

Original Article

ANTI-INFLAMMATORY ACTIVITY OF AQUEOUS EXTRACTS OF *GRACILARIA*

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ABSTRACT

**Objective:** In the present investigation an attempt was made to examine the anti-inflammatory potential of aqueous extracts of *Gracilaria salicornia* C. Ag., *Gracilaria edulis* (Gmelin) Silva, *Gracilaria corticata* J. Ag., *Gracilaria fergusonii* J. Ag., and *Gracilaria verrucosa* (Hudson) Papenfus from Mandapam, *Gracilaria edulis* (Gmelin) Silva., *Gracilaria verrucosa* (Hudson) Papenfus from Pulicate Lake, *Gracilaria fergusonii* J. Ag., *Gracilaria corticata* J. Ag. and *Gracilaria corticata* J. Ag. var. *cylindrica* from Tuticorin using heat induced haemolysis of RBC. In addition, the present study is focused to reveal the locality specific anti-inflammatory activities of selected *Gracillaria* species.

**Methods:** The fresh seaweeds were boiled with distilled water (1:20 W/V) for 2 h. The slurry was filtered through Whatman No. 41 filter paper and condensed. The semi-solid crude extracts were used for anti-inflammatory analysis.

**Results:** An aqueous extracts of studied *Gracilaria* species were effective in inhibiting the heat induced haemolysis at different concentrations. The results showed the dose dependent protection. The percentage of anti-inflammatory activity of studied *Gracillaria* was varied from 43.81 to 95.55. The highest percentage (95.55%) of activity was observed in 250 µg/ml of *G. edulis* aqueous extracts. The anti-inflammatory activity of studied *Gracillaria* species at 250 µg/ml were as follows *G. edulis* (Mandabam) > *G. corticata* (Mandabam) > *G. verucosa* (Mandabam) > *G. salicornia* (Mandabam) > *G. ferugosonii* (Tuticorin) > *G. ferugosonii* (Mandabam) > *G. edulis* (Pulicate) > *G. corticata* (Tuticorin) > *G. verucosa* (Pulicate) > *G. corticata* var. *cylindrica* (Tuticorin).

**Conclusion:** The results of the present study clearly explained the anti-inflammatory potential of the studied *Gracillaria* species. The aqueous Oextracts of *Gracillaria* collected from Mandapam showed the highest percentage of protection activity. This study results confirm the existence of active principle responsible for the anti-inflammatory activity. Further studies are required to isolate the active principles without any side effects.

**Keywords:** *Gracillaria*, Seaweeds, Anti-inflammatory

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INTRODUCTION

Due to the presence of a rich source of biologically active metabolites the seaweeds are a valuable pharmaceutical potential candidate for the drug development. The seaweed derived metabolites possess various biological activities especially with anti-inflammatory activities [1, 2]. Previous research on seaweeds polysaccharides revealed the anti-inflammatory properties and employed as an alternate candidate with anti-inflammatory [3, 4]. Several species of marine seaweeds are reported with anti-inflammatory activities viz., *Gracilaria textorii* and *Gracilaria verrucosa* [5, 6], *Caulerpa* [7], *Caulerpa racemosa*, *Cystoseira crinita* [8], *Sargassum swartzii* and *Ulva reticulata* [9], *Dichotomaria obtusata* [10], *Gracilaria cornea* [11], *P. tetrastomatica* and *P. gymnospora* [12], *Dictyota menstrualis* [13], *Padina tertastomatica*, *Sargassum wightii*, *Gracilaria edulis* and *Caulerpa racemosa* [14], *Spatoglossum schroederi* [15], *Cladophora indica* [16], *Gracillaria edulis* [17], *Undaria Pinnatifida*, *Laminaria Japonica*, *Sargassum fulvellum* and *Hizikia fusiforme* [18] and *Lobophora variegata* [19]. Only very few studies were reported from the coast of Tamil nadu [14, 17, 19, 20]. But there is no report on the anti-inflammatory activities of fresh aqueous extracts of *Gracillaria* species from Tamil Nadu. In the present investigation an attempt was made to examine the anti-inflammatory potential of aqueous extracts of *Gracilaria salicornia* C. Ag., *Gracilaria edulis* (Gmelin) Silva, *Gracilaria corticata* J. Ag., *Gracilaria fergusonii* J. Ag., and *Gracilaria verrucosa* (Hudson) Papenfus from Mandapam, *Gracilaria edulis* (Gmelin) Silva., *Gracilaria verrucosa* (Hudson) Papenfus from Pulicate Lake, *Gracilaria fergusonii* J. Ag., *Gracilaria corticata* J. Ag. and *Gracilaria corticata* J. Ag. var. *cylindrica* from Tuticorin using heat induced haemolysis of RBC. In addition, the present study is focused to reveal the locality specific anti-inflammatory activities of selected *Gracillaria* species.

MATERIALS AND METHODS

Plant material

The *Gracilaria salicornia* C. Ag., *Gracilaria edulis* (Gmelin) Silva, *Gracilaria corticata* J. Ag., *Gracilaria fergusonii* J. Ag., and *Gracilaria verrucosa* (Hudson) Papenfus were collected from Mandapam coast, *Gracilaria edulis* (Gmelin) Silva., *Gracilaria verrucosa* (Hudson) Papenfus from Pulicate Lake, *Gracilaria fergusonii* J. Ag., *Gracilaria corticata* J. Ag. and *Gracilaria corticata* J. Ag. var. *cylindrica* from Tuticorin, India. Further the species are identified using *Phycologia Indica* by Srinivasan [21]. The fresh seaweeds were used for extraction. The fresh seaweeds were boiled with distilled water (1:20 W/V) for 2 h. The slurry was filtered through Whatman No. 41 filter paper and condensed. The semi-solid crude extracts were used for anti-inflammatory analysis. For anti-inflammatory analysis, 10 mg crude extracts were dissolved in 10 ml of distilled water (W/V). From this, the aliquots 50, 100, 200 and 250 µg/ml were prepared.

Membrane stabilization

Preparation of red blood cells (RBCs) suspension

The Blood was collected from a healthy human volunteer who has not taken any NSAIDs (Non-Steroidal Anti-Inflammatory Drugs) for 2 w prior to the experiment and transferred to the centrifuge tubes. The tubes were centrifuged at 3000 rpm for 10 min and were washed three times with equal volume of normal saline. The volume of blood was measured and re constituted as 10% v/v suspension with normal saline [22, 23].

Heat induced haemolysis [22, 24]

The reaction mixture (2 ml) includes 1 ml of different concentrations of selected *Gracilaria* species aqueous extracts (50, 100, 200 and 250

µg/ml) and 1 ml of 10% RBCs suspension, instead of test sample the only saline was added to the control test tube. Aspirin was employed as a positive control. The reaction mixtures were incubated in water bath at 56 °C for 30 min. At the end of the incubation, the tubes were cooled under running tap water. The reaction mixtures were centrifuged at 3000 rpm for 5 min and the absorbance of the supernatants was measured at 560 nm. The experiment was performed in triplicates for all the test samples.

The Percentage inhibition of Haemolysis was calculated as follows:

Percentage inhibition = (Abs control – Abs sample) / Abs control X 100

Statistical analysis was performed using SPSS 21 software. Analysis of variance and pair wise Pearson correlation test was performed. The  $p < 0.05$  was considered significant.

## RESULTS

Aqueous extracts of studied *Gracillaria* species were effective in inhibiting the heat induced haemolysis at different concentrations. The results showed the dose dependent protection (table 1). The

percentage of anti-inflammatory activity of studied *Gracillaria* was varied from 43.81 to 95.55 (table 1). The highest percentage (95.55%) of activity was observed in 250 µg/ml of *G. edulis* aqueous extracts. The anti-inflammatory activity of studied *Gracillaria* species at 250 µg/ml were as follows (table 1) *G. edulis* (Mandabam) > *G. corticata* (Mandabam) > *G. verrucosa* (Mandabam) > *G. salicornia* (Mandabam) > *G. fergusonii* (Tuticorin) > *G. fergusonii* (Mandabam) > *G. edulis* (Pulicate) > *G. corticata* (Tuticorin) > *G. verrucosa* (Pulicate) > *G. corticata* var. *cylindrica* (Tuticorin) (table 1). The anti-inflammatory/protection activity of the aqueous extracts of studied *Gracillaria* species was significant at  $p < 0.005$ . Among the three localities studied, the *Gracillaria* species collected from Mandabam showed more activity than other two studied localities. This may be due to soil nutritional composition and ecological conditions of the coast. A Pearson moment-correlation was run to determine the relationship between the individual concentrations and their protection ability. There was a strong positive correlation between the concentration and protection ability, which was statistically significant at  $p < 0.05$  level (2-tailed) (table 1). The correlation results clearly explained the dose dependent protection of the studied *Gracillaria* species.

Table 1: Anti-inflammatory activity of *Gracillaria* species

Species/ concentration	Locality	% of protection				Pearson correlation (r)
		50	100	200	250	
<i>G. fergusonii</i> .	Tuticorin	63.98*	66.62*	70.10*	79.97	0.912
<i>G. corticata</i>		43.81	52.71	64.39*	71.07*	0.849
<i>G. corticata</i> var. <i>cylindrica</i>		57.16	58.83*	66.34*	67.87*	0.862
<i>G. edulis</i>	Pulicate Lake	45.06	59.94*	69.68*	74.55	0.968
<i>G. verrucosa</i>		47.15	57.44	59.53*	68.15*	0.974
<i>G. salicornia</i>	Mandabam	52.57	73.99*	79.97	82.75	0.975
<i>G. edulis</i>		81.50	82.20	83.59	95.55	0.974
<i>G. corticata</i>		68.85*	86.65	89.85	90.54	0.949
<i>G. fergusonii</i>		56.61	61.47	74.83*	77.47*	0.995
<i>G. verrucosa</i>		69.82*	78.58*	80.39	87.90	0.958

\*indicates the mean differences are significant at  $P < 0.05$  level.

## DISCUSSION

In the present study, RBC membrane stabilization of method was used to determine the anti-inflammatory properties of *Gracillaria* species. Similar to that Dependra and Bisu [25] also employed the RBC membrane stabilization of method to determine the anti-inflammatory property. The varied frequency (43.81 to 95.55 %) of protection/activity depends on the phytochemical composition of the aqueous extracts of the studied *Gracillaria* species. The occurrence of flavonoids was observed in *G. salicornia* and *G. corticata* [26]. Krishnaveni and Johnson [27] reported the existence of alkaloids, saponins, steroids and tannins in the aqueous extracts of *G. corticata*. Flavonoids and triterpenoids occurrence was reported in *G. edulis* [28]. The existence of these metabolites may be responsible for the activity. Vijayalakshmi [17] observed more frequency 58.2% of anti-inflammatory activity in the aqueous extracts of *G. edulis*. The results of the present study also supplemented and coincided with Vijayalakshmi observations. But in the present study, *G. edulis* aqueous extracts showed 95.55% of activity. The results of the present study clearly explained the anti-inflammatory potential of the studied *Gracillaria* species. The aqueous extracts of *Gracillaria* collected from Mandapam showed the highest percentage of protection activity. This study results confirm the existence of active principle responsible for the anti-inflammatory activity. Further studies are required to isolate the active principles without any side effects. The result is identified an alternate source for the isolation of anti-inflammatory agent.

## CONFLICT OF INTERESTS

Declare none

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