

Antibacterial activity of *Anabaena circinalis* isolated from fresh water

Hanan. M. Abobaker and Hamida. EL. Elsalhin

Abstract

Cultures of the blue green algae (cyanobacteria) *Anabaena circinalis* were identified and isolated from freshwater and their antimicrobial effect was studied. The extract of *A. circinalis* was tested to investigate its efficiency against four bacterial strains (*Achromobacter xylosoxidans*, *Staphylococcus aureus*, *Escherichia coli*, *Shigella dysenteriae*). Antimicrobial test was determined by disk diffusion method. Different concentrations of algal extracts (25, 50, 75 and 100%) were tested. Results showed that the highest level of antimicrobial activity was recorded against *Shigella-S. dysenteriae* at 100% concentration followed by 25% extract concentration against the same bacteria.. In comparison with two antibiotics Ampicillin (AMP) , ~~(OXA)~~ oxacilina (OXA), AMP was the most effective on *Shigella-S. dysenteriae* ~~followed by OXA.~~ *S. taphylococcus aureus* and *Escherichia-E. coli* were resistant to both antibiotics while they were sensitive to *A. nabaena circinalis* extracts at even at low concentrations (25% and 50%)-. Thus the In the present study revealed that, extracts of Anabaena circinalis extract is then would be a promising natural source, for novel antibioticsantibiotics, hence worthy for more investigations.

Key words: *Anabaena circinalis*, Antimicrobial, Algal extract, Ampicillin ,oxacilina.

Introduction

Algae are important organisms in the aquatic ecosystems and are the primary source of food. Algae are also one of the richest sources of bioactive compounds, including antibacterial and antifungal compounds [1, 2, 3]. Secondary metabolites obtained from algae have important properties. In recent years, the interest in biological activities obtained from cyanobacteria molecules has increased [4, 5]. In addition, cyanobacterial secondary metabolites have been shown to have hypocholesterolemic properties, enzyme inhibiting, and other pharmacological effects. These natural products are not only used as raw drug material, but also as structural models in the production of synthetic molecules [6]. These organisms are an excellent sources for investigation by the ecologists, physiologists, biochemists, pharmacists and molecular biologists. Biologically active substances were extracted from cyanobacteria [7, 8]. Microalgae have meanwhile been found to produce antibiotics: a large number of microalgal extracts and/or extracellular products have proven antibacterial, antifungal, antiprotozoal and antiplasmodial [9,10]. The antimicrobial activity of microalgae has been attributed to compounds belonging to several chemical classes including indoles, terpenes, acetogenins, phenols, fatty acids and volatile halogenated hydrocarbons [11,12]. An increasing number of such metabolites are being found to be directed against oxygenic photosynthetic processes, which, in the microbial world, are unique to algae and cyanobacteria, and are potentially useful as biochemical tools, and as herbicidal or biocontrol agents [13]. Bacterial infections are among worldwide and important diseases that cause high mortality rates in humans. Antimicrobial agents are commonly used in the treatment of bacterial infections. However, bacteria can become resistant to available drugs. Therefore, discovering of new antibacterial compounds is required. However, the increasing popularity of traditional medicine has led researchers to investigate the natural compounds [14]. Phenolic compounds in particular are considered as one of the most important classes of natural antioxidants. ~~Their~~ These molecules are formed by one or more aromatic rings with one or more hydroxyl groups. Chemically, polyphenols can be divided into several classes, such as phenolic acids, flavonoids, isoflavonoids, stilbenes, lignans, and phenolic polymers. Bioavailability within polyphenols differs considerably, as far as some compounds are concerned, it also depends on their form in their respective dietary sources. Generally, their primary function in plants is as ~~protection-protectants~~ against ultraviolet radiation and pathogens [15]. Phenolic compounds found in algae also include the phlorotannins which are found in brown algae and in lower amounts in some red algae, and reported with a wide array of their therapeutic properties-biological activities (anticancer, antioxidative, antibacterial, anti-allergic, anti-

diabetes, anti-aging, anti-inflammatory and anti-HIV activities) [16,17]. Our research was designed to investigate the antibacterial activity of blue-green alga *A. circinalis* extract against four selected pathogenic bacteria.

Material and Methods

Isolation and culture of *Anabaena circinalis* :

Algae samples were taken from spring water in the city of Shahat. The medium used throughout the maintenance and experimental studies was ~~medium~~-MBL medium [18]. MBL medium consists of stock solutions of macro and ~~miconutrients~~-micronutrients as given in Table 1. ~~It consists mainly of the following: Macronutrient stock solutions (each g/L distilled water)-~~

Macronutrient stock solutions (each g/L distilled water)		Micronutrient stock solution (all g/L distilled water)	
CaCl ₂ . 2H ₂ O	36.76	Na-EDTA	4.36
MgSO ₄	36.97	FeCl ₃ .6H ₂ O	3.15
NaHCO ₃	12.60	CuSO ₄ .5H ₂ O	0.01
K ₂ HPO ₄	8.71	ZnSO ₄ .7H ₂ O	0.022
NaNO ₃	85.01	CoCl ₂ .6H ₂ O	0.01
Na ₂ SiO ₃ . 9H ₂ O	28.42	MnCl ₂ .4H ₂ O	0.18
		Na MoO ₄ .2H ₂ O	0.006

Table 1:
.....(Title)

Formatted: Highlight

The nutrient medium was prepared by using one ml of each of the stock macronutrient solutions and one ml of the micronutrient stock solution and making it up to one liter of distilled water. The final pH was adjusted to 7.2. Potassium phosphate solution was autoclaved separately and then added aseptically to the sterilized medium to avoid phosphate precipitation. The isolation of the algae was carried out using the moist plate method according to (Jurgensen, M.F. and C.B. Davey, 1968) [19].

Microalgae culture

A. circinalis was cultivated in MBL medium and the experiments were carried out in 500 ml Erlenmeyer pyres-glass flasks containing 200 ml of culture under controlled conditions of ambient air and at room temperature. Light was provided by cool-white fluorescent lamps at 4000 Lux with a dark/light cycle of 16:8 h for 14 days.

Cultures preparation

After culturing, the cells of *A. circinalis* were centrifugated at 5000 rpm for 30 min using angle rotor centrifuge. The supernatants were discarded. The remaining pellets were then used to test the effect of ~~their~~ the algal extracts ~~on~~ some bacteria strains [20].

Algal extraction

Dried algae biomass was mixed in a glass flask with methanol: acetone: diethyl ether 5:2:1 volumes, respectively, and shaken for 3 days at about 20°C. The mixture was separated by filtration. Then, the combined solvents were air dried to dryness and the residue ~~was~~ re-dissolved in 2 ml distilled water to form a stock solution at 50 mg/ml [21].

Microorganism strains:

For antimicrobial tests, 4 bacterial strains (*Achromobacter xylosoxidans*, *Staphylococcus aureus*, *Escherichia coli*, *Shigella dysenteriae*) were ~~shosen~~. These bacteria were obtained from the Department of Microbiology El- Bayda Hospital and were used as indicator pathogens for this study.

Antimicrobial Aactivity test

Indicator bacteria ~~are~~ swabbed on the air dried nutrient agar plates by using sterile cotton swabs [22, 23]. Sterile discs (whatman filter paper) ~~are~~ loaded with varying concentration of *A. circinalis* (25%, 50%, 75%, 100%) , Alga extract was diluted in distilled water. The sterile disc loaded with ~~discs of algal~~ extracts ~~are were~~ placed on the nutrient agar plates ~~surface~~. The discs ~~were~~ loaded with 150 µl only ~~and~~ the appropriate solvents ~~are were~~ used as controls. Plates are incubated at 37°C for 18-24 hours. Experiments were carried out under aseptic conditions. The antimicrobial activity was evaluated by ~~mesearing~~ the inhibition diameter zone (in mm) around the disc [24].

Determination of total ~~phenols~~ phenolic content

Aliquots of the extracts (1 ml) were taken in a 10 ml flask and made up to a volume of 3 ml with distilled water. Then 0.5 ml folin ciocalteu reagent (1:1 with water) and 2 ml Na₂CO₃ (20%) were added. The test solutions were warmed for 1 minute, cooled and absorbance was measured at 650 nm against the reagent used as a blank [25].

Formatted: Line spacing: Multiple 1.15 li

Formatted: Font: (Default) +Headings CS, Highlight

Formatted: Highlight

Formatted: Highlight

Formatted: Highlight

Formatted: Highlight

RESULTS:

A. circinalis extracts showed the highest level of antimicrobial activity against *Shigella dysenteriae* where was (with an inhibition zone of 1.7mm) inhibition zone. The alga extract at a concentration (100%) of 100 % was the most effective against indicator bacteria, followed by the 25% concentration (25%) where was with a zone of inhibition (of 1.2mm) of against the same bacteria. Whereas, On the otherhand, *Staphylococcus aureus* was inhibited by the extract at a concentration of 25% where was (zone of inhibition of 0.1mm) and *Achromobacter xylosoxidans* at a concentration of 50% where was (zone of inhibition of 0.2mm). This indicated that these two pathogens are were the most resistant microorganisms. The results also it shows that there is a contradiction since you mention that *Staphylococcus aureus* was inhibited by the extract at a concentration of 25% and *Achromobacter xylosoxidans* was inhibited by the extract at a concentration of 75%. The results were are illustrated in figure (1).

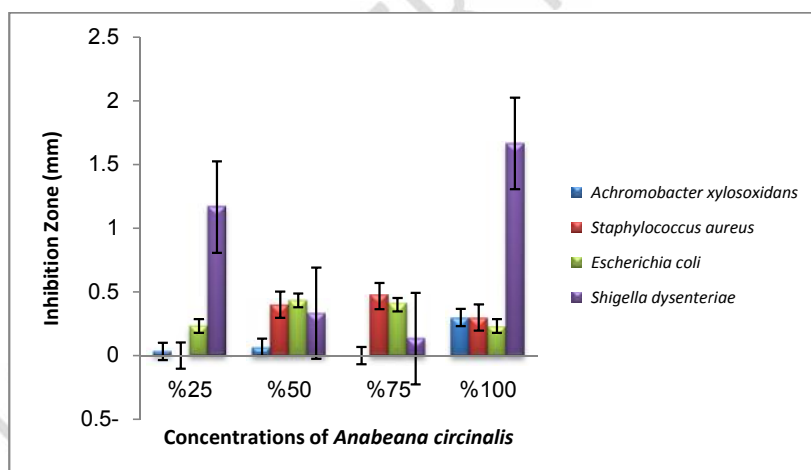


Figure 1: Inhibition zone diameter values of the *Anabaena circinalis* extracts on test microorganisms

The results also showed that the effect of out of AMP and OXA, the effect on *Shigella dysenteriae* was highest for AMP followed by OXA. While OXA was more effective than AMP against *Achromobacter xylosoxidans*. Whereas, —However, *Staphylococcus aureus* and *Escherichia coli* were resistant to both antibiotics tested (Fig. 2).

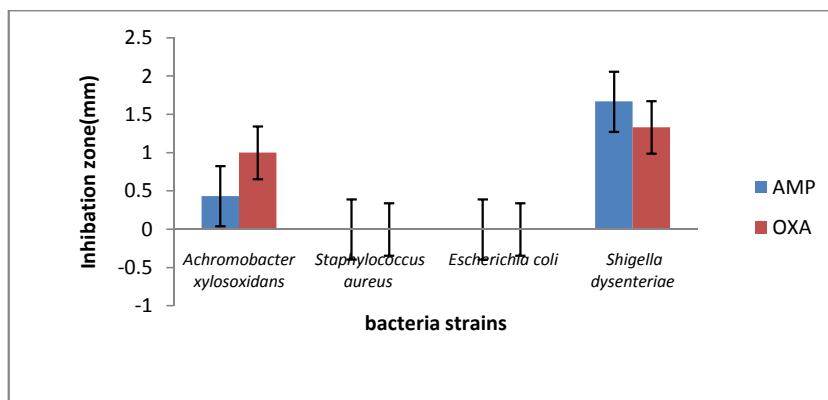


Figure (2): Inhibition zone diameter values of the AMP, OXA on test microorganisms

The results also showed that the content of total phenolic compounds in *A. circinalis* was 28.76ppm.

Discussion

Antibiotic-resistant bacteria species seriously threaten animal and human health. Clinical studies on the resistance mechanism has allowed for the defining of clinical uses of all antimicrobials [26]. Sensitive bacterium develops resistance against antimicrobials through either intrinsic or extrinsic factors. Intrinsic resistance to antibiotics is an innate property given by the bacterial genome and includes the existence of resistance genes, transformation of toxic compounds, impermeability and biofilms. Bacterial strain can also acquire resistance (Extrinsic factors) either by the uptake of exogenous genes or by mutation. The use of antimicrobial substances beyond the therapeutic dosage is one of the most common reasons for bacterial resistance [27]. Because of the growing bacterial resistance against the commercial standard and reserve antibiotics, the search for new active substances with antibacterial activity is of increasing importance [28, 29]. Most previous studies on antimicrobials from natural sources have been focused on various Cyanobacteria species such as *Spirulina platensis*, *Chroococcus* sp, *Oscillatoria* sp, *Synechocystis aquatilis*, *Anabaena* sp, *Oscillatoria limosa*, *O. limnetica* (Synonymous *Pseudoanabaena limnetica*), *Phormidium tenue*, and *Spirulina major* [30, 31]. In our study, *A. circinalis* extract at 100%

concentration, was the most active against *Shigella dysenteriae* compared with other concentrations. Whereas the same extract at 25% concentration showed the lowest activity against the same bacteria. In agreements with (Farag *et al* 2014) revealed that, aqueous and ethanol extracts of the blue green alga *A. nabaena* *circinalis* exhibited antibacterial activity against *Serratia marcescens* and *Escherichia coli* [32]. Algae are a source of many biologically ~~active-functional~~ substances, ~~including that include~~ phenolic compounds, ~~therefore algal also have~~ considerable amounts of total phenolic content ~~could be found in algal species~~. However, it is needed to keep in mind that all algal polyphenols are responsible for the health benefits. Previous study ~~was found-revealed~~ that *A. nabaena* *circinalis* and *Nostoc entophyllum* isolated from benthic and pelagic habitats at Ye ilirmak River (Turkey) the most effective cyanobacteria species in terms of antimicrobial activity[33]. Sánchez- Saavedra *et al* [34] demonstrated that all extracts of algae (*A. nabaena* *circinalis* and *N. ostoc-entophyllum*) inhibited ~~the growth of~~ *Bacillus subtilis* ~~growth~~. In the present study, antibacterial activity may vary with bacterial strain and algal extract concentration. In line with these findings, Jyotrimayee *p et al*(2014) the mechanism of action of antimicrobial agents was based on bacteria structure [35] . Similar results were reported by (Jyotrimayee *p et al* 2014 and P. S. Syed Shabudeen *et al* 2013) [35] , [36] for *Chlorella vulgaris* these authors ~~demonstarateddemonstrated that~~ the antimicrobial activity was carried out to determine inhibition against some of the common pathogen like *E. Coli*, *Klebsilla sp.*, *Bacillus sp.* and *Pseudomonas sp.* Microalgae possess the extra advantage of a substantial metabolic plasticity, dependent on their physiological state (i.e. stressed vs. non stressed); likewise, their secondary metabolism can easily be triggered by most forms of externally applied stress [37]. Regard that algae is a very interested natural source of new compounds and many of them ~~are-possess~~ antioxidant, antimicrobial and antiviral activities. Algae are very interested natural source of new compounds [38]. They are able to produce a wide range of biologically active substances with antibacterial, antiviral, antifungal, enzyme inhibiting immunostimulant cytotoxic and antiplasmodial activities [38, 39]. In this study, the effect of OXA and AMP antibiotics, was less interesting than that of algal extracts. ~~in-In~~ addition using these antibiotics may develop resistant by several pathogenic microbes. The use of algae extracts as a natural source of antibacterial compounds is an interesting alternative because of their low side effect. In a study by (Jyotrimayee *p et al*) , ~~freshwater microalgae contained bioactive antimicrobial compounds [35]. Algae are the source of many biologically active substances including phenolic compounds. In this study it has been shown that algae also have large amounts of total phenolic content and thus many antioxidants have been~~

Formatted: Highlight

Comment [MN1]: This sentence is incomplete

Formatted: Highlight

Formatted: Highlight

Comment [MN2]: Arrange these references in proper way

Formatted: Highlight

Comment [MN3]: Arrange this properly

Formatted: Highlight

~~found to have high phenol content reached to 28.76 ppm Polyphenols total phenolic content known as antioxidant agents In blue bacteria.~~ So that *A. circinalis* may also be a viable candidate for the production of compounds.

Formatted: Font: Italic

Conclusion

This study found that there is inhibition effect of alga extract on some pathogenic bacteria compared with some antibiotics. In some cases, this effect was stronger than the effect of the tested antibiotics. This effect may be due to the presence of effective compounds against tested bacteria and recommended study follow-up research in this area to identify the composition of these materials.

References:

[1]. Borowitzka M.A. (1988a). Vitamins and fine chemicals from micro-algae. In: Borowitzka M.A., Borowitzka L.J., Eds, Micro- Algal Biotechnology, Cambridge University Press, Cambridge, pp. 211-217.

[2]. Borowitzka M.A. (1988b). Fats, oils and hydrocarbons. In: Borowitzka M.A., Borowitzka L.J., Eds, Micro-Algal Biotechnology, Cambridge University Press, Cambridge, pp. 257-287.

[3]. Borowitzka M.A. (1995). Microalgae as sources of pharmaceuticals and other biologically active compounds. J. Appl. Phycol., 7: 3-15.

[4]. Nunnery JK, Mevers E and Gerwick V. Biologically active secondary metabolites from marine cyanobacteria. Curr Opin Biotechnol 2010; 21: 787-793.

Formatted: Highlight

[5]. El-Sheekh MM, Osman MEH, Dyab MA, Amer MS. Production and characterization of antimicrobial active substance from the cyanobacterium *Nostoc muscorum*. Environ Toxicol Pharmacol. 2006; 21: 42-50.

Formatted: Font: Italic

Formatted: Highlight

[6]. Gault PM and Marler HJ. Handbook on Cyanobacteria: Biochemistry, Biotechnology and Applications. New York: Nova Science Publishers 2009.

Formatted: Highlight

[7]. Volk R.B., Furkert F.H. (2006). Antialgal, antibacterial and antifungal activity of two metabolites produced and excreted by cyanobacteria during growth. Microbiol. Res., 161: 180-186.

[8]. Mundt,S.;Kreitlow,S.;Nowotny,A.and Effmert,U.(2001).Biochemical and pharmacological investigation of selected cyanobacteria. International J. Hygien and Environmental Health,203(4):327-334.

Formatted: Highlight

[9] Kellan SJ, Walker JM. Antibacterial activity from marine microalgae. British Journal of Phycology. 1989;23:41-44.

Formatted: Highlight

Formatted: Highlight

[10] Ghasemi Y, Yazdi MT, Shafiee A, Amini M, Shokravi S, Zarrini G. Parsiguine, a novel antimicrobial substance from *Fischerella ambigua*. Pharmaceutical Biology. 2004;42:318-322.

Formatted: Highlight

[11] Mayer AMS, Hamann MT. Marine pharmacology in 2001–2002: marine compounds with antihelmintic, 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. Comparative Biochemistry and Phycology, Part C. 2005;140:265-286.

[12] Cardozo KHM, Guaratini T, Barros MP, Falcão VR, Tonon AP, Lopes NP, Campos S, Torres MA, Souza AO, Colepicolo P, Pinto E. Metabolites from algae with economical impact. Comparative Biochemistry and Physiology. Part C, Toxicology & Pharmacology. 2007;146:60-78.

Formatted: Highlight

[13]. Smith G.D., Doan N.T. (1999). Cyanobacterial metabolites with bioactivity against photosynthesis in cyanobacteria, algae and higher plants. J. Appl. Phycol., 11: 337-344.

[14]. E.Taskin*, M. Ozturk, E. Taskin and O. Kurt.(2007). Antibacterial activities of some marine algae from the Aegean sea (Turkey). African Journal of Biotechnology Vol. 6 (24), pp. 2746-2751.

Formatted: Highlight

[15] . Manach, C.; Scalbert, A.; Morand, C.; Rémésy, C.; Jiménez, L. Polyphenols: Food sources and bioavailability. Am. J. Clin. Nutr. 2004, 79, 727–747.

Formatted: Highlight

[16]. Li, Y.X.; Wijesekara, I.; Li, Y.; Kim, S.K. Phlorotannins as bioactive agents from brown algae. *Process Biochem.* 2011, 46, 2219–2224.

Formatted: Highlight

[17]. Thomas, N.V.; Kim, S.K. Potential pharmacological applications of polyphenolic derivates from marine brown algae. *Environ. Toxicol. Pharmacol.* 2011, 32, 325–335.

Formatted: Highlight

[18]. Nichols, H. W. (1973). In *Handbook of Phycological Methods*, Ed. J. R. Stein, pp 16-17. Camb. Univ. Press. (R. R. L. Guillard, personal communication).

[19]. Jurgensen, M.F. and C.B. Davey, 1968. Nitrogen fixating blue-green algae in acid forest and nursery soils. *Can. J. Microb.*, 14: 1179-1179.

Formatted: Font: 14 pt, Highlight

[20]. Rippka R, Deruelles J, Waterbury J, Herdman M, Stanier R (1979). Generic assignments, strain histories and properties of pure cultures of cyanobacteria. *J. Gen. Microbiol.*, 111: 1-6.

[21]. H. Al-Wathnani, Ismet Ara*, R. R. Tahmaz, T. H. Al-Dayel and M. A. Bakir.(2012) Bioactivity of natural compounds isolated from cyanobacteria and green algae against human pathogenic bacteria and yeast . *Journal of Medicinal Plants. Research* Vol. 6(18), pp. 3425-3433

[22].L. Drago, B. Mombelli, deVecchi E., M.C. Fassina, L. Tocalli, M.R. Gismondo In vitro antimicrobial activity of propolis dry extract *J. Chemotherapy*, 12 (2000), pp. 390-395.

Formatted: Highlight

[23]. Bauer, A.W., Kirby, W.M., Sherris, J.C. and Turck, M., 1996. Antibiotic susceptibility testing by a standardized single disk method.

Formatted: Highlight

[24]. Sharma N, Gruszewski HA, Park SW, Holm DG, Vivanco JM. Purification of anisoform of patatin with antimicrobial activity against *Phytophthora infestans*. *Plant Physiol Biochem* 2004; 42: 647-655.

[25] . Wangensteen, H., A.B. Samuelsen and K.E. Malterud, 2004. Antioxidant activity in extracts from coriander. *Food Chem.*, 88: 293-297.

Formatted: Highlight

[26] . Helms M, Vastrup P, Gerner-Smidt P, Molbak K. Excess mortality associated with antimicrobial drug-resistant *Salmonella typhimurium*. *Emerg Infect Dis* 2002; 8: 490-495.

Formatted: Font: Italic

Formatted: Highlight

[27]. McDermott PF, Walker RD, White DG. Antimicrobials: modes of action and mechanisms of resistance. Int J Toxicol 2003; 22: 135-143.

Formatted: Highlight

[28]. Service R.F. (1995). Antibiotics that resist resistance. Science, 270: 724-727.

[29]. Mundt S., Kreitlow S., Jansen R. (2003). Fatty acids with antibacterial activity from the cyanobacterium *Oscillatoria redekei* HUB 051. J. Appl. Phycol., 15: 263-276.

[30]. Özdemir G, Karabay NU, Dalay MC, Pazarba B. Antibacterial activity of volatile component and various extracts of *Spirulina platensis*. Phytother Res 2004; 18: 754-757.

Formatted: Highlight

Formatted: Highlight

[31]. Demiriz T, Çökmü C, Pabuçcu K. Antimicrobial activity of some algal species belonging to cyanobacteria and chlorophyta. Chem Asian J 2011; 23 (3): 1384-1386.

[32] Farag A. Shaieb 1, Ahmed A. Issa 2*, Ahmad Meragaa(2014). Antimicrobial activity of crude extracts of cyanobacteria *Nostoc commune* and *Spirulina platensis*. Arch.of Biomed Sci ; 2 (2): 34-41.

Formatted: Highlight

[33] Yücer1, T, D , Beyat, Yı12, Pabuçcu3, K.(2018) The antiproliferative and antimicrobial effects of cultivated *Anabaena circinalis* Rabenhorts ex Bornet and Flahault and *Nostoc entophytum* Bornet and Flahault. Tropical Journal of Pharmaceutical Research; 17 (8): 1571-1577.

[34]. Sánchez-Saavedra MP, Licea-Navarro A, Bernáldez- Sarabia J. Evaluation of the antibacterial activity of different species of phytoplankton. Rev Biol Mar Oceanog 2010; 45: 531-536.

Formatted: Highlight

[35]. Jyotrimayee p, Sachidandndda D and Bastanta K.D(2014):Antibacteria activity of fresh water algae.academic journals Vol8(32)PP809.818..

Formatted: Font: Highlight

[36]. P. S. Syed Shabudeen, M. Soundrarajan and P. Indumathi, "Algae biomass growth kinetic study in wasteWater medium using spectroscopic analysis", Journal of Environmental Research And Development, vol. 7, no. 4A, April-June (2013).

Formatted: Font: Font color: Black, Highlight

[37]. Guedes AC, Amaro HM, Malcata FX. Microalgae as sources of high added-value compounds—a brief review of recent work. Biotechnology Progress. 2011; 27: 597-61

[38]. Plaza M, Santoyo S, Jaime L (2010). Screening for bioactive compounds from algae. J. Pharm. Biomed. Anal. 51:450-455.

[39]. Gasemi Y, Yazdi MT, Shafiee A, Amini M, Shokravi S, Zarrini G.(2004). Parsiguine ,a novel antimicrobial substance from *Fischerella ambigua* .Pharm. Biol. 42:318-322.

UNDER PEER REVIEW