WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.453
Volume 14, Issue 04, 777-789. Research Article ISSN 2277–7105

EVALUATION OF IN-VITRO ANTI-INFLAMMATORY ACTIVITY FROM CORAL *JUNCEELLA DELICATA* COLLECTED FROM MADH ISLAND, WEST COAST OF MUMBAI

Borate M. P. and Zodape G. V.*

Department of Zoology, Chikitsak Samuha's Sir Sitaram and Lady Shantabai Patkar College of Arts and Science and V. P. Varde College of Commerce and Economics, S. V. Road, Goregaon West, Mumbai-400104, Maharashta, India.

Article Received on
24 December 2024,

Revised on 14 Jan. 2025,
Accepted on 04 Feb. 2025

DOI: 10.20959/wjpr20254-35537

***Corresponding Author**
Dr. Zodape G. V.
Department of Zoology, Chikitsak Samuha's Sir Sitaram and Lady Shantabai Patkar College of Arts and Science and V. P. Varde College of Commerce and Economics, S. V. Road, Goregaon West, Mumbai-400104, Maharashta, India.

ABSTRACT

The Gorgonia *Junceella delicata* was collected during low tides. The crude extract was prepared by adding an equal volume of MeOH: DCM (1:1) for 24 hours in the water bath at 45° C. The sample was filtered through Whatman filter paper No. 1 and was subjected to concentrate in a rotary vacuum evaporator at 45° C. The extract obtained was used to investigate the anti-inflammatory properties of the coral *Junceella delicata* and its potential as an alternative to standard drugs. Membrane stabilization and in-vitro anti-inflammatory activity were assessed using Heat-induced hemolysis, inhibition of albumin denaturation and proteinase action assays. The crude extract of coral *Junceella delicata* showed strong inhibition of RBC hemolysis (up to 89.13%), inhibition of protein denaturation (up to 72.04%), and proteinase inhibition (up to 88.60%), comparable to the standard drug diclofenac sodium. The study suggests that the coral *Junceella delicata* has potential anti-inflammatory properties. These findings may contribute to the search for effective biomedical property.

KEYWORDS: Anti-inflammatory, Coral, Inflammation, *Junceella delicata.*

INTRODUCTION

Inflammation is indeed a natural defense mechanism in the body that helps protect it from various external threats. Inflammation is frequently characterized by redness, swelling,

warmth, and, at times, pain and immobility (Dhara, 2016). Inflammation can be caused by various factors such as chemical and physical agents, immunological stimuli, viruses, bacteria and neoplasms which is triggered by biochemicals, cytokines, and hormones produced by different types of surveillance cells in the body. (Denko, 1992). Inflammation is classified into two types: acute inflammation and chronic inflammation, also known as systemic inflammation. Acute inflammation is a rapid response to tissue damage caused by trauma, microbial invasion, or exposure to noxious compounds, characterized by severe symptoms that may last for a few days, whereas chronic inflammation is a slow, long-term response lasting for several months to years (Pahwa, 2023). While steroidal and nonsteroidal anti-inflammatory drugs are commonly used to treat inflammatory disorders, their long-term use often leads to significant side effects (Kapoor et al., 2019). The search for safe and effective anti-inflammatory drugs has always been a major focus of biomedical research (Li *et al.*, 2021).

Corals are known for their anti-inflammatory properties. Diterpenes are a class of molecules that have been isolated from corals and have shown promising therapeutic benefits, particularly in their anti-inflammatory properties (Cooper *et al.*, 2014). *Nephthea sp.* has anti-inflammatory activity due to the presence of steroids and terpenoids such as nebrosteroids A-H and columnariols A and B (Sahidin *et al.*, 2023). Excavatolide B is a briarane diterpenoid isolated from coral *Briareum excavatum* that shows displays anti-inflammatory activity both in vitro and in vivo (Gonzalez *et al.*, 2015). In a study conducted on *Eunicea fusca*, a gorgonian coral native to Florida, three diterpenes were isolated and found to have superior anti-inflammatory effects when compared to indomethacin, a commonly used anti-inflammatory medication (Marchbank *et al.*, 2012). One more diterpene, fucoside E, isolated from the same species of coral, has shown anti-inflammatory and antimicrobial activity (Reina *et al.*, 2011).

MATERIALS AND METHODS

a) Sample collection

The coral *Junceella delicata* was collected from Patwadi Village, Madh Island, Malad West Mumbai- 400061, Maharashtra, India (19^0 8’ 47652” N, 72^0 47’ 17.8116” E) during the low tide. The debris was removed during collection. The sample collected was washed twice with sea water and then rinsed three times with distilled water and stored in ice cubes until they were transferred to the deep freezer at 8^0 C at the Department of Zoology, S.S. & L.S. Patkar

College of Arts & Science, and V. P. Varde College of Commerce & Economics, Goregaon West, Mumbai-400104.

b) Identification of coral

Preliminary identification was done by examining the shape and size of the sclerites and by reviewing the literature. The confirmation of identification was done by Dr. Swapnaja Mohite, Professor and Head, Department of Fisheries Biology, College of Fisheries, Shirgaon, Ratnagiri, Maharashtra.

c) Preparation of crude extract

The coral sample was removed from the deep fridge and blotted with blotting paper and kept in shed dried for 48 hours. After 48 hours the sample was grinded with blender and then macerated by adding an equal volume of MeOH: DCM (1:1) for 24 hours in the water bath at 45^0 C. The aliquot mixture obtained was filtered through Whatman filter paper No. 1. The homogenate was centrifuged at 10,000 rpm for 15 minutes in cold centrifuge (Remi centrifuge serial No. VCDX- 5983) at -8^0C and supernatant was collected. The aliquot was concentrated in a rotary vacuum evaporator at 45^0 C. The resultant compound was subjected to Millipore filter system and finally dried in vacuum desiccator and stored in the refrigerator at -20 ^{0} C till further use.

d) Ethical approval

Ethical approval was sought from the Principal Chief Conservator of Forest, Nagpur (Desk-22(8)/Res/CR-25(22-23)/1431/(22-23) and final approval was taken from the Maharashtra State Biodiversity Board, Nagpur (MSBB/Desk-5/825/2022-23) for collection of *Junceella delicata* samples. The voucher specimen of *Junceella delicata* was submitted to the repository at the Zoological Survey of India, Western Regional Office, Pune (ZSI-WRC Misc/18), India.

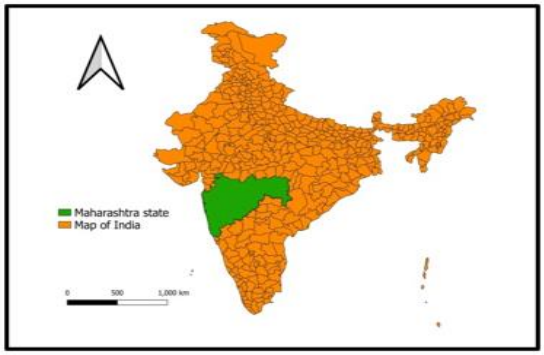
e) Drugs: Diclofenac Sodium (1 mg/mL) was used as a standard drug (Anand Chemist, Goregaon West, Mumbai)

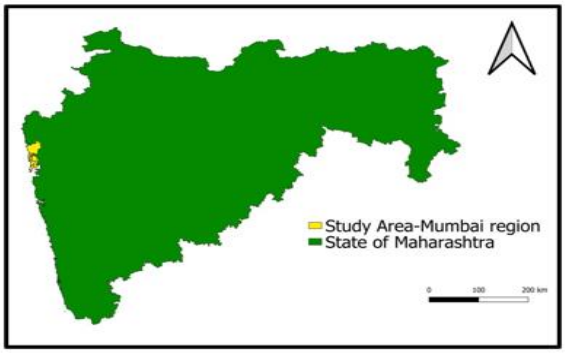
f) Classification of coral

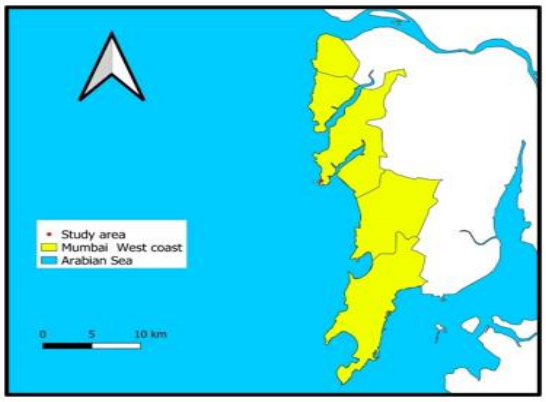
Coral sample: *Junceella delicata* (Grasshoff, 1999)

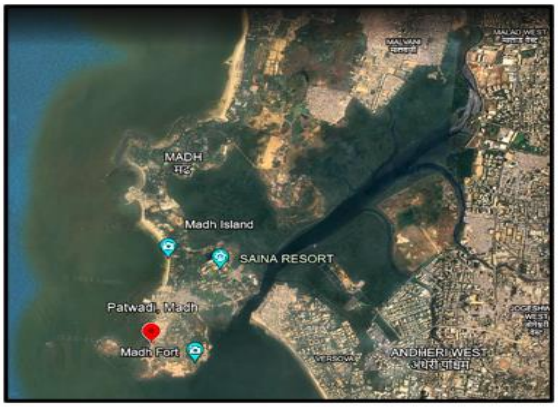
Systematic position
Kingdom: Animalia
Phylum: Cnidaria
Class: Anthozoa
Order: Gorgonacea
Sub-order: Calcaxonia
Family: Ellisellidae
Genus: *Junceella*
Species: ***Junceella delicata*** (Grasshoff, 1999)

Map of Mumbai showing the site of collection (Source - QGIS software) and Satellite image (Source - Google Earth).









In-vitro anti-inflammatory activity

a) Inhibition of albumin denaturation

The anti-inflammatory potential of crude extracts can be assessed in vitro by evaluating their ability to inhibit egg albumin (protein) denaturation. The inhibitory effect of *Junceella delicata* crude extract on albumin denaturation was tested using a modified version of the

method described by Matulja *et al.* (2021). In each tube, 1 mL of Bovine serum albumin (1% in PBS, pH 6.4) was added, followed by the addition of a series of test samples (crude extract) at concentrations ranging from 100 to 500 µg/mL. The total volume of 2 mL for each sample was achieved by adding deionized water. For the negative control, 1 mL of BSA was mixed with 1 mL of deionized water. The positive control was prepared by adding 1 mL of BSA and 1 mL of Diclofenac sodium, with the standard drug concentration varying from 100 to 500 µg/mL. All the test tubes 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 of protein denaturation was calculated as follows.

$$\textit{Percentage inhibition} = \frac{Absorbance\ of\ Control - Absorbance\ of\ Sample}{Absorbance\ of\ Control}\ x\ 100$$

b) Anti-proteinase action / Proteinase inhibitory activity

The experiment was conducted with slight modifications using Jyothilakshmi *et al.* (2017) and Patel and Desai (2016). In this experiment, trypsin was utilized as the enzyme, and Bovine Serum Albumin was served as the protein. The reaction mixture (total volume of 2 mL) was prepared by adding 0.06 mg of trypsin to 1 mL of 20 mM Tris-HCl buffer (pH 7.4). Then, 1 mL of the test sample at concentrations ranging from 100-500 µg/mL was introduced into the mixture. For positive control, instead of test sample, Diclofenac sodium (standard drug) was added.For the negative control, 0.06 mg of trypsin was mixed with 1 mL of 20 mM Tris-HCl buffer (pH 7.4) and 1 mL of deionized water instead of the test sample. The mixture was incubated at 37°C for 5 minutes, followed by the addition of 1 mL of 4% (w/v) Bovine Serum Albumin (BSA). After addition, the sample was incubated for 20 minutes and to it 2 mL of 70% perchloric acid was added to stop the reaction. The cloudy suspension was centrifuged, and the absorbance of the supernatant was recorded at 660 nm. The experiment was performed in triplicate, and the percentage inhibition of proteinase activity was calculated.

$$\textit{Percentage inhibition} = \frac{Absorbance\ of\ Control - Absorbance\ of\ Sample}{Absorbance\ of\ Control}\ x\ 100$$

Membrane stabilization

The membrane stabilization test was conducted following the method described by Govindappa (2011).

a) Preparation of red blood cell: (RBC) suspension

To prepare a red blood cell (RBC) suspension, 10 mL of fresh whole human blood was collected and placed in centrifuge tubes. The tubes were then centrifuged at 3000 rpm for 10 minutes at -8°C using a cold centrifuge. The resulting pellet was collected, washed three times with an equal volume of normal saline, and reconstituted into a 10% v/v suspension using normal saline after measuring the final blood volume.

b) Heat-induced hemolysis

The reaction mixture was prepared by combining 1 mL of the 10% RBC solution with 1 mL of the test sample at varying concentrations (100-500 µg/mL). The positive control was prepared in a similar manner, using 1 mL of the 10% RBC solution and 1 mL of Diclofenac sodium, a standard drug, at concentrations ranging from 100-500 µg/mL. For negative control, only 1 mL of saline was used instead of the test sample along with 1 mL of a 10% RBC solution. All centrifuge tubes were incubated for 30 minutes in a water bath set at 56°C. After incubation, the tubes were cooled under running tap water. The reaction mixture was then centrifuged for 5 minutes at 3000 rpm, and the absorbance of the supernatants was measured at 560 nm. The experiment was performed in triplicates. The percentage of hemolysis was calculated using the following formula.

$$\textit{Percentage inhibition} = \frac{Absorbance\ of\ Control - Absorbance\ of\ Sample}{Absorbance\ of\ Control}\ x\ 100$$

RESULTS AND DISCUSSION

Table No. 1: Showing the effect of crude extract of coral *Junceella delicata* 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 coral *Junceella delicata* at 660 nm	% inhibition of albumin denaturation of crude extract of coral *Junceella delicata*
100 µg/mL	0.24	74.19 %	0.47	49.46%
200 µg/mL	0.23	75.26 %	0.38	59.13%
300 µg/mL	0.22	76.34 %	0.35	62.36 %
400 µg/mL	0.18	80.64%	0.32	65.59 %
500 µg/mL	0.13	86.02%	0.26	72.04 %

Negative control = 0.93 nm

Table No. 2: Showing the effect of crude extract of coral *Junceella delicata* 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 coral *Junceella delicata* at 660 nm	% inhibition of enzyme proteinase in anti-proteinase activity of crude extract of coral *Junceella delicata*
100 µg/mL	0.17	78.48 %	0.18	77.21 %
200 µg/mL	0.13	83.54 %	0.16	79.74 %
300 µg/mL	0.12	84.81 %	0.15	81.01 %
400 µg/mL	0.11	86.07 %	0.13	83.54 %
500 µg/mL	0.06	92.40 %	0.09	88.60 %

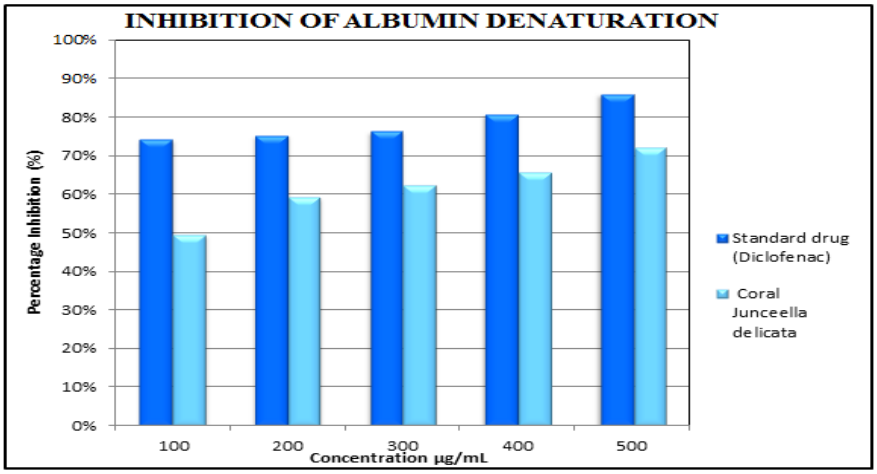
Negative control = 0.79

Membrane stabilization test

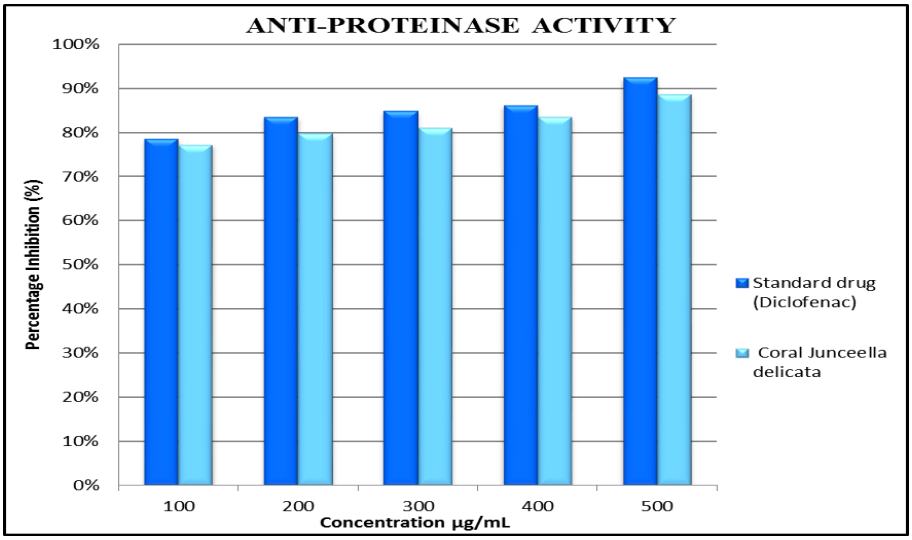
Table No. 3: Showing the effect of crude extract of coral *Junceella delicata* and standard drug diclofenac sodium on Heat-induced hemolysis (Membrane Stabilization test).

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 coral *Junceella delicata* at 560 nm	% inhibition of RBC hemolysis of crude extract of coral *Junceella delicata*
100 µg/mL	0.35	23.91 %	0.22	52.17 %
200 µg/mL	0.30	34.78 %	0.19	58.69 %
300 µg/mL	0.29	36.95 %	0.13	71.73 %
400 µg/mL	0.24	47.82 %	0.07	84.78 %
500 µg/mL	0.20	56.52 %	0.05	89.13 %

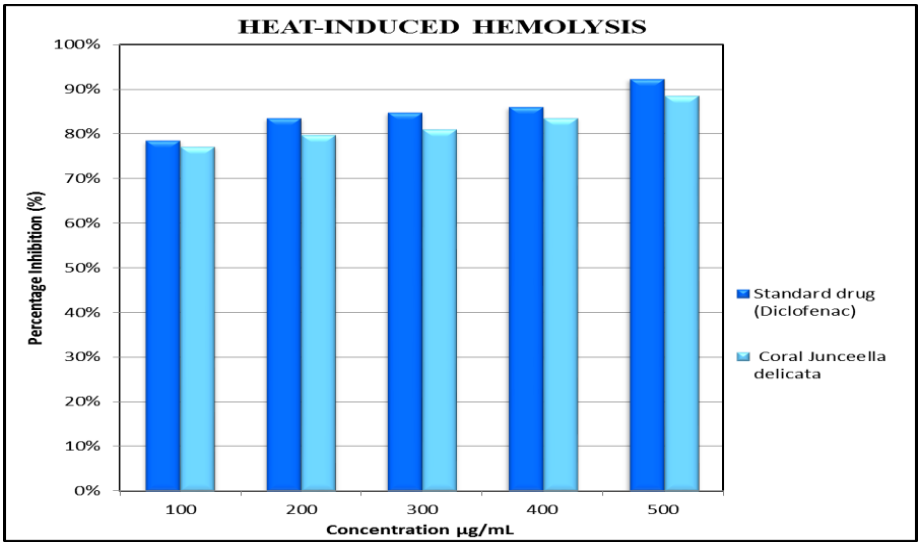
Negative control = 0.46



Graph No. 1: Showing the effect of crude extract of coral *Junceella delicata* and standard drug diclofenac sodium on inhibition of albumin denaturation.



Graph No. 2: Showing the effect of crude extract of coral *Junceella delicata* and standard drug diclofenac sodium on anti-proteinase activity.



Graph No. 3: Showing the effect of crude extract of coral *Junceella delicata* and standard drug Diclofenac sodium on Heat-Induced Hemolysis (Membrane stabilization test).

Soft corals have become an increasingly important source of anti-inflammatory compounds. Research has highlighted a range of bioactive molecules, such as sphingosine derivatives, cembrenoid diterpenes, and sterols, all of which exhibit strong anti-inflammatory effects. (Radhika *et al.*,2005; Putra *et al.*, 2016; Fernando *et al.*,2017). These compounds have shown effectiveness in both in vitro and in vivo models, where they inhibit critical inflammatory mediators including nitric oxide (NO), prostaglandin E2 (PGE2), inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), and pro-inflammatory cytokines (Fernando *et al.*,2017; Tsai *et al.*,2015). A sterol-rich fraction derived from *Dendronephthya gigantea*

demonstrated notable anti-inflammatory activity in both cell culture and zebrafish embryo models (Fernando *et al.*,2017). Structure-activity relationship analysis of cembranoids from *Sinularia* species indicated that compounds containing a seven-membered lactone ring at C-1 hold significant potential as anti-inflammatory agents (Tsai *et al.*,2015). These findings highlight the potential of soft coral-derived compounds in developing new anti-inflammatory therapeutics and cosmeceuticals.

As per the experiment, the evaluation of the activity was carried out through three in vitro assays. As highlighted in the study by Williams *et al.* (2008), in vitro assays offer significant advantages by reducing the need for animal testing, particularly when working with crude extracts or limited sample quantities for research purposes.

a) Inhibition of albumin denaturation

Protein denaturation is the loss of a protein's structure and function due to external stress like heat, strong acids or bases, organic solvents, or concentrated inorganic salts. This process is well-known to cause inflammation.

Table No. 1 and Graph No. 1 shows the effect of the standard drug diclofenac sodium and the crude extract of coral *Junceella delicata* against inhibition of albumin denaturation. Albumin denaturation is an important biochemical process, and it is essential to inhibit this process to evaluate the protective properties of different substances such as anti-inflammatory medications and natural extracts. The standard drug diclofenac sodium shows average inhibition of albumin denaturation at 78.61%. Whereas, crude extract of coral *Junceella delicata* shows average inhibition of albumin denaturation at 61.71%. Thus, it confirms that the crude extract of coral *Junceella delicata* showed inhibition of albumin denaturation property.

b) Inhibition of proteinase

Leukocyte proteases, which are richly produced by neutrophils and localized at lysosomes, have been reported to contribute to tissue damage during inflammatory reactions, but proteinase inhibitors can provide significant protection.

Table No. 2 and Graph No. 2 shows the effect of standard drug diclofenac sodium and the crude extract of coral *Junceella delicata* against proteinase inhibition. From the above results, diclofenac sodium shows average proteinase inhibition at 85.06 %. Whereas, the crude

extract of *Junceella delicata* shows average proteinase inhibition at 82.02 %. From the above result, it is confirmed that the crude extract of *Junceella delicata* has proteinase inhibition properties.

Membrane stabilization

a) Heat-induced hemolysis

The HRBC (Human Red Blood Cell) membrane stabilization assay has been widely used to assess in vitro anti-inflammatory activity, as it can stabilize erythrocyte membranes, which may also suggest its potential to stabilize lysosomal membranes. By stabilizing lysosomes, the extract helps prevent the release of harmful lysosomal components, such as bacterial enzymes and proteases, from activated neutrophils. These enzymes, if released, can exacerbate tissue inflammation and lead to either acute or chronic inflammatory conditions. Non-steroidal anti-inflammatory drugs (NSAIDs) typically work by inhibiting these lysosomal enzymes or stabilizing the lysosomal membrane to reduce inflammation (Patel, 2016).

Table No. 3 and Graph No. 3 showing the effect of standard drug diclofenac sodium and the crude extract of coral *Junceella delicata* against membrane stabilization. In the case of membrane stabilization, we have evaluated the heat induced hemolysis against standard drug and the crude extract of coral *Junceella delicata.* From the above result it was found that the average heat induced hemolysis was recorded in the standard drug 39.99 %, whereas the average heat induced hemolysis was found in the crude extract of coral *Junceella delicata* was 71.3%. From the above result it is concluded that the crude extract of coral *Junceella delicata* showed membrane stabilization by inhibiting the heat induced hemolysis.

From the above result it is found that the crude extract of coral *Junceella delicata* has strong anti-inflammatory activity. This activity gets confirm by separating the crude extract of coral *Junceella delicata* by using preparative TLC. The isolated compound was found as alkaloid, steroids and terpenoids which shows strong anti-inflammatory response as cited in the literature. Therefore, we have determined the structures of isolated compounds and their biological properties were studied and it was found that the compounds Gly-Gly, 2-(2-Pyridyl)-4 methylthiazole-5-carboxylic acid, 7-Methoxy-2-methylquinolin-4-ol, Fraxidin, α-Methylcinnamic acid, 4-Ethoxycoumarin, 3-Hydroxycoumarin, 2,4,7,9-Tetramethyl-5-decyne-4,7-diol, 2,2-Bis(3-allyl-4-hydroxyphenyl) propane, Dodecanedioic acid shows strong anti-inflammatory response. Thus, it may be suggested that isolated compounds may be

further processed to prepare drugs which have anti-inflammatory, anti-cancer, neurological disorders and many others for the treatment of different diseases.

CONCLUSION

The study found that the coral *Junceella delicata* has anti-inflammatory properties, particularly in its ability to inhibit RBC hemolysis and inhibition on proteinase and to provide protection against tissue damage during inflammatory reactions.The bioactive compounds responsible for the activities need to be identified, and functional characterization of the compounds is required. Increasing the number of bioactive compounds extracted from marine soft coral could lead to the search for effective biomedical property and therapeutic applications in the future.

Conflict of interest

Authors do not have any conflict of interest.

ACKNOWLEDGEMENT

The authors thank Dr. Swapnaja Mohite, Professor and Head, Department of Fisheries Biology, College of Fisheries, Shirgaon, Ratnagiri, for final species identification and confirmation. Thanks are also due to The Director, Principal Chief Conservator of Forest, Nagpur and The Director, Maharashtra State Biodiversity Board, Nagpur, for permitting the collection of species.

REFERENCES

1. Cooper, E. L., Hirabayashi, K., Strychar, K. B., & Sammarco, P. W. Corals and their potential applications to integrative medicine. *Evidence-based Complementary and Alternative Medicine*, 2014; *2014*(1). https://doi.org/10.1155/2014/184959
2. Denko, C. W. A role for neuropeptides in inflammation. In *Springer eBooks, 1992;* 177–181. https://doi.org/10.1007/978-94-011-2996-1_9
3. Dhara, Patel. Phytochemical screening, in vitro anti-microbial and anti-inflammatory activity of methanolic extract of aster lanceolatus willd leaves. International Journal of Medicine Research, 2016; 1: 26-30.
4. Fernando, I., Sanjeewa, K., Kim, H., Kim, S., Lee, S., Lee, W. W., & Jeon, Y. Identification of sterols from the soft coral Dendronephthya gigantea and their anti-inflammatory potential. *Environmental Toxicology and Pharmacology*, 2017; *55*: 37–43. https://doi.org/10.1016/j.etap.2017.08.003

5. Gonzalez, Y., Torres-Mendoza, D., Jones, G. E., & Fernandez, P. L. Marine diterpenoids as Potential Anti-Inflammatory Agents. *Mediators of Inflammation, 2015;* (1). https://doi.org/10.1155/2015/263543
6. Govindappa, M., Channabasava, R., Sowmya, D., Meenakshi, J., Shreevidya, M., Lavanya, A., Sadananda, T. Phytochemical Screening, Antimicrobial and in vitro Anti-inflammatory Activity of Endophytic Extracts from Loranthus sp. *Pharmacognosy Journal*, 2011; *3*(25): 82–90. https://doi.org/10.5530/pj.2011.25.15
7. Jyothilakshmi, M., Jyothis, M., Narayanan, G. N. H., and Latha, M. S. Antidermatophytic and Protease-inhibiting Activities of Zerumbone: A Natural Sesquiterpene from the Rhizome of. *PubMed*, 2017; *13*(49): 2-6. https://doi.org/10.4103/0973-1296.197649
8. Kapoor, S., Nailwal, N., Kumar, M., & Barve, K. (2019). Recent Patents and Discovery of Anti-inflammatory Agents from Marine Source. *Recent Patents on Inflammation & Allergy Drug Discovery*, 2019; *13*(2): 105–114. https://doi.org/10.2174/1872213x13666190426164717
9. Li, C. Q., Ma, Q. Y., Gao, X. Z., Wang, X., & Zhang, B. L. Research Progress in Anti-Inflammatory Bioactive Substances Derived from Marine Microorganisms, Sponges, Algae, and Corals. *Marine Drugs*, 2021; *19*(10): 572. https://doi.org/10.3390/md19100572
10. Marchbank, D. H., Berrue, F., & Kerr, R. G. Eunicidiol, an Anti-inflammatory Dilophol Diterpene from Euniceafusca. *Journal of Natural Products*, 2012; *75*(7): 1289–1293. https://doi.org/10.1021/np300149y
11. Matulja, D., Grbcic, P., Bojanic, K., Topic-Popovic, N., Coz-Rakovac, R., Laclef, S., Smuc, T., Jovic, O., Markovic, D., & Pavelic, S. K. Chemical Evaluation, Antioxidant, Antiproliferative, Anti-Inflammatory and Antibacterial Activities of Organic Extract and Semi-Purified Fractions of the Adriatic Sea Fan, Eunicellacavolini. *Molecules*, 2021; *26*(19): 5751. https://doi.org/10.3390/molecules26195751
12. Pahwa, R., Goyal, A., & Jialal, I. Chronic Inflammation. In *StatPearls*. Stat Pearls Publishing, 2023.
13. Patel, D., and Desai, S. Phytochemical screening, in vitro anti-microbial and anti-inflammatory activity of methanolic extract of aster lanceolatus wild leaves. *International Journal of Applied Research*, 2016; *1*(1): 26-30. https://www.medicinesjournal.com/archives/2016/vol1/issue1/1-1-18.1

14. Putra, M. Y., Wibowo, J. T., Murniasih, T., & Rasyid, A. Evaluation of antibacterial activity from Indonesian marine soft coral Sinularia sp. *AIP Conference Proceedings*, 2016; *1744*: 020039. https://doi.org/10.1063/1.4953513
15. Radhika, P., Rao, P. R., Archana, J., and Rao, N. K. Anti-inflammatory Activity of a New Sphingosine Derivative and Cembrenoid Diterpene (Lobohedleolide) Isolated from Marine Soft Corals of Sinulariacrassa TIXIER-DURIVAULT and Lobophytum species of the Andaman and Nicobar Islands. *Biological and Pharmaceutical Bulletin*, 2005; *28*(7): 1311-1313. https://doi.org/10.1248/bpb.28.1311
16. Reina, E., Puentes, C., Rojas, J., García, J., Ramos, F. A., Castellanos, L., Ospina, L. F. Fuscoside E: A strong anti-inflammatory diterpene from Caribbean octocoral Euniceafusca. *Bioorganic & Medicinal Chemistry Letters*, 2011; *21*(19): 5888–5891. https://doi.org/10.1016/j.bmcl.2011.07.092
17. Sahidin, I., Sadarun, B., Wahyuni, W., Muhammad J, L. O., Rahmatika, N. S., Malaka, M. H., Fristiohad, A. In vivo Anti-Inflammatory and Immunomodulatory Activity of Soft Coral Nephthea sp. from Southeast Sulawesi. *Pakistan Journal of Biological Sciences*, 2023; *26*(8): 403–408. https://doi.org/10.3923/pjbs.2023.403.408s
18. Tsai, S., Zheng, Z., Nguyen, N. Guide-seq enables genome-wide profiling of off-target cleavage by CRISPR-Cas nucleases. *Nat Biotechnol*, 2015; 33: 187-197. https://doi.org/10.1038/nbt.3117
19. Williams, L. A., O'Connar, A., Latore, L., Dennis, O., Ringer, S., Whittaker, J. A., Conrad, J., Vogler, B., Rosner, H., and Kraus, W. The in vitro anti-denaturation effects induced by natural products and non-steroidal compounds in heat treated (immunogenic) bovine serum albumin is proposed as a screening assay for the detection of anti-inflammatory compounds, without the use of animals, in the early stages of the drug discovery process. *The West Indian medical journal*, 2008; *57*(4): 327-331.