SCREENING OF ANTI-INFLAMMATORY AND ANALGESIC ACTIVITIES OF *CAULERPA RACEMOSA* FROM THE BAY OF BENGAL, BANGLADESH

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ABSTRACT

Caulerpa spp; is a remarkably potential seaweed due to its pharmacological importance, high nutritional compositions, biological activities, and human health benefit. It is a species of ulvophyte green algae inhabiting mostly in the intertidal and shallow sub-tidal coastal regions in the Asia-Pacific and commonly consumed in South-East Asia for its high contents of vitamins and minerals. Besides its culinary uses, it is well-reported for its antibacterial, antifungal, diabetes, blood pressure, and lipid-lowering properties. However, to date, no extensive assessment of the diversity, distribution, and biological activity assay of these seaweeds found on the Bangladesh coast has been carried out. This study aimed at anti-inflammatory and analgesic studies of 50% ethanolic extract of *Caulerpa racemosa.* collected from St. Martin's Island, Bangladesh. Consequently, secondary metabolites such as steroids, flavonoids, glycoside, and saponin were also detected in the phytochemical assay. In the present study, 25 & 50mg/kg body weight of ethanolic extract of *C. racemosa* was used in hot plate, acetic acid, formic acid, and carrageenan-induced paw edema in Swiss albino mice showing *C. racemosa* extract has significant analgesic and anti-inflammatory properties.

KEYWORDS: *Caulerpa racemose,* Ethanolic extract, Anti-inflammatory activity, Analgesic activity, HPLC, Phytochemical.

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Introduction

Marine macroalgae are an emerging source of functional foods due to their richness of minerals, essential amino acids, and fiber as well as several bioactive components such as polysaccharides, proteins, and lipids (Nguyen, Ueng and Tsai, 2011). There are over 9200 seaweed species on the earth, but only around 221 are commercially significant (Nguyen, Ueng and Tsai, 2011).

Caulerpa spp are green seaweeds that belong to the phylum Chlorophyta and is a famous edible species that grow all over the world but is particularly widespread in the Indo-Pacific area. It is also known as sea grape or green caviar (Nagappan and Vairappan, 2014; Yap *et al.*, 2019). There are around 75 distinct species of siphonous green macroalgae *Caulerpa spp*. The natural habitat of the sea grape *Caulerpa spp*. is sandy to the muddy lagoon and reef flats of shallow and transparent water with normally quiet currents (Tapotubun *et al.*, 2020).

It is widely distributed in shallow temperate and tropical and subtropical waters worldwide including the eastern Mediterranean Sea, South China Sea, Caribbean Sea, Southeast Asia, Japan, Taiwan, and Oceania (Preez *et al.*, 2020; Zhang, Ma, *et al.*, 2020; Nash *et al.*, 2022). It is consumed fresh as a snack, in salads and sushi, or in salt form and also becoming increasingly popular due to its high concentrations of polysaccharides, dietary fiber, protein, minerals (calcium and magnesium), folic acid, ascorbic acid, and various vital unsaturated fatty acids (Islam Md. *et al.*, 2019; Zhang, Zhao, *et al.*, 2020)

Recent research on the bioactivity of *Caulerpa spp* has shown that it may have anti-diabetic, antihypertensive, antioxidant, antibacterial anticoagulant, and anticancer characteristics (Tapotubun *et al.*, 2020), as well as anti-tumor and therapeutic microbial effects (Ghosh *et al.*, 2004; Zhang, Zhao, *et al.*, 2020). Numerous research has demonstrated that marine algal polysaccharides have the potential for a wide variety of biological actions including antioxidant, anticoagulant, anticancer, antiviral, antifungal, and immunological stimulatory effects (Yap *et al.*, 2019; Chaiklahan *et al.*, 2020; Dissanayake *et al.*, 2022; Pitchai *et al.*, 2022; Sanger *et al.*, 2023).

Natural compounds with anti-inflammatory effects include alkaloids, terpenes, sterols, and glycosides (Buwono, Yenny and Sulastri, 2018). Among all marine bio-products, sulfated

polysaccharides of seaweed wall-matrix have been identified as possible candidates for the treatment of thrombosis, nociception, and inflammation (de Araújo *et al.*, 2016). Sulfated polysaccharides are a class of heterogeneous macromolecules with a wide range of biological activities, including antiviral, immune-modulating, anticancer, anticoagulant, antioxidant, inflammatory, anti-nociceptive, and pro-inflammatory properties (Carneiro *et al.*, 2014).
However, little study has been conducted on the effectiveness of sulfated polysaccharides of green algae *in vivo* as analgesic and anti-inflammatory medicines.

The vast tropical water of the Bay of Bengal holds huge marine living resources consisting of fish, shellfish, marine algae, etc. *C. racemosa* occurs very frequently around 2-15m depth around St. Martin's Island, Bangladesh in the northeastern part of the Bay of Bengal from January to May (Sharif, 2020).

Despite the fact that this seaweed contains unique and potential features, they are relatively underutilized and there is no information on the chemical screening and biological activities of *Caulerpa spp* in Bangladesh (Islam Md. *et al.*, 2019) Understanding the secondary metabolites and qualities of *C. racemosa* will aid in their utilization in the cosmetics and pharmaceuticals sector. As a result, the objective of the research was to investigate the bioactive components of *C. racemosa* extract for its phytochemical characteristics, analgesic and anti-inflammatory activity (de Araújo *et al.*, 2016; Buwono, Yenny and Sulastri, 2018; Chaiklahan *et al.*, 2020)

The nutritional characteristics of *C. racemosa* including lipid and fatty acid profile, protein and amino acids, carbs and fiber, minerals, vitamins, pigments as well as the antioxidant profile of *C. racemosa have* been reported previously (Aroyehun *et al.*, 2020; Ullah *et al.*, 2020; Pangestuti *et al.*, 2021; Palaniyappan *et al.*, 2023). The high nutritional profile of C. *racemosa* has increased its appeal and led to its widespread planting around the world. As it can aid in global food and nutrition security, more studies need to be done in the future on creating efficient feed and farming methods for *C. racemosa*.

Previous research reported the cardioprotective qualities (such as anti-hypertensive and hypolipidemic), antibacterial, anticancer, anti-coagulation, anti-hyperglycemic, anti-diabetic, anti-inflammatory, antioxidative, antipyretic, chelating agent, and immunostimulatory capabilities of *Caulerpa spp* (Vinayak *et al.*, 2014; Ganesan, Tiwari and Rajauria, 2019; Sun *et al.*, 2020; Meinita, Harwanto and Choi, 2022). In-depth investigation of isolates and extracts from *C. racemosa* needs to be done in the future especially for nutraceutical and pharmaceutical purposes emphasizing its commercial feasibility and comprehending its bioactivity and mechanisms of action.

Materials and Methods

Sample collection and processing

Fresh *C. racemosa* was collected in December 2021 at low tide from the St. Martin's Island shore in Bangladesh, which is 13 kilometers away from the mainland. An internet gateway was used to identify the obtained sample (Agardh, 1837; *Algae Base*, 1913; *World Register of Marine Species*, 2020; *Macroalgal Herbarium Portal Homepage*, 2020). After being cleaned with fresh salt water, the sample was meticulously brushed to remove any epiphyte's unwanted impurities, such as adhering sand particles, animal castings, etc. To remove excessive salt rinsed with distilled water and preserved in 50% ethanol in a light protective jar and the sample was dried in a shade drawer (Model: JSON-030S,69, Geomsanggogae-GIL, Gongju-SI, Korea,32598) at 37°C after reaching the lab. Using a mortar pestle, the dry sample was crushed into powder and kept at -20° C until further use (Putri *et al.*, 2019; Alim *et al.*, 2021; Rahman *et al.*, 2021; Alim, Zaman and Hossain, 2023).

Extract preparations

The algal extract was maintained for 7 days at 25°C in a shaking incubator revolving at 150 rpm using 50% ethanol at a concentration of 1gm/10mL (1:10). After maceration, samples were filtered using double-layer filter paper (Whatman® qualitative filter paper, Grade 1 circles, diameter.15 mm) (Alim *et al.*, 2021; Rahman *et al.*, 2021; Akter, Shohag and Hossain, 2023; Alim, Zaman and Hossain, 2023)

Phytochemical Screening

A qualitative phytochemical analysis of phenolic compounds, tannins, alkaloids, steroids, steroidal glycosides, flavonoids, and saponins was carried out using traditional techniques, including the led acetate test, Mayer's test, Salkowski's test, ferric chloride test, alkaline reagent test, and foaming test (Vimalkumar *et al.*, 2014; Daisy A *et al.*, 2016; Ahsan, Islam and Hossain, 2020; Islam *et al.*, 2020; Alim *et al.*, 2021; Rahman *et al.*, 2021; Akter, Shohag and Hossain, 2023; Alim, Zaman and Hossain, 2023).

Experimental Animals

This study employed female Swiss albino mice (age 4-5 weeks, average weight 25- 30 grams) collected from International Center for Diarrheal Disease Research, Bangladesh (ICDDR,B). The animals were acclimated at the marine biotech laboratory at Bangabandhu Sheikh Mujibur Rahman Maritime University, Department of Genetic Engineering and Biotechnology, Mirpur, Dhaka-1216, Bangladesh. They were kept in ordinary polypropylene cages and kept at a constant temperature (25°C, humidity 55-60%) with a 12-hour light-dark cycle. They were fed a regular pellet diet and were given unlimited access to purified water (Bin Emran, 2015; Alemu *et al.*, 2018; Tajrin *et al.*, 2020; Faisal *et al.*, 2022).

Anti-inflammatory Test

The anti-inflammatory test was performed according to a previously described procedure with minor modifications (Emran *et al.*, 2015; Alemu *et al.*, 2018; Yasmeen *et al.*, 2021; Faisal *et al.*, 2022). The mice were divided into five groups of five animals each. Group 1 represents control group without any treatment, group 2 is positive control with the treatment of diclofenac (10mg/kg body weight), group 3 represents test group with treatment with 100mg extract of 50% ethanol/kg body weight, the group 4 represent test group with treatment with 50 mg extract of 50% ethanol /kg body weight and lastly, the group 5 represent negative control group with treatment with only 50% ethanol. Prior to all the treatment, the size of

the mice's left hind paws was measured three times with a plethysmometer in mL. The mice were given a vehicle (10% DMSO), a standard (Diclofenac at 10mg/kg bw), and extracts at 50 and 100mg/kg bw orally. Thirty minutes after the treatment were introduced, 1% carrageenan suspension (0.03 ml per mouse was injected into left hind paw of each mouse of all groups. For measuring the reduction of paw swelling/edema, the volume displacement of left hind paw was measured for each mouse again with the plethysmometer after ½ hour, 1 hour, 2 hours, 3 hours, 4 hours, and 5 hours, 6 hours, and 8 hours after carrageenan induction.

The reduction of swelling data of group 5 using only 50% ethanol was subtracted from the data of group 3 and 4 to measure the accurate anti- inflammatory effect of the *C. racemose* extract.

In contrast, the right hind paw functioned as a non-inflamed reference paw. The average % increase in paw volume was determined and compared to the control group. The following formula was used to compute the percentage of inhibition:

% inhibition of paw edema= [(Vc– Vt)/ Vc] x 100

Where, Vc and Vt represent the average paw volume of the control and treated animal respectively.

Hot plate test in mice

To conduct the hot plate analgesic test, a slightly modified approach was used as described by (Emran *et al.*, 2015; Karimzadeh *et al.*, 2020; Akter, Shohag and Hossain, 2023). There were five groups of five mice in each group as described before. To monitor the mice's reaction to electrical heat-induced pain stimuli, a beaker containing the mice was put on a hot plate. Jumping out of the beaker or licking its paws was recorded as an indication of the animal's reaction to the heat-induced pain stimuli. The time of licking paws or leaping out of the beaker was calculated to assess the nociceptic response time. Prior to treatment, the reaction time was assessed once and the mean of response time represented the initial reaction time before treatment. Following that, the test mice were given control and negative control of only water and 50% ethanol respectively, positive control diclofenac sodium (10mg/kg body weight), and 50% ethanol extract of *C. racemosa* at dosages of 50mg/kg and 25mg/kg body weight orally through syringe. Thirty minutes after treatment, the response time of each group was measured five times in one-hour intervals. Negative control was subtracted from extract groups to measure the accurate analgesic effect of the extract.

The analgesic score was computed as a percentage (%) as follows:

Percentage (%) of analgesic effect = (Tb-Ta/Tb) × 100

Where, Tb= Reaction time (in second) before drug administration. Ta = Reaction time (in seconds) after drug administration.

Acetic acid induced writhing test in mice

To induce pain in the experimental animals, acetic acid was injected intraperitoneally. To test the analgesic properties of *C. racemosa* extract, acetic acid-induced writhing in mice was used as described by (Akter, Shohag and Hossain, 2023) and (Bin Emran, 2015; Karimzadeh *et al.*, 2020) with some modifications. After an overnight fast, the two test group of Swiss albino mice was given the 50% ethanolic algal extract orally in two dosages (50 and 25mg/kg body weight). Positive control group was given diclofenac sodium (10mg/kg body weight). After 30 minutes, all of the animals in the various groups received intraperitoneal injections of 0.7% acetic acid (10ml/kg bw) solution. The writhes were recorded for 20 minutes starting five minutes after the acetic acid was administered. Animals in the treatment groups were given algal extract 15 minutes before being injected with acetic acid. All trials were carried out between 08:00 and 16:00h in the laboratory at 25° C as described by (Gupta *et al.*, 2015).

Negative control was subtracted from extract groups.

The following formula was used to calculate the percentage of pain inhibition:

% inhibition = (1-VT/VC) ×100

Where VT means the number of writhing motions in drug-treated mice, VC =number of writhing motions in the control group of mice. The Animal Ethical Committee approves animal experimentation.

Formic acid induced analgesic test in mice

For evaluating the analgesic effects of the extract, another method using formic acid induced pain was used with minor modifications reported by (Akter, Shohag and Hossain, 2023). After 30 minutes of 50% ethanolic algal extract in two dosages (50 and 25mg/kg body weight) of two test group, mice in all groups received 0.5% v/v formic acid injection into the subplantar region of their right hind paw. Diclofenac (10mg/kg body weight) was used as a positive control. Water was used as a control and same volume of 50% ethanol as test group was used as a negative control. Negative control was subtracted from extract groups to measure the accurate analgesic effect of the extract.

The biting and licking of the injected paw were interpreted as a pain reaction. The response was measured from 0 to 5 minutes in the early phase and from 15 to 30 minutes in late phase.

Percent inhibition was calculated as:

%inhibition = [(Nb– Na) ÷ Nb] ×100

Here, Nb is the number of responses before the drug administration, Na is the number of responses after drug administration.

Statistical analysis

The data obtained from the anti-inflammatory test and analgesic tests were statistically analyzed. For triplicate data, the mean ±SD was utilized, and p-values of <0.05 were used to determine significance. The data were analyzed using one-way analysis of variance (ANOVA) and then Dunnett's *t*-test as the significance test.

UV-Visible spectroscopy analysis

At room temperature, a UV-Visible spectrophotometer (Shimadzu UV 1900) with a slit width of 1.0 nm was used to perform spectrophotometric analysis on the *C. racemosa* extract. For proximate analysis the extract was studied under visible and UV light at wavelengths ranging from 190 to 1100nm. The extract was centrifuged at 3000 rpm for 10 minutes after being filtered through Whatman No. 1 filter paper for UV-VIS spectrophotometer analysis. The sample was diluted to 1:10 with 50% ethanol as the sample solvent. The base line was corrected using the same solvents. The UV-

VIS machine was left on for warmup for 30 minutes prior to this study (Jain *et al.*, 2016).

Results

Phytochemical Screening

The phytochemical screening is the initial step in determining seaweed bioactivity since components differ qualitatively not only across species but also between samples of the same species depending on solvents, factors, and storage conditions. The existence of several phytochemicals was investigated in a 50% ethanol extract of *C. racemosa*. Steroids, Glycoside, Flavonoids, and Saponin were found to be present in significant amounts (Table-1).

Table 1. 50% ethanolic extract of *C. racemose was* qualitatively tested for presence of phytochemicals. The table indicates (-): not detectable, (+): low quantities, (++): moderate quantities, (+++): high quantities.

50% ethanolic sample extract	Phenol	Flavonoid	Saponin	Glycoside	Steroids	Tannins	Alkaloid
	-	+	+++	++	++	-	-

In-vivo Experiments

Screening for anti-inflammatory activity using carrageenan-induced paw edema

Tables 2 and 3 show the determined effective values for each mice group. The sub-plantar injection of carrageenan-induced local edema in control animals grew progressively to a maximum severity of 5 hours after the administration of phlogistic agent. *C. racemosa* 50% ethanolic extract of 50mg/kg body weight exhibited a better anti- inflammatory activity than 100mg 50% ethanolic extract/kg body weight. At six hours of investigation, the highest inhibition of edema was determined to be 63.1% and 155.60% for 50% ethanolic extract of 100 and 50mg/kg body weight doses, respectively (Tables 3).

Table 2. The ethanolic extract of *C. racemosa* showed anti-inflammatory activity on carrageenan-induced mice paw edema

Anti-inflammatory effect of 50% ethanolic extract of *C. racemosa*									
Time =	**0 Hr**	**1/2 Hr**	**1 Hr**	**2 Hrs**	**3 Hrs**	**4 Hrs**	**5 Hrs**	**6 Hrs**	**8 Hrs**
Group1: Control	0.27±0.01	0.38±0.01	0.37±0.008	0.36±0.01	0.33±0.01	0.35±0.005	0.26±0.002	0.48±0.03	0.4±0.01
Group2: Diclofenac	0.28±0.05	0.36±0.07	0.32±0.05	0.30±0.04	0.27±0.03	0.30±0.03	0.25±0.03	0.25±0.07	0.21±0.06
Group3: Extract 100mg/kg bw	0.27±0.038	0.42±0.024	0.37±0.02	0.31±0.037**	0.26±0.031**	0.26±0.036***	9.33±11.44*	0.18±0.041***	0.16±0.035***
Group4: Extract 50mg/kg bw	5.56±0.055	-0.019±0.054	-0.02±0.027	-0.06±0.022	-0.07±0.048	-0.11±0.027	-0.03±0.048	-0.27±0.010	-0.21±0.067

The unit of mice paw edema reduction is measured in mL by digital plethysmometer. (n=5 animals per group) Values were given as mean ±SD. p value <0.05, **p value <0.01, ***p value <0.001. *t* –test was used to compare the mean of extract with the control group.

Table 3. Percent inhibition of mice paw edema by diclofenac and algal extract concentrations

% Inhibition									
Time	**0Hrs**	**1/2Hr**	**1 Hrs**	**2 Hrs**	**3 Hrs**	**4 Hrs**	**5Hrs**	**6 Hrs**	**8 Hrs**
Diclofenac	0±0.05	6.25±0.07	14.37±0.05	20.80±0.04	19.80±0.03	14.20±0.03	7.52±0.03	48.13±0.07	47.5±0.06
Extract (100mg/kg bw)	0±0.03	-9.90±0.02	0±0.02	14.21±0.03	20.96±0.03	23.87±0.036	0±11.43	63.10±0.04	58.5±0.03
Extract (50mg/kg bw)	0±0.03	104.67±0.02	107.44±0.02	116.40±0.03	122.75±0.03	131.25±0.036	113.53±11.43	155.60±0.04	152.5±0.03

(n=5 animals per group) Values were given as mean ±SD.* p value <0.05, **p value <0.01, ***p value <0.001. *t* -test comparing the extract with the control group.

Analgesic activity

Effect of C. racemosa extract on the hot plate test

The 50% ethanolic extract of *C. racemosa* revealed a statistically significant (p<0.05) analgesic effect on the same group of mice in the hot plate analgesic assay. Based on these findings, we can conclude that the greatly enhanced the reaction time of mice in a dose-dependent way. At 50 and 25 mg/kg body weight, the dose-dependent impact was 58.8% and 97.64% and respectively (Table 04 and 05).

Table 4. The analgesic impact of the 50% ethanolic extract of *C. racemosa* in hot plate assay

The average response of Hot plate						
Time	0 hour	½ Hour	1 Hour	2 Hours	4 Hours	6 Hours
Control	21.5±1.6**	21.75±1.2	29.5±1.2*	23.75±1.1	21.25±1.1	24±1.0
Diclofenac	36.6±1.3	25.8±2.06	23±1.2	23.8±1.4	21.4±1.4	24.4±0.6
C.racemose extract 50mg/kg bw	32.2±0.9*	32±4.9	36±4.1*	30.8±4.3	20.8±1.2	26.8±1.7
C.racemosa extract 25mg/kg bw	40.25±3.0	29.75±3.9	44.5±7.9	30±4.0	36±2.4**	9.75±6.2

Table 5. At 0 hr, the percentage inhibition of the standard (diclofenac) and two different concentrations of the extract (50 and 25 mg/kg of *C. racemosa*) compared to their respective means (analgesic activity)

%Inhibition of hot plate method				
Time	Control	Diclofenac	*C. racemosa* extract (50mg/kgbw)	*C. racemosa* extract (25mg/kgbw)
0 Hr	-39.2%	-2.92%	-10%*a	14.1%*a
1/2Hr	-1.8%	6.25%	28.9%	33.25%
1Hr	30.6%	14.36%	58.8%*a, **b	97.64%*b
2Hrs	4%	20.76%	35%	32.6%
4Hrs	0%	14.20%	0%	0%
6Hrs	0.22%	48.13%	17.13%	-57.95%**a,b

(n=5 animals per group) Values were given as mean ±SD. *p value <0.05, **p value <0.01, ***p value <0.001. "a"=*t*-test was used to compare the extract with the control, and "b"= extract with the diclofenac.

Acetic acid induced writhing test in mice

The results of acetic acid-induced writhing responses in mice show that the ethanol extract of *C. racemosa* is more effective at numbing pain than the control. The extract and diclofenac considerably ($p<0.05$) suppressed the writhing responses caused by acetic acid at the tested levels. When compared to the control, oral administration of the extract at dosages of 50 and 25 mg/kg body weight revealed a dose-dependent and substantial reduction in the number of abdominal constrictions generated by intraperitoneal injection of 0.7% acetic acid (Table 06).

Table 6. The analgesic activity of an ethanolic extract of *C. racemosa* was tested using acetic acid-induced writhing test method in mice

Treatment	Mean±SD	% Inhibition
Control	247.75±48.42	-
Diclofenac	133.75±15.40	43.14±15.76
Extract 50mg/kg	-28.75±130.20	118.72±52.91*
Extract 25mg/kg	-150.75±165.82	174.28±72.03**

(n=5 animals per group) Values were given as mean ±SD. *p value <0.05, **p value <0.01, ***p value <0.001. *t* –test was used to compare the mean of extract with diclofenac.

Formic acid method

Formic acid generates pain through modulating nociceptors in the early phase and inflammatory responses in the late phase (Akter, Shohag and Hossain, 2023). The treatment with the algal extract was successful in both periods. It greatly reduced the quantity of formic acid-induced pain when compared to the control and diclofenac group (licking and biting in the injected paw). The control group's results were used to compute the percent (%) inhibition. *T*-test revealed that the data collected using this strategy was statistically significant (Table 07 and 08).

Table 7. Effects of *C. racemose* extract on the formic acid-induced pain method

Effects of *C. racemose* extract on the formic acid-induced pain.		
	Early phase (0 -5min)	Late Phase (15-30 min)
Control	21.8±13.4	77.8±87.5
Diclofenac	52±17.3	171.8±83.0
Extract(50mg/kg)	-38.2±37.1	-42.8±64.7
Extract(25mg/kg)	-8.6±23.4	-48.6±74.4

Table 8. *% Inhibition of C. racemosa on formic acid test.* (n=5 animals per group). Values were given as mean ±SD. *p value<0.05, **p value <0.01, ***p value <0.001. *t*-test was used to compare the mean of extract with diclofenac

% Inhibition of *C. racemosa* on formic acid test		
	Early phase (0 -5min)	Late Phase (15-30 min)
Control	-81.4±83.5	-786±1050.4
Diclofenac	187.56±78.5	139.7±76.19
Extract(50mg/kg)	-34.14±147.0	65.79±76.19
Extract(25mg/kg)	100±0	100±0

UV-Visible spectroscopy analysis

The 50% ethanolic extract of *C.racemosa* underwent UV-VIS analysis to identify the phytoconstituents. The chromophores, aromatic rings, and chemical compounds with σ-bonds, π bonds and lone pair electrons and many more were all identified using the UV-Visible spectroscopy. Due to the clarity of the peak and adequate baseline, the qualitative UV-VIS profile of 50% ethanolic extract of *C.racemosa* was collected at wavelength ranging from 190 to 1100 nm. Peaks at 271, 197.50 and 254.50 nm with respective absorption values of 0.358, 2.933 and 0.252 were visible in profile. The absorption spectrum of *C.racemosa* extract is shown in table-09, and it is particularly transparent in the wavelength range of 190 to 1100 nm can be seen in the UV-VIS spectrum.

However, the inherent challenges of attributing the absorption peaks to any particular system elements limit the use of UV-VIS spectrophotometry in the research of complex media. To enable extract characterization and ingradient identification, UV-VIS findings must be supported with another analytical technique, such as GC/MS. The result of the UV-VIS spectroscopic study in the 50% ethanolic extract of *C.racemosa* was shown in table-09.

Table 9. Compound detection of *C. racemosa* extract

Compound detection of *C. racemosa* extract on UV-Vis Spectrophotometer				
Wavelength range	Scanning Speed	Sample Interval	Detection	Absorption
190-1100nm	Slow	0.5	271.00	0.358
			197.50	2.933
			254.50	0.252

Discussion

C. racemosa has analgesic and anti-inflammatory activities in both *in vitro* and *in vivo* models. They also contain a considerable quantity of phytochemicals. Individual algal sources' pharmacological effects are heavily influenced by phytochemical components. Thus, chemical screening is carried out to enable the localization and targeted isolation of novel or valuable phytochemical components with potential activity. This approach detects the presence of recognized phytochemical metabolites in extracts at the early phases of separation, which is critical in an economic environment.

The type of solvent used in the extraction technique has a substantial impact on the successful prediction of chemical constituents from algal material. In our study, *C. racemosa* employed largely 50% ethanol as the solvent, which offered more consistent analgesic and anti-inflammatory efficacy. As a result, we decided to continue our studies using ethanol extract. These findings can be explained by the polarity of the molecules extracted by each solvent, as well as their inherent bioactivity (Kumar Dey *et al.*, 2010).

The presence of phytochemicals like flavonoids, steroids, saponins, and glycoside in *Caulerpa spp* indicates very prospective features of the seaweed of Bangladesh and the seaweeds from this region could be potentially rich sources of antioxidants for food and pharma.

Flavonoids often present in ethanol extracts of *C. racemosa*, suggests that they may include potential antibacterial substances (Dwivedi, 2007; Xie *et al.*, 2014). It has a variety of biological effects, including anti-arrhythmic, anti-cholinergic, anti-tumor, vasodilating, anti-hypertensive, cough expectorant, anesthetic, analgesic, muscle relaxant, anti-pyretic, anti-malarial, and anti-protozoal activities (Abalaka M, 2012; Asif, 2013; Jafari *et al.*, 2013; Emran *et al.*, 2015).
The ability of flavonoids to alter viruses, carcinogens, and allergens suggests the possibility that they may operate as biological "response modifiers." It can also be utilized as an anti-allergy, anti-inflammatory, anti-microbial, and anti-cancer treatment (Wu *et al.*, 2008). Additionally, it also has neuroprotective and cardio-protective effects (Ullah *et al.*, 2020) and several well-known applications in food (Ruiz-Cruz *et al.*, 2017).

Seaweeds with various phytochemicals have long been used to benefit people all over the world. The secondary metabolite saponin has received considerable attention because of its various biological activities including hepatoprotective, anti-ulcer, anti-tumor, antimicrobial, adjuvant, and anti-inflammatory activities (Moghimipour and Handali, 2015). Commonly reported benefits of other phytochemical glycosides are anti-oxidants and anti-inflammatory which have possible applications in disease management and prevention (Kytidou *et al.*, 2020). Glycoside is also abundant in our sample *C. racemose.*
Tannins have anticancer activity and can be used in cancer prevention (Ekundayo, Adeboye and Ekundayo, 2011). The presence of tannins in *C. racemosa* extract has been previously published (Singh, Maurya and Singh, 2005) though we did not find tannins in our sample.
All these observations therefore support the use of *C. racemosa* in herbal remedies and as a dietary supplement. For further study, all phytoconstituents need to be detected using advanced techniques.

The analgesic impact of an ethanol extract of *C. racemosa* was assessed using the hot plate method, which is one of the most commonly used procedures for determining drug or compound analgesic effectiveness. Mice's paws are extremely sensitive to heat at temperatures that are not harmful to the skin. Shaking, leaping, paw withdrawal, and paw licking are all reactions. Following the injection of centrally active analgesics, the period before this reaction is extended (Bin Emran, 2015). The extract of *C. racemosa* at 50 and 25 mg/kg bw doses increased delay time significantly ($p<0.05$) compared to the control. At a dosage of 10 mg/kg bw, the positive control diclofenac Na demonstrated considerable ($p<0.05$) analgesic efficacy.

According to (Akter, Shohag and Hossain, 2023) Formic acid-induced paw pain is a well-established in-vivo pain model that may be used to study analgesics. Distinct analgesics may have different effects in the early and late stages of the formic acid test; therefore, both phases must be performed. As a consequence, the test may be used to understand how a possible analgesic might operate to relieve pain. The anti-nociceptive effect of *C. racemosa* extract may be due to its peripheral action, and it performs better than the reference drug, as demonstrated by its inhibitory impact on the nociceptive response in the early and late phases of the formic acid test.
These findings suggest that an ethanolic extract of *C. racemosa* has considerable analgesic properties.

Acetic acid induced writhing is a sensitive approach for evaluating analgesics with peripheral action. In the acetic acid-induced writhing test animal, an ethanol extract of *C. racemosa* has analgesic properties. Acetic acid-induced

writhing in mice has received a lot of interest in the search for analgesic medicines. Acetic acid induced abdominal writhing and the visceral pain model both produce prostaglandin via the cyclooxygenase pathway, which plays a role in the nociceptive process. The prostaglandin pathway and the acid-sensing ion channels of peritoneal mast cells are hypothesized to mediate this form of response. In other words, acetic acid-induced writhing has been linked to higher levels of PGE2 and PGF2 in peritoneal fluids as well as lipoxygenase products (Emran *et al.*, 2015; Akter, Shohag and Hossain, 2023). Prostaglandin levels rise in the peritoneal cavity and then increase capillary permeability, which intensifies inflammatory pain. According to the findings of the current studies, the significant analgesic effect of the ethanol extract of *C. racemosa* may have resulted from the inhibition of the synthesis of the arachidonic acid metabolite.

The production or release of mediators at the damaged site contributes to carrageenan-induced edema. Serotonin, histamine, bradykinins, leukotriene's, prostaglandins—particularly those from the E series—and prostaglandins all contribute to pain, and fever (Erlund, 2004; Emran *et al.*, 2015; Akter, Shohag and Hossain, 2023). Inflammation and associated symptoms are often reduced when these mediators are prevented from entering the wounded site or from exerting their pharmacological effects (Emran *et al.*, 2015). In the current investigation, it was observed that the carrageenan-induced paw edema could be significantly reduced by the ethanol extract of *C. racemose.*

Conclusion

The results of the current study suggested that the 50% ethanol extract of *C. racemosa* has the potential to be used in pharmacological purposes for its significant anti-inflammatory and analgesic properties. However, further studies are needed to isolate the effective bioactive compounds, determine their chemical structure, and optimize the formulation and application methods.

Authors Contribution

MNH made the hypothesis, designed the experiments, supervised the work, analyzed the data, and wrote and revised the final version of the manuscript; KRC conducted the experiments, and analyzed the data; MAA wrote the initial draft of manuscript and analyzed the data. NRZ did manuscript editing. AN, EMA, and PM helped in experiments and data collection.

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