Advances in Pharmacology and Pharmacy 13(3): 399-410, 2025 http://www.hrpub.org
DOI: 10.13189/app.2025.130311

Anti-Inflammatory Activity of Marine Red Algae through Dual Inhibition of Cyclooxygenase-2 and 5-Lipoxygenase

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Received August 21, 2024; Revised November 14, 2024; Accepted April 21, 2025

Cite This Paper in the Following Citation Styles

(a): *[1] Tejaswini Ullakula, Vani Mathakala, Umamaheshwari Amineni, Umakanth Naik Vankadoth, Uma Maheshwari Devi Palempalli , "Anti-Inflammatory Activity of Marine Red Algae through Dual Inhibition of Cyclooxygenase-2 and 5-Lipoxygenase," Advances in Pharmacology and Pharmacy, Vol. 13, No. 3, pp. 399 - 410, 2025. DOI: 10.13189/app.2025.130311.*

(b): *Tejaswini Ullakula, Vani Mathakala, Umamaheshwari Amineni, Umakanth Naik Vankadoth, Uma Maheshwari Devi Palempalli (2025). Anti-Inflammatory Activity of Marine Red Algae through Dual Inhibition of Cyclooxygenase-2 and 5-Lipoxygenase. Advances in Pharmacology and Pharmacy, 13(3), 399 - 410. DOI: 10.13189/app.2025.130311.*

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Abstract In the present study, two marine brown algae namely *Sargassum vulgare*, *Sargassum longifolium* and red algae *Gracilaria cearensis* were screened for their anti-inflammatory and anti-oxidant activity. The anti-inflammatory activity of algal samples was measured through the inhibition of haemolysis induced by heat and hyposaline. DPPH radical scavenging assay and hydrogen peroxide scavenging assay were adopted to detect the anti-oxidant potential of algal samples. Among the three marine algae, *G.cearensis* exhibited potential anti-inflammatory and anti-oxidant activity. GC-MS analysis was followed to capture the phytochemical picture of *G.cearensis*. The detected bioactive ligands were docked with inflammatory proteins such as COX-2, 5-LOX, mPGES-1, iNOS, TNF-α and NF-κB using Maestro 13.0 module of Schrodinger. The standard diclofenac exhibited docking affinity of -3.597 kcal/mol and -2.262 kcal/mol with the protein receptors COX-2 and 5-LOX respectively. While among all the bioactive compounds, 4-methyl-3-heptanone (dialkyl ketone) showed the highest docking energy of -6.608 kcal/mol and -4.205 kcal/mol with COX-2 and 5-LOX receptors respectively. Overall the bioactive ligands namely beta-sitosterol acetate, 4-methyl-3-heptanone, diacetone alcohol and 1-allyl-1,2-cyclohexanediol demonstrated potential binding affinity with the inflammatory markers. Thus the results state that *Gracilaria cearensis* exhibit dual inhibition against COX-2 and 5-LOX and can be used to develop novel anti-inflammatory drugs.

Keywords Inflammation, *Gracilaria cearensis,* Cyclooxygenase (COX), Lipoxygenase (LOX), Dual Inhibitors, Molecular Docking

1. Introduction

Inflammation, a complex physiological and pathological process triggered by a variety of stimuli such as bacterial infection, chemical injury, and environmental pollution is the leading cause for several diseases [1]. Complications from inflammatory disorders might impact many bodily components and malignancies. The diseases like type 2 diabetes, cardiovascular disease, rheumatoid arthritis, inflammatory bowel disease, asthma and chronic obstructive pulmonary diseases are highly connected with chronic inflammation [2,3]. Arachidonic acid metabolism

plays a central role in the inflammatory mechanism through cyclooxygenase (COX) and lipoxygenase (LOX) which produces prostaglandins (PGs) and leukotrienes respectively [4,5]. Pro-inflammatory PGs are produced and released at sites of inflammation more effectively when arachidonic acid is converted to prostanoids by COX-2 enzymes. Because of the upregulation of COX-2 in inflammatory tissues, COX-2-selective inhibitors are very useful for inflammatory conditions as they control the generation of proinflammatory PGs downstream [6]. COX-2 is an essential mediator of inflammation and can affect the NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) signalling pathway. While indirect activation encourages the release of inflammatory cytokines like TNF-α (tumor necrosis factor-alpha) and IL-1β (interleukins), which in turn increases COX-2 expression and maintains inflammation, direct activation of NF-κB by prostaglandins, especially PGE2, improves inflammation transcription and cell survival. The LOX enzymes, specifically 5-LOX, play a pivotal role in pro-inflammatory mechanisms by the conversion of arachidonic acids to leukotrienes [7]. 5-LOX has an indirect effect on NF-κB signalling via regulating cytokine and chemokine production, which in turn triggers NF-κB. The co-inhibition of COX-2 and 5-LOX has been considered to be an effective mechanism for controlling inflammatory responses. Progression of inflammatory disorders is also associated with both COX-2 and iNOS (Inducible nitric oxide synthase) [8]. PGE2, the key pro-inflammatory mediator, is synthesized from arachidonic acid through catalytic action of prostaglandin E synathase. Among the three PGE synthases, mPGES-1 (Microsomal prostaglandin E synthase-1) is the major mediator of inflammation which converts PGH2 into PGE2 and acts as a therapeutic target. Targeted by NF-κB, inducible nitric oxide synthase (iNOS) catalyses the oxidative deamination of L-arginine, which produces nitric oxide (NO), an essential pro-inflammatory mediator, concurrently with the COX enzyme family [9].

Non-steroidal anti-inflammatory drugs (NSAIDs) have long been recognised to reduce the inflammation by sabotaging the activity of cyclooxygenases (COX) enzymes [10] and the dual inhibitors of COX-2/5-LOX are promising anti-inflammatory agents compared to NSAIDs. The search for potent pain and inflammation remedies with the fewest possible adverse effects from prescription drugs is motivating researchers to investigate natural substitutes for NSAIDs, such as marine algae [11].

Macroscopic algae, or seaweeds, are valuable marine resources that are also environmentally benign and have medicinal uses [12]. Macroalgae like *Sargassum* sps. and *Gracilaria* sps. are some of the most significant and effective in the treatment of chronic diseases like rheumatoid arthritis, gastric ulcer, hypertension, cancer and asthma [13]. Considering that combination therapy targeting both COX-2 and 5-LOX may reduce or prevent the adverse effects associated with inhibiting PGD2, an anti-inflammatory mediator produced by COX-1. Keeping in view the importance of COX-2 and 5-LOX in inflammation, we screened three marine algae with the goal to detect potential dual inhibitors of COX-2 and 5-LOX.

2. Material and Methods

2.1. Preparation of Marine Algal Samples

Two brown and one red algal samples were collected along the coast line of Rushikonda (17.7825° N, 83.3851° E), bheemunipatnam (17.8555° N, 83.4162° E) and ramakrishna beach (17.7142° N, 83.3237° E) during low-tide periods in December and January at Visakhapatnam, Andhra Pradesh. The samples were cleaned, shade dried and then powdered. About 10 grams of each sample were extracted with solvents (1:10 v/v) such as ethanol, ethyl acetate and water for 48hrs at room temperature with intermittent shaking. The resulting extracts were filtered and concentrated in rotary evaporator and the obtained extracts were utilized for experimental analysis.

2.2. Qualitative Screening for Phytochemicals of Marine Algae

Ethyl acetate, ethanol and aqueous extracts of brown and red algal samples were used to detect the phytochemical profiles by adopting standard methods. Concisely, the alkaloids in the extract were analyzed by mixing 2ml of filtrate of the respective algal extract and 2N hydrochloric acid with Wagner's reagents and the appearance of reddish brown colour was treated as the presence of alkaloids [14]. Flavonoids were confirmed with magnesium tanning with pink red colour [15]. The content of tannins was confirmed with the formation of green-black colour after the addition of 5% $FeCl_3$ [16]. The presence of saponins was confirmed by formation of black green precipitate by mixing 2ml of filtrate, 1ml ammonia solution and 1ml lead acetate [17]. Cardiac glycosides were confirmed using Keller-Killiani test [18] and phenols were identified with the formation of precipitate upon the addition of a few drops of 5% aqueous $FeCl_3$ solution to the mixture of 1ml of extract and 1ml of lead acetate [19]. Similarly, terpenoids were identified by Salkowski Test [20] and Anthraquinones by Borntrager’s test respectively [19].

2.3. *In-vitro* Anti-Inflammatory Potential of Algal Extracts

2.3.1. HRBC Membrane Stabilization Assay

The anti-inflammatory potential of collected algal samples was analysed by HRBC membrane stabilization assay using heat induced haemolysis [21] and hypotonicity-induced haemolysis [22]. Briefly, heat

induced haemolysis was carried out with the reaction mixture comprising 1ml of algal extract with different concentrations (50-200 µg/ml) and 1ml of 10% RBC's suspension. Saline and diclofenac sodium were used as control and standard respectively. The anti-inflammatory activity was calculated based on the percentage of RBC lysis.

In hypotonicity-induced haemolysis different concentrations of extracts (50-200µg/ml) were mixed with 1ml of phosphate buffer, 2ml of hyposaline and 0.5ml of RBC's suspension. Diclofenac sodium (100µg/ml) was used as a standard drug. All the reaction mixtures were incubated at 37ºC for 30 minutes and centrifuged at 3000rpm. The percentage haemolysis was estimated at 560λmax by considering 100% haemolysis in the control.

2.4. Anti-Oxidant Activity of Algal Extracts

The free radical scavenging activity of the marine algal extracts was examined in-vitro using DPPH radical as described by Rashmi [23] and the inhibition percentage for scavenging DPPH radical was measured at 517nm by using ascorbic acid as standard. The ability of the extracts to scavenge hydrogen peroxide was determined according to the method of Vani [24] and the percentage was calculated by measuring the absorbance at 230nm.

2.5. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of Red Algae

The phytochemical profile of ethyl acetate extract of red algae was mapped using GC-MS analysis (Perkin-Elmer Clarus 680). Electron Ionisation (Turbo massver 5) as the ionisation source with 40 to 600 Da scan range was chosen for fragmentation and the component spectrums were compared with GC-MS NIST library (2008).

2.6. Insilico Analysis for Anti-Inflammatory Activity

2.6.1. Preparation of Inflammatory Protein Receptors

The crystalline structure of the inflammatory proteins such as COX-2, 5-LOX, mPGES-1, iNOS, TNF-α and NF-κB was derived from protein data bank with PDB ID: 1PXX, 3V99, 3DWW, 4NOS, 3GIO and 2O61 respectively. Water molecules and co-crystallized structures were modified to improve the binding efficiency of the proteins with ligands.

2.6.2. Preparation of Ligands from Red Algae

The bioactive compounds of *G.cearensis* such as beta-sitosterol acetate, 4-methyl-3-heptanone, diacetone alcohol, 1-allyl-1,2-cyclohexanediol, allyl butyrate, isoamyl nitrite and standard drugs (Diclofenac sodium salt, Ethyl Isothiourea) were chosen as ligands. The structure date file format of pubchem database was used to generate the three dimentional structures of ligands.

2.6.3. Molecular Docking of Inflammatory Proteins with Bioactive Ligands of *G.cearensis*

The programme Maestro 13.0 module of Schrodinger was performed for predicting interaction between beta-sitosterol acetate, 4-methyl-3-heptanone, diacetone alcohol, 1-allyl-1,2-cyclohexanediol, allyl butyrate, isoamyl nitrite and target proteins such as COX-2, 5-LOX, mPEGS1, iNOS, TNF-α and NF-κB. The grid box of target proteins was created for flexible docking with ligands and also to improve the binding of biologically active ligands in the active pockets of the target proteins by reducing torsion angles.

2.7. Statistical Analysis

The data presented were mean and standard deviation of three independent experiments. One-way analysis of variance (ANOVA) was adopted to determine the significance of the differences between the groups. Duncan's multiple range test was used as a post-hoc test to confirm the statistical significance between the groups.

3. Results and Discussion

3.1. Identification and Extraction of Algal Samples

The brown and red algal samples were botanically identified as *Sargassum vulgare* (ID: 827), *Sargassum longifolium* (ID: 16158), and *Gracilaria cearensis* (ID: 11082) respectively based on the morphological features and species identification from AlgaeBase bibliography database (1934).

The species of *Sargassum* is characterised by a holdfast with one to many major stipes and primary branches with specific morphological characteristics such as vesicles, receptacles, and laterals that resemble blade like leaves [25]. Vesicles have a muticous or coronal blade-like end, ranging from subspherical to somewhat oblong, and the receptacles include spines [26]. Each of these features varies in size, shape, or quantity, forming characteristics that are typically employed to distinguish between species of *Sargassum*. The characteristic sinuous and spirally twisted primary blades, and secondary blades with slightly dentate-serrate margins were found in *Sargassum vulgare* (Figure 1A), while *Sargassum longifolium* has been identified based on elongated smooth blades with central stipe and vesicles (Figure 1B). The morphological features such as strap-shaped flattened blades, vivid colours with a red tint, a delicate and lubricous texture, dichotomous to polychotomous branching, and small proliferations at the thallus's base of 12 mm confirmed the *Gracilaria cearensis* [27] (Figure 1C).

3.2. Phytochemical Profile of Algal Extracts

Among all the samples, the phytochemicals are

abundant in red algae namely *Gracilaria cearensis* when compared with brown algae namely *Sargassum vulgare* and *Sargassum longifolium* (Table 1). The three algal samples were extracted with aqueous, ethanol and ethyl acetate solvents out of which the ethyl acetate extracts showed best results with all the algae samples.

Ethyl acetate extract of *G.cearensis* showed the presence of higher levels of alkaloids, flavonoids, phenols, saponins and tannins along with terpenoids, anthraquinones, cardiac glycosides and our results are intune with earlier reports [28,29].

Aqueous extracts of all the three algal samples showed minor proportions of phytochemical constituents possibly due to the agar formation upon heating while ethanolic extracts showed relative amounts of phytochemicals. The ethyl acetate was found to be ideal solvent for the extraction of secondary metabolites from marine algal samples. Hence, ethyl acetate extract of all the three algal samples was selected for further experimental analysis due to the abundance of secondary metabolites [30].

3.3. Inhibition of Heat Induced Haemolysis by Marine Algal Extracts

Stabilization of HRBC membrane is an important mechanism to investigate the anti-inflammatory property [31]. Stabilising the membranes of neutrophils and other cells at the site of inflammation is crucial to control the inflammatory response and anti-inflammatory drugs stabilise cell membranes and reduce the release of lysosomal contents [32]. Ethyl acetate extract of all the algal extracts showed good anti-inflammatory potential when compared with the standard. The aqueous extracts of all the samples showed very low activity indicating that the secondary metabolites are not well extracted by water while the ethanol extracts showed fairly good haemolysis activity. The anti-inflammatory potential of *S.vulgare, S.longifolium* and *G.cearensis* increased with the increase in the concentration of the samples (50-200µg/ml). Ethyl acetate extract of *G.cearensis* (GCEA) showed the highest inhibition activity of 94% ($p<0.0001$) followed by *S.vulgare* (SVEA) and *S.longifolium* (SLEA) with inhibition of 70% and 69% respectively at 200µg/ml concentration. Diclofenac sodium standard showed an inhibition of 92% at 200µg/ml concentration (Figure 2). GCEA showed higher inhibition activity compared to standard diclofenac which indicates its potential anti-inflammatory activity at all concentrations starting from 50µg/ml. The IC_{50} value of GCEA and Diclofenac was recorded as 33.78 µg/ml and 30.48 µg/ml respectively.

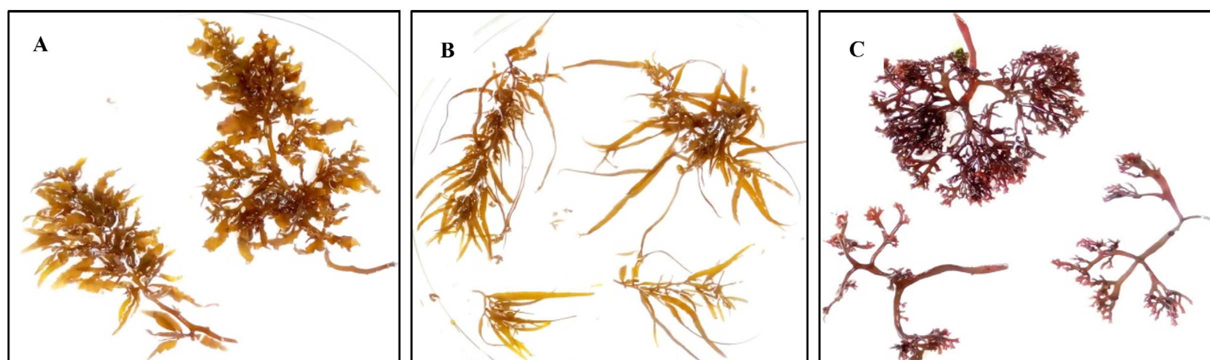


Figure 1. A. Sargassum vulgare, B. Sargassum longifolium, C. *Gracilaria cearensis*

Table 1. Phytochemical profiles of Sargassum vulgare (SV), Sargassum longifolium (SL) and *Gracilaria cearensis* (GC)

S.No	Phytochemical constituents	Aqueous extract			Ethanol extract			Ethyl acetate extract		
		SV	SL	GC	SV	SL	GC	SV	SL	GC
1.	Alkaloids	-	-	-	+	+	+	-	+	++
2.	Flavonoids	-	-	-	+	-	+	+	-	++
3.	Phenols	-	-	-	-	+	+	+	+	++
4.	Saponins	+	+	+	+	+	+	+	+	++
5.	Tannins	-	-	-	+	-	+	+	-	++
6.	Terpenoids	+	+	+	-	+	+	+	+	+
7.	Anthraquinones	-	-	-	+	+	+	+	-	+
8.	Cardiac glycosides	-	-	-	-	+	+	-	+	+

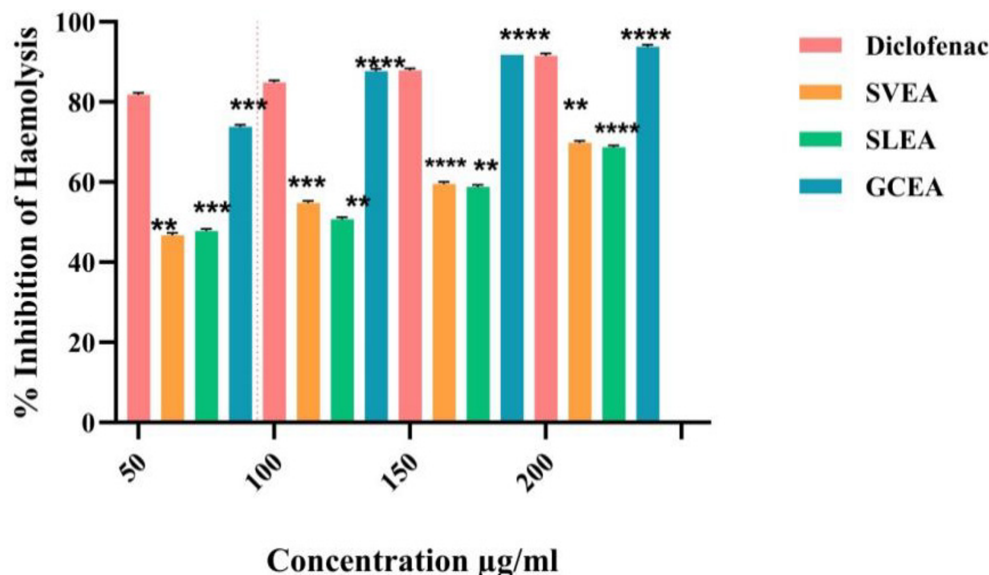


Figure 2. Anti-inflammatory activity of marine brown and red algae. Values are given as mean ± SEM of three independent experiments. The statistical significance ** p<0.01, *** p<0.001, **** p<0.0001

3.4. Regulation of Haemolysis by Algal Extracts

Effect of ethyl acetate extracts of *S.vulgare* (SVEA), *S.longifolium* (SLEA) and *G.cearensis* (GCEA) on hypotonicity induced haemolysis of RBC is represented in Figure 3. The protection of RBC membrane was found to be concentration dependant and the highest percentage of protection was noticed with GCEA (92%) (p<0.0001) and diclofenac (92%) followed by SVEA (69%) and SLEA (68%) at 200µg/ml concentration. According to prior research, flavonoid chemicals found in algae may aid in anti-inflammatory responses [33]. Each bar in the figure represents mean ± SEM of three independent experiments.

3.5. DPPH Radical Scavenging Activity

DPPH free radical scavenging assay is a remarkable model to analyse the antioxidant activity [34]. A comparison has been made between the algal extracts and standard ascorbic acid in scavenging the free radicals. The extracts demonstrated their ability to decrease the stable radical DPPH to diphenyl picrylhydrazine, which has a yellow colour, in the DPPH assay Ethyl acetate extracts of *G.cearensis* (GCEA), *S.vulgare* (SVEA) and *S.longifolium* (SLEA) showed the highest inhibition activity of 88% (p<0.0001), 65% and 63% respectively at 200µg/ml concentration on comparison with ascorbic acid (89%) (Figure 4). Mohammad Ali [35] reported dose-dependent anti-oxidant activity of marine algal samples. The values in the graph are expressed as mean ± SEM of three independent experiments.

3.6. Hydrogen Peroxide Scavenging Capacity

The scavenging potential of *G.cearensis,* GCEA (85%) (p<0.0001) was found to be nearly equal to the standard ascorbic acid (89%) followed by *S.vulgare* (SVEA) (60%) and *S.longifolium* (SLEA) (51%) at 200µg/ml concentration (Figure 5). The phenolic and flavonoid content of algal samples contributes to their antioxidant activity [36]. The hydroxyl group of phenols is essential for their capacity to scavenge free radicals.

Among the three algal extracts, *Gracilaria cearensis* exhibited remarkable anti-inflammatory and anti-oxidant activities compared with ethyl acetate extracts of *Sargassum longifolium* and *Sargassum vulgare*. Thus, GC-MS analysis was adopted to determine the phytochemical picture of *G.cearensis*.

3.7. GC-MS Analysis of *Gracilaria cearensis*

The GC-MS profile of ethyl acetate extract of *G.cearensis* showed six major peaks at 15.0, 16.5, 17.9, 17.9, 19.0, 27.0 (Figure 6). The compounds were identified as 4-methyl-3-heptanone, diacetone alcohol, 1-allyl-1,2-cyclohexanediol, Isoamyl nitrite, allyl butyrate, beta-sitosterol acetate (Table 2) after validation with NIST data library. Among the phytocompounds, beta-sitosterol acetate and Isoamyl nitrite were found to be significantly at higher levels.

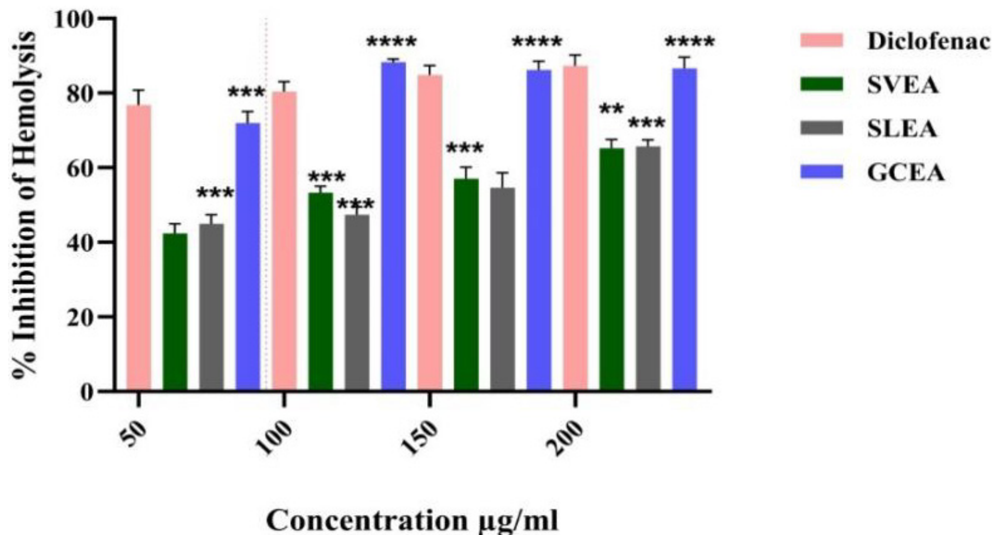


Figure 3. Anti haemolytic activity of brown and red algae. Statistical significance ** p<0.01, *** p<0.001, **** p<0.0001

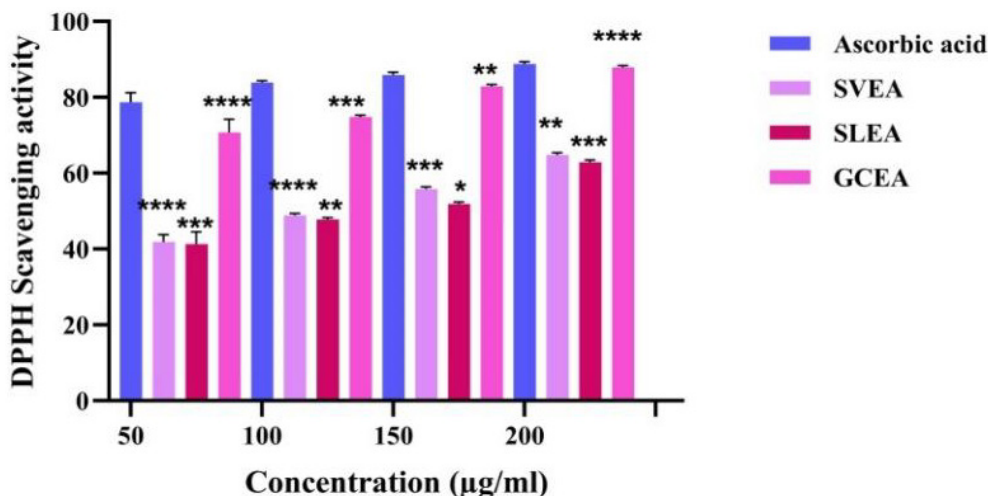


Figure 4. DPPH radical scavenging activity of brown and red algae. The statistical significance * means p<0.3, ** p<0.01, *** p<0.001, **** p<0.0001

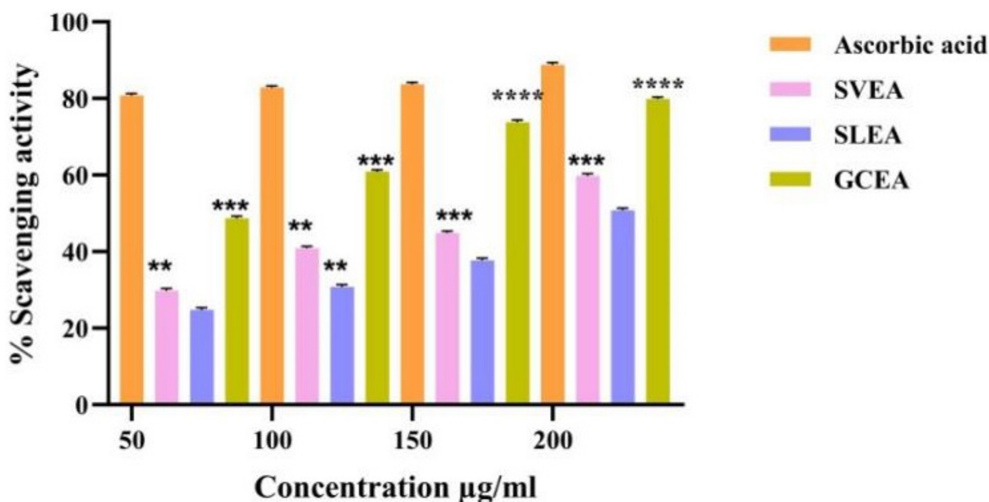


Figure 5. Hydrogen peroxide scavenging capacity of brown and red algae. Values are given as mean ± SEM of three independent experiments. The statistical significance ** p<0.01, *** p<0.001, **** p<0.0001

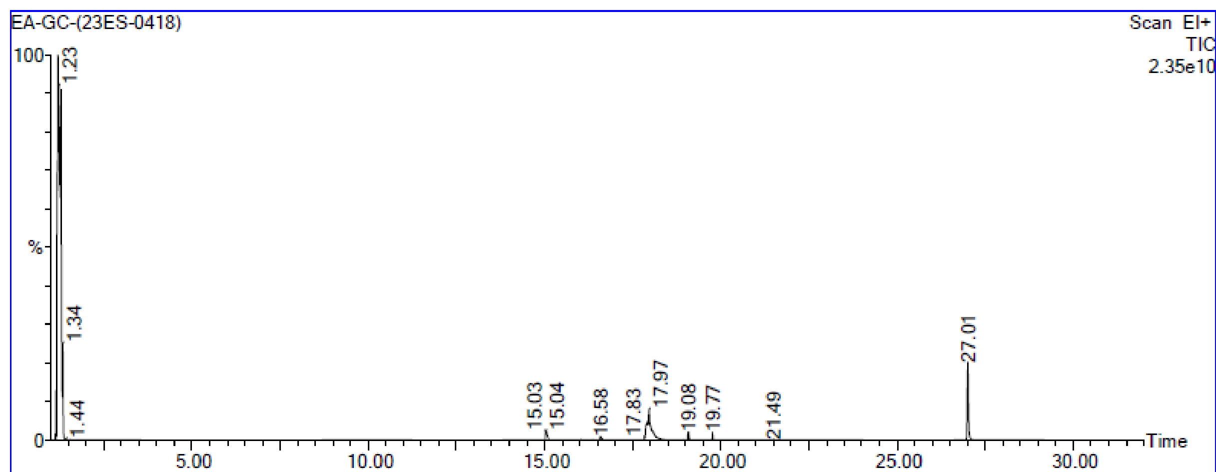


Figure 6. GC-MS spectrum of ethyl acetate extract of *G.cearensis*

Table 2. GC-MS phytochemical profile of ethyl acetate extract of *G.cearensis*

S.no	Retention time	Area %	Compound name	Molecular formula	Molecular weight	Structure
1	15.039	7.358	4-methyl-3-heptanone (dialkyl ketone)	C8H16O	128	
2	16.585	2.204	Diacetone alcohol	C6H12O2	116	
3	17.905	11.973	1-allyl-1,2-cyclohexanediol	C9H16O2	156	
4	17.965	33.464	Isoamyl nitrite	C5H11O2N	117	
5	19.081	2.877	Allyl butyrate	C7H12O2	128	
6	27.009	42.124	Beta-sitosterol acetate	C31H52O2	456	

3.8. Insilico Analysis for the Bioactive Compounds for Anti-Inflammatory Activity

In Mastero 13.O, typical anti-inflammatory drugs such as ethyl isothiourea and diclofenac sodium salt and bioactive compounds of *G.cearensis* such as beta-sitosterol acetate, 4-methyl-3-heptanone, diacetone alcohol and 1-allyl-1,2-cyclohexanediol were chosen as ligands to examine the interaction with inflammatory markers such as COX-2, 5-LOX, mPGES-1, iNOS, TNF-α and NF-κB (Figures 7, 8 and Table 3).

The catalytic amino acid residues such as Ser-530 and Tyr-385 are the key players in the activity of COX-2 [37]. Among the four ligands, diacetone alcohol mimicked the standard diclofenac in hydrogen binding pattern with Ser-530 and Tyr-385 with docking energy -4.241 kcal/mol and -3.597 kcal/mol respectively on the other hand, 4-methyl 3-heptanone found to be potential among all the four ligands, with -6.608 kcal/mol docking energy by hydrogen bonding with Ser-530 (Figure 8a).

Similarly, both diclofenac and diacetone alcohol demonstrated hydrogen interaction with Phe-177 and Ile-406 of 5-LOX. Phe-177, key amino acid dictates the binding of either substrate or inhibitor in the active site of 5-LOX [38]. The other ligands, 4-methyl 3-heptanone and beta-sitosterol acetate exhibited higher docking affinity -4.205 kcal/mol and -3.145 kcal/mol respectively and represented halogen interactions with the receptor. Thus, the anti-inflammatory activity of *G.cearensis* may be attributed to the dual inhibition of COX-2 and 5-LOX by the potential compounds of *G.cearensis*. The COX-2/5-LOX are the primary targeted proteins in the design of anti-inflammatory drugs as they posses a broad spectrum of anti-inflammatory properties.

With its ability to lower PGE2 levels and hence lower inflammation, targeting mPGES-1 may be a viable strategy for the development of anti-inflammatory medications [39]. Using mPGES1, docking was used to further validate the anti-inflammatory properties of the bioactive ligands. Diclofenac revealed hydrogen bonding with Lys-41, Arg-61 while 1-allyl-1,2-cyclohexanediol (Figure 8c), 4-methyl 3-heptanone and diacetone alcohol displayed hydrogen interactions with Arg-381.

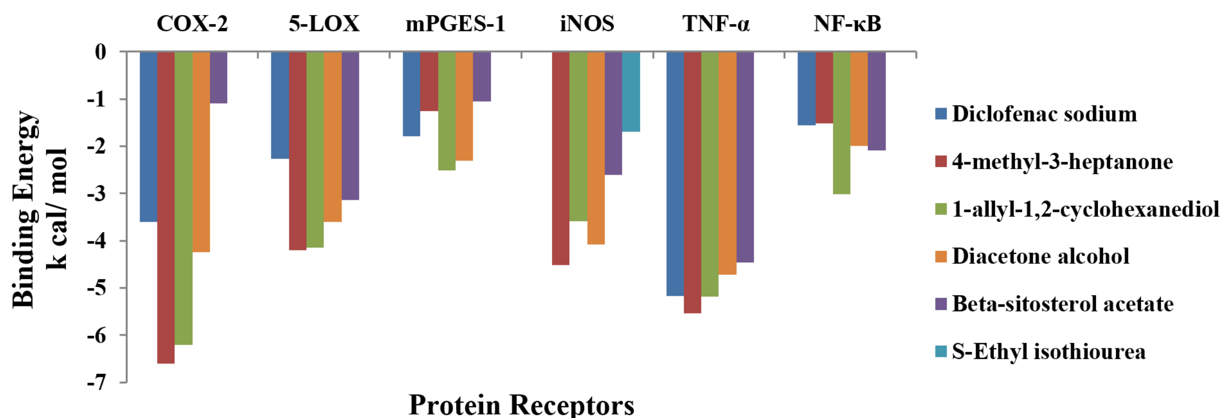
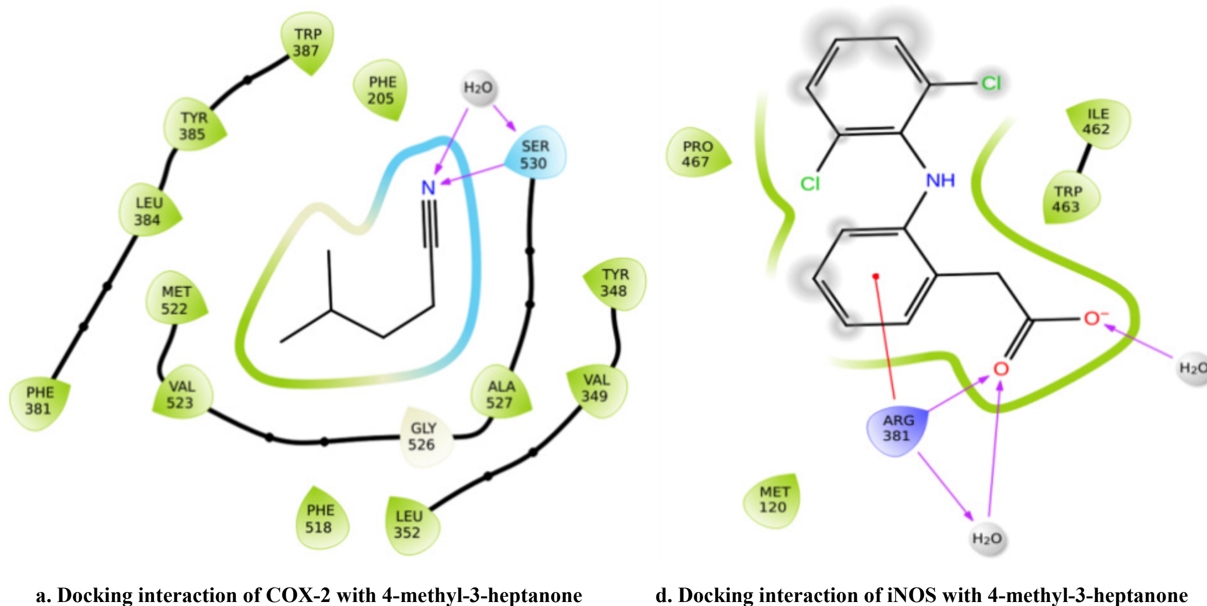
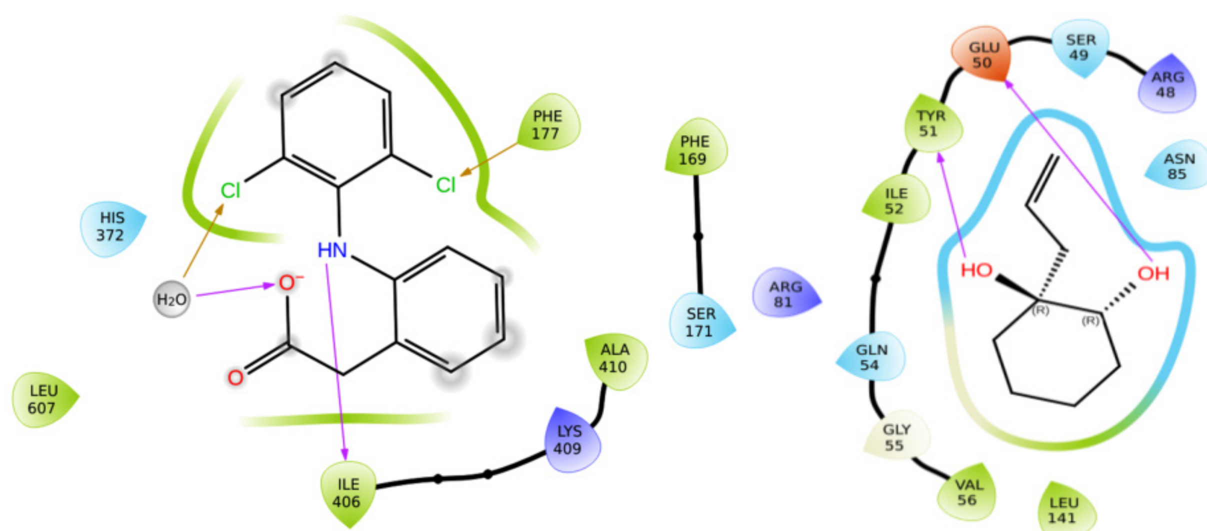


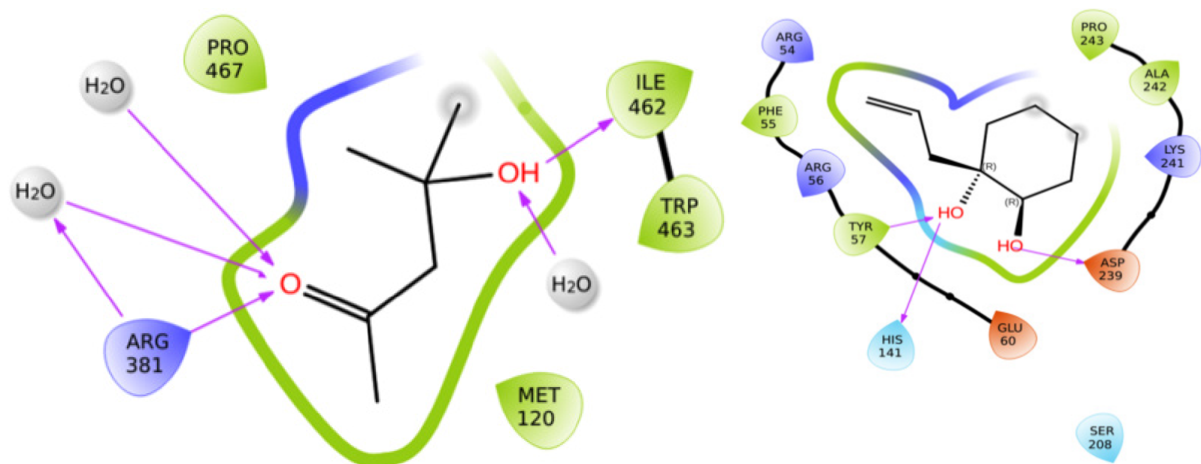
Figure 7. Docking energies of standard drugs and the bioactive compounds from *G.cearensis*



a. Docking interaction of COX-2 with 4-methyl-3-heptanone **d. Docking interaction of iNOS with 4-methyl-3-heptanone**



b. Docking interaction of 5-LOX with 4-methyl-3-heptanone **e. Docking interaction of TNF-α with 4-methyl-3-heptanone**



c. Docking interaction of mPGES-1with 4-1-allyl-1,2-cyclohexanediol **f. Docking interaction of NF-κB with 4-1-allyl-1,2-cyclohexanediol**

Figure 8. Molecular Docking of potential ligands with inflammatory proteins

Table 3. Active site amino acids of potential ligands with inflammatory proteins

Protein receptor	Ligand	Active site amino acids
COX-2	4-methyl-3-heptanone	Ser-530
5-LOX	4-methyl-3-heptanone	Phe-177 Ile-406
mPGES-1	1-allyl-1,2-cyclohexanediol	Arg-381 Ile-462
iNOS	4-methyl-3-heptanone	Arg-381
TNF-α	4-methyl-3-heptanone	Tyr-51 Glu-50
NF-κB	1-allyl-1,2-cyclohexanediol	Tyr-57 His-141 Asp-239

Another important enzyme in inflammation is the iNOS, which generates NO, a signalling molecule that affects immunological response and cellular processes. The results demonstrated that both beta-sitosterol acetate and standard S-ethyl isothiourea interacted strongly with Arg-199, Ile-201, Glu-377 amino acids and were effective against iNOS with docking energy of -2.603 kcal/mol and -1.697 kcal/mol respectively [40]. While 4-methyl-3-heptanone, diacetone alcohol and 1-allyl-1,2-cyclohexanediol showed bonding with Arg-381 with docking scores higher than S-ethyl isothiourea.

One vital cytokine that controls inflammation and immunological responses is TNF-α, which is important for the initiation as well as continuation of inflammatory processes. The diclofenac sodium with TNF-α demonstrated hydrogen binding to Ser-49, Glu-50, Arg-81 with docking energy -5.174 kcal/mol, while 4-methyl-3-heptanone and 1-allyl-1,2-cyclohexanediol exhibited hydrogen binding with Tyr-51, Glu-50 and Ser-49, Glu-50 respectively. They also showed docking energy of -5.541 kcal/mol and-5.181 kcal/mol respectively that are higher than the standard.

The essential transcription factor NF-κB controls immunological responses and inflammation, and it is essential for the body's reaction to inflammatory signals, cytokines, and stress. The 1-allyl-1,2-cyclohexanediol with NF-κB demonstrate mimicking properties similar to standard diclofenac with regard to hydrogen bond formation with two catalytic amino acids, Tyr-57 and His-141 and docking affinity of -3.01 kcal/mol and -1.556 kcal/mol respectively (Figure 8f). Other bioactive compounds displayed halogen bond interactions with the protein receptor and exhibited docking energy higher than the standard diclofenac.

All the four bioactive compounds demonstrated their anti-inflammatory activity actively by interacting with the inflammatory markers. Among all ligands, 4-methyl-3-heptanone exhibited higher docking affinity with COX-2/5-LOX and other receptors than the beta-sitosterol acetate which was revealed to be in higher levels in *G.cearensis*.

4. Conclusions

In the present study, two brown algae of genus *Sargassum* and one red algae of genus *Gracilaria* were screened for dual inhibitors of COX-2 and 5-LOX. Among the three algae, *Gracilaria cearensis* showed higher levels of anti-inflammatory and anti-oxidant activity. The phytochemical profiling of GCEA by GC-MS analysis revealed the abundance of beta-sitosterol acetate, 4-methyl-3-heptanone, diacetone alcohol and 1-allyl-1,2-cyclohexanediol. Insilico analysis for anti-inflammatory activity, demonstrated the inhibition of primary inflammatory markers, COX-2, 5-LOX by 4-methyl-3-heptanone and 1-allyl-1,2-cyclohexanediol of *Gracilaria cearensis*. In addition, 4-methyl-3-heptanone showed tremendous binding energy with iNOS and TNF-α. *Gracilaria cearensis* with comparision to that of standard drugs like diclofenac and S-ethyl isothiourea against inflammatory markers showed potential results. Further, the anti-inflammatory activity of *G.cearensis* was established through the modulation of the key inflammatory transcription factor NF-κB. Thus data confirm that *Gracilaria cearensis* may be exploited for the development of novel anti-inflammatory agents targeted towards dual inhibition of 5-LOX and COX-2.

Acknowledgements

The author U.Tejaswini, is thankful to DST-Inspire fellowship for financial support. The author also extends her sincere thanks to DBT-Builder, Sri Padmavati Mahila Visvavidyalayam for providing laboratory facilities in conducting experimental analysis.

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