International Journal of Pharmacy and Biological Sciences
ISSN: 2321-3272 (Print), ISSN: 2230-7605 (Online)
IJPBS™ | Volume 8 | Issue 3 | JUL-SEPT | 2018 | 1125-1129
Research Article | Biological Sciences | Open Access | MCI Approved| ज्ञान-विज्ञान विमुक्तये
|UGC Approved Journal |

ANTIBACTERIAL ACTIVITY OF MARINE RED ALGA *GRATELOUPIA LITHOPHILA* BOERGESEN

Priya, P[1], Murugesan, S[2*] Kotteswari, M[2], Shanthi, N[2] and Sivamurugan, V[3]

[1]PG and Research Department of Zoology, Unit of Parasitology, Pachaiyappa's College, Chennai– 600 030
[2]Division of Algal Biotechnology and Bionano Technology, PG and Research, Dept.of Botany, Pachaiyappa's College, Chennai – 600 030
[3]PG and Research Department of Chemistry, Pachaiyappa's College, Chennai – 600 030

***Corresponding Author Email: priyaponmudi79@gmail.com**

ABSTRACT

The present study was undertaken to investigate the methanol extract of marine red alga Grateloupia lithophila Boergesen collected from Kovalam Beach, Tamilnadu for its potential activity against human bacterial pathogens viz. Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa and Staphylococcus aureus by disc diffusion method. The methanol extract of G. lithophila at a maximum (750µg/mL) concentration showed a zone of inhibition (16 mm) against E.coli, P. aeruginosa and S. aureus whereas, in minimum concentrations (250 µg/mL) showed inhibition zone of 12 mm against E.coli and S. aureus. The methanol extract of G. lithophila displayed potential activity against bacterial pathogens tested; however, studies are in progress to explore the novel antibacterial bioactive molecules.

KEY WORDS

Antibacterial activity, Grateloupia lithophila, Pathogenic bacteria.

INTRODUCTION

One of the major causes for high mortality in humans and aquaculture organisms has been ascribed to infections caused by pathogenic bacteria (Kandhasamy and Arunachalam, 2008). The use of antibiotics has significantly increased now a days due to heavy infections. However, indiscriminate use of these antibiotics has resulted in the bacterial resistance against several antibiotics with an alarming rate. To overcome this problem, there is a pressing need to develop new bactericidal agents of natural origin with attention focusing on the marine seaweed resources.

Oceans are the greatest medicinal resources that have not been fully explored. Various natural antimicrobial compounds have been recorded more in the marine environment than those in the terrestrial one (Ireland *et al.,* 1988). In recent years, the natural compounds derived from the macroalgae (seaweeds) are gaining importance due to their unique pharmacological and biological activities. Different secondary metabolites contained in the green, brown and red algae are endowed with cytostatic, antibacterial, antiviral and antifungal properties (Chakraborthy *et al.,* 2010; Thennarasan *et al.,* 2015 and Subbiah Murugesan *et al.,* 2017).

The antibacterial activity of the marine algal extracts appears to be largely restricted to members of Phaeophyceae and to a certain extent among the members of Rhodophyceae and Chlorophyceae (Kotteswari *et al.,* 2015; Vinoth kumar *et al.,* 2015). Most of the studies are confined to screening these algae for antimicrobial activity. Yet, the commercial exploitation of marine flora and fauna in the field of medicine depends on the successful isolation and

characterization of the bioactive compounds from these sources.

There are a number of reports available on the antimicrobial activity of several marine algae or seaweed species (Nair *et al*., 2007; Sasidharan *et al.*, 2010). However, there is a paucity of information on the antibacterial potential of *Grateloupia lithophila*. Hence, an attempt has been made in the present study to investigate the antibacterial activity of methanol extract of marine red alga *G. lithophila*, against four human pathogenic bacteria.

MATERIALS AND METHODS

Collection of algae

Healthy, submerged thalli of the marine red alga *Grateloupia Lithophilia Borgesan* free from epiphytes were collected from Kovalam Beach, Tamilnadu during low tides in November 2017 and identified by standard manual (Desikachary *et al.,* 1990). The seaweed samples were thoroughly washed with many changes of fresh seawater, followed by rapid rinse in distilled water to remove the salt on the surface. Excess water was removed with blotting paper, shade dried and used for further experiments.

Preparation of algal extract

Dried seaweed was powdered and soaked in methanol (1:20, w/v) overnight, filtered and then concentrated to obtain the crude methanolic extract.

Bacterial cultures

The pure strains of *Escherichia coli, Klebsiella pneumoniae Pseudomonas aeruginosa* and *Staphylococcus aureus* were obtained from the American type culture collection (ATCC) and maintained on agar slopes at 4°C and sub cultured for 24 hrs before use.

Culture media

The composition of the medium was:

Peptone – 20 g
Sodium taurocholate – 5 g
Water – 1 litre
Agar – 20 g
Neutral Red Solution (2% in 50% ethanol) – 3.5 mL
Lactose (10% aqueous solution) – 100 mL

The bacterial cultures thus maintained were screened and tested for their purity and identity by cultural characteristics and standard biochemical tests.

Inoculum preparation

The strains were maintained on nutrient agar slants at 4°C. A loopful of each bacterial strain was added to a 50 mL sterile nutrient broth in a 100 mL conical flask. The flasks were then incubated on a rotary shaker for 24 hrs to activate the strains.

Antibacterial assay

The antibacterial activity of the algal extract was evaluated by the disc diffusion method (Bauer *et al*., 1996). A sterile cotton swab was dipped into the bacterial suspension and then was evenly streaked over the entire surface of a sterile Mueller-Hinton agar plate to obtain uniform inoculums. Sterile disc (6 mm diameter) loaded with various concentrations of algal extract (250 to 750 µg/mL) was impregnated onto the plates and incubated overnight at 37°C. Streptomycin was incorporated as the positive control. The antibacterial activity was determined by measuring the diameter of the zone of inhibition (mm).

Statistical analysis

The zone of inhibition of test extract at various concentrations viz., 250, 500 and 750 µg/mL was expressed as mean ± standard deviation. Data obtained were analyzed statistically using the statistical software SPSS (Version 17.0), to determine the degree of significance using a one-way analysis of variance (ANOVA).

RESULTS

The preliminary screening of the antibacterial activity of methanolic extract of marine red alga *Grateloupia lithophila* tested against the four pathogenic bacteria strains viz. *E. coli, K. pneumoniae, P. aeruginosa* and *S. aureus* are summarized in Table.1.

The study clearly showed that the extract was effective in inhibiting the growth of the bacterial pathogens. At the highest concentration of 750 µg/mL, the diameter of the zone of inhibition was 16 mm for *E. coli*, *P. aeruginosa* and *S. aureus*; while 14mm for *K. pneumoniae*. The zone of inhibition for the four bacterial strains ranged between 12 mm-14 mm at 500 µg/mL. Inhibition in the growth of the bacteria in least concentration (250 µg/mL) were recorded as 12 mm for *E. coli* and *S.aureus*; 11 and 10 mm for *K. pneumoniae* and *P. aeruginosa* respectively (Fig.1).

Table.1 Antibacterial activity of methanol extract of *Grateloupia lithophila.*

S.No	Name of the microorganisms	Zone of inhibition in mm on human pathogen (mean ± SD of n = 3) *				
		Control	250 µg/mL	500 µg/mL	750 µg/mL	Streptomycin
1	*E.coli*	-	12 ± 0.62	14 ± 0.36	16 ± 0.62	18 ± 0.10
2	*K.pneumoniae*	-	11± 0.12	12 ± 0.62	14 ± 0.12	20 ±0.01
3	*P.aeruginosa*	-	10 ± 0.10	12 ± 0.62	16 ± 0.11	20 ± 0.03
4	*S.aureus*	-	12 ± 0.62	14 ± 0.22	16 ± 0.24	18 ± 0.04

*** Zone of inhibition among different concentrations of *G. lithophila for* each bacterial strain is significantly different (P < 0.05)**

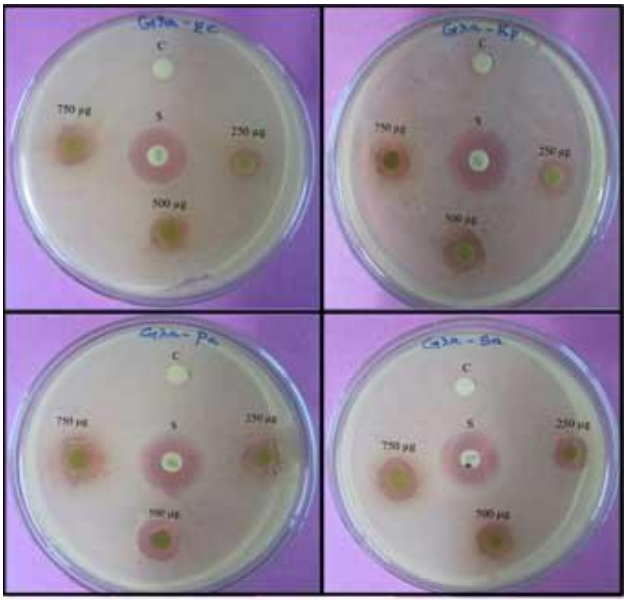

Fig.1 Antibacterial activity of the methanol extract of *G.lithophila.*

DISCUSSION

Marine organisms are a rich source of structurally novel and biologically active metabolites. Secondary or primary metabolites produced by these organisms are isolated, investigated and used to develop new pharmaceuticals (Freitas, 2002). The current study envisaged the antibacterial activity of the red alga, *Grateloupia lithophila.* The methanol extract was active against four pathogens such as *E. coli*, *K. pneumoniae*, *S. aureus* and *P. aeruginosa.*

The study corroborates with earlier reports on the inhibitory activity of crude marine algal extracts against pathogenic bacteria (Hornsey and Hide,1974). Salvador *et al.* (2007) studied the antimicrobial activities of 82 marine algae in fresh and lyophilized forms and reported the members of the red algal order, Bonnemaisoniales were the most active.

Generally gram-positive bacterial strains were more susceptible to seaweed extract than gram-negative bacterial strains (Ballantine *et al*., 1987). Tuney *et al*., (2006) also reported the effective control of gram-positive bacteria by the algal extracts used in their study when compared to gram-negative bacteria. The results of the present study also revealed that gram-positive organisms were more susceptible to the crude extracts of algae used. Taskin *et al*., (2001), also made similar observations, indicating that the more susceptibility of gram-positive bacteria to the algal extract was due to the differences in their cell wall structure and their composition (Tortora *et al*., 2001). Differences between the results of the present investigation and the results of other studies may be due to the production of bioactive compounds related to the seasons, assay method organic solvents used for extraction of bioactive compounds and the bacterial strain *S. aureus* is responsible for food poisoning, suppurating located infections and blood poisoning for debilitated subjects. This study has demonstrated that algae extracted by a

methanol solvent showed the greatest inhibition diameters for *E. coli, K. pneumoniae, P. aeruginosa* and *S. aureus*. Extracts prepared from fresh material of *Ulva rigida* showed remarkable inhibitor activity to S. *Aureus* while dried samples exhibited no antibacetrial activity (Tuney *et al.,*2007). Several reports pertaining to the antibacterial activity of several marine seaweed species against pathogenic bacterial strains are on record (Taskin *et al*., 2007; Kandhasamy and Arunachalam, 2008; Kotteswari *et al.,* 2015 and Subbiah Murugesan *et al.,* 2017).

Some studies concerned with the effectiveness of extraction methods highlight that methanol extraction yields the highest antimicrobial activity than n-hexane and ethyl acetate (Sastry and Rao, 1994). However, extraction with organic solvents always provides a higher efficiency for antimicrobial activities as compared to aqueous extracts (Lima-Filho *et al.,* 2002). The sequential extraction of marine algae using solvents from a low polar to highly polar always yielded a variety of antibacterial components.

The antibacterial property exhibited by *G. lithophila* in the current study may be due to the different active components contained in the extract. The exact mechanism and the compound responsible for the antimicrobial activity are still in progress in our laboratory.

Marine natural products contain a wide range of novel bioactive compounds or antibiotics with distinctive complex structures because of which they have developed unique metabolic and physiological capability. The antibacterial activity of *G. lithophila* explored in the current study suggests its use in the field of pharmaceuticals which may help in discover new antimicrobial compounds of natural origin in near future.

CONCLUSION

The present investigation has proved the antibacterial efficacy of methanol extract of marine red alga *Grateloupia lithophila* against *E. coli*, *K. pneumoniae*, *S. aureus* and *P. aeruginosa*. The marine red alga *G. lithophila* represent a potential source of bioactive compounds and must be studied for the production of natural antibiotics. Further work on the isolation, purification and characterization of the compound from *G. lithophila* are still in progress.

REFERENCES

Ballantine, D.L, Gerwick W.H, Velez, S.M, Alexander, E, Guevara, P. 1987. Antibiotic activity of lipid-soluble extracts from Caribbean marine algae. *Hydrobiologia,* 151/152: 463-469.

Bauer, A.W., Kirby, W.M.M., Sherris, J.C and Turok, M. 1996. Antibiotic susceptibility testing Berlin. pp.523.

Chakraborthy, K, Lipton, A.P, Paulraj, R, Vijayan K.K. 2010. Antibacterial diterpernoids of *Ulva fasciata* Delile from South-western coast of Indian Peninsula. Food Chem., 119, 1399-1408

Desikachary, T.V, V. Krishnamurthy and M.S. Balakrishnan. Madras Science Foundation, Chennai. 1990; pp. 279.

Freitas, A.M. 2002. Antibacterial activity of extracts of six macroalgae from the Northeastern Brazilian Coast. *Brazilian J. Microbiol*. 33: 311-313.

Hornsey, I.S, Hide, D.1985. The production of antimicrobial compounds by British Marine Algae. IV Variation of antimicrobial activity with algal generation. *Br. Phycol. J.* 20: 21-25.

Ireland, C., Roll, D., Molinsk, T., Mckee, T., Zarbriske, T., Swersey,J., 1988. Uniqueness of the marine environment: categories of marine natural product from invertebrates. In: Fautin, D.G. (Ed.), Biomedical Importance of Marine Organisms. California Academy of Sciences, San Francisco, pp. 41–58.

Kandhasamy, M and Arunachalam, K.D. 2008. Evaluation of *in vitro* antibacterial property of seaweeds of southeast coast of India. *Afr. J. Biotechnol*. 7: 1958-1961.

Kotteswari, M., Shanthi, N., Elamvaluthi, M and Murugesan, S. 2015. Antibacterial activities of *Caulerpa scalpelliformis* (R. Brown ex Turner) C.Agardh from the Gulf of Mannar South East Coast of India. *EJPMR.* 2(4):900-907.

Lima-Filho, J.V.M., A.F.F.U. Carvalho, S.M. Freitas and V.M.M. Melo. 2002. Antibacterial activity of extracts of six macroalgae from the Northeastern Brazilian Coast. *Brazil. J. Microbiol*., 33: 311-313.

Nair R, Chabhadiya R, Chanda S. 2007. Marine algae: Screening for a potent antibacterial agent. *Journal of Herbal Pharmacotherapy*. 7:73-86.

Salvador, N, A. Gomez-Garreta, L. Lavelli and L. Ribera, 2007. Antimicrobial activity of Iberian macroalgae. *Sci. Mar*. 71: 101-113.

Sasidharan, S, Darah, I, Noordin M.K.M.J. 2010. *In vitro* antimicrobial activity against *Pseudomonas aeruginosa* and acute oral toxicity of marine *algae Gracilaria changii. New Biotechnol*., 27(4): 390-396.

Sastry and Rao, V. S and G.R.K.Rao. 1994. Antibacterial Substances from Marine Algae: Successive Extraction Using Benzene, Chloroform and Methanol. *Botanica Marina* 37(4):357-360

Subbiah Murugesan, Sundaresan Bhuvaneswari, U.S. Mahadeva Rao, Vajiravelu Sivamurugan. 2017. Screening of Phytochemicals and Antibacterial Activity of Marine Red Alga

Portieria hornemannii (Lyngbye) P. C. Silva. *Research Journal of Pharmacology and Pharmacodynamics.* 9(3):131-136.

Taskin E, Ozturk M, Taskin E and Kurt O. 2007.Antibacterial activities of some marine algae from the Aegean Sea (Turkey). *African Journal of Biotechnology.* 6(24):2746-2751.

Thennarasan, S and Murugesan. S. 2015. Antibacterial activity of crude methanolic extract of marine brown alga *Lobophora variegata* (J.V.Lamouroux). *World Journal of Pharmaceutical Research*.1714-1722.

Received:07.05.18, Accepted: 06.06.18, Published:01.07.2018

Tortora GJ, Funke BR Case CL.2001. Microbiology: An Introduction. Benjamin Cummings. San Francisco, p. 88.

Tuney, I, Cadirci BH, Unal D, Sukatar A.2006.Antimicrobial activities of the extracts of marine algae from the Coast of Urla (zmir, Turkey). *Tur. J. Biol*. 30: 1-5.

Vinoth Kumar, R, Murugesan, S, Bhuvaneshwari, S, Thennarasan, S. 2015. *In vitro* antibacterial effects of red alga *Champia parvula* (C. Agardh) of various solvents against human pathogenic bacteria. *International Journal of Advances in Pharmaceutics.* 4 (6): 111-116.

***Corresponding Author:**
Priya, P*
Email: **priyaponmudi79@gmail.com**