



iJRASET

International Journal For Research in
Applied Science and Engineering Technology



INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY

Volume: 7 Issue: IV Month of publication: April 2019

DOI: <https://doi.org/10.22214/ijraset.2019.4018>

www.ijraset.com

Call:  08813907089

E-mail ID: ijraset@gmail.com

Validation of Antimicrobial and Anti-inflammatory Activities in *Chondrococcus hornemanni* and *Pocockiella variegata*

Maria Catherrine. T¹, Jeyachandran R²

¹Coordinator, ²PG Biotechnology

Department of Plant Biology And Biotechnology, Loyola College, Chennai-34.

Abstract: In this article the study on validation of the bioactive principles and its efficacy (antibacterial and antifungal) and anti inflammatory activity was done with the solvent extract of two different groups of algae that is, *Chondrococcus hornemanni* and *Pocockiella variegata*. The extract was prepared using solvents such as aqueous, hexane, chloroform and chloroform: methanol(2:1) mixture. The extracted sample was tested in vitro for their antimicrobial activities against different types of bacteria using disc diffusion method. Chloroform extract of *Pocockiella* against *Bacillus subtilis* and *Chondrococcus* against *Pseudomonas aeruginosa* showed maximum inhibitory zone than other solvents. Antifungal activity was done using poisoned food method in which no significant antifungal activity was observed in all the extracts. The determination of anti inflammatory activity was done using SRBC method and characterized under UV-VIS spectrophotometer under 560nm, where the maximum inhibition of 52% was observed in 400mg/ml of chloroform: methanol extract of *Chondrococcus*.

Keywords: Antimicrobial activity, Anti inflammatory activity, Disc Diffusion method, Poisoned food method, SRBC(Sheep Red Blood Cells) method.

I. INTRODUCTION

Macroalgae can be classified as red algae (rhodophyta), brown algae (phaeophyta) or green algae (chlorophyta) depending on their nutrient and chemical composition. Red and brown algae are mostly used as an important human food source.[1]. They serve as an important source of bioactive natural substances. Seaweeds have been used as food stuff in the Asia diet for centuries as it contains carotenoids, dietary fibers, proteins, essential fatty acids, vitamins and minerals.[3]. Different varieties of sea weeds were found to possess useful untapped biochemical compound which might be potential source of drug in the future[5]. Currently, only few marine derived products are in market and several of them are in clinical trials. People mostly use synthetic drugs to prevent or control the infectious diseases caused by microbes. Regular use of these drugs leads to development of resistance by the microbes against the drugs. Plant derived natural products and antibiotics are found to be effective and alternative recognized from natural resource[6][7]. Fungal infections of skin and its appendages are more prevalent in India due to favorable climatic conditions like temperature and humidity. The increased use of antifungal drugs, often for prolonged periods, has led to acquired antifungal resistance. Therefore, there is a need to determine the antifungal susceptibility of isolates to available drugs. In such cases antifungal susceptibility testing would obviously be beneficial.[4]. Inflammation is a local response of living tissue of our body when injured. It can be either acute or chronic. Acute inflammation is the initial response of the body to harmful stimuli. Chronic inflammation can lead to number of diseases such as hay fever, rheumatoid arthritis and gallbladder carcinoma. The chronic inflammatory disease is one of the major health issues which starts with discomfort in the biological system of human body[8]. Nowadays, the usage of synthetic drugs is dominating. It also causes side effects such as peptic ulcer, perforation and bleeding associated with these drugs. [9]The coastal region of Tamilnadu support a rich vegetation of marine algae. These studies have shown a great diversity in the macro algal community of the marine algal vegetation of the region.[2]. Among the macro algae of the region, the brown algae and red algae grow in abundance as dominant communities in the shores of Ramanthapuram Districts of Tamilnadu state, India. In the present study, antibacterial, antifungal and anti inflammatory activities of the extracts of red and brown algae using different solvent such as aqueous, hexane, chloroform, chloroform : methanol have been investigated.

II. MATERIALS AND METHODS

A. Sample Collection

Two seaweed samples *Pocokiella* (brown algae) and *Chondrococcus* (red algae) were received from Mahatma Gandhi medical college (CIDRF) which was cultivated and imported from Rameshwaram. The dried algae was powdered using electric blender.



A) Habit of *Chondrococcus hornemanni*

B) Habit of *Pocockiella variegata*

B. Preparation of Extract

Both red and brown algae were dried and powered using electric blender .5 grams of algal powder was submerged in 50 ml of distilled water, hexane ,chloroform, chloroform: methanol and placed in a shaker for 24 hrs and filtered using filter paper. Filtrate was concentrated by evaporated using water bath .

C. Assay of Antibacterial activity using Disc Diffusion method

Antibacterial activity was carried out using disc diffusion method. Petri plates were prepared with 20 ml of Muller Hinton agar (MHA). The test bacteria culture (*Bacillus subtilis* and *Pseudomonas aeruginosa*) were swabbed on the top of the solidified medium . The discs were loaded with different crude extracts and were placed on the well. The control (Streptomycin[standard]) were also placed on the wells . The plates were allowed for incubation for 24 hrs at 37°C. Zone of inhibition was recorded.

D. Determination of Anti-fungal activity using Poisoned Food Method

Poisoned food technique was employed to screen the antifungal efficacy of seaweed extracts. The different concentrations were made by taking 1 and 5 ml of the stock preparation and dissolved in distilled water to give 1% and 5% concentration. Potato dextrose agar media amended with different concentration of seaweed extracts and poured into sterile petri plates. Fungal strains (*Aspergillus niger*) were teased and placed in the well aseptically on PDA plates poisoned with seaweed extracts. The medium without incorporating the extract served as control. The inoculated plates were incubated at 25 °C and colony diameter was measured and recorded 48 and 72 hours after incubation. The percent inhibition of mycelial growth was calculated using formula.

$$\text{Percentage inhibition} = \frac{(C-T)}{C} \times 100$$

C

Where, C= colony diameter (mm) of the control ; T=colony diameter (mm) of the test plate.

E. Anti-inflammatory activity using Hemolysis method

The blood was collected from sheep (slaughter house). About 4ml of blood was collected and centrifuged at 3000 rpm for 20 minutes. The centrifuged sample was then washed thrice using isosaline (0.90%). Samples were prepared in different concentrations of 200 and 400mg /ml each. To each 2ml of 10% SRBC suspension , equal volume of Alsever's solution was added and centrifuged at 3000 rpm for 20 mins. The centrifuged sample was then washed thrice using isosaline. To each sample, 2 different concentration of algal extract was added. 1ml of phosphate buffer , 2ml of hypo saline (0.25%) was added to the sample extract. The sample was incubated for half an hour and then centrifuged at 3000rpm for 20 minutes. Diclofenac was used as reference control and the percentage of protection was calculated using the formula.

$$\text{Percentage of protection} = 100 - [\text{OD of sample} / \text{OD of control}] \times 100.$$

III. RESULTS

A. Antibacterial Activity

The screening of antibacterial activity was performed using disc diffusion method with two bacteria and activity was observed by zone formation in the bacterial culture. The maximum activity was seen at Chloroform extract of *Pocockiella* (brown algae) was effective against *Bacillus subtilis* and also at Chloroform extract of *Chondrococcus* (red algae) was effective against *Pseudomonas aeruginosa*.

B. Anti Fungal Activity

The screening of antifungal activity was performed using poisoned food method with one fungal strain and the activity was observed by mycelial growth. No antifungal activity was seen on both algal samples against *Aspergillus niger*.

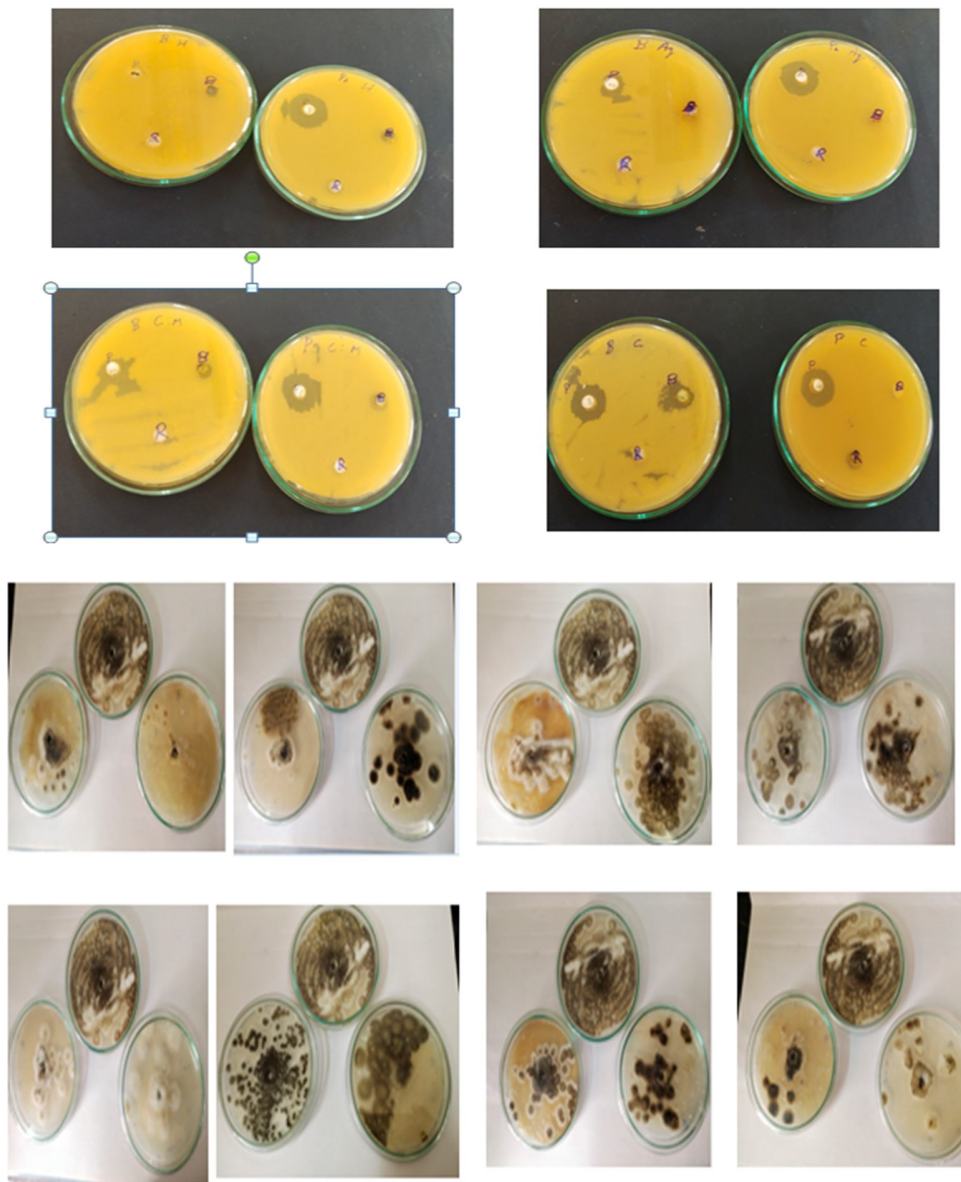


Fig 1. Effect of algal extracts of red and brown against *P.aeruginosa* and *B.subtilis* [R-Red algae, B-Brown algae, P-Positive control(Streptomycin)].

Tab .1.Antimicrobial activity of algal extracts against *B.subtilis*,*P.aeruginosa* and *A.niger*

EXTRACTS	<i>B.subtilis</i>	<i>P.aeruginosa</i>	<i>A.niger</i>
RED –AQUEOUS(mm)	0	0	-
BROWN-AQUEOUS(mm)	0	0	-
RED-HEXANE(mm)	1	1	-
BROWN-HEXANE(mm)	10	10	-
RED-CHLOROFORM(mm)	1	10	-
BROWN-CHLOROFORM(mm)	14	1	-
RED-CHLOROFORM:METHANOL(mm)	1	1	-
BROWN-CHLOROFORM:METHANOL(mm)	11	10	-

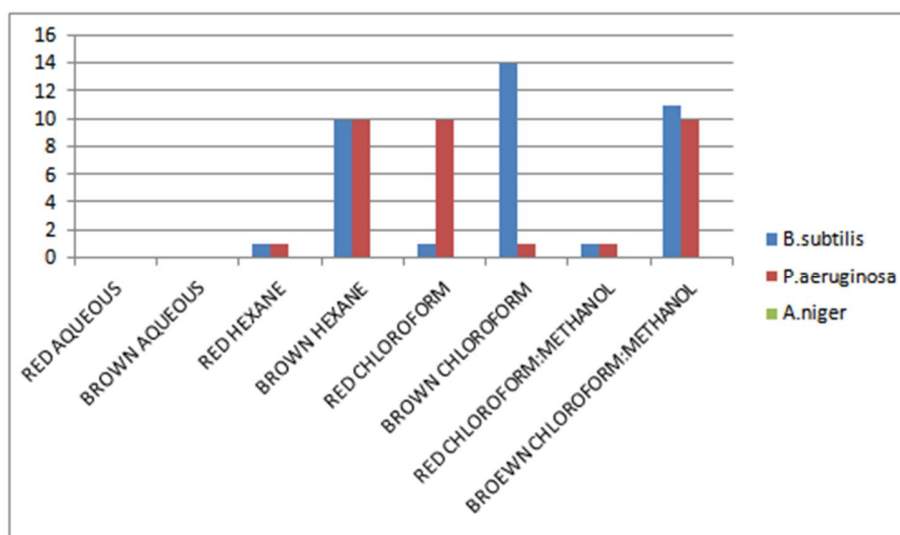


Fig 1. Histogram showing Antimicrobial activity of both red and brown algae

C. In-Vitro Anti Inflammatory Activity(Hemolysis method) :

The determination of anti inflammatory activity was performed using SRBC method. Diclofenac was used as reference control and percentage of protection was calculated. The maximum activity was seen in The determination of anti inflammatory activity of marine algal extracts against RBC using SRBC method was done and observed. The maximum inhibition of 52% was observed in 400mg/ml of chloroform: methanol extract of red algae

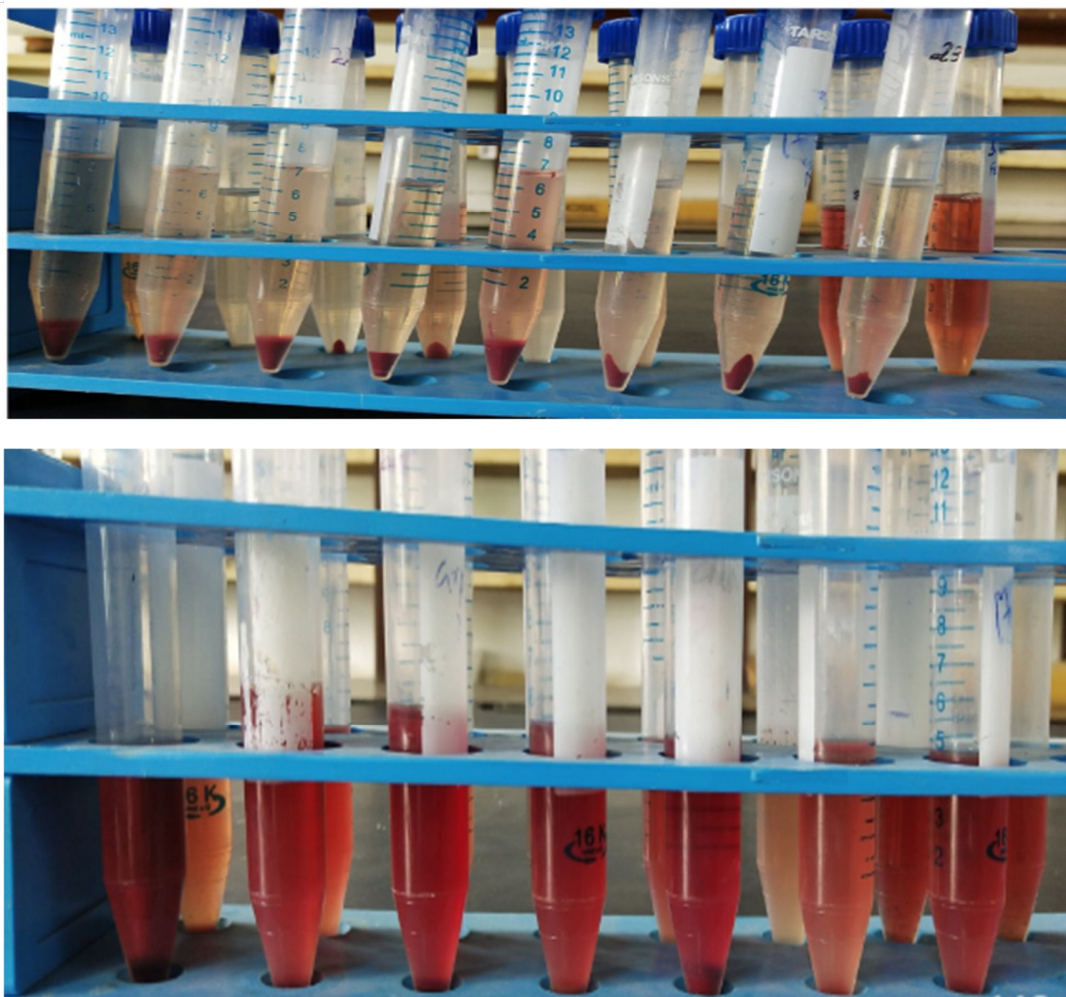


Fig 3. Anti inflammatory studies on red and brown algal extract using SRBC method (before and after incubation).

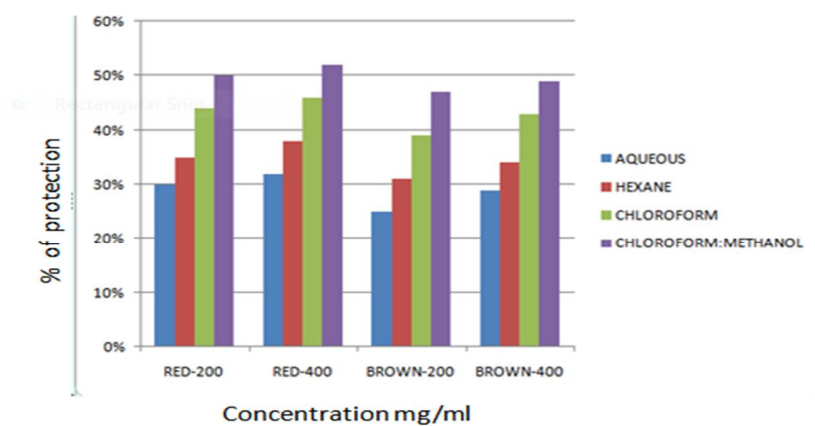


Fig 3 Histogram showing Anti inflammatory activity of red and brown algae.

Tab .3.Anti inflammatory activity (% of protection) of brown and red algal extract:

Concentration(mg/ml)	Absorbance at 560 nm(brown algae)	% of protection (brown algae)	Absorbance at 560 nm(red algae)	% of protection (red algae)
Aqueous-200	0.178	25%	0.169	30%
Aqueous -400	0.172	29%	0.164	32%
Hexane -200	0.166	31%	0.158	35%
Hexane -400	0.160	34%	0.148	38%
Chloroform -200	0.147	39%	0.136	44%
Chloroform -400	0.138	43%	0.134	46%
Chloroform: methanol -200	0.127	47%	0.120	50%
Chloroform : methanol-400	0.123	49%	0.117	52%
Diclofenac (5mg/ml)	0.110	54%	0.110	54%

IV. DISCUSSION

The Rhodophyta (red algae) are a distinct eukaryotic lineage characterized by the accessory photosynthetic pigments phycoerythrin, phycocyanin and allophycocyanins arranged in phycobilisomes, and the absence of flagella and centrioles. This is a large assemblage of between 2500 and 6000 species in about 670 largely marine genera that predominate along the coastal and continental shelf areas of tropical, temperate and cold-water regions. **Red Algae** are ecologically significant as primary producers, providers of structural habitat for other marine organisms, and their important role in the primary establishment and maintenance of coral reefs. Some red *algae* are economically important as providers of food and gels. For this reason, extensive farming and natural harvest of red algae occurs in numerous areas of the world. Red marine algae have been a valued food in Asia for thousands of years due to its highly nutritious qualities. Carrageenans, a family of polysaccharide compounds extracted from *algae*, have been studied for their unique properties. In vitro studies show that carrageenans aid in a cell's natural defense by significantly minimizing the binding of unfriendly proteins to the cell's surface.

In the present study, Chloroform extract of *Pocockiella* against *B.subtilis* and *Chondrococcus* against *P.aeruginosa* showed broader inhibitory zone than other solvents. The microbial effect of algae may be due to phenolic, terpenoids or unsaturated fatty acids. The species with antibacterial activity were active only against Gram positive. There was no significant antifungal activity both in *Pocockiella* and *Chondrococcus* it can be due to low concentration of algal sample. The comparison of dried and fresh algae was done and observed between where test organism where more effective in fresh algae. Chloroform : methanol extract of *Chondrococcus* exhibited greater anti-inflammatory activity. Among different algal samples red algae showed remarkable anti-inflammatory activity.



REFERENCES

- [1] Botryolins A and B, two tetramethyl sequalene triethers from the green microalga *Botryococcus braunii*. *Phytochemistry*, 59:839-843
- [2] Caccamese, S., R.M. Toscana, G. Furnari and M. Cormaci, 1985. Antimicrobial activities of red and brown algae from southern Italy coast. *Botanica Marina*, 28: 505-508.
- [3] De Campos-Takaki, G.M., M.B.S. Du, M.I. Koenig and E.C. Pereira, 1988. Screening of marine algae from Brazilian north east coast for antimicrobial activity. *Botanica Marina*, 31: 375-377.
- [4] Govenk, M.B. and S. Wahidulla, 2000. Constituents of *Chondria armata*. *Phytochemical*, 54: 979-981.
- [5] Juicy Abraham, et. al., Hepatoprotective and anti inflammatory activity of marine red algae. *Bioscience Discovery*, 9(2);232-236, April-2018.
- [6] Murata, M. and Nakazoe, J. (2001) Production and Use of Marine Algae in Japan. *Japan Agricultural Research Quarterly*, 35, 281-290.
- [7] Naqvi, S.M., V. Solimabi, S.Y. Kamal, L. Fernandes and C.V.G. Reddy, 1981. Screening of some marine plants from the India coast for Biological activity. *Botanica Marina*, 24: 51-55.
- [8] Somepalli Venkateswarlu, Gopala K. Panchagnula, Aditya L. Gottumukkala, Gottumukkala V. Subbaraju, 2007. Synthesis, structural revision, and biological activities of 4-chloroaurone, metabolite of marine brown alga *Spatoglossum variabile*, *Tetrahedron*, 63, 29, 6909-6914.
- [9] Vidyavathi, N. and K.R. Sridhar, 1991. Seasonal and Geographical variation in the antimicrobial activity of seaweeds from the Mangalore coast of India. *Botanica Marina*, 34: 279-284.



10.22214/IJRASET



45.98



IMPACT FACTOR:
7.129



IMPACT FACTOR:
7.429



INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY

Call : 08813907089  (24*7 Support on Whatsapp)