferric Reducing / Antioxidant Power Assay: The reducing power of the samples was assessed by measuring the absorbance of a blue-colored complex, which forms when ferric 2,4, 6-tripyridyl-s-triazine (Fe^{3+}-TPTZ) is reduced to its ferrous form (Fe^{2+}-TPTZ) in the presence of antioxidants[11]. The FRAP reagent was freshly prepared each day by combining 2.5 mL of 10 mM TPTZ (dissolved in 40 mM HCl), 2.5 mL of 20 mM $FeCl_3$, and 25 mL of 0.3 M acetate buffer at pH 3.6. The reagent was then heated to 37°C to optimize reaction conditions. Samples were prepared at a concentration of 2 mg/mL by dissolving them in methanol and performing serial dilutions to achieve final concentrations of 100, 200, 300, 400, and 500 µg/mL. For the assay, 200 µL of each sample was mixed with 1.8 mL of the FRAP reagent, and the mixture was incubated for 10 minutes. Absorbance was measured at 593 nm using a spectrophotometer. Trolox was used as a standard for calibration, and the results were reported as milligrams of Trolox equivalent (TE) per sample.

Assessment of Anti-Inflammatory Activity:

Albumin Denaturation: To assess the anti-inflammatory properties of crude extracts, their ability to inhibit the denaturation of egg albumin

(protein) was evaluated in vitro. The inhibitory effect of the crude extract on albumin denaturation was tested based on the method described by [12], with some adjustments to the volume following optimization procedures. The reaction mixture included 1000 µL of bovine serum albumin (1% in PBS, pH 6.4) and an equal volume of the tested sample or standard, with a final concentration range of 100-500 µg/mL. Deionized water (1000 µL) was used as the negative control, representing 100% albumin denaturation. The mixtures were incubated at 37°C for 15 minutes, followed by heating at 60°C for 3 minutes to induce albumin denaturation. After cooling, the turbidity of the samples was measured at 660 nm. The percentage inhibition was calculated using the formula:

Percentage inhibition = (Absorbance of Control-Absorbance of Sample) / (Absorbance of Control) x 100

Proteinase Inhibitory Activity: Proteinase plays a key role in tissue damage associated with inflammatory responses. Proteinases are extensively distributed in neutrophil lysosomal granules. As a result, proteinase inhibitors are essential in regulating the level of activity [13]. For this assay, various enzymes and proteins can be utilized. Proteinases like trypsin and casein or bovine serum albumin (BSA) serve as proteins in the reaction. The reaction mixture (2 ml) contains 0.06 mg of either proteinase or trypsin, 1 ml of 20 mM Tris-HCl buffer (pH 7.4), and 1 ml of the test sample or standard drug, Diclofenac sodium, at concentrations ranging from 100 to 600 µg/ml. The mixture is incubated at 37°C for 5 minutes. Then, 1 ml of 0.8% (w/v) casein or 4% (w/v) BSA is added, and the incubation continues for an additional 20 minutes. To stop the reaction, 2 ml of 70% perchloric acid or 5% trichloroacetic acid (TCA) is added. The resulting cloudy suspension is then centrifuged at 3000 rpm for 10 minutes (or 2500 rpm for 5 minutes). The absorbance of the supernatant is measured at 660 nm using the buffer as a blank. The assay is performed in triplicate, and the percentage inhibition of proteinase activity is determined using the following equation [14].

Percentage inhibition = (Absorbance of Control-Absorbance of Sample) / (Absorbance of Control) x 100

Membrane Stabilization:

Preparation of RBC Suspension: The membrane stabilization test was carried out to assess anti-inflammatory effects by measuring the inhibition of heat-induced hemolysis [15]. A suspension of red blood cells (RBC) was created by collecting 10 mL of fresh human blood, which was then centrifuged at 3000 rpm for 10 minutes at -8°C. The pellet obtained was washed three times with normal saline and resuspended in normal saline to make a 10% (v/v) RBC suspension.

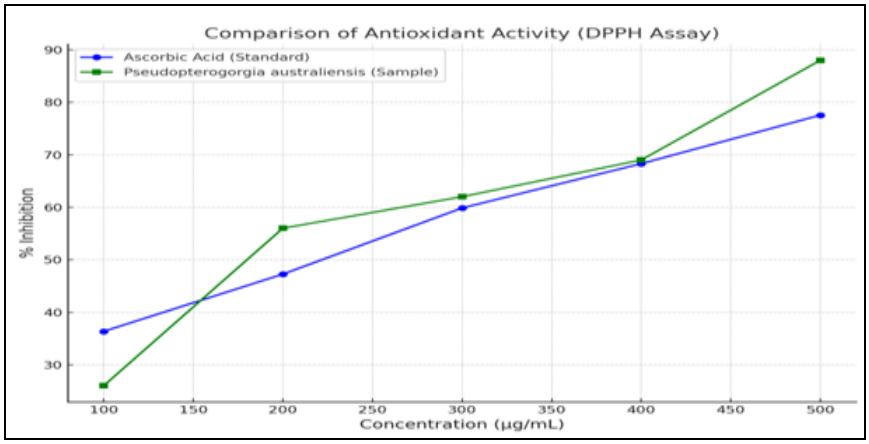
Heat-Induced Hemolysis Assay: For the heat-induced hemolysis test, reaction mixtures were prepared by mixing 1 mL of the 10% RBC suspension with 1 mL of the test samples at concentrations ranging from 100 to 500 µg/mL. A positive control was set up using Diclofenac sodium at the same concentrations, while saline served as the negative control. All mixtures were incubated in a water bath at 56°C for 30 minutes and then cooled under running tap water. Afterward, the mixtures were centrifuged at 3000 rpm for 5 minutes, and the absorbance of the supernatants was measured at 560 nm. The test was performed in triplicate to ensure consistency in the results. The percentage inhibition of hemolysis was determined using the following formula

Percentage inhibition = (Absorbance of Control-Absorbance of Sample) / (Absorbance of Control) x 100

RESULTS AND DISCUSSION:

TABLE 1: SHOWING THE EFFECT OF ASCORBIC ACID AND *PSEUDOPTEROGORGIA AUSTRALIENSIS* ON DPPH ASSAY – COMPARATIVE DATA OF MEAN ABSORBANCE AND PERCENTAGE INHIBITION AT 517 NM

Concentration (µg/mL)	Absorbance (Standard) at 517 nm	% Inhibition (Standard)	Absorbance (Sample) at 517 nm	% Inhibition (Sample)
100	0.2077	36.35%	1.58	26 %
200	0.1780	47.25%	1.28	56 %
300	0.1357	59.85%	1.22	62 %
400	0.1100	68.30%	1.15	69 %
500	0.090	77.55%	0.76	88 %



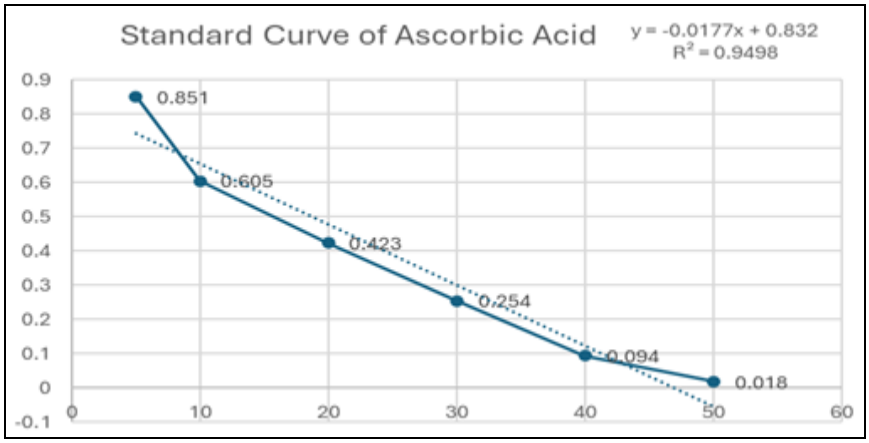
GRAPH 1: SHOWING ANTIOXIDANT ACTIVITY OF ASCORBIC ACID AND CRUDE EXTRACT

TABLE 2: SHOWING THE EFFECT OF ASCORBIC ACID ON ABTS ASSAY

Ascorbic Acid Concentration (µg/mL)	Absorbance at 734 nm	% Inhibition
5	0.851	19.41
10	0.605	40.25
20	0.423	55.68
30	0.254	70.00
40	0.094	90.34
50	0.018	99.83

TABLE 3: SHOWING THE EFFECT OF *PSEUDOPTEROGORGIA AUSTRALIENSIS* ON ABTS ASSAY

Sample Concentration (µL/mL)	Absorbance at 734 nm	% Inhibition	Concentration Equivalent to Ascorbic Acid (µg)
10X Diluted ABTS	1.501	-	-
10 ul	0.513	65.82	18.02
25 ul	0.129	91.42	39.73
50 ul	0.071	95.26	42.98
75 ul	0.037	97.51	44.89
100ul	0.025	98.36	45.62



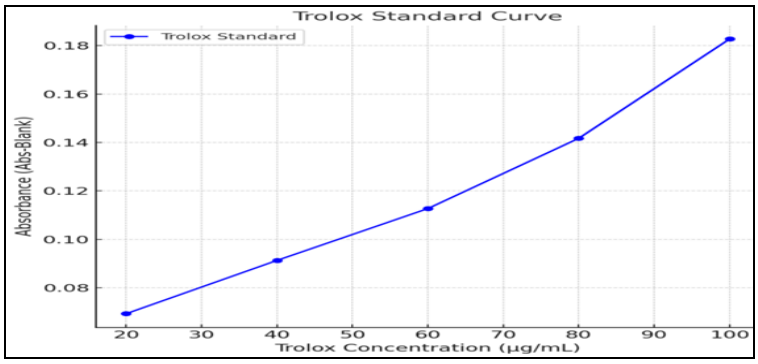
GRAPH 2: SHOWING THE EFFECT OF ASCORBIC ACID ON ABTS ASSAY

TABLE 4: SHOWING THE EFFECT OF TROLOX ON FRAP ASSAY

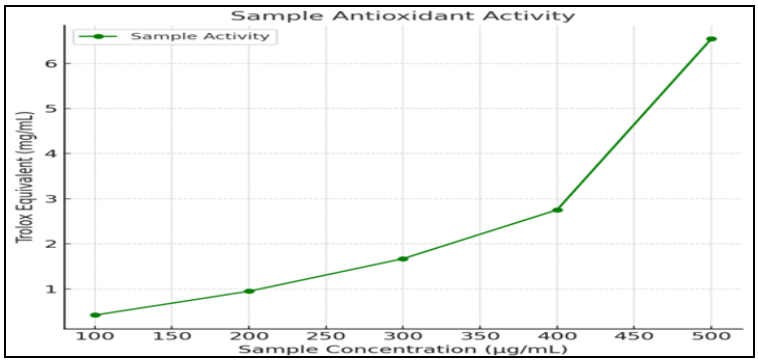
Trolox Concentration (µg/mL)	Mean Absorbance at 593 nm	Absorbance - Blank (After Reducing Blank)
Blank	-	-
20 µg/mL	0.0843	0.0693
40 µg/mL	0.1063	0.0913
60 µg/mL	0.1277	0.1127
80 µg/mL	0.1567	0.1417
100 µg/mL	0.1977	0.1827

TABLE 5: SHOWING THE EFFECT OF *PSEUDOPTEROGORGIA AUSTRALIENSIS* ON FRAP ASSAY

Sample Concentration (µg/mL)	Mean Absorbance of Crude Extract at 593 nm	Absorbance - Blank (After Reducing Blank)	Concentration Equivalent to Trolox (µg)
100 µg/mL	0.0630	0.0480	0.419
200 µg/mL	0.0777	0.0627	0.948
300 µg/mL	0.0977	0.0827	1.670
400 µg/mL	0.1277	0.1127	2.753
500 µg/mL	0.2327	0.2177	6.544



GRAPH 3: SHOWING THE EFFECT OF TROLOX ON FRAP ASSAY



GRAPH 4: SHOWING THE EFFECT OF *PSEUDOPTEROGORGIA AUSTRALIENSIS* ON FRAP ASSAY

TABLE 6: SHOWING THE EFFECT OF CRUDE EXTRACT OF *PSEUDOPTEROGORGIA AUSTRALIENSIS* AND STANDARD DRUG DICLOFENAC SODIUM ON INHIBITION OF ALBUMIN DENATURATION

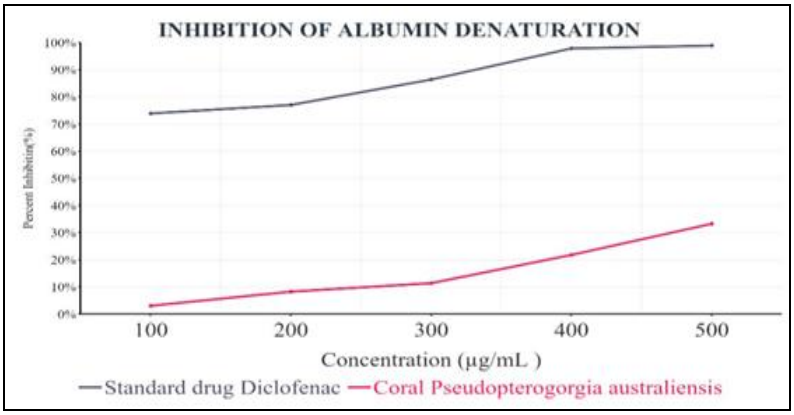
Concentration of sample and standard	Absorbance of standard drug diclofenac sodium at 660 nm	% inhibition of albumin denaturation of standard drug diclofenac sodium	Absorbance of crude extract of *Pseudopterogorgia australiensis* at 660 nm	% inhibition of albumin denaturation of crude extract of *Pseudopterogorgia australiensis*
100 µg/mL	0.25	73.95 %	0.93	3.12%
200 µg/mL	0.22	77.08 %	0.88	8.33%
300 µg/mL	0.13	86.45 %	0.85	11.45%
400 µg/mL	0.02	97.91 %	0.72	21.87%
500 µg/mL	0.01	98.95 %	0.64	33.33%

Control = 0.96 nm

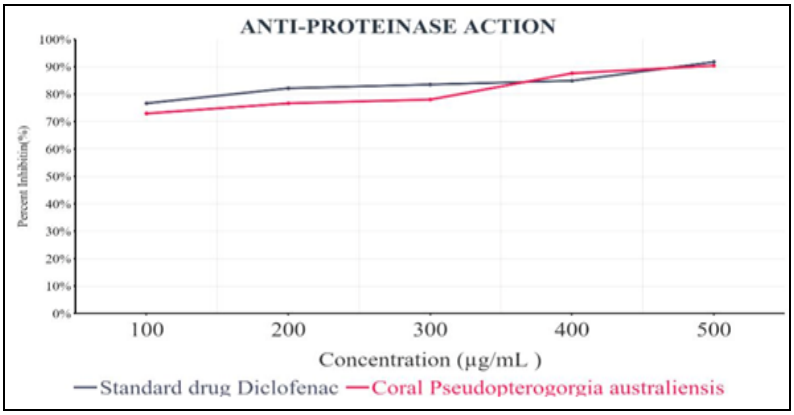
TABLE 7: SHOWING THE EFFECT OF CRUDE EXTRACT OF *PSEUDOPTEROGORGIA AUSTRALIENSIS* AND STANDARD DRUG DICLOFENAC SODIUM ON ANTI-PROTEINASE ACTIVITY

Concentration of sample and standard	Absorbance of standard drug diclofenac sodium at 660 nm	% inhibition of enzyme proteinase in anti-proteinase activity of standard drug diclofenac sodium	Absorbance of crude extract of *Pseudopterogorgi aaustraliensis* at 660 nm	% inhibition of enzyme proteinase in anti-proteinase activity of crude extract of *Pseudopterogorgi aaustraliensis*
100 µg/mL	0.17	76.71 %	0.20	73%
200 µg/mL	0.13	82.19 %	0.17	76.71%
300 µg/mL	0.12	83.56 %	0.16	78.08%
400 µg/mL	0.11	84.93 %	0.09	87.67%
500 µg/mL	0.06	91.78 %	0.07	90.41%

Control = 0.73 nm



GRAPH 5: SHOWING THE EFFECT OF DICLOFENAC AND *PSEUDOPTEROGORGIA AUSTRALIENSIS* ON INIHIBITION OF PROTEIN DENATURATION

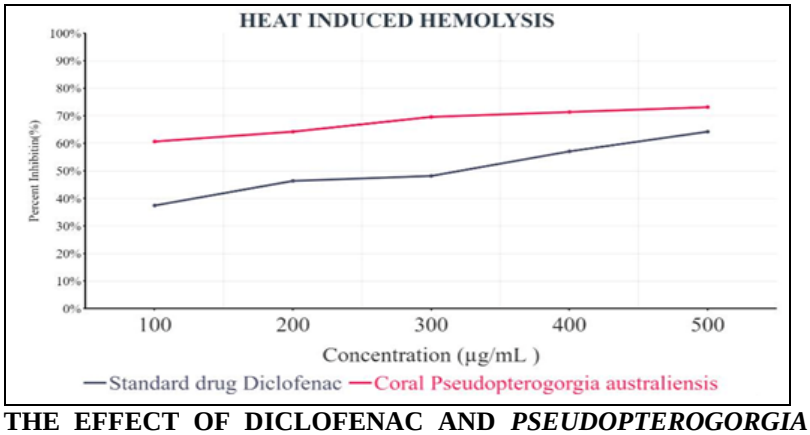


GRAPH 6: SHOWING THE EFFECT OF DICLOFENAC AND PSEUDOPTEROGORGIA AUSTRALIENSIS ON PROTEINASE INHIBITORY ACTIVITY

TABLE 8: SHOWING THE EFFECT OF CRUDE EXTRACT OF *PSEUDOPTEROGORGIA AUSTRALIENSIS* AND STANDARD DRUG DICLOFENAC SODIUM ON HEAT-INDUCED HEMOLYSIS (MEMBRANE STABILIZATION TEST)

Concentration of sample and standard	Absorbance of standard drug diclofenac sodium at 560 nm	% inhibition of RBC hemolysis of standard drug diclofenac sodium	Absorbance of crude extract of *Pseudopterogorgia australiensis* at 560 nm	% inhibition of RBC hemolysis of crude extract of *Pseudopterogorgi aaustraliensis*
100 µg/mL	0.22	37.5%	0.35	60.71%
200 µg/mL	0.20	46.42%	0.30	64.28%
300 µg/mL	0.17	48.21%	0.29	69.64%
400 µg/mL	0.16	57.14%	0.24	71.42%
500 µg/mL	0.15	64.28%	0.20	73.21%

Negative control = 0.46



GRAPH 7: SHOWING THE EFFECT OF DICLOFENAC AND *PSEUDOPTEROGORGIA AUSTRALIENSIS* ON PROTEIN DENATURATION

Antioxidant Assays: The antioxidant potential of the crude extract from *Pseudopterogorgia australiensis* was assessed using three commonly used methods: DPPH, ABTS, and FRAP. These assays provided distinct information on the extract's capacity to neutralize free radicals, mitigate oxidative damage, and lower reactive species, suggesting its promise as a natural antioxidant.

DPPH Assay: The results from the DPPH assay exhibited a clear dose-dependent increase in antioxidant activity for both the crude extract and ascorbic acid. The percentage inhibition in this assay reflects the ability of a substance to neutralize or "scavenge" DPPH radicals. DPPH is a stable free radical, and when an antioxidant is introduced, it donates electrons or hydrogen atoms to neutralize the radicals, leading to a reduction in absorbance. As the concentrations of ascorbic acid and the extract were raised, the percentage inhibition of DPPH radicals also increased. Ascorbic acid demonstrated a more pronounced scavenging effect, as shown in **Graph 1**. According to **Table 1**, at a concentration of 100 µg/ml. ascorbic acid achieved 36.35% inhibition, rising to 77.55% at 500 µg/ml, indicating strong antioxidant activity. Similarly, the extract displayed 26% inhibition at 100 µg/ml, increasing to 88% at 500 µg/ml. highlighting its significant antioxidant capacity, although it was less potent than ascorbic acid.

ABTS Assay: The antioxidant potential of the crude extract was assessed using the ABTS radical cation decolorization assay, with ascorbic acid serving as the reference standard. A standard curve, shown in **Graph 2**, was constructed using ascorbic acid at concentrations of 5, 10, 20, 30, 40, and 50 µg/mL. As presented in Table No. 2, the percentage of inhibition increased proportionally with concentration, ranging from 19.41% at 5 µg/mL to 99.83% at 50 µg/mL. The crude extract was tested at concentrations of 10, 25, 50, 75, and 100 µL/mL, as detailed in **Table 2** & **3**. The mean absorbance of the diluted ABTS solution was 1.501. Significant antioxidant activity was demonstrated by the crude extract, with inhibition percentage rising with concentration. At a concentration of 10 µL/mL, the mean absorbance was 0.513, resulting in a 65.82% inhibition, equivalent to 18.02 µg of ascorbic acid. At higher concentrations, inhibition reached 91.42% at 25 µL/mL, 95.26% at 50 µL/mL, 97.51% at 75 µL/mL, and 98.36% at 100 µL/mL. The ascorbic acid equivalency of the extract ranged from 18.02 µg at 10 µL/mL to 45.62 µg at 100 µL/mL.

FRAP Assay: The outcomes of the FRAP (Ferric Reducing Antioxidant Power) assay are summarized in **Table 4** and **5**, and **Graph 3** & **4**, comparing the antioxidant activity of various samples to Trolox, a well-known antioxidant. The blank reading was 0.015, which was subtracted from all sample readings to eliminate background interference. A standard series of Trolox concentrations (20–100 µg/mL) was used to create a calibration curve, with absorbance values ranging from 0.0843 for 20 µg/mL to 0.1977 for 100 µg/mL. These absorbance values were corrected by subtracting the blank absorbance to obtain "Abs-Blank" values, which allowed the calculation of concentrations equivalent to Trolox. For the tested samples, the absorbance values ranged from 0.0630 for the 100 µg/mL sample to 0.2327 for the 500 µg/mL sample. The Trolox equivalent concentration varied across the samples, with the 100 µg/mL sample showing an equivalent concentration of 0.419 µg/mL, while the 500 µg/mL sample had a much higher value of 6.544 µg/mL. The antioxidant activity of the samples increased with concentration, with the highest activity seen at the 500 µg/mL concentration. The FRAP assay measured the extract's ability to reduce Fe^{3+} to Fe^{2+}, revealing a clear dose-dependent increase in reducing power, peaking at the highest concentration tested.

The DPPH assay mechanism is consistent with the work [16], which highlighted the effectiveness of DPPH in assessing the antioxidant activity of various bioactive compounds. In a similar study, Kardile [17] examined the antioxidant potential of soft corals from the Persian Gulf, including *Junceella juncea* and *Menella sp.*, using the DPPH assay. Among these, the white variety of Menella sp. exhibited the strongest activity. The ABTS assay is highly adaptable, as it can measure the scavenging abilities of both hydrophilic and lipophilic antioxidants, making it especially suitable for marine-derived extracts. These findings suggest that *Pseudopterogorgia australiensis* possesses significant radical scavenging properties, aligning with previous reports on other gorgonian

corals like *Eunicella cavolini* and *Pseudopterogorgia elisabethae*, which exhibited similar bioactivities [18, 19]. This supports the idea that the extract's ability to donate electrons, a key characteristic of antioxidants, is essential. Other marine species, such as *Sinularia* sp. and *Lobophytum crassum*, have also demonstrated comparable reducing power, which is attributed to secondary metabolites like diterpenes and phenolics [20, 21].

Anti-Inflammatory Activity: The anti-inflammatory effects of the crude extract from *Pseudopterogorgia australiensis* were evaluated using assays to measure albumin denaturation, proteinase inhibitory activity, and membrane stabilization. These tests provided valuable information about the extract's potential to inhibit processes linked to inflammation, which is essential for the development of effective anti-inflammatory treatments.

Inhibition of Albumin Denaturation: The results, as shown in **Table 6** and **Graph 5**, highlight the difference in the inhibition of protein denaturation between the test sample and the standard. Diclofenac, the standard drug, displayed a high level of inhibition across all concentrations tested, with the highest dose (500 µg/mL) achieving nearly complete inhibition at 98.95%. This suggests that Diclofenac has significant anti-inflammatory activity by effectively preventing protein denaturation. In comparison, the test sample showed much lower inhibition rates, with the maximum inhibition of 33.33% at the highest concentration (500 µg/mL).

Proteinase Inhibitory Activity: Table 7 and **Graph 6** illustrate the results for proteinase inhibitory activity. Diclofenac demonstrated a high degree of inhibition at all concentrations, with the highest inhibition (91.78%) occurring at 500 µg/mL. This indicates that Diclofenac is highly effective at inhibiting protein denaturation, a key factor in proteinase activity. The test sample also exhibited substantial inhibition, although slightly lower than Diclofenac, with the peak inhibition reaching 90.41% at 500 µg/mL.

Heat-Induced Hemolysis: The heat-induced hemolysis test was performed to assess the anti-inflammatory potential of the crude extract from *Pseudopterogorgia australiensis* in comparison with Diclofenac sodium, a commonly used anti-inflammatory drug. The results, presented in **Table 8** and **Graph 7**, show a clear dose-dependent inhibition of protein denaturation for both the crude extract and Diclofenac sodium. For Diclofenac sodium, the inhibition of protein denaturation increased progressively with higher concentrations. At 100 µg/mL, inhibition was 37.5%, rising to 46.42% at 200 µg/mL, 48.21% at 300 µg/mL, 57.14% at 400 µg/mL, and reaching a peak of 64.28% at 500 µg/mL. In comparison, the crude extract of *Pseudopterogorgia australiensis* exhibited greater inhibition of protein denaturation than Diclofenac sodium at similar concentrations. At 100 µg/mL, inhibition was 60.71%, increasing to 64.28% at 200 µg/mL, 69.64% at 300 µg/mL, 71.42% at 400 µg/mL, and peaking at 73.21% at 500 µg/mL. These results underscore the potent anti-inflammatory activity of the crude extract, which not only exhibited dose-dependent inhibition but also achieved higher inhibition percentages than Diclofenac sodium at all tested concentrations.

The albumin denaturation assay assessed the extract's capacity to prevent protein denaturation, a key factor in inflammation. Protein denaturation is closely linked to inflammation, as it disrupts protein structure and function, initiating immune responses. Substances that stabilize proteins can effectively reduce inflammation [22].

The results of the albumin denaturation assay align with previous research on marine-derived compounds. Madhuranga [23] reported significant inhibition of albumin denaturation by fractions from *Eunicella cavolini*, which was attributed to steroids and terpenoids, compounds that are likely present in *Pseudopterogorgia australiensis*. Sumi [24] demonstrated that squid ink inhibited protein denaturation by 71.2%, showcasing the potential of marine resources for anti-inflammatory drug development. Although the result was slightly lower than the standard, it still indicates considerable proteinase inhibitory activity. This strong inhibitory effect suggests that the extract can stabilize proteins and prevent their breakdown by proteolytic enzymes, which is a crucial mechanism in controlling inflammation [25].

These findings are supported by similar research on other marine organisms. Su [26] showed that papain hydrolysates from marine crab protein exhibited 78.55% inhibition of protein denaturation, comparable to the activity observed in *Pseudopterogorgia australiensis.* Furthermore, [27] reported that sterols from the gorgonian *Pinnigorgia* species effectively inhibited inflammatory mediators like COX-2 and iNOS, reinforcing the potential of marine-derived compounds in managing inflammation.

CONCLUSION: The findings underscore the potential of *Pseudopterogorgia australiensis* as a rich source of natural antioxidants. Future studies should focus on isolating and characterizing the active compounds to better understand their mechanisms of action and evaluate their efficacy in therapeutic applications. By contributing to the growing evidence on marine-derived antioxidants, this research highlights the importance of conserving marine biodiversity as a reservoir of bioactive compounds with promising health benefits. The findings also underscore the Importance of marine biodiversity as a source of novel anti-inflammatory agents. By leveraging the unique bioactive properties of marine organisms, it is possible to develop safer and more effective alternatives to conventional non-steroidal anti-inflammatory drugs (NSAIDs), which are often associated with adverse effects. This study contributes to the growing body of evidence supporting the pharmaceutical potential of marine-derived compounds in inflammation management.

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