*Journal of Applied Biological Sciences*
*E-ISSN: 2146-0108 16(1): 115-127, 2022*
*DOI: https://doi.org/10.71336/jabs.965*
*Research Article*

# EFFICACY OF ANTI-MICROFOULING AND TOXICITY FROM RED SEAWEED - *Portieria hornemannii* (Lyngbye) P.C.Silva 1987

Kavipriya Sakthivel*, Anantharaman Perumal

[1]*Annamalai University, Faculty of Marine Sciences, CAS in Marine Biology, Parangipettai, India*

**Corresponding Author:*
*E-mail: sakthivelkavipriya@gmail.com*

*(Received 26th June 2021; accepted 15th September 2021)*

**ABSTRACT.** Biofouling on drenched structures causes foremost monetary losses in the aquatic system. The point of this work was to screen the phytochemicals and antifouling capability of the different solvent extracts from seaweed *Portieria hornemannii* against fouling bacteria. Our methodology joins *in-vitro* toxicity bioassay, GC-MS and FT-IR analysis were carried out. The main target was to explore the biological activities of this species and to investigate the presence of chemical constituents. Primarily phytochemical analysis deduced the presence of the alkaloids, terpenoids, steroids, tannins, saponins, flavonoids, phenols, coumarins carbohydrates and glycosides. The antibacterial activities were tested against ten biofilm-forming bacteria using hexane, ethyl acetate, acetone and methanol extract of *P. hornemannii*. The methanol extract revealed the highest inhibition zone against *Bacillus flexus* (15.4 mm) and lower inhibition recorded in the acetone extract showed the zone of inhibition against the *Bacillus aryabhattai* (8.5 mm). The toxicity assay was analyzed against *Artemia* nauplii, the 50% inhibitory concentration (LC-50) value of methanol extract was recorded as low toxic (500 µg/ml). The Gas Chromatography-Mass Spectrometry (GC-MS) analysis of methanol fraction had shown the presence of bioactive compounds such as Oxirane, n-Hexadecanoic acid, 25-methyl-methyl ester and Lauroyl peroxide. Fourier Transform-Infrared Spectroscopy (FT-IR) reveals the presence of functional groups in the methanol extract of *P. hornemannii*. The present work recommended that the methanol extract of *P.hornemannii* might be further explored for testing biological activities after the isolation of individual components. The mixture and various combinations of these chemicals may hint at actual potent agents which may be novel against vast varieties of biofilm creatures.

**Keywords:** *FT-IR, GC-MS, phytochemicals, seaweed, toxicity analysis*

## INTRODUCTION

In an aquatic environment, bio-fouling is a major problem on submerged man-made structures such as fishing nets, engineering structure underwater pipelines, ship's vessels and hulls [1-3]. Biofouling accumulation in marine environs is made up of thousands of marine creatures including bacteria, fungi, phytoplankton, polychaetes, algae, barnacles, mollusks and ascidians [4, 5]. These fouling communities are very complex and socially organized processes [6]. These are natural processes that can have a significant environmental and economic impact on maritime. For example, corrosion, distortion and alteration of the immersed surface, increased drag leading to the reduction in watercrafts speed and increases the fuel consumption more than 45% [7-9]. The incremental fuel consumption leads to arouses the emission of carbon dioxide, sulfur oxide and nitrogen

oxide [10]. In the aquaculture and coastal industries, hundreds of millions to billions of dollars are spent annually on the anti-fouling process [6].

Tributyltin (TBT) containing antifouling paint was widely used in commercial vessels to control biofouling [11]. However, the use of TBT caused an environmental problem as it is more toxic to non-target marine organisms at low concentrations [12-14]. Because of the environ concern over the utilization of TBT, the International Maritime Organization (IMO) and Marine Environment Protection Committee (MEPC) prohibited the use of TBT for maritime solicitations from January 2008 [15, 16]. In addition, many other compounds are also commonly used as antifouling biocides like irgarol-1051 [17], sea-nine211, chlorothaloril [18], copper pyrithione, diuron, chlorothalonil [19], zinc pyrithione [20], tralopyil, triphenylborane, pyridine, capsaicin [21], maneb, thiram and zineb [22].

Natural products are suggested as an alternative to toxic biocides in antifouling paints for controlling biofouling [23]. Current research to control biofouling is focused on producing non-toxic, effective antifouling natural agents to replace toxic synthetic molecules [24]. In biofouling research, many natural extracts of terrestrial and marine sources have been tested as replacements for toxic antifoulants [25, 26]. Among these investigations, marine natural products have been highlighted as promising environmental friendly antifoulants [27]. Many organisms including marine seaweeds, sponges, corals and ascidians are believed to produce rich natural antifouling substances that could get rid of fouling organisms [28-33].

Among all marine organisms, the marine macroalgae are the most important living resources of the ocean with containing more bioactive compounds such as amino acids, terpenoids, phlorotannins, steroids, phenolic compounds, halogenated ketones and alkanes [34-41]. In this context, the present study was aimed to perform the anti-fouling activity of solvents extracts of *Porteria hornemannii* (Lyngbye) P.C. Silva to find out potential active antifouling agents.

## MATERIALS AND METHODS

### *Microorganisms*

Biofouling bacteria such as *Bacillus cohnii*-MT071882 (MBF1), *Bhargavaea cecembensis*-MT071889 (MBF2), *Virgibacillus dokdonensis*-MT071890 (MBF3), *Bacillus idriensis*-MT071893 (MBF4), *Fictibacillus phosphorivorans*-MT071896 (MBF5), *Bacillus sp*.-MT075845 (MBF6), *Bacillus aryabhattai*-MT075846 (MBF7), *Bacillus flexus*-MT075851 (MBF8), *Bacillus megaterium*-MT075850 (MBF9) and *Alcaligenes faecalis*-MT071952 (MBF10) were utilised for this study.

### *Seaweed sample collection*

The seaweed of *Portieria hornemannii,* was collected from the Gulf of Mannar, region (9.284 N, 79.178 E). The collected seaweed sample was cleaned using the running tap water followed by double distilled water to remove the unwanted biota. This sample was dried at room temperature. Finally, this seaweed sample was ground as fine particles.

### *Preparation of seaweed extraction*

Seaweed powder was subjected to sequential extraction with different solvents such as hexane, ethyl acetate, acetone and methanol at the ratio of 1:5 by the maceration method.

The seaweed extract was filtered by the Whatman No.1 filter paper and stored at -20°C for further examination [42].

### *Phytochemical screening*

The crude extracts phytochemicals analysis by a standard method to determine the presence of alkaloids, terpenoids, steroids, tannins, saponins, flavonoids, phenols, coumarins carbohydrates and glycosides [43-45]. The formation of precipitate or any changes of colors is a positive reaction to these investigates.

*Test for Alkaloids:* For alkaloid detection, 2 mL of concentrated HCl was added to 2 mL algal extract. After that, a few drops of Mayer's reagent were added. The presence of alkaloids is indicated by the presence of green or white precipitate.

*Test for Terpenoids:* Chloroform (2 mL) and concentrated Sulphuric acid were added to 0.5 mL of algal extract for terpenoids identification. The presence of terpenoids is shown by the formation of a reddish-brown hue at the contact.

*Test for steroids:* For identify the steroids, 0.5 mL of the algal extract was mixed with 2 mL of chloroform and 1 ml sulphuric acid ($H_2SO_4$). Steroids are detected by the appearance of a reddish-brown ring at the interface.

*Test for tannins:* 1 mL of algal extract mixed with 1 mL ferric chloride (5 %). The presence of tannins is indicated by the formation of a dark blue or greenish-black color.

*Test for saponins:* 2 mL distilled water was added to 2 mL algal extract and agitated for 15 minutes lengthwise in a graduated cylinder. The presence of saponins is indicated by the formation of a 1 cm layer of foam.

*Test for flavonoids:* 2 mL algal extract was mixed with 1 mL 2N sodium hydroxide (NaOH). The presence of flavonoids was indicated by the formation of a yellow color.

*Test for phenol:* 1 mL of algal extract was mixed with 2 mL of distilled water and a few drops of 10% ferric chloride. The presence of phenols is indicated by the formation of a blue/green color.

*Test for coumarins:* 1 mL of 10% NaOH was added to 1 mL of algal extract for coumarin identification. The presence of coumarins is indicated by the formation of a yellow color.

*Test for carbohydrate:* In a test tube, add 5 mL Fehling's solution with 2 mL seaweed extract and bring to a boil. The presence of carbohydrates in a sample is indicated by the formation of a yellow or brownish-red cuprous oxide precipitate.

*Test for glycosides:* 3 mL chloroform and 10% ammonium solution were added to 2 mL algal extract for glycosides detection. The presence of glycosides is indicated by the formation of pink color.

### *In-vitro antibacterial activity*

The Antifouling activity was evaluated by the agar well technique in petri dishes by using Zobell Marine Agar (ZMA). Bacterial isolates were spread on ZMA plates with sterile effusion and extracts (100 µl) were loaded. These plates were placed on an incubator at 37°C for 24 h. After incubation clear zone around a well was proof of antimicrobial activity. Diameters of the zones of inhibition were evaluated in millimeter. Each test was repeated in triplicate [46].

### *Toxicity assay*

The toxic activity assay method was followed by Ravichandran et al., 2018, different concentration of *P.hornemannii* seaweed extract was treated with *Artemia* nauplii in the 24 well plates that contain the filtered marine water and matured *Artemia* nauplii (50 numbers). In this lethality assay, triplicate was maintained. After 24 hours the survival rate of *Artemia* nauplii was recorded [47].

Percentage of *Artemia* survival =No. of live *Artemia*÷ Initial number of live *Artemia*×100

### *Gas Chromatography-Mass spectrometry [GC-MS]*

Gas Chromatography-Mass spectrometry (GC-MS) investigation was carried with the Perkin Gas Chromatography-Mass spectrum analyser. The program of the GC was maintained at the temperature of 60ºC with a hold for 5 minutes, followed by a gradual increase rate to 300ºC at 5ºC per minute for 15 minutes. Then 1 µl of sample was injected in the split mode at the ratio of 10:1. The compounds were recognized based on the retention time on the capillary column and matching them with the NIST library database.

### *Fourier Transform Infrared Spectroscopy [FT-IR]*

The Fourier Transform Infrared Spectroscopy (FT-IR, Shimadzu, IR Affinity1 Japan), analysis used to detecting the functional groups (Chemical bonds). The extract powder was compressed with KBr pellet (ratio of 1:10). Then the prepared sample was placed in FTIR Spectrum with a scan range from 400 to 4000 $cm^{-1}$.

## RESULTS AND DISCUSSION

### *Phytochemical Analysis*

In the current investigation, qualitative phytochemical analysis was conducted in *P.hornemannii* with hexane, ethyl acetate, acetone and methanol. The outcomes exposed the profile of metabolites such as alkaloids, terpenoids, steroids, tannins, saponins, flavonoids, phenols, coumarins carbohydrates and glycosides. Among the ten extracts, hexane extract indicated the existence of five phytoconstituents such as terpenoids, tannins, flavonoids and phenols and glycosides. The ethyl acetate extract exhibited the presence of seven compounds such as alkaloids, terpenoids, tannins, saponins, phenols, coumarins and glycosides. Acetone extract showed six compounds without tannins, phenols, carbohydrates and glycosides. The methanolic extract showed the seven compounds such as terpenoids, tannins, saponins, flavonoids, phenols, coumarins, and carbohydrates (Table 1).

***Table 1***. *Phytochemical analysis of Portieria hornemannii using different solvent extract [M-Methanol, H- hexane, EA-Ethyl Acetate, A-Acetone, present [+], absent [-]*

| Sample. No | Phytochemicals | Solvents | | | |
|---|---|---|---|---|---|
| | | M | H | EA | A |
| 1. | Alkaloids | + | - | + | + |
| 2. | Terpenoids | - | + | + | + |
| 3. | Steroids | - | - | - | + |
| 4. | Tannins | + | + | + | - |
| 5. | Saponins | - | - | + | + |
| 6. | Flavonoids | + | + | - | + |
| 7. | Phenols | + | + | + | - |
| 8. | Coumarins | + | - | + | + |
| 9. | Carbohydrates | + | - | - | - |
| 10. | Glycosides | + | + | + | - |

Seaweeds are known to contain rich phytochemicals include alkaloids, glycosides, saponins, tannins and steroids related active metabolites which are of great medicinal and economical value [48-51]. Many reports discovered the presence of phytochemicals in marine algae have been investigated for their antimicrobial activity [52-54]. Similarly, Prabhakaran et al., 2012 also reported the phytochemical of the seaweed must potential of anti-biofilm activity [55].

### *In-vitro bacterial Activity*

The bactericidal efficacy of hexane, ethyl acetate, acetone and methanol extract of *Portieria hornemannii* were investigated against biofilm forming bacteria (Fig. 1). Among the four extracts, Hexane, showed maximal bactericidal efficacy against the MBF1 strain (13.5 mm) and MBF2 strain (13 mm) and minimal zone of clearance was noted for BF5 strain (8.9 mm). Ethyl acetate extract, showed maximal antibacterial efficiency against the MBF1 strain (13.1 mm) and MBF3 strain (12.9 mm), minimal inhibition was noted for MBF9 strain (8.5 mm). Acetone extract, showed maximal antibacterial activity against MBF3 (13 mm) and MBF5 (12mm), minimum zone of inhibition was observed against MBF7 (8.3 mm). Finally, the Methanol extract presented the maximal antibacterial activity against MBF8 (15.4 mm) and minimum zone of inhibition was observed against MBF4 (9.9 mm).

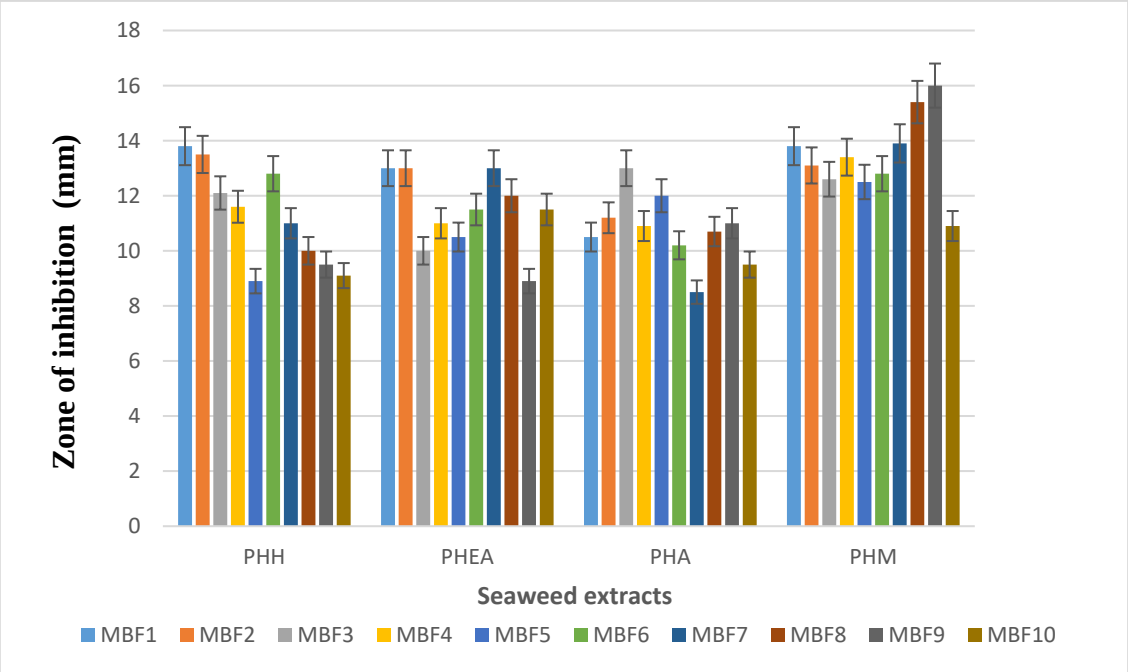


***Fig.1.*** *In-vitro antibacterial potential of different solvent extract of P. hornemannii [PHH- Hexane extract of P. hornemannii, PHEA- Ethyl Acetate extract of P. hornemannii, PHA- Acetone extract of P. hornemannii, PHM-Methanol extract of P. hornemannii].*

In general, seaweed species belonging to Rhodophyceae have a significant antibacterial activity [56]. As the result of the bacterial growth inhibition assay, methanol extract display potential efficacy against all gram-positive bacteria, which is also correlated with previous reports [57-59]. Similarly, Sasidharan., *et al*., 2009 reported that higher antimicrobial activities using methanol extraction yielded seaweed extract [60]. However, the red seaweed of methanol extract was unable to exhibit efficacy against *Bacillus* sp (0mm) [61]. Nevertheless, in this study, methanol extract of red seaweed of *P.hornemannii* exhibits good bactericidal efficacy against *Bacillus* sps (>8mm).

### *In vitro toxicity assay*

In the cytotoxic activity assay, different solvent extracts of *P. hornemannii* seaweed was tested using *Artemia* nauplii (50 numbers). In the control group, no mortality was recorded. At a similar time the percentage of mortality rates against *Artemia* using hexane, ethyl acetate, acetone and methanol solvent at different concentrations (1, 10, 100, 500, 750 and 1000 µg $mL^{-1}$). The $LC_{50}$ value was registered at more than 500 µg $mL^{-1}$ (Fig. 2). This experiential lethality analysis point out the existence of effective toxicity. Based on the outcomes, the hexane, ethyl acetate, acetone and methanol extract of *Portieria hornemannii* are low toxic.

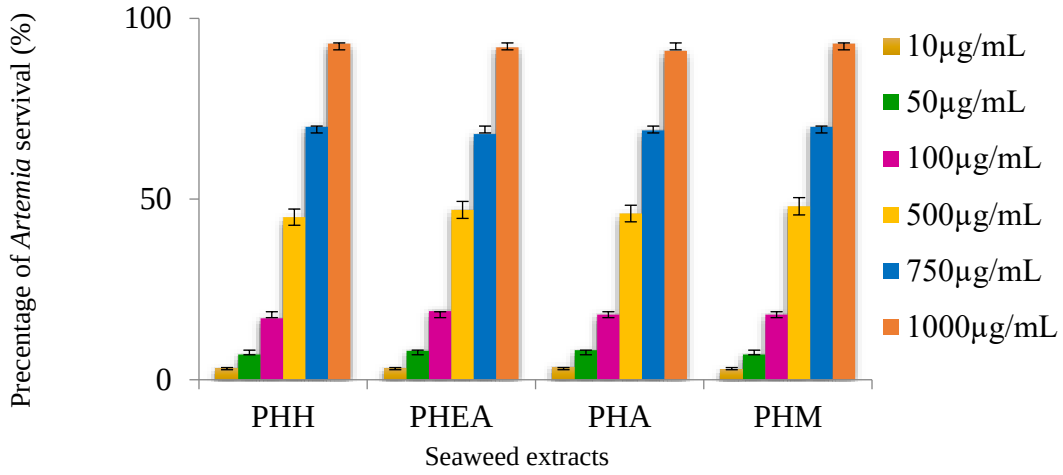


***Fig.2.*** *Toxicity analysis using different solvent extracts of P. hornemannii seaweed was tested using Artemia nauplii.*

Different model systems such as fathead minnows, zebrafish embryos, copepod, Daphnia and rainbow trout have been reported [62]. In addition, this is essential to establish better, rapid and convenient methods to predict the toxic effects of biomolecules on biological organisms. Till now, a few investigations had been described the toxicity effect of seaweed on *Artemia* [63,64].

Finally, evidence of antimicrobial efficiency against a wide range of biofouling bacteria from the methanol extracts obtained from the *P. hornemannii*, along with the lack of toxic effects against *Artemia* sp.

### *Fourier Transform Infrared Spectroscopy*

In the present study, FT-IR is used to identify the functional group in the seaweed (Fig 3 and Table 2).They showed the presence of prominent peaks at 3504 $cm^{-1}$, 1767 $cm^{-1}$, 1532 $cm^{-1}$, 903 $cm^{-1}$, 839 $cm^{-1}$, 708 $cm^{-1}$ and 618 $cm^{-1}$. The peak at 3504 $cm^{-1}$ represents the O-H stretching vibration of hydroxyl groups. The spectral peak at 1767 $cm^{-1}$ was assigned to C=O stretching of a carboxyl group. The peak at 1532 $cm^{-1}$ was assigned to C=C stretching vibration on the aromatic group. The peak 839 $cm^{-1}$ was response for C-H of aromatic ring. The signal 708 $cm^{-1}$ belongs to C=Cl stretching vibration of alkyl halides and 618 $cm^{-1}$ corresponding for C-Br stretching of alkyl halides (65-67)

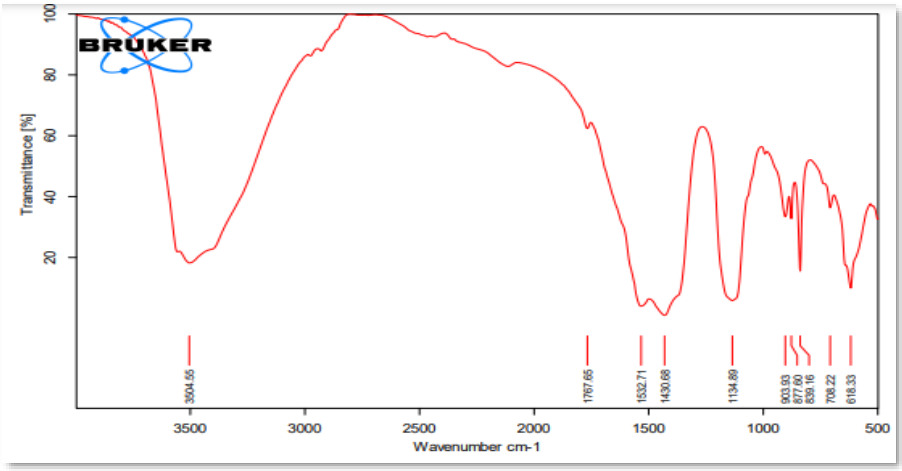


***Fig. 3**. FTIR analysis of methanolic extract of Portieria hornemannii*

***Table 2.** Functional group of methanol extract of Portieria hornemannii obtained through FTIR.*

| S. No | Wave Number | Bond | Functional Group |
|---|---|---|---|
| 1. | 3504 | OH Stretch | H-bond |
| 2. | 1767 | C=O Stretch | Carboxyl |
| 3. | 1532 | C=C Stretch | Aromatic |
| 4. | 839 | C-H | Aromatic |
| 5. | 708 | C-Cl Stretch | Alkyl halides |
| 6. | 618 | C-Br Stretch | Alkyl halides |

### *Gas Chromatography-Mass Spectrometry*

The presence of bioactive compounds in the methanol extract of *P. hornemannii* was identified through the GC MS [Fig. 3 and Table 2].

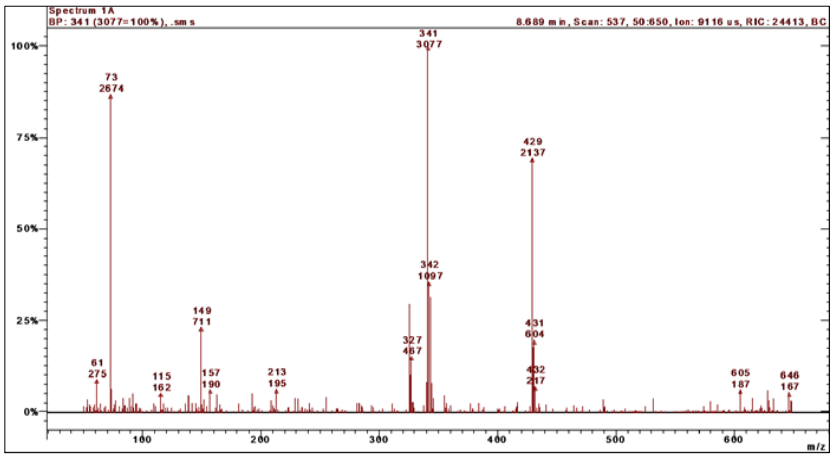


***Fig. 3.** GC-MS analysis of methanol extract of P. hornemannii*

***Table 2.*** *Chemical constituents of methanol extract of Portieria hornemannii obtained through GC-MS*

| S.No | Retention time | Name of the compound | Molecular formula | Peak area% |
|---|---|---|---|---|
| 1. | 3.4 | Oxirane | $C_{19}H_{38}O_2$ | 24.2 |
| 2. | 9.82 | n-Hexadecanoic acid | $C_{16}H_{32}O_2$ | 99.9 |
| 3. | 10.1 | Heptadecanoic acid, 25-methyl-methyl ester | $C_{29}H_{58}O_2$ | 42.8 |
| 4. | 12.3 | Lauroyl peroxide | $C_{24}H_{46}O_2$ | 67.3 |

In this study, Oxirane, n-Hexadecanoic acid, Heptacosanic acid, 25-methyl-methyl ester and Lauroyl peroxide are present in the methanol extract of *P. hornemannii*. Previously reported the antibacterial activities of seaweeds were reported because of the presence of bioactive compounds [68,69]. Oxirane was well reported in the red seaweeds with good biological efficacy and evaluated the antimicrobial activity of synthesised oxirane [70]. Oxirane and n-Hexadecanoic acid were revealed superior inhibition activity against microbes and good antioxidant properties [71,72].

## CONCLUSION

In summary, this study demonstrated that hexane, ethyl acetate, acetone and methanol extract of *P. hornemannii* have numerous phytochemicals. Nevertheless, it has defensive effects against biofilm-forming bacteria. Thus, this marine alga might be a proper source of potential bioactive compounds. Moreover, this study concludes that the methanol extract has a potential antibacterial efficacy with low toxic effects. Therefore, advanced studies should be performed on the isolation and identification of the potential bioactive compounds. So they may be utilized in antifouling paint formulation.

**Acknowledgment.** The Authors gratefully acknowledge the higher authorities of Annamalai University and Dean and Director in CAS, Marine Biology, Faculty of Marine Sciences, Parangipettai, Tamilnadu, for facilitating this experiment.

**Conflict of Interest.** The authors declare no potential conflicts of interest with regard to this research, authorship, and/or publication of this article.

**Authorship Contributions.** K.S., Data collection, analysis and interpretation; P.A., Literature search and corrections.

**Financial Disclosure.** This research received no grant from any funding agency/sector.

## REFERENCES

[1] Flemming, H.C. (2002): Biofouling in water systems–cases, causes and countermeasures. Applied microbiology and biotechnology 59(6):629-640.

[2] Schultz, M.P., Bendick, J.A., Holm, E.R. and Hertel, W.M. (2011): Economic impact of biofouling on a naval surface ship. Biofouling, 27(1) pp.87-98.

[3] Bannister, J., Sievers, M., Bush, F. and Bloecher, N. (2019): Biofouling in marine aquaculture: a review of recent research and developments. Biofouling 35(6):631-648.

[4] Coetser, S.E. and Cloete, T.E. (2005): Biofouling and biocorrosion in industrial water systems. Critical reviews in microbiology 3(4):213-232.
[5] Dobretsov, S., Dahms, H.U. and Qian, P.Y. (2006): Inhibition of biofouling by marine microorganisms and their metabolites. Biofouling 22(1):43-54.
[6] Fitridge, I., Dempster, T., Guenther, J. and De Nys, R. (2012): The impact and control of biofouling in marine aquaculture: a review. Biofouling 28(7):649-669.
[7] Schultz, M.P. (2007): Effects of coating roughness and biofouling on ship resistance and powering. Biofouling 23(5):331-341.
[8] Davidson, I.C., Brown, C.W., Sytsma, M.D. and Ruiz, G.M. (2009): The role of containerships as transfer mechanisms of marine biofouling species. Biofouling 25(7):645-655.
[9] Oliveira, I.B., Groh, K.J., Schönenberger, R., Barroso, C., Thomas, K.V. and Suter, M.J.F. (2017). Toxicity of emerging antifouling biocides to non-target freshwater organisms from three trophic levels. Aquatic Toxicology 191:164-174
[10] Shi, W., Xiao, Y., Chen, Z., McLaughlin, H. and Li, K.X. (2018): Evolution of green shipping research: themes and methods. Maritime Policy & Management 45(7):863-876.
[11] Yebra, D.M., Kiil, S. and Dam-Johansen, K. (2004): Antifouling technology—past, present and future steps towards efficient and environmentally friendly antifouling coatings. Progress in organic coatings 50(2):75-104.
[12] Magin, C.M., Cooper, S.P. and Brennan, A.B. (2010): Non-toxic antifouling strategies. Materials today 13(4):36-44.
[13] Cao, S., Wang, J., Chen, H. and Chen, D. (2011): Progress of marine biofouling and antifouling technologies. Chinese Science Bulletin 56(7):598-612.
[14] Amara, I., Miled, W., Slama, R.B. and Ladhari, N. (2018): Antifouling processes and toxicity effects of antifouling paints on marine environment. A review. Environmental toxicology and pharmacology 57:115-130.
[15] Jones, G. (2009): The battle against marine biofouling: a historical review. Advances in marine antifouling coatings and technologies19-45.
[16] Kyei, S.K., Darko, G. and Akaranta, O. (2020): Chemistry and application of emerging ecofriendly antifouling paints: a review. Journal of Coatings Technology and Research 1-18.
[17] Kottuparambil, S., Lee, S. and Han, T. (2013): Single and interactive effects of the antifouling booster herbicides diuron and Irgarol 1051 on photosynthesis in the marine cyanobacterium Arthrospira maxima. Toxicology and Environmental Health Sciences, 5(2):71-81
[18] Onduka, T., Ojima, D., Ito, M., Ito, K., Mochida, K. and Fujii, K. (2013): Toxicity of the antifouling biocide Sea-Nine 211 to marine algae, crustacea, and a polychaete. Fisheries science 79(6):999-1006
[19] Almond, K.M. and Trombetta, L.D. (2016): The effects of copper pyrithione, an antifouling agent, on developing zebrafish embryos. Ecotoxicology 25(2):389-398
[20] Soon, Z.Y., Jung, J.H., Jang, M., Kang, J.H., Jang, M.C., Lee, J.S. and Kim, M. (2019): Zinc Pyrithione(ZnPT) as an Antifouling Biocide in the Marine Environment—a Literature Review of Its Toxicity, Environmental Fates, and Analytical Methods. Water, Air, & Soil Pollution 230(12):310.
[21] Oliveira, I.B., Groh, K.J., Schönenberger, R., Barroso, C., Thomas, K.V. and Suter, M.J.F. (2017): Toxicity of emerging antifouling biocides to non-target freshwater organisms from three trophic levels. Aquatic Toxicology 191:164-174
[22] Zhao, W. and Wang, X. (2015): Antifouling based on biocides: From toxic to green. In Antifouling Surfaces and Materials, Springer, Berlin, Heidelberg. 105-134.
[23] Ohji, M. and Harino, H. (2017): Comparison of toxicities of metal pyrithiones including their degradation compounds and organotin antifouling biocides to the Japanese killifish Oryziaslatipes. Archives of environmental contamination and toxicology 73(2):285-293.

[24] Agostini, V.O., Macedo, A.J., Muxagata, E., da Silva, M.V. and Pinho, G.L.L. (2020): Non-toxic antifouling potential of Caatinga plant extracts: effective inhibition of marine initial biofouling. Hydrobiologia 847(1):45-60.
[25] Qian, P.Y., Xu, Y. and Fusetani, N. (2009): Natural products as antifouling compounds: recent progress and future perspectives. Biofouling 26(2):223-234
[26] Salta, M., J. A. Wharton, S. P. Dennington, P. Stoodley, Stokes K. R. (2013): Anti-biofilm performance of three natural products against initial bacterial attachment. International Journal of Molecular Sciences 14(11): 21757–21780
[27] Wang, L., Yu, L. and Lin, C. (2019): Extraction of Protease Produced by Sea Mud Bacteria and Evaluation of Antifouling Performance. Journal of Ocean University of China 18(5):1139-1146.
[28] Proksch, P., Putz, A., Ortlepp, S., Kjer, J. and Bayer, M. (2010): Bioactive natural products from marine sponges and fungal endophytes. Phytochemistry Reviews 9(4):475-489.
[29] Da Gama, B.A., Pereira, R.C., Carvalho, A.G., Coutinho, R. and Yoneshigue-Valentin, Y. (2002): The effects of seaweed secondary metabolites on biofouling. Biofouling 18(1):13-20.
[30] Armstrong, E., Boyd, K.G. and Burgess, J.G. (2000): Prevention of marine biofouling using natural compounds from marine organisms.
[31] Ortlepp, S., Pedpradap, S., Dobretsov, S. and Proksch, P. (2008): Antifouling activity of sponge-derived polybrominateddiphenyl ethers and synthetic analogues. Biofouling 24(3):201-208.
[32] Zhang, J., Liang, Y., Wang, K.L., Liao, X.J., Deng, Z. and Xu, S.H. (2014): Antifouling steroids from the South China Sea gorgonian coral Subergorgiasuberosa. Steroids 79:1-6.
[33] Trepos, R., Cervin, G., Hellio, C., Pavia, H., Stensen, W., Stensvåg, K., Svendsen, J.S., Haug, T. and Svenson, J. (2014): Antifouling compounds from the sub-arctic ascidian Synoicumpulmonaria: Synoxazolidinones A and C, pulmonarins A and B, and synthetic analogues. Journal of natural products 77(9):2105-2113.
[34] Nedumaran, T. and Arulbalachandran, D. (2015): Seaweeds: a promising source for sustrainable development. In Environmental Sustrainability 65-88.
[35] Eggertsen, M. and Halling, C. (2020): Knowledge gaps and management recommendations for future paths of sustrainable seaweed farming in the Western Indian Ocean. Ambio 1-14.
[36] Casado-Amezúa, P., Araújo, R., Bárbara, I., Bermejo, R., Borja, Á., Díez, I., Fernández, C., Gorostiaga, J.M., Guinda, X., Hernández, I. and Juanes, J.A. (2019): Distributional shifts of canopy-forming seaweeds from the Atlantic coast of Southern Europe. Biodiversity and conservation 28(5):1151-1172.
[37] Amorim, A.M., Nardelli, A.E. and Chow, F. (2020): Effects of drying processes on antioxidant properties and chemical constituents of four tropical macroalgae suitable as functional bioproducts. Journal of Applied Phycology 1-15.
[38] Uribe, E., Vega-Gálvez, A., García, V., Pastén, A., López, J. and Goñi, G. (2019): Effect of different drying methods on phytochemical content and amino acid and fatty acid profiles of the green seaweed, Ulva spp. Journal of Applied Phycology 31(3):1967-1979.
[39] Uribe, E., Vega-Gálvez, A., Vargas, N., Pasten, A., Rodríguez, K. and Ah-Hen, K.S. (2018): Phytochemical components and amino acid profile of brown seaweed Durvillaeaantarctica as affected by air drying temperature. Journal of food science and technology 55(12):4792-4801.
[40] Farvin, K.S., Surendraraj, A., Al-Ghunaim, A. and Al-Yamani, F. (2019): Chemical profile and antioxidant activities of 26 selected species of seaweeds from Kuwait coast. Journal of Applied Phycology 31(4):2653-2668
[41] Rasool, S. and Mohamed, R. (2016): Understanding agarwood formation and its challenges.In Agarwood 39-56.
[42] Thanigaivel, S., Hindu, S.V., Vijayakumar, S., Mukherjee, A., Chandrasekaran, N. and Thomas, J. (2015): Differential solvent extraction of two seaweeds and their efficacy in

controlling Aeromonassalmonicida infection in Oreochromismossambicus: A novel therapeutic approach. Aquaculture 443:56-64.

[43] Harborne, A.J. (1998): Phytochemical methods a guide to modern techniques of plant analysis. springer science & business media.

[44] Mujeeb, F., Bajpai, P. and Pathak, N., 2014. Phytochemical evaluation, antimicrobial activity, and determination of bioactive components from leaves of Aegle marmelos. BioMed research international *2014*.

[45] Mir, M.A., Sawhney, S.S. and Jassal, M.M.S., 2013. Qualitative and quantitative analysis of phytochemicals of Taraxacum officinale. Wudpecker Journal of Pharmacy and Pharmocology *2*(1), pp.001-005.El Shafay, S.M., Ali, S.S. and El-Sheekh, M.M. (2016): Antimicrobial activity of some seaweeds species from Red sea, against multidrug resistant bacteria. The Egyptian Journal of Aquatic Research 42(1):65-74.

[46] Ravichandran, A., Subramanian, P., Manoharan, V., Muthu, T., Periyannan, R., Thangapandi, M., Ponnuchamy, K., Pandi, B. and Marimuthu, P.N., (2018): Phyto-mediated synthesis of silver nanoparticles using fucoidan isolaed from Spatoglossumasperum and assessment of antibacterial activities. Journal of Photochemistry and Photobiology B: Biology 185:117-125.

[47] AfzalRizvi, M. and Shameel, M. (2005): Pharmaceutical biology of seaweeds from the Karachi coast of Pakistan. Pharmaceutical biology 43(2):97-107.

[48] Abu-Khudir, R., Ismail, G.A. and Diab, T. (2020): Antimicrobial, antioxidant, and anti-Tumor activities of Sargassumlinearifolium and Cystoseiracrinita from Egyptian Mediterranean Coast. Nutrition and cancer 1-16.

[49] Antony, T. and Chakraborty, K. (2020): Anti-inflammatory polyether triterpenoids from the marine macroalga Gracilariasalicornia: Newly described natural leads attenuate pro-inflammatory 5-lipoxygenase and cyclooxygenase-2. Algal Research 47:101791.

[50] Liu, L., Heinrich, M., Myers, S. and Dworjanyn, S.A. (2012): Towards a better understanding of medicinal uses of the brown seaweed Sargassum in Traditional Chinese Medicine: A phytochemical and pharmacological review. Journal of ethnopharmacology 142(3):591-619.

[51] El-Din, S.M.M. and El-Ahwany, A.M. (2016): Bioactivity and phytochemical constituents of marine red seaweeds (Janiarubens, Corallinamediterranea and Pterocladiacapillacea). Journal of Taibah University for Science 10(4):471-484.

[52] Wijesinghe, W.A.J.P. and Jeon, Y.J. (2012): Biological activities and potential industrial applications of fucose rich sulfated polysaccharides and fucoidans isolated from brown seaweeds: A review. Carbohydrate Polymers 88(1):13-20.

[53] Aarland, R.C., Bañuelos-Hernández, A.E., Fragoso-Serrano, M., Sierra-Palacios, E.D.C., Díaz de León-Sánchez, F., Pérez-Flores, L.J., Rivera-Cabrera, F. and Mendoza-Espinoza, J.A. (2017): Studies on phytochemical, antioxidant, anti-inflammatory, hypoglycaemic and antiproliferative activities of Echinacea purpurea and Echinacea angustifolia extracts. Pharmaceutical biology 55(1):649-656.

[54] Prabhakaran, S., Rajaram, R., Balasubramanian, V. and Mathivanan, K. (2012): Antifouling potentials of extracts from seaweeds, seagrasses and mangroves against primary biofilm forming bacteria. Asian Pacific Journal of Tropical Biomedicine 2(1):316-S322.

[55] Bansemir, A., Blume, M., Schröder, S. and Lindequist, U. (2006): Screening of cultivated seaweeds for antibacterial activity against fish pathogenic bacteria. Aquaculture 252(1):79-84.

[56] Val, A., Platas, G., Basilio, A., Cabello, A., Gorrochategui, J., Suay, I., Vicente, F., Portillo, E., Río, M., Reina, G. and Peláez, F. (2001): Screening of antimicrobial activities in red, green and brown macroalgae from Gran Canaria (Canary Islands, Spain). International Microbiology 4(1):35-40.

[57] Morales, J.L., Cantillo-Ciau, Z.O., Sánchez-Molina, I. and Mena-Rejón, G.J. (2006): Screening of antibacterial and antifungal activities of six marine macro algae from coasts of Yucatan peninsula. Pharmaceutical biology 44(8):632-635.

[58] Li, Y., Sun, S., Pu, X., Yang, Y., Zhu, F., Zhang, S. and Xu, N. (2018): Evaluation of antimicrobial activities of seaweed resources from Zhejiang Coast, China. Sustrainability 10(7):2158.

[59] Sasidharan, S., Darah, I. and Noordin, M.K.M.J. (2009): Screening antimicrobial activity of various extracts of Gracilariachangii. Pharmaceutical Biology 47(1):72-76.

[60] Alwarsamy, M. and Ravichandran, R., 2014. In-Vitro antibacterial activity of Kappaphycus alvarezii extracts collected from Mandapam coast, Rameswaram, Tamil Nadu. International Journal of Innovative Research in Science, Engineering and Technology *3*(1), pp.8436-40.

[61] Rajabi, S., Ramazani, A., Hamidi, M. and Naji, T. (2015): Artemiasalina as a model organism in toxicity assessment of nanoparticles. DARU Journal of Pharmaceutical Sciences 23(1):20.

[62] Ara, J., Sultana, V., Ehteshamul-Haque, S., Qasim, R. and Ahmad, V.U. (1999): Cytotoxic activity of marine macro-algae on Artemiasalina(Brine shrimp). Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives 13(4):304-307.

[63] Kanjana, K., Radtanatip, T., Asuvapongpatana, S., Withyachumnarnkul, B. and Wongprasert, K. (2011): Solvent extracts of the red seaweed Gracilariafisheri prevent Vibrio harveyi infections in the black tiger shrimp Penaeusmonodon. Fish & shellfish immunology 30(1):389-396.

[64] Meenakshi, S., Umayaparvathi, S., Arumugam, M. and Balasubramanian, T. (2011): In vitro antioxidant properties and FTIR analysis of two seaweeds of Gulf of Mannar. Asian Pacific Journal of Tropical Biomedicine 1(1): S66-S70.

[65] Baseri, M.K. and Baker, S. (2011): Identification of cellular components of medicinal plants using FTIR. Rom. J. Biophys 21: 277-284.

[66] Doh, H., Lee, M.H. and Whiteside, W.S. (2020): Physicochemical characteristics of cellulose nanocrystals isolated from seaweed biomass. Food hydrocolloids 102: 105542.

[67] Pádua, D., Rocha, E., Gargiulo, D. and Ramos, A.A. (2015): Bioactive compounds from brown seaweeds: Phloroglucinol, fucoxanthin and fucoidan as promising therapeutic agents against breast cancer. Phytochemistry Letters 14:91-98.

[68] Moshfegh, A., Salehzadeh, A., Shandiz, S.A.S., Shafaghi, M., Naeemi, A.S. and Salehi, S. (2019): Phytochemical analysis, antioxidant, anticancer and antibacterial properties of the Caspian Sea red macroalgae, Laurenciacaspica. Iranian Journal of Science and Technology, Transactions A: Science 43(1):49-56.

[69] Thirunarayanan, G. and Vanangamudi, G. (2016): Synthesis, spectral studies, antimicrobial and insect antifeedant potent ketooxiranes. Arabian Journal of Chemistry 9:269-S277.

[70] Saravanakumar, K., Chelliah, R., Ramakrishnan, S.R., Kathiresan, K., Oh, D.H. and Wang, M.H. (2018): Antibacterial, and antioxidant potentials of non-cytotoxic extract of Trichodermaatroviride. Microbial pathogenesis 115:338-342.

[71] Aher, N.G., Pore, V.S., Mishra, N.N., Shukla, P.K. and Gonnade, R.G. (2009): Design and synthesis of bile acid-based amino sterols as antimicrobial agents. Bioorganic & medicinal chemistry letters 19(18):5411-5414.