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In Vitro Antioxidant and Anti-Inflammatory Activity of Ethanolic Extract of *Sargassum Ilicifolium* (Turner) C.Agardh.

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ABSTRACT

The present study was focused to assess traditional claims of *Sargassum ilicifolium* Turner C.Agardh towards anti-inflammatory and antioxidant property by *invitro* studies. In this study 70% of ethanolic extract of *sargassum ilicifolium* was prepared and subjected to preliminary phytochemical constituent screening followed by invitro antioxidant activity byDPPH assay using concentration 25-200µg/ml and *invitro* anti-inflammatory studies by Human RBC stabilization method using the concentration of 12.5-200µg/ml. The phytochemical analysis revealed the presence of alkaloids, terpenioids, steroids, phenolic compounds, fats, oils, and tannins. In DPPH assay the free radical scavenging activity of the extract was determined by its reduction in yellow colored reduced DPPH formation and IC_{50} value was found to be 164.98µg/ml and *invitro* HRBC anti-inflammatory study, the extract was effective in inhibiting the hemolysis induced by hypotonic solution added to RBC cells and % reduction of haemolysis was found to be 52.08% at concentration of 12.5µg/ml and 16.86% for 200µg/ml and standard drug Diclofenac showed 32.68 and 4.28 % reduction of heamolysis at concentration of 12.5 and 200µg/ml respectively. The result shown effective action in dose dependent manner and was statistically proved.

Keywords: *Sargassum ilicifolium*, DPPH assay, Human RBC Stabilization, Diclofenac.

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INTRODUCTION

Sargassum ilicifolium is the brown macro algae belong to family *Sargasaceae.* Brown algae are unique is having photosynthetic pigments such as chlorophyll, carotene, xanthophyll's and also food reserves such as mannitol, polysaccharides, alginic acids. The marine algae are popularly called as gulf weed sargassum with almost 50 species. It is most common in the warmer seas of southern hemisphere. About 12 species have been reported from India. Sargassum is preferably available in west cost of mandabam, coast of kannyakumar, gulf of mannar. Marine algae recognized as rich sources of structurally diverse biologically active compounds with great pharmaceutical and biomedical potential. Researches have reported that marine algae have various biological activities such as "cholinesterase inhibitory activity[1], neuroprotective activity[2], anti-pyretic[3], analgesic[3], anti-coagulant[4], anti-viral[5], and anti-cancer activity[6]." Our present study for "*in-vitro* anti-inflammatory activity on Human Red Blood Cell Stabilization (HRBC) and anti-oxidant activity on DPPH, suggest about their mechanism for their therapeutic activity.

MATERIALS AND METHOD

Plant collection and Authentication

The marine specimen *Sargassum ilicifolim (*Turner) C. Agardh for the proposed study was collected form Mandabam, coast, Rameswaram and authentified by botanist Professor, Jeyaraman, Director Plant anatomy research Centre, Tambaram, Chennai.

Extraction

About 1kg of air-dried plant material was extracted in Sozhlet assembly using ethanol (70% v/v) and was filtered. The extract was concentrated by using rotary vacuum evaporator and percentage yield was calculated in terms of dried weight of the material. The colour and consistency of the extract was also noted. All the solvents used for this entire work were analytical reagent grade (Merck, Mumbai).

In vitro anti-inflammatory activity[7]

In vitro anti-inflammatory activity of *sargassum ilicifolium* was performed by using Human Red Blood Cell Stabilization (HRBC) method. The blood was collected from healthy human volunteer who was not taken NSAID'S for 2 weeks prior to the experiment. The blood was mixed with equal volume of Alsever solution (2% dextrose, 0.8 sodium citrate, 0.5% citric acid, 0.42% sodium chloride) and centrifuged at 3000rpm. The packed cells were washed with isosaline and 10% suspension was made. Various concentration of plant extract were prepared (12.5µg/ml, 25µg/ml, 50µg/ml, 100µg/ml, 200µg/ml). To each concentration 1ml of phosphate buffer, 2ml of hypo

saline was added. Diclofenac sodium was used as the reference drug. Instead of hypo saline 2ml of distilled water was used in control. Mixture was incubated at 37^{o}C for 30 minutes and centrifuged. The hemoglobin content in the supernated solution was estimated by UV analysis at 560nm.

***In vitro* antioxidant activity**[8]

This is based upon 1, 1 dipheny 2-picryl hydrazyl (DPPH)· Due to the presence of odd electron gives a strong absorption maximum at 517nm. A methanolic solution of test sample was prepared at different concentration (100,200,400,800, &1000µg/ml). To a set of test tube DPPH solution (100mg in ethanol) and various concentration of test sample were added and set aside for 20 minutes. After 20 minutes the absorbance was measured at 517nm. Percentage scavenging activity was calculated by comparing the absorbance between test mixture and control.

RESULTS AND DISCUSSION

In vitro* Anti-inflammatory activity of *Sargassum ilicifolium

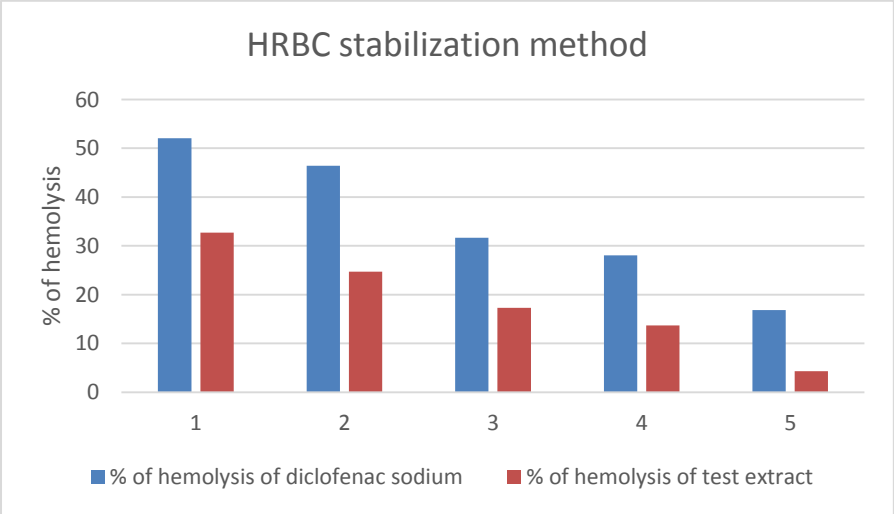
The result of *in vitro* anti-inflammatory activity of *Sargassum ilicifolium* on human red blood cell membrance were given in graph 1. *In vitro* anti-inflammatory activity of *Sargassum ilicifolium* was performed by using human red blood cell membrane stabilization method. The extract showed significant anti-inflammatory activity in a concentration dependent manner. The extract at concentration of 12.5, 25, 50,100,200 µg/ml showed 32.68%, 24.72%, 17.28%, 13.66%, 4.28% of hemolysis inhibition All the result were compared with standard Diclofenac at 12.5,25,50,100,200 µg/ml which showed 52.08%,46.44%,31.64%,28.02%,16.863% inhibition of hemolysis.

In vitro* antioxidant activity of *Sargassum ilicifolium

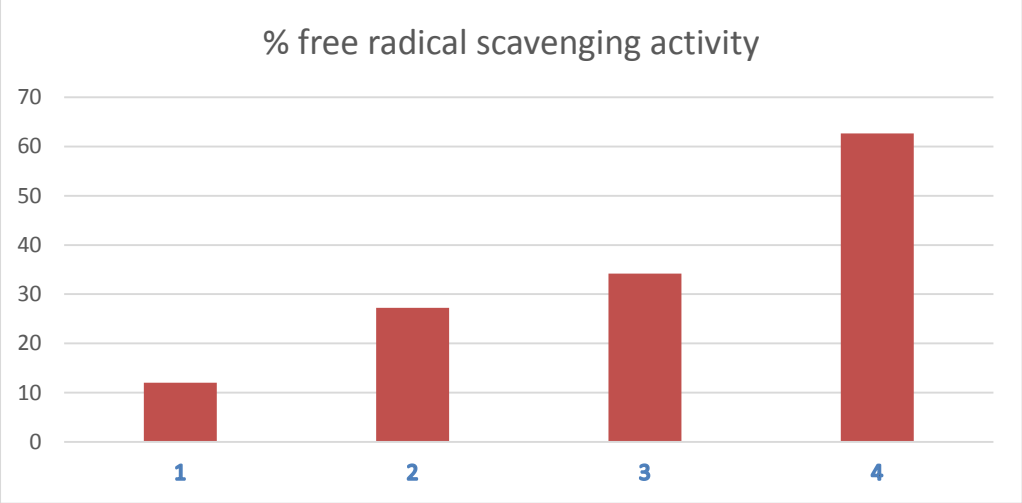
The result of *in vitro* antioxidant activity of *Sargassum ilicifolium* on free radical scavenging activity were posted in graph 2. *In vitro* antioxidant activity of extract was performed by using DPPH assay. The extract showed significant anti-oxidant activity in a concentration dependent manner. The extract at 25, 50,100,200µg/ml showed 12.24%, 27.22%, 34.20%, 62.63% inhibition of free radical scavenging.

Inflammation is a common phenomenon and it is reaction of living tissues towards injury. HRBC method was selected for the *in vitro* anti-inflammatory activity because the erythrocyte membrane is analogous to the lysosomal membrane. Stabilization of lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil. The result indicated that the extract of *Sargassum ilicifolium* at various concentration has significant anti-inflammatory property. Dpph is a stable free radical and accepts an electron or hydrogen radical to be come a stable diamagnetic molecule. A freshly prepared DPPH solution

exhibited a deep purple colour with a maximum absorption at 517 nm. The purple colour disappear when an antioxidant is present in the medium. Thus anti-oxidant molecuels can quench DPPH free radicals and convert them to a colourless product, resulting in decrease in absorbance at 517nm.



Graph 1: HRBC stabilization method.



Graph 2: DPPH assay.

CONCLUSION

Sargassum ilicifolium possess potent anti-inflammatory activity by inhibiting the release of prostaglandins or other inflammatory mediators from cell membrane by stabilizing membrane, and antioxidant activity is one of the possible pathway of prevent free radical formation. By further studies it can be possible to formulate natural anti-inflammatory, antioxidant drugs of *Sargassum ilicifolium.*

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