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ANTI-INFLAMMATORY ACTIVITIES OF SOME SEAWEED COLLECTED FROM THE Gulf Of Mannar COAST, TUTICORIN, SOUTH INDIA.

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ABSTRACT

Methanol extracts of four seaweeds namely *Padina tertastomatica, Sargassum wightii, Gracilaria edulis* and *Caulerpa racemosa* were subjected to paw edema anti-inflammatory test. The extracts were injected into albino rats and the percentage inhibition of paw edema was calculated. Results revealed that all the seaweed extracts had equally good anti-inflammatory effects in the carageenan induced paw edema compared to those of standard drug (Diclofenac) ($P<0.05$). Maximum inhibition of 35.38% was found in the first hour after carageenen injection in *Caulerpa racemosa* extract. Similar percentage of inhibition (29.23 and 23.07) was also found in *Padina tertastomatica* and *Sargassum wightii,* after the first phase. The anti-inflammatory activity had its peak in the first phase and deceased as the hours increased. In *Gracilaria edulis* anti-inflammatory activity showed a high level after third hour.

KEYWORDS: Methanolic extract, anti-inflammation, Padina tertastomatica, Sargassum wightii, Gracilaria edulis and Caulerpa racemosa, paw edema.

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INTRODUCTION

Among marine organisms, seaweed is a promising candidate for drug production because it is relatively easy to obtain-adequate, reliable, and most importantly, renewable supplies by aquaculture. They are to have a rich source of structurally diverse bioactive compounds with valuable pharmaceutical potential[1, 2]. Several metabolized products of PUFAs, called oxylipins, resemble human eicosanoid hormones, which carry out a range of physiologically important functions. The anomalous production of these compounds underlies several diseases related to inflammation and thus eicosanoids and their derivatives have received wide attention in the search for anti-inflammatory drugs [3, 4]. Inflammation is the normal physiological and immune response to tissue injury. Increased blood supply, enhanced vascular permeability and migration of immune cells occur at damaged sites. The inflammatory process is a protective response that occurs in response to trauma, infection, tissue injury or noxious stimuli. It can be identified by tumor (swelling); Robor (redness), Calor (heat) and Dolar (pain) [5-7]. Seaweed is one of the potential objects for the extraction of anti-inflammatory agents. The anti-inflammatory activity of ω-3 polyunsaturated fatty acids (ω-3 PUFAs) *in vivo* and *in vitro* had been affirmed [8]. The anti-inflammatory substances with different nature have been separated from marine algae (sterol glycoside from *Undaria pinnatifida* and *Enteromorpha linza)* [9]. Nowadays, searches of natural medicinal herbs against inflammatory diseases, especially from marine organisms including marine algae with certain advantages are attracting the attention of many scientists and other countries in the world. The stages of the acute phase of inflammation in carageenan-induced edema are biphasic; the first phase begins within one hour after the injection to the release of histamine and serotonin. The second phase begins at first hour and remains through third hour to the potentiating effects release of bradykinin and the delayed phase releases potentiating effects of prostaglandins [10, 11]. NSAIDs inhibit the synthesis of prostaglandins (PGs) and cause edema to pass by the cyclooxygenase (COX) pathway from the arachidonic acid metabolism[10].

MATERIALS AND METHODS

Seaweed (*Padina tertastomatica, Sargassum wightii, Gracilaria edulis* and *Caulerpa racemosa*) were collected from Tuticorin coast, Tamilnadu, India. The samples were washed with seawater and freshwater to remove salt, epiphytic forms and other suspended materials. The clean algae were frozen and lycophilized. The dry material was stored at -20°C.

Preparation of extract

Extracts of the freeze dried and powdered biomass were prepared using methanol as solvent using a soxhlet apparatus. The resultant crude extracts were filtered and then concentrated in a rotary evaporator at a temperature less than 40°C. The crude extracts were weighed and deep frozen (-20°C) until further use.

ANTI INFLAMMATORY ACTIVITY

Materials required

1. Animals. Species: Albino Rat.
2. Carageenan powder.
3. 25 –gauge hypodermic needles 5-8 inches long.
4. Plethysmometer.

Method

In the present study, anti-inflammatory activity was determined in albino rats according to the method described by Winters *et. al*., (1962).

1) Albino rats of either sex were divided into 6 groups of four each.

2) Group I served as control and received saline at a dose of 1 ml/kg p.o
3) Group II served as standard and treated with diclofenac sodium 10 mg/kg.
4) Group III was treated with methanolic extract of *Caulerpa racemosa* 200 mg/ kg p.o.
5) Group IV was treated with ethanolic extract of *Padina tetrastomatica* 200 mg/ kg p.o.
6) Group V was treated with ethanolic extract of *Gracilaria edulis* 200 mg/ kg p.o.
7) After 30 minutes 0.1 ml of 1% (w/v) carageenan was injected in the plantar region of the left paw of all groups of animals. The right paw served as reference non – inflamed paw for comparison. The paw volume of both legs of all groups of animals at 1, 2, 3 hrs after carageenan challenge was measured by pleythysmometer.

The percent inhibition of edema for each group was calculated as:

$$\% \text{ inhibition of paw edema} = \frac{(Vt - Vo)\text{Control} - (Vt - Vo)\text{ Treated}}{(Vt - Vo)\text{ control}} \times 100$$

Vt = paw volume after carageenan administration.
Vo = paw volume before carageenan administration.
The percentage of inhibition was carried out for different time interval.

Table 1
Showing the effect of seaweeds against percentage inhibition of paw edema.

Drug		Time After Treatment (hrs)			
		% of Inhibition of Paw edema			
		1	2	3	4
Diclofenac		0.12±0 63.07	0.18±0.016 62.5	0.26±0.016 62.85	0.38±0.048 56.06
Caulerpa racemosa		0.21±0.025 35.38	0.40±0.034 15.62	0.54±0.04 22.85	0.78±0.032 9.82
Padina tetrastromatica		0.23±0.025 29.23	0.42±0.034 11.45	0.53±0.047 07.14	0.81±0.025 6.35
Sargassum wightii		0.25±0.025 23.07	0.43±0.025 10.41	0.55±0.025 21.42	0.71±0.025 17.91
Gracilaria edulis		0.29±0.025 10.76	0.42±0.036 12.5	0.57±0.025 18.57	0.73±0.025 15.60
One Way Anova	F	29.42	18.217	23.121	58.32
	DF	8,3	8,3	8,3	8,3
	P	<0.05	<0.05	<0.05	<0.05

Statistical analysis

All assays were done in triplicate. All data are expressed as means ± S.D. Data were analyzed by an analysis of variance ($P < 0.05$) and the means separated by one-way ANOVA with Dunnet's t-test. The data were calculated by computer programs: Microsoft Excel and SPSS version 17.0.

RESULTS AND DISCUSSION

The percentage inhibition of paw edema was calculated for a control, a standard drug (Diclofenac) and four extracts of seaweeds namely *Caulerpa racemosa*, *Padina tetrastromatica, Sargassum wightii* and *Gracilaria edulis*. The paw volume after the administration of carageenan was measured at each hour and the reduction in paw thickness was noted (Table 1). The results show that all the seaweeds effectively controlled the paw thickness of albino rats at the end of each hour. These results were comparable with that of control and the maximum percentage of inhibition of paw thickness was found at 1st hour in *Caulerpa racemosa* (35.38%) of carageenan injection. In the present study, seaweed extracts had equally good anti-inflammatory effects in the carageenan induced paw edema than those of standard drug (Diclofenac) ($P<0.05$) Table.1. Earlier findings reported that the alcoholic extract of *Ulva fasciata* collected from Gujarat coast exhibited antiviral and anti-inflammatory activity [12].The most widely used primary test to screen new anti-inflammatory agent is to measure the ability of the extract to reduce ear edema induced in mice paw by injection of an irritant agent . The initial phase of inflammation was observed around first hour after injection of irritant. In this phase the extract of *Caulerpa racemosa*, had a high percentage of inhibition. The second phase is the accelerating phase. It is the phase of swelling due to the release of prostaglandins like substances which was prominently observed in the control but slightly appeared in the test species. This phase is reported to be sensitive to both clinically careful steroidal and NSAID.

In the present study, *Gracilaria edulis* extract exhibited a high level of percentage of inhibition at each hour after carageenan injection (0.42±0.036), (0.57±0.025) and (0.73±0.025). Similar results were obtained by other researchers. In their work, *G. folifera* showed high activity (89.12%) which is almost equal to 250 mg/kg in Ibuprofen group. They concluded that this significant anti-inflammatory activity may be due to the presence of PUFA. [13-17] Marine algae are rich in PUFA [18-20] and are of potential value as source of essential fatly acids, important in the nutrition of humans of animal.[21, 22]. *Gracilaria edulis* is red algae and they are said to be rich in sulfated polysaccharides, fucanoids and ω-3 fatty acids which are commonly associated with anti inflammatory activity. Extracts as well as structurally diverse compounds obtained from marine red algae have been shown to inhibit inflammation. Marine red alga *G. marginata* displayed anti-inflammatory activity in its apolar extract, which was ten-fold more potent than the apolar substance obtained from *L. farinose.*[23, 24] Sulfated polysaccharides present in algae were shown to posses anti-inflammatory properties. *Ulva lactuca* the green alga available in Tuticorin coast was found to show anti-inflammatory effect as evidenced by the reduction in the inhibition of edema at the 4th day of the experiment compared with the positive control drug and control[25].

Conflict of Interest:, Conflict of interest declared none.

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