J. Indian bot. Soc. (2022) Vol. 102 (3) : 218-228
https://doi: 10.5958/2455-7218.2022.00049.3

e-ISSN:2455-7218, ISSN:0019 - 4468
www.indianbotsoc.org

RESEARCH ARTICLE

In vitro antioxidant and antibacterial activity of *Scenedesmus obliquus* collected from Similipal biosphere reserve, Odisha, India

Prajna Paramita Bhuyan[1], Emamee Sahoo[1], Sukumar Bhakta[2], Biswajita Pradhan[3], Mrutyunjay Jena[3] and Akshaya Kumar Bastia[1,*]

Abstract: Microalgae have the ability to produce natural bioactive chemicals that are difficult to synthesize chemically. Natural antioxidants derived from microalgal sources may be effective substitutes for manufactured antioxidants due to their antibacterial action and low side effects. The influence of several solvents on phytochemical screening, antioxidant, and antibacterial properties of *Scenedesmus obliquus*, collected from Similipal Biosphere Reserve, was investigated in this study. The results revealed the presence of various phytoconstituents such as phenols, tannins, flavonoids, saponins, terpenoids, sterols, glycosides, and alkaloids, which were extracted using organic solvents of various polarities. For qualitative identification of phytochemicals, chloroform extract performed better than petroleum ether, acetone, methanol, and aqueous. Owing to their free radical scavenging and total antioxidant activity, total phenolic, total flavonoid, and total antioxidant activity of different solvent extracts of *S. obliquus* were examined. The highest phenolic content was found in chloroform and the lowest in aqueous extract. Flavonoid concentration was highest in chloroform extract and lowest in petroleum ether and acetone. Similarly, methanol extract displayed higher DPPH radical scavenging activity and lower total antioxidant activity than aqueous. Furthermore, agar cup diffusion testing of antibacterial activity against eight pathogenic bacteria revealed that all solvent extracts have the ability to block at least two of the test pathogens, with the exception of aqueous, which showed no action. The highest zone of inhibition (12 mm) was observed with chloroform and acetone extracts against *Shigella dysenteriae*, moderate (10 mm) with petroleum ether, chloroform, and acetone extracts against *Bacillus brevis*, and *Bacillus licheniformis* with petroleum ether and methanol extracts. The results showed that extracts of the green microalga *S. obliquus* in chloroform, acetone, and methanol have regained antioxidant and antibacterial activity, which could be useful for medicinal applications.

Key words: *Scenedesmus obliquus*, Phytochemicals, Antioxidant, Antibacterial, Similipal Biosphere Reserve.

Introduction

Algae has a diverse range of biological properties, including anticancer, antioxidant, anti-inflammatory, and antibiotic substances (Pradhan *et al.* 2021b, Pradhan e*t al.* 2021d). Proteins, vitamins, omega-3 fatty acids, and antioxidants are all abundant in algae (Mohanty *et al.* 2020, Pradhan *et al.* 2022a). Microalgae include a wide range of bioactive chemicals, including antioxidants, carotenoids, phenolics, and flavonoids (Mohanty *et al.* 2020). Although several research have reported on the antioxidant capabilities of macroalgae and their consequences on a variety of free radical disorders (Pradhan *et al.* 2021c, Pradhan *et al.* 2020c), the antioxidant activities of microalgae, notably *Scenedesmus obliquus* from the Biosphere Reserve, have received less attention.

Microalgae were recently identified as a natural source of a variety of biologically and pharmacologically active chemicals with structurally composite molecules (Pradhan *et al.* 2021d). In today's food sector, there is a clear trend toward the development and manufacture of functional items (Mendiola *et al.* 2008). Several studies reported that microalgae are excellent sources of natural antioxidants, antibacterial, and antifungal activity (Najdenski *et al.* 2013, Battah *et al.* 2014). Microalgal bioactive compounds have unique properties like inhibitory activities against growth and development of microorganisms such

✉ Akshaya Kumar Bastia
bastianou@gmail.com

[1]Department of Botany, Maharaja Sriram Chandra Bhanja Deo University, Baripada-757003, India
[2]Botanical Survey of India, Western Regional Centre-7, Pune-411001, India
[3]Algal Biotechnology and Molecular Systematic Laboratory, Post Graduate Department of Botany, Berhampur University, Bhanja Bihar, Berhampur-760007, India

Received : 08 March 2022 Accepted : 30 May 2022 Published online : 30 September 2022

as bacteria, antiviral, antifungal, anti-allergic, antioxidant, antidiabetics, anticancer activities (Kim *et al.* 2011, Devi *et al.* 2011, Pradhan *et al.* 2021a, Pradhan *et al.* 2022b, Pradhan *et al.* 2022f). Both marine and fresh water possess a rich of algae biodiversity and donates a wide variety of chemically bioactive secondary metabolites which counter act as a chemical defense against predators and herbivores under environmental stress(Behera *et al.* 2020, Behera *et al.* 2021, Dash *et al.* 2020, Dash *et al.* 2021, Maharana *et al.* 2019, Pradhan *et al.* 2021a).

Antioxidant has the ability to scavenge the free radicals and minimise the ROS produced in the stress condition. As electron or hydrogen donors, antioxidants serve as free radical scavengers, producing a large number of stable intermediate radicals (Pradhan *et al.* 2020b). They also prevent oxyradical formation to reduce oxidative stress, as well as delaying and inhibiting the oxidation of oxidizable components (Pradhan *et al.* 2020b). Artificial antioxidants are generally Phenolic and Flavonoids compounds such as BHA (butylated hydroxyanisole) and BHT (butylated hydroxytoluene) and causes several adverse health effects on human health (Pradhan *et al.* 2021c). As a result of their lower toxicity, natural antioxidants derived from plant and algae extracts have gotten a lot of interest. Recent studies have focused on developing new antioxidants to counteract or prevent diseases caused by reactive oxygen species (ROS). Algae have stronger antioxidant activity than other plants because they comprise of more non-enzymatic antioxidants like ascorbic acid, reduced glutathione, phenols, and flavonoids (Wu *et al.* 2010). The ability of algae to produce antimicrobial substances could be used not only as a defense agent (against pathogens) but also inhibit the growth of bacteria (Wu *et al.* 2010). Though much is known about the chemistry and the antimicrobial action of several phytochemicals, very few reports are available on the possible mechanism of action (Pradhan *et al.* 2022d, Pradhan *et al.* 2022e).

Currently, industrial and commercial use of microalgal biomass and extracts of biomass has gained a strong foot hold in various sectors of human life due to the presence of different useful compounds in them. Numerous studied suggest that many microalgae have good antimicrobial activity. Some microalgae species such as *Chlorella* sp. and *Scenedesmus* sp. have valuable sources of bioactive compounds. The rich metabolic content of *Scenedesmus* sp. is being exploited for use in food, aquaculture, bioremediation, cosmetics and pharmaceutical industries (Becker 2004, Toyub *et al.* 2008).

Studies also reported the antimicrobial activity of different microalgae against different pathogenic bacteria (Marrez *et al*. 2019, Pradhan *et al.* 2022c). Although some author reported that the evaluation of phytochemical constituents and antioxidant activity of *Chlorella vulgaris* and *Spirulina platensis* have been done (A El-Chaghaby *et al.* 2019) but no research has been carried out in case of *S. obliquus* from Similipal Biosphere Reserve, Odisha owing to the antibacterial activity. Keeping this in view, the present investigation aims to assess the evaluation of phytochemical constituents and antioxidant activity of *S. obliquus* with special reference to antibacterial efficacy. The findings of this study could lead to the identification of a possible source of future therapeutic compounds for disease-causing microorganisms, as well as its use as a dietary supplement during treatment and leads to new drugs discovery.

Materials and methods

Reagents and chemicals used

1,1-Diphenyl-2-picrylhydrazyl (DPPH), Rutin, Hydrogen peroxide (H_2O_2), Gallic acid, Ascorbic acid, Sodium salicylate, Ferrous sulfate ($FeSo_4$), Ammonium molybdate, Sodium carbonate (Na_2CO_3), Ferric chloride ($FeCl_3$), Folin-Ciocalteu's reagent, Ammonia solution (NH_3), Hydrochloric acid (HCl), Sulfuric acid (H_2SO_4), Fehling's solutions A and B, Sodium carbonate, Mercury potassium iodide, Aluminum chloride ($AlCl_3$), Sodium hydroxide (NaOH), Glacial acetic acid, Chloroform, acetone, Petroleum ether and Methanol were purchased from Sigma-Aldrich, Merck and Himedia.

Sample collection and isolation of algal pure strain

Algal samples were collected from

freshwater habitat of Similipal Biosphere Reserve situated in the central part of the Mayurbhanj district of Odisha. The collected species is cultured to its pure form and maintained at the algal research laboratory of MSCBD University. Repeated microscopic observation and subculture was carried out for purification. The pure microalgal strain was identified as *Scenedesmus obliquus* via morphological enumeration by using published literature. The strain number was assigned as MSCBDACC11 and photographed (fig. A).

Algal culture and preparation of crude extract

The green algal pure cultures were maintained in algal culture room in cultures rack at a controlled temperature of 25 ± 2 ˚C under 7.5 W/m^2 cool fluorescent light of 16-hour light:8 hour-dark cycle. The liquid cultures were hand shaken thrice daily to provide uniform light and aeration to culture suspension and to prevent sticking to the side of glass vessels. Fresh algal biomass was harvested through filtration from the late exponential phase to stationary phase cultures and shade dried in the laboratory. The dried biomass was blended in to a coarse powder. During the extraction, a known amount of powdered green algal biomass (1 gm) was taken in a tube and metabolites were extracted successively with petroleum ether, chloroform, acetone, methanol and aqueous for 24 hours. Then all the solvent crude extracts were centrifuged, filtered and evaporated by rotary evaporator. Then the crude extract was collected in a pre-weighed vial and dried and final weight was taken to know the percentage of yield. The percentage of the yield of algal crude was calculated and was stored at –20 ºC for future analysis. Different solvent extraction of dried biomass of *S. obliquus* (fig. B) and solvent extracts of *S. obliquus* dissolved in DMSO and preserved in vials (fig. C).

Qualitative screening of phytochemicals

The qualitative phytochemical screening of green algal extracts was carried out following standard methods such as test for phenols (Ferric chloride test), tannins (Braymer's test), flavonoids (Alkaline reagent test), saponins (Foam test), terpenoids and sterols (Salkowski's test), glycosides (Legal's test) and alkaloids by Wagner's test by using the standard protocol.

Estimation of total phenol content (TPC)

The spectrophotometric technique was used to determine the total phenolic content of the algal crude extract (OD; 765 nm taking gallic acid as standard) according to (Pradhan *et al.* 2021e). After three separate trials, the TPC concentration in the extract was calculated and represented in milligram gallic acid equivalent dry weight (mg GAE g-1 DW) of the sample.

Estimation of total flavonoid content (TFC)

The spectrophotometric technique was used to determine the total flavonoid content of the algal crude extract (OD; 415 nm taking rutin as standard) (Pradhan *et al.* 2021d). After three separate trials, the TFC in the extract was calculated using the algal sample's milligram rutin equivalent dry weight (mg of RUE g^{-1} DW).

Total antioxidant capacity

The phosphomolybdenum technique, as reported (Pradhan *et al.* 2020a), was used to assess total antioxidant activity (OD; 695 nm taking ascorbic acid as standard). Ascorbic acid (10-500 g ml^{-1}) was used as the positive control in the standard curve calibration. The quantity of gram equivalents of ascorbic acid was used to calculate total antioxidant activity.

DPPH free radical scavenging assay

Free radical scavenging assay activity was measured by DPPH scavenging activity. The algal crude extract's DPPH free radical scavenging activity was determined as described (Patra *et al.* 2020) (OD; 517 nm using ascorbic acid as a standard) and the percentage of DPPH decolorization was calculated using the formula:

Screening of antibacterial activity

The agar cup diffusion method was performed as described (Du Toit and Rautenbach 2000) was used to test the antimicrobial activity of petroleum ether, chloroform, acetone, methanol, and aqueous extracts of green microalgal strains. The antibacterial efficacy of nutrient agar plates inoculated with test organisms was tested in vitro. Four gram-negative bacteria, such as *Pseudomonas*

aeruginosa (S1), *Escherichia coli* (S2), *Salmonella typhimurium* (S5), *Shigella dysenteriae* (S21), and four gram-positive bacteria, such as *Staphylococcus aureus* (S3), *Staphylococcus epidermidis* (S4), *Bacillus licheniformis* (S6), and *Bacillus brevis* (S22) were used for antibacterial activity. For screening, a known amount of extract was dissolved in DMSO. Using crude extract (100 µl) and standard inoculums of 105 CFU/ml of bacterial cells, the agar cup method was used.

Statistical analysis

Experiments were done by taking each sample in triplicate and results were expressed as mean ± standard deviation (SD). All the experiments were repeated in triplicate and represented as values ± SD.

Results

Taxonomic enumeration of microalgal isolated strain

Scenedesmus obliquus (Turpin) Kützing

Table 1: Phytochemical analysis of *S. obliquus*

Solvent used	Phenols	Tannins	Flavonoids	Saponins	Terpenoids	Sterols	Glycosides	Alkaloids
P. Ether	+	-	-	++	-	-	+	+
Chloroform	++	-	+	++	-	-	-	+
Acetone	++	-	+	++	-	-	-	-
Methanol	+	-	+	++	-	-	-	-
Aqueous	+	-	-	++	-	-	++	-

Philipose, 1967, P.248, fig.159.

The identified character is, Cell unicellular, colonial, colony 2 to 4 cells, cells sickle to oval in shape, tapering acutely toward each end, cell 15 µm in long, 5 µm in broad, auto spores sick shaped, 7 µm in long, 2.5µm in broad (fig. A).

The antioxidant activity of active phytochemicals in different solvent extract of *S. obliquus* was assessed

After extraction, all the extractions of green microalga *S. obliquus* were subjected to estimate the % of yield and calculated. Further the phytochemical screening may be suitable and helpful for revealing of bioactive compounds and subsequently may lead to the drug discovery and pharmacological design. The phytochemical screening would be helpful for screening of bioactive compounds which may lead to the drug discovery. The phytochemical screening of green alga *S. obliquus* under different solvents e.g., petroleum ether, chloroform, acetone, methanol and aqueous is given in table 1. The phytochemical analysis of *S. obliquus* extracts revealed the presence of phytoconstituents such as phenols, tannins, flavonoids, saponins, terpenoids, sterols, glycosides and alkaloids. The petroleum ether extract confirms the presence of phenols, saponins, glycosides alkaloids and negative to tannins, flavonoids, terpenoids and sterols. Saponins are present in more amount in petroleum ether extract. The chloroform extract showed the presence of phenol, saponins, alkaloids and absence of tannins, terpenoids, sterols, glycosides. The acetone extract gave positive results for phenols, flavonoids, saponins and negative to tannins, terpenoids, sterols, glycosides and alkaloids. Methanol extract of *S. obliquus* displayed positive result for phenols,

Table 2: Antioxidant potential of *S. obliquus*

Solvent used	Total phenols mg/1gm dry biomass	Total flavonoids mg/1gm dry biomass	DPPH Scavenging %	Total Antioxidant mg/1gm dry biomass
P. Ether	0.946154 ± 0.002	0.73125 ± 0.007	62.28288 ± 0.009	2.055 ± 0.035
Chloroform	2.053846 ± 0.009	11.68125 ± 0.061	62.03474 ± 0.013	10.59 ± 0.056
Acetone	1.361538 ± 0.004	8.75625 ± 0.044	61.64481 ± 0.012	11.25 ± 0.035
Methanol	1.361538 ± 0.004	5.2875 ± 0.048	63.16909 ± 0.027	21.72 ± 0.076
Aqueous	0.403846± 0.002	5.2875 ± 0.048	60.15597 ± 0.018	0.345 ± 0.01

Table 3: Zone of inhibition (in mm) formed by crude extract of *S. obliquus* against different pathogenic bacteria

Solvent used	S1	S2	S3	S4	S5	S6	S21	S22
P. Ether	-	-	-	-	-	10	10	10
Chloroform	-	-	-	-	-	-	11	10
Acetone	-	-	-	-	-	-	11	10
Methanol	-	-	-	-	-	10	-	-
Aqueous	-	-	-	-	-	-	-	-

flavonoids, saponins and negative to others. Aqueous extracts showed the presence of phenols, saponins, glycosides and absence of rest of the phytochemicals are displayed (Table 1) and the phytochemicals present test Fig. D.

Different solvent extract of *S. obliquus* displayed potent source of Phenolics and flavonoids contents

Phenolics and flavonoids play a very crucial role in antioxidant activity. The presence of TPC in the crude extract was measured by Folin-Ciocalteu reagent and was represented in terms of gallic acid equivalent (R^2= 0.9815). The result showed that the highest phenolic content was found in chloroform extract (2.053846 ± 0.009 mg/g gallic acid equivalent) and the lowest phenolic content was observed in aqueous extract (0.403846± 0.002 mg/g gallic acid equivalent) in table 2 (Fig. E 1). Further, the TFC content algal crude solvent extracts were estimated and expressed in terms of rutin equivalent (R^2 = 0.9904). The total flavonoid contents of five solvent extracts of the microalgae *S. obliquus* showed that the maximum flavonoid content was found in chloroform extract (11.68125±0.061 mg/g) and the lowest was found in p. ether (0.73125±0.007 mg/g) and aqueous extract (0.73125±0.191 mg/g) (Fig. E2). Thus, the organism is a massive reservoir of phytochemicals with potent antioxidant activity.

Active phytochemicals in different solvent extract of *S. obliquus* displayed potent antioxidant activity and in vitro radical scavenging activity

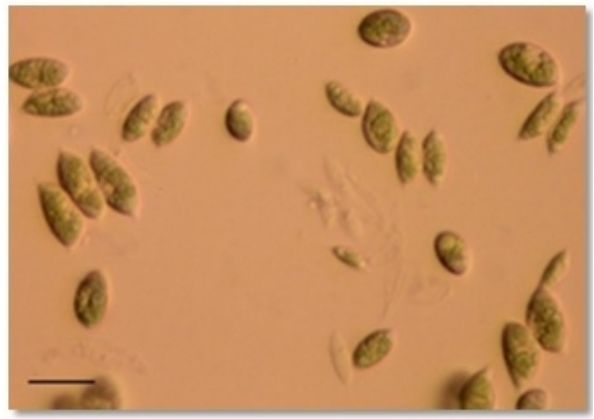

Figure A: Microscopic photograph of isolated *S. obliquus.*

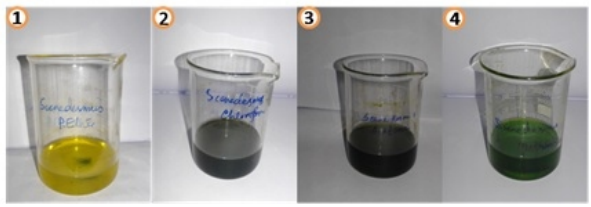

Figure B: Solvent extraction of dried biomass of *S.obliquus.* (1). Petroleum ether, (2). Chloroform, (3). Acetone and (4). Methanol.

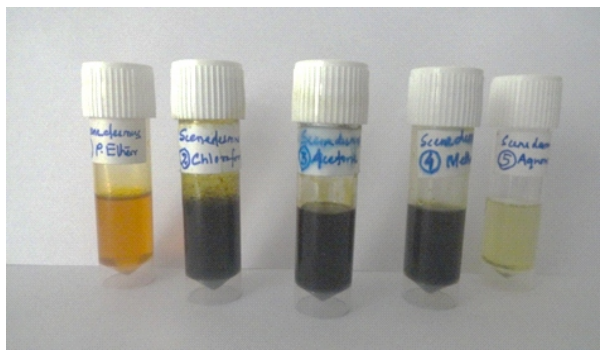

Figure C: Solvent extracts of *S. obliquus* dissolved in DMSO and preserved in vials

Further, phenolic and flavonoids are responsible for antioxidant activity. The total antioxidant activity was assessed by phosphomolybdenum assay and the results were estimated in terms of mg AAE g^{-1}. The total antioxidant activity of different solvent extracts of the microalga *S.obliquus* showed highest antioxidant activity in methanol extract (21.72 ± 0.076 mg/g ascorbic acid equivalent). The lowest activity was noticed in the aqueous extract (0.345 ± 0.01 mg/g ascorbic acid equivalent) (Fig. E3).

The quantification of DPPH exhibited in

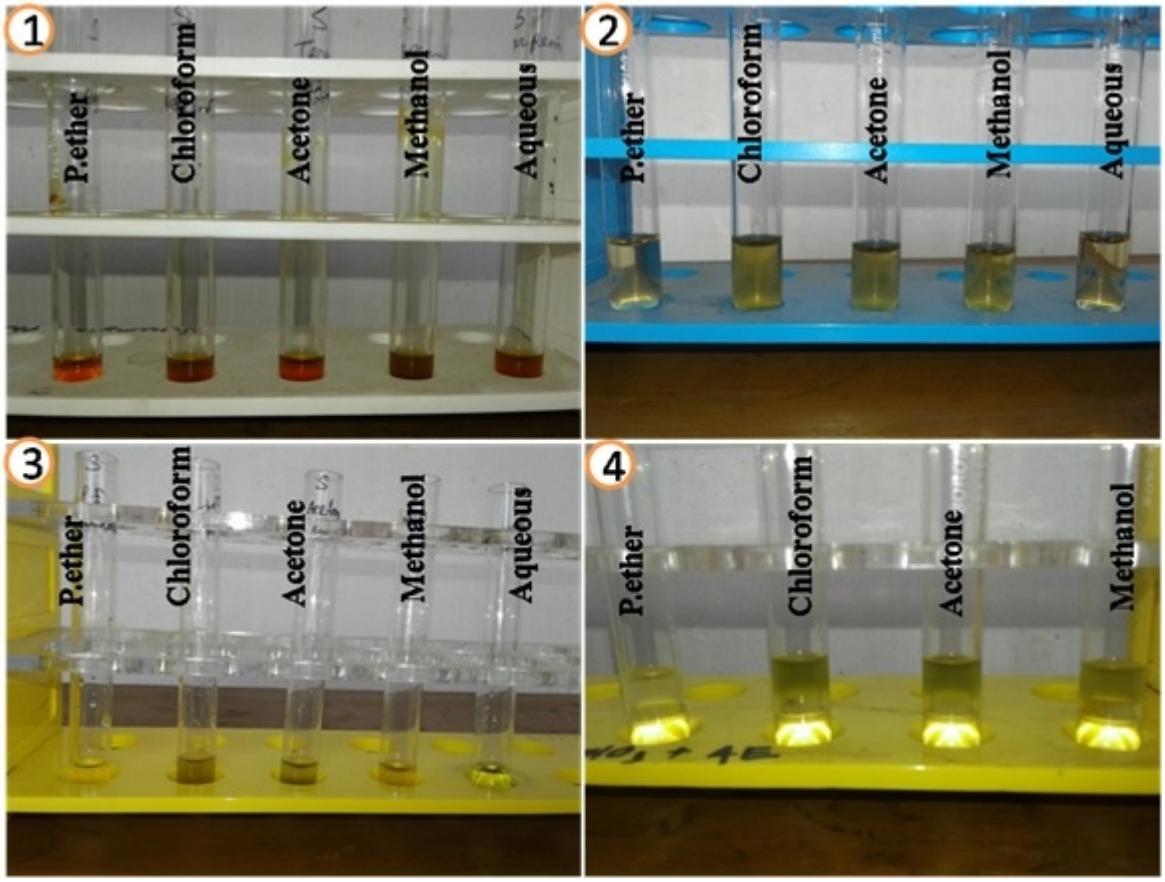

Figure D: Phytochemical analysis of *S. obliquus.* **1.** alkaloids, **2.** glycosides, **3.** phenols and **4.** steroids.

radical scavenging capacity. The DPPH antioxidant scavenging activities of algal crude different solvent extract were calculated as % of inhibition and to be (63.16909±0.027). DPPH radical scavenging activities of different extract of microalgae *S. obliquus* presented in this table 2. All these solvent extracts possessed the ability to scavenge the DPPH at various degrees, the DPPH radical scavenging activity was found to be hike in methanol extract and expressed in the form of % of inhibition (63.16909±0.027). The aqueous extract exhibited the minimum DPPH radical scavenging activity (60.15597±0.018) (Fig. E4). An analysis of our results, indicate that the solvent extract i.e., chloroform was found to be the best solvent for phytochemical extraction along with antibacterial and antioxidant screening of *S. obliquus*.

The antibacterial activity of active phytochemicals in different solvent extract of *S. obliquus* was assessed

To evaluate the antibacterial activity of methanolic extract of different solvent extract of *S. obliquus*, we evaluated the zone of inhibition in different bacterial strain. The intracellular metabolites were extracted with the organic viz. solvents e.g., petroleum ether, chloroform, acetone, methanol and aqueous. The results on antimicrobial activity of green algal extracts revealed that *Bacillus brevis* (S22) *and Shigella dysenteriae* (S21) are very sensitive to three of the solvent extracts that were petroleum ether, chloroform and acetone showing zone of inhibition between 10-11mm. In addition, *Bacillus licheniformis* (S6) was found sensitive to petroleum ether and methanol extract showing the zone of inhibition 10 mm. Additionally, it was found that aqueous extract of *S. obliquus* had no activity against any of the pathogen (Table 3). Moreover, maximum zone of inhibition (11mm) was obtained against the pathogen *Shigella dysentriae* (S21) in chloroform and acetone extract and minimum zone of inhibition (10mm) worked against *Bacillus brevis* (S22) in petroleum ether,

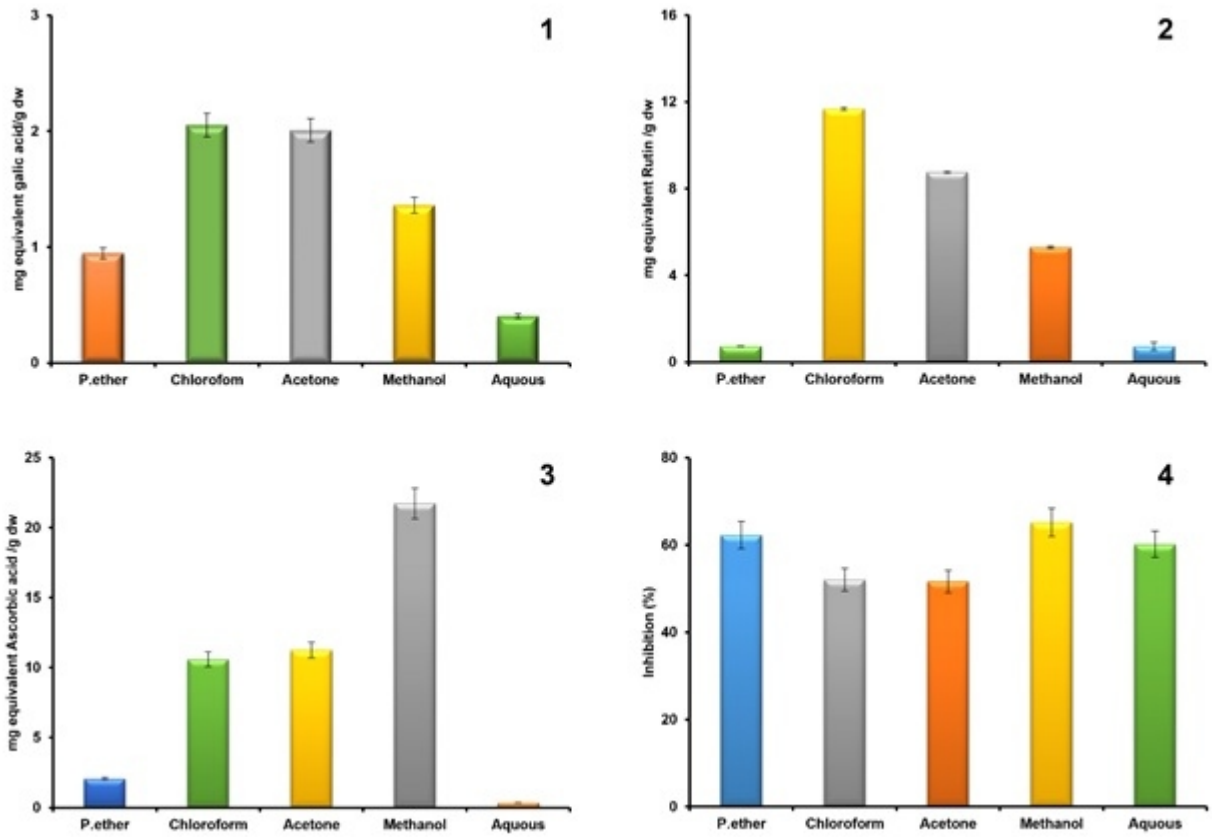

Figure E: Different solvent extract of *S. obliquus* displayed, **(1).** Total phenolic contents; **(2).** Total flavonoid contents; **(3),** Total antioxidant activity; **(4).** DPPH free radical scavenging.

chloroform and acetone and *Bacillus licheniformis* (S6) in petroleum ether, methanol (Table 3) (fig. F).

Discussion

Phytochemicals are currently receiving more attention in the creation of novel pharmaceuticals for potential medicinal pharmacophores (Pradhan *et al.* 2020c). The principal algal bioactive substances reported in various algae genera include alkaloids, sugars, proteins, glycosides, flavonoids, phenols, saponins, tannins, coumarins, and terpenoids. All of the above phytochemicals were found in (Pradhan *et al.* 2020a) S. obliquus, with the exception of carbs or reducing sugar, which are listed in Table 1. The plant's phenolic and flavonoid components have significant antioxidant activity as well as a variety of biological functions, including anthelmintic, analgesic, anti-inflammatory, antibacterial, anticancer, and anti-allergic effects (Behera 2019). The presence of phenolics, flavonoids, and tannins in most common phytochemicals confers antioxidant effects (Chaudhuri *et al.* 2014). Phenolics are the most well-known antioxidant compounds, and they work by adding hydrogen atoms to free radicals to neutralise them. These phytochemicals inhibit the generation of singlet oxygen species by scavenging reactive oxygen species (Balboa *et al.* 2013). According to the findings, the methanol extract had the strongest antioxidant activity, which is corroborated by earlier research. This could be because methanol is a dipolar solvent.

Furthermore, biochemical assays have been used to assess antioxidants' ability to scavenge free radicals. As a result, the DPPH test was utilised to determine the ability of active antioxidants in algal extracts to scavenge free radicals. By taking an electron or hydrogen radical and converting to a diamagnetic stable molecule, antioxidants in the microalgal extract cause the deep violet hue DPPH solution to fade yellow (Behera 2018). The entire

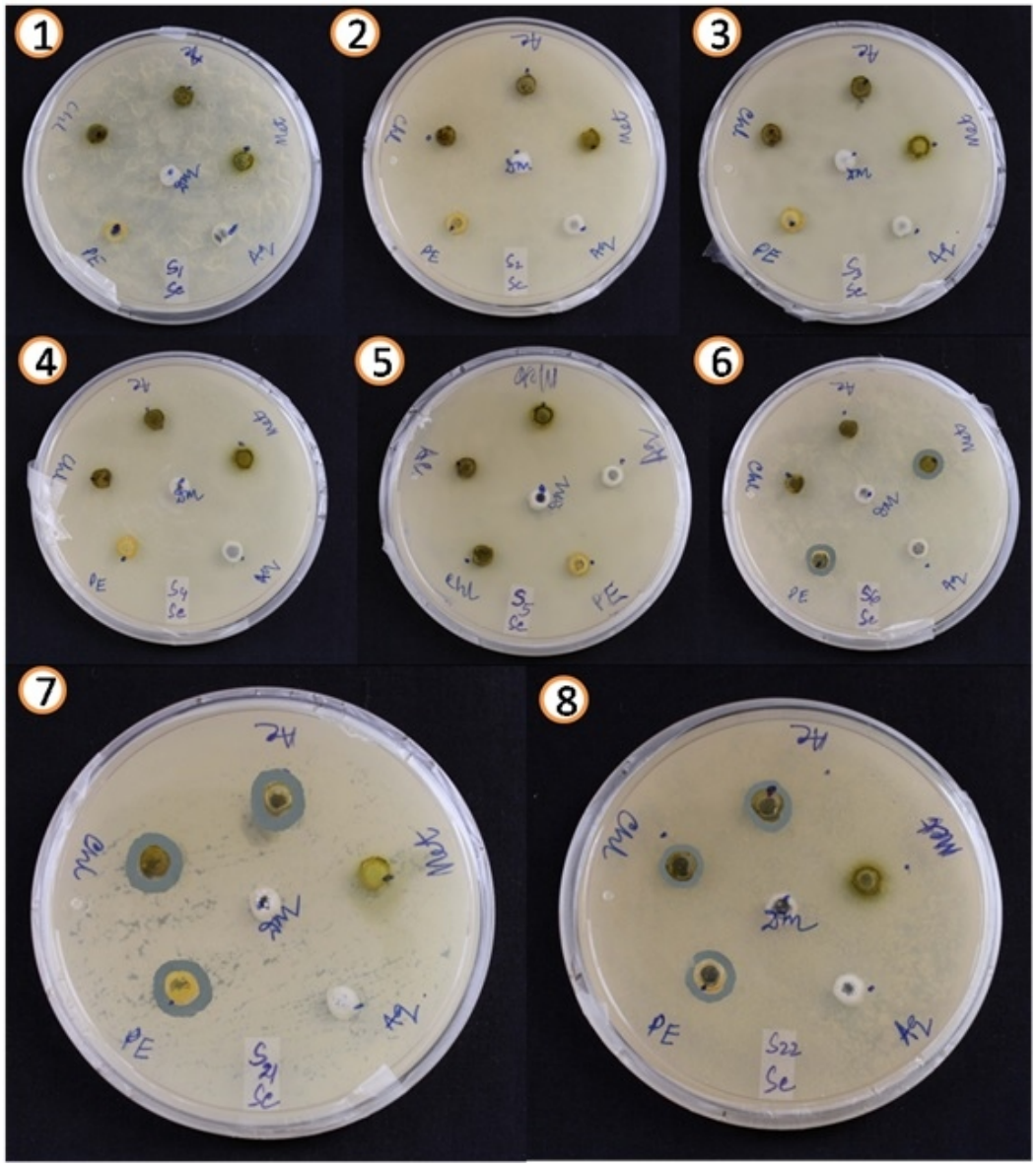

Fig. F: Nutrient agar plate showing antibacterial activities of crude extract of *S. obliquus* against 1. *Pseudomonas aeruginosa* (S1), 2. *Escherichia coli* (S2), 3. *Staphylococcus aureus* (S3), 4. *Staphylococcus epidermidis* (S4), 5. *Salmonella typhimurium* (S5), 6. *Bacillus licheniformis* (S6), 7. *Shigella dysenteriae* (S21) and 8. *Bacillus brevis* (S22). PE= petroleum ether, Chl = Chloroform, Ac = Acetone, Met = Methanol, Aq = Aqueous, DM = DMSO

extract's DPPH radical scavenging activity was significantly inhibited, confirming its strong antioxidant action. This conclusion is consistent with prior reports (Pradhan *et al.* 2020b, Pradhan *et al.* 2021d). Free radicals are short-lived and can produce in the cell. These radicals, on the other hand, are extremely reactive and can damage a variety of essential macromolecules in cells, making them detrimental to human health. As a result, the cell's redundant free radical neutralizer is critical (Pradhan *et al.* 2021e). As a result, the DPPH free radical scavenging activity of all solvent algal extracts was investigated, and the results showed that the methanol extract inhibited microalgal extracts more than the others, as previously reported(Pradhan *et al.* 2021c).

As a natural source of bioactive compounds, microalgae hold a lot of appeal. Because they have a high potential for producing bioactive chemicals that are difficult to make by

chemical synthesis (Goud *et al.* 2007, Kaushik and Chauhan 2008). According to the results of the aforesaid experiment, the green alga *S. obliquus* is a rich source of phytochemicals, resulting in the appearance of possible antibacterial activity. The presence of important phytoconstituents like phenols, flavonoids, saponins, glycosides, and alkaloids without tannins, terpenoids, and sterols in all extracts led to antimicrobial activity, according to phytochemical screening of different solvent extracts such as petroleum ether, chloroform, acetone, methanol, and aqueous. Algal antimicrobial compounds comprise phytoactive compounds with phenolic and flavonoid groups that have been structurally designed (Fayzi *et al.* 2020). *Pseudomonas aeruginosa* (S1), *Escherichia coli* (S2), *Staphylococcus aureus* (S3), *Staphylococcus epidermidis* (S4), *Salmonella typhimurium* (S5), *Bacillus licheniformis* (S6), *Shigella dysenteriae* (S21), and *Bacillus brevis* (S22) were employed to test antibacterial activity. In petroleum ether, chloroform, acetone, and for *Bacillus licheniformis* (S6) in petroleum ether, methanol, chloroform and acetone extracts exhibited maximal zone of inhibition (11mm) against *Shigella dysenteriae* (S21) and moderate zone of inhibition (10 mm) against *Bacillus brevis* (S22). The chloroform and acetone extract of *S. obliquus* were found to be highly efficient against *Shigella dysenteriae*, and somewhat effective against *Bacillus brevis* and *Bacillus licheniformis*, resulting in a zone of inhibition and reduced cell viability. The findings of this study were backed up by a study of an ethanolic extract of *Nostoc calcicola* that showed significant antibacterial activity against *E. coli* and *Staphylococcus aureus* (Agrawal 2016). Because of the beginning of oxidative imbalance after pharmacological therapy, the zone of inhibition is feasible (Pradhan *et al.* 2021d). Following medication treatment, a zone of inhibition was visible, as was the presentation of antimicrobial activity, which led to ROS-mediated cell death.

Conclusion

Due to the presence of phenolic and flavonoids ingredients in microalgal bioactive compounds, they are effective scavengers of intracellular free radicals. The efficiency of a methanol solvent extract of *S. obliquus* as a powerful antioxidant activity in vitro was discovered in this investigation. The methanolic extract of *S. obliquus* was found to be a more effective free radical ROS scavenger than other extracts due to the effective reduction of DPPH radicals. Furthermore, it modulates bacterial infections via a zone of inhibition. According to the results of this study, the green algae *S. obliquus* has important secondary metabolites with antibacterial and antioxidant properties. Phytochemicals that operate as natural sources of antioxidants and antimicrobials were found in a variety of solvent extracts. More research is needed to isolate and discover bioactive chemicals from *S. obliquus*, a microalgal species that could be beneficial for therapeutic applications. Further research into the design and development of prospective ROS scavengers from this species will ensure the synthesis of viable synthetic pharmacophores with improved bioavailability and therapeutic efficacy.

List of abbreviations

DPPH: 1,1-Diphenyl-2-picrylhydrazyl; TPC: Total phenol content; TFC: Total flavonoid content; TAA: Total antioxidant activity; SOD: *S. obliquus: Scenedesmus obliquus*.

Acknowledgments

The authors are thankful to Maharaja Sriram Chandra Bhanja Deo University, Baripada, Odisha for providing the necessary facilities to carry out this piece of research work.

References

A El-Chaghaby G, Rashad S, F Abdel-Kader S, A Rawash E-S and Abdul Moneem M 2019 Assessment of phytochemical components, proximate composition and antioxidant properties of *Scenedesmus obliquus, Chlorella vulgaris* and *Spirulina platensis algae extracts*. *Egyptian Journal of Aquatic Biology and Fisheries* **23 (4)** 521-526.

Agrawal M K 2016 Antimicrobial Activity of *Nostoc calcicola* (Cyanobacteria) isolated from central India against human pathogens. *Asian Journal of Pharmaceutics* **10** (04).

Balboa E M, Conde E, Moure A, Falqué E and Domínguez H 2013 In vitro antioxidant properties of crude extracts and compounds from brown algae. Food chemistry **138 (2-3)** 1764-1785.

Battah M G, Ibrahim H A, El-Naggar M M, Abdel_Gawad F K and Amer M S 2014 Antifungal agent from *Spirulina maxima*: extraction and characterization. *Global Journal of Pharmacology* **8 (2)** 228-236.

Becker W 2004 18 microalgae in human and animal nutrition. In: Handbook of microalgal culture: biotechnology and applied phycology, vol 312. Wiley Online Library.

Behera C, Dash S R, Pradhan B, Jena M and Adhikary S P 2020 Algal Diversity of Ansupa lake, Odisha, India. *Nelumbo* **62 (2)** 207-220.

Behera C, Pradhan B, Panda R, Nayak R, Nayak S and Jena M 2021 Algal Diversity of Saltpans, Huma (Ganjam), India. *J. of Indian Bot. Soc.* **101 (1 &2)** 107-120.

Behera S K 2018 Phytochemical screening and antioxidant properties of methanolic extract of root of *Asparagus racemosus* Linn. *International Journal of Food Properties* **21 (1)**2681-2688.

Behera S K 2019 Phytochemical Analysis and Antioxidant Activities of Gymnema sylvestre R. Br. Leaf Extracts. Free Radicals & Antioxidants **9** (1).

Chaudhuri D, Ghate N B, Deb S, Panja S, Sarkar R, Rout J and Mandal N 2014 Assessment of the phytochemical constituents and antioxidant activity of a bloom forming microalgae *Euglena tuba*. *Biological research* **47** 24. doi:10.1186/0717-6287-47-24.

Dash S R, Pradhan B, Behera C and Jena M 2020 Algal Diversity of Kanjiahata Lake, Nandankanan, Odisha, India. **99 (1 & 2)** 11-24.

Dash S R, Pradhan B, Behera C, Nayak R and Jena M 2021 Algal Flora of Tampara Lake, Chhatrapur, Odisha, India. *J.Indian Bot. Soc.* **101 (1)** 1-15.

Devi G K, Manivannan K, Thirumaran G, Rajathi F A A and Anantharaman P 2011 In vitro antioxidant activities of selected seaweeds from Southeast coast of India. *Asian Pacific journal of tropical medicine* **4 (3)** 205-211.

Du Toit E and Rautenbach M 2000 A sensitive standardised micro-gel well diffusion assay for the determination of antimicrobial activity. *Journal of microbiological methods* **42 (2)** 159-165.

Fayzi L, Askarne L, Cherifi O, Boufous E and Cherifi K 2020 Comparative antibacterial activity of some selected seaweed extracts from Agadir Coastal Regions in Morocco. *International Journal of Current Microbiology and Applied Sciences* **9** 390-399.

Goud M J P, Seshikala D and Charya M S 2007 Antibacterial activity and biomolecular composition of certain fresh water microalgae collected from River Godavari (India). *International Journal on Algae* **9 (4)**.

Kaushik P and Chauhan A 2008 In vitro antibacterial activity of laboratory grown culture of Spirulina platensis. *Indian Journal of Microbiology* **48 (3)** 348-352.

Kim S-K, Thomas NV and Li X 2011 Anticancer compounds from marine macroalgae and their application as medicinal foods. *Advances in food and nutrition research* **64** 213-224.

Maharana S, Pradhan B, Jena M and Misra M K 2019 Diversity of Phytoplankton in Chilika Lagoon, Odisha, India. Environ Ecol **37** 737-746.

Marrez D A, Naguib M M, Sultan Y Y and Higazy A M 2019 Antimicrobial and anticancer activities of *Scenedesmus obliquus* metabolites. Heliyon **5 (3)** e01404.

Mendiola J, Rodríguez-Meizoso I, Señoráns F, Reglero G, Cifuentes A and Ibáñez E 2008 Antioxidants in plant foods and microalgae extracted using compressed fluids. *Electronic Journal of Environment, Agricultural and Food Chemistry* **7** 3301-3309.

Mohanty S, Pradhan B, Patra S, Behera C, Nayak R and Jena M 2020 Screening for nutritive bioactive compounds in some algal strains isolated from coastal Odisha. *Journal of Advanced Plant Sciences* **10 (2)** 1-8.

Najdenski H M, Gigova L G, Iliev I I, Pilarski P S, Lukavský J, Tsvetkova I V, Ninova M S and Kussovski V K 2013 Antibacterial and antifungal activities of selected microalgae and cyanobacteria. *International journal of food science & technology* **48 (7)** 1533-1540.

Patra S, Bhol C S, Panigrahi D P, Praharaj P P, Pradhan B, Jena M and Bhutia S K 2020 Gamma irradiation promotes chemo-sensitization potential of gallic acid through attenuation of autophagic flux to trigger apoptosis in an NRF2 inactivation signalling pathway. Free radical biology & medicine **160** 111-124. doi:10.1016/j.freeradbiomed.2020.06.022.

Philipose M T 1967 Chlorococcales. Indian Council of Agricultural Research, Krishi Bhawan, New Delhi.365p.Pradhan B, Baral S, Patra S, Behera C, Nayak R, MubarakAli D and Jena M 2020a Delineation of gamma irradiation (60Co) induced oxidative stress by decrypting antioxidants and biochemical responses of microalga, Chlorella sp. Biocatalysis and Agricultural Biotechnology **25** 101595.

doi:https://doi.org/10.1016/j.bcab.2020.101595.

Pradhan B, Bhuyan P P, Patra S, Nayak R, Behera P K, Behera C, Behera A K, Ki J-S and Jena M 2022a Beneficial effects of seaweeds and seaweed-derived bioactive compounds: Current evidence and future prospective. Biocatalysis and Agricultural Biotechnology **39** 102242. doi:https://doi.org/10.1016/j.bcab.2021.102242.

Pradhan B, Kim H, Abassi S and Ki J-S 2022b Toxic Effects and Tumor Promotion Activity of Marine Phytoplankton Toxins: A Review. Toxins **14 (6)**397.

Pradhan B, Maharana S, Bhakta S and Jena M 2021a Marine phytoplankton diversity of Odisha coast, India with special reference to new record of diatoms and dinoflagellates. Vegetos. doi:10.1007/s42535-021-00301-2.

Pradhan B, Nayak R, Bhuyan P P, Patra S, Behera C, Sahoo S, Ki J-S, Quarta A, Ragusa A and Jena M 2022c Algal Phlorotannins as Novel Antibacterial Agents with Reference to the Antioxidant Modulation: Current Advances and Future Directions. Marine Drugs **20 (6)** 403.

Pradhan B, Nayak R, Patra S, Bhuyan P P, Behera P K, Mandal A K, Behera C, Ki J-S, Adhikary S P, MubarakAli D and Jena M 2022d A state-of-the-art review on fucoidan as an antiviral agent to combat viral infections. Carbohydrate Polymers:119551. doi:https://doi.org/10.1016/j.carbpol.2022.119551.

Pradhan B, Nayak R, Patra S, Bhuyan P P, Dash S R, Ki J-S, Adhikary S P, Ragusa A and Jena M 2022e Cyanobacteria and Algae-Derived Bioactive Metabolites as Antiviral Agents: Evidence, Mode of Action, and Scope for Further Expansion; A Comprehensive Review in Light of the SARS-CoV-2 Outbreak. Antioxidants **11 (2)** 354.

Pradhan B, Nayak R, Patra S, Jit B, Ragusa A and Jena M 2021b Bioactive Metabolites from Marine Algae as Potent Pharmacophores against Oxidative Stress-Associated Human Diseases: A Comprehensive Review. Molecules **26 (1)** 37.

Pradhan B, Patra S, Behera C, Nayak R, Jit B P and Ragusa A 2021c Preliminary Investigation of the Antioxidant, Anti-Diabetic, and Anti-Inflammatory Activity of *Enteromorpha intestinalis* Extracts. Molecules **26 (4)**. doi:10.3390/molecules26041171.

Pradhan B, Patra S, Behera C, Nayak R, Patil S, Bhutia S K and Jena M 2020b *Enteromorpha compressa* extract induces anticancer activity through apoptosis and autophagy in oral cancer. Molecular Biology Reports **47** 9567-9578.

Pradhan B, Patra S, Dash S R, Nayak R, Behera C and Jena M 2021d Evaluation of the anti-bacterial activity of methanolic extract of *Chlorella vulgaris* Beyerinck [Beijerinck] with special reference to antioxidant modulation. *Future Journal of Pharmaceutical Sciences* **7 (1)** 1-11

Pradhan B, Patra S, Dash S R, Satapathy Y, Nayak S, Mandal A K and Jena M 2022f In vitro antidiabetic, anti-inflammatory and antibacterial activity of marine alga *Enteromorpha compressa* collected from Chilika lagoon, Odisha, India. *Vegetos*. doi:10.1007/s42535-022-00359-6.

Pradhan B, Patra S, Maharana S, Behera C, Dash S R and Jena M 2021e Demarcating antioxidant response against aluminum induced oxidative stress in *Westiellopsis prolifica* Janet 1941. **23 (3)**238-251. doi:10.1080/15226514.2020.1807906.

Pradhan B, Patra S, Nayak R, Behera C, Dash S R, Nayak S, Sahu B B, Bhutia S K and Jena M 2020c Multifunctional role of fucoidan, sulfated polysaccharides in human health and disease: A journey under the sea in pursuit of potent therapeutic agents. International journal of biological macromolecules **164** 4263-4278.

Toyub M, Miah M, Habib M and Rahman M 2008 Growth performance and nutritional value of Scenedesmus obliquus cultured in different concentrations of sweetmeat factory waste media. *Bangladesh Journal of Animal Science* **37 (1)** 86-93.

Wu S-C, Wang F-J and Pan C-L 2010 The comparison of antioxidative properties of seaweed oligosaccharides fermented by two lactic acid bacteria. *Journal of Marine Science and Technology* **18 (4)** 537-545.