INTRODUCTION
Due to the presence of a rich source of biologically active metabolites, 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], Dictotomaria obtusata [10], Gracilaria cornea [11], P. tertrastomatica and P. gymnospora [12], Dictyota menstrualis [13], Padina tetrastomatica, Sargassum wightii, Gracilaria edulis and Caulerpa racemosa [14], Spatoglossum Schroederi [15], Chudophora indica [16], Gracilaria edulis [17], Undaria Pinnatifida, Laminaria japonica, Sargassum fulvellum and Hizikia fusiiforme [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 Gracilaria corticata 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 Pulicat Lake, Gracilaria fergusonii J. Ag., Gracilaria corticata J. Ag. and Gracilaria verrucosa 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 Gracilaria 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 from Mandapam, Gracilaria edulis (Gmelin) Silva, Gracilaria verrucosa (Hudson) Papenfus from Pulicat Lake, Gracilaria fergusonii J. Ag., Gracilaria corticata J. Ag. and Gracilaria verrucosa J. Ag. var. cylindrica from Tuticorin were used for the study. The fresh seaweeds were boiled with distilled water (1:20 W/V) for 2 h. The slurry was filtered through Whatman No. 1 filter paper and condensed. The semi-solid crude extracts were used for anti-inflammatory analysis.

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) in 96 well microtiter plates [25]. Heat induced haemolysis (HIH) was performed by adding different concentrations of the aqueous extracts in the reaction mixture. The reaction mixture was then incubated at 37°C for 30 min, followed by centrifugation at 3000 rpm for 10 min. The absorbance of the supernatant was measured at 414 nm.
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:

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\text{Percentage inhibition} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 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 *Gracilaria* 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 *Gracilaria* 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 *Gracilaria* species at 250 µg/ml were as follows (table 1) G. edulis (Mandabam)>G. corticata (Mandabam)>G. verrucosa (Mandabam)>G. salicornia (Mandabam)>G. fergusoni (Tuticorin)>G. fergusoni (Mandabam)>G. edulis (Pulicate)>G. corticata (Tuticorin)>G. verrucosa (Pulicate)>G. corticata var. cylindrica (Tuticorin) (table 1). The anti-inflammatory/mediation activity of the aqueous extracts of studied *Gracilaria* species was significant at p<0.005. Among the three localities studied, the *Gracilaria* 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 Table 1). The correlation results clearly explained the dose dependent protection of the studied *Gracilaria* species.

**DISCUSSION**

In the present study, RBC membrane stabilization of method was used to determine the anti-inflammatory properties of *Gracilaria* 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 *Gracilaria* species. The occurrence of flavonoids was observed in *G. salicornia* and *G. corticata* [26]. Krishnaveni and Johnson [27] reported the existence of alkaloids, saponins, sterols 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 *Gracilaria* species. The aqueous extracts of *Gracilaria* collected from Mandabam 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

**REFERENCES**


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

<table>
<thead>
<tr>
<th>Species/concentration</th>
<th>Locality</th>
<th>% of protection</th>
<th>Pearson correlation (r)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>G. fergusoni</td>
<td>Tuticorin</td>
<td>63.98*</td>
<td>66.62*</td>
</tr>
<tr>
<td>G. corticata</td>
<td>Mandabam</td>
<td>43.81</td>
<td>52.71</td>
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<td>G. corticata var. cylindrica</td>
<td>Pulicate Lake</td>
<td>57.16</td>
<td>58.83*</td>
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<tr>
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<td>45.06</td>
<td>59.94*</td>
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<td>57.44</td>
</tr>
<tr>
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<td>Mandabam</td>
<td>52.57</td>
<td>73.99*</td>
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<tr>
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<td>81.50</td>
<td>82.20</td>
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<tr>
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<td>Tuticorin</td>
<td>68.85*</td>
<td>86.65</td>
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<tr>
<td>G. fergusoni</td>
<td>Tuticorin</td>
<td>56.61</td>
<td>61.47</td>
</tr>
<tr>
<td>G. verrucosa</td>
<td>Tuticorin</td>
<td>69.82*</td>
<td>78.58*</td>
</tr>
</tbody>
</table>

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


How to cite this article