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Screening and Anti-Inflammatory Activity of Methanolic and Aqueous Extracts of Seaweed *Gracillaria Edulis*

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Abstract: Seaweeds were collected from coastal area (Mandabam, Tamilnadu, India).Out of 9 species *Gracillaria edulis* expressed both antimicrobial and anti inflammatory activity. In this study, the aqueous and methanolic extracts of seaweeds were subjected to anti inflammatory activity using experimental animal model,in the presence of the positive control drugs.The inflammation was induced by carrageenan. The aqueous extract of *Gracillaria edulis* showed highest activity for anti-inflammation. However the methanolic extract did not exhibited any appreciable activity. The aqueous extract of seaweed contains compound may be a novel drug for anti-inflammatory activity.

Keywords: Anti-inflammatory, animal model, carrageenan, methanolic extract, aqueous extract, seaweed.

1. Introduction

Seaweeds are widely distributed in the marine world and these contains medicinal properties like medicinal plants.Seaweeds are producing novel compounds for antimicrobial and pharmacological activities.They act as a good source of bioactive compounds as they are able to produce a greatvariety of secondary metabolitescharacterized by a broad spectrum of biological activities [1].Coumpounds with antioxidant, antiviral, antifungal and antimicrobial activities have been detected in brown, red and green algae [2].

The bacteria, fungi, algae, sponges, soft corals, tunicates, molluscs and bryozoans are the most interesting organisms of pharmacological significance inhabiting the complex ecosystems of the environment [3].Numerous substances were identified as antimicrobial agents from algae: chlorellin derivatives, acrylic acid, halogenated aliphatic compounds, terpenes, sulphur containing heterocyclic compounds, phenolic inhibitors, etc [4]. Antibacterial activity was found to vary with season [5].

A wide range of useful drugs including antibiotics, analgesic, anti-inflammatory, anti-coagulants, CNS depressants etc. have been isolated from marine organisms.Currently,only few marine derived products are in the market and several of them are in clinical trials. Inflammation involves a complex sequence of biochemical events closely associated to the pathogenesis of various diseases such as rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, acute gout, migraine, etc [6].In vivo anti-inflammatory activity in polyphenolic extracts from the red alga *Laurencia undulate* resulting in significant inhibition of asthmatic reactions [7].

In the present study,the seaweeds were allowed to determine the antimicrobial and anti inflammatory activities. Out of 9 species *Gracillaria edulis* exhibited highest activity for antimicrobial and also exhibited for anti-inflammatory activity. This compound may be novel metabolites. Therefore, In future study, further compound identification is needed.

2. Materials and Methods

2.1. Collection of Seaweeds

Seaweeds totally 9 species (*Gelidiella acerosa, Gracillaria edulis, Gracillaria crassa,Gracillaria oliifera, Gracillaria verrucosa, Sargassum spp., Turbinaria spp.,Enteromorpha compressa and Ulva lactuca*) were collected from the Mandabam coastal area (Tamilnadu, India).Collected samples were brought to the laboratory in aseptic conditions (Using sterile bags).

2.2. Antibacterial activities of Seaweeds

Seaweed were washed with tap water to remove the loosely attached microbes and allowed to dry in sun shade.The dried seaweeds were powdered using mechanical grinder and extracted with ethyl acetate using soxlet apparatus. Compounds were concentrated by using rotary vacuum evaporator.Crude compounds were allowed to determine the anti bacterial activity against five human pathogens (*E.coli,Salmonella sp.,Vibrio sp.,Staphylococcus sp.,and Streptococcus sp.,*)were collected from Christian Medical college (CMC, Vellur, Tamilnadu, India) by disc diffusion assay method [8].

2.3. Antibiotic disc preparation

The discs were prepared using whatman No.1 filter paper and extracts of bacterial cultures were impregnated into the discs separately.Approximately 50µg of the crude extracts were impregnated subsequently. Muller hinton agar plates were swabbed by muller hinton broth containing 16 hrs pathogens separately.The prepared antibiotic discs were placed on the inoculated plates separately.The plates were allowed to incubation at 37°C for 24 hrs.After

incubation the results were recorded.The active metabolite producing seaweed (*Gracillaria edulis*) extract was screened for anti inflammatory activity.

2.4. Extraction process

2.4.1 Aqueous extract preparation

The collected seaweeds were allowed to dry in sunshade. The dried seaweeds were reduced to fine powder with a mechanical grinder.500 ml of distilled water was added to 100g of algae powder and vortexed in a shaker for 24 hours at room temperature.The resulting material was centrifuged (1000 g for 15 min).The supernatant was collected and centrifuged again at 8000 g for 1 hour to obtain a clarified mixture. The supernatant was lyophilized and stored at 4ºC until use [6].

2.4.2. Methanolic extract preparation

The seaweed powder (200 g) was extracted by methanol and maceration for 40 hrs, and concentrated to dryness using a rotary evaporator attached to a vacuum pump and their use it immediately or stored at a temperature of -4º for further use [9].

2.4.3. Anti-inflammatory activity

Albino rats (HA strain) of 150-200 g were obtained from laboratory Animal Resource Section, Arulmigu Kalasalingam College of Pharmacy, Pharmacology Research Lab at Anand nagar ,Srivilliputhur. The animals were kept in polypropylene cages in an air conditioned area at 25 ± 2ºC in 10:14 hr light dark cycle.They were provided with Amrut brand balanced feed and tap water *ad libitum (*Seaweed extracts were administered orally by gavages in distilled water at different dose levels).

Anti inflammatory activity was measured using carrageenan induced rat paw oedema assay [9-11].Groups of five rats of both sexes (pregnant females excluded) were given a dose of the extract. After 1 hour,0.1 ml,1% carrageenan suspension in 0.9% Nacl solution was injected into the sub planter tissue of the right hind paw. The linear paw circumference was measured at hourly interval for 4 hrs .Two groups of drug treated rats and one control group were used each test day, the mean paw oedema value for the test group being compared with its mean value for the control group for that day. Anti-inflammatory activity [12] was measured as the percentage reduction in oedema level when drug was present, relative to control as shown in table 1.

Activity=100-(100x average drug treated/average for control).

Indomethacin (10 mg/kg) was administered orally as reference drug while distilled water was used as negative control.

2.4.4. Statistical analysis

All data were expressed as mean ± SEM and the student's t-test was applied to determine the significance of the difference between the control group and mice treated with the test compounds.

3. Results and Discussion

The aqueous extract of *Gracillaria edulis* exhibited highest activity for anti-inflammatory than methanolic extracts (Table-1).Carrageenan induced rat paw oedema is used widely as a working model of inflammation in the search for new anti inflammatory drug [13] and appeared to be the basis of the discovery of indomethacin anti inflammatory drug[10].The anti inflammatory activities of the aqueous and methanolic extracts of *Gracillaria corticata* were evaluated by carrageenan induced rat paw oedema method [9-11].

Seaweeds are considered as source of bioactive compounds and produce a great variety of secondary metabolites characterized by a broad spectrum of biological activities. Compounds with cytostatic, antiviral, anti-helminthic, antifungal and antibacterial activities have been detected in green ,brown and red algae [14].The algal extracts such as *Enteromorpha ramulosa* (Smith) Carmichael and *Dictyopteris membranacea* (Stackhouse) Batters were active against Gram positive and Gram negative bacteria [15].

Table 1: Anti-inflammatory activity of the aqueous and methanolic extract of the seaweed *Gracillaria edulis*

Extracts	Dose(mg/kg)	Change in paw oedema mean (mm)	% of paw oedema inhibition relative to control at the 4th hour
Control	0.3 ml	2.75±1.35	-
Indomethacin	10	1.12±0.12*	59.10
Aqueous extract	100µg	1.33±0.48*	51.50
	200 µg	1.15±0.25*	58.20
Methanolic	100µg	2.20±0.35	20.10
extract	200 µg	2.10±0.58	23.64

Values are mean ± S.E.M.N=5, * significant values at $p<0.05$

Red algae are considered the most important source of many biologically active metabolites in comparison to other algal classes [16].Many studies have found interesting biological activities in polar fractions from marine algae [17-19].Anti-inflammatory activity of the crude extracts and fractions of the Mediterranean sponge *Spongia officinalis* in the in vivo rat carrageenan-induced paw oedema assay [20].In this study,the aqueous extracts of the seaweed *Gracillaria edulis* showed highest activity. This may be a novel metabolite for anti-inflammatory activity.

4. Conclusion

Seaweeds out of 9 species only one species expressed highest antimicrobial activity.The same species showed high anti-inflammatory activity.It may synthesis novel drug for pharmacological activities especially anti-inflammatory activity.Further chemical analysis on the composition of *Gracillaria edulis* aqueous extract is necessary to isolate and identify bioactive compounds that may have applications in therapeutic fields of inflammation and pain.

5. References

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