

ANTIMICROBIAL EVALUATION OF THE ORGANIC EXTRACTS OF SARGASSUM WIGHTII (BROWN ALGAE) AND KAPPAPHYCUS ALWAREZII (RED ALGAE) COLLECTED FROM THE COAST OF MEEMESAL, TAMILNADU

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ABSTRACT

Marine organisms are rich source of structurally novel and biologically active metabolites. Secondary or primary metabolites produced by these organisms may be potential bioactive compounds of interest in the pharmaceutical industry. The secondary metabolites of seaweed sources from oceans are also equally effective like the marine fauna. The present study was investigated to explore the antimicrobial effect of the organic extracts of the two seaweeds *Sargassum wightii* (Brown algae) and *Kappaphycus alvarezii* (Red algae). The solvent systems used for the preparation of the extracts are diethyl ether, acetone, petroleum ether and acetic acid. The extracts of both the seaweeds are concentrated in a rotary evaporator and the concentrates were stored in refrigerator for further use. Trace amount of protein and strong positive results for carbohydrate content of the extracts were qualitatively analyzed. The extracts showed the presence of phytochemical constituents like alkaloids, phenols and sugars. Among the tested extracts, the maximum activity of 10mm was observed with acetic acid extract of *S. wightii* and almost all the tested microbes showed activity against the tested extracts. The presence of activity might be due to the presence of phyto components alkaloids, phenols and sugars.

Keywords: Seaweeds, *Sargassum wightii*, *Kappaphycus alvarezii*, antimicrobial activity.

INTRODUCTION

Pharmaceutical market is growing rapidly and continuously. But, still the demand for new drug discovery is encouraged. The reason behind this motivation can be the growing numbers of drug-resistant infectious disease and more and more upcoming disorders. The terrestrial resources have been greatly explored and thus academic and industry researchers are striving to get lead molecules from the inner space of oceans¹.

Marine organisms are rich source of structurally novel and biologically active metabolites. Secondary or primary

metabolites produces by these organisms may be potential bioactive compounds of interest in the pharmaceutical industry². To date, many chemically unique compounds of marine origin with various biological activities have been isolated, and some of them are under investigation and are being used to develop new pharmaceuticals. The sea source includes various organisms such as sponges, tunicates, seaweed, marine micro organisms, and symbionts etc. All the marine invertebrates are studied extensively because of their wealth of metabolites, which display many biological activities like anti inflammatory, anti

tumors, anti hypersensitive and antimicrobial effects³.

The secondary metabolites of seaweed sources from oceans are also equally effective like the marine fauna. Seaweeds belong to a group of plants known as algae. They grow almost exclusively in the shallow waters at the edge of the world's oceans. They provide home and food for many different sea animals, lend beauty to the underwater landscape, and are directly valuable to man as a food and industrial raw material. Seaweeds are classified as Rhodophyta (red algae), Phaeophyta (brown algae) or Chlorophyta (green algae) depending on their nutrient, pigments and chemical composition. Seaweeds are considered as a source of bioactive compounds as they are able to produce a great variety of secondary metabolites characterized by a broad spectrum of biological activities⁴.

The present study was investigated to explore the antimicrobial effect of the organic extracts of the two seaweeds *Sargassum wightii* (Brown algae) and *Kappaphycus alvarezii* (Red algae).

MATERIALS AND METHODS

Collection of Seaweeds

The seaweed samples *Sargassum wightii* (Brown algae) [Fig 1] and *Kappaphycus alvarezii* (Red algae) [Fig 2] were collected at the depth of 1-2m by scuba divers from the areas of Memesal coast, Pudukottai district, Tamil Nadu during the month of August. Algal samples were cleaned from epiphytes, extraneous matter and necrotic were removed. Samples were collected in sterilized polyethylene bags, and put in an ice box, then transferred to the laboratory immediately until the experimental work was done. Samples were washed thoroughly with sea water then sterile distilled water, air dried, cut into small pieces and then ground in a tissue grinder until reach fine powder.

Extraction of Seaweeds

Solvent extraction

The powdered seaweed was soaked in 25ml of organic solvents viz., Diethyl ether, Petroleum ether, Acetone and Acetic acid and incubated for 1hr to homogenize the

sample. Then it was filtered through Whatman No.1 filter paper. To the filtrate again add 25ml of respective solvents and incubate it for 1hr and then filter again. Collect the filtrate and concentrate by using rotary evaporator. Store the concentrated extract in refrigerator for further use.

Biochemical Characterization

Test for Carbohydrate

The presence of carbohydrate in the extracted seaweeds was tested using Molisch's test. Add two drops of Molisch's reagent to 2ml of seaweed extract in a test tube. Mix thoroughly. Add two drops of concentrated sulfuric acid by the side of the test tube slanting the tube. Then erect the test tube slowly. The formation of reddish violet ring at the junction of two liquids indicates the presence of carbohydrate.

Test for Protein

The presence of protein in the seaweed extract is determined by using Biuret test method. To 2ml of seaweed extract add 1ml of 40% NaOH solution and 1 or 2 drops 1% CuSO₄ solution. The formation of violet color indicates the presence of peptide linkage of the extract.

Phytochemical Characterization

Tests for Alkaloids

0.5g of extract was stirred with 5ml of 1 per cent aqueous hydrochloric acid on a water bath from that 1ml portion was treated with Dragendorff's reagent. Turbidity of precipitation showed the presence of alkaloids in the extract.

Test for Tannin

A few ml of the sample was taken in a boiling tube and vanillin reagent was added to it. Lack of color change showed the absence of tannin.

Test for Flavonoid

A few ml of sample was taken in a boiling tube and treated with magnesium turnings and few drops of concentrated hydrochloric acid. The lack of appearance of blue colour confirms the absence of flavonoids in the extract.

Test for Phenol

The sample was taken in small quantity that was treated with alcoholic ferric chloride. The yellow color formation indicates the presence of phenol.

Test for Sugar/Glucosides

A few ml of sample was taken in a boiling tube and mixed with equal quantity of anthrone reagent. Then it was treated with 2 drops of concentrated sulfuric acid and heated in a boiling water bath gently. The green color formation indicates the presence of sugar.

Test for Coumarine

A small quantity of sample was taken in boiling tube and treated with alcoholic sodium hydroxide. The formation of yellow color indicates the presence of coumarine and no yellow color formation showed the absence of coumarine.

TLC for seaweed extracts

The seaweed extracts were subjected to TLC in the solvent system toluene: ethyl acetate (9:1). The subjected TLC sheet was then observed under UV light. After that vanillin was sprayed on it and dried in the hot plate.

The crude extracts showed a number of compounds separated in TLC plates. The RF values for the separated compounds were noted as 0.667, 0.550, 0.400 for SW and 0.583, 0.413, 0.650 for KA. On comparing with standards the compounds separated are Phenols, Sugars and Alkaloids.

Evaluation of Antimicrobial activity**Agar Well Diffusion Method**

Antibacterial activity was determined against the above bacteria using the well bore assay method. The sterile bore was impregnated with different extracts (50 mg/ml). Agar plates were surface inoculated uniformly from the broth culture of the tested microorganisms. In all cases, the concentration was approximately 1.2×10^8 CFU/ml. The impregnated extracts were placed on the Muller Hinton medium suitably spaced apart and the plates were incubated at 37°C for 24h. Organic solvents were used as a negative control while commercial antibiotics (chloramphenicol,

10 mg/well and tetracycline, 30 mg/well) were used as a positive control⁵. The diameter of the growth inhibition halos caused by the organic extracts of marine organisms was measured by a ruler and expressed in millimeter.

RESULT AND DISCUSSION

The organic extracts of two seaweeds were assayed for antimicrobial activity by using agar diffusion method. Antimicrobial activity of these extracts is shown in the following table. (Table: 1)

In this investigation, Antimicrobial activity of *Sargassum wightii* extracts and *Kappaphycus alvarezii* extracts were found to active against all tested microorganisms. The maximum activity of diethyl ether extract of SW was shown against *Bacillus aureus*(9mm), *E.coli*(8mm), *Pseudomonas aeruginosa*(8mm), *Aspergillus flavus*(8mm), *K.Pneumonia*(7mm), and *Aspergillus fumigatus*(7mm). The maximum activity of diethyl ether extract of *Kappaphycus alvarezii* was shown against *S.aureus*(7mm), *Trichophyton rubrum*(8mm), *Microsporum gypsum*(7mm) and *E.coli*(7mm). The solvent extracts of KA also showed a very good antioxidant activity against some of the free radicals commonly present in our body⁷.

The maximum activity of *Kappaphycus alvarezii* were against some fungal pathogens and hopefully it can be used in the treatment of the fungal infections. The activity of the diethyl ether extracts of both the seaweeds were depicted in the graph (Fig: 5).

Effect of Petroleum ether extracts

The petroleum ether extracts of the seaweeds *S.wightii* and *K.alvarezii* also showed promising effects on all the tested bacterial and fungal pathogens. The maximum activity of the K.A extracts were measured on *E.coli* (9mm), *Streptococcus pneumonia*(8mm), *Vibrio cholerae*(8mm), *K.pneumoniae*(8mm), and the fungal pathogens *Aspergillus niger* and *A.flavus*(8mm). The maximum activity of 8mm were measured with the bacterial strains *Bacillus aureus*, *S.aureus*, *proteus* and the fungal strain *A.fumigatus* with S.W extracts. The *Sargassum* extracts of different

species were also found to be effective against *Bacillus subtilis*, *E.coli*, and *S.aureus*⁸.

The minimum activity of the petroleum ether seaweed extracts were found with the bacterial strains *Bacillus subtilis*, *Citrobacter*, and *P.aeruginosa*.(Fig: 6)

Effect of Acetone extracts of seaweeds

The acetone extract of KA showed maximum activity of 8mm against *Citrobacter* and the fungal strains *Microsporum gypsum* and *Trichophyton rubrum*. The SW acetone extract showed maximum activity of 8mm against *Bacillus aureus*, *P.aeruginosa* and *A.flavus*.The minimum activity of both the seaweeds were measured with some of the few bacterial and fungal strains (Fig: 7). *Microchaete tenera*, *Nitella tenuissima* seaweeds extract were also found to be effective against *A.flavus*, *Bacillus aureus* and *P.aeruginosa*⁹.

Effect of Acetic acid extracts of Seaweeds

The acetic acid extracts of the *Kappaphycus alvarezii* and *Sargassum wightii* are also found to be effective against most of the tested bacterial strains and fungal strains with the maximum activity of 10mm and 8mm. The results can be interrupted from the Table and the graph (Fig: 8)

CONCLUSION

The seaweeds extracts that were tested for its antimicrobial effect successfully showed the maximum zone of inhibition against most of the tested microorganisms. All the four solvent extracts were found to be effective against the pathogens. The maximum zone of inhibition was shown by the acetone extract of KA against *Pseudomonas aeruginosa* and diethyl ether extract of SW against *E.coli*. The scopes of using seaweeds in the development of new pharmaceutical agents are having a new hope in the present study. It will be better developed with the screening of phytochemical constituents for its antimicrobial research.

Table 1: Inhibition halo diameter of various organic extracts of some marine algae (*Kappaphycus alvarezii* and *Sargassum wightii*) against microorganisms

S. No.	Organisms	Zone of inhibitions of various organic extracts in mm							
		Diethyl ether Extract		Petroleum ether Extract		Acetone Extract		Acetic acid Extract	
		K.A	S.W	K.A	S.W	K.A	S.W	K.A	S.W
1	<i>Streptococcus faecalis</i>	5	7	6	5	5	5	6	5
2	<i>Streptococcus pneumonia</i>	6	5	8	6	5	6	7	8
3	<i>Bacillus subtilis</i>	6	5	6	4	5	7	5	5
4	<i>Bacillus aureus</i>	6	9	5	8	7	8	6	7
5	<i>Vibrio cholerae</i>	5	7	8	6	5	7	7	8
6	<i>Vibrio parahaemolyticus</i>	6	6	7	5	7	5	5	6
7	<i>E. coli</i>	7	8	9	6	6	7	7	6
8	<i>S. aureus</i>	5	7	7	8	5	6	6	6
9	<i>K.pneumonia</i>	6	7	8	7	7	6	5	8
10	<i>P.aeruginosa</i>	5	8	6	5	7	8	7	10
11	<i>Proteus</i>	7	5	7	8	6	5	6	7
12	<i>Citrobacter</i>	6	4	5	5	8	7	6	5
13	<i>Microsporum gypsum</i>	7	6	7	6	8	5	8	7
14	<i>Aspergillus niger</i>	5	6	6	5	6	5	6	8
15	<i>Aspergillus flavus</i>	5	8	8	5	6	8	7	7
16	<i>Trichophyton rubrum</i>	8	5	6	7	8	5	5	6
17	<i>Aspergillus fumigatus</i>	5	7	8	8	5	6	7	8



Fig. 1: *Sargassum wightii*



Fig. 2: *Kappaphycus alvarezii*

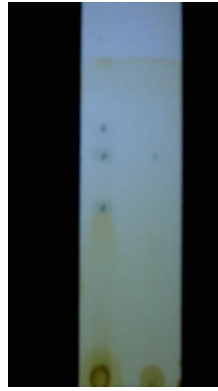


Fig. 3: TLC of seaweed extracts of *Sargassum wightii*



Fig. 4: *Kappaphycus alvarezii*

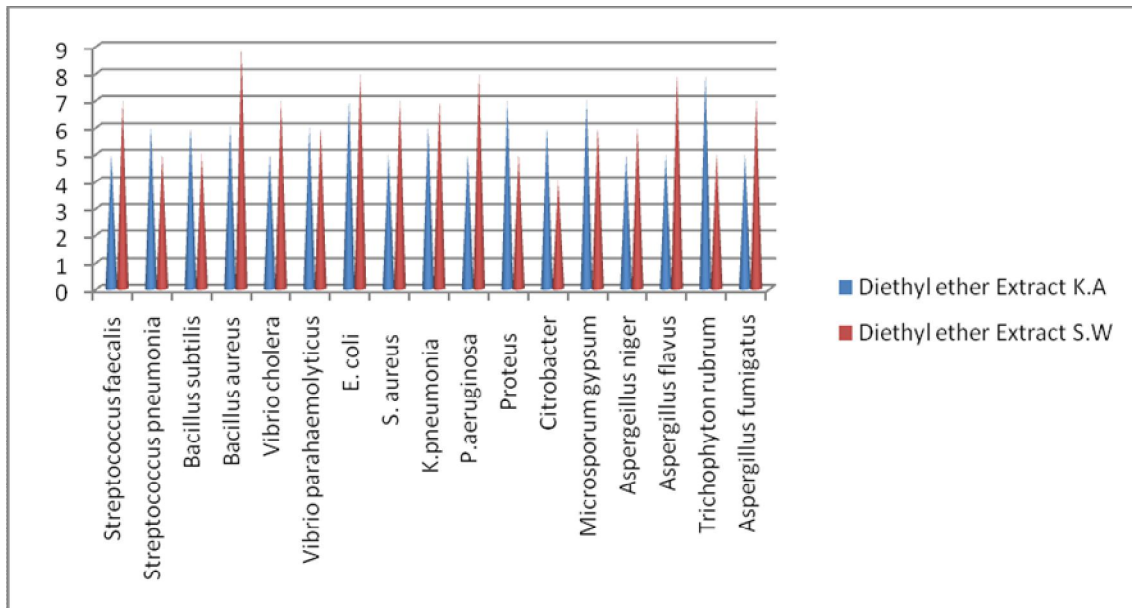


Fig. 5: Zone of inhibition (mm) of Diethyl ether extracts of *Kappaphycus alvarezii* (K.A) and *Sargassum wightii* (S.W) against tested organisms

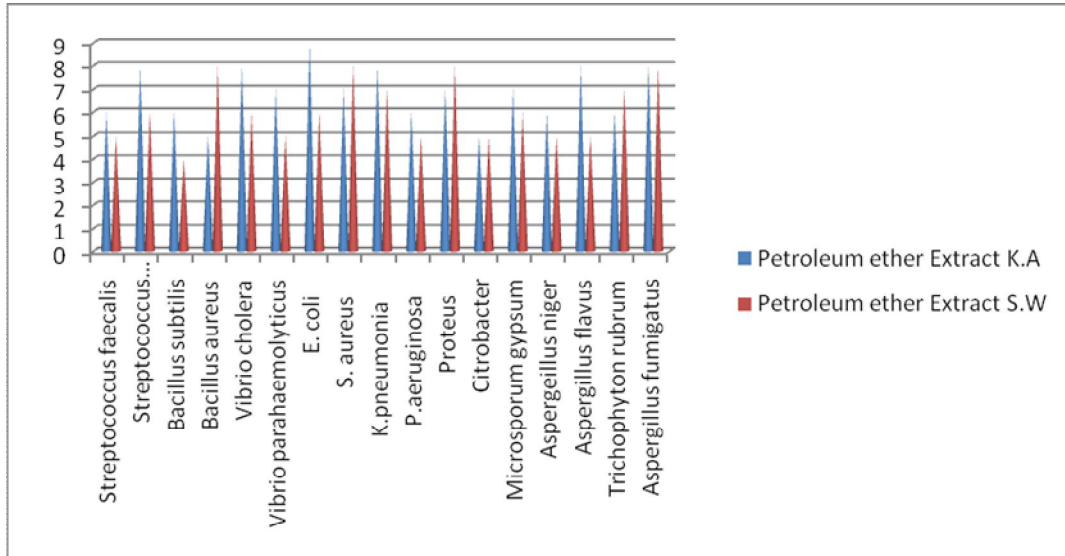


Fig. 6: Zone of Inhibition of Petroleum ether extracts of *Sargassum wightii* and *Kappaphycus alvarezii*

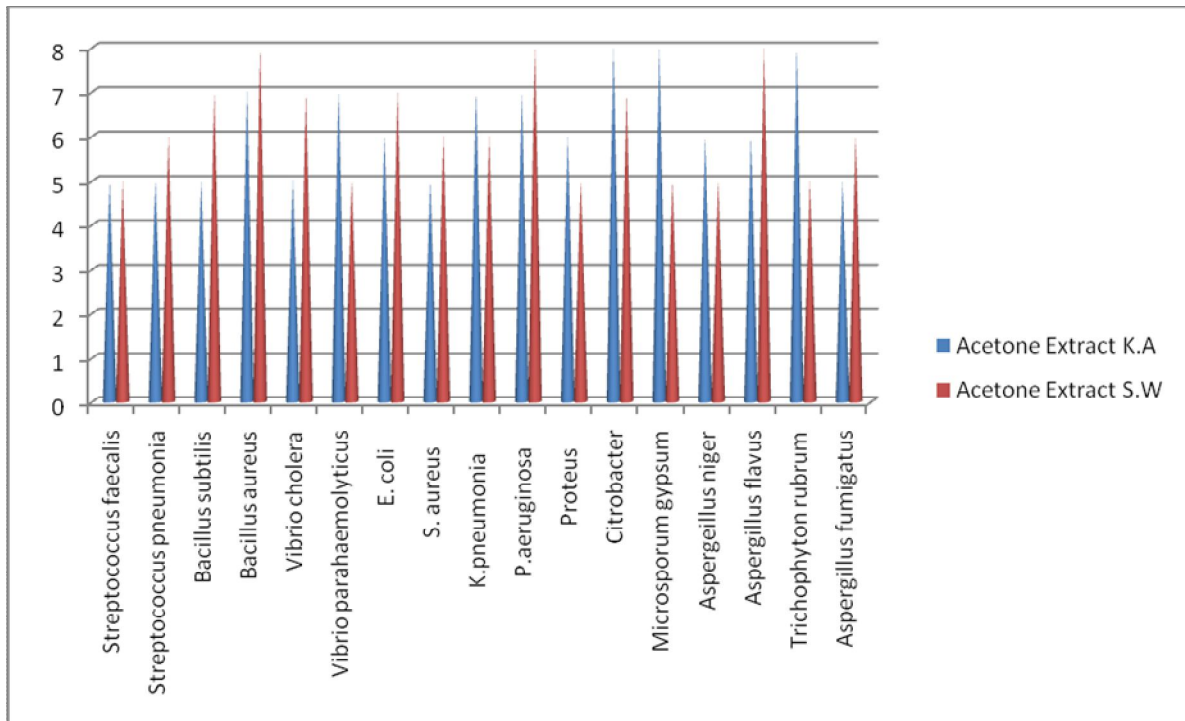


Fig. 7: Zone of Inhibition of Acetone extract of *Sargassum wightii* and *Kappaphycus alvarezii*

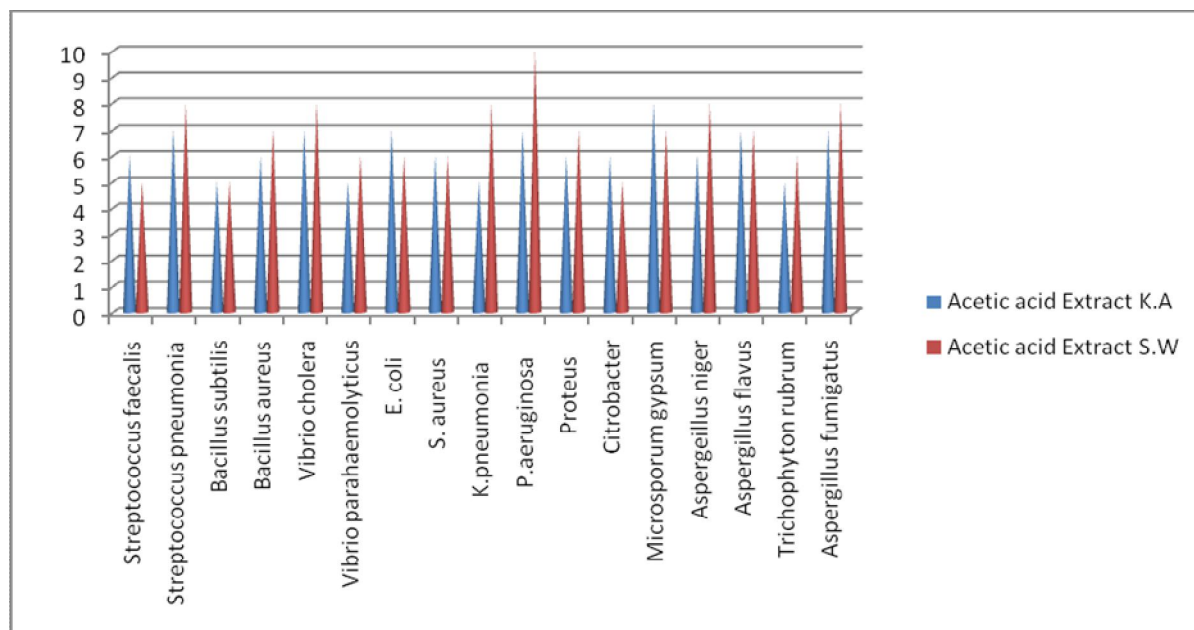


Fig. 8: Zone of Inhibition of Acetic acid extract of *Sargassum wightii* and *Kappaphycus alvarezii*

REFERENCES

1. Baker J.T. Seaweeds in pharmaceutical studies and applications. *Hydrobiologia*, 1984 116/117:29-40.
2. Santhanam, Shanmughapriya.et.al, Antimicrobial activity of seaweeds extracts against multiresistant pathogens. *Annals of Microbiology*, 2008, 58 (3) 535-541.
3. K. Vallinayagam, R. Arumugam, R. Ragupathi Raja Kannan,G. Thirumaran and P. Anantharaman .Antibacterial Activity of Some Selected Seaweeds from Pudumadam Coastal Regions. *Global Journal of Pharmacology*, 2009, 3 (1): 50-52.
4. P. Rajasulochana, R. Dhamotharan, P. Krishnamoorthy, S. Murugesan. Antibacterial Activity of the Extracts of Marine Red and Brown Algae .*Journal of American Science* 2009:5(3) Pg no: 20-25.
5. E.Taskin, M.Ozturk, E.Taskin and O.Kurt.Antibacterial Activity of some marine algae from the Aegaen sea(Turkey). *African Journal of Biotechnology*, 2007, Vol.6(24),pp.2746-2751.
6. B. Hebsibah Elsie, M.S.DhanaRajan. Evaluation of antimicrobial activity and phytochemical screening of *Gelidium acerosa*. B.Hebsibah Elsie et al /*J. Pharm. Sci. & Res.*2010, Vol.2 (11),704-707.
7. K. Suresh Kumar, K. Ganesan, P.V. Subba Rao. Antioxidant potential of solvent extracts of *Kappaphycus alvarezii* (Doty) Doty – An edible seaweed, *Food Chemistry* 107 2008, 289–295.
8. Patra JK, Rath SK, Jena K, Rathod VK, Thatoi H. Evaluation of antioxidant and antimicrobial activity of seaweed (*Sargassum* sp.) extract: A study on inhibition of Glutathione-S-Transferase activity. *Turkish Journal of Biology*. 2008;32:119-125
9. Prashantkumar P, Angadi SB, Vidyasagar GM. Antimicrobial activity of blue-green and green algae. *Indian Journal of Pharmaceutical Sciences*. 2006; 68:647-648.
10. Pandurangan Aruna, Periasamy Mansuya, Sekaran Sridhar.

- Pharmacognostical and Antifungal activity of selected seaweeds from Gulf of Mannar region. Recent Research in Science and Technology 2010, 2(1): 115-119.
11. V. Vinoy Kumar and P. Kaladharan. Amino acids in the seaweeds as an alternate source of protein for animal feed. J.Mar.biol.asst.india, 49(1):35-40.
 12. Hee-Jung Bae .et.al, In vitro antibacterial and anti-inflammatory properties of seaweed extracts against acne inducing bacteria, *Propionibacterium acnes*. J. Environ. Biol. 2011, 32, 313-318.