Indian Journal of Ecology (2023) 50(4): 1188-1191
DOI: https://doi.org/10.55362/IJE/2023/4034

Manuscript Number: 4034
NAAS Rating: 5.79

Screening of Seaweeds for Antibacterial and Antifungal Activities

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***Abstract*:** Commonly occurring seaweeds, *Caulerpa scapelliforms, Ulva lactuca*, *Padina tetrastromatica, Steochspermum marginatum* and *Acanthophora spicifera* collected from the Tuticorin coast, Tamil Nadu (India) were evaluated for antibacterial and antifungal activity by agar diffusion method. Four different solvents *viz.* petroleum ether, benzene, chloroform, and methanol wereused for extraction. By using commercial medicine, Amikacin-AK30-30mcg/disc, Cephalexin-CN30-30mcg/disc, Ciprofloxacin-CIP5-5mcg/disc and Fluconazole-FLC25-25mcg/disc as control and zones of inhibition were compared. The seaweed extracted using petroleum ether as a solvent showed the best antifungal activity and was more effective than commercial antibiotic, fluconazole. None of the extracts were active against *Staphylococcus aureus*, a gram-positive bacterial pathogen. The methanol extract was active against *Salmonella typhi, Pseudomonas aeruginosa, Proteus vulgaris,* and *Escherichia coli* except for *Caulerpa scapelliforms* and *Ulva lactuca*. All the four extracts of *Acanthophora spicifera* showed antibacterial activity.

***Keywords*:** Seaweeds, Antibacterial, Antifungal, Solvents

The marine environment has a rich biological and chemical diversity (Dayanidhi et al 2021). This diversity of marine organisms and habitat offers a wide variety of natural marine compounds. Several oceanic compounds show pharmacological activities and are helpful in the invention and discovery of novel bioactive compounds, primarily for deadly diseases like Cancer, Acquired immunodeficiency syndrome (AIDS) and Arthritis (Samuel et al 2011, Pham-Huy and Huy 2022). Many other compounds have been developed as analgesics or to treat inflammation (Giriwono et al 2019). Seaweed (macroalgae), a marine organismis widely distributed in the coastal regions of all the continents. Several scientists have worked on the seaweed extracts, exhibiting antibacterial and antifungal activity (Hellio et al 2000, Lima-Filho et al 2002, Taskin et al 2007, Seenivasan et al 2010). Moreover, crude extracts of Indian seaweeds were found to be active only against Gram-positive bacteria whereas, majority of the pathogenic bacteria are gram-negative. Therefore, keeping this in view, the present work was designed to study the antibacterial and antifungal activity of four different solvents extracts of five seaweeds collected from Tuticorin coast, Tamil Nadu.

MATERIAL AND METHODS

Collection and preparation of selected seaweeds: Fresh seaweed samples of *Caulerpa scalpelliformis, Ulva lactuca, Padina tetrastromatica, Stoechospermum marginatum* and *Acanthophora spicifera* were handpicked from the intertidal zone of Hare Island, Tuticorin (TN) located at 8.8°N, 78.3°E The collected seaweed samples were cleaned with seawater and freshwater, dried in the shade, and powdered to extract antimicrobial compounds.

Preparation of organic seaweed extracts: The organic seaweeds extracts were prepared by following the method of El Shafay et al (2016). Powdered seaweed sample (5g) were soakedin 50 mL of four different solvents (analytical grade) i.e. petroleum ether, benzene, chloroform and methanol for three days. Then soaked samples were filtered and concentrated in a rotary evaporator at 35^0C. The residual water was then removed with the help of vacuum pump (Agilent vacuum pump PVL401), followed by suspension of weighted crude extract in dimethyl sulfoxide (DMSO) to the final concentration of50 mg/mL and stored in a refrigerator.

Bioassay: For bioassay, fungal pathogen *Candida albicans* ATCC 90028 and five gram-negative bacterial pathogens namely *Salmonella typhi* ATCC 35640, *Pseudomonas aeruginosa* ATCC27853, *Proteus vulgaris* ATCC 33420, *Klebsiella pneumoniae* ATCC 700603, *Escherichia coli* ATCC 25922 and one pathogenic gram-positive bacteria namely *Staphylococcus aureus* ATCC 25923 were used. A loop full of the microorganism was inoculated in nutrient broth from the 24 hours incubated nutrient agar slant of each test organism at pH-7.4 to activate the bacterial strains used as test organisms. The broths were kept for incubation at 37°C for 24 hours so that the microorganism can grow till the log phase. The nutrient broth was maintained as control without inoculating the test organisms. These bacteria were obtained from Hi-Media Ltd., Mumbai. Amikacin-AK30-30mcg/disc,

Cephalexin-CN30-30mcg/disc, Ciprofloxacin-CIP5-5mcg/disc and Fluconazole-FLC25-25mcg/disc were usedas control medicine. The bioassay was carried out under a sterile condition in a clinical laboratoryby following the agar diffusion method as described by Perez et al (1990). Inoculums in the exponential phase of growth, equivalent to a 0.5 McFarland standard were swabbed on to the surface of Muller Hinton agar media. Different crude extract (2 mL) was transferred to a sterile 6 mm Whatman No.1. Filter paper disc (E-760) and after drying it was placed in the seeded agar plate, along with the control discs which was prepared with the solvents alone. The inhibition zone was observed after 24 hours of incubation at 37°C.

RESULTS AND DISCUSSION

Among five different seaweeds (*Caulerpa scalpelliformis, Ulva lactuca, Padina tetrastromatica, Stoechospermum marginatum* and *Acanthophora spicifera*) collected, the extracts of all seaweeds except *Caulerpa scalpelliformis* showed specific activity against the pathogens (bacteria i.e. *Salmonella typhi, Pseudomonas aeruginosa, Proteus vulgaris, Klebsiella pneumoniae* and *Escherichia coli* and fungi i.e. *Candida albicans)* (Table 1, 2). The present study showed that petroleum ether extract of *Ulva lactuca*is active against fungal pathogen, *Candida albicans,* in accordance with the findings of Oranday et al (2004). Further, the petroleum ether extract of *Caulerpa scalpelliformis*, *Ulva lactuca,* and *Padina tetrastromatica*; the benzene extract of *Stoechospermum marginatum* were active against fungal pathogen *Candida albicans*. The different extracts of the red alga, *Acanthophora spicifera* were not found active against fungal pathogen, *Candida albicans*. The zone of inhibition indicates that extracts of all the seaweeds exhibit antifungal activities except *Acanthophora spicifera* which are quite comparable with the commercial antifungal agent i.e. fluconazole (Table 2). The petroleum ether extract of *Caulerpa scalpelliformis* was more effective (32mm) than the commercial antifungal agent fluconazole (27mm). Similarly, Kaur et al (2016) observed *Inula racemosa* root extract were active against phyto-pathogenic fungi (*Dreschlera oryzae*). Tuney et al (2006) found that the methanol extract of *Ulva rigida* collected from the coast of Urla (Izmir, Turkey) hadno antifungal activity against *Candida albicans*. Karthick et al (2014) reported that *Caulerpa scalpelliformis* contains

Table 1. Antibacterial activity of sea weeds extracts from Hare Island of Tuticorin coast

Name of the bacterial pathogens	Control medicine			*Caulerpa scalpelliformis*				*Ulva lactuca*				*Acanthophora spicifera*				*Padina tetrastromatica*				*Stoechospermum marginatum*			
	AK30	CN30	CIP5	PE	BZ	CF	MN	PE	BZ	CF	MN	PE	BZ	CF	MN	PE	BZ	CF	MN	PE	BZ	CF	MN
Salmonella typhi ATCC 35640	16	20	21	-	-	-	-	-	-	-	-	15	-	-	-	-	-	-	-	-	-	-	16
Pseudomonas aeruginosa ATCC 27853	14	15	14	-	-	-	-	-	-	-	-	-	-	-	14	17	-	-	14	-	14	-	-
Proteus vulgaris ATCC 33420	16	20	21	-	-	-	-	16	-	-	-	-	-	-	-	-	-	-	15	-	-	-	-
Klebsiella pneumoniae ATCC700603	26	29	30	-	-	-	-	-	-	23	-	-	20	24	-	-	-	-	-	-	-	-	-
Escherichia coli ATCC 25922	16	20	22	-	-	-	-	-	-	-	-	-	-	-	16	-	-	-	-	-	-	-	16

*PE-Petroleum ether; BZ-Benzene; CF-Chloroform; MN-Methanol; FLC25-Fluconazole; AK30-Amikacin; CN30-Cephalaxine; CIP5-Ciprofloxacin
*Zone in mm indicates the distance from the border of the disc to the edge of the clear zone.
(-): hyphen indicates no clear zone has been observed

Table 2. Antifungal activities of sea weeds extracts from Hare Island of Tuticorin coast

	Control medicine	*Caulerpa scalpelliformis*				*Ulva lactuca*				*Acanthophora spicifera*				*Padina tetrastromatica*				*Stoechospermum marginatum*			
	FLC25	PE	BZ	CF	MN	PE	BZ	CF	MN	PE	BZ	CF	MN	PE	BZ	CF	MN	PE	BZ	CF	MN
Candida albicans ATCC90028	27	32	-	-	-	24	-	-	-	-	-	-	-	22	-	-	-	-	22	-	-

See Table 1 for details

tannin, flavonoids, glycosides, phenols, saponins, and terpenoids. This indicated that in the particular case that petroleum ether could be able to successfully extract antifungal compounds in an effective concentration.

The petroleum ether extracts of *Acanthophora spicifera*was sensitive against Gram-negative bacterial pathogen *Salmonella typhi* but comparatively lower than the commercial antibiotic agents i.e. Amikacin, Cephalexin and Ciprofloxacin. The methanol extract of *Stoechospermum marginatum* was active against Gram-negative bacterial pathogen *Salmonella typhi.* The methanol extract of a *Padina tetrastromatica* and benzene extract of *Stoechospermum marginatum* showed similar activities like Ciprofloxacin and Amikacin against Gram-negative bacterial pathogen *Pseudomonas aeruginosa*. However, petroleum ether extracts of *Padina tetrastromatica* was more effective (17 mm) than commercial antibiotics. The methanol extract of *Ulva lactuca* showed antibacterial activity against Gram-positive bacterium *Staphylococcus aureus* and Gram-negative bacterium *Escherichia coli* (Selvi et al 2001, Oranday et al 2004). The methanol extract of *Ulva lactuca* did not show such antibacterial activity against all these pathogenic bacteria whereas petroleum ether extract of *Ulva lactuca* was active against Gram-negative bacterial pathogen *Proteus vulgaris*. This could be attributed to the presence of lipophilic and phenolic compounds, especially steroids fatty acids, in *Ulva lactuca* organic extract towards antimicrobial activity (El-Baky et al 2008). The zone of inhibition (16 mm) indicates that the methanol extract of *Padina tetrastromatica* was similarly active against *Escherichia coli* as commercial antibiotic i.e. Amikacin. The chloroform extract of *Ulva lactuca* and *Acanthophora spicifera* and the benzene extract of *Acanthophora spicifera* showed lesser activity than needed against Gram-negative bacterial pathogen *Klebsiella pneumoniae*. The methanol extract of *Stoechospermum marginatum* and *Acanthophora spicifera* were active against Gram-negative bacterial pathogen *Escherichia coli*. All the extracts studied were inactive against the Gram-positive bacterial pathogen *Staphylococcus aureus* which might be attributed to the inhibited entry of antimicrobial agents into cell due to the presence of high percentage (90-95%) peptidoglycan, lipopolysaccharides and phospholipids in the bacterial cell wall. Marke et al (2022) also tested *Tinospora cordifolia* (natural herbal shrub) extracts for assessing antibacterial activity and suggested that phytochemical compounds present in this natural herbal shrub can be utilized for formulation of drugs against some gastrointestinal pathogens like *Staphylococcus aureus, Escherichia coli, Vibrio cholera, Salmonella typhi* and *Shigella*.

During the present study, different extracts of *Caulerpa scalpelliformis* were inactive against all the studied pathogenic bacteria whereas Selvi et al (2001) reported that the methanol extract of *Caulerpa scalpelliformis* showed antibacterial activity against Gram-positive bacterium *Staphylococcus aureus* and Gram-negative bacterium *Salmonella typhi, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Escherichia coli*. Non-conformance of the findings might be due to different natural factors such as environmental conditions (light, temperature or salinity), reproductive state, age of the seaweed, geographic location and seasonality (Perez et al 2016). Hence, information made during the present study revealed that seaweed extracts with different solvents exhibit antibacterial and antifungal properties which could be further utilized forproduction of organic pharmaceutical products. Moreover, this created a quest also for the discovery of antibiotic compounds of natural origin.

CONCLUSION

Seaweeds have huge potential for discovery of compounds with various bioactivities. Seaweeds collected from the coastal areas of Tuticorin exhibit antibacterial and antifungal properties. The test microorganism showed resistance towards seaweed extracts prepared with different organic solvents, which is indicating the availability of biologically active compounds at altered concentrations in seaweeds. The study is signifying the need to explore the possibilities of having bioactive compounds in seaweeds with pharmaceutical importance.

REFERENCES

Dayanidhi DL, Thomas BC, Osterberg JS, Vuong M, Vargas G, Kwartler SK and Somarelli JA 2021. Exploring the diversity of the marine environment for new anti-cancer compounds. *Frontiers in Marine Science* **7:** 6147-6166.

El Shafay SM, Ali S and El-Sheekh MM 2016. Antimicrobial activity of some seaweeds species from Red sea, against multidrug resistant bacteria. *The Egyptian Journal of Aquatic Research* **42**(1): 65-74.

El-Baky HA, El Baz FK and El Baroty GS 2008. Evaluation of marine alga *Ulva lactuca* L. as a source of natural preservative ingredient. *American-Eurasian Journal of Agricultural and Environmental Science* **3**: 434-444.

Giriwono PE, Iskandriati D, Tan CP and Andarwulan N 2019. Sargassum seaweed as a source of anti-inflammatory substances and the potential insight of the tropical species: A review. *Marine Drugs* **17**(10): 590.

Hellio GB, Bremer AM, Pons G, Cottenceau Y, Le Gal and Borgougnon N 2000. Antibacterial and antifungal activities of extracts of marine algae from Brittany France. Use as antifouling agents. *Applied Microbiology and Biotechnology* **54**: 543-549.

Karthick N, Fathimal MA, Ramesh K, Sridhar H, Natrajan M, Divya VV and Umamaheswari S 2014. Screening of phytochemicals and antimicrobial activity of Caulerpa scalpelliformis collected from Manapad Coast, Tuticorin District, Tamilnadu, South India. *Journal of Coastal Life Medicine* **2**(2): 107-111.

Kaur R, Urvashi, Chahal KK and Kaur J 2016. Antifungal potential of *Inula racemosa* root extract and *Vetiveria zizanioides* root essential oil against some phytopathogenic fungi. *Indian Journal of Ecology* **43**(2): 631-36.

Lima-Filho JVM, Carvalho AF, Freitas SM and Melo VM 2002. Antibacterial activity of extracts of six macroalgae from the northeastern Brazilian coast. *Brazilian Journal of Microbiology* **33**(4): 311-314.

Marke V, Shete P and Dhundale V 2022. *Tinospora cordifolia*: A valuable plant in Ayurveda. *Indian Journal of Ecology* **49**(1): 38-41.

Mohanta TK, Patra JK, Rath SK, Pal DK and Thatoi HN 2007. Evaluation of antimicrobial activity and phytochemical screening of oils and nuts of Semicarpus anacardium Lf. *Scientific Research and Essays* **2**(11): 486-490.

Oranday MA, Verde MJ, Martinez-Lozano SJ and Waksman NH 2004. Active fractions from four species of marine algae. *Pyton* 165-170.

Patra JK, Rath SK, Jena K, Rathod VK and Thatoi H 2008. Evaluation of antioxidant and antimicrobial activity of seaweed (*Sargassum sp*.) extract: A study on inhibition of Glutathione-S-Transferase activity. *Turkish Journal of Biology* **32**(2): 119-125.

Perez C, Paul M and Bazerque P 1990. Antibiotic assay by agar-well diffusion method. *Acta Biologiae et Medicinae Experimentalis* **15**: 113-115.

Pérez MJ, Falqué E and Domínguez H 2016. Antimicrobial action of compounds from marine seaweed. *Marine Drugs* **14**(3): 52.

Pham-Huy C and Huy BP 2022. *Food and Lifestyle in Health and Disease*. CRC Press.

Samuel P, Prince L and Prabakaran P 2011. Ocean: The inviolated source of pharmaceutical leads and drug metabolites. *World Journal of Science and Technology* **10**: 74-91.

Seenivasan R, Indu H, Archana G and Geetha S 2010. The antibacterial activity of some marine algae from south east coast of India. *Journal of Pharmacy Research* **8**: 1907-1912.

Selvi M, Selvarj R and Chidambaram A 2001. Screening for antibacterial activity of macro algae. *Seaweed Research and Utilization* **23**: 59-63.

Taskin E, Ozturk M and Kurt O 2007. Antibacterial activities of some marine algae fromthe Aegean Sea (Turkey). *African journal of Biotechnology* **6(**24): 27-46.

Tuney I, Cadirci BH, Unal D and Sukatar A 2006. Antimicrobial activities of the extracts of marine algae from the coast of Ural (Izmir, Turkey) Brittany France. *Turkish Journal of Biology* **30**: 171-175.

Received 16 February, 2023; Accepted 20 July, 2023