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Review on Bioactive Potential in Seaweeds (Marine Macroalgae): A Special Emphasis on Bioactivity of Seaweeds Against Plant Pathogens

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INTRODUCTION

The term 'bioactive compound' is an expression in common use and includes compounds, which at low concentrations, may be either beneficial or harmful to living organisms. Generally, the term refers to secondary metabolites that have attracted the attention of both scientists and industrialists. Seaweeds have been identified as a rich source of bioactive compounds. Seaweeds constituting an important renewable marine resource occur generally on the rocky substratum in the intertidal and sub tidal regions of the coastal waters. They are the only source for the production of agar, algin and carrageenan. Ocean has been recognized as a store house of fine chemicals. Besides their traditional use as phycocolloids and liquid fertilizer, seaweeds also known to possesses compounds exhibiting antimicrobial potential against the pathogenic microbes of medical, agricultural and environmental importance. Pharmacologists, physiologists and chemists have been paying increasing attention to the marine organisms particularly on seaweeds for screening bioactive substances. Several works have been undertaken on crude and purified compounds obtained from seaweeds for evaluating their bioactive potential (Faulkner, 1992).

Works done for the last 3 decades were mainly on screening biologically active compounds in different seaweeds against various human pathogenic viruses, bacteria and fungi (Rao and Parekh, 1981; Glombitza and Klapperich, 1985; De Campos-Takaki *et al.*, 1988; Rao, 1990; Vidyavathi and Sridhar, 1991; Premnathan *et al.*, 1992; Kamat *et al.*, 1992; Robles-Centeno *et al.*, 1996; Sastry and Rao, 1994). In the present review, potential of crude and purified compounds obtained from the seaweeds tested against pathogens causing diseases in human and plant pathogens are compiled. Besides, a special emphasis on bioactive potential of seaweeds against the plant pathogen is addressed in order to exploit this renewable resource for crop protection in agriculture.

BIOACTIVITY OF CRUDE EXTRACTS

Hornsey and Hide (1974) screened 151 species of seaweed for the production of antibiotics. Of these, *Asparagopsis armata*, *Bonnemaisonia asparagoides*, *Bonnemaisonia hamifera*, *Chondrus crispus*, *Dilsea carnosa*, *Gloiosiphonia capillaris*, *Sphondylothamnion multifidum*, *Desmaretia aculeata*, *Desmaretia liqulata*, *Laminaria digitata*, *Dictyopteris membranacea*, *Dictyota dichotoma*, *Halidrys siliquosa* and most members of the family Rhodomelaceae exhibited high antibacterial potential against the test bacteria such as *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Streptococcus pyogenes* and *Proteus morganii*. The crude extracts obtained from the green alga *Chloroccocum humicoum* (Chlorophyceae) showed bioactivity against the pathogenic bacteria such as *Cryptococcus neoformans*, *Bacillus pumillus*, *Escherichia coli*, *Sarcina lutea*, *Bacillus subtilis* and *Staphylococcus aureus* (Pande and Gupta, 1977). Caccamese *et al.* (1979) reported the antibacterial potential of *Hypnea musciformis* against *Bacillus subtilis*. Rao and Parekh (1981) found that the crude extracts obtained from the green seaweeds such as *Caulerpa taxifolia*, *Caulerpa scalpelliformis*, *Halimeda tuna* and *Enteromorpha intestinalis*; brown seaweeds such as *Padina gymnospora* and *Dictyota dichotoma* and red seaweeds such as *Gelidiella acerosa*, *Gracilaria corticata*, *Chondria armata*, *Acanthophora delile*, *Laurencia papillosa* and *Coralline officinalis* showed considerable bioactive potential. Rao and Parekh (1981) reported that the crude extracts of brown seaweeds such as *Dictyota dichotoma* and *Dictyota* sp. were active against the Gram positive bacteria such as

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Bacillus megatherium and *Staphylococcus aureus* but not active against Gram negative bacteria tested. The crude extracts of *Zanardinia prototypus* and *Cystoseira balearica* exhibited the best antimicrobial and antiviral activities among the seaweeds tested, while extract obtained from *Lophocladia lallemandii* did not active against the test bacteria but had high antiviral potential. Reichelt and Borowitzka (1984) found that majority of the algal extracts showing antibacterial activity against Gram positive bacteria.

The crude extracts obtained from the red seaweeds such as *Falkenbergia rufolanosa* and *Laurencea obtusa* possessed strong inhibitory effect against several pathogenic bacteria tested whereas the extract of *Hypnea musciformis* showed a very weak activity against the test bacteria (Pesando and Carm, 1984). Some fractions obtained from the crude extracts of red, brown and green seaweeds showed optimum antibacterial activity against the test bacteria such as *Pseudomonas aeruginosa* and *Proteus vulgaris*. Unsaponifiable part of the lipid extracted in diethyl ether and saponifiable part of the lipid obtained in benzene from the brown alga *Sargassum johnstonii* exhibited more antibacterial activity against Gram positive and Gram negative bacteria tested (Parekh *et al*., 1984). Different concentration of the fractions isolated from the seaweeds showed antibacterial activity against the test bacteria *Staphylococcus aureus* and *Escherichia coli* (Parekh,1985). The distribution of bioactive potential in seaweeds was evaluated against some human pathogenic Gram positive and Gram negative bacteria (Ballantine *et al*., 1987). Among the seaweeds evaluated 63% of *Rhodophyta*, 71% *Phaeophyta* and 66% *Chlorophyta* showed antibacterial activity and 83% of species within the order *Dictyotales* showed bioactivity (Ballantine *et al*., 1987). Extracts of 12 different species of *Sargassum* were separated into two fractions and tested against 9 human pathogenic bacteria. Both fractions of *Sargassum vulgare* showed good antibacterial activity against Gram positive and Gram negative bacteria (Rao *et al*., 1988). Of the 35 seaweeds collected along the coast of Sri Lanka and screened against the human pathogenic bacteria (*Staphylococcous aureus* and *Escherichia coli*) and fungi (*Cladosporium cladosporoides* and *Candida albicans*), 26 species exhibited antibacterial and/or antifungal activity (Bandara *et al*., 1988). Crude extracts obtained in the diethyl ether from various parts, viz., fronds, ‘stems’ and air ‘bladders’ of *Sargassum johnstonii* screened for their antibacterial potential and found that the extracts of the frond portion showed more bioactivity than the stem and air bladders (Rao, 1990).

Studies conducted on 30 species of seaweeds collected along the coast of Mandapam, Tamil Nadu for their hemolytic and antimicrobial potential. Results indicated that extracts obtained from the seaweeds such as *Enteromorpha compressa*, *Cladophoropsis zoolingeri*, *Padina gymnospora*, *Sargassum wightii* and *Gracilaria corticata* showed antibacterial activity against the Gram negative bacteria and Gram positive cultures of *Bacillus*. A strong hemolytic activity was shown by the extract of *Cladophoropsi zoolingeri* and *Grateloupia lithophila* (Rao *et al*., 1991). Ethanolic extracts of Indian marine macro algae belonging to the Rhodophyceae, Phaeophyceae and Chlorophyceae were tested for anti-semiliki Forest (SFV), Ranikhet disease (RDV) and vaccinia viruses (vv). In the primary screening of 31 seaweeds, 17 showed biologically activity of which seven were anti-SFV and 10 were antivaccinia. None of them showed any activity against RDV. The antiviral activity observed in *Codium elongatum* and the two species of *Hypnea* was attributed to the polysaccharides. The palmitoyl ester amide of dihydroxy sphingosine was found to be the antiviral principle of *Ulva fasciata* (Kamat *et al*., 1992).

Seventy one species of marine macrophytes found along the coast of Central Mediterranean screened for the production of antibacterial, antifungal, antiviral, cytotoxic and antimitotic potential. Of the 71, 65 species displayed some kind of activity and most of them were active on more than one pathogens or cell tested. Antifungal activity was the most widespread (70% of the plants), whilst the incidence of antibacterial activity was extra ordinarily low (6% of the plants). Of the plants tested, 21% showed antiviral activity, 35% were cytotoxic and nearly 50% had antimitotic property. The maximum level of activity was found among the *Chlorophyta* and some members of the *Bryopsidales* (*Flabellia petiolata, Caulerpa prolifera, Halimeda tuna*) were most active species. Most of the dominant species in Mediterranean phytobenthic algae such as *Corallina elongate, Lithophyllum lichenoides, Phyllophora cripsa, Cystoseria* sp., *Halopteris* spp., *Codium* sps., *Halimeda tuna, Valonia utricularis, Posidomia oceanica, Zostera noltii* and *Cymodocea nodosa* exhibited strong antifungal properties (Ballesteros *et al*., 1992). Extracts of Seaweeds, seagrasses and mangroves from the Southeast coast of India have been tested *in vitro* for antiviral activity against New castle disease, vaccinia, Semliki Forest, encephalomyocarditis and hepatitis B viruses. In the preliminary test, among the 73 crude extracts examined, 43 exhibited antiviral activity (>50%) against at least anyone of the viruses

(Premnathan *et al*., 1992). Antimicrobial potential of six marine green algae found along the coast of Tanzania was screened against three bacterial species viz., *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and a yeast, *Candida albicans* using a disk assay method. A brine shrimp bio-assay using newly hatched *Artemia salina* larvae was used for cytotoxicity study of crude extracts from three algal species. Of the six species tested, extract of *Valonia aegrophila* was most active against all the pathogens tested and its extract was even more active against the test bacteria than penicillin G at a concentration of 2.5 μg mol^{-1}. The extracts of *Halimeda opuntia* and *H.tuna* showed mild activity against all pathogens tested. The extract of *Ulva pertusa* was more active against the bacteria such as *Staphylococcus aureus* and *Bacillus subtilis* but less active against *Escherichia coli* and was not active against the fungus *Candida albicans* . The extract of *Caulerpa mexicana* was inactive against all the pathogens tested. Occasional development of antimicrobial resistance colonies within the inhibition zones were observed from the extracts of *Halimeda opuntia* and *H. tuna* when they were assayed against *Candida albicans* and *Escherichia coli* (Mtolera and Semesi, 1996).

Ethanolic and lipophilic extracts of 21 marine algal species *(Rhodophyta*-9, *Phaeophyta*-2 and *Chlorophyta*-10) occurring along the coast of Yucatan, Mexico were evaluated for antibacterial potential against the human pathogenic microbes (4 Gram positive bacteria, 5 Gram negative and one fungus). Extracts of all the seaweeds exhibited antibacterial activity. The lipid soluble extract of *Ceramium nitens* exhibited the highest bioactivity among the seaweeds screened. Bioactivity of extracts obtained from different regions of the thallus (apical, basal and stolon) of *Caulerpa* sps. (*C. ashmeadii*, *C. paspaloides* and *C. prolifera*) was evaluated. It was observed that the stolon of *Caulerpa* has the highest antibacterial potential (Freile-Pelegrin and Morales, 2004). Antibacterial potential of seaweeds such as *Gracilaria foloferia*, *Padina tetrastomatica*, *Caurelrpa recemosa* and *Ulva lactuca* was evaluated against both Gram negative and Gram positive human pathogenic bacteria. Methanol extracts of all seaweeds test exhibited broad spectrum of antibacterial activity. Green algal members showed higher antibacterial activity than red. *Escherchia coli* alone resistant to all the seaweed extracts except *Sargassum tencerium* (Kandhasamy and Arunachalam, 2008).

EXTRACTION OF BIOACTIVITY

Methanol extracts obtained from the brown algae such as *Laminaria angustata*, *Undaria pinnatifida*, *Rhodomela larix* and *Sargassum gracilis* found along the coast of Japan inhibited the several kinds of pathogenic bacteria. The extract prepared from *Sargassum gracilis* strongly inhibited the growth of *Bacillus mesentericus* (Saito and Nakamura, 1951). Naqvi *et al*. (1981) tested the antimicrobial potential of ethanolic extracts of marine algae and found the highest efficiency in the brown seaweeds. Hornsey and Hide (1974) extracted antimicrobial compounds using acetone from British marine alga *Codium fragile*.

Ethanolic extracts of eight species of seaweeds showed bioactivity against Gram positive and Gram negative bacteria against pathogenic fungi (De Campos-Takaki *et al*., 1988).Crude extracts obtained from the whole plant, stem, leafy- portion, receptacle and vesicle of brown alga *Sargassum weightii* found along the South east coast of India were tested for their antibacterial activity. Extracts of leafy- portion and whole plant exhibited good antibacterial activity (Rao, 1990). Three different solvents viz., benzene, chloroform and methanol were used successively for the extraction of bioactive substances in 5 seaweeds. These extracts were tested against both Gram positive and Gram negative bacterial strains for their antibacterial activity. The chloroform extract exhibited the greatest antibacterial activity (Sastry and Rao, 1994). Eight southern African seaweeds (*Phaeophyta*-5,*Rhodophyta*-2 and *Chlorophyta*-1) were evaluated for antibacterial potential. The extracts were prepared in ethanol from the selected portions such as meristem, stripe and blade(in the case of member of the *Laminariales*) and tested for anti bacterial activity against 10 Gram positive and Gram negative food-associated bacteria by agar diffusion method. Extracts of the intercalary meristem of *Ecklonia radiate* showed significantly greater ($p<0.1$) antibacterial activity than corresponding blade and stripe extracts whereas the extracts of *Laminaria pallida* possessed uniform distribution of antimicrobial activity throughout its thallus against all the test bacteria. Those seaweeds showing apical growth, the antibacterial activity of meristem extracts was either significantly greater ($p<0.1$) or equal to that of thallus extracts. Thallus extracts never exhibited greater antibacterial activity than meristem extracts (Vlachos *et al*., 1999). Ethanol extracts obtained from 22 seaweeds (red-3, brown-13 and green-6) collected from the Karachi coast were investigated for marine shrimp cytotoxity. Of the algal extracts, extracts of brown seaweeds such as *Stoechospermum marginatum*, *Sargassum swatzii*, *S. binderi*, *Spatoglossum aspermum*, *Stokeyia indica* and a green alga *Caulerpa racemosa* showed significant bioactivity. The n-hexane soluble fractions of the ethanolic extracts of *Sargassum marginatum* and *S. swartzii* found to be responsible for the activity whereas methanol soluble fractions of *Sargassum aspermum* and *S. binderi* were most active. The water extract of *S. indica* and *Caulerpa racemosa* exhibited the most prominent bioactivity (LC_{50} value below 70 $\mu g\ mL^{-1}$) when compared with the ethanol extracts and their fractions. Cytotoxic activity may be due to the presence of compounds with polarity (Ara *et al*., 1999).

The ethanol extracts of *Sargassum asperum* and *S. swatzii* and methanol and chloroform soluble fractions of *S. variable* inhibited the growth of all the test bacteria. However, methanol soluble fractions of *S. binderi*, *S. tenerrimum*, *S. variegatum*, *S. marginatum* and *B. leptopoda* inhibited all the test bacteria except *Psedomonas aeruginosa* (Ara *et al.,* 2002). The crude extracts obtained in hexane, chloroform and ethanol from six seaweeds belongs to *Rhodopyta* and *Chlorophyta* occurring along the coast of North Ceara Coast (Northeast Brazil) were evaluated for antibacterial activity through single disc method. Extract obtained in hexane from *Amansia multifida* strongly inhibited the growth of enteric Gram negative strains such as *Enterobacter aerogenes*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *S. choleraesuis*, *Serratia marcescens*, *Vibro cholerae* and the Gram positive bacteria *Bacillus subtils* and *Staphylococcus aureus* (Lima-Filho *et al*., 2002).

DYNAMICS OF BIOACTIVITY

Numbers of factors influenced on the bioactive potential of seaweeds are reproductive stage (Moreau *et al*., 1984; Hornsey and Hide, 1985), location (Almodovar, 1964; Moreau *et al*., 1984) and seasonality (Almodovar, 1964; Hornsey and Hide, 1974; Combaut *et al*., 1981; Moreau *et al.*, 1984). The crude extract of most of the seaweeds showed high bioactive potential during their fertility period (Pesando and Carm, 1984). Variation in antimicrobial activity as well as in concentration of the active halogenated sesquiterpenes from various collection of *Laurencia obtusa* were differed (Olesen *et al*., 1963; Caccamese *et al*., 1979, 1980). Hexane, ethyl acetate and methanol extracts of *Sargassum desfontainesii*, *Halopteris scoparia*, *Stypopodium zonale*, *Codium intertextum* and *Ulva rigida* collected from the littoral of Tenerife during both autumn and winter) were screened for antibacterial and antifungal activities. The methanol extracts showed very high antibacterial activity, particularly against *Bacillus cereus* and *B. subtilis*. The methanol extracts of brown and green seaweeds collected during autumn did not exhibit antifungal activity. Whereas methanol extract of *Codium intertektum* collected in winter showed inhibitory effect against *Saccharomyces cerevisiae* and three species of *Candida* (Febles *et al*., 1995). Extracts of 14 different species of seaweed were tested against the pathogenic bacteria. The extracts of seaweed collected at one location were more active than same species collected at another location. Extracts of seaweeds collected from the post monsoon season were much more active than they collected from other seasons. The antimicrobial potential of seaweeds collected during the post monsoon season was the highest in case of green alga *Chaetomorpha antennina*, whereas it was least in red alga *Hypnea valentiae.* Among the bacteria tested *Staphylococcus aureus* was most sensitive and *Salmonella paratyphi* B was least sensitive. Fully-grown red seaweed *Gracilaria corticata* showed maximum antibacterial potential against *Staphylococcus aureus* compared to medium and young stages of growth. The middle part of the thalli of fully-grown alga showed maximum bioactive potential compared with the terminal part and the basal part with hold fast (Vidyavathi and Sridhar, 1991). Seaweeds collected from the backwaters of Muttukadu possessed higher antibacterial potential than the same species collected from coastal waters. Significant seasonal differences in antibacterial activity of seaweeds were found in specimens collected at seven localities along the coast of Tamil Nadu, India (Arunkumar and Rengasamy, 2000a).

BIOACTIVITY AGAINST PLANT PATHOGEN

Fenical *et al.* (1973) reported two sesquiterpenes, zonarol and isozonarol from a brown alga, *Dictyopteris zonarioides*. Neither substances possessed antibacterial properties, but strongly inhibited the growth of ten species of pathogenic fungi causing diseases in plants. Lipid extracts of more than twenty algae found along the coast of Eastern Sicily inhibited the growth of some plant pathogenic bacterium *Xanthomonas malvaciarum* and Tobacco Mosaic Virus under *in vitro* (Caccamese *et al*., 1980).

Extracts obtained from seaweeds sprayed on plants reduced the incidence of *Botrytis cinerea* (graymould) on straw berries, *Erysiphe polygoni* (powdery mildew) on turnips and damping off of tomato seedlings (Kulik, 1995). The biological activity of ethanolic extracts of some commonly occurring seaweed found along the coast of kwazulu-Natal, South Africa was conducted *in vitro* against two phytopathogenic fungi such as *Verticillium*, sps. and *Rhizoctonia solani*. Extracts of *Caulerpa filiformis*, *Ulva rigida*, *Zonaria toumefortii*, *Hypnea spicifera*, *Gelidium alottiorum* and *Osmundaria serrata* inhibited the fungal growth by more than 50%. Whereas the extracts of red seaweeds, *Spyridia cupressian* and *Beckerella pinnatifida* showed minimum antifungal activity (Barreto *et al*., 1997).

Ethanolic extracts of seventeen species of seaweeds were tested against the plant pathogenic root infecting fungi *Marcophomina phaseolina*, *Rhizoctonia solani*, *Fusarium solani* and *F. oxysporum.* Extracts of *Spatoglossum asperum* and *Spatoglossum* inhibited the radial growth of the fungi such as *Marcophomina phaseolina*, *Rhizoctonia solani* and *Fusarium solani in vitro* when used at 6 mg^{-1} disc. Extracts obtained from the brown alga *Stoechospormum marginatum* and green alga *Codium iyengarii* control only the growth of *F. solani* at the same concentration (Ara *et al*., 1998). A meroditerpenoid metabolite has been isolated from the brown alga *Cystoseira tamariscifolia* and characterized as Methoxybifurcarenone. Methoxy bifurcarenone posses antifungal activity against three tomato pathogenic fungi, *Botrytis cinerea*, *Fusarium oxysperium* sp. *mycopersici* and *Verticillium alboatrum* and antibacterial activity against *Agrobacterium tumefaciens* and *Escherichia coli* under *in vitro* (Bennanmara *et al*., 1999).

Eleven seaweeds were collected from seven different sites-one from the backwaters of Muttukadu, Chennai and six regions along the coast,Tamil Nadu,India and tested for antibacterial potential against the plant pathogenic bacterium *Xanthomonas oryzae pv.oryzae* bacterial blight in rice. Unsaponified fractions of red and green seaweeds exhibited maximum antibacterial activity followed by petroleum ether extracts, lipophilic fractions, diethyl ether extracts, saponified fractions, chloroform extracts and methanol extracts. However, methonal extracts of brown seaweeds showed the highest antibacterial activity followed by lipophilic fractions, unsaponified fractions, ethanol extracts, saponified fractions, chloroform: methanol (2:1v/v) extracts and chloroform extracts. The antibacterial potential of the seaweeds was in the following order: *Enteromorpha flexuosa*>*Sargassum wightii*>*Turbinaria conides*>*Padina boergesenii*> *Gracilaria edulis*>*G.blodgettii*>*Hypnea valentiae* = *H.musciformis*>*Spyridia insignis*>*Chnoospora minima*> *Ulva lactuca* (Arunkumar and Rengasamy, 2000a). Petroleum ether extracts and unsaponified fractions of red and green seaweeds and methanol extracts, lipophlic fractions and unsaponified fractions of brown seaweeds separated on TLC were tested for their efficacy against the plant pathogenic bacterium *Xanthomonas oryzae pv.oryzae* causing bacterial blight in rice. Two active zones from red seaweeds, one to five from brown seaweeds and two to three from green seaweeds were isolated through TLC profiles. Among eleven seaweeds, *Gracilaria edulis*, *Sargassum wightii* and *Enteromorpha flexuosa* showed high antibacterial activity. The Rf 0.30 substance obtained from unsaponified fractions of *Enteromorpha flexuosa* showed the maximum antibacterial activity against the test bacterium *Xanthomonas oryzae* pv. *oryzae* (Arunkumar and Rengasamy, 2000b). Fatty acids predominant with palmitic acids obtained from the petroleum ether extract of green alga *Enteromorpha flexuosa* exhibiting antibacterial activity against the plant pathogenic bacterium *Xanthomonas oryzae* pv. *oryzae* (Arunkumar *et al*., 2001). The sulphoglycerolipid 1-0-palmitoyl-3-0(6′-sulpho-α-quinovopyranosyl)-glycerol isolated from the methanolic extract of the brown seaweed *Sargassum wightii* inhibited the growth of *Xanthomonas oryzae* pv. *oryzae* which causes bacterial blight of rice (Arunkumar *et al*., 2005). Extract obtained from the red alga *Soleria robusta* amended at 0.5 or 1% w/w individually or incombination with plant growth promoting bacterium *Pseudomonas aeruginosa* showed significant ($p<0.05$) control on the growth of plant pathogenic fungi such as *Macrophomina phaseolina*, *Rhizoctonia solani* and *Fusarium solani* infected in pepper roots. Combined use of *Pseudomonas aeruginosa* and extract of *Soleria robusta* showed better control of *Macrophomina phaseolina* infection than they used individually. The brown seaweed *Padina pavonia* was found effective against *Macrophomina phaseolina* and *Fusarium solani* when used with *Pseudomonas aeruginosa* and against *Rhizoctonia solani* either used alone or with *Pseudomonas aeruginosa.* The extract of brown alga *Stokeyia indica* also reduced the infection of *Rhizoctonia solani* and *Fusarium solani*. Combined use of compatible strain of *Pseudomonas aeruginosa* with seaweed holds promising effect. Seaweeds therefore could be used for the control of root infecting fungi affecting peppers (Sultana *et al*., 2005). Antimicrobial screening of 12 different seaweeds extracts namely *Chaetomorpha antennina*, *Laurencia obtusa*, *Gracilaria corticata*, *Gracilaria verucosa*, *Grateloupia lithophila*, *Padina boergesenii*, *Sarassum wighti*, *Turbinaria conoieds*, *Halimeda tuna* and *Ulva lactuca* was carried out. The crude extracts were tested against the phytopathogenic bacterium *Pseudomonas syringae* causing leaf spot disease of the medicinal plant *Gymnema sylvestre*. The methanolic extracts of *Sargassun wightii* showed maximum activity followed by ethyl acetate compared to that of other organic solvent extracts (Kumar *et al.*, 2008).

BIOACTIVITY OF FRACTIONATED COMPOUNDS

The compounds purified through High Performance Liquid Chromatography (HPLC) from three distinct TLC zones obtained from the crude lipid extracts of red alga *Spyridia filamentosa* were tested against five human pathogenic microorganisms. Of these, 19 showed detectable peaks (seven HPLC peaks from TLC zone 5, five peaks from TLC zone 9 and 7 peaks from TLC zone 12). Activities by single HPLC peaks were generally against one or two assay microorganisms. The total number of biologically active compounds in a given alga is shown to be substantially greater than can be ascertained by assaying whole algal extracts (Tovar and Ballantine, 2000). The polar and non-polar extracts obtained from the seaweeds such as *Gracilria tikvahiae*, *Ulva lactuca*, *Ulva fasciata* and *Sargassum fluitans* were screened for antibacterial and antifungal potential against seven microbial pathogens by the agar diffusion method. Non-polar extracts of *Gracilaria tikvahiae* inhibited the growth of more than four microorganisms. Extracts separated using column chromatography were tested against the bacterium *Staphylococcus aureus* and fungus *Candida albicans*. There are eight fractions obtained in the petroleum either from the crude extract of *Sargassum fluitans* exhibited high bioactivity against *C.albicans* as MIC 0.16 μg mL^{-1} (Oranday *et al.*, 2004).

CHEMISTRY OF BIOACTIVE COMPOUNDS

Acrylic acid and its related compounds: Acrylic acid is the common antibacterial components occur in many red, brown and green seaweeds as well as in various species of phytoplankton (Challenger *et al.*, 1957; Katayama, 1962; Sieburth, 1960). Katayama (1964) isolated bioactive acrylic acid from seaweeds. Hodgkin *et al.* (1966) isolated dipotassium 2,3-dibromobenzyl alcohol and 4,5-disulfate (lanosol disulfate) from the red alga *Polysiphonia lanosa*.

Dimethyl sulfide, acrylic acid, CH2=CH-COOH isolated from the seaweeds such as *Polysiphonia fastigiata*, *Ulva lactuca*, *Enteromorpha* sp. displayed antibacterial property (Haas, 1935; Challenger and Simpson, 1948; Bywood and Challenger, 1953; Cantoni and Anderson, 1956; Armstrong and Boalch, 1960; Allen and Dawson, 1960). Acrylic acid isolated from the *Gracilaria corticata* and *Ulva lactuca* was responsible for the antimicrobial activity (Bandara *et al.*, 1988).

Lipids, fatty acids and their related compounds: Pratt *et al.* (1944) first isolated an antibacterial fatty acid *Chlorellin* from the unicellular green alga, *Chlorella vulgarize*. Algal lipids contain peptides, toxins, sterols, hydrocarbons, growth factors, fatty acids, antibiotics, triglycerides (Fogg, 1962; Lefevre, 1964; Sieburth, 1969; Hellebust, 1974). Lipid extracts of brown seaweeds such as *Neria filiformis* and *Sporochnus pedenculatus* inhibited the growth of several pathogenic bacteria tested and no efficacy was found in the extract of green seaweeds (Pesando and Carm, 1984). Lipid extracts of 24 red and brown seaweeds mainly from Eastern Sicily were tested for antimicrobial activity against four microorganisms. Some of the extracts showed activity against bacteria, while none was active against yeast and fungi (Caccamese *et al.*, 1985). A novel long chain fatty ester, pentyl hentriacontanoate 1 and an orange red pigment, caulerpin 2 were isolated and characterized from a red alga *Chondria armata.* The pigment, Caulerpin hitherto known to be a constituent of green algal genus *Caulerpa* sps also reported here for the first time from the red alga (Govenkar and Wahidulla, 2000). The chemical composition of the Bery brown seaweed *Cystoseira erinita* collected along the Eastern Mediterranean coast isolated 19 compounds from volatile fractions and 15 compounds in the polar fraction and they were main lipid classes and their fatty acids showed bioactivity (Kamenarska *et al.*, 2002).

Phenols and their related compounds: Mautner *et al.* (1953) reported that the bromophenol from ethanol extracts of *Rhodomelalerin* and *Symphocladia gracilics* exhibited bioactivity against several pathogenic bacteria. The bioactive brominated phenols, polyphenols, tannins and tannic acid were isolated from seaweeds (Ogino and Taki, 1957; Peguy, 1964; Sieburth and Conover, 1965; Craigie and Gruenig, 1967). Volatile organic acids called Sarganin complex was isolated from the *Cympolin birbata* and *Sargassum natans* showed broad spectrum antibacterial activity (Martinez, *et al.,* 1964, 1966). *Laurnin* and *laurentin* from red alga *Laurencia glandulifera and Laurinterol* from *Laurencia intermedia* showed biological activity (Irie *et al.*, 1965). A novel brominated phenolic derivative 2, 3-dibromo benzyl alcohol and 4, 5-disulphate potassium salt were isolated from red alga *Polysiphonia lanora* exhibiting antibacterial activity (Hodgkin *et al.*, 1966). Phenols extracted from seaweeds posesses antibacterial properties against both Gram positive and Gram negative bacteria (Stoffelen *et al.*, 1972; Glombitza, 1974). An antiviral halogenated acetylene and chondriol was isolated in the red alga *Laurencia* sp. (Fenical *et al.*, 1974; Fenical and

Norris, 1975). Devon and Scott (1972) identified a similar compound as phytol constituent of Chlorophyll from the active fractions exhibiting bioactivity from the members of Rhodophyceae, Chlorophyceae, Phaeophyceae and Cyanophyceae. Fusetani and Hashimoto (1975) isolated palmitic acid from *Ulva pertusa* showing antibacterial property. Ohta (1977) isolated debromolaurinterol in the calciphileus alga, *Marginisporum aberrans* which inhibit the bacterial pathogen *Bacillus subtilis*. Burreson *et al.* (1976) detected similar compounds in the *Asparagopsis taxiformis*.

Organic acids and phenolic compounds, especially polyphenols and tannins have also been shown to have antimicrobial activities (Glombitza, 1979; Chuyen *et al.*, 1982). Volatile compounds from seven species of brown algae wide spread in the Black sea were obtained by distillation extraction and investigated by GC/MS Different groups of compounds were identified: hydrocarbons alkylated benzenes, alcohols, phenols, aldehydes, ketones,acids, esters, terpenes, sulfur and chlorine- containing compounds. On the basis of their qualitative and semi-quantitative composition some conclusions about the taxonomy and evolution of the species investigated are proposed. The functions of some volatile compounds are discussed (Kamenarska *et al.*, 2002).

Terpenes and their related compounds: Seaweeds are an extensive and prolific source of secondary metabolites, among which the diterpenoids constitute one of the most abundant groups' attendant with profound ecological and pharmacological significance. Over 170 diterpenoids belonging to about 32 skeleted pattern, some of which are of unprecedented and unexpected variation are known. The bio-activities attributed to some of their metabolities include ichthyotoxicity, phyotoxicity, antibiosis, cytotoxicity, antiviral, antifungal, insecticidal antifeedant properties in addition to activity against the Eatumor in mice. The logical bio-genetic derivation from acyclic precursor for some important groups and the role of diterpenoids in chemical defense and chemotaxonomy are noted (Bheemasankara Rao *et al.*, 1994).

Katayama (1962) observed that terpenes isolated from seaweeds inhibit the growth of *Staphylococcus aureus* and *Eschericia coli.* Chesters and Scott (1956) observed antibacterial properties of ether extracts of *Halidrys siliquosa*, *Pelvetia canaliculata*, *Laminaria digitata* and *Polysiphonia fastigata*. Fenical *et al.* (1972) reported two sesquiterpenes, zonarol and isozonarol from a brown alga, *Dictyopteris zonarioides*. Neither substance possessed antibacterial activity, but strongly inhibited the growth of ten species of pathogenic fungi causing diseases in plants. Fenical *et al.* (1974) isolated a multitude of halogenated acetones and butenones from *Asparagopsis taxiformis*, which strongly inhibit the growth of bacteria *Staphylococcus*, *Fusarium* and *Vibrio*. Fenical *et al.* (1973, 1974) found that chondriol and cycloeudesmol isolated in *Chondria oppositiclada* and rhodophytin isolated in *Laurencia pacifica* inhibited the growth of *Staphylococus aureus*, *Salmonella choleraesuis*, *Mycobacterium smegmtis* and *Candita albicans.* Fenical *et al.* (1972), reported two sesquiterpenes, zonarol and isozonarol from a brown alga, *Dictyopteris zonarioides*. Neither substances exhibited antibacterial activity, but strongly inhibited the growth of ten species of pathogenic fungi causing diseases in plants.

Faulkner *et al.* (1977) extracted diterpene, pachidictyol as bioactive compounds. Fattorusso *et al.* (1976) extracted the bioactive diterpenes, Dictyol A and Dictyol B from the red alga *Dictyota dichotoma* var. *implexa.* Caccamese *et al.* (1980) isolated bioactive non-isoprenoid cyclic ether, Laurencyenine from *Laurencea obtusa*. Caccamese *et al.* (1980) identified the antibacterial compounds in the red alga *Laurencia decidua*. Obtusadiol isolated from *Laurencia obtusa* found to be active against *Bacillus subtilis* (Howard and Fenical, 1978). Caulerpenyne, a sesquiterpene was active only against Gram positive bacteria was isolated from the green alga *Caulerpa prolifera* (Amico *et al.*, 1978). Oligoanyl ether, diphlorethol and oligoaryls often substituted with aryl ether (Fucophlorethols) have all been isolated by Glombitza (1979) in Fucaceae. Pullaiah *et al.* (1985) isolated Dictyoxetane, a novel diterpene from the brown alga *Dictyota dichotoma* found in the Indian Ocean. Parekh (1985) reported that extracts of fraction A obtained from the crude extract of green alga *Caulerpa taxifolia* and *Enteromorpha intestinalis* inhibited the growth of *Staphylococcus aureus*.

Seven new diterpenes and one already known diterpene were isolated from the brown alga, *Dictyota divaricata*, found along the Great Barrier Reef region of northern Australia. Of the seven new diterpenes, five are xenicane derivatives and two are hydroazulenoid diterpenes (Konig *et al.*, 1991). The bioactive deoxylapachol was isolated from the New Zealand brown alga, *Landsburgia quercifolia* (Perry *et al.*, 1991). Amentadione-1'-methyl ether and a new meroterpenoid identified as 11- hydroxyamentadione were isolated from the chloroform - methanol extract of *Cystoseira usneoides* (Urones *et al.*, 1993). A new xenicane-type diterpene called dipopholide and four known diterpenoids such as acetoxyerenulide, acetylcoriacenone, isoacetylcoriacenone and hydroxyacetyldictyolal were isolated from the brown alga *Dilophus ligulatus* (syn. *spiralis* (Montagne) Hamel). They exhibit cytotoxic activity against several types of mammalian cells: human nasopharynse carcinoma cells (KB), human ling carcinoma cells (NSCLC-N6), murine leukemia cells (P-388) and murine leukemia cells expressing the multi-drug-resistance gene, mdr (P-388/Dox) (Noureddine and Pauel, 1993).

Bioactive three terpenoids were isolated from the methanol extract of the brown alga *Padina tetrastromatica* (Parameswaran *et al*., 1996). Two new monoterpenes called usneiodones E and usneiodones Z isolated from the brown seaweed *Cystoseria usneoides* compounds exhibited antitumoural and antiviral activities (Urones *et al*., 1992). 1'-dimethyl cystalgerone and a new monoterpene, identified as Cyclo-1'-dimethyl cystalgerone isolated from the brown alga *Cystoseria baccata* showed bioactivity. The bioactive amentadione-1'-methyl ether and 6-cis–amentadione-1'-methyl ether were isolated from the brown alga *Cystoseria abies-marina* (Basabe *et al*., 1992).

A diterpenoid showing antimicrobial property was isolated from the brown seaweed *Dictyota baratayresii* (Norris and Fenical, 1982). De Silva *et al*. (1982) found that the antibacterial activity of methanolic extract of the brown seaweed *Stoechospermum marginatum* was active against *Staphylococcous aureus*. The active constituents were spatane, diterpenoids, 19-acetoxy, 5, 15, 18-trihydroxyspata-13, 16-diene. Amico *et al*. (1982) isolated (2'E, 6'E) -2 -(10', 11' -Dihydroxy geranial geranial)-6 Methylquinol and (2'E, 6'E)-2-(10', 11'-dihydroxy-geranyl geranyl)-6-methyl-1, 4-benzoquinone from the brown alga *Cystoseira stricta*. Enoki *et al*. (1983) identified four new diterpines with 9-membered ring named as dictytriene A and B, dictyone and dictyriol reported from the brown seaweed *Dicyota dichotoma*. Caulerpenyne was very active against KB as well as against the Gram-positive test bacteria (Hodgson, 1984). A high bioactivity was found in the extracts of red alga *Chondrococcous hornemanni* and the active component identified in the extract was dihalogenated monoterpenes (Bandara *et al*, 1988).

The ethyl acetate extract of *Caulerpa racemosa* yielded two new red pigments which are Cauleripin analogues and a new sesquiterpenoid was also identified as 10-keto -3, 7, 11-trimethyldodecanoic acid (Anjaneyulu *et al*., 1991). Three new diterpenes and 14 known brominated and non-brominated diterpenes have been isolated from the red alga *Laurenicia saitoi* collected along the Isoyake areas of Japan coast. Some of these diterpenes showed significant inhibitory activity against young abalone (*Haliotis discuss hannai*) and young sea urchins (*Strongylocentrotus nudus* and *S.intermedius*), thus suggesting that these metabolites provide a chemical defense against marine herbivorous animals in Isoyake areas (Kurata *et al*., 1998). Six new bromotriterpene polyethers, armatol A-F (1-6), with a re- arranged carbon skeleton were isolated from the Indian Ocean red alga *Chondria armata*. The structure was characterized by spectroscopic techniques, in particular ID – and 2D- NMR. (Ciavatta *et al*., 2001). As like previously known elatol and iso-obtusol, 2 halogenated C_{15} acctogenins, named as lembyne-A and lembyne-B isolated from an unrecorded *Laurencia* sps. collected along the coast of Malaysian showed bioactivity against some marine bacteria (Vairappan *et al*., 2001).

Sterols and its related compounds: Irie *et al*. (1966, 1970) isolated laurinterol and debromolaurinterol from red alga *Laurencia intermedia*, which inhibited the growth of *Bacillus subtilis*. Sims *et al*. (1971, 1973) isolated pacifenol, johnstonol and prepacifenol from the red alga *Laurencia* sp. which inhibited the growth of *Staphylococcus aureus* and *Mycobacterium segmantis*. Glomnbitza *et al*. (1973) reported the presence of phloroglucinol in the brown algal genera such as *Cladostephus*, *Dictyota*, *Fucus*, *Chorada*, *Laminaria* and *Saccorhiza*. Glombitza *et al*. (1973, 1975) isolated trifuhalol (in *Halidrys siliquosa*), diphlorethol, bifuhalol (in *Cystoseira tamariscifolia*), difucol, trifucol and tetrafucol (in *Fucus vesiculosus*). Hirschfeld *et al*. (1973) isolated the bioactive substance Pachydictyol A from *Pachydictyon coriacerum*. Hager *et al*. (1966) isolated halogenated and brominated organic compounds exhibiting antimicrobial activity against human pathogenic Gram positive bacteria from the red alga *Laurencia* species. Rao and Pullaiah (1982) isolated 6, 10, 14-trimethyl-pentadecan-2-one (I), trans-Phytol (II), Fucosterol (III) and digiprolactone or loliolide, (1,3-dihydroxy-3,5,5-trimethyl cyclohexylidene -4 acetic acid lactone, (IV) from the brown alga *Padina tetrastromatica*. They also isolated 6,10,14-trimethyl Pentadecane -2-one (O), Plytol (II) and Clinasterol (III) from the green alga *Caulerpa taxifolia*.

Three new sterols named as decortinol, isodecortinol and decortinone have been isolated from the ethyl acetate soluble portion of the extract of a green alga *Codium decorticatum*. Clerosterol and 3-0-β-D-galacto- Pyranosyl clenrosterol were also extracted for the first time from this alga (Ahmad *et al*., 1993). The chemical composition of the brown alga *Cystoseira erinita* Bery from the Eastern Mediterranean was investigated. Fourteen sterols have been identified, five of them for the first time in algae. The structure of one new sterol was established. The origin of seven sterols with short side chains was discussed (Kamenarska *et al*., 2002).

Alkaloids and other compounds: Martinez *et al*. (1964) isolated an antibacterial principle termed *Sarganin* complex from the ether extract of a brown alga, *Sargassum natans* and red alga *Chondria lithoralis* which exerted a wide range of bioactivity against human pathogenic bacteria, fungi and KB tumor cells. Aguilar-Santos and Doty (1968) isolated *Caulerpicin* and *Caulerpin* as toxic components from green algae such as *C. racemosa var. clarifera*, *C.sertularioides* and *C. serrulata*.

The water soluble chlorophyll derivatives, terpenes, phenols of seaweeds inhibited the growth of pathogenic bacteria (Blaauw-Jansen, 1954; Jorgensen, 1962; Aubert *et al*., 1966; Sieburth and Conover, 1965) showed that tannin found in the brown alga *Sargassum natan* inhibited the growth of *Psendomonas* and *Vibrio* but not the Sarganis complex. They also pointed out that tannic acid extracted from the brown alga *Sargassum natan* inhibited the growth of pathogenic bacteria at concentration greater than 0.01%. Martinez *et al*. (1963) reported that benzene and ethyl-ether were suitable solvents for extracting the antibiotic principle from the fresh and dry *Sargasasum natans* (L) J. Meyen and *Chondria littoralis* Harvey to obtain the bioactive *Sarganin* and *Chonalgin*. Wratten and Faulkner (1976) isolated a mixture of cyclic polysulfides and their oxidation products showing bioactivity from the red algae *Chondria californica*. Shelat (1979) found methanol and dimethyl-formamide extracts of *Sargassum plagiophyllum* (Mert) C.Ag. active against Gram positive bacteria while acetone extract of *Sargassum tenerriaum* J.Ag. showed activity against all the strains tested. Shelat (1979) reported highest content of fatty acid in red algae *Hypnea muciformis* and lowest in *Cystoseira indica*. The peptide known as aurantiamide acetate and a new diastereoisomer of this dipeptide (di-aurantiamide acetate) was identified from the red alga *Acanthophora spicifera* showing bioactivity (Wahidulla *et al*., 1991). The fucoxanthin, a biologically active carotenoid identified as fucoxanthin was isolated from a brown alga *Scytosiphon lomentaria* (Mori *et al*., 2004).

In conclusion, Seaweeds occurring along the coast of the world would be a potential and promising source of bioactive compounds. This renewable resources could also be utilized as a source of bioactive compounds to control the pathogens causing diseases in crops.

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