

## Antioxidant And Anti-Inflammatory Properties Of The Marine Crab Tumidodromia Dormiahemolymph Lectin

Benikca Prakash<sup>1</sup>, J. Vinoliya Josephine Mary<sup>2\*</sup>, S. Mary Mettilda Bai<sup>2</sup>, J. Jovila<sup>3</sup>

<sup>1</sup>Research Scholar (21213042192005), Department of Zoology, Holy Cross College (Autonomous), Nagercoil. (Affiliated to Manonmaniam Sundaranar University, Tirunelveli, Tamil Nadu, India.)

Email ID : [ammubeni16@gmail.com](mailto:ammubeni16@gmail.com).

<sup>2</sup>Associate Professor, Department of Zoology, Holy Cross College (Autonomous), Nagercoil, (Affiliated to Manonmaniam Sundaranar University, Tirunelveli, Tamil Nadu, India.)

<sup>2</sup>[vinoliya75@gmail.com](mailto:vinoliya75@gmail.com), <sup>2</sup>[metti.silvester@gmail.com](mailto:metti.silvester@gmail.com)

<sup>3</sup>Research Scholar (21213042192006), Department of Zoology, Holy Cross College (Autonomous), Nagercoil. (Affiliated to Manonmaniam Sundaranar University, Tirunelveli, Tamil Nadu, India.)

Email ID : [jovilaj@gmail.com](mailto:jovilaj@gmail.com)

### Corresponding author –

J. Vinoliya Josephine Mary

Associate professor, Department of Zoology, Holy Cross College (Autonomous), Nagercoil, Tamil Nadu, India.

Email ID : [vinoliya75@gmail.com](mailto:vinoliya75@gmail.com),

Cite this paper as Benikca Prakash, J. Vinoliya Josephine Mary, S. Mary Mettilda Bai, J. Jovila (2025) Antioxidant And Anti-Inflammatory Properties Of The Marine Crab Tumidodromia Dormiahemolymph Lectin. *Journal of Neonatal Surgery*, 14, (3) 337-345

### ABSTRACT

The marine environment is a source of unique bioactive components that display great potential as therapeutic agents. The study on antioxidant and anti-inflammatory activities comprehensively examines the properties of purified lectin isolated from the marine crab *Tumidodromia dormia*. The lectin exhibits ABTS radical scavenging with an IC<sub>50</sub> value of 19.56 µg/ml, outperforming ascorbic acid and inhibiting lipid peroxidation more effectively than Trolox, with moderate activity in DPPH and FRAP assays. The presence of anti-inflammatory properties was confirmed through cyclooxygenase (COX) and lipoxygenase (LOX) inhibition, with each having an IC<sub>50</sub> value of 59.5 µg/ml and 61.7 µg/ml, respectively. These results elucidate the multifunctional potential of the marine crab lectins as a template for novel drug development for oxidative and inflammatory diseases..

**Keywords:** *Marine crab lectin, ABTS, COX, LOX, DPPH, FRAP, LPIC.*

### 1. INTRODUCTION

Inflammation is a vital protective response that enables the body to manage harmful stimuli, including invading pathogens, chemical agents, and physical trauma. This process is driven by a series of well-orchestrated events—such as dilation of the microvasculature, enhanced vascular permeability, and the directed movement of immune cells into affected tissues (Gerber et al., 2002). Although acute inflammation is beneficial and supports tissue healing, a failure to resolve this response can lead to a chronic state. Persistent inflammation is strongly linked to the development and progression of several major health disorders, including diabetes, cardiovascular disease, autoimmune conditions, cancer, and inflammatory bowel disease (Steer et al., 2002; Bhatia et al., 2003). One of the key contributors to chronic inflammation is oxidative stress, which arises when the production of reactive oxygen species (ROS) exceeds the capacity of endogenous antioxidant defences (Di Matteo and Esposito, 2003). Elevated ROS levels trigger lipid peroxidation, protein modification, and DNA damage, all of which intensify pro-inflammatory signalling. Antioxidants help counter these reactions by neutralising free radicals, and their role in protecting biological systems from oxidative injury is well documented (Krishnaiah et al., 2011). Due to the interactions between oxidative stress and inflammation, naturally occurring antioxidants have garnered significant interest. They are alternatives to synthetic antioxidants like BHA and BHT, which face increasing scrutiny due to possible adverse effects, including carcinogenicity (Gerber et al., 2002). Given the close relationship between oxidative imbalance, inflammation, and the onset of chronic diseases, the search for safe, natural therapeutic agents remains a priority.

The marine environment is a rich reservoir of structurally diverse secondary metabolites, shaped by evolutionary adaptations to extreme and fluctuating conditions such as high salt, pressure, and dynamic microbial communities (Blunt et al., 2018; Monteiro et al., 2012). Among these bioactive molecules, lectins—a category of carbohydrate-binding proteins—stand out due to their unique structure, robustness, high selectivity, and pharmacological versatility (Ogawa et al., 2011). Animal lectins play a key role in the immune system and exhibit both antioxidant and anti-inflammatory properties. They modulate inflammation by recognising specific carbohydrates present on the surface of cells, interfering with the binding sites thereby reducing leukocyte migration, production of cytokines and interaction with the immune receptors. Marine lectins have gained attention for their antioxidant, anti-inflammatory, antimicrobial, and anticancer properties (Cheung et al., 2015). Chronic redox imbalance and inflammation are central to many human diseases, making multitarget compounds like marine lectins attractive candidates for therapeutic development (Elbandy et al., 2022).

Crabs have been identified as a rich source of lectins as they are produced in response to pathogens by the innate immune system (Ravindranath et al., 1985). Marine-derived lectins from crabs exhibit unique glycan binding, high stability, and adaptive functions that distinguish them from terrestrial counterparts (Brinchmann et al., 2022). Crab lectins mediate host defense through several mechanisms: non-self-recognition, pathogen binding and neutralization, immune modulation, and, in some cases, regulation of apoptosis and cell proliferation (Pangestuti and Kim, 2011; Qiu et al., 2021). These activities are largely due to their interaction with cell-surface glycans, modulation of reactive oxygen species (ROS), and orchestration of immune cell responses (Cheung et al., 2015; Narayanasamy et al., 2020).

Several studies have shown the antioxidant potential of marine crab lectins. The shell extract of *Charybdis natator* demonstrated antioxidant and anti-inflammatory effects by reducing proinflammatory markers (TNF- $\alpha$ , NF- $\kappa$ B) while enhancing antioxidant enzyme activities such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) in zebrafish models (Galal-Khalla et al., 2024). Similarly,  $\beta$ -1,3-glucan binding protein ( $\beta$ -GPB) from the rice paddy crab *Paratelphusa hydrodromus* reduced DPPH radicals and restrained albumin denaturation, indicating antioxidant activity. Marine crab lectins and peptides exert anti-inflammatory effects by reducing inflammatory mediators and modulating key pathways such as NF- $\kappa$ B. They can inhibit multiple stages of inflammation, including COX and LOX activity, thereby reducing the release of proinflammatory mediators like prostaglandins and leukotrienes (Silva et al., 2022; Patel et al., 2013; Ogura et al., 2015).

Crab-derived peptides can also exert antinociceptive effects, playing a role in pain control by acting as COX-2 inhibitors in vitro (Narayanasamy et al., 2020). Lectins' anti-inflammatory effects are often linked to their carbohydrate-binding specificity, which affects neutrophil migration, cytokine generation, and vascular adhesion. Vargila et al. (2023) reported the anti-inflammatory property of the lectin of crab *Oziotelphusa naga* (NagLec) which inhibited COX and LOX activities by 49.43% and 61.81% at a dose of 100  $\mu$ g/mL, highlighting the role of lectins in influencing major inflammatory enzymes. Similar anti-inflammatory effects have been reported in other invertebrates such as *Holothuria grisea*, *Lamellidens marginalis*, (Moura et al., 1984; Chakraborty et al., 2017). Lectins from marine algae further support the therapeutic potential of these molecules. A lectin from the red alga *Solieria filiformis* reduced neutrophil migration in peritonitis models and decreased paw oedema in mice without systemic toxicity (Abreu et al., 2016). A lectin from the green seaweed *Caulerpa cupressoides* var. *lycopodium* decreased carrageenan-induced paw oedema, neutrophilic infiltration, and inhibited expression of IL-1 $\beta$ , IL-6, TNF $\alpha$ , and COX-2 (Lino et al., 2015).

Overall, marine lectins, particularly from crabs, represent a unique class of bioactive molecules with substantial pharmaceutical potential. Their antioxidant, anti-inflammatory, and immunomodulatory properties position them as promising candidates for developing novel therapeutic agents targeting oxidative stress, inflammation, and immune dysfunction (Ahmed et al., 2022). The current research evaluates the antioxidant and anti-inflammatory properties of lectin purified from *T. dormia*, expanding the known pharmacology of marine lectins and exploring their therapeutic promise for human health.

## 2. MATERIALS AND METHODS:

### Collection of samples:

The crab *Tumidodromia dromia* was collected from the coastal area of Kela Manakudi, Kanniayakumari, Tamil Nadu, India.

### Preparation of samples:

The hemolymph was extracted from the third walking leg of the marine crab *Tumidodromia dormia*. The lectin was isolated from the hemolymph by affinity chromatography.

### Antioxidant activities

#### ABTS radical scavenging activity:

The ABTS radical cation (ABTS $^{+}$ ) was generated following the procedure described by Re et al. (1999), with minor modifications. A 7 mM solution of 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) disodium salt (ABTS) was prepared in distilled water and reacted with potassium persulfate at a final concentration of 2.45 mM. The reaction mixture was maintained in the dark at room temperature for 12–16 h to facilitate the complete generation of the radical cation. Before

analysis, the ABTS solution was diluted with ethanol (approximately 1:89, v/v) and equilibrated at 30°C to obtain an absorbance of  $0.70 \pm 0.02$  at 734 nm using a 1-cm path length cuvette. For the assay, 1 mL of the diluted ABTS<sup>+</sup> solution was added to 10  $\mu$ L of the sample (20, 30, 40  $\mu$ g/mL-purified lectin) or Trolox standard (final concentration range: 0–15  $\mu$ M) in ethanol. The decrease in absorbance at 734 nm was recorded exactly 30 min after the initial mixing at 30°C. The total antioxidant activity (TAA) was calculated and expressed as  $\mu$ mol Trolox equivalents per gram of the sample, corresponding to the concentration of sample that produced 20–80% inhibition of the control absorbance.

The percentage inhibition of ABTS radical cation was calculated using the following formula:

$$\text{Percentage of inhibition} = \frac{[(\text{Absorbance of control} - \text{Absorbance of sample})]}{(\text{Absorbance of control})} \times 100$$

#### DPPH radical scavenging activity

The free radical scavenging activity of the fractions was evaluated in vitro using the 2,2'-diphenyl-1-picrylhydrazyl (DPPH) assay as described by Williams et al. (1995), with slight modifications. A stock solution of DPPH (24 mg) was prepared in 100 mL of ethanol and stored at 20°C until use. The working solution was obtained by diluting the stock with ethanol to yield an absorbance of approximately  $0.98 \pm 0.02$  at 517 nm. For the assay, 3 mL of the DPPH working solution was mixed with 1 mL of the sample at varying concentrations (20, 30, and 40  $\mu$ g/mL). The reaction mixture was vortexed and incubated in the dark at room temperature for 15 minutes. The absorbance was then measured at 517 nm against ethanol as the blank.

$$\text{Percentage of inhibition} = \frac{[(\text{Absorbance of control} - \text{Absorbance of sample})]}{(\text{Absorbance of control})} \times 100$$

#### Ferric Reducing Antioxidant Power (FRAP) Assay:

**Ferric reducing antioxidant power (FRAP)** was evaluated following the procedure of Stephanie et al.,(2009) with slight modifications. The FRAP reagent was freshly prepared each day by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ in 40 mM HCl, and 20 mM FeCl<sub>3</sub>·6H<sub>2</sub>O in a 10:1:1 (v/v/v) ratio, and warmed to 37°C before analysis. For the assay, 100  $\mu$ L of the lectin sample (20, 30, 40  $\mu$ g/mL) and 300  $\mu$ L of deionised water were combined with 4 mL of the reagent. The mixture was incubated at 37°C for 30 minutes, and absorbance was recorded at 593 nm against a reagent blank.

$$\text{Percentage of inhibition} = \frac{[(\text{Absorbance of control} - \text{Absorbance of sample})]}{(\text{Absorbance of control})} \times 100$$

#### Lipid peroxidation inhibition capacity (LPIC) assay:

Lipid peroxidation inhibition capacity was evaluated using the standard thiobarbituric acid reactive substances (TBARS) method (Shabbir et al.,2013). Briefly, egg yolk (1 g) was homogenised in 100 mL of 100 mM Tris–HCl buffer (pH 7.4) to prepare the lipid substrate. Different concentrations of the test sample were mixed with 100  $\mu$ L of 15 mM ferrous sulfate, followed by the addition of 3 mL of the egg-yolk homogenate. The reaction mixture was incubated for 30 min at room temperature to allow lipid peroxidation to occur. After incubation, 0.1 mL of the mixture was transferred to a tube containing 1.5 mL of 10% trichloroacetic acid (TCA) and allowed to stand for 10 min. The solution was then centrifuged at 3000 rpm for 10 min. The resulting supernatant was mixed with 1.5 mL of 0.67% thiobarbituric acid (TBA) prepared in 50% acetic acid, and the tubes were heated in a boiling water bath for 30 min to allow formation of the pink MDA–TBA chromogen. The absorbance of the developed colour was measured at 532 nm, and the extent of lipid peroxidation inhibition was calculated based on the decrease in TBARS formation.

The percentage inhibition of lipid peroxidation was calculated using the formula:

$$\text{Percentage of inhibition} = \frac{[(\text{Absorbance of control} - \text{Absorbance of sample})]}{(\text{Absorbance of control})} \times 100$$

#### Anti-inflammatory activity:

##### Cell culture:

RAW 264.7 macrophages were procured from the National Centre for Cell Sciences (NCCS), Pune, India, and maintained in Dulbecco's Modified Eagle's Medium (DMEM; Sigma-Aldrich, USA) supplemented with 10% fetal bovine serum, L-glutamine, sodium bicarbonate (Merck, Germany), and antibiotics (penicillin 100 U/mL, streptomycin 100  $\mu$ g/mL, amphotericin B 2.5  $\mu$ g/mL). Cells were cultured at 37°C in a humidified 5% CO<sub>2</sub> incubator (NBS Eppendorf, Germany) and allowed to reach approximately 60% confluence. Following this, cells were stimulated with lipopolysaccharide (LPS, 1  $\mu$ g/mL) and treated with lectin at concentrations of 25, 50, and 100  $\mu$ g/mL for 24 hours. After treatment, cells were collected, and the lysates were prepared for subsequent anti-inflammatory assays.

#### Cyclooxygenase (COX) activity

Cyclooxygenase activity was determined following the method of Walker and Gierse, (2010). In brief, 100  $\mu$ L of cell lysate was incubated with Tris-HCl buffer (pH 8), 5 mM glutathione, 5 mM haemoglobin, and arachidonic acid at 25°C for 1 minute. The reaction was initiated by adding 200 mM arachidonic acid and allowed to proceed at 37°C for 20 minutes, after which it was terminated with 200  $\mu$ L of 10% trichloroacetic acid in 1 N HCl. Following centrifugation, 200  $\mu$ L of 1% Thio barbituric acid was added, and the mixture was boiled for 20 minutes. After cooling and a brief centrifugation, the absorbance of the resulting chromogen was measured at 632 nm to assess COX activity, and the percentage inhibition of COX activity was determined as per the following formula:

$$\text{Percentage of inhibition} = ((\text{Absorbance of control} - \text{Absorbance of test}) / \text{Absorbance of control}) \times 100$$

### Lipoxygenase (LOX) activity

The determination of LOX activity was done as per the method of Axelrod et al. (1981). Briefly, the reaction mixture (2 ml final volume) contained Tris-HCl buffer (pH 7.4), 50  $\mu$ L of cell lysate, and sodium linoleate (200  $\mu$ L). The LOX activity was monitored as an increase of absorbance at 234 nm (Agilent Cary 60), which reflects the formation of 5-hydroxyeicosatetraenoic acid.

$$\text{Percentage of inhibition} = ((\text{Absorbance of control} - \text{Absorbance of test}) / \text{Absorbance of control}) \times 100$$

### Result

Hemolymph lectin (TdL) of the marine crab *Tumidodromia dormia*. was isolated using affinity chromatography (unpublished data) and the purified lectin was used to assess the antioxidant and anti-inflammatory activity.

#### Antioxidant activity

The antioxidant potential of the lectin was evaluated using ABTS radical scavenging activity, DPPH radical scavenging activity, Ferric Reducing Antioxidant Power (FRAP) assay and Lipid Peroxidation Inhibition Capacity (LPIC) assay.

#### ABTS Radical Scavenging Activity

The antioxidant activity of the purified lectin was assessed using the ABTS radical scavenging assay, a widely employed method for evaluating the free radical quenching potential of bioactive compounds. The lectin exhibited a concentration-dependent increase in radical scavenging activity, with percentage inhibition values of 51.25%, 74.07%, and 98.99% at concentrations of 20, 30, and 40  $\mu$ g/mL respectively (Table-1). The half-maximal inhibitory concentration (IC<sub>50</sub>) of the lectin was determined to be 19.56  $\mu$ g/mL, which is markedly lower than that of the reference antioxidant, ascorbic acid (IC<sub>50</sub> = 31.27  $\mu$ g/mL). The lower IC<sub>50</sub> value of the lectin compared to the standard clearly demonstrates its superior radical scavenging efficiency.

**Table 1:Antioxidant activity of purified crab lectin measured by the ABTS radical**

cation decolourization assay.

Sample	Concentration			IC <sub>50</sub> Value $\mu$ g/mL
	20 $\mu$ g/ ml	30 $\mu$ g/ ml	40 $\mu$ g/ ml	
<b>TdL</b>	51.252 $\pm$ 0.038	74.071 $\pm$ 0.031	98.987 $\pm$ 0.094	<b>19.56</b>
<b>Standard</b>	34.892 $\pm$ 0.018	43.317 $\pm$ 0.015	65.889 $\pm$ 0.515	<b>31.27</b>

Values are expressed as mean  $\pm$  SD of triplicate measurements. TdL = purified lectin from *T.dormia*. IC<sub>50</sub> values were calculated based on concentration-response curves. Ascorbic acid was used as the reference antioxidant.

#### DPPH Radical Scavenging Activity

The DPPH radical scavenging assay was employed to further evaluate the antioxidant potential of the purified lectin, based on its ability to donate hydrogen atoms or electrons to stabilize the DPPH free radical. In contrast to the ABTS assay, the lectin exhibited comparatively lower DPPH radical scavenging activity, showing percentage inhibition values of 4.98%, 10.41%, and 15.63% at concentrations of 20, 30, and 40  $\mu$ g/mL, respectively. The IC<sub>50</sub> value was determined to be 104.00  $\mu$ g/mL, which is considerably higher than that of the reference antioxidant, ascorbic acid (IC<sub>50</sub> = 27.62  $\mu$ g/mL). The relatively higher IC<sub>50</sub> value indicates a moderate ability of the lectin to neutralise DPPH radicals. This difference in scavenging capacity between the ABTS and DPPH assays may be attributed to variations in the solubility and reaction kinetics of the respective radicals, as well as the distinct mechanisms of electron or hydrogen transfer involved.

**Table 2:DPPH free radical scavenging activity of purified crab lectin at different concentrations.**

Sample	Concentration			IC 50 Value ( $\mu$ g/ ml)
	20 $\mu$ g/ ml	30 $\mu$ g/ ml	40 $\mu$ g/ ml	
TdL	4.978 $\pm$ 0.093	10.409 $\pm$ 0.016	15.625 $\pm$ 0.049	<b>104.505</b>
Standard	36.69 $\pm$ 0.299	58.442 $\pm$ 0.113	64.967 $\pm$ 3.078	<b>27.619</b>

Values are expressed as mean  $\pm$  SD of triplicate measurements. TdL = purified lectin from *T.dormia*. IC<sub>50</sub> values were calculated based on concentration-response curves. Ascorbic acid was used as the reference antioxidant.

#### Ferric Reducing Antioxidant Power (FRAP) Assay

The ferric reducing antioxidant power (FRAP) assay was conducted to assess the electron-donating capacity of the purified lectin, which reflects its potential to act as a reducing agent by converting ferric (Fe<sup>3+</sup>) to ferrous (Fe<sup>2+</sup>) ions. The lectin demonstrated relatively low reducing activity, with FRAP values of 1.98, 2.46, and 4.00 at concentrations of 20, 30, and 40  $\mu$ g/mL, respectively. The IC<sub>50</sub> value was determined to be 496.72  $\mu$ g/mL, which is notably higher than that of the standard ascorbic acid (IC<sub>50</sub> = 350.46  $\mu$ g/mL). The higher IC<sub>50</sub> value indicates that the lectin possesses limited ferric ion reducing power and a relatively weak electron-donating capacity compared to the other antioxidant activities.

**Table 3: Ferric reducing antioxidant power (FRAP) of the purified crab lectin evaluated at varying concentrations.**

Sample	Concentration			IC 50 Value ( $\mu$ g/ ml)
	20 $\mu$ g/ ml	30 $\mu$ g/ ml	40 $\mu$ g/ ml	
TdL	1.980 $\pm$ 0.014	2.461 $\pm$ 0.006	4.002 $\pm$ 0.039	<b>496.723</b>
Standard (Ascorbic acid)	7.76 $\pm$ 0.312	15.52 $\pm$ 0.059	44.28 $\pm$ 0.120	<b>350.457</b>

Values are expressed as mean  $\pm$  SD of triplicate measurements. TdL = purified lectin from *T.dormia*. IC<sub>50</sub> values were calculated based on concentration-response curves. Ascorbic acid was used as the reference antioxidant.

#### Lipid Peroxidation Inhibition Capacity (LPIC) Assay

The lipid peroxidation inhibitory potential of the purified lectin was evaluated to assess its ability to protect biomembranes from oxidative damage. The lectin exhibited substantial inhibition of lipid peroxidation, with percentage inhibition values of 35.69%, 37.28%, and 38.72% at concentrations of 20, 30, and 40  $\mu$ g/mL, respectively. These values were markedly higher than those of the reference standard, Trolox, which showed 8.69%, 11.44%, and 12.60% inhibition at the corresponding concentrations. The higher inhibitory activity of the lectin compared to Trolox indicates its strong protective effect against oxidative degradation of lipids.

**Table 4: Lipid peroxidation inhibition capacity (LPIC) of the purified *T. dormia* lectin compared with Trolox.**

Sample	Concentration		
	20 $\mu$ g/ ml	30 $\mu$ g/ ml	40 $\mu$ g/ ml

<b>TdL</b> <b>% of Inhibition</b>	35.69 %	37.28 %	38.72 %
<b>Standard</b> <b>% of Inhibition</b>	8.69 %	11.44 %	12.60 %

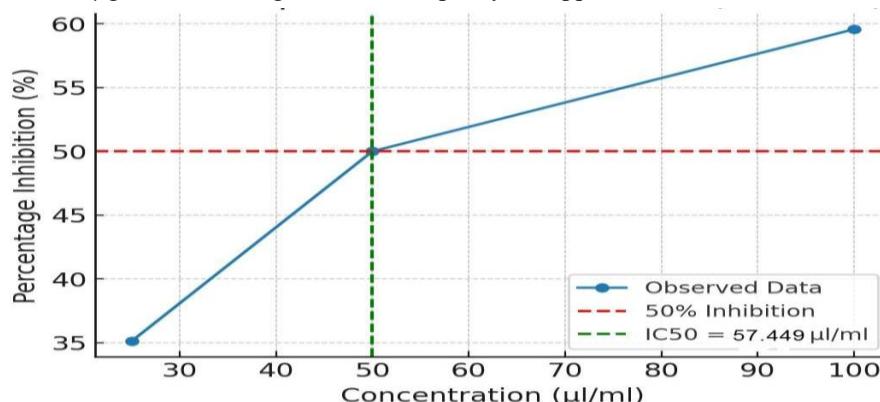
Values are expressed as percentage inhibition (mean  $\pm$  SD, n = 3). TdL = purified lectin from *T. dormia*. LPIC was determined using the TBARS method, where lower malondialdehydeformation indicates higher inhibitory activity. Trolox was used as the reference antioxidant.

#### Anti-inflammatory Activity

The anti-inflammatory potential of the sample was evaluated using RAW 264.7 macrophage cells by inhibiting key inflammatory enzymes, cyclooxygenase (COX) and lipoxygenase (LOX) activities.

#### Cyclooxygenase (COX) Inhibition Activity

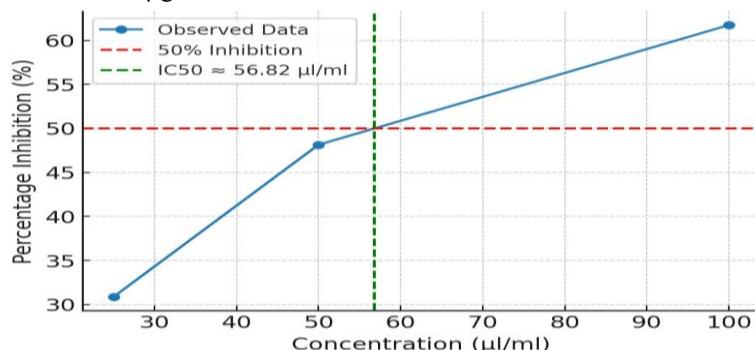
The anti-inflammatory potential of the purified lectin was assessed using the cyclooxygenase (COX) inhibition assay (Fig. 1). The lectin exhibited concentration-dependent inhibitory effects on COX activity, with percentage inhibition values of 35.11%, 50.00%, and 59.57% at concentrations of 25, 50, and 100  $\mu$ g/mL, respectively. The  $IC_{50}$  value for COX inhibition was determined to be 57.50  $\mu$ g/mL, indicating a moderate capacity to suppress COX-mediated inflammatory pathways.



**Fig 1: Graphical representation depicting the Anti-inflammatory activity (COX) of the sample. Along the Y axis, Percentage of Inhibition, alongthe X axis, varied concentration of the sample.**

#### Lipoxygenase (LOX) Inhibition Activity

In parallel with COX inhibition, the purified lectin demonstrated dose-dependent inhibitory activity against lipoxygenase (LOX) (Fig. 2). The percentage inhibition increased from 30.86% at 25  $\mu$ g/mL to 48.15% at 50  $\mu$ g/mL, reaching 61.73% at the highest tested concentration of 100  $\mu$ g/mL. The  $IC_{50}$  value for LOX inhibition was calculated as 56.82  $\mu$ g/mL.



**Fig 2: Graphical representation depicting the Anti-inflammatory activity (LOX) of the sample. Along the Y axis, the Percentage of Inhibition, and along the X axis, the varied concentration of the sample.**

### 3. DISCUSSION

The present study demonstrated the antioxidant and anti-inflammatory properties of the purified lectin from the hemolymph of the marine crab *Tumidodromia dormia*, highlighting its potential as a multifunctional bioactive molecule. The lectin (TdL) showed strong ABTS radical scavenging activity with an  $IC_{50}$  of 19.56  $\mu\text{g}/\text{ml}$ , outperforming the standard ascorbic acid. This suggests that the crab lectin exhibits an effective hydrogen-donating ability and is highly reactive towards the ABTS radical cation, indicating its potential to efficiently neutralise reactive oxygen species (ROS) (López-García et al., 2022). The observed activity is due to the presence of carbohydrate-binding domains capable of stabilising free radicals, as previously reported for other marine-derived lectins (Cheung et al., 2015; Pangestuti and Kim, 2018).

Compared to the ABTS assay, the DPPH radical scavenging activity of the purified lectin was moderate, showing  $IC_{50}$  of 104  $\mu\text{g}/\text{ml}$ . This difference can be attributed to the solvent polarity and radical type involved. DPPH assays typically favour lipophilic antioxidants, whereas ABTS can evaluate both hydrophilic and lipophilic molecules (Minarti et al., 2024). Although the lectin displayed lower reactivity towards the DPPH radical, its observable scavenging potential still reflects the presence of functional groups capable of participating in redox reactions. These results suggest that the lectin possesses a moderate level of antioxidant activity under the DPPH system, complementing its stronger ABTS radical scavenging performance.

The FRAP results indicated a limited ferric-reducing potential ( $IC_{50} = 496.7 \mu\text{g}/\text{ml}$ ), which shows that the lectin may act predominantly through radical quenching rather than direct electron donation (Ahmed et al., 2022). This reduced activity is attributed to the absence or lower abundance of specific redox-active functional groups within the lectin structure that facilitate electron transfer reactions. Despite this, the lectin showed a distinguished lipid peroxidation inhibition capacity (LPIC), exceeding that of the Trolox standard. This suggests strong membrane-protective potential by reducing malondialdehyde formation, a key indicator of lipid oxidation (Mourão, 2015). This pronounced lipid peroxidation inhibition suggests that the lectin can effectively scavenge lipid-derived free radicals and interrupt oxidative chain reactions, thereby contributing to membrane stabilisation and cellular protection. These findings highlight the lectin's potential as a natural antioxidant with promising applications in the prevention of lipid peroxidation-related disorders.

The antioxidant efficiency observed aligns with findings from other marine crab lectins like lectins isolated from *Scylla serrata* and *Oziotelphusa naga*, which exhibited notable ABTS scavenging and lipid peroxidation inhibition, further supporting the antioxidant roles of marine-derived proteins (Fredrick and Ravichandran, 2012; Vargila et al., 2024). Such bioactivity is crucial in mitigating oxidative stress, which plays a central role in chronic diseases such as cardiovascular disorders, neurodegeneration and cancer (Elbandy, 2023). The strong radical quenching capacity of the lectin highlights its potential as a natural antioxidant agent with possible applications in pharmaceutical and nutraceutical formulations aimed at combating oxidative stress and associated pathophysiological disorders.

In addition to its antioxidant potential, the lectin demonstrated notable anti-inflammatory activity, inhibiting both cyclooxygenase (COX) and lipoxygenase (LOX) pathways with  $IC_{50}$  values of approximately 57  $\mu\text{g}/\text{mL}$  and 56.8  $\mu\text{g}/\text{mL}$ , respectively. The inhibition of these enzymes indicates a broad-spectrum anti-inflammatory effect. Since COX and LOX catalyse the synthesis of prostaglandins and leukotrienes—key mediators of inflammation—simultaneous inhibition provides enhanced therapeutic efficacy with potentially fewer side effects (Ranjbar et al., 2016; Irrera & Bitto, 2017). Similar dual inhibition mechanisms have been identified in  $\alpha$ -amyrin derivatives, suggesting that compounds with combined COX/LOX modulation could serve as promising drugs for anti-inflammation (Ranjbar et al., 2016).

Marine lectins have been increasingly recognised for their diverse biological roles, including modulation of immune responses, apoptosis regulation, and ROS balance (Qiu et al., 2021; Napoleão et al., 2025). The findings of this study reinforce the multifunctional nature of these compounds, particularly their potential as natural therapeutic agents. The inhibition of inflammatory enzymes observed in *T. dormia* lectin may stem from its ability to interact with specific glycan motifs on immune cells, leading to downstream suppression of pro-inflammatory mediators such as TNF- $\alpha$  and NF- $\kappa$ B, as previously observed in *Charybdis natator* extracts (Galal-Khalla et al., 2024). Overall, this study establishes that the lectin from *Tumidodromia dormia* possesses potent ABTS scavenging and lipid peroxidation inhibition activities along with dual COX and LOX enzyme inhibition. These results highlight its therapeutic promise as a natural marine compound capable of addressing oxidative stress and inflammation simultaneously. Further research, including structure-function characterisation, molecular docking, and in vivo studies, would help elucidate its precise mechanisms and expand its pharmaceutical applications.

### 4. CONCLUSION:

The lectin demonstrated antioxidant activity depending on the specific assay employed. It exhibited strong ABTS radical scavenging activity and significant inhibition of lipid peroxidation, indicating a high capacity to neutralise free radicals and protect against oxidative damage. Moderate activity was observed in the DPPH radical scavenging assay, while the FRAP assay revealed relatively low electron-donating capacity. In the anti-inflammatory evaluations, the lectin displayed concentration-dependent inhibitory effects against both cyclooxygenase (COX) and lipoxygenase (LOX) enzymes. The present study suggests that the lectin is capable of modulating multiple inflammatory pathways, highlighting its potential as

a natural bioactive compound with both antioxidant and anti-inflammatory properties.

## REFERENCES

- [1] Gerber, M., Boutron-Ruault, M.-C., Hercberg, S., Riboli, E., Scalbert, A., & Siess, M.-H. (2002). Food and cancer: State of the art about the protective effect of fruits and vegetables. *Bulletin du Cancer*, 89(3), 293–312.
- [2] Steer, P., Millgård, J., Sarabi, D. M., et al. (2002). Cardiac and vascular structure and function are related to lipid peroxidation and metabolism. *Lipids*, 37(3), 231–236.
- [3] Bhatia, S., Shukla, R., Madhu, S. V., Gambhir, J. K., & Prabhu, K. M. (2003). Antioxidant status, lipid peroxidation and nitric oxide end products in patients of type 2 diabetes mellitus with nephropathy. *Clinical Biochemistry*, 36(7), 557–562.
- [4] Di Matteo, V., & Esposito, E. (2003). Biochemical and therapeutic effects of antioxidants in the treatment of Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis. *Current Drug Targets – CNS & Neurological Disorders*, 2(2), 95–107.
- [5] Krishnaiah, D., Sarbatly, R., & Nithyanandam, R. (2011). A review of the antioxidant potential of medicinal plant species. *Food and Bioproducts Processing*, 89(3), 217–233.
- [6] Blunt, J. W., Copp, B. R., Keyzers, R. A., Munro, M. H. G., & Prinsep, M. R. (2018). Marine natural products. *Natural Product Reports*, 35(1), 8–53.
- [7] Monteiro, M. C., Morais, J. S., Costa, C. S., Reyes, F. G., & Rodrigues, A. E. (2012). Biologically active compounds from marine organisms. *Marine Drugs*, 10(7), 1584–1617.
- [8] Ogawa, T., Ueda, M., & Nakamura, T. (2011). Lectins from marine organisms: Structural characteristics and functions. *Marine Drugs*, 9(11), 1998–2018.
- [9] Cheung, R. C. F., Wong, J. H., Pan, W., Chan, Y. S., Yin, C., Dan, X., & Ng, T. B. (2015). Marine lectins and their medicinal applications. *Applied Microbiology and Biotechnology*, 99(9), 3755–3773.
- [10] Elbandy, M. (2023). Anti-inflammatory effects of marine bioactive compounds and their potential as functional food ingredients in the prevention and treatment of neuroinflammatory disorders. *Molecules*, 28(1), 2. <https://doi.org/10.3390/molecules28010002>
- [11] Vargila, F., Mary Mettilda Bai, S., Vinoliya Josephine Mary, J., & Citarasu, T. (2024). Isolation, characterisation and antimicrobial properties of hepatopancreas lectin of the freshwater crab *Oziotelphusa naga*. *Protein Expression and Purification*, 222, 106536. <https://doi.org/10.1016/j.pep.2024.106536>
- [12] Brinchmann, M. F., Olsen, R. E., & Nilsen, F. (2022). Marine-derived lectins: Recognition and antiviral functions of crustacean lectins. *Frontiers in Marine Science*, 9, 847324.
- [13] Pangestuti, R., & Arifin, Z. (2018). Medicinal and health benefit effects of functional sea cucumbers. *Journal of Traditional and Complementary Medicine*, 8(3), 341–351.
- [14] Qiu, J., Li, Y., Zhang, J., Yang, X., & Wang, Y. (2021). Therapeutic potentials of marine lectins in disease treatment. *International Journal of Molecular Sciences*, 22(8), 4231.
- [15] Narayanasamy, A., Balde, A., Raghavender, P., Shashanth, D., Abraham, J., Joshi, I., & Nazeer, R. A. (2020). Isolation of marine crab (*Charybdis natator*) leg muscle peptide and its anti-inflammatory effects on macrophage cells. *Biocatalysis and Agricultural Biotechnology*, 25, 101577.
- [16] Narayanasamy, P., Kim, J. S., & Kim, S. K. (2020). Molecular mechanisms of anticancer activity of marine lectins from crabs. *International Journal of Molecular Sciences*, 21(15), 5152.
- [17] Galal-Khalla, A., Boyce, M. W., & Al-Sarawi, A. (2024). Antioxidant and anti-inflammatory properties of invasive crab *Charybdis natator* shell extract in zebrafish. *Frontiers in Marine Science*, 11, 1376768.
- [18] Silva, A. W. A., Lopes, B. S., Francisco, L. W. G. M., Lima, E. R., Rodrigues, M. C. A., Wlissess, A. S., Pinheiro, R. C., Farias, R. C., Henrique, E. S. B., Izabel, M. F. G., Orlando, E. P. T. F., Shiniti, C. N., Holanda, A. S., & Anderson, B. M. R. (2022). *Codium isthmocladum* lectin 1 (CiL-1): Interaction with N-glycans explains antinociceptive and anti-inflammatory activities in adult zebrafish. *International Journal of Biological Macromolecules*, 208, 1082–1089.
- [19] Patel, K., Jain, A., & Patel, D. K. (2013). Medicinal significance, pharmacological activities, and analytical aspects of anthocyanidins 'delphinidin': A concise report. *Journal of Acute Disease*, 2, 169–178.
- [20] Ogura, N., & Kondoh, T. (2015). Molecular aspects in inflammatory events of temporomandibular joint: Microarray-based identification of mediators. *Japanese Dental Science Review*, 51, 10–24.
- [21] Moura, M. R., Aragao, K. S., de Melo, A. A., Carneiro, R. F., Osorio, C. B., Luz, P. B., de Queiroz, A. F., dos

Santos, E. A., de Alencar, N. M., & Cavada, B. S. (2013). *Holothuria grisea* agglutinin (HGA): The first invertebrate lectin with anti-inflammatory effects. *Fundamental & Clinical Pharmacology*, 27, 656–668.

[22] Chakraborty, M., Bhattacharya, S., Bose, M., Sasmal, S. K., Gupta, M., & Mishra, R. (2017). Anti-inflammatory activity of a COX-2 inhibitory anionic protein fraction from *Lamellidens marginalis*. *Indian Journal of Experimental Biology*, 55, 88–97.

[23] Abreu, T. M., Ribeiro, N. A., Chaves, H. V., Bezerra Jorge, R. J., Bezerra, M. M., Monteiro, H. S. A., Vasconcelos, I. M., Mota, E. F., & Barros Benevides, N. M. (2016). Antinociceptive and anti-inflammatory activities of the lectin from marine red alga *Solieria filiformis*. *Planta Medica*, 82, 596–605.

[24] Lino de Queiroz, I. N., Quindere, A. L. G., Rodrigues, J. A. G., Vanderlei, E. D. S. O., Ribeiro, N. A., da Conceicao Rivanor, R. L., Coura, C. O., Pereira, K. M. A., & Chaves, H. V. (2015). Dual effects of a lectin from green seaweed *Caulerpa cupressoides* on inflammatory mediators. *Inflammation Research*, 64, 971–982.

[25] Ahmed, I. A. M., Babiker, E. E., Al-Juhaimi, F. Y., & Bekhit, A. E. D. A. (2022). Clove polyphenolic compounds improve the microbiological status, lipid stability, and sensory attributes of beef burgers during cold storage. *Antioxidants*, 11(7), 1354.

[26] Re R., Pellegrini N., Proteggente A., Pannala A., Yang M. and Rice Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic. BioMed.* 1999; 26: 1231-37.

[27] Williams, BW, Cuvelier, ME & Berset, CLWT 1995, 'Use of a free radical method to evaluate antioxidant activity', LWT-Food science and Technology, vol. 28, no. 1, pp. 25-30.

[28] Stephanie, D.; Xavier, V.; Philippe, C.; Marion, W.; Jean-Michel, M. Comparative study of antioxidant properties and total phenolic content of 30 plant extracts of industrial interest using DPPH, ABTS, FRAP, SOD, and ORAC assays. *Journal of Agriculture and Food Chemistry* 2009, 57, 1768–1774.

[29] Shabbir, M., Khan, M. R., & Saeed, N. (2013). Assessment of phytochemicals, antioxidant, anti-lipid peroxidation and anti-hemolytic activity of various fractions of *Maytenus royleanus* leaves. *BMC Complementary and Alternative Medicine*, 13, 143.

[30] Walker MC, Gierse JK. In vitro assays for cyclooxygenase activity and inhibitor characterization. *Methods Mol Biol.* 2010;644:131–44.

[31] Axelrod, B., Cheesbrough, T. M., & Laakso, S. (1981). Lipoxygenases in soybean. *Methods in Enzymology*, 71, 441–451.

[32] López-García, G., Dublan-García, O., Arizmendi-Cotero, D., & Gómez-Oliván, L. M. (2022). Antioxidant and antimicrobial peptides derived from food proteins. *Molecules*, 27(4), 1343. <https://doi.org/10.3390/molecules27041343>

[33] Minarti, M., Ariani, N., Megawati, M., Hidayat, A., Hendra, M., Primahana, G., & Darmawan, A. (2024). Potential antioxidant activity methods DPPH, ABTS, FRAP, total phenol and total flavonoid levels of *Macaranga hypoleuca* leaves extract and fractions. *E3S Web of Conferences*, 503, 07005.

[34] Mourão, P. A. (2015). Perspective on the use of sulfated polysaccharides from marine organisms as a source of new antithrombotic drugs. *Marine Drugs*, 13(5), 2770–2784.

[35] Fredrick, R. S., & Ravichandran, S. (2012). Isolation and characterisation of scyllin, a lectin from the mud crab *Scylla serrata* with antimicrobial properties. *Marine Biotechnology*, 14(5), 600–609.

[36] Ranjbar, M. M., Assadolahi, V., Yazdani, M., Nikaein, D., & Rashidieh, B. (2016). Virtual dual inhibition of COX-2/5-LOX enzymes based on binding properties of alpha-amyrins, the anti-inflammatory compound as a promising anti-cancer drug. *EXCLI Journal*, 15, 238–245.

[37] Irrera, N., & Bitto, A. (2017). Evidence for using a dual COX 1/2 and 5-LOX inhibitor in neurodegenerative diseases. *Neural Regeneration Research*, 12(7), 1077–1078. <https://doi.org/10.4103/1673-5374.211185>

[38] Napoleão, T. H., Lira, T. L. D. S., Pontual, E. V., Ferreira, G. R. S., & da Silva, P. M. (2025). Lectins as natural antibiofilm agents in the fight against antibiotic resistance: A review. *Molecules*, 30(16), 3395. <https://doi.org/10.3390/molecules30163395>

[39] Ravindranath, M. H., Higa, H. H., Cooper, E. L., & Paulson, J. C. (1985). Purification and characterization of an O-acetylsialic acid-specific lectin from a marine crab *Cancer antennarius*. *Journal of Biological Chemistry*, 260(15), 8850–8856..