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RESEARCH ARTICLE

AN INVESTIGATION ON BIOCHEMICAL AND ANTIMICROBIAL ACTIVITY OF MARINE RED ALAGE *Polysiphonia dIchotama*

N.SIVAKUMAR[1] AND M. THANIGAIMALAI[2]

[1]Assistant Professor, Department of Biology, College of Science, Sultan Qaboos University, P. O. Box 36,PC 123, Al-Khodh, Muscat, Sultanate of Oman
[2]Department of microbiology, King nandhivarman College of Arts and Science, Thellar – 604 406. T.V.malai, Tamil Nadu, India.
. Corresponding Author: apnsiva@squ.edu.om/apnsiva@yahoo.com

Abstract

The present investigation were made in the Red seaweed of *Polysiphonia dichotoma* is commonly found in East Coast region of Muthupet mangrove.The antibacterial properties of acetone extracts of *Polysiphonia dichotoma* were used against 4 types of ram positive and Gram negative bacterial strains and 3 types of fungal strains. The fresh and dried algal sample were used for obtaining extracts with the help of suitable Solvent.(Acetone, petroleumether, and benzene) . In this study solvent extract of algae were used in disc diffusion, agar well method against the pathogenic bacteria and fungi. The solvent extract of(Acetone, petroleum ether, benzene) fresh sample of *Polysiphonia dichotoma* in disc diffusion method shows a prominent inhibitory zone ranging from 17 –18mm in diameter against *Staphylococcus aureus* and *Salmonella typhi*. The solvent extracts of dry sample of *Polysiphonia dichotoma* by disc diffusion method, of the anti-fungal activity was found to be maximum for *A. fumigatus* and *A. sulphreus* . In agar well method, the dry ethanol extract of *Polysiphonia dichotoma* shows the prominent anti-fungal activity against for *Aspergillus sulphureus.*

Keywords: *Polysiphonia dichotoma ,* solvent extract, anti-bacterial, anti- fungal activity.

Introduction

Algae are a major source of food in the aquatic environment. It is commonly used as a food supplements to the humans and feed materials to the domestic animals. Further, it has been used as a source of biogas production and raw materials for the salad preparation. They are commonly distributed on shallow and sheltered areas of coastal regions. About 40% of marine environment covered by algal population. It includes green, red and brown algae. More than 1, 50000 species of macro algae are found in the oceans of the globe but only few of them were isolated from fresh water ecosystem. Some members of algae produced secondary [or] primary metabolites and this organism have to potentially enrich the bioactive compounds of interest to the pharmaceutical industry. Now days special attention have been made by many phychologist in the seaweeds for getting antiviral, antibacterial and antifungal compounds related to controlling of pathogenic microbes.

Majority of Rhodophyceae are marine, and mostly found in sublittoral zones. When water is clear they can penetrate several meters of depth. Certain species of *Gigartina, Laurencia, Lomentaria* from extensive belts in littoral zones, C*atenella* and B*ostrychia* are found in saline waters.

IJCRCPS

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Several Rhodophyceae are present on the roots of mangroves vegetation. The red algae are commonly found in the warmer seas of Australia and Asia. Some species of red algae are found in polar seas in the depth of 30 to 92 metres. Even few red algae have been reported from the depth of 200 metres. Majority of red algae are lithophytes and some of epiphytes e.g., *polysiphonia violacea* is epiphytic on Fucus vesiculosus. True parasitic forms have also been reported from red algae for example, *Harveyella bachyderma* is parasitic upon G*racilaria confervoides*. Polysiphonia fastigiata is parasitic upon A*scophyllum nodosum*. The photosynthatic tissue is greatly reduced in such forms some times they are quite colourless. About 50 species of 19 or more genera are fresh waters in habit.

Yamomoto *et al.,* (1983) was reported that the fucodin fraction of brown seaweed which has activity against Leukemia infections. Zeng Chengkui and Zhang Junfu (1984) observed the seaweed used as a herbal medicine in China. The large scale screening of red, green and brown seaweed were also studied. Rechelt and Borowwitzka (1984) was showed prominent antibacterial activity in brown algae extracts. Biological activity of sodium alginate, Fucoidan and Laminarin in mice has been studied by Mayer and Panick (1984). They also have reported that *Macrocystis pyrifera*, brown algae from which the above mentioned bioactive compounds found in their extracts and tested against antitumor, cytotoxic and humoral response in mice. Oliver and Wieir (1985) studied the effect of *Pseudomonas* alginate in rat alveoli. According to Woods and Bryan (1985) were studied that the effect of brown seaweed alginate compounds against *Pseudomonas aeruginosa* infections.

Skf Hagul *et al., (*1996) was studied in broad spectrum antimicrobial activity against Gram negative, Gram positive bacteria, yeast and fungi. Aithal *et al*., (1996) was studied the *invitro* antibacterial activity of the complex on *E.coli* and *Staphylococcus aureus* was better then that of plain drug as evidenced from the reduction in MIC value. Silva *et al*., (1996) proposed the use of Muller Hinton Agar for the assay of antibacterial activity of plant extract. Taylor *et al*., (1996) suggested that tannins are considered at least partially responsible for the antibiotic activity of methanolic extracts of the some medicinal plants.

Nakano and Noro,(1997) described the intropromoting activity of human β-interferon production by extracts of marine algae in Japan. Sujatha G Dastidar *et al*., (1997) studied Antimicrobial action of penicillin and some of its derivatives including Fosfomycin was studied with respect to 225 strains of Gram positive and Gram negative bacteria. Suresh Gunasekaran and Muthukumaran Gunasekaran(1997) was studied antifungal activity of *Aspergillus fumigatus* to a series of Alfa,beta-unsaturated styryl ketones known to be thiol-alkylaters was examined, and the result were compared with those obtained for *Candida albicans*.

Yoshikawa *et al*., (1997) studied a antibiotic named as korormicin was isolated from the marine bacterium, *Psedoalteromonas* sp.f-420. The strain was isolated from the surface of the macro alga of *Halimeda sp*. Mearns-spragg, *et al*., (1998) was described the production of antimicrobial compounds by marine bacteria can be induced by the presence of terrestrial bacteria .

M.Naviner *et al*., (1998) was studied the active compounds, partialy purified from an organic phase,were tested against some pathogens of shellfish or fish. Several species of the genus *vibrio* appeared to be inhibited. Vikas Dhingra *et al*., (1999) was Studied the Artimisinic acid and arteannuin B are biogenetic precursors of artimisinin, an important type of antimalarial compounds produced by the herb *Artemisia*

IJCRCPS

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annua. These compounds have been screned for antimicrobial activity against the different range of organisms. Osterhage *et al., (*2000). Studied the ascosalipyrrolidinone A(1) has antiplasmodial activity toward *falciparum* strains K1 and NF 54 ,as well as antimicrobial activity and inhibiting tyrosine kinase p561ck.

Numerous synthetic drugs are used to cure various microbial diseases of man. Allopathic medicinal system offer immediate cure against disease due to the presence of active principle in it. But at the same time it has adverse actions. Homeopathic medicine did not give immediate remedies but gives permanent solution. Over dosage of drugs can produce side effects but algae did not give any side effects. Algae are promising strains for the production of feed, fine chemicals and pharmaceutical compounds. The present study was initiated to find out the biological active compounds and their antimicrobial activity with the following objectives: Culturing of marine red algae *P.dichomata,* Analysis of biologically active compounds such as Protein, Carbohydrate, Vitamin B2 (riboflavin) and Amino acid (Lysine);and To study the antibacterial activity of solvents extract of algae using Disc diffusion and agar well methods at various concentrations.

Materials and methods

Collection of marine red algae

The marine red algae *Polysiphonia dichotoma* was collected from the mangrove area of Muthupet at Thiruvarur district. During the time of sample collection, the temperature of water and pH were recorded. The fresh algae were attached on the submerged rock area. The *Polysiphonia dichotoma* were carefully collected from the substratum by using the sterile scalpel and placed in sterile polythene bags and brought into the laboratory. The algal sample was thoroughly washed several times with distilled water and stored in refrigerator for further study.

Identification marine red algae

The identified algal samples were comes under the group of Alginophytes, namely *Polysiphonia dichotama*.

Analysis of biological compounds of *Polysiphonia dichotoma*

Protein estimation (Lowry's *et al*., 1951)

Extraction of sample:

500mg of the sample was grounded with a pestle and mortar in 5-10ml of the buffer. The sample was centrifuged and the supernatant was used for protein estimation.

Estimation of protein:

0.2ml of the sample was taken in the test tube. The volume made up to 1ml with distilled water. 5ml of alkaline copper solution was added. The test tube was mixed and allowed to strand for 10minutes. 0.5ml of folin reagent was added and mixed well. The tube was incubated at room temperature in the dark for 30minutes. The reading was taken at 660nm.

Estimation of lysine (Mertz *et al*., 1975)

100mg of sample and 5ml of papain solution were incubated overnight at 65°C. The solution was cool to room temperature and centrifuged. The clear digest was decanted. 1ml of digest was taken and centrifuged with 0.5ml of carbonate buffer and 0.5ml of copper phosphate suspension. The mixture was shaken for 5minutes in a vortex and centrifuged, To 1ml of supernatant.0.1ml pyridine reagent was added and mixed well. 5ml of 1.2N HCl was added and mixed. The solution was extracted with 5ml ethyl acetate for 3 times and discard the top layer.The absorbance was read at 390nm.

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Estimation of total cabohydrate by phenol sulphuric acid method (Dubois *et al.,*1956)

100mg of the sample was weighted into boiling tube. Hydrolyse by keeping it in boiling water bath for three hours with 5ml of 2.5N- HCl and cool to room temperature. Neutralize it with solid sodium carbonate until the effervescence ceases. The volume made up to 100ml and centrifuged. 0.2ml of the supernatant was taken in test tube. 1ml of phenol solution was added. 5ml of 96% sulphuric acid was added. The content was shaken for 10minutes and placed in a water bath at 25-30°C for 20minutes. The colour was read at 490nm.

Estimation of Riboflavin (AACC,1976)

2g of sample were placed in conical flask containing 75ml of H_2SO_4. The flask was immersed in boiling water bath for 30minutes. The flask was shaken for every 5 minutes. 5ml of sodium acetate solution was added and mixed. The volume made up to 100ml with distilled water. The content was filtered through medium- fast paper (Whattman No.2). 10ml of the sample was taken and 2ml of water was added. 0.5ml of potassium permanganate was added. After 2 minutes, 0.5ml of hydrogen peroxidase was added. The fluorimeter was set and fluoroscence was measured.

Antimicrobial activity study of *Polysiphonia dichotoma*

Bacterial cultures used

In our study strains Four bacterial members were used among these bacteria culture out of two in Gram-positive bacteria and another two are Gram negative bacteria. Gram positive bacteria: *Bacillus subtilis, Staphylococcus aureus* , Gram negative bacteria *Escherichia coli, Salmonella typhi*

Preparation of algal extract

2g of fresh algae were weighed and cleaned with distilled water.Then it was grounded by using morter and pestle with 10ml of all the solvents.The algal extract were obtained by filtration with Whatmann No.1 filter paper and the filtered extract were concentrated by exposing them in a laminar air flow chamber and kept at refrigerator for further study.

Preparation of dry algal extract

The algal samples were shade dried and make a fine powder by using morter and pestle.2g of dry algal powder were mixed with 10 ml of suitable solvent. (Acetone, Benzene and Petroleum ether). They are kept in two days at room temperature.The extracts were filtered by whatmann No.1 filter paper. The collected extracts were concentrated by employing in a laminar airflow chamber and kept in a room temperature. The filtrates were used for the disc preparation.

Preparation of antibiotic disc:

The extracts of seaweed samples were incorporated into sterile disc, punching machine. Each sterile disc was incorporated individually with 50, 100,150 and 200µl of the extract by using micropipette. This can be achieved by adding small quantity of extracts and the disc were allowed to dry in laminar airflow chamber. Another dose of the extract was applied to already prepared disc. Control was maintained by adding extracting solvent on the discs.

Assay of antimicrobial activity:

Assay of antibacterial activity of the algae extracts were done by Disc diffusion, Agar well techniques. Antibacterial activity of extract by Disc diffusion method: (Kirby-Bauer *et al*., 1960)

IJCRCPS

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Sterile Muller Hinton agar medium was prepared in sterile petriplates. After solidification, the test bacterial cultures were inoculated by means of agar swab method using sterile cotton swab. The prepared extract discs were placed on the surface of the plates of seeded bacterial cultures. Control (Acetone) and standard (Ampicillin) were also maintained. The plates were incubated at 37°C for 24 hours. After incubation the zone of inhibition was measured and expressed as mm in diameter.

Antifungal activity of solvents extracts by agar well method

Sterile MHA plates were prepared. After solidification, the test bacterial cultures were inoculated by means of swab method using sterile cotton swab. Using sterile cork borer, the agar well (5mm size) was prepared at respective place in Muller Hinton Agar plates. The prepared extract of different microlitre (50, 100,150 and 200µl) were loaded in well containing Muller Hinton Agar plates by using micropipette. Control was also maintained with the solvent acetone. The plates were incubated at 37°C for 24 hours. After incubation the zone of inhibition was measured and expressed in mm in diameter.

Results

The present study was revealed that the red algae *Polysiphonia dichotoma* (Plate. 1) were used for the morphological, phytochemical and antimicrobial activity.

Morphological features of *P.dichotoma*

Polysiphonia morphologically shows various in colour from red to purple. They have two type of thallus system namely (1) creeping basel system (2)vertical (or) erect system. The *Polysiphonia* is a member of marine red algae. They differ from their size and colour to one species to another. It contains discoid chromatophores. They stored food material is namely floridean starch. After the photosynthetic activity. Polysiphonia reproduce either vegetatively or sexually.

Bio- chemical features of *P. dichotoma*

The member of *Polysiphonia* contain different type of cell wall components, growth regulators and bio-chemical contents. The algal cells have a distinct cell wall, which made up of pectin substances. The inner region of the cell wall consist of alginic acid, fucodian granules and laminarin etc., The component and fucodian fractions of *P.dichotoma* having antitumor activity. The present study was carried out to analyze the biological compounds of *P.dichotoma* and its antibacterial activity against bacterial pathogens.

Analyses of biological compounds:

Analysis of Protein, Carbohydrate, Crude Fiber, Vitamin B2 and Lysine were determined from *P.dichotoma* and tabulated in Table.1 and Figure.1.

Protein content: The protein content of *P.dichotoma* was found to be 65mg/g.

Carbohydrate content: The Carbohydrate content of *P.dichotoma* was found to be 18 mg/g.

Vitamin B12 Content:The Vitamin B12 content of *P.dichotoma* was found to be 106 mg/kg.

Lysine content: The lysine content of *P.dichotoma* was found to be 31.50 g/kg.

Antimicrobial activity in *Polysiphonia dichotoma*

The antagonistic effects of the extracts in *Polysiphonia dichotoma* against different pathogenic group of bacteria and fungi. It includes both Gram positive and Gram

IJCRCPS

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www.ijcrcps.com Volume 1 Issue 1. 2014 Pages: 112-124
(pISSN: 2348 – 5213; eISSN: 2348 - 5221)

Table.1 Analysis of biological compounds:

Biological compounds	Amounts in mg & g
Protein	65 mg/g
Carbohydrate	18mg/g
Vitamin B2 (Riboflavin)	106mg/ kg
Lysine	31.50g/kg

Table.2 Antibacterial activity in acetone extract of fresh *Polysiphonia dichotoma* by disc diffusion technique

S.No	Name of Bacteria	Control	Zone of inhibition (Represented by mm in diameter)			
			50«l	100«l	150«l	200«l
1	*Bacillus subtilis*	--	8	12	14	12
2	*Staphylococcus aureus*	-	--	10	9	13
3	*Escherchia coli*	-	7	7	8	11
4	*Salmonella tuphi*	-	8	9	8	9

Table.3 Antibacterial activity in petroleum ether extract of fresh *Polysiphonia dichotoma* disc diffusion method

S.No	Name of Bacteria	Control	Zone of inhibition (Represented by mm in diameter)			
			50«l	100«l	150«l	200«l
1	*Bacillus subtilis*	--	--	9	12	16
2	*Staphylococcus aureus*	--	8	12	17	14
3	*Escherichia coli*	--	9	18	12	9
4	*Samonella typhi*	--	--	15	18	18

Table.4 Antibacterial activity in benzene extract of fresh *Polysiphonia dichotoma* by disc diffusion technique.

S.No	Name of Bacteria	*Control*	Zone of inhibition (Represented by mm in diameter)			
			50«l	100«l	150«l	200«l
1	*Bacillus subtilis*	--	8	11	10	10
2	*Staphylococcus aureus*	--	9	11	12	10
3	*Escherichia coli*	--	7	8	8	12
4	*Salmonella typhi*	--	8	14	15	18

IJCRCPS

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Table.5 Anti-fungal activity in acetone extract of dry *Polysiphonia dichotoma* by disc diffusion method.

S.No	Name of the fungi	Control	Zone of Inhibition (Represented by mm in diameter)			
			50«l	100«l	150«l	200«l
1	*Aspergillus fumigatus*	--	18	21	20	23
2	*Aspergillus sulphreus*	--	16	20	18	21

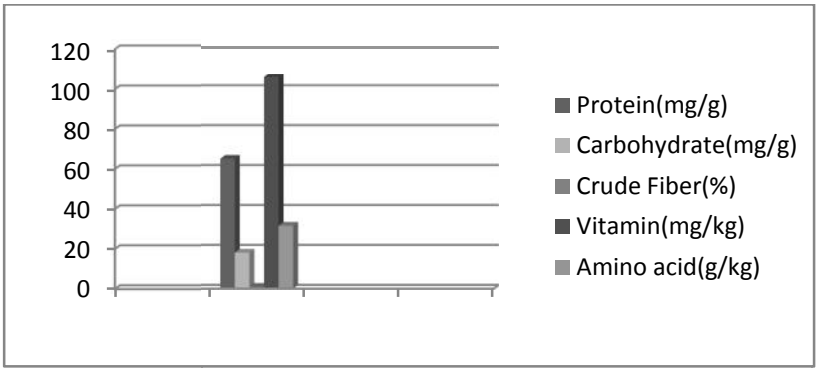
Table.6 Anti-fungal activity in petroleum ether extract of dry *Polysiphonia dichotoma* by disc diffusion technique.

S.No	Name of fungai	Control	Zone of inhibition (Represented by mm in diameter)			
			50«	100«	150«	200«
1	*Aspergillus fumigatus*	--	12	18	17	18
2	*Aspergillus sulphreus*	--	16	20	18	17

Table.7 Anti-fungal activity in benzene extract of dry *Polysiphonia dichotoma* by disc diffusion method.

S.No	*Name of the fungi*	Control	Zone of inhibition (Represented by mm in diameter)			
			50«L	100«L	150«L	200«L
1	*Aspergillus fumigatus*	-	12	18	17	18
2	*Aspergillus sulphreus*	-	16	20	18	17

Figure. 1 Analysis of biological compounds



International Journal of Current Research in Chemistry and Pharmaceutical Sciences
www.ijcrcps.com Volume 1 Issue 1. 2014 Pages: 112-124
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Figure 2: antibacterial activity in acetone extract of fresh *Polysiphonia dichotoma* by disc diffusion technique

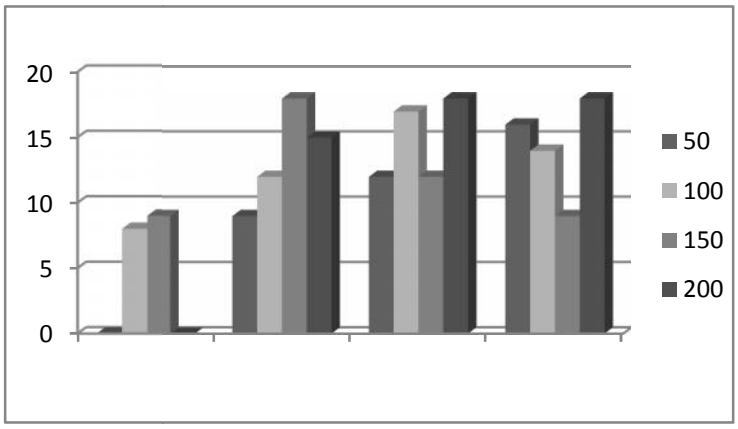

Figure 3: antibacterial activity in petroleum ether extract of fresh *Polysiphonia dichotoma* by disc diffusion method

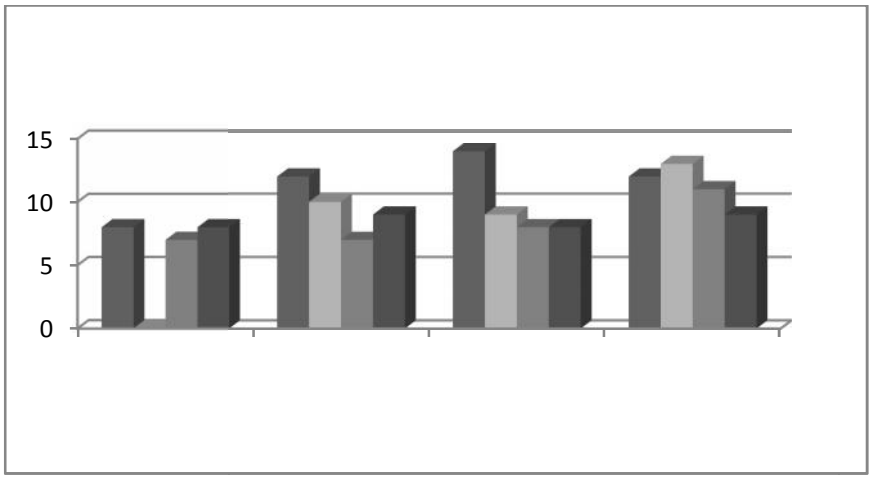

Figure 4: antibacterial activity in benzene extract of fresh *Polysiphonia dichotoma* by disc diffusion technique.

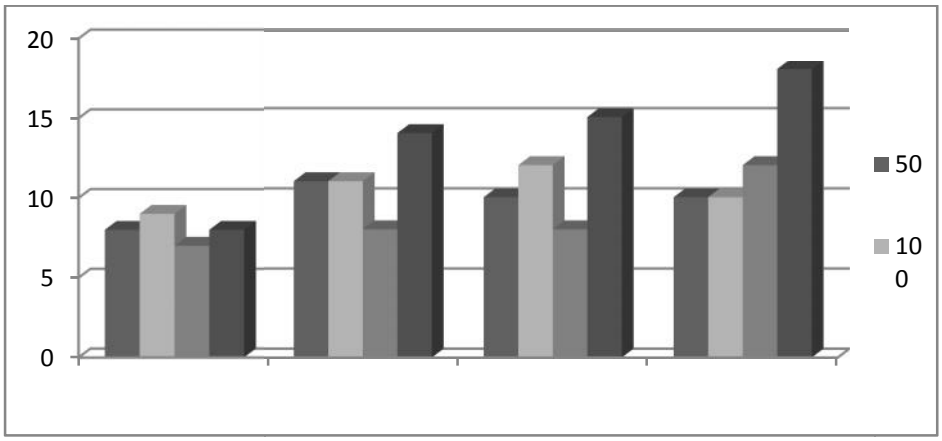

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Figure :5 anti-fungal activity in acetone extract of dry *Polysiphonia dichotoma by disc diffusion method.*

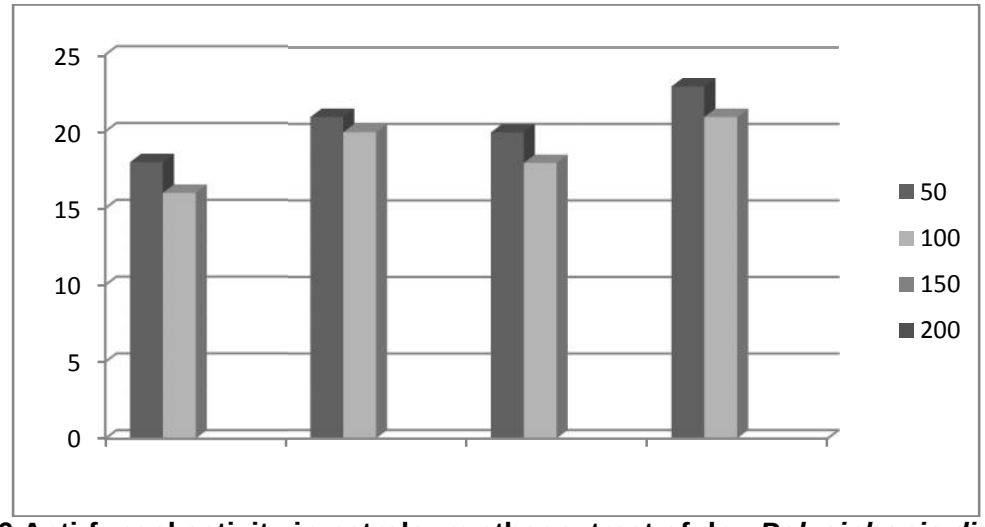


Figure. 6 Anti-fungal activity in petroleum ether extract of dry *Polysiphonia dichotoma* by disc diffusion technique.

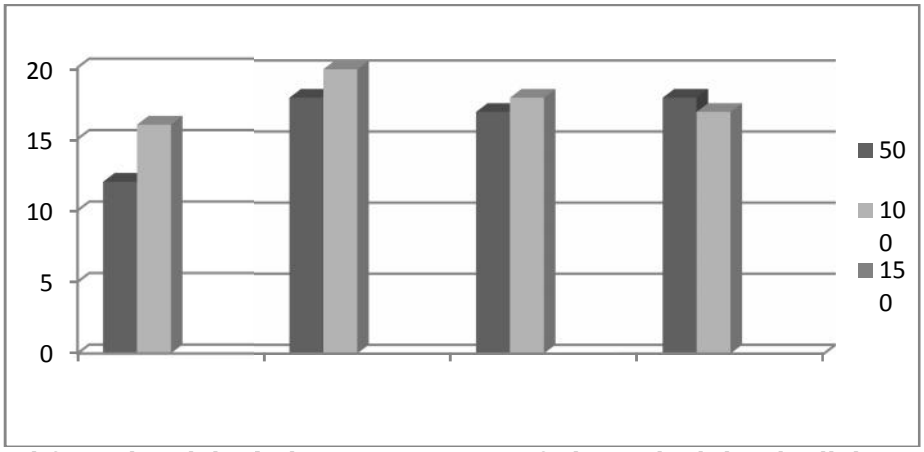
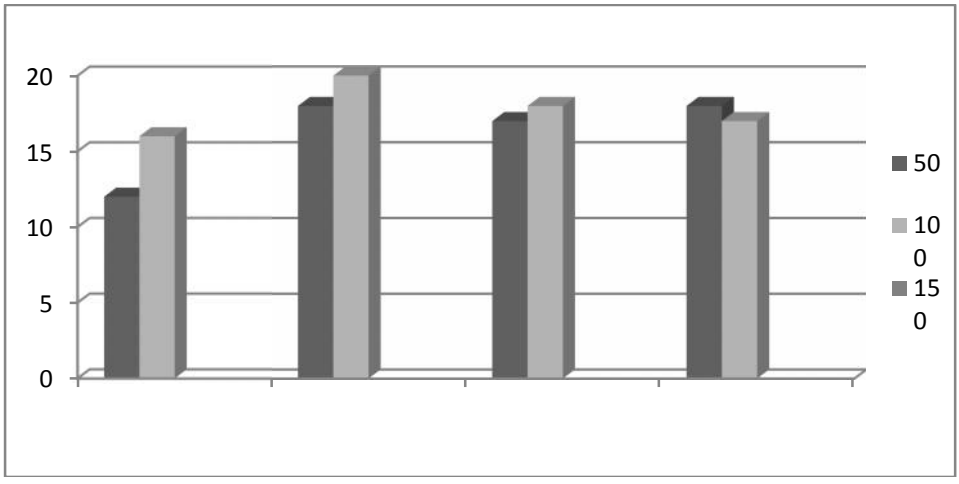


Figure :7 Anti-fungal activity in benzene extract of dry *Polysiphonia dichotoma* by disc diffusion method.



IJCRCPS

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negative bacterial cultures and *Aspergillus* and *Penicillium* cultures were used. Comparative studies on the bacterial and fungal activity were made by using crude fresh and dried algal extracts of *Polysiphonia*. The antimicrobial activity were observed from algal samples by using disc diffusion and agar well method (Plate 2 & 3).

Antibacterial activity of acetone extract of *Polysiphonia dichotoma* (fresh samples)

The crude extract of P*olysiphonia dichotoma* in disc diffusion method shows a prominent inhibitory zone ranging from 16 – 18mm in diameter against *Staphylococcus aureus* and *Salmonella typhi*. The same algal crude extracts were showed lowest inhibitory activity for *Staphylococcus aureus* (17mm) and *Escherichia coli* (18mm). This was represented in Table 2.Fig 2.

Antibacterial activity were performed by fresh petroleum ether extract of *Polysiphonia dichotoma*.

The crude extract of *Polysiphonia dichotoma* assayed in disc diffusion method. It shows a prominent inhibitory zone ranging from (9-14)mm in diameter against *Bacillus subtilis* and *Salmonella typhi* and algal crude extract were showed lowest inhibitory activity for *Staphylococcus aureus* (13mm)and *Escherichia coli* (11mm). (Fig. 2 & 3) This was represented in table 3.fig 3. 5.4.3. Antibacterial activity were observed from fresh benzene extract of *Polysiphonia dichotoma.*

The extract of *Polysiphonia dichotoma* in disc diffusion method shows a prominent inhibitory zone ranging from (12-18)mm in diameter against *Salmonella typhi* and *Staphylococcus aureus* the same algal crude extract were showed lowest inhibitory activity for *Bacillus subtilis* (11mm) and *Escherichia coli* (12mm). This was represented in table 4.Fig 4.

Anti-fungal activity in acetone extract of dry sample of *Polysiphonia dichotoma*

The acetone extract in dried samples of *Polysiphonia dichotoma*, the anti-fungal activity was found to be maximum zone of inhibition against *A.fumigatus* (23mm). The lowest activity was found (21mm) in diameter for *A.sulphreus* respectively (Table.5, Fig 5). Anti-fungal activity in petroleum ether extract of dry sample on *Polysiphonia dichotoma.* The petroleum ether extract of dried of *Polysiphonia dichotoma* shows the anti –fungal activity was found to be maximum zone of inhibition against *A.sulphreus* (20mm). The lowest was showed (18mm) in diameter for *A.fumigatus* respectively (Table 6, Fig 7).

Anti-fungal activity in benzene extract of dry sample on *Polysiphonia dichotoma*

The benzene extract of dried sample for *Polysiphonia dichotoma* shows anti-fungal activity was showed maximum zone of inhibition against *A. sulphreus* (20mm) diameter. The lowest anti-fungal activity was found in (18mm) diameter for *A. fumigatus* (Table 7. Fig 7).

Discussion

The present investigation was correlated with the studies done by Padmini sreenivasa Rao (1998) extracted antibacterial compounds from marine algae showed antibacterial activity against pathogenic bacteria. Certain marine algae collected from the rocky shore of Vishakhapatanam which have been screened for their antimicrobial activity by disc diffusion method. The crude extract of Algal species at 5 mg concentration formed zone of inhibition ranging from 10- 15mm against *Salmonella typhimurium* were reported by Vinella and Elizabeth (2005).

Screening of the antibacterial activity of phlorotannins extracted from brown algae

and tested against food borne illness-causing bacteria. The phlorotannins which are oligomers of phloroglucinol compounds extracted from thalli of brown algae *Ecklonia kurome* was reported by Kokinagayama *et al*., (2002).

Sargassum pilgiophylum and *S.tenerrimum* have best antimicrobial activities against several test bacteriaexperimentally proved by Naqui *et al*., (1981). Suresh Gunasekaran and Muthukumaran Gunasekaran(1997) was studied antifungal activity of *Aspergillus fumigatus* to a series of Alfa,beta-unsaturated styryl ketones known to be thiol-alkylaters was examined, and the result were compared with those obtained for *Candida albicans*.

In our study various degrees of activity were present in 18 out of the 24 algae extracts. The highest activity was rhodophyta diameter of the inhibited zone ranged from 10- 22mm the lowest was found in chlorophyta 8-12mm. Mahasneh.l *et al*., (1995). In our study activity the present in east and fungi.. MIC of the antibiosis varied from 20-100 mg/ ml.(Hagul *et al.,* 1996). In the study of in vitro antibacterial activity of the complex on *E.coli* and *Staphylococces aureus* was better than that of plain drug as evidenced from the reduction in MIC value. (Aithal *et al*.,1996). Antimicrobial action of penicillin and some of its derivatives including fosfomycin in our study used gram positive and gram negative bacteria (Sujatha G Dasdiar *et al.,*1997). In our study used for antifungal activity of *Aspergillus fumigatus* to a series of series of result were compared with those obtained for *Candida albicans* . Suresh Gunasekaran (2002).

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