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RESEARCH ARTICLE

DETERMINATION OF ANTIMICROBIAL, ANTIOXIDANT PROPERTIES AND POLYPHENOLIC COMPOUNDS OF SEAWEEDS ORIGINATED FROM SOUTH COAST REGION OF MAHARASHTRA

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ABSTRACT

The present study demonstrates the antimicrobial, polyphenolic compounds and antioxidant potential of brown marine algae collected from the south coast region of Maharashtra. The methanolic extracts were tested against gram positive, gram negative bacteria in an attempt to be used as an alternative to commonly used antibiotics. Both brown seaweed species *Sargassum wightii* and *Ascophyllum nodosum* methanolic extracts were found to be active against gram positive as well as gram negative bacteria. *Ascophyllum nodosum* methanolic extract gave the highest inhibitory activity against *Klebsiella sp*. The present work shows a comparable therapeutic potency of tested seaweed members *Sargassum* and *Ascophyllum* extracts in treating human microbial pathogens to synthetic chemical antibiotics. A remarkable higher antioxidant DPPH free radical scavenging effect was recorded with *Ascophyllum nodosum* extract compared to *Sargassum sp*. Total flavonoid and phenol contents were determined by colorimetric methods using querecitin and gallic acid standards. FTIR Infrared Spectrometer analysis together with the high performance liquid chromatography provided a detailed description of possible functional constituents and major chemical components present in the marine macroalgae particularly in brown seaweeds to be mainly of phenolic nature to which the potent antimicrobial activity is being attributed.

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INTRODUCTION

Macroscopic marine algae, popularly known as 'seaweeds', are one of the important living resources of the ocean marine micro algae. Seaweeds are neurotic organisms that live in salty water and recognized as potential source of radioactive natural products.

Classification

Seaweeds are classified into four groups, Chlorophyce (Green algae), Phaeaphyceae (Brown algae), Rhodophyceae (Red algae), Cynophyceae (Blue-Green algae) based on type of pigments, morphological anatomical reproductive structures. Seaweeds are rich in vitamins A, E, C, and vitamin B12 and B. pantothenic acid, folic acids are generally higher in greens and reds than browns.

Sources of seaweeds: A seaweed may belong to one of several group of multicellular algae, Red, Brown, Green algae.

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Seaweeds most commonly inhibit the littoral zone and with in that zone more frequently on rocky shores than on sand or shingle. Red seaweeds – Red seaweeds have had a diverse evolution than the green and brown. Red seaweeds such as polysiphonia, lanasa are epiphyte, these are plants that grow on other plants for physical support. Many red algae are used in a manufacture of the important agar, widely as a growth medium for microorgsanisms and other biotechnologiocal and food applications. Brown seaweeds – Brown seaweeds consumed raw, boiled or dried material with sweetened green beans, jelly, crushed ice, and coconut milk in southern vietnam. Green seaweeds – Green seaweeds are found on both sandy and rocky beaches. The green colour of the seaweed is due to the green pigment Chlorophyll required for the photosynthesis of light. Using only Chlorophyll means that green seaweeds required good levels of light and therefore will not thrive in shadowed areas or too any depth.

MATERIALS AND METHODS

Collection of seaweeds

Seaweeds were collected by hand picking from the Kashid beach and Alibaug beach District Raigad, Maharashtra, India.

The individually collected samples were transferred to the laboratory in sealed ice packed boxes for immediate analysis and extraction. Seaweed thus collected were kept for drying for 7 days and extraction was done by solvent extraction method by using methanol as a solvent. Two identified species *Ascophyllum nodosum* and *Sargassum wightii* extracts were then screened for the evaluation of their antimicrobial, antioxidant activities and phenol and flavonoid contents.

Determination of antimicrobial activity

The antimicrobial activity was determined by the agar well diffusion method. Overnight grown bacterial cultures of *Eschericia coli, Psuedomonas aeuginosa, Proteus sp, Klebsiella sp., Staphylococcus aureus, Salmonella typhi* were transferred to sterile plate with muller hinton agar medium (Hi Media Laboratories Limited, Mumbai, India) and was spread with sterile spreader to create lawn. Wells of 6mm were punched into the previously seeded MH agar plates using sterile cork borer. About 20,40,60,80 µl of the different seaweed extract (red is solved as mentioned above) was placed in the wells and allowed to diffuse for 2h at 4ºc and then plates were incubated at 37º c for 24 h. The activity was determined by measuring the diameter of the inhibition zones for each well and expressed in diameter

Determination of minimum inhibitory concentration

To determine the minimum inhibitory concentration (MIC) of the crude extracts *S.wightii* and *A.nodosum* tube dilution technique was employed. This test was done to determine the lowest concentration of crude extracts that inhibit the growth of bacteria. A loopful of exponential phase bacterial culture corresponding to 0.5 Macfarlands opacity was inoculated into nutrient broth with different concentrations of extracts ranging from 20,40,60 and 80 µg/ml. The tubes were then incubated at 37°c for 24 hour. Turbidity was observed after the incubation period.MIC was defined as the lowest concentration of crude extract that completely inhibited the visible growth of the test organisms

Minimum Bactericidal concentrations

To determine minimum bactericidal concentration (MBC), one loopful of sample corresponding to MIC and the next higher concentrations were streaked individually on nutrient agar and incubated at 37°c for 24 h. The lowest concentration of extract that showed no bacterial growth on agar plate represents the MBC value of the particular extract.

DPPH free radical scavenging assay

The antioxidant potency of the different algal extracts was determined using 2,2-diphenyl-1-picrlyhydrazyl (DPPH) free radicals scavenging technique. 150 ll DPPH solution (4.3 mg dissolved in 3.3 ml methanol) was added to 3 ml methanol and the absorbance was taken immediately at 517 nm for control reading. Different volumes of test sample (20, 50,100, and 150 ll) were taken and diluted with methanol up to 3 ml to be screened with 150 ll DPPH solution added to each test tube. The mixture was vortexed and kept at room temperature for 0 and 5 min in the dark. Absorbance was taken at 517 nm spectrophotometrically using methanol as a blank. The percentage of DPPH free radicals scavenging activity was calculated with the following formula:

$$\text{PI (\% inhibition)} = \left[1 - \frac{[A2 - A1]}{A0} \times 100\right]$$

where A0 control absorbance (methanol), A1 sample absorbance(methanol + sample +DPPH) and A2 test absorbance(methanol +sample without DPPH). All reactions were carried out in duplicates and the degree of purple color formation and decolorization indicates the free radial scavenging activity of the algal extracts (Viturro *et al.*, 1999). The antioxidant effects of the tested extracts will then be compared to that produced by ascorbic acid as standard antioxidant.

RESULTS

Antimicrobial activity

Table 2 demonstrates the in vitro antimicrobial activity of the dried seaweeds; *Sargassum wightii* and *Ascophyllum nodosum*, methanol extracts. It can be easily can be easily noticed that the *Sargassumwightii* extract exhibited higher antimicrobial activity compared to that obtained by *Ascophyllum* extract except the antimicrobial activity showed in *Klebsiella sp.*, indicated as + or – signs where + + is relative to an inhibiton zone > 10mm; + indicating a measurement > 6mm or equal to 6mm. Among these dried brown seaweed extracts, *Ascophyllum nodosum* methanolic extract was the most effective showing largest inhibition zone particularly with *Klebsiella sp.* followed by *Sargassum wightii* particularly with *Escherecia coli* and *Pseudomonas aeruginosa*. Methanol extract showed variable antibacterial effect against some isolates but significant when compared with the standard antibiotic disks being used.

Minimum inhibitory concentration

Table 3 demonstrates the minimum inhibitory concentration of the dried brown seaweed extracts; *Sargassum wightii* and *Ascophyllum nodosum*, methanolic extracts on the basis of turbidity in the specific concentration. In MIC assay, *Klebsiella sp., Escherichia coli* and *Salmonella typhi* were found most sensitive for *Sargassum wightii* extract at lower concentrations as compared to other pathogens. On the other side *Klebsiella sp*., and Psuedomonas *aeruginosa* were found most sensitive for *Ascophyllum nodosum* extract at lower concentration as compared to other pathogens. The results of the present study revealed that gram positive bacteria are more susceptible than gram negative bacteria.

Minimum inhibitory concentrations achieved by *Sargassum wightii* and *Ascophyllum nodosum* extracts against several bacterial strains

Minimum bactericidal concentratin

Table 4 demonstrates the minimum bactericidal concentration of dried brown seaweed *Sargassum wightii* and *Ascophyllum nodosum*, methanolic extracts on the basis of total colonies observed on the subcultured agar plates from the MIC concentration that do not contained test agent. In MBC assay, *Klebsiella sp.*, and *Salmonella typhi* were found most sensitive for *Sargassumwightii* extract at lower concentrations as compared to other pathogen which completely killed the bacteria and no colonies were observed.

Table 1. Protocol for the preparation of final volumes of 20,40,60,80,100 µg/ml

Sea weed extract (µg/ml)	Diluent (ml)	Final Concentration (µg/ml)	Suspension of *Escherichia coli, Psuedomonas aeruginosa, Klebsiella sp., Proteus sp, Staphylococcus aureus, Salmonella typhi.*
0.2	0.8	20	0.1
0.4	0.6	40	0.1
0.6	0.4	60	0.1
0.8	0.2	80	0.1
1.0	0	100	0.1

Table 2. Antimicrobial activity showed by *Sargassum wightii* and *Ascophyllum nodosum*

Sr no	Pathogen Bacterial isolates	*Sargassum wightii* (µl) 20 40 60 80	*Ascophyllum nodosum* (µl) 20 40 60 80
1	*Escherichia coli*	- - + +	- - - +
2	*Pseudomonas aeruginosa*	- + ++ ++	- - + +
3	*Proteus sp.*	- - - -	- - - +
4	*Klebsiella sp.*	- + + ++	- + ++ ++
5	*Staphylococcus aureus*	- - + +	- + + ++
6	*Salmonella typhi*	- - + +	- - + +

Table 3. MIC achieved by seaweed extracts

Sr no.	Pathogens Bacterial isolates	MIC *Sargassum wightii* seaweed extract (µl)	MIC *Ascophyllum nodosum* seaweed extract (µl)
1	*Escherichia coli*	60 µl	80 µl
2	*Pseudomonas aeruginosa*	80 µl	60 µl
3	*Proteus sp.*	80 µl	80 µl
4	*Klebsiella sp.*	40 µl	60 µl
5	*Staphylococcusaureus*	80 µl	80 µl
6	*Salmonella typhi*	60 µl	80 µl

Table 4. MBC Showed by seawee extracts

Sr no.	Pathogens Bacterial isolates	MBC *Sargassum wightii* seaweed extract (µl)	MBC *Ascophyllum nodosum* seaweed extract (µl)
1	*Escherichia coli*	80	100
2	*Pseudomonas aeruginosa*	100	80
3	*Proteus sp.*	-	-
4	*Klebsiella sp.*	80	80
5	*Staphylococcusaureus*	100	80
6	*Salmonella typhi*	100	100

On the other hand Psuedomonas aerugninosa and Klebsiella sp. were found most sensitive for Ascophyllum extract at lower concentrations as compared to other pathogens which completely killed bacteria and no colonies were observed.

Minimum bactericidal concentration achieved by *Sargassum wightii* and *Ascophyllum nodosum* seaweed extracts.

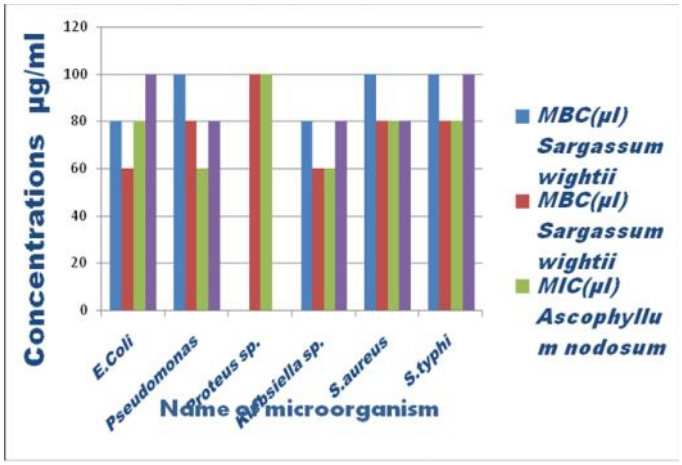


Fig. 1. Graphical representation of MIC and MBC achieved by *S.wightii and A.nodosum*

DPPH free radical scavenging assay

Screening the antioxidant activity by free radical scavenging assay showed that crude extracts of the seaweeds, revealed a comparable antioxidant activity. Increased activity was observed with increased algal extract concentration and increased time (5 min interval), where maximal values were obtained with S. latifolium B at a 150 lg/ml compared to the C. socialis samples. Results indicated 66% scavenging activity of the S. latifolium B crude extracts followed by C. socialis (65%) S. platycarpum A (60%) compared with the reference control ascorbic acid having a 92% free radical scavenging activity (Fig. 7). It was noted as well that higher antioxidant activity was observed with dried samples than with fresh ones (59%); supporting as such the finding of dried samples having a significant antimicrobial activity correlated to the high phenolic Constituent presence and to an increased scavenging free radical activity.

Determination of Phenolic content and Flavonoid content

Total phenolic compounds (TPC) of algal extracts was determined by Folin-Ciocalteu reagent according to the method of Antolovich *et al.* (2002) (17) with minor modifications. In Brief, 20 µL of extracts were mixed with 100 µL of 1:10 Folin-Ciocalteu reagent followed by the addition of Na2CO3 (80 µL, 7.5%).

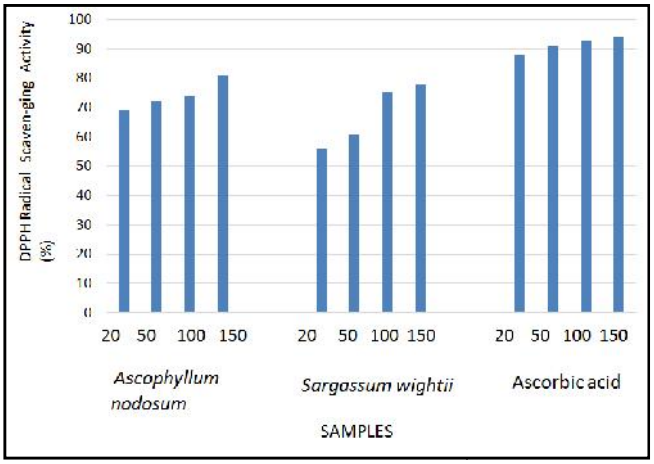


Fig. 2. DPPH Radical Scavenging Activity of Seaweed Extracts

The assay was carried out in microplate. After incubation at room temperature for 2 hours in dark, the absorbance at 600 nm was recorded. Gallic acid was used as the standard reference. TPC was expressed as mg Gallic acid equivalents per gram of dried extract (mg GAE g-1). Flavonoid content of each extract was determined by following colorimetric method (18). Briefly, 20 µL of each extract were separately mixed with 20 µL of 10 % aluminium chloride, 20 µL of 1 M potassium acetate and 180 µL of distilled water, and left at room temperature for 30 min. The absorbance of the reaction was recorded at 415 nm. The calibration curve was prepared by using Rutin methanolic solutions at concentrations of 12.5 to 100 µg mL-1. FC was expressed as mg Rutin equivalents per gram of dried extract (mg RE g-1)

Statistics

Data were expressed as means ± standard errors of three replicate determinations. All statistics analyses were carried out using SPSS 16.0 for Windows. To determine whether there were any differences among the means, one way analysis (ANOVA) and the Duncan's new multiple range test were applied to the result. p-values < 0.05 were regarded to be significant. The Pearson correlation analysis was performed between antioxidant activity and total phenolic and flavonoids, and also between total phenolic and flavonoid contents.

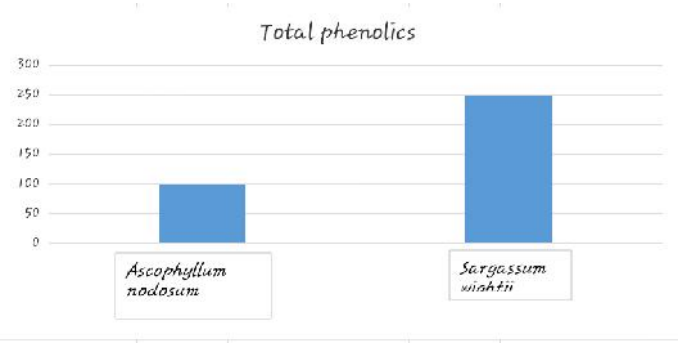


Fig.3. Total Phenol content

Total Flavonoids content

Total flavonoid content was calculated as quercetin equivalents (QE) as shown in fig .Total flavonoid content of the crude extract of *Ascophyllum nodosum* and its fractions varied noticeably. This study showed the significant flavonoid content in *Ascophyllum nodosum* i.e. 0.056 mg QE/100 mg of sample, follwed by Sargassum wightii i.e. 0.04 mg QE/100 mg of sample.

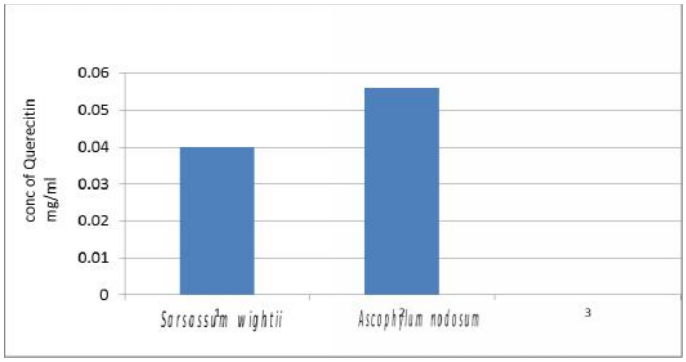
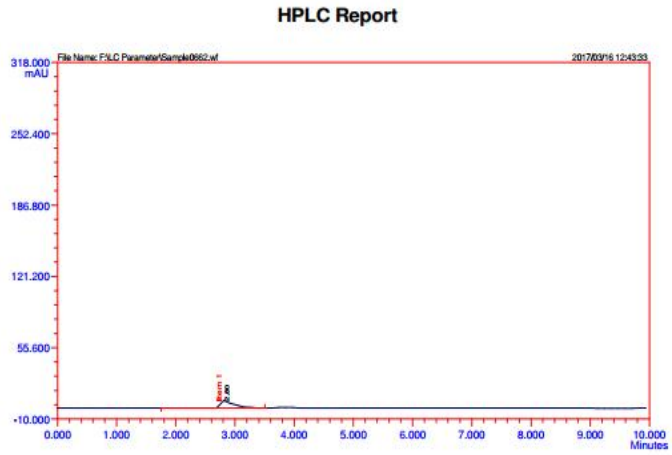


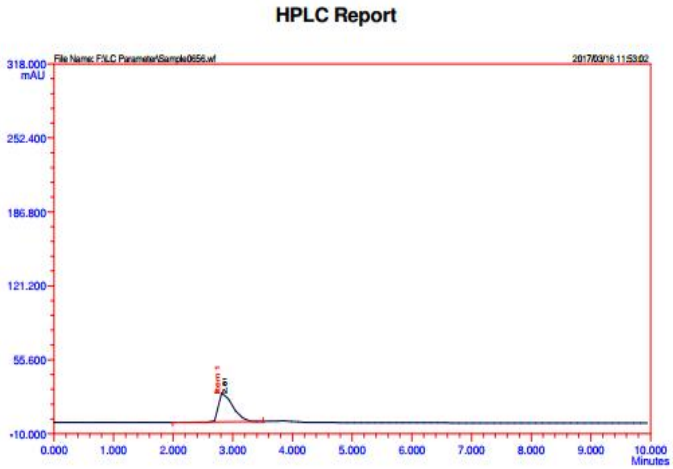
Fig 4. Total Flavonoids content (mg/100mg of extract) of *Ascophyllum* and *Sargassum* extract. Data are expressed as mean ± S.D

HPLC analysis

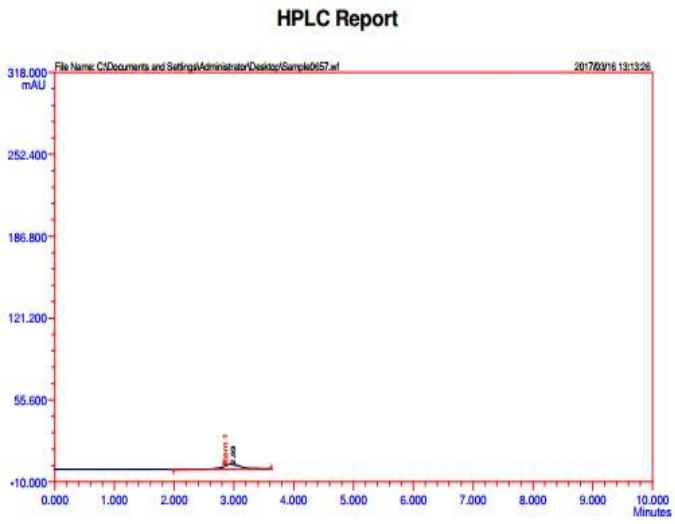
Qualitative analysis of the extract and fractions was carried out by using reverse phase HPLC and their chromatographic profile was compared with the retention times and absorption spectrum of reference standards (catechins, caffeic acid and querecitin). It was observed that the *A.nodosum* as well as *S.wightii* extracts contains phenol and it may be quericitin among other polyphenols



A.HPLC profile of standard quercitin of 20µg/ml



B.HPLC profile of methanol fraction *of Ascophyllum nodosum*



C. HPLC profile of methanol fraction of *Sargassum wightii*

Fig. 5. HPLC profiles of A) standard quercitin B) *Ascophyllum nodosum* C) *Sargassum wightii*

The Fourier Transform Infrared Spectrometer (FTIR) analysis

The exact mechanism and the compounds responsible for this antimicrobial activity are still unclear. Many studies suggested that the higher phenol content of marine macroalgae may affect bacterial growth and metabolism; they could have an activating or inhibiting effect on the microbial growth according to their distribution and concentration Reguant *et al.*, 2000; Alberto *et al.*, 2001).

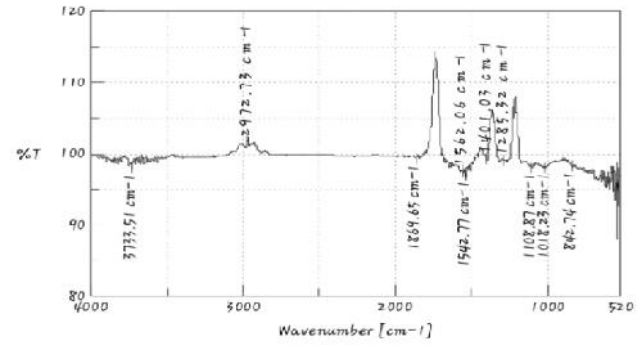


Fig. 6. *Sargassum wightii* FTIR spectrum showing highest peaks of phenolic, aromatic and of nitrite nature responsible for the potent antimicrobial activity

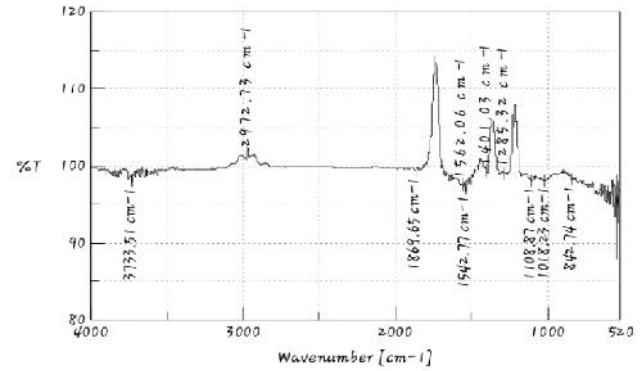


Fig. 7. *Ascophyllum nodosum* FTIR analysis indicating phenolic and nitrite groups to be the major chemical constituent of this seaweed extract revealed by highest peak

This was emphasized by analyzing the major chemical constituents of both algal cells being processed using the FTIR which indicated highest O-H absorption ranging between 3700 cm^{-1} and 3300 cm^{-1} related to the main chemical groups phenols namely hydroxyl amide between 2000 cm^{-1} and 1600 $cm^{-1,}$ and the nitrogen dioxide compounds absorption ranging between 1600 cm^{-1} and 1400 cm^{-1} on the cell walls, mainly of *Sargassum wightii* as well as *Ascophyllum nodosum*

DISCUSSION

Marine macroalgae are a rich source of novel and biologically active metabolites, producing potential bioactive compounds of interest in the pharmaceutical industry. Solvent extracts and constituents of the various algae have been shown to have in vitro antibacterial activity against gram positive and gram negative bacteria. Higher antimicrobial activity were obtained with methanol brown seaweed *Sargassum* species followed by the *Ascophyllum* extracts, a potent inhibitory effect was precisely observed on gram negative than on gram positive bacteria, except with *Proteus sp*. DPPH free radical scavenging effect of the tested extracts at concentration of 20, 50, 100, and 150 µl on the DPPH radical decreased in the order of: Ascophyllum nodosum (81%) > Sargassum nodosum (78 %). Higher radical scavenging activity was found in Ascophyllum nodosum (brown algae) having the greater phenolic constituent (Duan *et al.*, 2006; Nahas *et al.*, 2007) in agreement with our findings where higher phenolic content and increased antioxidant activity were observed in brown algae Ascophyllum species. Moreover our study reveals that the *Sargassum* has high phenolic contents and antioxidant potential due to high electron donors' attribute (Shahidi *et al.*, 1992). This study, therefore, suggests that the best reducing power of *Sargassum* and *Ascophyllum* might be due to the presence of its phenolic contents which is in agreement with the report of (Li *et al.*, 2009) Our study indicated that methanol fraction contains significant amounts of phenolic and aromatic compounds having good antioxidant potential and their beneficial effects on human nutrition and health are considerable. HPLC is the best way for chemical profiling of plant extract, therefore simple, rapid, reproducible and specific RP-HPLC fingerprinting was also established in the current research work for quantification of three major polyphenols (gallic acid, catechins and quercetin) in the *Ascophyllum nodosum and Sargassum wightii.* Caffeic acid reduces the acute immune and inflammatory response (Huang *et al.*, 1998) and catechin is the class of flavonoids with potent antioxidant and cancer chemo preventive properties (Weyant *et al.*, 2001). FTIR Chemical analysis revealed that the major constituents in *Sargassum* species were of phenolic nature (Reguant *et al.*, 2000; Alberto *et al.*, 2001) to which both potent antimicrobial and antioxidant activities are associated.

Conclusion

To our knowledge, this is the first study of its kind to investigate in details the antimicrobial and antioxidant properties of seaweed coriginating from Alibaug (south coast region of maharashtra).The antimicrobial activity of *Sargassum wightii* and *Ascophyllum ndodosum* extract were determined in both gram positive (*S.aureus*) and gram negative (*Klebsiella sp., E.coli, Salmonella typhi, Proteus sp., Pseudomans aeruginos*a) bacteria. It is concluded that methanol fraction of the both extracts contained significant amount of flavanoid and phenolic, aromatic contents and

exhibited significant antioxidant potentials. Among other polyphenols, to the best of our knowledge querecitin have been reported to be present In *A.nodosum* and *S. wightii.*

REFERENCES

Alberto, M.R., M.E. Faryas, M.C. Manca de Nadra, 2001. Effect of gallic acid and catechin on *Lactobacillus hilgardii* growth and metabolism of organic compounds. *J. Agric. Food Chem.*, 49, pp. 4359–4363

Anantharaman, P. 2002. Manual on Identification of Seaweed. All India Coordinate Project on Survey and Inventorization of Coastal and Marine Biodiversity. *Journal of the Marine Biological Association of India*, 29, 1-9

Berghe, V.A. and A.J. Vlietinek, 1991. Screening methods for antibacterial and antiviral agents from higher plants Methods for Plant Biochemistry, vol. 6, pp. 47–48

Caccamese, S. and Azzolina R. 1979. Screening for antimicrobial activities in marine algae from Eastern Sicily. Planta Med. 37, 333-339

Chang, C.C., M.H. Yang, H.M. Wen, J.C. 2002. Chern Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *J. Food Drug Anal.*, 10, pp. 178–182

Jeeva, S., Antonisamy, J. M., Domettila, C., Anantham, B. and Mahesh, M. 2012. Preliminary phytochemical studies on some selected seaweeds from Gulf of Mannar. *India Asian Pacific Journal of Tropical Biomedicine.*, 30-33.

Kandhasamy and Arunachalam, M. Kandhasamy, K.D. 2008. Arunachalam Evaluation of in vitro antibacterial property of seaweed of Southeast coast of India. *Afr. J. Biotechnol.*, 7, pp. 1958–1961.

Kanwal, S., N. Ullah, Ihsan-ul-Haq, I. Afzal, B. Mirza, 2011. Antioxidant, antitumor activities and phytochemical investigation of *Hedera nepalensis* K. Koch, an important medicinal plant from. *Pakistan Pak. J. Bot.*, 43, pp. 85–89

Kessler, M., G. Ubeaud, L. Jung, 2003. Anti- and pro-oxidant activity of rutin and quercetin derivatives. *J. Pharm. Pharmacol.*, 55, pp. 131–142

Kim, E. J., Park, S. Y., Lee, J. Y. and Park, J. H. 2010. Fucoidan present in brown algae induces apoptosis of human colon cancer cells. *BMC Gastroenterol.*, 10: 96.

Kolanjinathan, K. and Saranraj, P. 2014. Pharmacological efficacy of marine seaweed Gracilaria edulis extracts against clinical pathogens. *Global Journal of Pharmacology*, 8(2): 268-274

Kolanjinathan, K., P., Ganesh and P. Saranraj, 2014. Pharmacological importance of seaweeds, department of microbiology, Annamalai university, Chidambaram Tamilnadu, ISSN 2078-4589

Kolodziej, H. and Kiderlen, A. F. 2005. Antileishmanial activity and immunemodulatory effects of tannins and related compounds of leishmania parasitized RAW 264.7cells. *Phytochem.*, 66(17): 2056-2071

Laila Jafri, Samreen Saleem, Ihsan-ul-Haq, Nazif Ullah, Bushra 2004. Mirza In vitro assesment of antioxidant potential and determination of polyphenolic compounds of *Hedera nepalensiis* K.*Koch Arabian Journal Of Chemistry.*

Madhavi D. Rane, Kailash Doke, 2013. Physicochemical Properties, Antioxidant Features of Honey Samples from Ecological Niches of Western Maharashtra, *Journal of Science and Research (IJSR)*, Volume 3 Issue 8, August 2014 ISSN (Online): 2319-7064

Mandlik, R., Naik, S., Tatiya, A., Maseeh, A., Ghayal, N., 2014. B. Analysis of phytocomponents of Seaweed, Caulerpa racemosa. *International Journal of Pharma Research and Review*, 3(11): 39-45

Manilal, A., S. Sujith, B. Sabarathnam, G.S. Kiran, J. Selvin, C. Shakir, A. Ghandhimathi, P. 2009. Lipton Antimicrobial potential and seasonality of red algae collected from the Southwest coast of India tested against shrimp, human and phytopathogens. *Ann. Microbiol.*, 59, pp. 207–219

Maria Jose Perez, Elena Falque and Herminia Dominguez, 2015. Antimicrobial Action of compounds from Marine seaweed, Departmento de Enxeneria, Facultadde Ceincies. de Vigo, 32004, Spain.

Maruyama, H., Taumachi, H., Lizuka, M. and Nakano, T. 2006. The role of NK cells in antitumor activity of dietary fucoidan from Undaria pinnatifida sporophylls (Mekabu). Planta Medica, 72: 1415–1417.

Mohanty, D. and Adhikary, S. P. 2013. Assessment of changes in the algal diversity of Chilika lagoon after opening of new mouth to Bay of Bengal. *Journal of Water Resource and Protection*, 5: 611-623

Nadine m.s. et al. 2016. Nadine M.S.Moubayed,Hadeel Jawad Al Houri, Manal M. Al Khulaifi, Dunia A. Al Farraj, 2017. Antimicrobial, antioxidant properties and chemical composition of seaweeds collected from Saudi Arabia, *Saudi Journal of Biological Sciences*, 24, 162-169

Padmini Sreenivasa Rao, P. Sreenivasa Rao, S.M. 1986. Karmarkar Antibacterial substances from brown algae II. Efficiency of solvents in the evaluation of antibacterial substances from *Sargassum johnstonii* Setchell et Gardner Bot. Mar., 29, pp. 503–507

Park, H. K., Kim, I. H., Kim, J. and Nam, T. J. 2012. Induction of apoptosis by laminarin, regulating the insulin-like growth factor-IR signaling pathways in HT-29 human colon cells. *International Journal of Molecular Medicine.*, 30: 734–738.

Pavia, H. and Aberg, P. 1996. Spatial variation in polyphenolic content of Ascophyllum nodosum (Fucales, Phaeophyta). *Hydrobiologia*., 327: 199–203.

Ryu, M. J., Kim, A. D., Kang, K. A., Chung, H. S., Kim, H. S., Suh, I. S., Chang, W. Y. and Hyun, J. W. 2013. The green algae Ulva fasciata Delile extract induces apoptotic cell death in human colon cancer cells. *Biology Animations.*, 49: 74–81

Shon, M.Y., S.D. Choi, G.G. Kahng, S.H. Nam, N.J. 2004. Sung Antimutagenic, antioxidant and free radical scavenging activity of ethyl acetate extracts from white, yellow and red onions. *Food Chem. Toxicol.*, 42, pp. 659–666

Springfield *et al.*, E.P. Springfield, P.K.F. Eagles, G. 2005. Scot Quality assessment of South African herbal medicines by means of HPLC fingerprinting. *J. Ethnopharmacol.*, 101, pp. 75–83

Velioglu, *et al.*, Y.S. Velioglu, G. Mazza, L. Gao, B.D. 1998. Oomah Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. *J. Agric. Food Chem.*, 46, pp. 4113–4117

Zhuang, C., Itoh, H. and Mizuno, T. 1995. Antitumor active fucoidan from the brown seaweed umitoranoo (Sargassum thubergii). Biosci. *Biotechnology Biochem Journal*, 59: 563–567.

Zu, C. Li, Y. Fu, C. 2006. ZhaoSimultaneous determination of catechin, rutin, quercetin, kaempferol and isorhamnetin in the extract of sea buckthorn (*Hippophae rhamnoides* L.) leaves by RP-HPLC with DADJ. Pharm. Biomed. Anal., 41 (2006), pp. 714–719

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