Marine Microalgae (*Chlorella Sp.*,) – A Source Of Nutritional Supplements

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Abstract
Microalgae plays a diverse role in aquaculture and *chlorella sp.* is a predominant microalga involves in the aquatic food chain. The present study focus on the preparation of microalgal fish feed, study of proximate composition and control of bacterial infection in fish. For the present study tilapia was selected which is most consumed and easily infected by pathogen. In the current study phytochemical analysis reveals that the petroleum ether and ethanol was the best solvent for the extraction of bioactive constituents. The antibacterial activity of ethanol extract of micro algae showed highest effect against *staphylococcus aureus, klebsiella sp, shigella sp* and *E coli*. The highest antibacterial activity was noted against *staphylococcus* with the zone of inhibition of 1.2 ± 0.1mm. The antioxidant activity showed that the ethanol extract of *C. vulgaris* possess high scavenging activity of 40.5%. The extracts showed good reducing power ability in a dose dependent manner. A medicated algal feed was prepared and administered to the fish to study its pharmacological activity. It was noted that the amount of proximate composition was drastically increased after fed with medicated feed. To study the efficacy of feed to control staphylococcal infection, the fish was injected with *staphylococcus*. Tilapia fed with medicated feed showed higher survival than the control group and it could effectively control the fish bacterial pathogen *staphylococcus*. The present study revealed that the microalgae fish feed is an excellent source of nutritional supplements for the fishes.

Keywords: Microalgae, *Chlorella vulgaris*, Aquaculture, Proximate composition, Fish feed

INTRODUCTION

Microalgae are microscopic organisms present not only in aquatic but also in terrestrial ecosystem and it represents a huge variety of species, which can live in a wide range of environment. Microalgae is a rich source of carbon compounds, which can be utilized in biofuels, health supplements, pharmaceuticals, and cosmetics (Das *et al*.,2011). Microalgae produce a wide range of bioproducts, including polysaccharides, lipids, pigments, proteins, vitamins, bioactive compounds, and antioxidants (Brennan *et al*.,2010). Microalgae possess an efficient biological system capable of utilizing sunlight to produce organic compounds (Richmond *et al.*,2004). Microalgae are commercially used as human nutrition, animal, and aquatic feed, in cosmetic products, pigments, biofertilizer for extracting high-value molecules, stable isotope biochemicals, and for the synthesis of antimicrobial, antiviral, antibacterial and anticancer drugs (Suganya.,2016).

Micro-algae can increase the nutritional value of human and animal feed, and they also perform a vital part in aquaculture. The growth of the aquaculture industry has gone hand in hand with the growth of the population worldwide to ensure that fish supply as a part of protein sources can be fulfilled. According to Melba & Rohana, 2008 the aquaculture industry is expected to solve food security and nutritional well-being, reduce poverty, and develop the economy. Microalgae are an alternative to sustainable aquaculture practices by playing roles in wastewater treatment and ingredient replacement in fish feed. The development of fish feed with the inclusion of microalgae is widely studied because of its encouraging effects on farmed fish. However, microalgae must be easily cultured and non-toxic to be used in aquaculture (Spolaore *et al.*, 2006).

Research on microalgae, such as *S. platensis* and *C. vulgaris*, their effects in enhancing the immunity and reproduction performance of fish which possess antiviral, anticancer, anti-HIV, several neurological and antimicrobial activities (Apt *et al.*,1999). Microalgae can produce a variety of biochemicals, which are used for food and medical research. Becker *et al*.,(2007) said that Chlorella is rich in proteins (51–58% dry weight), carotenoids, as well as different vitamins. According to Barrow and Shahidi (2008), Chlorella has many advantages in the health sector Barrow and Shahidi *et al*.,2007). Chlorella is mostly sold as a fish food and as a healthy food. The by-products of Chlorella are used as fruit and vegetable

preservative (Hills *et al*.,1978). improve immune response, improve fertility, control weight, improve skin and a lustrous coat (Pulz *et al*.,2004). Microalgae for aquaculture Food chain is a major source of nutrients for aquaculture animals. Microalgae are the primary producer in the food chain. Microalgae have the nutrients which are essential for the growth and survival for larva and adult animals.

Certik *et al*., (1999) studied about the physiological growth of microalgae and external appearance of aquatic animals. The most widely used species for aquatic animal feed are Chlorella, Pavlova, Phaeodactylum, Chaetoceros,Nannochloropsis, Skeletonema, and Thalassiosira (Muller-Feuga ,2000). Until now, microalgae are successfully used as a food source and feed additive for both freshwater and marine aquatic animals. In aquaculture, microalgae used as fish feed (Brown *et al*.,1997), for stabilization, improvement of culture medium quality (Chuntapa *et al*.,2003), and inducing important biological activities in red aquatic species ((Muller-Feuga ,2000)) , and for improving immune systems of fish (Pulz *et al*.,2004). Several studies have shown that microalgae can be used as a feed supplement (Becker *et al*.,2004). Worldwide, 30% of the current algal production is used to produce animal feed (Becker *et al*.,2004). Tilapia have been used as biological controls for certain aquatic plant problems. They prefer a floating aquatic plant, duckweed (*Lemna* spp.), but also consume some filamentous algae. Nagl *et al*.,(2001) in Kenya, investigated about tilapia were introduced to control mosquitoes, which were causing malaria, because they consume mosquito larvae, consequently reducing the numbers of adult female mosquitoes, the vector of the disease (John.,2013). These benefits are, however, frequently outweighed by the negative aspects of tilapia as an invasive species (Petr *et al*.,2000). The microalgae (chlorella vulgaris) fish feed plays an important role in aquaculture which helps to improve the immune system in fish and decreases the disease rate in fish community. A report from the Kyle *et al*., (1991) indicating that animals fed with microalgae in the diet clearly improved the weight, immunity, fatty acids, vitamins, and mineral profiles. Azaza *et al*. (2008) showing that the tilapia feed formulated with microalgae as ingredient augments the protein efficiency and growth performances. In the current study an investigation was carried out to analyse the proximate composition of fish and determination of median lethal dose of algal extract.

MATERIALS AND METHODS

COLLECTION AND CULTURE OF *Chlorella vulgaris*

The *chlorella vulgaris* was collected from CMFRI, Tuticorin. For algal cultivation 25ml of culture was added to a flask containing 250ml of mineral media ($1MgSo_4.7H_2O$, 10 K_2HPO4, 4 $NaNO_3CaCl_2$, 16 $FeCl_3O$, 32 EDTA-Na_2, 4 NaCl, 30 Na_2CO_3, $8MnCl.4H_2O(NH4)$, $6Mo7O_{24}.4H_2O$, $ZnSO_4.7H_2O$, $CuSO_4.5H_2O$, $COCL_4.6H_2O$, H_3BO_3) and incubated under specific laboratory condition for 28 days. After incubation the culture was again inoculated to 1000 ml of mineral media and incubated for another 28 days (Kassim *et al*.,1999).

PREPARATION OF ALGAL EXTRACT

The phytochemical constituents of microalgae (2gm) were extracted in a soxhlet apparatus with 200 ml of solvent (methanol and hexane 1:1). The solvent was evaporated at room temperature and the extract was utilized for further analysis (Fajardo *et al*., 2007).

BIOCHEMICAL AND PHYTOCHEMICAL SCREENING

The biochemical constituents such as total carbohydrate, protein, lipids and aminoacids were analyzed by standard methodology (Hedge and Hofreiter, 1962, Lowry *et.al*.,1951). The extracts of samples were then subjected to phytochemical screening for the presence of different bioactive constituents (Prasanth *et al.,* 2011).

PURIFICATION OF BIOACTIVE COMPOUNDS FROM MICROALGAE

COLUMN CHROMATOGRAPHY

Microalgae extract was subjected to column chromatography for the extraction of bioactive compounds. Microalgae extracts of 10ml was chromatographed over silica gel (100-200mesh) using solvents with increasing polarity. The mixture was packed on a silica gel column (Merck, India) with 100% Hexane and the extract was eluted by increasing the polarity using Chloroform, Ethyl acetate, Ethanol and Methanol in the ratio of 90:10, 80:20, 70:30 and 50:50. The collected fractions were subjected to further analysis (Nakasnishi *et al*., 1998).

ANTIBACTERIAL ACTIVITY OF *Chlorella vulgaris*

Antimicrobial activity of Microalgae (*Chlorella*) extracts was screened based on Kirby Bauer agar diffusion method. Bacterial test organism was obtained from microbial type culture collection (MTCC). The strains used for antibacterial activity was *Staphylococcus aureus, Klebsiella sp, Shigella sp.and e coli.* The filter paper disc (1.0mm) of microalgae column extract was placed on the surface of microorganism seeded nutrient agar plates. Each plate was labeled with the pathogenic organisms inoculated and incubated incubation at 26-30∘C for 24hrs. (Bauer *et.al.*,1966).

ANTIOXIDANT ACTIVITY OF *Chlorella vulgaris*

DPPH free radical scavenging assay

DPPH free radical scavenging was determined according to the standard procedure described by Ul-Haq *et al*., (2012). In a glass tube 800 µl of 0.1mM methanolic DPPH solution was mixed with 100 µl of test sample. Each sample was assayed

in triplicate and kept away from the light at 37º C for 60 minutes. The tubes were centrifuged at 3000 rpm for 5 mins after incubation. Then the change in color (from deep violet to light-yellow) of DPPH free radical was measured by taking the absorbance of the reaction mixtures at 517 nm on a UV spectrophotometer. The percentage of scavenging of DPPH free radical of test sample was calculated by using the following formula:

$$\% \text{ Inhibition} = \left[\frac{\text{Absorbance of control - Absorbance of test sample}}{\text{Absorbance of control}} \right] \times 100$$

Total antioxidant capacity (TAC) assay

A test sample of 0.2 ml of various extracts was mixed with 3 ml of phosphomolybdenum reagent. Then the sample was incubated for 60 mins in a boiling water bath at 95° C. After that, the samples were cooled at room temperature and the absorbance was measured at 695 nm against a blank (0.2 ml solvents without extracts). The total antioxidant capacity (TAC) was determined by Ahmad *et al.* (2013).

PREPARATION AND EVALUATION OF PHARMACOLOGICAL PROPERTIES OF MICROALAGAE FISH FEED

The sub cultured microalgae were taken for the preparation of fish feed. For the feed preparation 6 gm of alum was added to 1000ml of microalgae culture. The preparation was mixed thoroughly and centrifuged at 5000 rpm for 30 minutes. After centrifugation the extracted sediment was separated air dried and formulated as fish feed. For the pharmacological analysis microalgae fish feed the Tilapia fish was selected. For the current research blue tilapia was selected and the length and weight of fish was noted as 45.7cm and 2.01kg respectively. (Andreas and Ulrich, 2013).

PROXIMATE COMPOSITION ANALYSIS OF FISH

Fresh tilapia fish (*Oreochromis niloticus*) was washed, cleaned with cooled water again, and drained for twenty minutes on a sterile stainless-steel plate, as illustrated by Etemadian *et al.*2012**.** For the analysis 1gm of fish was weighed and homogenated with 50ml of phosphate buffer and the extract was centrifuged at 10000 rpm for 10 minutes. After centrifugation the extract was stored in refrigerator for further use.

Proximate composition of fish was determined using AOAC methods (1990). All analysis was done in triplicate. Moisture content was measured by weighing differences before and after hot air oven drying at 100°for 16h. Lipid determination was carried out using the standard Bligh and Dyer procedure (1959). Crude protein was determined according to AOAC procedure (1990). Total fat content was determined by acid digestion prior to continuous extraction using petroleum ether (b.p., 40—60°C) in Soxtec system AOAC, 1990).

DISEASE INJECTION AND DETERMINATION OF MEDIAN LETHAL DOSE (MLD) OF ALGAL EXTRACT

Staphylococcus aureus is a gram-positive, round-shaped bacterium that is a member of the Firmicutes, and it is a usual member of the microbiota of the body. *Staphylococcus aureus* usually acts as a communal bacterium, asymptomatically colonizing about 30% of the human population**.** Nile tilapia fish were randomly assigned in to 3 groups: Group 1 fish was maintained as control. Group 2 & 3 were intravenously injected with *staphylococcus aureus* bacteria. Group 1 & 2 fish were fed with algal feed while group 3 was fed with normal fish feed. Electric aerators and an underwater internal power filter were used to maintain the oxygen level in all aquarium tanks. Daily siphoning of waste material and feces was performed to maintain water quality. The immediate reflexes and mortality were observed every hour for the first 6 h and every 24 h for 6 days.

RESULT

BIOCHEMICAL AND PHYTOCHEMICAL ANALYSIS OF MICROALAGE

The preliminary biochemical analysis was performed based on standard methodology. The amount of carbohydrate (Anthrone method) present in microalgae was estimated as 0.93 ± 0.005 mg/ml. The amount of protein present was estimated (Lowry's method) as 0.20 ± 0.005 mg/ml. The amount of lipid present was estimated as 0.69 ± 0.005 mg/ml (table1).

Table 1 : Preliminary analysis of microalgae

Biochemical test	Concentration (mg/ml)
Carbohydrate	0.93 ± 0.005
Protein	0.20 ± 0.005
Lipid	0.69 ± 0.005

PHYTOCHEMICAL SCREENING Of *Chlorella vulgaris*

Phytochemical screening of *Chlorella vulgaris* showed the presence of important phytoconstituents such as alkaloid, protein, carbohydrate, cardiac glycosides, tannin and saponin. Highest phytoconstituents was observed in ethanol and petroleum ether (Table 2).

Table 2: Quantitative analysis of phytochemicals of microalgae extract

Sl. No	Tests	Ethanol	chloroform	methanol	Petroleum ether	Aqueous extract
1	Alkaloid	+	-	+	+	-
2	Phenol	-	-	-	-	-
-3	Protein	+	+	+	+	-
4	Cardiac glycosides	+	+	+	+	-
5	Phyto sterol	-	-	-	+	-
6	Saponin	+	+	_	+	-
7	Coumarin	-	+	+	-	-
8	Flavanoids	-	-	+	-	-
9	Tannin	+	-	-	+	-
10	carbohydrates	+	-	+	+	-

'+'- positive
'- '- negative

ANTIBACTERIAL ACTIVITY OF MICROALGAL EXTRACT

In antibacterial screening the highest activity was noted in ethanol extract against *Staphylococcus* (1.2 ± 0.1 mm) followed by *Klebsiella* (0.9± 0.2 mm), *E. coli* (0.733±0.15 mm) and S*higella* (0.7± 0.66 mm). After ethanol the highest activity was noted in chloroform extract against S*taphylococcus* and the zone of inhibition was 1.1 ± 0.96mm. The chloroform extract shows moderate zone of inhibition against almost all other bacterial pathogens. The lowest activity was noted in petroleum ether and no zone of inhibition was recorded in aqueous extract (Table 3).

Table 3: The inhibition zone diameter (mm) of different concentration of crude algal extract against *Stapylococcus, Klebsiella, Shigella*

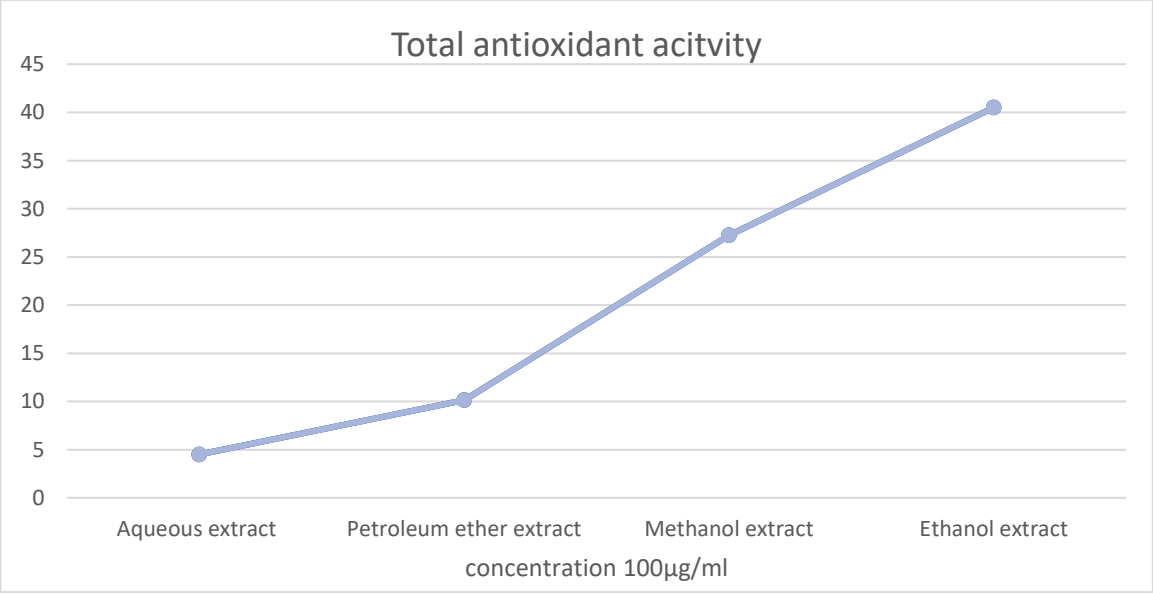
Bacteria	Ethanol	chloroform	methanol	Petroleum ether	Aqueous extract
Staphylococcus	1.2 ± 0.1	1.1± 0.96	0.66±0.05	0.03±0.05	0.0
Klebsiella	0.9± 0.2	0.5±0.4	0.2±0.1	0.03±0.05	0.0
Shigella	0.6± 0.1	0.7±0.66	0.7±0.1	0±0	0.0
E coli	0.733±0.15	0.6±0.5	0.2±0.1	0.06±0.05	0.0

ANTIOXIDANT ACTIVITY OF MICROALGAL EXTRACT

Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

In antioxidant activity the ethanol extract of *C. vulgaris* showed the highest scavenging activity of 40.5%, followed by the methanol extract with 27.23% and petroleum ether extract with 10.12%. The water extract recorded the lowest scavenging activity 4.5% (Fig 1).

Fig.1: Total antioxidant capacity of the different extracts of Chlorella vulgaris



Analysis of Proximate composition of formulated microalgae fish feed

After six days of feeding with microalgae fish feed considerable changes were recorded in fish proximate composition compared to those fed with normal diet (Table 5). Proximate analysis showed that the carbohydrate, protein and lipid content was drastically increased after administering microalgal fish feed. After fish feed the carbohydrates was increased as 0.68%, the protein and lipid was increased as 0.14% & 0.09 % respectively.

Feed type	Carbohydrate (%)		Protein (%)		Lipid (%)	
	Before	After	Before	After	Before	After
Normal fish feed	0.34	0.52	0.045	0.11	0.022	0.04
Microalgae fish feed	0.34	0.68	0.045	0.14	0.022	0.09

Evaluation of microalgae fish feed to control the *Staphylococcal* infection.

To check the efficacy of microalgae fish feed, the fish mortality rate was measured after disease injection from day 1 to day 6. In the control there was no mortality rate observed. In group 2 on 6th day 1 fish was died and all others recovered from the staphylococcal infection and survived with improved health. In Group 3 all the fishes died from 3rd day to 5th day. The results suggested that the microalgae possess antibacterial activity against *Staphylococcus* infection (Table 4).

Table 4 : Evaluation of microalgae fish feed to control the *Staphylococcal* infection.

Group	Days (mortality rate)					
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
1	-	-	-	-	-	-
2	-	-	-	-	-	1
3	-	1	2	2	1	-

1- Control, 2- Microalgae Feed administered fish, 3-Normal feed administered fish

DISCUSSION

Marine microalgae constitute attractive sources of novel and active metabolites, comprising proteins, enzymes, pigments and polyunsaturated fatty acids (PUFA) that could be exploited in pharmaceutical, food, feed and cosmetic industries (Surendhiran et *al*., 2014). Compounds with pharmaceutical characteristics, such as antioxidative, anti inflammatory, antimicrobial or antitumoral properties, have been identified; some of them have been in the clinical trial state (Guedes *et al*., 2011). Antimicrobial activities are among the most researched features in natural extracts as they have been attributed to different compounds, including, indoles, terpene derivatives, acetogenins, phenols, fatty acids and hydrocarbons (Bhakuni and Rawat, 2005). There has been increasing demand for new antimicrobial compounds in response to continuous evolution of microbial pathogens in antibiotic-resistance. Marine environment has been considered among the most promising sources of antimicrobial compounds, as numerous sea organisms produce bioactive metabolites in response to environmental stress and develop chemical strategy for defense and survival (Madhavi et al., 2012). Many new active antimicrobial compounds have been isolated from marine sources. But the majority of these compounds has not been yet characterized (Sanmukh et al., 2014).

Algae have a wide span of ecosystems contributes to the innumerable chemical compounds that they can synthesize. Bioactive compounds released by microalgal cells are either bactericidal or bacteriostatic (Falaise *et al.*, 2016). In the present study the microalgal extract showed the presence of biochemicals and phytochemicals which was extracted in petroleum ether and ethanol solvents. A large number of microalgal extracts and/or extracellular products have been proven antibacterial, antifungal, antiprotozoal and antiplasmodial activity (Mayer and Hamann, 2005; Cardozo *et al*., 2007). Marine algae were reported to produce a wide variety of bioactive secondary metabolites as antimicrobial, antifeedant, antihelmintic and cytotoxic agents. In the present study the different extract of Chlorella vulgaris was subjected to antibacterial activity against the bacteria *staphylococcus aureus, klebsiella sp, shigella sp.and E coli*. The highest activity of microalgae extract was recorded in ethanol extract against *staphylococcus* with the inhibition zone 1.2 ± 0.1 mm, while the lowest inhibition zone was recorded in petroleum ether extract. In the other extract such as ethanol, methanol and petroleum ether also showed moderate antibacterial activity against most of the bacterial pathogens. From this study using organic solvents always provides a higher efficiency in extracting compounds for anti-microbial activities. Antibacterial suggests that the ethanol extract shows the highest efficacy against Staphylococcus *aureus, Klebsiella pneumonia and shigella sps.,*

The commercial cultivation of microalgae began with the cultivation of *Chlorella* in Japan in the 1960s followed by the cultivation of *Spirulina* in Mexico, the USA and China in the 1970s. During the last four decades the biotechnological industry of photosynthetic microorganisms has grown and become much diversified. The most important commercially produced microalgae are *Spirulina, Chlorella* and *Dunaliella*. This is achieved because of their growth in highly selective media and hence they remain relatively free from contamination by other microorganisms (Borowitzka, 1999). In contrast, promising research and development is being taken up by various academic and government laboratories to understand the algae cultivation with small-scale closed photobioreactors and scale up studies in open ponds as well (Radmann *et al*., 2007).

Chlorella diet can be easily prepared with crude extract. Therefore, purification strategies and consequent synthetic analogue development process need not be undertaken. As the extract was prepared from the dried material, such source material can easily be stored for 12 months. To sustain the host-defense system and relative protection against pathogenic invaders, the Chlorella medication can be used as a prophylactic agent for the entire culture period at low cost.

In the present study the microalgae extract was subjected to antioxidant activity and the Chlorella cultured samples were submitted to sequential extraction using as solvents: ether, methanol and water. When compared to other extracts Ethanol extract exhibited highest reducing power ability with most scavenging activity with an inhibition percentage of 40.5%, followed by the methanol extract (27.23%) and petroleum ether extract (10.12%) while the water extract showed the lowest activity. The extracts showed good reducing power ability in a dose dependent manner. Compounds having reducing power can donate electrons and can inhibit lipid peroxidation by reducing the oxidized intermediate. The reductones present in the extract exhibit antioxidant activity.

In addition, fish oil is a rich source of vitamins including vitamin A, D, E, and K, which are soluble in oil and must be taken on a regular basis because of their key roles in human health and metabolism (Kinsella, 1987). Principal composition of fish is 16-21% protein, 0.2-25% fat, 1.2-1.5% mineral, 0-0.5% carbohydrate and 66-81% water (Love, 1970). Furthermore, the variations in proximate composition of fish are closely related to the feed intake. During periods of heavy feeding, the protein content of muscle tissue increases slightly at first and then the fat content might show a marked and rapid increase. On the other hand, fish may have starvation periods for natural or physiological reasons (spawning or migration) or because of external factors such as shortage of food. In that case, fat content gradually decreases and then a decline in protein may also be seen (Huss, 1988; 1995). Therefore, it is important to know proximate composition of fish and variations throughout the year.

Rezaya *et al*.,2016 determined the proximate composition of fish feed ingredients based on this this study, proximate composition of Tilapia fish was determined before and after feed Proximate analysis showed that the carbohydrate, protein and lipid content was drastically increased after administering microalgal fish feed. After fish feed the carbohydrates was increased as 0.68% and the protein and lipid were increased as 0.14% & 0.09% respectively. Tilapia fed with medicated feed could effectively control the bacterial pathogens. The Tilapia treated with the microalgal fish feed gave higher survival than the control group. The fish mortality rate was noted after disease injection from day 1 to day 6. In the control there was no mortality observed. In group 2 on 6^{th} day 1 fish was died and all others recovered from the staphylococcal infection and survived with improved health. In Group 3 all the fishes died from 3^{rd} day to 5^{th} day. The results suggested that the microalgae possess antibacterial activity against staphylococcus infection. Based on the present findings it could be concluded that the microalgae were found to be a potent immune modulator and nutritionally rich diet which can be used as a fish feed to control staphylococcal infection.

SUMMARY

Chlorella sp. is an important microalga which contains lot of nutritional supplements such as protein, lipid, amino acids, fatty acids etc., It can be easily grown in chemically defined media and in sewage. The present study mainly focus on fish feed preparation, proximate composition analysis extraction of bioactive compound and screening of bioactive compound from marine microalgae Chlorella sp. The phytochemical analysis reveals that the petroleum ether and ethanol was the best solvent for the extraction of bioactive constituents. In the present study the different extracts of *Chlorella vulgaris* was screened against the bacteria *staphylococcus aureus, klebsiella sp, shigella sp and E coli*. The highest effect of extract was recorded in ethanol extract against *staphylococcus* with the inhibition zone of 1.2 ± 0.1mm. It is clear that using organic solvent always provides a higher efficiency in extracting compounds for pharmacological activities.The antioxidant activity suggest that the ethanol extract of *C. vulgaris* possess high scavenging activity of 40.5%.The extracts showed good reducing power ability in a dose dependent manner..In this study, proximate composition of Tilapia fish were determined before and after microalgal fish feed the amount of carbohydrate present in microalgae was estimated as 0.83mg/mL. The amount of protein and lipid present was estimated as 0.33mg/ml & 0.18mg/ml respectively. It was noted that the amount of proximate composition was drastically increased after fed with medicated feed. Based on the present findings, Tilapia fed with medicated feed could effectively control the bacterial pathogens challenged in this study. The Tilapia treated with the microalgal fish feed gave higher survival than the control group. The present study concluded that the microalgae fish feed is an excellent source to control the bacterial infection and also served as a potential nutritional supplement which increase the proximate composition of fish.

REFERENCE

1. Apt KE, Behrens PW. Commercial developments in microalgal biotechnology. J Phycol 1999;35:215–26.
2. Ahmad, Farah Saeed, Mehjabeen, Noor Jahan(2013). Evaluation of Insecticidal and Anti-oxidantactivity of Selected Medicinal Plants. Journal of Pharmacognosy and Phytochemistry; 2 (3): 153-158.
3. Andreas R Dunz , Ulrich K Schliewen(2013). Molecular phylogeny and revised classification of the haplotilapiine cichlid fishes formerly referred to as "Tilapia"; Mol Phylogenet Evol. 2013 Jul;68(1):64-80.

4. AOAC, 1990: Official analysis method of AOAC international (16th ed.). USA: AOAC.Bligh EG and Dyer WJ (1959): A rapid method for total lipid extraction and purification. Canadian Journal of Biochem and Physiology, 37, pp911 – 917.
5. Apt KE, Behrens PW. Commercial developments in microalgal biotechnology. JPhycol
6. Azaza, M. S., Mensi, F., Ksouri, J., Dhraief, M. N., Brini, B., Abdelmouleh, A., & Kraiem, M. M. (2008). Growth of Nile tilapia (Oreochromis niloticus L.) fed with diets containing graded levels of green algae ulva meal (Ulvarigida) reared in geothermal waters of southern Tunisia. Journal of Applied Ichthyology, 24(2): 202-207.
7. Barrow C, Shahidi F. Marine nutraceuticals and functional foods. CRC Press; 2007.
8. Barrow, F. Shahidi(2008).Marine nutraceuticals and functional foods., CRC Press, United State.
9. Bauer AW, Kirby WMM, Sherries JC, Tuck M (1966). Antibiotic susceptibility testing by standardized disc diffusion method. Am. J. Clin. Pathol. 45: 493-496.
10. Becker E.W. Micro-algae as a sourse of protein. *Biotechnol. Adv.* 2007;25:207–210. doi: 10.1016/j. biotechadv. 2006.11.002.
11. Becker W.Microalgae in human and animal nutrition. Handbook of Microalgal Culture:Biotechnology and Applied Phycology. 2004. p. 312.
12. Bhakuni, Diwan Rawat.(2005)Bioactive Marine Natural Products.,DOI:10.1007/1-4020-3484-9.
13. Borowitzka.Commercial production of microalgae((1999): ponds, tanks, tubes and fermenters.,Journal of Biotechnology.
14. Brennan L, Owende P. Biofuels from microalgae- a review of technologies for production,processing, and extractions of biofuels and co-products. Renew Sustain Energy Rev. 2010;14:557–77.
15. Brown M, Jeffrey S, Volkman J, Dunstan G. Nutritional properties of microalgae for mariculture. Aquaculture 1997;151:315–31.
16. Cardozo, Guaratini, Barros, Falcao and Tonon *et al*., 2007. Metabolites from algae with economical impact. Comp. Biochem. Physiol. C: Toxicol. Pharmacol., 146: 60-78.
17. Certik M, Shimizu S. Biosynthesis and regulation of microbial polyunsaturated fatty acid production. J Biosci Bioeng 1999;87:1–14.
18. Chuntapa B, Powtongsook S, Menasveta P. Water quality control using Spirulina platensis in shrimp culture tanks. Aquaculture 2003;220:355–66.
19. Composition of Artemisia annua L. Leaves and Antioxidant Potential of Extracts as a Function of Extraction Solvents. Molecules 2012, 17, 6020-6032; doi:10.3390/molecules17056020.
20. Das P, Aziz SS, Obbard JP. Two phase microalgae growth in the open system for enhanced lipid productivity. Renew Energy. 2011;36(9):2524–8.
21. Elsass, Kinsella . Social interaction following severe closed head injury. *Psychological Medicine*, *17*(1), 67–78.
22. Etemadian, B. Shabanpour, A. S. Mahoonak and A. Shabani, Combination effect of phosphate and vacuum packaging on quality parameters of Rutilus frisii kutum fillets in ice, *Food Res. Int.*, 2012, 45(1), 9–16.
23. Fajardo, A.R.; Cerdan, L.E.; Medina, A.R.; Fernandez, F.G.A.; Moreno, P.A.G. and Grima, E.M.(2007). Lipid extraction from the microalgae Pheaodactylumtricornutum. Eur. J. Lipid Sci. Technol. 109:120-126.
24. Falaise ,Cyrille François , Marie-Agnès Travers , Benjamin Morga , Joël Haure ,Réjean Tremblay, François Turcotte ,Pamela Pasetto(2016). Antimicrobial Compounds from Eukaryotic Microalgae against Human Pathogens and Diseases in Aquaculture., *Mar. Drugs* 2016, *14*(9), 159; https://doi.org/10.3390/md14090159.
25. Guedes CA, Amaro HM, Barbosa CR, Pereira RD , Malcata FX. Fatty acid composition of several wild microalgae and cyanobacteria, with a focus on eicosapentaenoic, docosahexaenoic and α-linolenic acids for eventual dietary uses. Food Res Int 2011; 44: 2721-2729.
26. Hedge, J., Hofreiter, 1962. In: Carbohydrate Chemistry, volume 17. Academic Press, New York.
27. Hills C, Nakamura H. Food from sunlight: planetary survival for hungry people, how to growedible algae and establish a profitable aquaculture [World Hunger Research Project]. Boulder, Creek: University of the Trees Press; 1978.
28. Huang, X.H.; Li, C.L.; Liu, C.W.; Wang, Z.D. and Chen, J.J. (2002). Studies on the N and Pnutrient demand if Nannochlorisoculata. J. Marine Sciences(China). 26(8): 13-17.
29. Huss, H.H. 1988. Fresh fish: quality and quality changes. FAO. Rome, Italy, 132 pp.
30. Huss, H.H. 1995. Quality and Quality Changes in Fresh Fish. FAO. Rome, 348 pp
31. John W. Cross (2013). "Aquatic Weed Control". Mobot.org. Archived from the original on 2012-09-21. Retrieved 2013-09-14.
32. Kassim,T.I.;Al-Saadi,H.;andSalman,N.A. 1999.Production of some phyto-and zooplankton and their use as live food for fish larva. IraqiJ.Agric.Proc.of 2nd Sci.Confer.Nov.4(5):188-201.
33. Kyle, D. J., & Gladue, R. (1991). Eicosapentaenoic acids and methods for their production. World Patent, vol. 9, pp. 114-427.
34. Love, R.M. 1970. The Chemical Biology of Fishes. Academic Press, Inc. London, U.K., 547 pp.
35. Lowry, O. H., Rosenberg, N. J., Farr, A. L., Randall, R. J. 1951. Protein measurement with the folin phenol reagent. The Journal of Biological Chemistry, 193(1):265–275.
36. Madhavi, Mallika, Lokanath, Vishnu (2012). A review on phytochemical and pharmacological aspects of Saussurea lappa. Int. J. Rev. Life. Sci., 2(1), 24-31.
37. Mayer, A.M.S. and M.T. Hamann, 2005. Marine pharmacology in 2001-2002: Marine compounds with anthelmintic, antibacterial, anticoagulant, antidiabetic, antifungal, anti-inflammatory, antimalarial, antiplatelet, antiprotozoal,

antituberculosis, and antiviral activities; affecting the cardiovascular, immune and nervous systems and other miscellaneous mechanisms of action. Comp. Biochem. Physiol. Toxicol. Pharmacol., 140: 265-286.
38. Muller-Feuga A. The role of microalgae in aquaculture: situation and trends. J Appl Phycol 20012:527–34.
39. Nagl, Sandra; Herbet Tichy; Werner E. Mayer; Irene E. Samonte; Brendan J. McAndrew; Jan Klein (2001). "Classification and Phylogenetic Relationships of African Tilapiine Fishes Inferred from Mitochondrial DNA Sequences". Molecular Phylogenetics and Evolution. 20 (3): 361–374. doi:10.1006/mpev. 2001. 0979. PMID 11527464.
40. Nakanishi ,Norio Ishizuka , Hiroyoshi Minakuchi , Naohiro Soga, Nobuo Tanaka.(1998) Designing monolithic double-pore silica for high-speed liquid chromatography., Journal of Chromatography A Volume 797, Issues 1–2, 27 , Pages 133-137.
41. Petr, T (2000).”Interactions between fish and aquatic marcrophytes in inland waters” A review. FAO Fisheries Technical Papers. 396.
42. Prashant, T., Bimlesh, K., Mandeep, K., & Gurpreet, K. (2011). Phytochemical screening and Extraction: A Review. Internationale Pharmaceutica Sciencia, 1(1),98-106.
43. Pulz O, Gross W. Valuable products from biotechnology of microalgae. Appl Microbiol Biotechnol 2004;65:635–48.
44. Radmann, Reinehr, Costa.Aquaculture; Volume 265, Issues 1–4, 1 May 2007, Pages 118-126.
45. Rezaya (2016)Determination of proximate composition of fish feed ingredients locally available in Narsingdi region, Bangladesh,nternational Journal of Fisheries and Aquatic Studies 2016; 4(3): 695-699.
46. Richmond(2004). Handbook of Microalgal Culture: Biotechnology and Applied Phycology.,Blackwell Science Ltd.
47. Ryckebosch, E.; Muylaert, K.; Foubert, I. Optimization of an analytical procedure for extraction of lipids from microalgae. J. Am. Oil Chem. Soc. 2012, 89, 189–198. [CrossRef].
48. Sanmukh, Benedict Bruno, Udhaya Ramakrishnan, Hebrew University of Jerusalem, Krishna Khairnar (2014). Bioactive Compounds Derived from Microalgae Showing Antimicrobial Activities, Journal of Aquaculture Research and Development 5(3).
49. Spolaore, C. Joannis-Cassan, E. Duran, A. Isambert, “Review: commercial applications of microalgae,” Journal of Bioscience and Bioengineering, vol. 101, pp. 87-96, 2006.
50. Suganya, Varman, Masjuki, Renganathan. Macroalgae and microalgae as a potential source for commercial application along with biofuels production;a biorefinery approach. Renew sustain Energy .Rev 2016;55:909-41.
51. Surendhiran Duraiarasan, Sirajunnisa Abdul Razack, Subramaniyan Thiruvengadam.(2014) A green synthesis of antimicrobial compounds from marine microalgae Nannochloropsis oculate. Journal of Coastal Life Medicine 2(11)DOI:10.12980/JCLM.2.2014APJTB-2014-0138.
52. Ul-Haq ,Iqbal , Umer Younas , Kim Wei Chan , and Maznah Ismail(2012). Chemical Chemical Composition of Artemisia annua L. Leaves and Antioxidant Potential of Extracts as a Function of Extraction Solvents. Molecules 2012, 17(5), 6020-6032; https://doi.org/10.3390/molecules17056020.